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Rifamycin SV production using immobilized cells of *Amycolatopsis mediterranei* OVA5-E7 in different matrices

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ABSTRACT

Rifamycin SV is abroad-spectrum antibiotic widely used in human drug therapy. Cell immobilization can enhance the productivity of secondary metabolites such as antibiotics. The purpose of the present work is to enhance the yields of rifamycin by immobilization of mutant strain of Amycolatopsis mediterranei OVA5-E7.Calcium alginate was found effective and suitable for improving the productivity of rifamycin SV under repeated batch fermentation compared to other matrices. Rifamycin SV production with immobilized A.mediterranei cells was better matric made of 2% alginate and 3% calcium chloride with 1h curing time and maintained at pH 7.0 while the temperature was maintained at 28ºC for a period of 6 days. The rifamycin SV production increased to 6396 mg/L which yielded 1.5 folds higher quantity of antibiotic than free cells cultured under similar conditions.The cell leakage from the Ca-alginate matric is 0.94 mg/ml enabling five repeated batch fermentations with each batch lasting for 6 days.Similarly, the yields from other immobilization matrices are 4865mg/L in k-Carrageenan, 4620 mg/L in agar-agar, 4552 mg/L in polyacrylamide and 4401 mg/L using gelatin.Compared to earlier scientific reports, Ca-alginate entrapped cells of Amycolatopsis mediterraneia long with fine-tuned fermentation parameters as reported here were found effective for repeated batch submerged fermentations towards maximizing the rifamycin SV yields.

Keywords: rifamycin SV production, *Amycolatopsis mediterranei*, cell immobilization, alginate matrice, repeated batch fermentation

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INTRODUCTION

In human drug therapy, antibiotic production is one of the key areas in the field of applied microbiology and modern medicine attracting the attention of scientific groups world-wide.Self immobilization of microorganisms on films, floccules and pellets is a wide spread occurrence or a common phenomenon observed in nature. The use of immobilized cells in bioprocesses however is a relatively recent development that took place globally. In recent times much interest is being focused on the use of immobilized cells; many organisms have been immobilized by various methods for enhancing the production of various compounds. Antibiotics being secondary metabolites are synthesized after exponential growth phase of microbial cells in case of free cell cultures [1-5]. It is interesting to note that immobilization uncouples growth and metabolite production without affecting secretion of antibiotics in to extracellular medium. There is a paucity of information on the application of immobilized cell in the production of antibiotics, which needs focus and scientific attention.

Presently, cell immobilization is a useful technique to investigate the potential of new bio-industrial production processes. This technique has been used to increase production of several kinds of antibiotics and may improve, occasionally, the yields. The application of immobilized cells so asto improve microbial antibiotic production is one of the main areas of modern biotechnology [1,2,3]. The potential of immobilization is to maintain a high

concentration of cells and provide the required condition for continuous production of antibiotic without the need to grow cells, further. Cell immobilization can provide many operational and economical advantages such as prolonged metabolic activities, reuse of biocatalyst and preventing washing out of cells at higher fermentation flow rates [1-5].

Rifamycin is one of the most potent and broad-spectrum antibiotic used against bacterial pathogens and is a key component of anti-tuberculosis and retro-virus related disease therapy. Rifamycin's (e.g., rifamycin B, SV, etc) are a family of antibacterial antibiotics produced by the soil bacterium, *Amycolatopsis mediterranei.* These rifamycins belong to a class of antibiotics known as ansamycins, which are medically important macrolide antibiotics produced by actinomycetes in which an ansachain is linked to two non-adjacent positions of an aromatic nucleus.

Earlier, we developed an efficient strain namely *Amycolatopsis mediterranei* OVA5-E7 by successive mutations with UV and EtBr. Now, to enhance the rifamycin yields further we tried to refine and evaluate different immobilization matrices and the fermentation conditions.

Immobilization and repeated batch fermentations for rifamycin production are well known [2, 5].

The purpose of the present investigation was to study the immobilization of mutant *Amycolatopsis mediterranei* OVA5-E7 cells for higher rifamycin SV production using different entrapment matrices namely calcium alginate, k-Carrageenan, polyacrylamide, agar-agar and gelatin gel. The reusability of immobilized cells for rifamycin SV production under repeated batch fermentation conditions was also investigated and the results are presented.

EXPERIMENTAL SECTION

Culture isolate

A mutant strain of *Amycolatopsis mediterranei* OVA5-E7, producer of rifamycin SV was used in the present study. The mother strain *A. mediterranei* (NCIM 5008) was obtained from National Collection of Industrial Microorganisms (NCIM) and Gene Bank, a national facility housed at Pune, India. The strain was mutated with UV radiations followed by chemical mutagenesis with Ethidium Bromide (EtBr) which eventually lead us to develop a new efficient strain, named as OVA5-E7.

