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Original Article

Production of Rifamycin using *Nocardia mediterranea* (*ATCC 13865*)

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ABSTRACT: The U.V. irradiation to *Nocardia mediterranea ATCC 13865* for 10, 20, 30, 40, 50, 60, 70, up to 120 minute the maximum (581µgml^{-1}) production of rifamycin was found developed S-10 strain for maximum production of rifamycin antibiotics (optimum time 15 minute) for further enhancement of rifamycin various parameter like age and size inoculum, pH of the culture medium, various carbohydrate, various amino acids, temperature, various Carbone sources, aeration were optimized. The maximum rifamycin conc. of 570 µgml^{-1} at 4.50 inoculums % with the age of 48 hrs. Inoculum was given even maximum rifamycin production with various age of inoculums (24, 36, 48, 60, 72 hrs.) with corresponding inoculums % (1.5, 2.5, 3.5, 4.5) in culture media, the optimum rifamycin 485 and 480 µgml^{-1} of rifamycin was producing M-6 and M-8 respectively medium amongst M-1, 2, 3, 4, 5, 6, 7, 8, 9 and M-10.

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INTRODUCTION

The empirical formula of rifamycin B is $C_{39}H_{49}N_{14}$. When it is submitted to either atmospheric oxygen or other mild oxidizing agents, it is transformed into rifamycin O ($C_{39}H_{47}NO_{14}$). This undergoes hydrolysis with the loss of one molecule of glycolic acid and produces rifamycin S (C_{37} H_{45} NO_{12}) [1]**.** Rifamycins are a group of antibiotics rifamycins A, B, C, D, E, S, SV, L, O, Y, G, R, P, O and Verde. The principal component is rifamycin B that can be chemically transformed into rifamycin O, S and SV. When rifamycin B($C_{39}H_{19}NO_{14}$) is submitted to either atmospheric oxygen or other mild oxidizing agent, it is transformed to Rifamycin O ($C_{39}H_{47}NO_{14}$). This undergoes hydrolysis with the loss of one molecule. Rifampicin has bactericidal activity against tubercle bacillus. It is particularly effective against mycobacteria that lies semidormant within cells. It has a wide range of antimicrobial activity and other uses include leprosy, severe Legionnaires' disease (with erthyromycin), and the chemoprophylaxis of meningococcal meningitis. It is well absorbed from the gastrointestinal tract. It penetrates well into most tissues.

Today, antibiotics occupy an established place in our medical system, providing sound and acceptable therapeutic agents for the successful treatment of a wide range of bacterial infections, and a major defense against secondary infections.

The present study is to evaluate the Production of Rifamycin B at definite level of inoculums, temperature Ph for industrial production in Bundelkhand region, abundantly. So, we have planned to prove it scientifically.

MATERIAL AND METHOD

Microorganism

Nocardia mediterranea ATCC 13865 was obtained from American type culture collection centre, USA and maintained on agar slants of medium containing(gl^{-1}) peptone-6, yeast extract-3, beef extract-1.5, dextrose-1, agar-15, pH-6.6 used for production of antibiotic rifamycin.

Inoculum Preparation

Vegetative inoculum was used to inoculate shake flasks containing 50 ml medium. The vegetative inoculum was developed on rotary shaker. The inoculum (1.50-4.50 % v/v) of different ages (24-72 hrs.) was used to inoculate fermentation medium. The fermentation broths were assayed for antibiotic potency after 120 hrs. of incubation [2, 3].

Fermentation medium

The initial fermentation medium for fermentation experiments containing (gl^{-1}) Glucose- 94, Soybean meal- 10, Peanut meal- 21.4, $CaC0_3$- 9.5, KH_2P0_4- 0.4, $MgS0_47H_20$- 1.0, Barbital- 2.0, Glucose- 70, Peptone- 30, Glycerol- 20, $CaC0_3$- 8.0, KH_2P0_4- 19, $MgS0_47H_20$- 1.0, Barbital- 2.0, $(NH_4)_2S0_4$- 3.0, $(NH_4)_2S0_4$- 8.0, $CuS0_4.5H_20$- 0.0165, $ZnS0_4.7H_20$- 0.004, $FeS0_4.2H_20$- 0.002, $MnS0_4.4H_20$- 0.001 was used and various other method as age and size inoculum, UV irradiation. Other chemical such as glucose, ammonium sulphate, KH_2P0_4 and calcium carbonate etc.were of analytical grade. Fermentation experiment were conducted in 250ml. shake flask with 50ml. medium for a period of 12 days by changing the various concentration of media component and optimizing the cultural condition of the strain to improve the rifamycin production [4, 5].

RESULT AND DISCUSSION

Production of Rifamycin

Screening of culture media

The composition of the basal medium greatly influences the production of antibiotics. The fermentation media used, by different workers were compared to produce antibiotic rifamycin B. About ten culture media were selected to produce rifamycin B (Table 1). 50 ml of each medium contained in 250 ml conical flask, was inoculated with 5 % (v/v) vegetative inoculum (48 hrs. old) under aseptic conditions and incubated on rotary shaker (150 rpm) at 28°C for 120 hrs. Among the various media tested, medium M-6 gave maximum antibiotic titre [6, 7, 8].

The medium M-6 consisted of ($g1^{-1}$), glucose 94, soybean meal 10, peanut meal 21.4, calcium carbonate 9.5, potassium dihydrogen phosphate 0.4, magnesium sulphate 1, ammonium sulphate 8, copper sulphate 0.0165, zinc sulphate 0.004, iron sulphate 0.002, manganese sulphate 0.001 and sodium di ethyl barbituric acid 2.0. After M-6, the medium M-8 was also found to be more effective than the other media. Both the media contained soybean meal but the highest yield of antibiotic rifamycin B in M-6 was probably due to presence of peanut meal (Figure 1). The defatted soybean meal in combination with peanut meal was found to enrich the medium M-6 for production. In subsequent studies therefore, M-6 was used to produce antibiotic rifamycin B. Figure 1 show the growth curve for *Nocardia mediterranea* in two different media.

A series of experiments were carried out to obtain the high yield of the antibiotic by changing the environmental conditions and different constituents of the fermentation medium M-6 in shake flasks [9].

TABLE 1: SELECTION OF CULTURE MEDIA TO PRODUCE RIFAMYCIN

Medium	Rifamycin (µg ml^{-1})
M-l	145
M-2	223
M-3	190
M-4	365
M-5	210
M-6	485
M-7	395
M-8	480
M-9	215
M-10	90

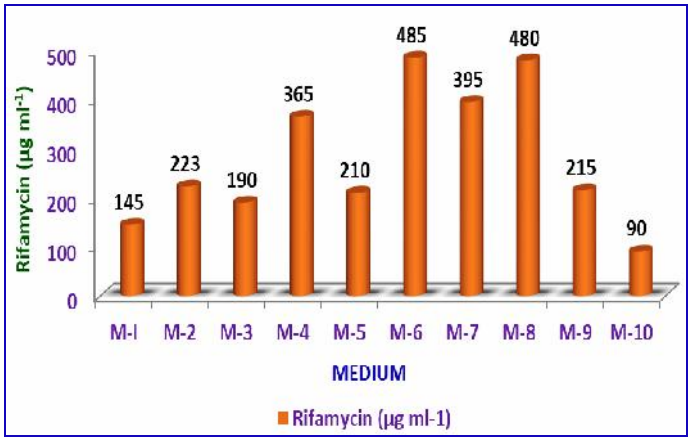

FIGURE 1: SELECTION OF CULTURE MEDIA TO PRODUCE RIFAMYCIN

Effect of age and size inoculum

In this study, the vegetative inoculum was used to inoculate shake flasks shows the relation between age of the vegetative mycelium and rifamycin yield. A considerable increase in rifamycin B production was observed when 48 hrs. old 4.50 % inoculum was employed, although 36 hrs. old 4.5% inoculum was potent to produce antibiotic rifamycin B. Further increase in the incubation time did not improve the antibiotic formation. Therefore, 4.5% inoculum was used in later experiments (Table 2).

The results of fermentation can be influenced considerably by the quality and quantity of vegetative inoculum. Best results were reported either with the use of 4.5% (volume of preculture/volume of fermentation) inoculum of a preculture grown for 24 hrs or with a 1.50% inoculum of a 40 hrs old culture [10].

