International Journal of Drug Development & Research | Jan-March 2010 | Vol. 2 | Issue 1 |
ISSN 0975-9344
Available online http://www.ijddr.com

Original Research Manuscript

STUDY ON THE DEVELOPMENT OF HIGH YIELDING NEOMYCIN RESISTANT STRAIN OF *STREPTOMYCES FRADIAE* FOR IMPROVED PRODUCTION OF NEOMYCIN BY USING OPTIMAL LEVELS OF MINERALS

R HAQUE*AND S MONDAL,

S MONDAL Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India.
R HAQUE* Department of Biotechnology Technology, Institute of Technology & Marine Engineering , DH Road, West Bengal,India

ABSTRACT

We have developed an efficient high yielding neomycin resistant Strain of Streptomyces fradiae for improved production of neomycin. Streptomyces fradie was submerged in the solution of10 ug/ml, 15 ug/ml, 20 ug/ml,30 ug/ml,40 ug/ml,50 ug/ml, 60 ug/ml,70 ug/ml, 80 ug/ml, for five day for screeing out high yielding Neomycin resistant Strain of Streptomyces fradiae . Streptomyces fradie in the solution of 70 ug/ml was the high yielding Neomycin resistant Strain of Streptomyces fradiae It was same observed as prevous authors that optimal levels of the elements Ca, Fe, and Zn per milliliter of a synthetic medium for neomycin production . K_2HPO_4 was required at a concentration of 0.1% for maximal yield of neomycin but observed improved production of neomycin. It was observed 252 ug/ml, neomycin production.
Key words: *Neomycin, Streptomyces fradia, high yielding Neomycin resistant Strain, Fermentation.*

Introduction

Development of high yielding neomycin resistant Strain of Streptomyces fradiae is required for industrial production. Although many studies of the mineral requirements of microorganisms for growth have been reported, there have been only a few detailed investigations on the requirements of actinomycetes for metals in the biosynthesis of antibiotics probably because of the difficulty of preparing a metal-free medium[1,2,3,4,5,6,7], and also because requirements of metals for antibiotic production vary with the basal medium[8]. Requirements for metals like Fe, Zn, Mg, and Ca for neomycin production were demonstrated [9,10] that in media containing a high concentration (2.0%) of glutamic acid. In the present work, an extensive study has been made on the mineral requirements of Streptomyces fradiae for neomycin biosynthesis in a synthetic medium. The strain was previously shown to produce 90% neomycin B and 10% neomycin C in the basal medium used in the study.In our present observation , high yielding neomycin resistant Strain of *Streptomyces fradiae* was shown higher production than prevous observation[7].

Correspondence: Dr R Haque, Jadavpur University, Kolkata,WB,Email id: ruuubil_haque@yahoo.com

Materials and Methods

In present work, we have used same media composition which was reported by previous authors. The neomycin-producing culture of S. fradiae [7,11] was used throughout the study for neomycin biosynthesis. Streptomyces fradia was submerged in the solution of 10 ug/ml, 15 ug/ml, 20 ug/ml,30 ug/ml,40 ug/ml,50 ug/ml, 60 ug/ml,70 ug/ml, 80 ug/ml, for five day for screening out high yielding neomycin resistant Strain *of Streptomyces*

