OPTIMIZATION OF POTENTIAL ANTIBIOTIC PRODUCTION BY SALT – TOLERANT ACTINOMYCETES *STREPTOMYCES SP.* – MSU29 ISOLATED FROM MARINE SPONGE

Krishnakumar S.[1], Premkumar J.[1], Alexis Rajan R.[2] and Ravikumar S.[3]

[1]Department of Biomedical Engineering, Sathyabama University, Chennai, India
[2]Centre for Marine Science and Technology, Manonmaniam Sundranar University, Rajakkamangalam, India
[3]Department of Oceanography and Coastal area studies, Alagappa University, Karaikudi, India
drkrishnakumar_phd@yahoo.com

ABSTRACT

Marine actinomycetes, *Streptomyces sp.* – MSU29 was isolated from marine sponge *Ircinia* sp. from Cape Comorin Coast, India. Attempts were made for optimization of culture conditions for antibiotic production by *Streptomyces. sp*. The isolate exhibited optimal antibiotic production at 3% NaCl. The antibiotic production was found to be at maximum levels after 120h of incubation and thereafter gradually declined. The culture medium adjusted to pH 7.0 supported the antibiotic production compared to other pH and optimum temperature for antibiotic production was found to be 30°C. The medium supplemented with glycerol and Soybean meal as sole carbon and nitrogen sources respectively was proved to be the best for bioactive metabolites production. Among minerals tested, K_2HPO_4 displayed higher antibiotic production by the strain compared to other mineral supplements. So far only a few salt – tolerant actinomycetes have been explored for media optimization for their bioactive potential. Hence an attempt has been made to optimize culture conditions for secondary metabolites production to meet industrial demand.

Key words: antagonism, actinomycetes, antibiotics, *Streptomyces sp.*, sponge, *Ircinia sp*.

I. INTRODUCTION

Actinomycetes from extreme environments have attracted considerable attention in recent years. Majority of the studies on extremophilic organisms, however, bacteria and actinomycetes are relatively less explored group [1]. Marine actinomycetes are capable of producing potential antibiotics [2]. It is widely accepted that alkaliphilic actinomycetes will provide a valuable resource for novel products of industrial interest, including enzymes and antimicrobial agents [3, 4]. Actinomycetes are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics [5]. Marine actinomycetes have also been recently reported for their antimicrobial activity [6, 7]. Some novel antitumour antibiotics, Chinikomycin were detected in marine *Streptomyces* spp. [8]. The search for novel molecules having unique therapeutic properties continues to be an attractive area of research. In this regards, studies on extremophilic actinomycetes would be a valuable addition.

The environmental factors such as incubation period, temperature and pH moreover the nutritional source like carbon, nitrogen and minerals are found to be playing a vital role on antibiotic production by actinomycetes [9] . In this context, the study focuses on the production of bioactive metabolites by salt – tolerant actinomycetes *Streptomyces sp.* strain MSU29. Optimization of culture conditions is essential to get high yields of the bioactive metabolites. Hence an attempt has been made to increase the productivity by optimizing the culture conditions of salt – tolerant actinomycetes *Streptomyces sp.* for the production of bioactive metabolites to meet the industrial demands.

II. MATERIALS AND METHODS

A. Sample collection

Marine sponge, *Ircinia sp*. (Class: Demospongiae, Order: Dictyoceratida, Family: Spongiidae; Genus: *Ircinia*) was collected by SCUBA diving from the coast of Cape Comorin, India. The sponge sample was gently rinsed and homogenized in sterile aged sea water for the enumeration of actinomycetes.

B. Microorganism and culture media

The actinomycetes, *Streptomyces sp.* was isolated from sponge *Ircinia sp*. by spread plate method using Starch Casein Agar (SCA) Hi-media, Mumbai, India in aged seawater and distilled water (1:1) supplemented with antibiotics (Nalidixic acid

20 μg ml^{-1}; Nystatin 25 μg ml^{-1}; Cycloheximide 100 μg ml^{-1};) to minimize the gram negative bacteria, fungal and yeast contaminants respectively [10] since actinomycetes are filamentous gram positive bacteria. The inoculated agar plates were incubated at 28°C for 7 days. A typical powdery colony was picked and sub-cultured on ISP2 medium (International *Streptomyces* project). The isolate was gram positive, having a long filamentous structure and are identified as *Streptomyces sp.*-MSU29 based on the morphological, physiological and biochemical characteristics on ISP2 media [11].

