JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2008. Vol. **2**(1), p. 211-214

Biotransformation of Rifamycin B to Rifamycin S with free and Immobilized Cells of *Curvularia lunata*

R.H. Patil*, S.V. Patil[1], J.A. Rajput[2], Javed Bhat[3], R.G. Chaudhary[4], U.K. Patil and S.B. Chincholkar[5]

*Department of Microbiology, R.C.Patel Arts, Commerce and Science College, Shirpur, India.
[1-4]Department of Microbiology, School of Life sciences,
North Maharashatra University, Jalgaon - 425 001, India.

(Received: 08 February 2008; accepted: 20 March 2008)

Studies on Biotransformation of rifamycin B to rifamycin S revealed that immobilized mycelia of *Curvularia lunata* are more efficient than free cells of organism with respect to time, cultural conditions and cost. Effect of various polymers revealed that carboxymethylcellulose has stimulatory effect on biotransformation whereas other polymers do not show significant effect on biotransformation. TLC analysis of transformed product shows two bands, one matching with standard rifamycin B while another with standard rifamycin S indicates significant transformation. Optimum transformation takes place at 40^{0}c and p^{H}7 respectively. UV-Visible spectrophotometric studies indicated that rifamycin B and rifamycin S has absorption maxima 425 and 525 respectively.

Key words: Biotransformation, rifamycin B, rifamycin S, TLC, *Curvularia lunata*.

Biotransformation of organic compounds to biologically more active derivatives is always considered as attractive area in researchers. Biotransformation of base compounds by whole cells and enzymes of microorganisms includes vast reactions. Microbial transformation is always considered as method of choice due to its enormous specificity, cost effectiveness and process simplicity (Vohra *et al.,* 1989).

* To whom all correspondence should be addressed.
Tel.: (+91) 02563-257328
E-mail: ravi_nmu@yahoo.com

Biotransformation involves steareospecific as well as regio specific modification of the base compound in chemically defined conditions. Biotransformation includes reactions like oxidation, hydroxylation and isomerization. Simpler extraction of transformed product makes biotransformation as a method of choice in industrialists. Biotransformation has been extensively used in obtaining the more potent derivatives of antibiotics, steroids and in some alkaloids. Rifamycins are obtained by microbial fermentation as well as by chemical synthesis. Rifamycins are biologically active against the Gram positive microrganisms and mycobacteria. Chemical transformation however is the method widely used in industry however it generates the

hazardous acidic water and also the process is not economically viable. Here in present work attempts were made to devise rapid biotransforming procedures in case of rifaycin, which is widely, used as cost effective drug sin our antituberculosis battle (Banarjee *et al.,* 1992)

MATERIAL AND METHODS

Standard Rifamycin B and Rifamycin S were obtained from lupin laboratories; Tarapur, as a gift sample. C*urvularia lunata* was isolated from the local soil. It was maintained on the medium contaning gL^{-1}; glucose 10, peptone 4.0, Yeast Extract 4.0 agar-agar 30. p^H of the medium was adjusted 6.5 with 0.1N HCL before sterilization (Banarjee *et al.,* 1992).

Spore suspension was prepared from 7-8 days old agar slant culture of *C. lunata.* 10 ml of sterile saline and 1-2 drops of tween-80 were added. Sterile glass beads were also added and shacked by using cyclomixer for 5 min. in order to shake the mixture for obtaining uniform spore suspension. Spore concentration was optimized to 3×10^4 spores /ml using nubor's chamber (Thaker *et al.,* 1986).

Method of Biotransformation

5.0 ml of uniformly mixed spore suspension was inoculated in 100ml of cell biomass production medium contaning yeast extract and peptone as carbon and nitrogen source respectively and incubated at 29°C for 48 hrs. After incubation cell mass was recovered by simple filtration and mycelium was dried by using filter paper and 1 gm of mycelial mass was inoculated in starvation medium containing 0.05 gm of Rifamycin B as substrate and incubated at 29°C and 120 rpm for 12 hours and biotransformation ability was checked every hours by using spectrophotometry and TLC method (Seong *et al.,* 1989). The change in colour of starvation medium from yellow to red indicates biotransformation of Rifamycin B to Rifamycin S after 24 hours (Thaker *et al.,* 1986).

Spectrophotometric assay

The spectrophotometric assay was carried out according to Seong *et al.,* 1983. Accurately 0.1 gm cell mass was added to Rif. B solution (2 ml in 0.1m phosphate buffer 6.5). After incubation at 29°C for 60 min., 0.5 ml sample diluted in 4.5 ml of 1:1 methanol phosphate buffer (0.1 m, pH 7.8). The mixture was boiled for