Inoculum preparation

Inoculum was prepared by transferring 5 ml suspension prepared from 5 days old slant culture, into 250 ml Erlenmeyer flasks containing 100 ml of sterile inoculum medium. The composition of the inoculum medium was (g/L): glucose 20, peptone 5.0, yeast extract 5.0, meat extract 5.0, enzymatic hydrolysate casein 2.5 and sodium chloride 1.5 with a pH of 7. The flasks were incubated on a rotary shaker at 200 rpm at 28ºC for 2 days. After incubation the culture was transferred into a sterile tube and centrifuged for 5 minutes at 1200 rpm. The supernatant was discarded and the culture pellet suspended in 0.1 M phosphate buffer.The obtainedvegetativecells were used to inoculate shake flask in immobilization studies.

Medium for rifamycin SV production by immobilization

Medium used for immobilization studies contains [g/L]: 20.0 glucose, 3.0 KH_2PO_4, 1.5 K_2HPO_4, 1.0 $MgSO_4$**.**7 H_2O**,** 0.016 $FeSO_4$.7 H_20, 0.001 Zn acetate, 5.0 yeast extract, 5.0 peptone. The pH was adjusted to 7.0 with 0.1 NaOH. A 50 ml of medium was distributed into each flask before autoclaving. Glucose was sterilized separately and added to the cultivation medium before inoculation.

Optimization for immobilization

Sodium alginate

Various concentrations of sodium alginate (0.5, 1, 1.5, 2, 2.5 and 3 w/v) were used to prepare the beads. Sodium alginate and calcium chloride were used to prepared alginate beads. Sodium alginate solution was prepared by dissolving appropriate amount of sodium alginate in 100 ml of hot distilled water separately. The contents were stirred vigorously for 10 min to obtain thick and uniform slurry without any un-dissolve clumps. The same was sterilized by autoclaving. Both alginate slurry and cell suspension (10% v/v) were mixed and stirred for 10 min to get uniform mixture. The slurry was taken into sterile syringe and then added drop wise into 3% calcium chloride solution from 5 cm height and kept it for curing at 4ºC for 1 h. Then the beads were washed with sterile water for 3 to 4 times and preserved at 4ºC if not used immediately. All the operations were carried out aseptically under laminar flow unit. The beads were transferred into production medium and incubated at 28ºC for 144 h. The cell mass and rifamycin SV production at different times of fermentation were estimated.

$CaCl_2$ concentration
The concentration of cationic solution has a significant effect on the gelling behavior of alginate. For the evaluation of bead stability, immobilization procedure was carried out with different concentration of calcium chloride solution (1, 2, 3 4 and 5 %). All the fermentation experiments and assays were carried out as per the general procedure described earlier.

Cationic solutions
Different cationic solutions such as $BaCl_2$, $SrCl_2$, in addition to $Cacl_2$ (3%) were used to prepare the alginate beads containing cells as described earlier, the success of cell immobilization in gels mainly depends upon the gel stability to withstand compactness problem during fermentation process. The fermentation and assay was carried as described earlier

Curing time
To study the effect of curing time of beads on rifamycin SV production, the beads were cured for different time periods (1, 2, 4, 6 and overnight). Equal number of beads from each curing time were transferred into production medium and incubated at 28ºC for 144 h. The fermentation and assay was carried as described earlier.

Polyacrylamide
Two ml chilled cell suspension was prepared in 10 ml chilled sterile water. To 10 ml of sterile 0.2 M phosphate buffer pH 7.0 the following chemicals were added; 2.85 g acrylamide, 0.15 g methylene bisacrylamide, 10 mg ammonium per sulphate and 1 ml TEMED. Then the chilled cell suspension and chilled potassium phosphate buffer were mixed well and poured into sterile flat bottom 4 inches diameter petriplates. After polymerization (solidification) the acrylamide gel was cut into equal size cubes ($5x5x5mm^3$) and transferred to 0.2 M phosphate buffer pH 7.0 and kept in a refrigerator for 1 h for curing. The cubes were washed 3 to 4 times with sterile distilled water and transferred to production medium in EM flasks. The flasks were incubated at 28ºC for 144 h.The cell mass and rifamycin SV production at different times of fermentation were estimated.

Agar-agar
Agar-agar was dissolved in 18 ml of 0.9% sodium chloride solution to get final concentration of 1% in boiling tubes and sterilized by autoclaving. To a molten agar tube maintained at 40ºC, a 10% level (2 ml) of cell suspension was added and mixed well for few seconds (without forming foam) and then poured into sterile flat bottom petriplates and allowed to solidify. After solidification the solidified agar was cut into equal size cubes ($5\times5\times5$ mm^3), and then added to sterile and transferred to 0.2 M phosphate buffer pH 7.0 and kept in a refrigerator for 1 h for curing. The cubes were washed 3 to 4 times with sterile distilled water and transferred into production medium in EM flakes. The flasks were incubated at 28ºC and the sample was collected after 144 hr.