C. Screening of antagonistic actinomycetes

Antagonistic activity of *Streptomyces sp.*- MSU29 was detected using Modified Nutrient Agar (MNA) medium containing glucose 5g, peptone 5g, beef extract 3g, sodium chloride 5g, agar 18g, aged sea water 500ml and distilled water 500ml was used. The final pH of the medium was adjusted to 7.0. The *Streptomyces sp.*-MSU29 was spotted on MNA medium and incubated at 30°C for 5 days still sporulation. After that, the molten Nutrient Agar (NA) (Himedia, Mumbai, India) with test organisms (10^6 cells), like gram positive organism such as *Staphylococcus aureus*, *Bacillus subtilis* and gram negative organism such as *Vibrio cholerae* and *Escherichia coli* and yeast *Candida albicans* was poured on already grown *Streptomyces sp.*-MSU29 plates separately. The inoculated plates were incubated at 37°C for 24 h. The zone of inhibition was measured for each test organism and expressed as mm in diameter. Bioactive metabolites production was determined in terms of their antimicrobial spectrum.

D. Effect of salinity and pH on the production of antibiotics

Optimum salt requirement and pH for the production of bioactive metabolites by the strain was studied by inoculating sporulated cultures spotted on MNA medium supplemented with different salt concentration ranges (0 – 5%) and pH ranges (6 – 8). After 5 days test organism in molten NA medium was poured on the plates and the zone of inhibition was measured after 24h incubation at 37°C.

E. Impact of incubation period and temperature on the production of antibiotics

The impact of incubation period and temperature for the production of antibiotic was studied by inoculating spore suspension of *Streptomyces sp.* - MSU29 on MNA medium. The effect of temperature ranges (20 – 40°C) and incubation period ranges (0 – 168h) on the production of bioactive compound was studied. At every 24h intervals under different temperature the plates were examined for the production of bioactive metabolites.

F. Role of carbon, nitrogen and mineral sources on bioactive metabolites production

To determine the role of carbon sources on antibiotic production by the strain, different carbon sources such as glycerol, dextrose, galactose, maltose, sucrose and lactose at a concentration of 1% (w/v) were added to the MNA medium. The nitrogen sources such as peptone, yeast extract, casein, soy bean meal and beef extract were amended to the MNA medium at a concentration of 1% (w/v) to determine the production of bioactive metabolites by the strain. The effect of various minerals such as K_2HPO_4, KH_2PO_4, $MgSO_4$, $ZnSO_4$, $CuSO_4$, $FeSO_4$ and $MnSO_4$ at a concentration of 0.1% (w/v) was studied by the strain on MNA medium for the production of bioactive metabolites. Final pH of the medium was adjusted to 7.0 and the cultures were incubated at 30°C.

III. RESULTS

The effect of various growth parameters of *Streptomyces sp.* - MSU29 on antibiotic production was examined on MNA medium. Optimum salinity required for antibiotic production was determined by inoculating *Streptomyces sp.* -MSU29 on MNA medium supplemented with different NaCl (0 – 5%) concentrations. Antibiotic production was started at 1% NaCl and reached maximum at 3% NaCl against all the tested organisms. When the percentage of salinity was increased, the antibiotic production was slightly decreased (Table 1).

Optimum incubation period required for maximum bioactive metabolites production was studied by the isolate to meet our demand. The antibiotic production was started after 72h of incubation and reached maximum level after 120h of incubation and thereafter gradually decreased (Table 2) against all the tested organisms.

Table 1. Effect of salinity on antibiotic production by *Streptomyces sp.* - MSU29

Salinity	Diameter of zone of inhibition (mm)				
	SA	BS	VC	EC	CA
0	–	–	–	–	–
1	7	8	9	10	8
2	13	15	17	18	14
3	20	21	24	25	20
4	18	19	22	23	18
5	12	15	18	19	13

SA – *Staphylococcus aureus,*
BS – *Bacillus subtilis, VC - Vibrio cholerae,*
EC – *Escherichia coli,* CA – *Candida albicans,*
"-" No activity

Table 2. Effect of incubation period (h) on antibiotic production by *Streptomyces sp.* - MSU29

