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Enhanced Production of Cephalosporin by Entrapment of *Cephalosporium Acremonium* Spores in Various Support Materials

A. Swaroopa Rani[1], H R V N Goutham Rao[2], A. Kiran Kumar[3], B. Hima[4], A. Ravali[5]

[1,2,4]Centre for Biotechnology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad – 500 085, Telangana state (India)

[3]Dept. of Chemistry, Nizam College, Osmania University, Basheerbhag, Hyderabad- 500001, Telangana State (India)

[5]Department of Biotechnology,Sreenidhi Institute of science & Technology, Ghatkesar, Hyderabad, Telangana (India)

Abstract: ***In order to know the effect of supports on cephalosporin production under similar experimental conditions, Cephalosporium acremonium cells were immobilized by entrapment with Carragenan, Alginate, Agar, chistosan, Agarose, gelatin & PVA as support materials. A set of experiment with free cells was maintained as control. Cephalosporin production by immobilized & free cells was estimated from 24 hrs to 168 hrs of fermentation. In all cases cephalosporin production was found to be high at 72 hrs when immobilized. Whereas, free cells showed high production at 120 hrs. Agar was found to be a better support material than other supports used for immobilization. From the results of repeated batch fermentation in shake flasks, a good level of antibiotic was maintained for a period of about 30 days using 2% agar as support material.***

Keywords: Cephalosporin, C.acremonium, entrapment, support materials, aga

1. Introduction

During recent years the interest for secondary metabolites of commercial value has increased considerably. Broad spectrum activity and resistance to β-lactamases make cephamycins more effective in treating many cephalosporin resistant isolates i.e *E-coli, klebsiella proteus* etc. the extended spectrum of cephamycins also includes such organisms as *serratia, proteus, and bacterroides*, which are resistant to most cephalosporins [1]. Cephamycin C, a β-lactam antibiotic is used as an intermediate for semisynthetic antibiotics such as cefoxitin, cefmetazole, and cefotetan. Cefoxitin and cefmetazole are being used as therapeutic agents. Many market free casters see cephalosporins and cephamycins taking over from penicillin's as the most important β-lactam products of the future [2]-[4]

Several reports are currently available on the ability of Immobilized cells to produce cephalosporin with various supports like polyacrylamide, calcium alginate, carrageenen, celite as in the case of the production of bacitracin [4], patulin [5], thienamycin [6], nikkomycin [7,8], and penicillin [9]. Cephalosporin production by calcium alginate immobilized cells of the fungus *C.acremonium* [10, 11]. These studies have shown that the immobilization of *C.acremonium* can improve its cephalosporin productivity under special conditions. Studies on the polyacrylamide gel [12] suggested that polymerization reagents individually inactivate the multienzyme systems in the mycelium. Such negative effects have also been encountered which are shown by [13], when entrapping viable streptomyces cells for cephalosporin production with polyacrylamide. However very few reports are available on agar immobilization for cephalosporin production.

In the present study of immobilization, *C.acremonium* spores by entrapment in various gels have been carried. The production of secondary metabolites by immobilized cells was higher than that for freely suspended cells under the same conditions. Thus immobilized cells produced upto 3-4 times as much cephalosporin as free counterparts. In our initial studies on immobilized spores of *C.acremonium* we choose to use entrapment method using various polymers because of its distinct advantages with this immobilization technique, including reusability of the immobilized cells, which enables the investigation of free suspended cells, subsequent to their confinement in the immobilized state. Here we describe studies on alternative methods for the immobilization of *C.acremonium* spores. All experiments have been carried out with spores of *C.acremonium*. After immobilization by entrapment in various gels the viability of gels as well as the ability of the immobilized cells to synthesize cephalosporin has been investigated.

2. Materials and Methods

2.1 Materials

Carragenan, Alginate, Agar, chistosan, Agarose, gelatin & PVA were purchased from commercial sources.