fradiae . *Streptomyces fradiae* in the solution of 70 ug/ml was the high yielding neomycin resistant Strain of *Streptomyces fradiae* The culture was maintained on a potato-dextroseagar slant at 28 C and was subcultured at monthly intervals. The effects of different minerals were studied in the medium consisting of: glucose, 10.0 g; glutamic acid, 2.0 g; K_2HP0_4, 1.0 g; NaCl, 5.0 g; $MgSO_47H_20$, 0.5 g; CaCl2-2 H_20, 0.04 g; $FeSO_4$ $7H_20$, 0.005 g; $MnSO_4$ $4H_20$, 0.005 g; ZnSO4 $7H_20$,0.005 g; water, 1,000 ml; pH 7 . Triple glass distilled water was used throughout the experiments. In a typical experiment, the inorganic salt under test was first omitted from and then added to the basal medium in graded doses in separate flasks to determine optimal concentration. In each subsequent experiment, the composition of the basal medium was so altered as to include an optimal amount of a mineral. Chemicals for the medium were of analytical-reagent grade and were obtained from E. Merck,Qualigens and High Media. Sugar, amino acid, and K_2HP0_4 were further made free from trace elements by the following method[12] 4 g of glutamic acid, 10 g of K_2HP0_4, and 10 g of glucose were each dissolved separately in 200 ml of triple glass-distilled water, and the resulting solution was shaken twice with a mixture of 0.1 g of 8-hydroxyquinoline and 5 ml of chloroform in a separating funnel, first at pH 7.2 and then at pH 5.2. After each extraction, the solution was washed three times with 5 ml and then once with 10 ml of chloroform to free the medium from traces of 8-hydroxyquinoline. Clean Pyrex glassware was used throughout the study. Solutions of glucose, glutamic acid, phosphate, and a mixture of other minerals were sterilized separately. The medium was dispensed in 20-ml volumes in 100-ml Erlenmeyer flasks and inoculated with 0.05 ml of spore suspension. Inoculum was obtained by making a suspension in 2 ml of sterile water of spores which were harvested from slant cultures of potato dextrose-agar grown at 28 C for 10 days. Flasks were inoculated at night and kept stationary; shaking was not started until early the 190 next morning[13]. This was done to minimize the adherence of spores at the flask wall and to facilitate formation of a uniform spore layer at the surface. Flasks were incubated at 28 C on a rotary shaking machine (65 rpm; eccentricity, 2.5 cm). After fermentation, the antibiotic potency of the broth was determined by a conventional agar diffusion (cup-plate) method of assay against the sensitive organism Bacillus subtilis (strain B3). results are expressed in terms of micrograms of neomycin per milliliter of broth, calculated with reference to a standard curve[7]. Growth was determined by measuring dry weights of cells. The mycelium was collected by filtration of the cultures through filter-paper discs which had previously been dried for 24 hr at 70 4 5 C. After filtration, the paper discs were dried again at 70 ± 5 C and weighed [14]. To check the variation of weight of filter paper, a blank was also prepared in each set. A digital pH meter was used for pH measurement.

Results and Discussion

The effects of salts, such as K_2HP0_4 and NaCl,on neomycin production and of the elements Ca, Mn, Fe, Zn, and Cu on growth of neomycin resistant strain of *Streptomyces fradiae* and neomycin production are shown in Tables 1 to 6. Since there were no appreciable changes in pH of the broth with increasing concentration of metals, except iron, the data on pH changes during the fermentation process have only been reported with respect to different concentrations of the iron[7]. Obsevation is same as prevous author but production of neomycin is much higher by use of Neomycin resistant Strain of *Streptomyces fradiae* The optimal concentration of K_2HP0_4 for neomycin formation in the synthetic

medium is 0.1 %, and NaCl is without any effect[7]. NaCl was included in the study because [15]showed that NaCl helps in the release of bound streptomycin from the mycelium. It is of interest that higher concentrations of phosphate interfere with neomycin synthesis.This type of behavior of phosphate has TABLE 1. Effect of $K_2HP0_4$and NaCl on neomycin production by Streptomyces fradiae .Concentration of neomycin (ug/ml) also been observed with regard to streptomycin biosynthesis [16].Tables 2 to 6 indicate that the elements Ca,Mn Fe, and Zn are required at 10.8, 1.250, 2.00 , and 0.02 ug/ml, respectively, for maximal neomycin production, whereas Mn and Cu have no effect either on growth of the organism or on neomycin formation .Our observation is same as prevous author but production is much higher by use of neomycin resistant Strain of *Streptomyces fradiae*.We have used same media compostion but the production is much higher by use of Neomycin resistant Strain of *Streptomyces fradiae* . some authors reported that ,Of the metals Ca, Fe, and Zn, only the first two are essential for growth of the organism, although the optimal concentrations for growth and antibiotic,production are different[7]. . One interesting feature of this investigation[7]. is the effect of iron on pH changes of fermentation broth at different periods of incubation. There was an increase in acidity of the medium with low iron concentrations up to the 7th day of fermentation, but there was no appreciable change in pH of the medium at a dose of 0.2 ,ug/ml or more of Fe . It is possible that there is a disturbance of normal metabolism of the organic acids at low levels of iron[23].We have observed same as prevous author. Such an acid accumulation was reported by some author [17] in case of an actinomycete, Nocardia opaca, in iron-deficient medium. Some authors reported that accumulation of acetic acid in certain S. fradiae strains grown poorly buffered medium[18].