Incubation period (h)	Diameter of zone of inhibition (mm)				
	SA	BS	VC	EC	CA
0	–	–	–	–	–
24	–	–	–	–	–
48	–	–	–	–	–
72	7	8	10	10	8
96	13	15	18	17	15
120	21	20	24	25	21
144	19	18	22	23	18
168	16	15	18	19	15

SA – *Staphylococcus aureus,*
BS – *Bacillus subtilis, VC - Vibrio cholerae,*
EC – *Escherichia coli,* CA – *Candida albicans,*
"-" No activity

When the nutrients depleted in the medium the microorganism produce extra cellular metabolites. The impact of different pH on antibiotic production was determined by inoculating *Streptomyces sp.* - MSU29 on MNA medium. Among the pH tested, pH 7.0 recorded maximum antibiotic productions against the tested organism is presented in Table 3.

Table 3. Effect of incubation period (h) on antibiotic production by *Streptomyces sp.* - MSU29

Incubation period (h)	Diameter of zone of inhibition (mm)				
	SA	BS	VC	EC	CA
0	–	–	–	–	–
24	–	–	–	–	–
48	–	–	–	–	–
72	7	8	10	10	8
96	13	15	18	17	15
120	21	20	24	25	21
144	19	18	22	23	18
168	16	15	18	19	15

SA – *Staphylococcus aureus,* BS – *Bacillus subtilis, VC - Vibrio cholerae,* EC – *Escherichia coli,* CA – *Candida albicans, "-" No activity*

The effect of temperature on antibiotic production by the strain *Streptomyces sp.* is presented in Table 4.

Table 4. Effect of temperature on antibiotic production by *Streptomyces sp.* - MSU29

Temperature (°C)	Diameter of zone of inhibition (mm)				
	SA	BS	VC	EC	CA
20	8	8	12	12	10
25	16	18	22	20	18
30	22	22	26	24	20
35	18	20	22	20	18
40	10	10	14	12	–

SA – *Staphylococcus aureus,*
BS – *Bacillus subtilis, VC - Vibrio cholerae,*
EC – *Escherichia coli,* CA – *Candida albicans,*
"-" No activity

The optimum temperature for maximum antibiotic production was noticed at 30°C against all the tested

organisms. When the temperature increased the antibiotic production was slightly decreased in the present study. The isolate was found to be strictly mesophilic for secondary metabolite production. The effect of carbon sources on antibiotic production by inoculating *Streptomyces sp.* on MNA plates having different sugars like glycerol, dextrose, galactose, maltose, sucrose and lactose at the concentration of 1% (w/v) was studied. The impact of different carbon sources on antibiotic production by the strain is presented in Table 5.

Table 5. Role of different carbon sources (1% w/v) on antibiotic production by *Streptomyces sp.* – MSU29

Carbon sources (1%w/v)l	Diameter of zone of inhibition (mm)				
	SA	BS	VC	EC	CA
Glycerol	24	26	30	28	22
Dextrose	20	22	26	24	20
Galactose	8	10	12	10	8
Maltose	18	20	22	20	10
Sucrose	10	12	10	12	–
Lactose	8	10	8	10	–

SA – *Staphylococcus aureus,* BS – *Bacillus subtilis, VC - Vibrio cholerae,* EC – *Escherichia coli,* CA – *Candida albicans, "-" No activity*

Among the carbon sources tested glycerol supplemented with MNA medium provided to be the best for antibiotic production by the strain followed by dextrose, maltose, and galactose against bacterial pathogens. Carbon sources like sucrose and lactose were found to be negative effect of antibiotic production by the strain against *Candida albicans*. The effect of nitrogen source on antibiotic production was examined on MNA medium having different organic N_2 sources like peptone, yeast extract, casein, soy bean meal and beef extract were studied. The role of different N_2 sources on antibiotic production by the strain is given in Table 6.

Table 6. Role of different N_2 sources (1% w/v) on antibiotic production by *Streptomyces sp.* – MSU29

N_2 sources (1%w/v)	Diameter of zone of inhibition (mm)				
	SA	BS	VC	EC	CA
Peptone	10	12	16	18	20
Yeast	12	14	22	22	26
Casein	8	10	12	12	14
Soybean meal	20	20	28	24	26
Beef Extract	12	10	18	16	18

SA – *Staphylococcus aureus,* BS – *Bacillus subtilis, VC - Vibrio cholerae,* EC – *Escherichia coli,* CA – *Candida albicans, "-" No activity*

Among the N_2 sources studied soy bean meal amended MNA medium provided to be the best for antibiotic production by the strain followed by yeast extract, peptone and beef extract. N_2 source like casein was found to be moderately supported the antibiotic production by the strain against the tested pathogens. The role of minerals on antibiotic production was determined by inoculating *Streptomyces sp.* - MSU29 on MNA medium is presented in Table 7.