2.2 Cultivation of *Cephalosporium acremonium* spores

C. acremonium ATCC 20339 spores were cultivated in the medium containing (in g/L); Soluble starch, 15; Yeast extract, 4.0; K_2HPO_4, 1.0; $MgSO_4$,1.0; pH: 6.5 at 27^0C.

2.3 Immobilization of *C. acremonium* spores

The immobilization of *C. acremonium* spores in various gels was carried out under sterile conditions in the following ways: Spores were entrapped in carragenan (2%), Alginate

(2%), Agar (2%), chistosan (2%), Agarose(2%), by following method [14]. Immobilization in a co.polymer of: chitosan (2%) & Gelatin (1%), Agarose (2%) & Gelatin (1%), Alginate (2%) & Gelatin (1), Agar (2%) & Gelatin (1%), PVA (2%) & Alginate (1%) were carried out [15, 16].

In all the above experiments equal amount of spore suspension 8.4×10^8spores/ml were taken for entrapment. These immobilized beads were grown in growth medium for 5 days containing (in g/l) : Peptone, 20; Malt extract, 20; Corn steep liquor, 5.0; $MgSO_4$, 0.25; K_2HPO_4, 0.5; KH_2PO_4, 1.0; $CaCl_2$, 0.1. pH maintained at 6.5 ± 0.2 with NaOH / HCl. Above beads were transferred to production medium containing (g/l): Sucrose, 80; Soya-bean meal, 60; $CaCO_3$, 1.5; DL-Methionine, 7.0; ammonia, 30. Sucrose was autoclaved separately; pH= 6.0.

Daily samples were collected from the fermented broth cultures and cephalosporin yield was estimated by biological assay [17] for each of the support matrices.

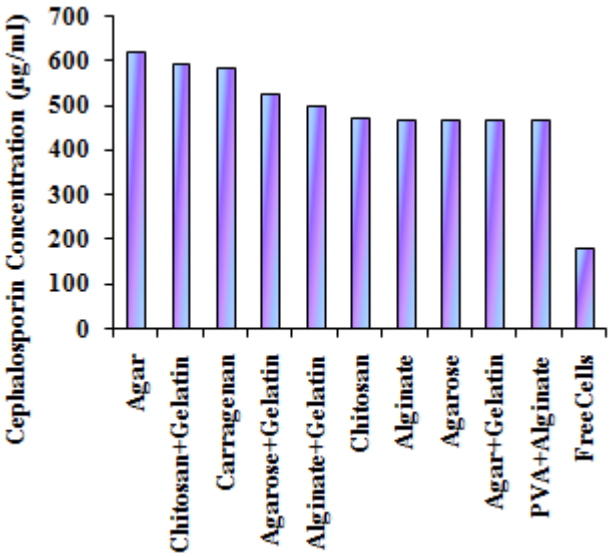

Figure 1: Effect of Various Polymers on Cephalosporin Production at the End of 72 hrs of Fermentation.

3. Results and Discussion

The present paper describes a comparative study of different support materials for immobilization of *C. acremonium* for enhancing cephalosporin production. 10 different experiments with various polymers are studied and compared with free cells. In this study spores of *C.acremonium* ATCC 20339 are entrapped into different polymers and allowed to grow for 5 days in growth media. This growth medium is selected based on our earlier studies carried out with different composition of growth medium. The amount of inocula is also optimized, since the higher level of spores reduced the lag period for growth, which was still slower for the cultures grown in presence of agar. Germinating the spores in a richer medium as mentioned above could further reduce this lag phase. After 5 days of incubation, beads from respective flasks were transferred into production medium; here the main carbon source is sucrose. The medium used is further optimized for higher production, by adding ammonia solution. Therefore this media composition is more favorable for eliciting cephalosporin from the entrapped beads.

From the preliminary work the concentrations of polymers containing; carragenan (2%), Alginate (2%), Agar (2%), chistosan (2%), Agarose(2%), & copolymers of chitosan (2%) + Gelatin (1%), