Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 4, Issue 2, 2012

Research Article

OPTIMIZATION OF PROCESS PARAMETERS FOR RIFAMYCIN B PRODUCTION UNDER SOLID STATE FERMENTATION FROM *AMYCOLATOPSIS MEDITERRANEAN* MTCC 14

BM VASTRAD[1], SE NEELAGUND[2*]

[1]Department of Pharmaceutical Biotechnology, S. E. T`s College of Pharmacy, Dharwad, [2]Department of PG Studies and Research in Biochemistry, Jnana Sahayadri Kuvempu University, Shankarghatta, Karnataka, India. Email: neelgund@gmail.com

Received: 18 February 2012, Revised and Accepted: 16 March 2012

ABSTRACT

Solid-state fermentation (SSF) was carried out for the production of extra-cellular rifamycin B by *Amycolatopsis Mediterranean* MTCC 14 using four agro-industrial substrates, coconut oil cake, groundnut oil cake , ground nut shell and rice husk. Coconut oil cake and ground nut shell showed the highest antibiotic yield compared to groundnut oil cake and rice husk. Maximum rifamycin B titres were produced when SSF was carried out with 60% initial moisture content of the substrate, 8% (w/w) inoculums, substrate particle size 1.4 and 1.6 mm, initial pH 8, 32^{o}C as incubation temperature, and day 10 of incubation period in all four substrates. External supplementation of fermentation medium with various carbons, organic and inorganic nitrogen sources was benefit for antibiotic production.

Keywords: Rifamycin B; *Amycolatopsis Mediterranean* MTCC 14; Solid state fermentation; Coconut oil cake; Optimization

INTRODUCTION

Rifamycins are clinically important ansamycin antibiotics, composed of a naphthalenic chromophore spanned by a long aliphatic ansa chain. The rifamycins derived from them exert their antibiotic activity by specific inhibition of bacterial DNA-dependent RNA polymerase of different microbial species[1,2,3]. At higher concentrations, these antibiotics also inhibit the RNA-dependent DNA polymerase of retroviruses as mono-therapy or in combination with other drugs[4,5,6]. Therefore, antibiotics of the rifamycin class such as rifampicin, rifabutin and rifapentine have been employed on a global basis in a number of well established combination regimes for the clinical treatment of tuberculosis, leprosy, AIDS-related mycobacterial infections and many other enteric infections [7, 8, 9,10]. Organisms known to produce rifamycins include *Nocardia mediterranei* [11] and *Amycolatopsis mediterranei*-CBS-42575 [12].

Solid-state fermentations(SSF) is generally defined as that in which microbial growth and product formation take place on solid substrate in absence of free water. Several reviews are available describing the techniques. In recent years, solid state fermentation technology has received increasing interest. This is partially because, as a fermentation technology, it has lower energy requirement and produces less waste water than alternative technology such as submerged technology. In addition, there are increasing environmental concern regarding the disposal of solid waste, and there use as substrate for the commercial production of microbial metabolites is becoming an alternative position [13].

The production of antibiotics by SSF has gained much attention in biotechnology studies for production of cephamycin [14, 15], oxytetracycline [16, 17], iturin [18, 19], neomycin [20, 21], cephalosporin C [22], penicillin [23] and rifamycins [24, 25]. The use of low cost agricultural solid residues, higher productivities, low energy requirements, lower wastewater production, extended stability of products and low production costs are some of the main advantages of SSF [13, 26]. The selection of a suitable microorganism is an important aspect of SSF for production of antibiotics [23]. The microorganism should be able to grow at low water activity, to be GRAS ("Generally Recognized as Safe") [27].

In the recent past, many agro-industrial byproducts such as wheat rawa [14], corn cobs [16], okara [18] barley [28] etc. have been screened as low-cost solid substrates for production antibiotics in SSF. A perusal of literature shows that some of the solid substrates possess higher potential as compared to the other solid substrates for supporting/inducing the rifamycin production by *Amycolatopsis sp.* RSP 3. For example, wheat bran, corn husks and corn cobs are reported to be superior as compared to the other tested solid substrates for optimum rifamycin production by many microorganisms under the identical SSF condition [24]. However, the exact reason(s) for such a higher antibiotic production by microbes while growing on some specific substrates has never been explored and till date no logical explanation has been provided for the above observed effect.

MATERIALS AND METHODS

Microorganism maintenance and seed preparation

Amycolatopsis mediterranei MTCC 14 [29] used in the present study was obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The culture was maintained on Q/2 agar slants. This medium consists of (g l^{-1}): yeast extract, 4.0; malt, 10.0; glucose monohydrate, 4.0; oat flakes, 20.0and agar, 20.0. The pH was adjusted to 7.6 before sterilization and the slants were incubated at 28 °C for8 days. Surface growth of the selected slant was harvested in flasks containing 100 ml of vegetative medium. The flasks were incubated at 28°C in a rotary incubator shaker at 100 rpm for 56 to 72 h. Flasks showing the following parameters were selected for inoculation of the fermentation medium: pH, 7.6-7.9; percentage packed mycelial volume [30] 9 – 12%; color, intense orange to red; time for methylene blue decolorization, 50 – 70 s.

