IJPSR (2013), Vol. 4, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 April, 2013; received in revised form, 20 May, 2013; accepted, 15 July, 2013; published, 01 August, 2013

PRODUCTION OF ANTIBIOTIC FROM ACTINOMYCETES ISOLATED FROM NAGPUR REGION AND OPTIMIZATION OF PARAMETERS TO INCREASE THE YIELD

Rita N. Wadetwar*[1] and Arun T. Patil [2]

J.L. Chaturvedi College of Pharmacy [1], Electronic Zone Building, Hingna Road, Nagpur 440016, Maharashtra, India
Department of Pharmaceutical Sciences R.T.M. Nagpur University [2], Nagpur 440033, Maharashtra, India

Keywords:

Nagpur region, Fermentation parameters, Antibiotic, *Streptomyces violaceorubidus*

Correspondence to Author:

Rita N. Wadetwar

Assistant Professor, J. L. Chaturvedi College of Pharmacy, Electronic Zone Building, Hingna Road, Nagpur 440016, Maharashtra, India

E-mail: nywadetwar@gmail.com

ABSTRACT: Actinomycetes species *Streptomyces violaceorubidus*, strain RC 1796 was isolated from Nagpur region which was identified from MTCC Chandigarh. The inoculum was prepared for fermentation using *Streptomyces violaceorubidus* RC 1796. Different fermentation parameters were studied to increase the yield which includes types of fermentation media, temperature, pH, agitation condition, time period of fermentation, inoculum size, and effect of different concentrations of fermentation media ingredients. The best media for fermentation was found to be yeast malt extract broth media. The optimum fermentation temperature was 28^{o}C, pH 7, agitation speed 150 rpm; time period of fermentation 7 days, inoculum size 10%, concentration of media ingredients like yeast extract concentration 0.4% and malt extract concentration 1.0%. Analysis for the extracellular or intracellular antibiotic production was carried out and it was found that there was extracellular production of antibiotic. Production of antibiotic was done on large scale using the optimum conditions and by using **y**east malt extracts broth for fermentation.

INTRODUCTION: *Streptomyces*, genus of the order actinomycetales constitute a distributed group of bacteria. They have many properties that favour their predominance among other saprophytic microorganisms.

They are best known for their economic importance as producers of antibiotics, vitamins and enzymes, and are certain to have a significant role in future biotechnology [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(8).3094-98

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(8).3094-98

Moreover, the majority of antibiotics in use today were discovered in the 1950's, 1000 antibiotics are known today and most of them (58%) are produced by actinomycetales especially the genus *Streptomyces* [2].

Some species of *Streptomyces* are causative agents of important human and animal diseases; plant pathogens and the rest are involved in the turnover of organic matter [1]. Revising the literature, Waksman and Henrici [3] revealed that *Streptomyces sp.* had been investigated and their antagonistic properties were known and some species produced famous antibiotics. Many antibiotics and enzymes from actinomycetes have been commercialized and marketed and lot of research is being carried out to obtain new metabolites.

In the process of commercialization of a metabolite, yield and production cost form two important parameters from point of view of company, scientist and a biochemical engineer. Thus the target is to obtain maximum metabolite in minimum cost of production. Optimization of parameters affecting production of a metabolite is one of the oldest, cheapest and easiest methods to have best possible yield without metabolic or genetic engineering. This research was aimed to isolate bacterial strains from soil samples around Nagpur region which were able to produce antibiotic and to optimize the parameters to increase the yield of antibiotic.

MATERIALS AND METHODS:

Preparation of inoculum for antibiotic production: Inoculum for antibiotic production was used in the form of spores. Actinomycetes species was isolated from soil samples near Nagpur region, was identified from MTCC Chandigarh as *Streptomyces violaceorubidus* strain RC 1796 and was inoculated on yeast malt extract agar (YMEA Yeast extract (0.4%), Malt extract (1.0%), Glucose (0.4%), $CaCO_3$ (0.2%), Agar (1.2%)) petriplates. The plates were incubated at 28^{o}C for 7 days. Spores from plates were harvested in sterile water. The suspension was used as inoculum for further fermentation [4].

Factors affecting production of antibiotic: Different parameters affecting the production of antibiotic were studied as follows:

1. **Environmental factors affecting production of antibiotic:**

a. **Effect of different types of media:** Antibiotic production depends on type of media used for fermentation. Different media used for fermentation were maltose yeast extract broth, yeast malt extract broth, starch casein broth and nutrient broth. 150 ml of each media was prepared in 4 conical flasks, sterilized and inoculated with the inoculum prepared. The flasks were kept for fermentation on rotary incubator shaker at 28^{o}C and 150 rpm for 7 days. Fermentation broth was centrifuged (Remi, RM12C, India) at 10,000 rpm, at 4^{o}C and supernatant was analyzed for antimicrobial and antifungal activity by cup plate method [5].

b. **Effect of Temperature**: Yeast malt yeast extract broth (150 ml) was prepared in 5 ml conical flasks, was sterilized and inoculated with spore inoculum. Flasks were incubated at 4^{o}C, 15^{o}C, 28^{o}C, 44^{o}C and 60^{o}C on rotary incubator shaker at 150 rpm for 7 days. Fermentation broth was centrifuged and supernatant was assayed for antimicrobial activity by using cup plate technique [6].

c. **Effect of pH:** Yeast malt extract broth (150 ml) with different pH 5, 6, 7, 8 and 9 was prepared in 5 conical flasks and was inoculated with spore inoculum. The conical flasks were incubated at 28^{o}C on rotary incubator shaker (150 rpm) for 7 days. After 7 days, fermentation broth was centrifuged at 10,000 rpm and supernatant was assayed for antimicrobial activity by using cup plate technique [7].

d. **Effect of Agitation condition:** To study the effect of agitation on fermentation, yeast malt extract broth (150 ml) was prepared in 5 conical flasks, sterilized and inoculated with spore inoculum. The flasks were kept for incubation on rotary incubator shaker at 50, 100, 150, 200 and 250 rpm for 7 days. Incubation temperature was 28^{o}C. After 7 days fermented broth was centrifuged at 10,000 rpm at 4^{o}C and supernatant was assayed for antimicrobial activity [8].

e. **Effect of time period of Fermentation:** Yeast malt extract broth (150 ml) was prepared in 12 conical flasks, sterilized and inoculated with spore inoculum. The conical flasks were kept for incubation on rotary incubator shaker at 28^{o}C and 150 rpm. One conical flask was removed daily; the fermentation medium was centrifuged and assayed for antimicrobial activity by using cup plate technique [9]. This procedure was repeated on 12 flasks for 12 days and observed for antimicrobial activity.

f. **Effect of Inoculum size:** Effect of inoculum size was studied on antibiotic production. Spore suspension was inoculated in 4, 6, 8, 10, 12 and 14% (v/v) proportion to the yeast malt extract broth medium. The flasks were incubated at 28^{o}C and 150 rpm on rotary incubator shaker for 7 days.

After 7 days fermentation broth was centrifuged and supernatants were assayed for antimicrobial activity [10].

2. **Effect of yeast extract and malt extract concentration on production of Antibiotic:** To study the effect of malt extract on antibiotic production, yeast malt extract broth was prepared with different concentrations of malt extract as 0.4, 0.6, 0.8, 1, 1.2 and 1.4 % (w/v). The medium was inoculated with spore inoculum and was incubated in rotary incubator shaker at 28^{o}C and 150 rpm for 7 days. After 7 days, fermentation medium was centrifuged and supernatants were assayed for antimicrobial activity.

   Effect of yeast extract was studied by adding yeast extract to the yeast malt extract broth in concentration of 0.1, 0.2, 0.4, 0.6, and 0.8% (w/v). Medium was sterilized and inoculated with spore inoculum. The flasks were incubated at 28^{o}C and 150 rpm for 7 days. After 7 days, fermentation media were centrifuged and supernatants were assayed for antimicrobial activity [11].

Analysis for intracellular/extracellular production of Antibiotic: The extracellular or intracellular antibiotic production was confirmed by following experiment. Spore inoculum of *Streptomyces violacerorubidus* was inoculated in 150 ml of sterile yeast malt extract broth. The media was incubated on rotary incubator shaker at 28^{o}C and 150 rpm for 7 days. After 7 days broth was divided into 2 parts. To check for extracellular production of antibiotic one part of broth was centrifuged at 10,000 rpm for 10 min at 4^{o}C and supernatant as well as pellet were checked for antimicrobial activity by cup plate method.

Plates were incubated at 25^{o}C for 4 days for fungi and 37^{o}C for 24 to 48 hrs for bacteria. After specific time, plates were observed for zone of inhibition. To check for intracellular production of antibiotic, the cells from another part of broth were subjected for lysis by ultrasonicator (PCI Analyticus Pvt Ltd.). After ultra-sonication cell suspension was centrifuged at 10,000 rpm for 10 min at 4^{o}C and supernatant was tested for antimicrobial activity by cup plate method [12].