Table 7. Role of minerals (1% w/v) on antibiotic production by *Streptomyces sp.* – MSU29

Minerals (0.1%w/v)	Diameter of zone of inhibition (mm)				
	SA	BS	VC	EC	CA
K_2HPO_4	24	22	26	26	20
KH_2PO_4	20	18	22	20	18
$MgSO_4$	22	20	24	24	18
$ZnSO_4$	–	–	–	–	–
$CuSO_4$	–	–	–	–	–
$FeSO_4$	10	12	18	16	14
$MnSO_4$	–	–	–	–	–

SA – *Staphylococcus aureus,* BS – *Bacillus subtilis, VC - Vibrio cholerae,* EC – *Escherichia coli,* CA – *Candida albicans, "-" No activity*

Among different minerals tested, K_2HPO_4 showed positive effect on antibiotic production followed by $MgSO_4$, KH_2PO_4 and $FeSO_4$ as a sole mineral source. Antibiotic production was totally absent in the medium supplemented with $ZnSO_4$, $CuSO_4$ and $MnSO_4$ by the isolate.

IV. DISCUSSION

The media optimization is an important aspect to be considered in the development of fermentation technology. This was achieved by a systematic study on the suitability of various nutritional and physical parameters required for the growing microbes. The isolate *Streptomyces* sp. characterized in our present study exhibited maximum antibiotic production at 3% NaCl concentration. The results of the study correlate with the earlier finding [12]. However, certain researchers had reported that the maximum antibiotic production for marine actinomycetes isolated from Bay of Bengal, India, exhibited maximum production at 5% NaCl in the production medium [13]. The antibiotic production by the isolate was started after 72h of incubation and reached maximum level after 120h of incubation. The *Streptomyces* sp. isolated from terrestrial soil exhibited maximum antibiotic production after 120h, which coincided with stationary phase of the culture [14]. Among the pH tested, pH 7.0 recorded maximum antibiotic productions against the tested organism. The pH of the medium plays an important role in the microorganism by causing certain morphological changes, enzymes and extracellular metabolites secretion [15]. The acidic pH affects the antibiotics production, since actinomycetes are neutrophiles for growth and antibiotics production in production medium [16,17]. When the pH increased to alkaline the antibiotic production gradually declined. The strain was found to be strictly neutrophilic for secondary metabolites production, extreme pH were unfavorable for antibiotic production. The new marine *Streptomyces* sp. BT- 408 showed optimal pH 7.2 for antibiotic production [18]. The optimum pH for antibiotic production by *S. hygroscopicus* DI-5 strain is 7.0 [19]. However some *Streptomyces* sp. isolated from soil recorded to secrete antibiotics against bacteria, fungi and yeast at alkaline pH [20]. The optimum temperature for maximum antibiotic production was noticed at 30°C in the present study by the strain. The antifungal antibiotic production was maximum at 30°C by *Thermomonospora* sp MTCC 3340 [21]. The growth and regulation of secondary metabolites in *Streptomyces* sp. isolated from virgin soil were recorded maximum antibiotic production at 30°C [22]. These findings correlated with our present study. A study on the production of antibiotics usually involves a search for optimal media. Media optimization is achieved by a systematic study of a large number of carbon, nitrogen and mineral sources. In the present study glycerol (1%), soy bean meal (1%) and K_2HPO_4 (0.1%) as a sole nutrient source showed positive effect on antibiotic production by the strain. Carbohydrates such as glycerol, maltose, mannose, sucrose and xylose have been reported to be interfering with the production of secondary metabolites in actinomycetes [23]. The antibiotic productions on synthetic media are unsatisfactory but the medium supplemented with a minimal amount of soy bean meal (0.5%) has been supported growth and antibiotic production by *Streptomyces* sp. [24]. 1% glycerol supported better chloramphenicol production by *Streptomyces sp*. [25]. This is also true of the strain *Streptomyces sp* – MSU29 used in the present study. Carbon and nitrogen sources influence for actinorhodin antibiotic production by *S coelicolor* [26]. Similarly, pristinamycin production by *S. pristinaespiralis* has been governed by N_2 sources [27]. The basal media amended with K_2HPO_4 was found to be suitable for maximum antimicrobial metabolites production by the strain *S. albidoflavus* [28]. Likewise, K_2HPO_4 is required for maximum yield of neomycin by *S. fradiae* [29] whereas, metals like $MnSO_4$, $ZnSO_4$ and $CuSO_4$ are no effect on neomycin production. This is also agreed by the strain *Streptomyces sp.* - MSU29 used in the present study.