Selection of the substrate

Various oil cakes such as coconut oil cake (COC) and groundnut oil cake (GOC) was obtained from an local oil mill, Dharwad, India and traditional agro-industrial residues such as ground nut shell (GNS) and rice husk (RH) was obtained from the local market of Dharwad, India were screened as substrates for rifamycin B production. These were ground in a mill to a particle size between 0.8-2.0 mm and dried at 55-60°C for 12 h.

Solid State Fermentation

Initially, the fermentation conditions reported by Mahalaxmi et al 2011 were used. Solid substrates were dried in an oven at 50 ^{o}C for 5 h. Five grams of the dried substrate was taken in conical flask and supplemented with (in mg) soya bean meal 250, calcium carbonate 75, potassium nitrate 100, barbital 20 and magnesium sulphate 1 with predetermined quantity of water [24]. The initial moisture level of the substrate was adjusted by adding adequate amount of distilled water. The thoroughly mixed substrate was autoclaved at 121 ^{o}C for 20 min and cooled to room temperature (28 ± 2 ^{o}C) before inoculation. After cooling, the substrate was inoculated with 1 ml of seed culture (4 % w/w), thoroughly mixed with glass rod and incubated at 28 ± 2 ^{o}C for 15 days.

Effect of moisture content

Effect of moisture content for the fermentation process was analyzed by using the above defined medium added to solid substrates for achieving varying levels of moisture (35, 40, 45, 50, 55, 60, 65, 70 and 75 %) . The process was carried out for 12 days at 32 °C. The moisture content suitable for maximum production was followed for subsequent set-ups [25].

Estimation of moisture content

Substrate moisture play an important role in SSF [32]. Solid substrates were soaked individually with desired quantity of water and the weight was noted. The soaked material was then dried (105 °C) to a constant weight and the weight of the dried material was recorded [21]. The percent moisture content was calculated as follows.

Percent of moisture content (initial) of solid medium = (wt. of the wet tea waste - dry wt.) X 100/dry wt.

Effect of incubation period

To study the effect of incubation period on rifamycin B production, flasks were incubated for varying periods (2–14 days). Other parameters were kept at their optimum levels.

Effect of initial pH of the culture medium

The effect of initial pH of culture medium on production of rifamycin B was studied by varying the pH of salt solution from pH 4.0–9.0. The pH was adjusted using 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. The pH of culture medium was measured as reported by Mahalaxmi et al 2011[24] and Venkateswarlu et al 2000 [25]. About 1 g of solid culture was stirred in 10 distilled water and pH was measured after settling the solid matter. The initial pH of culture medium supported maximum rifamycin B production was used for subsequent experiments.

pH Measurement

Five milliliters of distilled were added to 0.5 g of fermented material and the mixture was agitated vigorously. After 10 min, the pH of the supernatant was determined with a pH meter [31].

Effect of incubation temperature

While deriving the incubation temperature the fermentation was carried out at various temperatures ranging 24–36 °C. The optimum incubation temperature attained by this step was utilized for subsequent study.

Effect of inoculum level

To define the suitable level of inoculum for rifamycin B production, various levels of inoculum (2–8 w/w %) were used. Fermentation reaction was incubated at 32 °C while following the other conditions as described [24,25]. The inoculum level achieved by this step was followed for subsequent evaluation.

Effect of supplementary carbon sources

Different carbon sources: glucose, galactose, sucrose, lactose, maltose, sucrose and soluble starch were supplemented separately to a final concentration of 1% (w/v) in solid media. They were dissolved to required concentrations in 15 ml of distilled water, which was used as moistening agent per 25 g of solid substrate.

Effect of organic and inorganic nitrogen sources

Different organic and inorganic nitrogen sources: yeast extract, peptone, tryptone, glycine, urea, ammonium chloride, ammonium sulphate, sodium nitrate and potassium nitrate were supplemented separately to a final concentration of 1% (w/v) in solid media after dissolving them in distilled water used for adjusting the moisture content.

Antibiotic extraction

After incubation, 100 ml of distilled water was added to the flasks and the antibiotic was extracted from solid substrate by shaking at 200 rpm for 1 h. The extract was passed through muslin cloth and centrifuged at 10,000g for 5 min. Supernatant was stored at 4 1C for antibiotic assay.

Antibiotic assay

Supernatant containing antibiotic was diluted and stabilized by acetate buffer and was made collarless. This solution of antibiotic was determined spectrophotometrically as described by Pasqualucci et al. (1970) [33] using a Schimadzu UV spectrophotometer, Japan.

RESULTS AND DISCUSSION

SSF is a culture system that has been used in several countries since antiquity. One of the major advantages that SSF offers is in the utilisation of agro-industrial residues. India is one of the leading producers of oil cakes and husks is an easily available cheap commodity which finds use only as a animal feed and fuel in rural areas. Oil cake, especially coconut oil cake and ground nut oil cake, being rich sources of carbon and nitrogen, can thus serve as a suitable substrate for the growth of micro-organisms by SSF. Paddy and ground nuts are an agronomically versatile crop grown in many countries all over the world. Dry milling of rice in the separation of husk from paddy, the process in which husk is by-products. Coconut oil cake and ground nut oil cake find use as traditional animal feed, whereas ground nut serves as a rich source of oil. But by-products like ground nut oil cake and shell are utilized for any useful purpose.

Effect of various solid substrates

SSF offers a number of advantages over conventional submerged fermentation for antibiotic production [15]. The production medium is often simple, using agro-industrial by-products like wheat bran, rice bran or wheat straw as substrate [34]. Because the moisture level is low, the volume of medium per unit weight of substrate is low. Hence, antibiotic is usually very high yield [28]. Thus to achieve a given antibiotic productivity, fermentor volumes can be much smaller than in submerged fermentation systems [34]. Here in this study, we are reporting for the first time use of COC, GOC, GNS and RH s as solid substrates for the production of rifamycin B by *Amycolatopsis mediterranei* MTCC 14.

The selection of the substrate for SSF process depends upon several factors mainly related to the cost and availability. The substrate that provides all nutrients required by the microorganism growing in it should be considered as an ideal substrate. Since no substrate was reported for production of rifamycin B by *Amycolatopsis mediterranei* MTCC 14 using SSF, different solid substrates viz, COC, GOC, GNS and RH were screened for production of rifamycin B by *Amycolatopsis mediterranei* MTCC 14. The results presented in Fig. 1 showed that rifamycin B production by *Amycolatopsis mediterranei* MTCC 14 varied with type of the substrate. All the substrates supported the production of rifamycin B however, COC and GNS were found to be the best substrate giving a maximum yield of 1.46±0.073 mg/gds and1.32±0.066 followed by GOC and RH.

Effect of substrate particle size

The nature of solid substrate is the most important factor in SSF. This not only supplies the nutrients to the culture but also serves as an anchorage for the microbial cells. Therefore, the particle size and the chemical composition of substrate are of critical importance [35].The particle size and there for the specific area of the substrate is of importance in SSF [36], and usually smaller particles stimulate greater growth [37]. Maximum rifamycin B production was obtained with substrate particles of average size 1.4 and 1.6 mm, irrespective of the inoculum type. With smaller particle, the surface area for growth was greater but the inter-particle porosity was less. With the larger size, the porosity was greater but the saturated surface area was less. These two opposing factors probably interacted to give the value corresponding to optimum growth and product formation [36]. However, all other particle sizes started production on the third day of fermentation. The influence of substrate particle size, which determines the accessible surface area to the micro-organisms on product formation has been, emphasized earlier [38,39].

In the present study, the effect of all four type substrates particle size on rifamycin B production, with seven different sizes (0.8, 1.0,

1.2, 1.4, 1.6, 1.8 and 2.0 mm) were tried. The results indicate in Fig 2, that the intermediate (1.4 and 1.6 mm) size of the substrates were the best substrate for rifamycin B production (1.79-3.96 g/Kgds) than the fine (0.8, 1.0, and 1.2mm) size, which yielding (0.69-2.97 g/Kgds) and corse (1.8 and 2.0 mm) size substrates, which yielding(0.67-2.92 g/Kgds). The intermediate size of COC was found to be optimal size of the substrate for higher rifamycin B production. In the subsequent experiments, therefore intermediate size was used for the production of rifamycin B.

Effect of initial moisture content

The initial moisture level is one of the most important factors in SSF media. Five moisture levels ranging from 35% to 75% were established to study their effect on rifamycin B production and the results obtained are shown in Fig. 3. Significantly, the highest production was attained when the initial moisture levels were 55%–60%.

For solid state fermentation, moisture is a key parameter to control the growth of microorganism and metabolite production [40,13]. In SSF, the intensity of microbial growth generally depends on the initial moisture level and it indirectly affects the production titre. This result was similar to the findings of Mahalaxmi et al 2010 [24] who reported that initial substrate moisture content less than 40% gave less rifamycin B production, but that of 50–56% could give the highest rifamycin B production. Higher initial moisture in SSF leads to suboptimal product formation due to reduced mass transfer process and decrease in initial moisture level results in reduced solubility minimizes heat exchange, oxygen transfer and low availability of nutrients to the culture [41].

Effect of incubation period

Initially, a time profiling of rifamycin B production was studied in 250 ml flask using all four substrate independently. The profile for rifamycin B production passed through a maximum on day 10 of the experiments. The highest rifamycin B production observed was 4.89g/Kgds for COC**,** 3.78 g/Kgds for GOC, 4.45 g/Kgds for GNS and 3.33 g/Kgds for RH (Fig. 4). The loss of organic matter followed a typical saturation curve indicative of its relationship with biomass formation.

COC and GNS both served as good substrates for rifamycin B production. However, in view of easy availability, we used all COC, GOC, GNS and RH as the substrate. Maximum yield of antibiotic was obtained after day 6 of incubation at 28 ± 2 ^{o}C. Decrease in production after 12 day may be due to accumulation of end product which hampers rifamycin B production or may be due to accumulation of toxic metabolites secreted during fermentation [24]. Venkateshwarlu et al 2000 [25] reported maximum production after day 3 of incubation with *Amycolatopsis mediterranei* VA18 under solid state fermentation.