Gelatin
Two ml cell suspension was added to 18 ml molten sterile 4% gelatin solution at 45°C and the mixture was poured into a sterile petridish. The gel was over layered with 10 ml of 5% sterile gultaraldehyde solution for hardening at room temperature. The resulting film was cut into small cubes ($5\times5\times5mm^3$) and the cubes were washed thoroughly with sterile distilled water to remove excess gultaraldehyde and then added to sterile 0.2 M phosphate buffer pH 7.0 and kept in the refrigerator (1 h) for curing. After curing, phosphate buffer was decanted and the cubes were washed with sterile distilled water for 3 to 4 times. All the above operations were carried out under sterile conditions in a laminar flow unit. Then the cubes were transferred to production medium in EM flasks. The fermentation was allowed to take place by incubating the flasks at 28ºC on rotary shaker (200 rpm) for 120 h. The cell mass and rifamycin SV production at different times of fermentation were estimated.

κ-Carrageenan
About 800 mg was weighed and added to 18 ml of 0.9 % (w/v) NaCl. It was dissolved by gently heating and sterilized by autoclaving. To a molten κ-carrageenan tube at 40ºC, a 10 % cell suspension was added, mixed well and pour into flat bottom petriplates. After solidification the κ-carrageenan entrapped cells were cut into equal size cubes ($5x5x5$ mm^3) and added to sterile 2 % potassium chloride solution and kept at 4ºC for 1 h for curing. The cubes were washed for 2 to 3 times with sterile distilled water and transferred to production medium EM flasks. The fermentation was carried out at 28ºC for 120 h.The cell mass and rifamycin SV production at different times of fermentation were estimated

Repeated batch fermentation with immobilized cells
This is an important commercial parameter, because the main advantage of immobilized system is its usage for multiple batches. Hence an attempt was made to study the reusability of immobilized beads. The reusability of the immobilized beads was tested in batch system. Repeated batch fermentation with immobilized cells of four matrices

viz. alginate, polyacrylamide, k-carrageenan,agar-agar and gelatin which showed higher rifamycin SV production than that of free cells was carried out to evaluate the antibiotic production capacity. The beads/blocks were prepared and used for rifamycin SV production. The fermentation medium was replaced after every 120 h incubation for k-carrageenan, gelatin, and after 144 h for alginate, polyacrylamide, agar-agar immobilized cells. The immobilized cells were washed twice with 50 ml of sterile saline solution. 50 ml of fresh production medium was added and again incubated. The above process was repeated until there was no significant rifamycin yield. The antibiotic titers of different cycles were measured.

Analytical methods

Quantitative estimation of rifamycin is one of the important areas of pharmaceutical analysis; it was performed by microbiological assay using *E.Coli* 2345 and chromatographic techniques (HPLC).The column effluents were monitored at 254 nm. Samples were eluted with aqueous solutions contain potassium phosphate buffer : acetonitrile (55:45). Always 20 µl of sample was loaded for analysis at 25ºC. The purified test samples using reverse phase column RP-C18, 254 nm showed an identical peak to standard rifamycin SV.

RESULTS AND DISCUSSION

Optimization of fermentation parameters

Cell immobilization is one of the common techniques in fermentation process used to increase the overall cell concentration and productivity [1-7]. Here the separation of final product from immobilized cells is easier when compared to suspended free cell cultures. The purpose of this investigation was to study the effect of *Amycolatopsis mediterranei* OVA5-E7 cells immobilized in various matrices so as to enhance the yields of rifamycin SV. The five important matrices used are calcium alginate, κ-carrageenan, polyacrylamide, agar-agar and gelatin. The reusability of immobilized cells for rifamycin SV production under repeated batch fermentation was finally reported.

Sodium alginate concentrations

Ca-alginate was reported suitable for cyclosporinA production by immobilizing *Aspergillus terreus* [1].In order to evaluate the stability of Ca-alginate beads in rifamycin production as well various concentrations of alginate (0.5, 1, 1.5, 2 and 3 w/v) were used to immobilize *Amycolatopsis mediterranei* OVA5-E7. In the present study, our results indicated that Ca-alginate concentration plays a prominent role in rifamycin SV production. The results were shown in Fig. 1 and Table 1-3. The antibiotic titer reduced with increased alginate concentration above 2% (w/v). This may be due to reduced porosity in the beads limiting the nutrients and oxygen diffusion. It was found that 2% alginate and 6 days batch incubation period were optimum conditions to enhance the antibiotic out put. Rifamycin SV production increased to 6396 mg/L which accounts for 1.5 folds higher antibiotic than free cells under the given set of conditions. The cell leakage from the Ca-alginate matric is just 0.94 mg/ml compared to all other immobilization matrices. On the contrary, 4% alginate beads with gellan gum as hardening agent were earlier used by other scientific groups [4] to yield 2393 mg/L of rifamycin SV in 3 days fermentation period that could be repeated for 5 batches. By comparing the earlier reports [4] with our study, the influence and importance of porosity and fermentation period on rifamycin SV production or yields by *A. mediterranei* can be clearly visualised.