Production of antibiotic on large scale using the optimum conditions: Yeast malt extract broth for fermentation (7 L) was prepared in conical flask. The pH of media was adjusted to 7. The media was sterilized and inoculated with spore inoculum of *Streptomyces violacerorubidus* prepared by using same medium. Flask was incubated at 28^{o}C for 7 days at 150 rpm. After 7 days, broth was centrifuged (Remi, RM12C, India) at 10,000 rpm and 4^{o}C for 15 min to separate the mycelial biomass. The antibiotic was extracted from the supernatant.

RESULTS AND DISCUSSION:

Factors affecting production of Antibiotic:

1. **Environmental factors affecting production of antibiotic:** The best media for production of antibiotic was yeast malt extract broth. **Figure 1** shows effect of different types of media on antibiotic production. Optimum temperature for antibiotic production was 28^{o}C. Effect of different temperatures on antibiotic producing activity is shown in **Figure 2**.

   The optimum pH was found to be 7 for antibiotic production. The activity was relatively less at pH above and below 7.0 as shown in **Figure 3**.

   Maximum activity against *Candida albicans, B. cereus* and *E. coli* was observed at 150 rpm as shown in **Figure 4**. Time course of antibiotic production was observed throughout the growth phase of the species daily for 12 days. It was observed that maximum antibiotic was produced on 7th day of fermentation.

   Figure 5 clearly showed that the antibiotic production started from 3rd day of fermentation that corresponds to log phase and it slowly increased up to 7 days. Later on there was decrease in production of antibiotic. Varied volumes of spore inoculum were used for antibiotic production [10].

   Maximum activity was observed with 10 % inoculum size against *B. cereus*, which remained same with further increase in inoculum size. The results are shown in **Figure 6**.