An iron-containing respiratory pigment b-type cytochrome was obtained in S. fradiae [19,20,21]which could oxidize succinate in vitro. It was reported by [7,22] that ,It is probable that b-type cytochromes may have some role in utilization of some acids produced during oxidation of glucose. We found that acidity did not develop in an iron-deficient medium containing a small amount of glucose . Studies of the effect of Zn on neomycin biosynthesis show that a low dose of Zn has a remarkable stimulating effect on neomycin formation without affecting the growth of the organism. At higher concentrations of Zn (0.23 ,g/ml or more), there is a rapid destruction of neomycin after the 5th day of fermentation, whereas there is no such effect in the medium with low Zn contentWe have observed same result.The effect of Zn on the destruction of neomycin can also be observed from the results in Tables 1 to 4, as the basal medium used in the study contained high dose of Zn (1.15 ,ug/ml). It is possible that Zn has a role in the activity of the probable enzyme neomycinase which is responsible for the destruction of neomycin. Again, as a stimulator of neomycin synthesis, Zn may be involved in the enzyme system utilized at some stages of neomycin biosynthesis[7]. As the initial step in the investigation of neomycin biosynthesis, the present work has helped in the selection of a suitable synthetic medium, consisting of: glucose, 10.0 g; glutamic acid, $K_2HP0_4$2.0 g; , 1.0 g; $MgSO_4$-$7H_20$, 0.5 g; $CaCl_2$- $2H_20$,0.04 g; $FeSO_4$.7H20, 0.005 g; $ZnSO_4$-$7H_20$, 0.0005 g; water, 1,000 ml; It was observed maximum 252 ug/ml neomycin production.

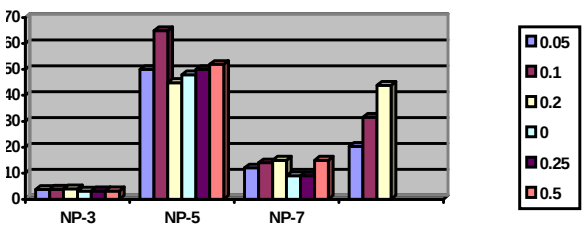
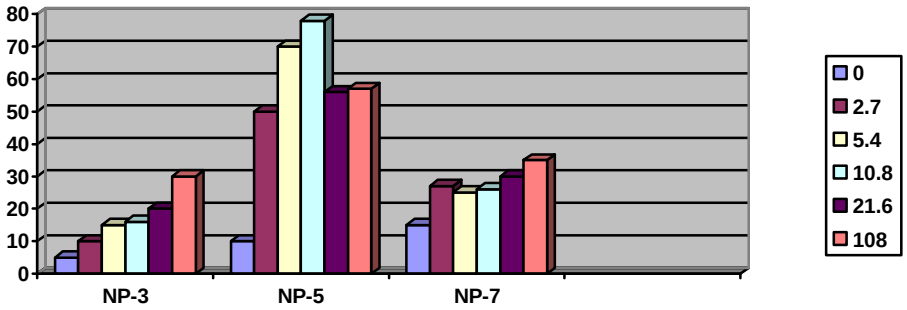


Fig 1. Effect of K2HP04 and Nacl on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Table 1: Effect of K2HP04 and Nacl on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Salt	Concentration (gm/100 ml)	Neomycin(*ug/ml*		
		3rd day	5th day	7th day
K2HP04	0.05	3.7	50	12
K2HP04	0.1	3.8	65	14
K2HP04	0.2	3.9	45	15
Nacl	0.0	3.10	48	9
Nacl	0.25	3.11	50	9
Nacl	0.5	3.12	52	15