Effect of initial pH

The initial pH of the fermentation media may change during fermentation because the substrates employed in SSF usually have the least buffering. Data in Fig. 5 indicated that rifamycin B production was significantly affected by initial pH variations, and *Amycolatopsis mediterranei* MTCC 14 was capable of producing rifamycin B in the initial pH range of 6.0–8.5, optimum being 8, with the yield of 4.89g/Kgds for COC**,** 3.98 g/Kgds for GOC, 4.32 g/Kgds for GNS and 3.74 g/Kgds for RH.

Rifamycin B production by microbial strains strongly depends on the extra-cellular pH because culture initial pH strongly influences many antibiotic production and transport of various components across the cell membranes, which in turn support the cell growth and antibiotic production [25]. Because the metabolic activities of microorganism were very sensitive to initial pH changes, the rifamycin B by *Amycolatopsis mediterranei* MTCC 14 was found to be affected when initial pH level was deviated from the optimum value. Similar initial pH trends have been observed by others using *Amycolatopsis mediterranei* VA18 on Corn husk [24].

Effect of incubation temperature

The results showed that the most suitable temperature for rifamycin B biosynthesis of *Amycolatopsis mediterranei* MTCC 14 was in the range of 28–32 ^{o}C (Fig. 6). It was found that the growth was very low when the temperature of the medium was below 26^{o}C. However, when the temperature was higher than 34 ^{o}C, rifamycin B activity decreased considerably.

Incubation temperature was shown to effect rifamycin B production. It was another critical parameter that has to be controlled which varies from organism to organism [25]. Higher temperature had some adverse effect on the metabolic activities of the microorganisms and it has been reported by various scientists that the metabolic activities of the microorganisms become slow at lower temperature. Hence, incubation temperature and its control in SSF process is crucial as the heat evolved during SSF processes is accumulated in the medium due to poor heat dessipation in solid medium. This results in reduced microbial activity, thereby decreasing the product yield [42,40].

Effect of inoculum level

The effect of inoculum level on the production of rifamycin B was observed using 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11% and 12% (w/w) of inoculum in the production medium. Maximal rifamycin B yield was obtained with 8 (w/w) % of inoculums level (COC for 4.89g/Kgds, GOC for 3.47 g/Kgds, GNS for 4.56 g/Kgds and RH for 3.37 g/Kgds), however, decreased rifamycin B activity was observed at lower and higher inoculum concentrations(Fig. 7).

Inoculum level was also an important factor for the production of rifamycin B. Lower levels of acid production were observed when an inoculum level higher or lower than the optimal inoculum was used. However, augmentation in biomass formation was attained by the increment in inoculum size. It is important to provide an optimum inoculum level in fermentation process [24, 25]. A low inoculum density may give insufficient biomass causing reduced product formation whereas higher inoculum may produce excessive biomass and deplete substrate for the nutrients necessary for product formation [43].

Effect of supplementary carbon source

The effect of various supplementary carbon sources on rifamycin B production was studied by employing these at 1% w/v level to the substrate. Results are presented in Fig.8. The results indicated that glucose showed maximum rifamycin B yield (COC for 4.94g/Kgds, GOC for 4.75g/Kgds, GNS for 4.7g/Kgds and RH for 4.75g/Kgds). Rifamycin B production with the addition of other carbohydrates was found to be low when compared with the control.

In the literature, contradictory evidence exists for the effects of the nature and concentration of the carbon source on rifamycin B production. Carbon rich conditions enhanced the production of the rifamycin B in *Amycolatopsis mediterranei* VA18 (Mahalaxmi et al 2010)24. Glucose supplementation gave the highest rifamycin B production in *Amycolatopsis Mediterranean* MTCC 14 for all four substrates. The role of these carbon compounds in the regulation of antibiotic synthesis depends not only on the physiology of the tested microbes but also on the medium composition, especially on presence of agro industrial by products [22] showed that some species, *Acremonium chrysogenum* produced the highest antibiotic production in a medium containing high carbon conditions.

Effect of supplementary organic and inorganic nitrogen source

Nitrogen can be an important limiting factor in the microbial production of antibiotics [44].Supplementation of medium with organic as well as inorganic nitrogen source resulted in improved in antibiotic production compared to control. It may be due to the fact that the substrate is already rich enough to supply the nutrients required for micribial growth and rifamycin B production. Mahalaxmi et al 2010 [24] has reported similar results for rifamycin B production under SSF using corn husk and wheat bran as the substrate.

Among the different inorganic nitrogen sources tested, ammonium chloride (1% w/v) was the best source for maximal antibiotic production (COC for 3.56g/Kgds, GOC for 3.65/Kgds, GNS for 3.12/Kgds and RH for 3.75/Kgds) (Fig. 10). Further, the inorganic nitrogen sources, compared to organic nitrogen sources, at the given

concentrations of nitrogen, promoted higher antibiotic yield, where a maximum production (COC for 4.98g/Kgds, GOC for 4.56/Kgds, GNS for 4.79g/Kgds and RH for 4.46g/Kgds) was observed with Yeast extract (Fig. 9). Probably the presence of additional nitrogen sources in the medium promoted enhanced growth and consequent antibiotic production.