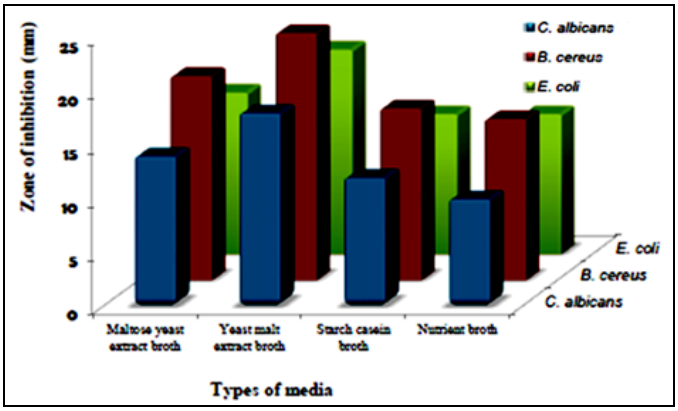

FIGURE 1: EFFECT OF DIFFERENT TYPES OF MEDIA ON PRODUCTION OF ANTIBIOTIC

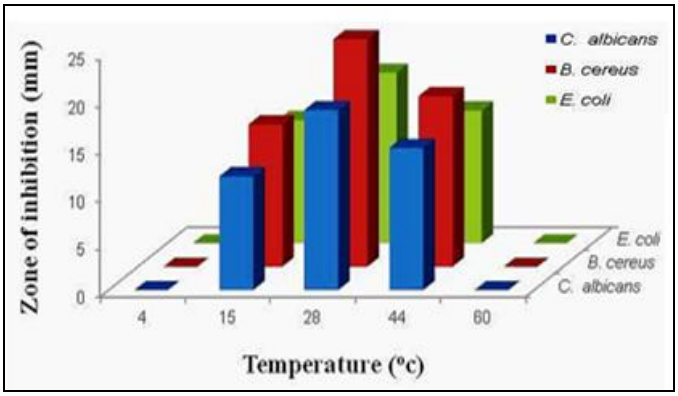

FIGURE 2: EFFECT OF TEMPERATURE ON PRODUCTION OF ANTIBIOTIC

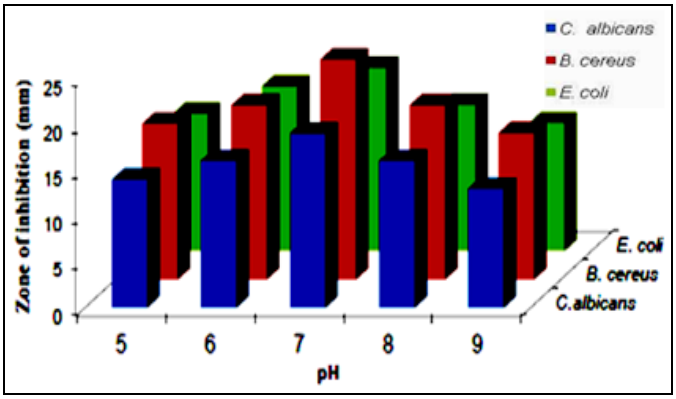

FIGURE 3: EFFECT OF pH ON PRODUCTION OF ANTIBIOTIC

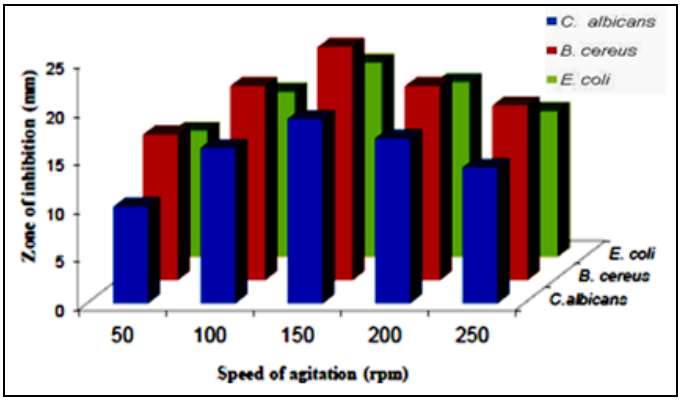

FIGURE 4: EFFECT OF AGITATION CONDITION ON PRODUCTION OF ANTIBIOTIC

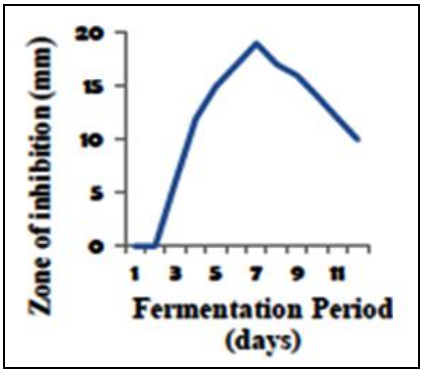

FIGURE 5: EFFECT OF FERMENTATION TIME PERIOD ON PRODUCTION OF ANTIBIOTIC

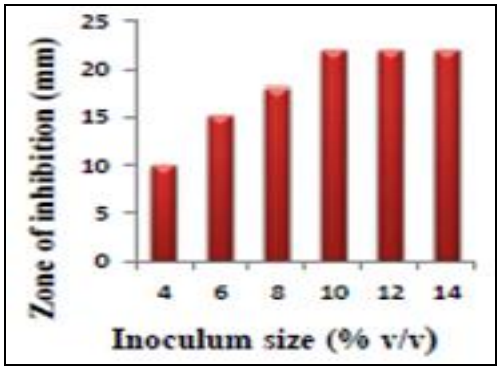

FIGURE 6: EFFECT OF INOCULUM SIZE ON PRODUCTION OF ANTIBIOTIC

ii) Effect of yeast extract and malt extract concentration on production of antibiotic:

Maximum antimicrobial activity was observed with 0.4 % (w/v) yeast extract and 1.0 % (w/v) malt extract concentration. Antimicrobial activity was relatively less at the concentrations other than these two concentrations. The results are shown in **Figure 7** and **Figure 8** respectively.

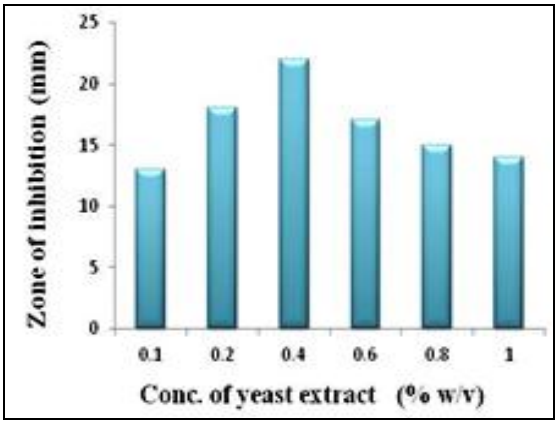

FIGURE 7: EFFECT OF YEAST CONCENTRATION ON PRODUCTION OF ANTIBIOTIC

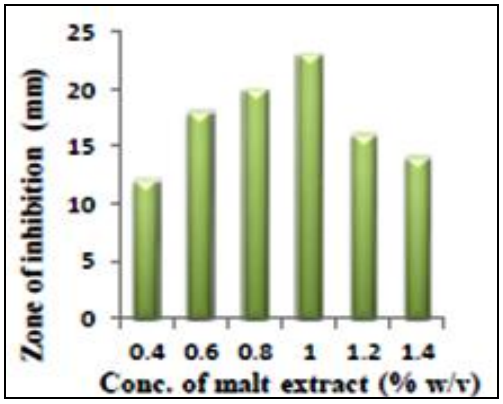

FIGURE 8: EFFECT OF MALT EXTRACT ON PRODUCTION OF ANTIBIOTIC

Analysis for intracellular/extracellular production of Secondary Metabolite: Experiments performed to check for extracellular or intracellular production of secondary metabolite showed that the supernatant of the fermentation broth was having the antimicrobial activity while the cells or cell pellet did not exhibit antibacterial as well as antifungal activity. From this result it was concluded that the antibiotic production was extracellular.