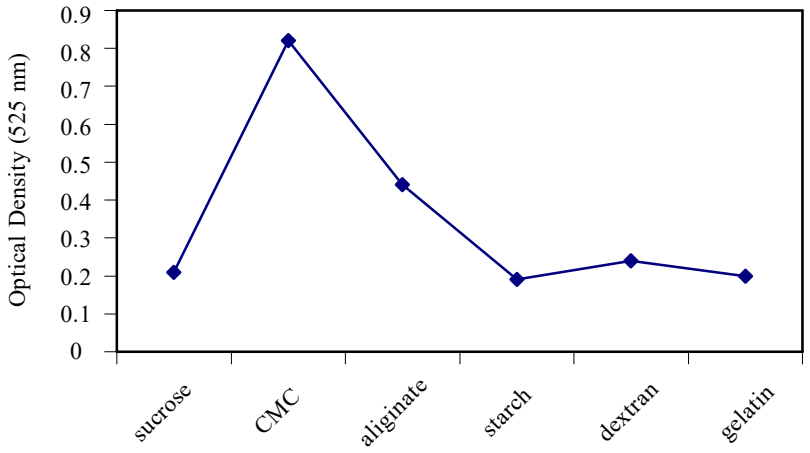

Fig. 1. Effect of various biopolymers on Biotransformation

3 min in water bath centrifuged 5 min at 4000rpm. The absorbance of Rifamycin S in supernatant was measured at 525 nm. Activity was defined as an equivalent of 1um rifamycin S produced in an hour under the specified condition (Seong *et al.,* 1989).

TLC analysis of biotransformation

The dried extract of the product was dissolved in methanol: chloroform (1:1 v/v). It was then used for spotting on Aluminium coated Silica gel sheets (Sd-fine Chemicals, Mumbai). The chromatogram was subjected for development in solvent systems containing Acetone: carbon tetrachloride 1:1. The spots were detected by observing different colour by observing the plate under UV light and RF values were determined (Sensi and Theimann, 1967.) For immobilization of mycelia Sodium Alginate 2% were well mixed by using magnetic stirrer and sterilized 1 gm of 24 hrs mycelium was mixed in alginate solution by using 0.2 mm standard sterile micro syringe, beads were prepared by dropping drops uniformly in 0.2 m each solution. Then beads cured in $CaCl_2$ solution for 48 hours.

Effect of polymers

Studies on the effect of different polymers were carried out with a view to know the effect of change in morphology of organism involved in the fermentation for Rifamycin biotransformation. Experiments on the effect of different polymers on Rifamycin biotransformation were designed accordingly (Han. and Varia, 1995) 100ml sucrose medium was separately supplemented with CMC, alginate, agar, starch and dextrin in 1 gm%. It was inoculated with 1% inoculum and incubated on rotary shaker at 120 rpm at room temperature. After 24 hrs interval, cell moss was harvested form each flask, washed and used for biotransformation and enzyme assay was carried out as per Banargee *et al.,*

Production of extracellular Rifamycin oxidase was reported from *C. lunata*_var. aeria grown in YPD medium at 28°C. Mycelial form of growth was found to produce higher enzyme levels than the pellet form (Dubey,1983). Studies on the influence of different factors on Rifamycin oxidase production revealed that 1% CMC was effective in producing mycelia growth which yielded was enzyme activity (Thaker *et al.,* 1986).

RESULTS AND DISCUSSION

TLC

The TLC chromatogram revealed that Rf values of transformed rifamycin B exactly match with that of standard rifamycin S indicating that complete transformation has occurred.

Spectrophotometry

Spectrophotometric analysis shows that a λmax value for rifamycin S was found to be 525 nm. which is similar to standard.

Immobilization

Biotransformation with immobilized system is found to be more rapid and efficient as compare to free mycelial system. Immobilized spores of *C. lunata* do not showed transformation.

Effect of polymers

Effect of various polymers shows that 1% CMC gives maximum biotransformation whereas other polymers do not show significant effect on biotransformation. (Fig.1).

CONCLUSION

The results of this work suggest that the biotransformation of Rifamycin B to Rifamycin S is efficient and rapid with immobilized mycelia. On the basis of experimental results it was concluded that biotransformation is maximum at 1% concentration of Carboxymethylcellulose.

REFERENCES

1. Vohra, R.M., Banerjee, U.C., Das, S and Dube S. Microbial transformation of rifamycin B: A new extracellular oxidase from *C. lunata*, *Biotechnol. Lett,* 1989; **12**: 851.
2. Banerjee, U. C. and Chankarbarti S, Production and properties of carboxymethylcellulose from *C. lunata, World J. Microbiol. Biotechnol.* 1992; **8**: 423.
3. Thaker, A.J., Ray, R.M and Patel, H.C. Xylanase production by fungus *T. longibacterium* and enhancement on enzymatic saccharification of celulosic waste, *Ind. J. Exptl. Biol.* 1986; **24**: 659.
4. Seong, B.L., Son, H.J., Wheen, T.I. and Han, M.H. Microbial transformation of rifamycin B, A synthetic approach to rifamycin

derivatices. *J. Antibiotics,* 1983; **36**: 1402-1404.

5. Dubey, H.C. An introduction to fungi. Vikas Publ. House Pvt. Ltd., N. Delhi. 1983; 403-415.
6. Seong, B.L. and Han, H.M., A facile preparation of rifamycin derivatives by use of manganese dioxide. *Chem. Lett.* 1982; 627-628.
7. Patil, B.B., Studies on the role of iron metabolism with special reference to hydroxylation of progesterone and oxidation of rifamycin B., Ph.D. thesis NMU, Jalgaon 1999.
8. Sensi, P. and Theimann, J.E. Production of Rifamycin. In: Progress in Indust. *Microbiol.* 1967; **6**: 21-59.
9. Han, J. and Varia, D., Rifamycin B oxidase from Monocillium sp. A new type of diphenol oxidase. *FEBS Lett.* 1995; **151**: 36-40.