Similar findings were also reported in the production of oxytetracycline by immobilized *Streptomyces rimosus* [6]. Other research groups cited that 4% alginate was better than other concentrations for the production oxytetracycline where there was a significant decrease in the cell leakage from the beads as the concentration of alginate was increased [4]. However, this was not true with our findings. In our study, 2%Ca-alginate concentration showed higher antibiotic productivity and beads intactness, which was similarly to xylanase production using *Bacillus pumilus* [3].

$CaCl_2$

The alginate beads were prepared with various concentrations of cation (1, 2, 3, 4, and 5 %) for rifamycin SV production. It is evident from the results shown in Fig. 2 that calcium chloride concentration (3%) has a significant effect on rifamycin SV production. When 1% $CaCl_2$ solution was used, the gelling was relatively slow and it required more time for curing. A central core was observed inside the bead. At higher concentrations of calcium chloride (5%), the gelling was instantaneous and hardening effect was noticed. The beads prepared with 1% and 2% calcium chloride solution were irregular in shape, whereas the beads prepared with 3% and higher concentrations of calcium chloride were spherical in shape. The antibiotic production from the beads prepared with 3% $CaCl_2$ was found to be higher when compared to the beads prepared with other higher concentrations. The beads prepared with 1% and 2% calcium chloride were disintegrated by the end of fermentation. Hence in our study, the beads prepared with 3% calcium chloride were found to be optimum for alginate gelling and maximized the rifamycin SV (6396 mg/L) production. Stability of the beads increased with an increase in calcium chloride concentration. Reports have

shown that Nisin production was enhanced using batch and fed-batch fermentation with Ca-alginate immobilized cell cultures [7], which is in concurrence with our findings.

Table.1: Rifamycin SV production with immobilized cells of *A. mediterranei* OVA5-E7 in different matrices

S.No	Matrices	No. of batchs	Rifamycin SV (mg/L)
1	Free cells	1	4328(±42.1)
2	Ca-alginate	6	6396(±32.8)
3	Poly acryl amide	3	4552(±82.5)
4	Agar-Agar	4	4621(±54.6)
5.	κ-Carrageenan	5	4865(±34.8)
6.	Gelatin	3	4401(±53.2)

Table.2: Rifamycin SV production in repeated batch fermentation using immobilized cells of *A. mediterranei* OVA5-E7 with different matrices

Matrices	Batch No.	Rifamycin SV (mg/L)	Cell leakage (mg/ml)
Alginate matric	1	6396	0.94
	2	6201	1.12
	3	5936	1.34
	4	5634	1.72
	5	4876	1.91
	6	*1976*	*3.5*
Polyacrylamide	1	4552	1.22
	2	4023	1.78
	3	2819	2.25
κ-Carrageenan	1	4865	1.06
	2	4237	1.21
	3	3821	1.85
	4	2854	2.13
	5	2256	2.41
Agar-agar	1	4620	1.27
	2	4021	2.67
	3	3128	3.09
	4	2013	2.86
Gelatin	1	4401	1.27
	2	3109	2.48
	3	1089	3.26

Table 3: Total Rifamycin SV yields in repeated batch fermentation with different immobilizing matrices

Matrices	Period/batch (days)	No. of batches to repeat	Total fermentation days	Total yield of Rifamycin SV (grams)
Free cells	9	1	9	4.328 ±0.421
Ca-alginate	6	5	30	29.043±0.33
Poly acryl amide	6	3	18	11.394±0.82
Agar-Agar	6	4	24	13.782±0.546
κ-Carrageenan	5	5	25	18.033±0.348
Gelatin	5	2	10	7.510±0.532

Cationic solutions

The alginate beads were prepared and evaluated with $CaCl_2$, $BaCl_2$ and $SrCl_2$ (3%) for the production of rifamycin SV and the results were shown in Fig. 3. Curiously, the results indicated that antibiotic production was higher with the calcium alginate beads (6038 mg/L) when compared with strontium alginate (3290 mg/L) and barium alginate (3451 mg/L). Lower antibiotic production with barium and strontium alginate beads was recorded because of reduced porosity. Presence of $CaCl_2$ resulted in better porosity and therefore led to higher production of antibiotic. Although the alginate beads prepared with $BaCl_2$ and $SrCl_2$ eventuallu yielded beads with good strength, $CaCl_2$ was used to prepare the beads in all further experiments because of its higher yield, better porosity and lower cost. The calcium alginate beads got disintegrated within 90 minutes, whereas the barium alginate and strontium alginate beads were stable until 48 h. Hence, our present investigation inferred that the gel stability was higher with $BaCl_2$ and $SrCl_2$ when compared with $CaCl_2$. However, $CaCl_2$ gave better porous beads and higher production of antibiotic as observed when compared with other two other cations in use.

Curing time on rifamycin SV production

The results shown in Fig. 4 indicated that the rifamycin SV production (5355 mg/L) with the beads cured for 1 h was higher than the beads cured for higher duration. The solubilization time of the beads increased with increased

curing time. Thus, from the results it was inferred that curing time of 1 h is optimum for the formation of stable calcium alginate beads which in turn improved the antibiotic production. Increase in curing time resulted in hard beads with less antibiotic production.

Fig. 1: Effect of various concentrations of sodium alginate on rifamycin SV production

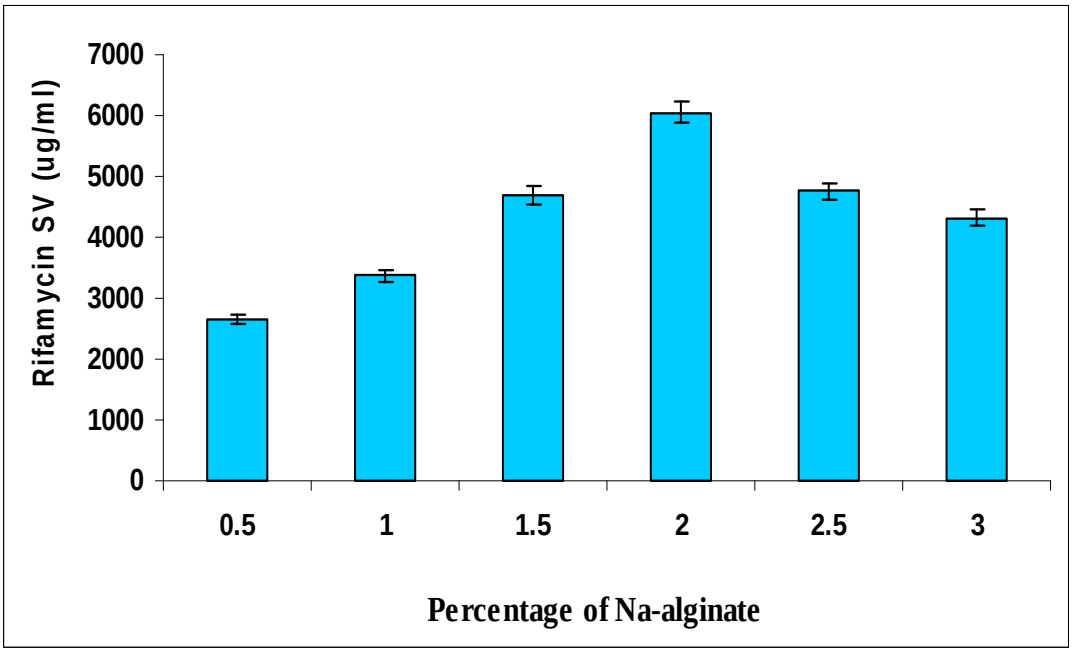
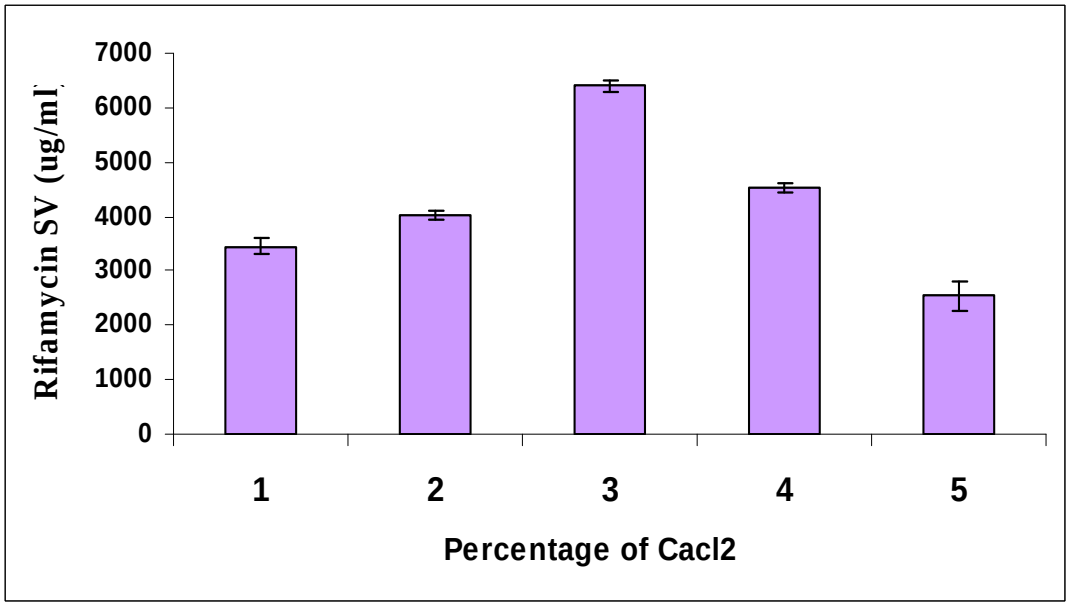