CONCLUSION: Hence, it was concluded that the antibiotic producing actinomycetes can be isolated from Nagpur and nearby area. The best media for fermentation was found to be yeast malt extract broth media. The optimum fermentation temperature was 28^{o}C, pH 7, agitation speed 150 rpm, time period of fermentation 7 days, inoculum size 10%, concentration of media ingredients like yeast extract concentration 0.4% and malt extract concentration 1.0%. Analysis for the extracellular or intracellular antibiotic production was carried out and it was found that there was extracellular production of antibiotic

ACKNOWLEDGEMENT: The authors are grateful to Dr. S.N. Umathe, Head, Department of Pharmaceutical Sciences R.T.M. Nagpur University, Nagpur for providing necessary infrastructural facilities for carrying out the work.

REFERENCES:

1. Goodfellow M, Haynes JA: Actinomycetes in marine sediments. In: Biological, biochemical and biomedical aspects of actinomycetes. Ortiz-ortiz L and Bojalil LF eds. Academic Press, London, 1984: 453-472.
2. Edward DI: History of Chemotherapy. In: Antimicrobial Drug Action. Mac Millan press Ltd. 1980: 1-9.
3. Waksman SA, Henrici: Family III Streptomycetaceae in Bergey's Manual of Determinative Bacteriology. Bread RS, Murray EGD and Simth NR eds. seventh edition, Baltimore, The Williams and Wilkins Co. 1957.
4. Imamura N, Nishijima M, Adachi K, Sano H: Novel antimycin antibiotics. Urauchimycins A and B produced by marine actinomycete. J Antibiot. 1993; 46(2): 241-246.
5. Kokare C, Rangari V, Chopade B, Mahadik K, Kadam S: Production of acetamide derivative from marine *Streptomyces* sp. isolated from west coast of India. Bioscience Biotechnology Research Asia. Res Microbiol. 2005; 3(2): 307-316.
6. Huddedar SB, Shete AM, Tilekar JN, Gore SD, Dhavale DD, Chopade BA: Isolation: characterization and plasmid pU1126-mediated indole-3-acetic acid production in *Acinetobacter* strains from rhizosphere of wheat. Appl Biochem Biotechnol. 2002; 102-103
7. Takahashi C, Takada T, Yamada T, Minoura K, Uchida K, Matsumura E, Numata: A Halichomycin, a new class of potent cytotoxic macrolide produced by an actinomycete from a marine fish. Tetrahedron Lett. 1994; 35(28): 5013-5014.
8. Bernan VS, Montenegro DA, Korshalla JD, Maiese WM, Steinberg DA, Greenstein M: Bioxalomycins, new antibiotics produced by the marine *Streptomyces* sp LL-31 F508: Taxonomy and fermentation. J Antibiot. 1994; 47(12): 1417-1423.
9. Cui XL, Mao PH, Zeng M Li E J, Zhang LP Xu LH, Jiang CL: *Streptomonospora* gen. nov., a new member of the family *Nocardiopsaceae*. Int J Syst Evolut. Microbiol. 2001; 51: 357-363.
10. Abbas S, Subhan M, Durrani F, Mehmood S, Khan H , Hameed: A Biosynthesis of Antibiotic through metabolism of Actinomycetes Strain Mh-9 through Shake Flask Fermentation. Sarhad J Agric. 2010; 26(1): 7-18.
11. Pusecker K, Laatsch H, Helmke E, Weyland H: Dihydrophencomycin methyl ester, a new phenazine derivative from a marine *Streptomycetes*. J Antibiot. 1997; 50(6): 479-483.
12. Omura S: Philosophy of new drug discovery. Microbiol Review. 1986; 59(3): 259-279.

How to cite this article:
Wadetwar RN and Patil AT: Production of Antibiotic from Actinomycetes isolated from Nagpur region and optimization of parameters to increase the yield. *Int J Pharm Sci Res* 2013: 4(8); 3094-3098. doi: 10.13040/IJPSR. 0975-8232.4(8).3094-98