NP-3 (Neomycin production(ug/ml) *3rd day),*
NP-5 Neomycin production(ug/ml*)* 5 th day
NP-7 Neomycin production(ug/ml*)*7 th day

Fig 2. Effect of calcium (added as CaC12.2H20) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Table 2. Effect of calcium (added as CaC12.2H20) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Concentration of Ca(*ug/ml)*	Neomycin production (*ug/ml)3rd day*	Neomycin production (*ug/ml)5 th day*	Neomycin production (*ug/ml) 7th day*
0.0	5.0	10	15
2.7	10	50	27
5.4	15	70	25
10.8	16	78	26
21.6	20	56	30
108	30	57	35

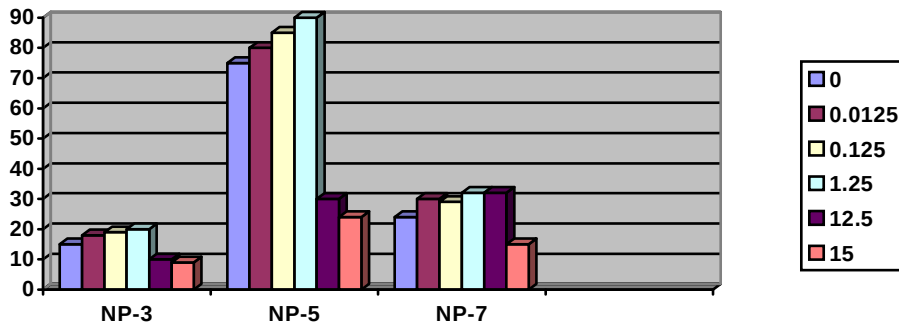


Fig 3. Effect of manganese (added as MnSO4,4H20) on neomycin production by

neomycin resistant strain Streptomyces fradiae at pH 7

Table 3. Effect of manganese (added as MnSO4,4H20) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Concentration Of Mn(*ug/ml)*	Neomycin production(*ug/ml) 3rd day*	Neomycin production(*ug/ml) 5 thd day*	Neomycin production(*ug/ml) 7th day*
0.000	15	75	24
0.0125	18	80	30
0.125	19	85	29
1.250	20	90	32
12.50	10	30	32
15.00	9	24	15

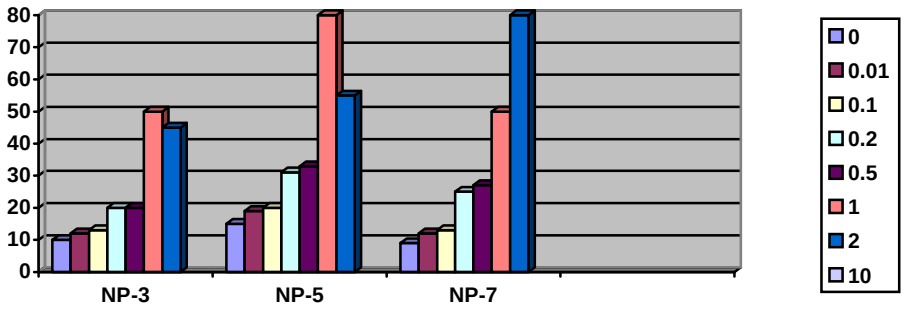


Fig 4.. Effect of iron (added as FeS04- 7H20) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Table 4. Effect of iron (added as FeS04- 7H20) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Concentration of Fe *(ug/ml)*	Neomycin production (3rd day)	Neomycin production (5th day)	Neomycin production (7th day)
0.00	10	15	9
0.01	12	19	12
0.10	13	20	13
0.50	20	31	25
1.00	20	33	27
2.00	50	80	50
10.0	45	55	30

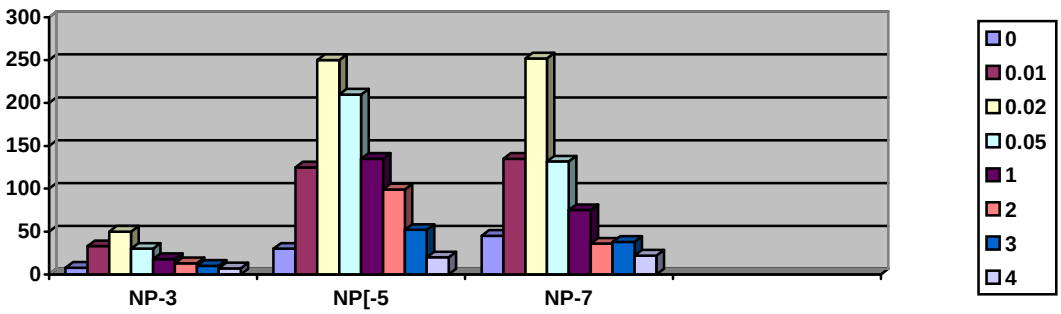


Fig 5. Effect of Zinc (added as $ZnS0_4$- $7H_20$) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Table 5. Effect of Zinc (added as $ZnS0_4$- $7H_20$) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Concentration of Zn (*ug/ml)*	Neomycin production (3rd day)	Neomycin production (5th day)	Neomycin production (7th day)
0.00	8	30	45
0.01	33	125	135
0.02	50	250	252
0.05	30	210	132
1.00	18	135	75
2.00	13	99	36
3.00	10	52	38
4.00	7	20	22

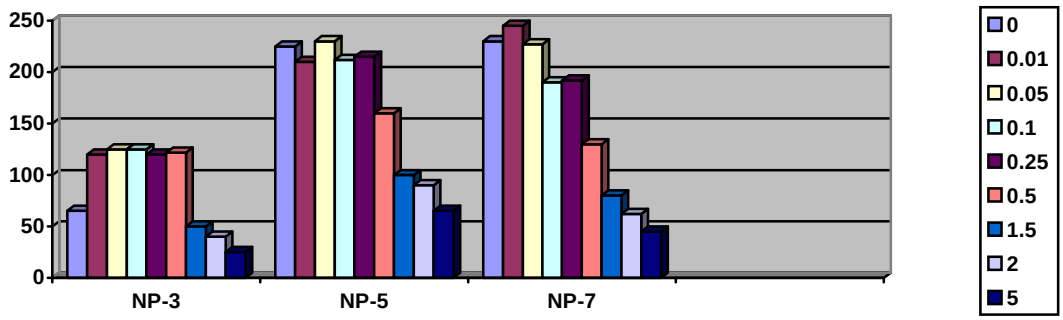


Fig 6.. Effect of Cu (added as $CuS0_4$- $5H_20$) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Table 6. Effect of Cu (added as $CuS0_4$- $5H_20$) on neomycin production by neomycin resistant strain Streptomyces fradiae at pH 7

Concentration of Cu (*ug/ml)*	Neomycin production (3rd day) (*ug/ml)*	Neomycin production (5th day(*ug/ml)*	Neomycin production (7th day) (*ug/ml)*
0.00	65	225	230
0.01	120	210	245
0.05	125	230	227
0.100	125	212	190
0.25	120	215	192
0.50	122	160	130
1.50	50	100	80
2.00	40	90	62
5.00	25	65	45

Acknowledgments

We wish to thank the Director Dr Professor S. Chattapadhaya of my institute The Institute of Technology & Marine Engineering for his moral support and blessings and I also express my heartful thanks to him for motivating me with strength and spirit.