TABLE 2: EFFECT OF AGE AND SIZE OF INOCULUM ON RIFAMYCIN PRODUCTION

Time (Hrs)	Amount of inoculums (%)				Remarks
	1.50	2.50	3.50	4.50	
24	212±6.60	280±5.50	348±6.58	467±6.50	s
36	253±7.50	310±6.50	477±2.13	571±6.57	h.s
48	471±5.40	510±6.45	530±4.70	572±3.55	s
60	490±4.30	315±3.45	345±5.34	415±3.85	s
72	325±3.30	280±2.45	210±4.74	220±3.75	s

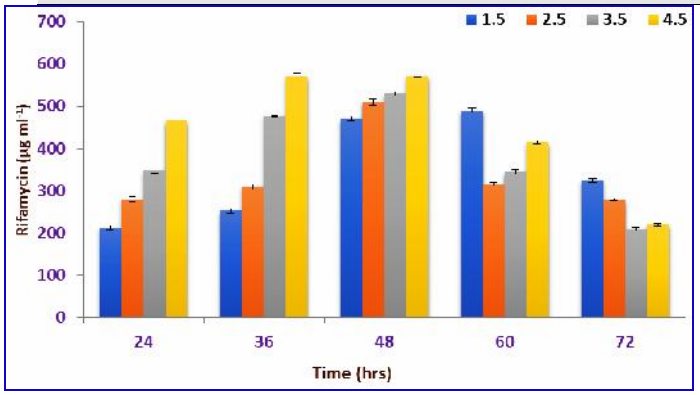

FIGURE 2: EFFECT OF AGE AND SIZE OF INOCULUM ON RIFAMYCIN PRODUCTION

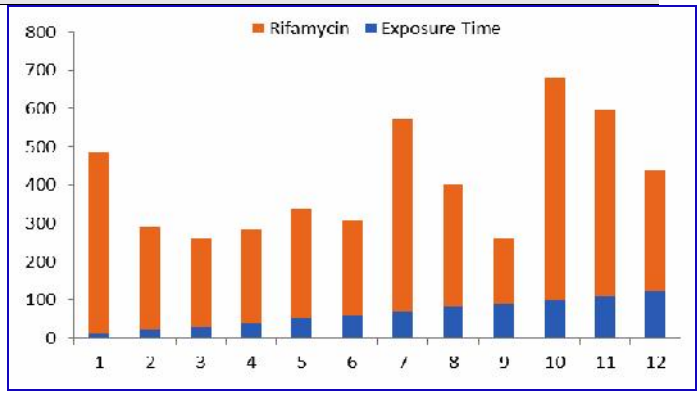

FIGURE 3: PRODUCTION OF RIFAMYCIN BY UV IRRADIATED MYCELIA OF *NOCARDIA MEDITERRANEA ATCC 13865*

Culture Improvement by UV Irradiation

The mycelial suspension of *Nocardia mediterranea* ATCC 13865 was treated with UV irradiation for improvement. Bacterial cells at the logarithmic growth phase were collected by centrifugation at 10,000 rpm. The cells were washed with saline water and diluted to about $2xl0^6$ to $IxlO^8$ cells ml^{-1}. Subsequently, U.V light irradiation with a 15 W U.V lamp at 20 cm distance was carried out for the induction of mutation. Twelve isolates of independent colonies on the agar plates, after irradiation for different time periods were picked up and maintained on complete medium (CM) agar slants. All the cultures were tested to produce rifamycin B in M-6 in shake flask experiments. The data of table 3 shows the biosynthesis of the rifamycin B by bacterial cell irradiated for 10-120 min. Treatment with different time intervals appeared to induce mutation [11].

TABLE 3: PRODUCTION OF RIFAMYCIN BY UV IRRADIATED MYCELIA OF *NOEARDIA MEDITERRANEA ATCC 13865*

Strain No.	Exposure Time	Rifamycin
1.	10	474
2.	20	269
3.	30	232
4.	40	246
5.	50	290
6.	60	248
7.	70	502
8.	80	320
9.	90	170
10.	100	581
11.	110	486
12.	120	320

The culture No. S-10 gave the highest production of antibiotic litre in the fermented broth i.e. 581 μgml^{-1}. For selection of mutant with high antibiotic potency, one of these strains, the S-10 with better yield was further irradiated with mild U.V light repeatedly and examined for antibiotic formation **(**Table 4**)**. The production of antibiotic by these culture strains was not increased, instead some of the strains were found to be very less antibiotic producing strains of *Nocardia mediterranea.* The U.V irradiation treatment was followed by NTG treatment as described by Nagavalli Mandali et al. (2006).