V. CONCLUSION

The objective of the present investigation was to optimize the culture conditions for the antibiotics production by salt – tolerant antinomycete *Streptomyces* sp. – MSU29 strain. The antibiotic production was reached maximum levels after 120h of incubation and pH at 7.0 with an optimum temperature of 30°C. The isolate exhibited optimal antibiotic production at 3% NaCl concentration. The media supplemented with glycerol (1%), soy bean meal (1%) and K_2HPO_4 (0.1%) showed positive effect on antibiotic production by the strain. Apart from normal actinomycetes, the salt – tolerant actinomycetes are much less explored group of microbes for their antimicrobial potential. The media optimization for

antibiotic production from marine forms is still infancy. Hence an attempt has been made the optimum level of culture conditions and composition of media for optimal antibiotic production by the strain *Streptomyces sp.* - MSU29 has been developed in the present study.

ACKNOWLEDGEMENT

The authors are thankful to Centre for Marine Science and Technology (CMST), Manonmaniam Sundranar University, Rajakkamangalam, India, for providing all the needed facilities to carry out this work successfully.

REFERENCES

[1] P.R. Jensen, and C. Mafnas, 2006. Biogeography of the marine actinomycete *Salinispora* . *Environ. Microbiol*. 8(11):1881-1888.

[2] N.K. Asha Devi, M Jeyarani and K Balakrishnan 2006. Isolation and Identification of Marine Actinomycetes and their Potential Antimicrobial activiy. Pak. J. Biol. Sci., 9(3): 470-472.

[3] S.Mitsuiki, M.Sakai, Y.Moriyama, M.Goto, and K.Furukawa, 2002 Purification and some properties of keratinolytic enzyme from an alkaliphilic *Nocardiopsis sp*. TOA-1. *Biosci. Biotechnol. Biochem*.. 66:164-167.

[4] H.Tsujibo, T.Kubota, M.Yamamoto, K.Miyamoto, and Y.Inamori, 2003. Characteristics of chitinase genes from an alkalophilic actinomycete, *Nocardiopsis prasina* OPC-131. *Appl. Environ Microbiol*. 69: 894-900.

[5] M.Valan Arasu, V.Duraipandiyan, P Agastian and S.Ignacimuthu 2009 In vitro antimicrobial activity of *Streptomyces* spp. ERI-3 isolated from Western Ghats rock soil (India) J. Medical Mycol. 19(1): 22-28.

[6] H. P Fiedler. et al., 2005. Marine actinomycetes as a source of novel secondary metabolites. Antonie Van Leeuwenhoek. 87: 37-42.

[7] C. R Kokare, K. R Mahadik, S. S Kadam, and B. A. Chopade, 2004. Isolation, chatacterization and antimicrobial activity of marine halophilic *Actinopolyspora* species AH1 from the west coast of India Curr. Sci., 86: 593-597.

[8] F Li, et al. 2005 Chinikomycins A and B; isolation, structure elutidation and biological activity of novel antibiotics from a marine *Streptomyces* sp isolate M045. J. Nat. Prod., 68: 349-353.

[9] M Himabindu, and A. Jetty, 2006. Optimization of nutritional requirements for gentamicin production by *Micromonospora echinospora*. Indian J. Exp. Biol., 44: 842-848.

[10] J.Ravel, M.J Amoroso, R.R Cowell and R.T. Hill, 1998 Mercurry resistant actinomycetes from the Chesapeake Bay . *FEMS Microbiol. Lett*.,. 162: 177 – 184.

[11] E. B Shirling and D. Gottileb, 1966. Methods for characterization of *Streptomyces* species. J. Syst. Bacteriol., 16: 313-340.