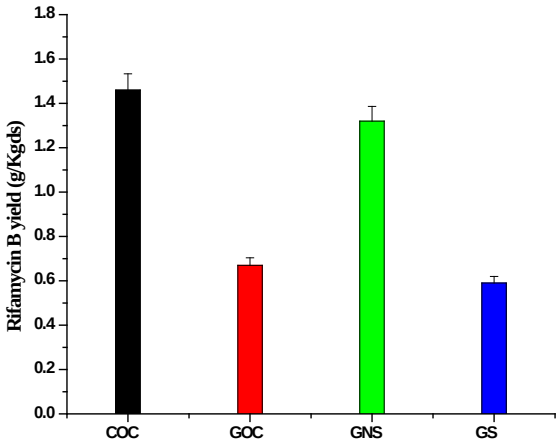

Fig. 1: Production of rifamycin B by *Amycolatopsis mediterranei* MTCC 14 by SSF in various solid substrates

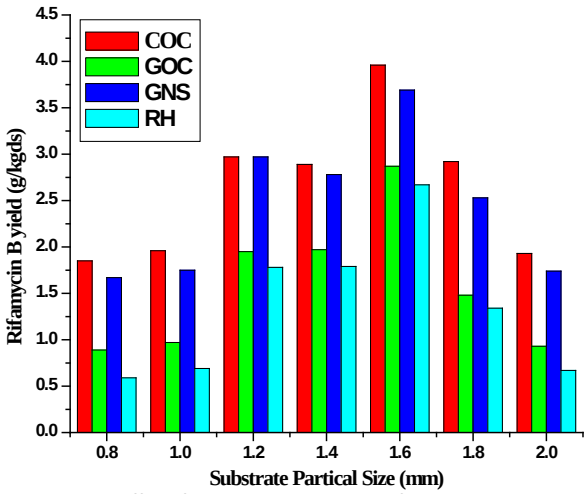

Fig. 2: Effect of substrate partical size on rifamycin B production by *Amycolatopsis mediterranei* MTCC 14 under SSF.

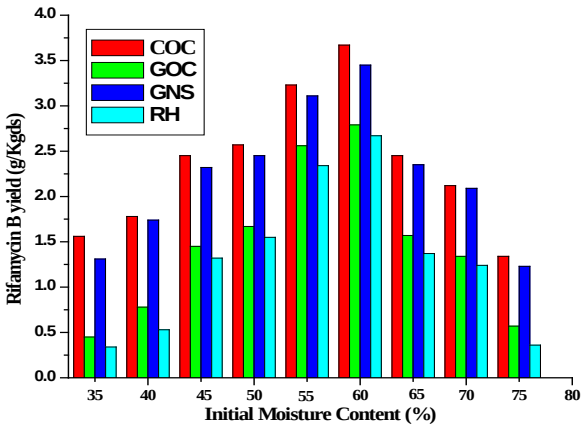

Fig. 3: The effect of initial moisture content in the production of rifamycin B by *Amycolatopsis mediterranei* MTCC 14 in SSF

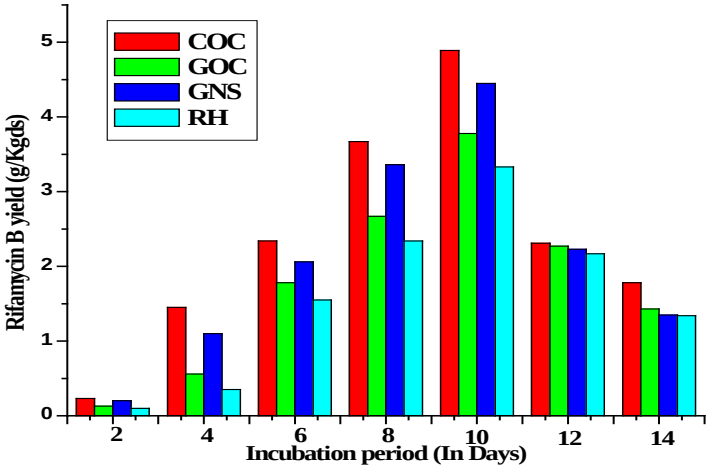

Fig. 4: Effect of incubation period on rifamycin B production by *Amycolatopsis mediterranei* MTCC 14 under SSF

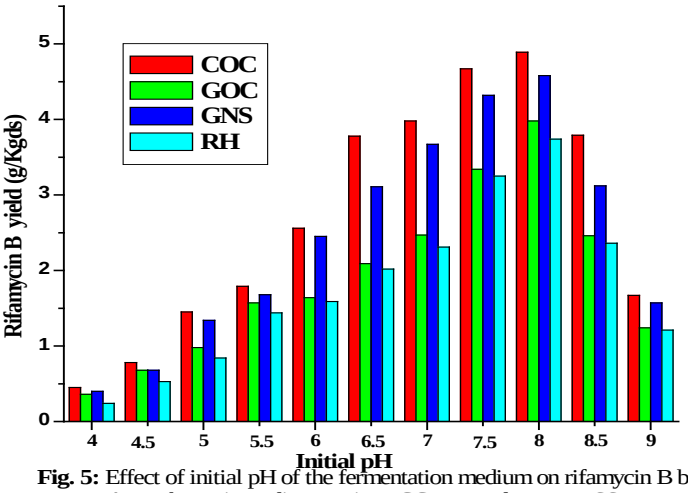

Fig. 5: Effect of initial pH of the fermentation medium on rifamycin B by *Amycolatopsis mediterranei* MTCC 14 production in SSF.

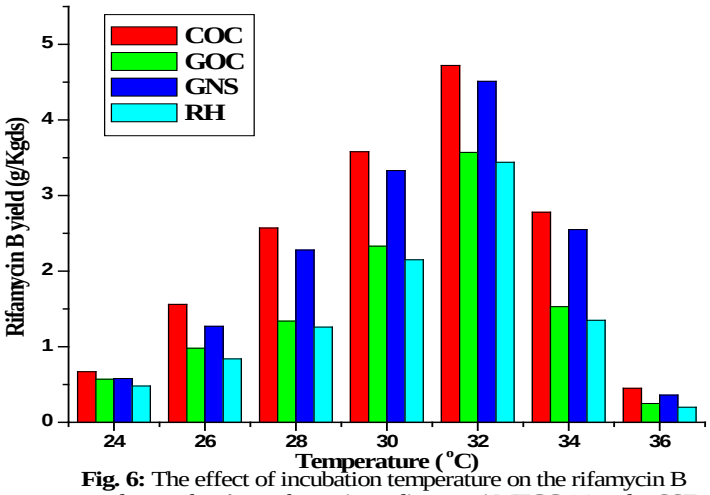

Fig. 6: The effect of incubation temperature on the rifamycin B production by *Amycolatopsis mediterranei* MTCC 14 under SSF

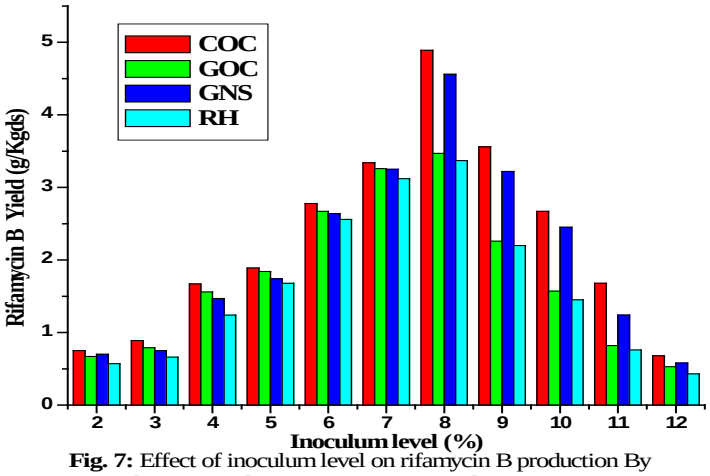

Fig. 7: Effect of inoculum level on rifamycin B production By *Amycolatopsis mediterranei* MTCC 14 Under SSF

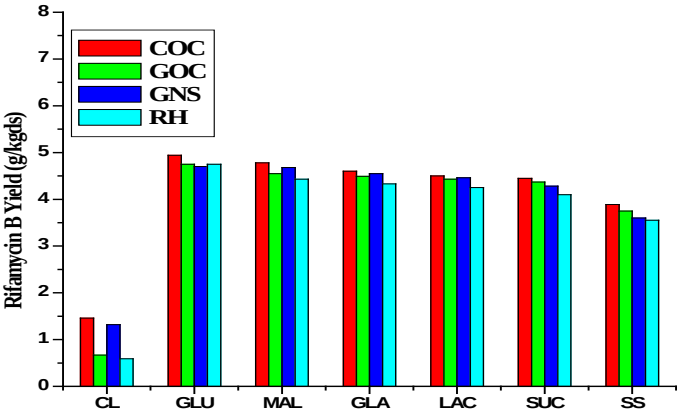

Fig. 8 : Effect of supplementary carbon sources (1% w/v) on rifamycin B production by *Amycolatopsis mediterranei* MTCC 14 under SSF. (CL, control only sustrates; GLU, glucose; GLA, galactose; SUC, sucrose; LAC, lactose; MAL,maltose and SS soluble starch)

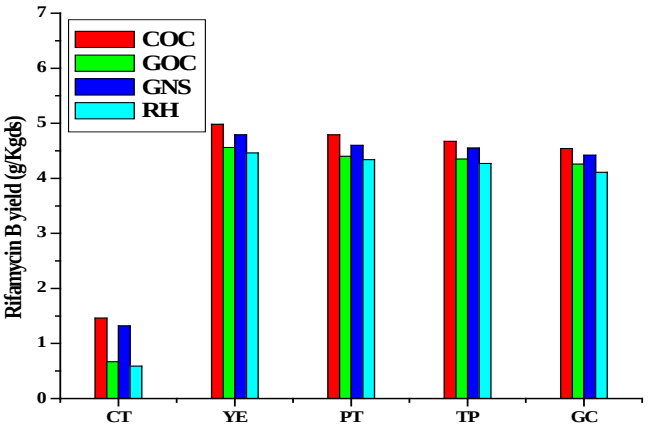

Fig. 9: Effect of suplimentary organic nitrogen (1% w/v) sources on rifamycin B by ***Amycolatopsis mediterranei*** MTCC 14 under SSF, (CL,control with substrate only; YE, Yest Extract; PT Peptone; TP, Tryptone; GC, Glycine)

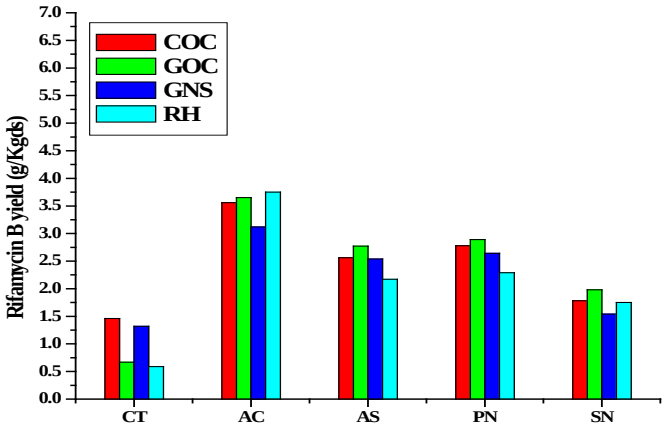

Fig. **10:** Effect of suplimentary inorganic nitrogen sources on rifamycin B production by *Amycolatopsis mediterranei* MTCC 14 under SSF.(CL, Con -trol only substrate; AC, Ammonium Chloride; AS, Ammonium Sulphate; PN, Potassium Nitrate; SN, sodium nitrate)

CONCLUSION

The results of the present study pointed to the importance of utilization of natural substrates, viz. coconut oil cake, groundnut oil cake, ground nut shell and rice husk for the production of rifamycin B antibiotic, which has therapeutically importance. The yields obtained in the present case would have to be further increased for its industrial importance but it has proved the feasibility of SSF and agro-industrial residues for rifamycin B production.

ACKNOWLEDGEMENTS

We grate full to principal SET`s College Of Pharmacy, Dharwad and Department of PG Studies in Biochemistry, Jnana Sahayadri Kuvempu University, Shankarghatta, Shivamogga-577451,Karnataka, India for supporting this project.

REFERENCE

1. Wehril W. Ansamycins chemistry, biosynthesis and biological activity Topics in Current Chemistry. 1977; **72:** 21.
2. Yee YC, Kisslinger B, Yu VL, Jin DJ. A mechanism of rifamycin inhibition and resistance in Pseudomonas aeruginosa. Journal of Antimicrobial Chemotherapy. 1996; 38: 133.
3. Yagura T, Kozu T, Seno T. Inhibition of DNA polymerases alpha and gamma by rifamycin derivative AF/013. Biochemistry Journal. 1981; 90**:**1397.
4. Kukielka E, Cederbaum AI. Stimulation of NADH-dependent microsomal DNA strand cleavage by rifamycin SV. Journal of Biochemistry. 1995; 307**:** 361.
5. Maenza J, Flexner C. Combination antiretroviral therapy for HIV infection. Amirican Family Physician. 1998; 57**:** 2789.
6. Szabo C, Bissel MJ, Calvin M. Inhibition of infectious Rous sarcoma virus production by rifamycin derivative. Journal of Virology. 1976; 18:445.
7. Huang DB, Dupont HL. Rifaximin–a novel antimicrobial for enteric infections. Journal of Infection. 2005; 50:97.
8. Kim IH, Combrink KD, Ma Z, Chapo K, Yan D, Renick P, Morris TW, Pulse M, Simecka JW, Ding CZ. Synthesis and antibacterial evaluation of a novel series of rifabutin-like spirorifamycins. Bioorganic Medicial Chemistry Letters. 2007; 17:1181.
9. Lawn SD. Tuberculosis and HIV Coinfection. Medicine. 2005; 33:112.
10. Sepkowitz KA, Rafalli J, Riley L, Kiehn TE, Armstrong D. Tuberculosis in the AIDS era. Clinical Microbiology Review. 1995; 8: 180.
11. Sensi, PA, Greco M, Ballotta R. Rifomycin 1. Isolation and properties of rifomycin B and rifomycin complex. Antibiot. Annu. 1960; 262-270.
12. El-Shahed KYL, Farid MA. Effect of soe parameters on the production of rifamycins B and SV by *Amycolotopsis mediterranei*. Pak. J. Sci.Ind.Res. 1994; 37:1458-461.
13. Ashok P. Solid state fermentation. Biochem. Eng. J. 2003; 13: 81–84.
14. Kota KP, Sridhar P. Solid state cultivation of *Streptomyces clavuligerus* for cephamycin C production. Process Biochem. 1999; 34:325-/8.
15. Kota KP, Sridhar P. Solid state cultivation of *Streptomyces clavuligerus* for production cephamycin C. J Sci Ind Res.1998; 58:587-/90.
16. Yang SS, Swei WJ. Oxytetracycline production by *Streptomyces rimosus* in solid state fermentation of corn-cob. World J Microbiol Biotechnol 1996; 12:43-46.
17. Yang SS. Antibiotics production of cellulosic wastes with solid state fermentation by *Streptomyces*. Renew Energy. 1996; 9:876-/979.
18. Ohno A, Ano T, Shoda M. Use of soybean curd residue, okara, for the solid state substrate in the production of lipopeptide antibiotic, iturin A by *Bacillus subtilis* NB 22. Process Biochem. 1996; 31:801-806./888
19. Ohno A, Ano T, Shoda M. Effect of temperature on production of lipopeptide antibiotics iturin A and surfactin by a dual producer, *Bacillus subtilis* RB 14, in solid state fermentation. J Ferment Bioeng. 1995; 80:517-519.
20. Adinarayana K, Ellaiah P, Srinivasulu B, Bhavani Devi R, Adinarayana G. Response surface methodological approach to optimize the nutritional parameters for neomycin production by *Streptomyces marinensis* under solid-state fermentation. Process Biochem. 2003; 38: 1565-1572.
21. Ellaiah P, Srinivasulu B, Adinarayana K. Optimisation studies on neomycin production by a mutant strain *Streptomyces marinensis* in solid state fermentation. Process Biochem. 2004; 39: 529-534.
22. Adinarayana K, Prabhakar, T, Srinivasulu V, Anitha Rao , Jhansi Lakshmi P, Ellaiah P. Optimization of process parameters for cephalosporin C production under solid state fermentation from *Acremonium chrysogenum*. Process Biochem. 2003; 39:171-/177.
23. Barrios-González J, Tomasini A, Viniegra-González G, López L. Penicillin production by solid state fermentation. 1988; 10:793-79.
24. Mahalaxmi Y, Sathish T, Subba Rao CH, Prakasham RS. Corn husk as a novel substrate for the production of rifamycin B by isolated *Amycolatopsis* sp. RSP 3 under SSF. Process Biochemistry. 2010; 45:47–53.
25. Venkateshwarlu G, Murali Krishna PS, Pandey A, Rao LV. Evaluation of *Amycolatopsis mediterranei* VA18 for production of rifamycin-B. Process Biochem. 2000; 36:305–9.
26. Holker U, Lenz J. Solid-state fermentation: are there any biotechnological advantages? Curr Opin Microbiol. 2005; 8:301–6.