Fig. 2: Effect of various concentrations of calcium chloride on rifamycin SV production



Polyacrylamide matric

Polyacrylamide was successfully used for immobilization of many enzyme systems [8]. The results obtained on rifamycin SV production using cells immobilized in polyacrylamide matric are presented in Table. 1. The data indicated that there was a gradual increase in rifamycin SV production from 48 h onwards upto 96 h. However, on further increase in incubation period no significantly increase in the production rate was observed. Maximum yield and steady production was observed between 96 to 144 h of incubation. Best rifamycin SV yield (4552 mg/L) was obtained after 120 h of incubation. Antibiotic yield achieved by this method was higher than the yield obtained by entrapment in gelatin. The incubation period per batch was 6 days. Cell leakage increased gradually with increase of fermentation period and was more (1.29 mg/ml) when compared to other matrices except for agar and gelatin.

Polyacrylamide matric was used successfully in the immobilization of cells for the production of primary metabolites [9]. However, there is a scarcity of information on the production of secondary metabolites especially antibiotics, using immobilized cells entrapped in polyacrylamide gel matrices. Whole cells of *Bacillus sp*. for production of bacitracin [10] by immobilizing in polyacrylamide gel prepared by using 5% total acrylamide (95% acrylamide monomer and 5% N, N'-methylene-bis-acrylamide).

Fig. 3: Effect of curing time on rifamycin SV production

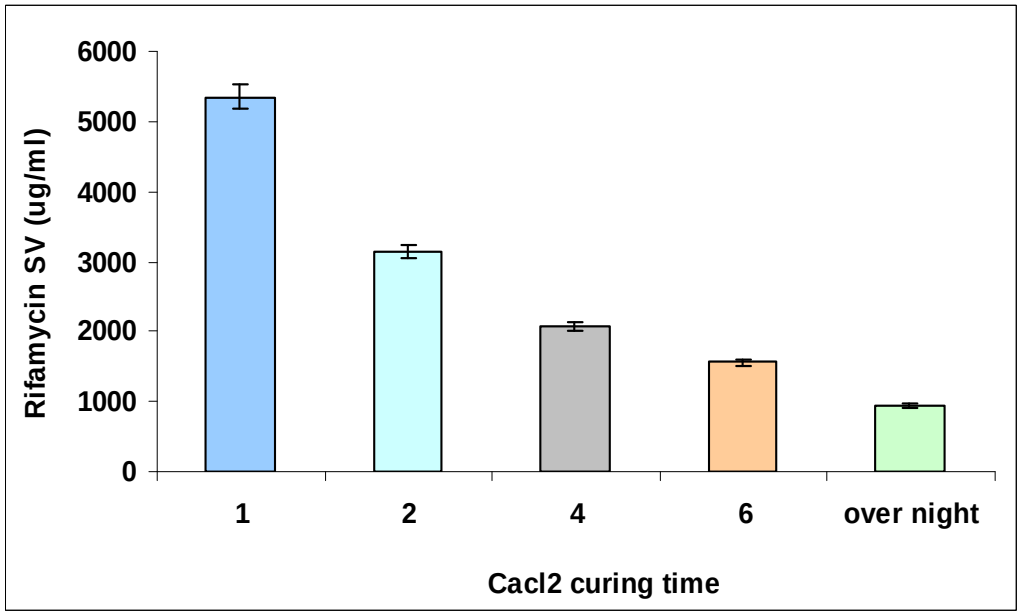
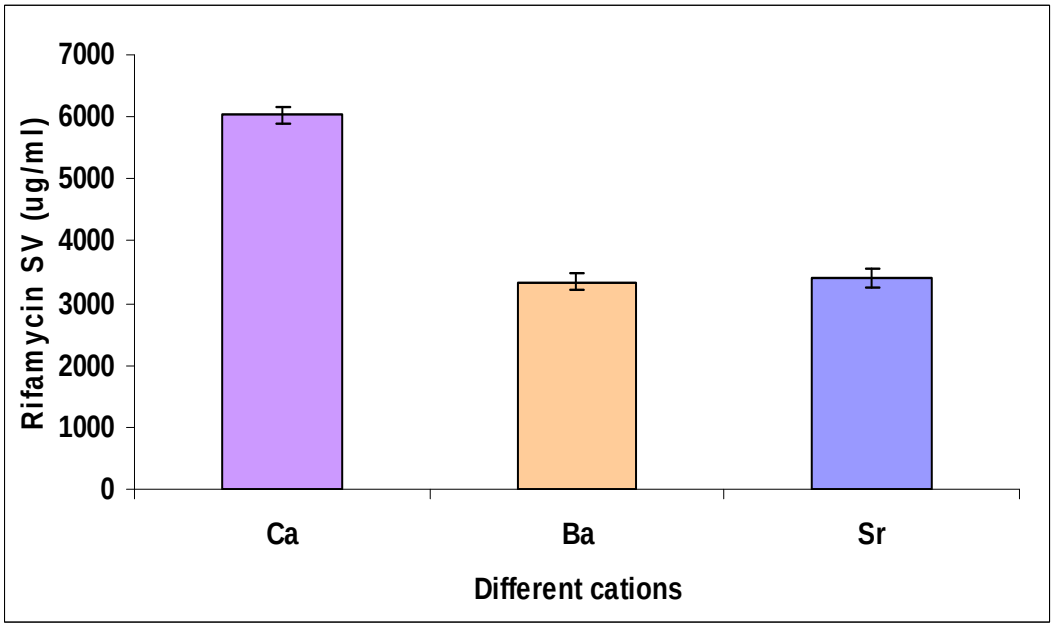


Fig. 4: Effect of various cationic solutions on rifamycin SV production

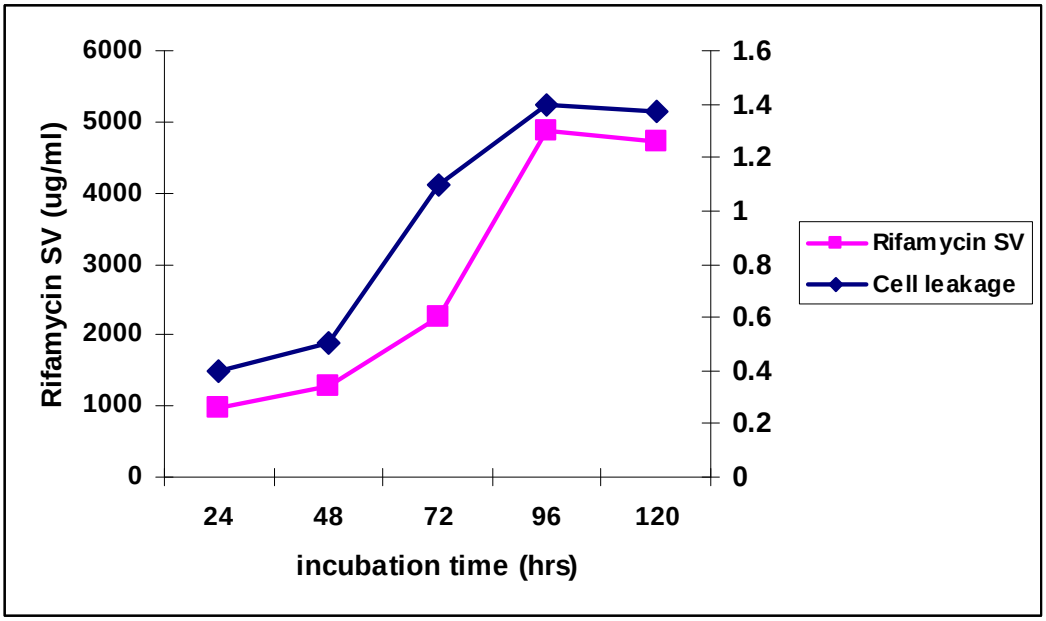


Agar-agar immobilization

Natural polymer like agar at 2% (w/v) is also employed for immobilization of *A. mediterranei*. The results indicated that the rifamycin SV production was initiated from 48 h onwards and reached a maximum level by 144 h (4620 mg/L) (Table 1-3). Rifamycin yield achieved by this method was lower than the yield obtained by entrapment in alginate and k-Carageenan. The incubation period for maximum antibiotic production (144 h) was however longer when compared to other matrices. Earlier [5], glass wool was used to reduce the fermentation period from 4 days to two days (half of the time period) to achieve the same quantity of rifamycin B and SV. Cell leakage in agar matric

started from 48 h onwards and increased gradually with increase in fermentation period. Cell leakage in agar matricwas more (1.29 mg/ml) when compared to other matrices used in our study, except for alginate.

Fig. 5: Rifamycin SV production using κ-carrageenan matric at different time intervals



Gelatin

The results showed that the rifamycin SV titer increased from 48 h of fermentation and reached a maximum level (4401 mg/L) by 120 h (Table 1). Our results also indicate that antibiotic yield was less when compared to free cell fermentation. The cell leakage increased gradually with increase of fermentation period. Cell leakage was also more (1.67 mg/ml) among all the matrices. Because of a very high leakage of cells from the matric, the growth of the organism and the antibiotic production was less. Probably, both gultaraldehyde (used for cross linking with gelatin) and polyacrylamide monomers were toxic for the cells. Relatively good titers of rifamycin SV were obtained with κ-carrageenan and agar. Calcium alginate beads appeared to be quite stable. These results are in accordance with earlier reports [11], where they used alginate immobilized cells for the production of tetracycline. The lower yields with κ-carrageenan and polyacrylamide blocks compared to alginate may be due to the low diffusibility of the nutrients and oxygen into the matrices. Lower production in agar matric could be attributed to higher cell leakage while gelatin may be due to toxicity of the cross linking agent gultaraldehyde.

κ-Carrageenan

Amycolatopsis mediterranei OVA5-E7 cells were immobilized in the κ-Carrageenan (4%) matric and used for the production of rifamycin SV (Fig. 5).The fermentation time period for each batch was fixed at 5 days. The results indicate that there is an increase in rifamycin SV production from 48 h onwards till 96 h (4865 mg/L) and later a negligible increase in rifamycin yields were observed. The maximum yield rifamycin SV was found to be 4865 mg/L at 120 h (Table 1-3). Antibiotic yield achieved by this method was lower than the yield obtained by entrapment in Ca-alginate. Cell leakage increased up to 120 h but the leakage was less (1.2 mg/ml) when compared to other matrices except alginate.There were a few reports were available using κ-Carrageenan as entrapment matric i.e. immobilization of *Pencillium chrysogenum* cells for the production of penicillin [10], respectively. The rate of cell leakage could be lowered by hardening the gel with potassium cations.

Repeated batch fermentation with immobilized cells

In repeated batch fermentation, a gradual decrease in rifamycin SV yield per batch was observed as the batches progress (Table 2 & 3).On the opposite, a gradual increase in cell leakage from the gel or matric was also observed during repeated fermentations. The beads entrapped in alginate matric were stable up to 5 batches and got disintegrated during the 6th batch operation, while in most other cases, matric was stable up to 3 batches and got disrupted during the 4th batch with an exception to agar and gelatin which showed cell leakage during the 2nd batch itself.

Our results again indicated that the productivity of rifamycin SV in alginate matric was higher than that of κ-carrageenan, polyacrylamide, agar-agar and gelatin in all the cycles. Low yields with gelatin and agar blocks may be

due to diffusional resistances of the nutrients and oxygen into the matrices for cell metobilization. Earlier, erythromycin was produced by *Streptomyces erythreus* entrapped in Ca-alginate beads under repeated batch fermentation (48 h of each batch) successfully for 12 batches (30 days) [12]. Similarly, good levels of oxytetracycline was produced for a period of 28 days (accounting for 7 batches) using *S. rimoses* cells immobilized in 4% Ca-alginate [4]. Further, immobilized cell stirring tank reactor produced penicillin for 25 days in a steady state [13].

The antibiotic production and cell leakage in five different matrices during the repeated batch fermentation were shown in Table 2. From the data obtained in present studies, it is clear, that the cells immobilized in Ca-alginate were efficient in rifamycin SV production in shake flask method of repeated batch fermentation when compared to the free cells and immobilized with other carriers. Additionally, the other advantage with Ca-alginate matric is that it is economical, non-toxic and just mild conditions are sufficient to prepare the biocatalyst.

CONCLUSION

The industrial production of rifamycin uses free cells in submerged fermentation which require longer incubation periods where most of the nutrients are utilised in microbial cell growth during the early phases. On the contrary, here the immobilization of mutant *Amycolatopsis mediterranei* with 2% alginate, 3% calcium chloride, 1 h curing time at pH 7.0 and temperature 28ºC for a period of 6 days was found to yield 6396 mg/L rifamycin SV which is 1.5 folds higher production than fermentation in free cell culture, under a given set of parameters. The cell leakage from Ca-alginate matric was nominal (0.94 mg/ml). Thus, in 30 days, 5 repeated batch fermentations can yield more than 29 g/L of rifamycin SV which is a costly broad-spectrum antibiotic. To our knowledge, this antibiotic yield is better than the previous reports published, so far.

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