[12] S.H Vasavada, J.T Thumar, S.P Singh, 2006 Secretion of a potent antibiotic by salt – tolerant and alkaliphilic actinomycete *Streptomyces sannanensis* strain RJT-1. *Curr. Sci*.. 9(10): 1393-1397.

[13] M Saha, *et al.,* 2005. Studies on the production and purification of an antimicrobial compounds and taxonomy of the producer isolated from the marine environment of the Sundarbans. Appl. Microbiol. Biotechnol., 66: 497-505.

[14] K.J.P Narayana, P. Prabhakar, M. Vijayalakshmi, Y.Venkateswarlu, and P.S.J Krishna, 2007. Biological activity of phenylepropionic acid from a terrestrial Streptomycetes. Polish J. Microbiol., 56: 191-197.

[15] Takashi Nakamura., Takuya Nihira., and Yasuhiro Yamodo, 2006.Identification of two metabolites induced by butyrolactone autoregulator IM-2 in *Streptomyces* sp.FRI-5.*FEMS Microbiol lett*. 124(3): 307-313.

[16] Mohamed I. Ali, S.Maged, I Ahmad, and Wael N Hozzein 2009. WA52-A Macrolide Antibiotic Produced by Alkalophile *Nocardiopsis Dassonvillei* WA52**.** Aust. J. Basic & Appl. Sci., 3(2): 607-616.

[17] K Suthindhiran, and K.Kannabiran, 2009. Cytotoxic and Antimicrobial Potential of Actinomycete Species *Saccharopolyspora salina* VITSDK4 Isolated from the Bay of Bengal Coast of India. Am. J. of Inf. Disease 5(2) : 90- 98.

[18] P.Sujatha, K. V. V. S. N. Bapiraju, and T. Ramana, 2005. Studies on a new marine Streptomycete BT – 408 producing polyketide antibiotic. Microbiol. Res., 160: 119-126.

[19] B.K.Bhattacharyya, S.C Pal, and S. K. Sen, 1998. Antibiotic production by *Steptomyces hygroscopicus* D1-5. Cultural effect. Rev. Microbiol., 29: 36-41.

[20] A.Basilio, I Gonzalez., M. F. Vicente, J.Gorrochategui, A. Cabello, A. Gonzalez, and O. Genilloud, 2003. Patterns of antimicrobial activities from soil actinomycetes isolated under different conditions of pH and salinity. J. Appl. Microbiol., 95: 814.

[21] G Monali, and K Pushpa, 2003. A study of antifungal antibiotic production by *Thermomonospora* sp MTCC 3340 using full factorial design. J. of Chem. Technol. & Biotechnol . 78 (6): 605-610.

[22] M.J. Bibbs, 2005. Regulation of secondary metabolites in Streptomycetes. Curr. Opin. Microbiol., 8: 208-215.

[23] A.L Demain, and A.Fang, 1995. Emerging concepts of secondary metabolites in actinomycetes. Actinomycetology, 9: 98-117

[24] V.Gesheva, V. Ivanova, and R. Gesheva, 2005. Effect of nutrients on the production of AK-111-81 macrolide antibiotic by *Streptomyces hygroscopicus*. Microbiol. Res., 160: 243-248.

[25] M Legator, and D. Gottlieb, 1953. The dynamics of chloramphenicol synthesis. Antibiot. Chemother., 3: 809- 817.

[26] G.Hobbs, C. M. Frazer, D. C. J Gardner, F Flett, and S. G. Oliver, 1990. Pigmented antibiotic production by *Streptomyces coelicolor* A3 (2): Kinetics and the influence of nutrients. J. Gen. Microbiol., 136: 2291-2296.

[27] V.Francois, and A. Stephane, 2001. Nitrogen source governs the patterns of growth and pristinamycin production in *Streptomyces pristinaespiralis*. Microbiology, 147: 2447-2459.

[28] K.J.P. Narayana and M.Vijayalakshmi, 2008. Optimization of Antimicrobial metabolites Production by *Streptomyces albidoflavus.* Res. J. Pharmacol., 2(1): 4-7.

[29] M.K. Majumdar and S.K Majumdar, 1965. Effect of minerals on neomycin production by *Streptomyces fradiae*. Applied Microbiology, 13: 190-193.