27. Schneider ALS. Estudo da produc¸ ˜ao de inulinase por *Kluyveromyces marxianus* ATCC 36907. D.Sc. Thesis, Universidade Federal de Santa Catarina, lorian´opolis, Brazil, 1998.
28. Balakrishna K, Pandey A. Production of biologically active secondary metabolites in solid state fermentation. J Sci Ind Res 1996; 55:365-72.
29. Ghisalba O, Auden JAL, Schupp T, Nufesch J. The rifamycins: properties, biosynthesis, and fermentation. In *Biotechnology of Industrial Antibiotics*, Edited by E. J. Vandamme, Marcel Dekker, New York & Basel. 1984; P.281-327.
30. Rho YT, Nam-Goong G, Lee RJ. Rheological characteristics of rifamycin B fermentation using *Nocardia mediterranei*. J. Microbiol. Biotechnol. 1991; 1: 70-74.
31. Moore RE, Johnson DB. In Chemical Analys Wood and Wood Products. USDA Forest Product Laboratory, Madison. WS. 1967.
32. Prior BA, Preez JCD, Rein PW. Evironmental parameters, in: Solid Substrate Cultivation, Elsevier, London. 1992, p. 65.
33. Pasqualucci CR, Vigevani A, Radaelli P, Gallo GG. Improved differential spectrophotometric determination of rifamycins. J. Pharm. Sci. 1970; 59: 685-687.
34. Mitchell DA, Lonsane, BK. In: Doelle, H.W.,Mitchell, D.A., Rolz, C.E. (Eds.), Solid Substrate Cultivation. Elsevier Science Publishers, London. 1992; p.1–16.
35. Lonsane BK, Ghildyal NP, Budiatman S, Ramakrishna SV. Engineering aspects of solid state fermentation. Enzyme Microb Technol. 1985; 7:258–65.
36. Muniswaran PKA, Charryalu NCLN. Solid substrate fermentationof coconut coir pith for cellulase production. Enzyme Microb Technol. 1994; 16:436–40.
37. Hunang SY, Waug HH, Wei C-J, Malaney GW, Tanner RD.Kinetic responses of the koji solid state fermentation processes. In:Wiseman A, editor. Topics in Enzyme and Fermentation Biotechnology, vol. 10. Chichester: Ellis Horwood, 1985, p88–108.
38. Hesseltine CW. Solid state fermentations. Biotechnol Bioeng 1972;14:217–532.
39. Knapp JS, Howell JA. Solid state fermentations. In: Wiseman A, editor. Topics in Enzyme and Fermentation Technology.Chichester: Ellis Horwood, 1980; p85–143.
40. Pandey A. Recent developments in solid-state fermentation. Process Biochem. 1992; 27:109–116.
41. Carrizales V, Rodriguez H. Determination of specific growth rate of moulds in semi solid cultures. Biotechnol. Bioeng. 1981; 23:321–333.
42. Rainbauet M, Alazrd D. Culture method to study fungal growth in solid state fermentation. Eur J Appl Microbiol Biotechnol 1980; 9:199 -/209.
43. Sabu A, Swati C, Pandey A. Tannase production by *Lactobacillus* sp. ASR-S1 under solid-state fermentation. Proc. Biochem. 2006; 41: 575–580.
44. Bussari B, Saudagar PS, Shaligram NS, Survase SA, Singhal, RS. Production of cephamycin C by *Streptomyces clavuligerus* NT4 using solidstatefermentation. J. Ind. Microbiol. Biotechnol. 2008; 35:49–58.