References

1) *Acker, R. F., and H. Lechevalier. 1954. Some nutritional requirements of Streptomyces griseus3570 for growth and candicidin production. Appl.Microbiol. 2:552-157.*

2) *Reynolds, D. M., and S. A. WaksmanA. 1948. Gresein, an antibiotic produced by certain strains of Streptomyces griseus. J. Bacteriol. 55:738-752.*

3) *Thornberry, H. H., 1948. The role of minerals in production of streptomycin by Streptomyces griseus. Phytopathol. 38:26.*

4) *Thronberry, H. H., and H. W. Andersor. 1948. Synthetic medium for Streptomyces griseus and the production of streptomycin. Arch. Biochem. 16:389-397.*

5) *Chesters, C. G. C., and G. N. Rolinson. 1951. Trace elements and streptomycin production. J. Gen. Microbiol. 5:559-565.*

6) *Gallicchio, V., and D. Gottlieb. 1958. The biosynthesis of chloramphenicol III. Effects of micronutrients on synthesis. Mycologia 50:490-496.*

7) *Katz, E., P. Ppienta, and A. Sivak. 1958. The role of nutrition in the synthesis of actinomycin.Appl. Microbiol. 6:236-41.*

8) *Majumdar M. K. , and Majumdar S. K..1965. Effects of minerals on neomycin production by Streptomyces fradiae. Appl. Microbiol. 13:190-193.*

9) *Villemin, P. F., H. A. Lechevalier, and S. A. Waksman. 1953. Antibiotics of actinomycetes with special reference to their role in the physiology of the organisms producing them. Symp.Actinomycetales, Rome, p. 168.*

10) *Dulmage, H. T. 1951. The production of neomycin by S. fradiae. Ph.D. Thesis, Rutgers Univ., New Brunswick, N.J.*

11) *Nickerson, W. J., and R. R. Mohan. 1953. Nutrition trition and metabolism of Streptomyces. Int. Congr. Microbiol., 6th, Rome, p. 137-47.*

12) *Waksman, S. A., and H. A. . Lechevalier. 1949. Neomycin, a new antibiotic active against streptomycin resistant bacteria, including tuberculosis organisms. Science 109:305-307.*

13) *Majumder, S. K., and S. K. Bose. 1960. Trace element requirements of Bacillus subtilis for Mycobacillin formation. J. Bacteriol. 79:564-565.*

14) *Ratledge, C., and F. G. Winder. 1962. The accumulation of salicylic acid by mycobacteria during growth on an iron-deficient medium. Biochem. J. 84:501-506.*

15) *Waksman, S. A. 1953. Neomycin, p. 41. Rutgers University Press, New Brunswick, N.J.*

16) *Perlman, D., and A. F. Langlykke. 1949. Methods for the extraction of streptomycin from fermentation media. Abstr. 116th Meeting Amer. Chem. Soc., p. 18A-19A.*

17) *16.Woodruff, H. B., and M. Ruger. 1948. Studies on the physiology of a streptomycin-producing strain of Streptomyces griseus on proline medium.*

18) *Webley, D. M. 1960. The effect of deficiency of iron, zinc and manganese on the growth and morphology of Nocardia opaca. J. Gen.Microbiol. 23:87-92.*

19) *Waksman, S. A., and D. A. Harris. 1949. Neomycin- production and antibiotic properties. J. Clin. Invest. 26:934-939.*

20) *Heim, A. H., W. S. Silver, and Y. Birk. 1957. Cytochrome composition of some strains of Streptomyces. Nature 180:608-609.*

21) *HeimI, A. H., and H. A. Llechevalier. 1956. Effect of iron, zinc, manganese and calcium on the growth of various strains of Streptomyces.Mycologia 48:628-36.*

22) *Birk, Y., W. S. Silver , and A. H. Heim. 1957. A b-type cytochrome from Streptomyces fradiae. Biochim. Biophys. Acta 25:227-228.*

23) *Reynolds, D. M., and S. A. WaksmanA. 1948. Gresein, an antibiotic produced by certain strains of Streptomyces griseus. J. Bacteriol. 55:738-752.*

24) *Winder, F. G., and C. O'hara. 1962. Effects of iron deficiency and of zinc deficiency on the composition of Mycobacterium smegmatis. Biochem. J. 82:98-108. J. Bacteriol. 56:315-321.*

****

Article History: ----------

Date of Submission: 23-01-10

Date of Acceptance: 25-03-10

Conflict of Interest: NIL

Source of support: NONE