TABLE 4: EFFECT OF UV IRRADIATION OF STRAIN NO. S10 OF *NOCARDIA MEDITERRNEA* ON RIFAMYCIN PRODUCTION

Strain No.	Exposure Time	Rifamycin
S-10	15	490
S-10	15	344
S-10	15	300
S-10	15	267
S-10	20	230
S-10	20	250
S-10	20	230
S-10	20	210
S-10	30	87
S-10	30	60
S-10	30	34
S-10	30	60

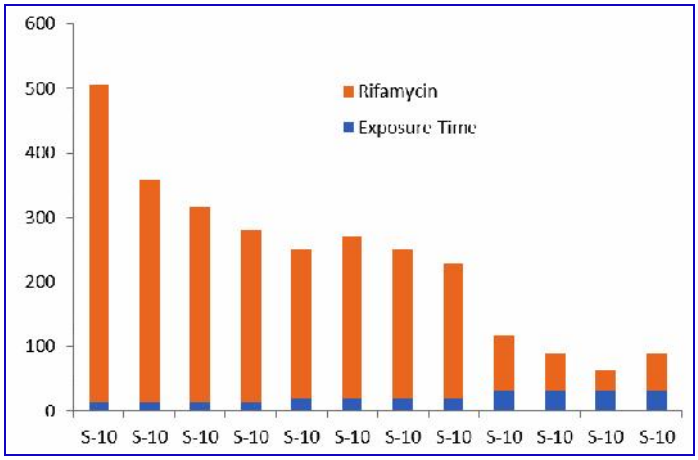

FIGURE 4: EFFECT OF UV IRRADIATION OF STRAIN NO. S10 OF NOCARDIA MEDITERRNEA ON RIFAMYCIN PRODUCTION

REFERENCES

1. Bapat PM, Wangikar PP, Optimization of Rifamycin B fermentation in shake flasks via a machine-learning-based approach. Biotechnol Bioeng, 2004; 86:201–208.
2. El Enshasy H.A., A.F. El Baz, and E.M. Ammar Simultaneous production and decomposition of different Rifamycins during *Amycolatopsis mediterranei* growth in shake flask and in stirred tank bioreactor Communicating Current Research and Educational Topics and Trends in Applied Microbiology *A. Méndez-Vilas. Formatax* (Ed.), 2005; 315-21.
3. Heinz G. Floss* and Tin- Wein Yu, Rifamycin Mode of Action, Resistance, and Biosynthesis. *Chemical Reviews* (ACS Publications) 2005,105(2):621-32. [View in PubMed]
4. Hewaida F El-Sedawy, Manal MM Hussein, Tamer Essam, Ossama M El-Tayeb and Fatma HA Mohammad Scaling Up for the Industrial Production of Rifamycin B Fed-Batch Production Mode in Shake Flasks and Bench-Scale *Fermentor Ferment Technol*, 2007;1(5):2167-72
5. Jin ZH, Lin JP, Xu ZN, Cen PL, Improvement of industry-applied rifamycin B-producing strain, *Amycolatopsis mediterranei*, by rational screening. J. *Gen. Appl. Microbiol*.2002 48(6): 329-334.
6. JIN Zhi-hua , LIN Jian-ping , CEN Pei-lin , Scale-up of rifamycin B fermentation with *Amycolatoposis mediterranei*. *Journal of Zhejiang University science*, 2004;5(12):1590-96
7. Nagavalli Mandali, V. Girijashankar, S. P. D. Ponamgi, Sujatha Peela and L. Venkateswar Rao Rifamycin SV production using immobilized cells of *Amycolatopsis mediterranei* OVA5-E7 in different matrices. *Journal of Chemical and Pharmaceutical Research*, 2014, 6(9):298-306
8. Ates S, M.Elibol, F. Mavituna, Production of actinorhodin by Streptomyces coelicolor in batch and fed- batch cultures - Science Direct, 2010, 32(4):273- 278.
9. Xuan-Tien Doan, Rajagopalan Srinivasan, Prashant M. Bapat, Pramod P. Wangikar, Detection of phase shifts in batch fermentation via statistical analysis of the online measurements: A case study with rifamycin B fermentation, *Journal of Biotechnology*, *2007*; 132(2):156-66.
10. Xuan-Tien Doan[a], Rajagopalan Srinivasan[a,b,*],Prashant M. Bapat[c], Pramod P. Wangikar[c], Detection of phase shifts in batch fermentation via statistical analysis of the online measurements: A case study with rifamycin B fermentation. *Current Trends in Biotechnology and Pharmacy*, 2010;3(1):153-61.
11. Mahalaxmi y, Ch. Subba Rao, G. Suvarnalaxmi, T. Satish, P. Sudhakar and R. S. Prakasham Rifamycin B Production Pattern in Nocardia RSP-3 Strain and Influence of Barbital on Antibiotic Production, *Current Trends in Biotechnology and Pharmacy*, 2008, 2 (1): 173-181.

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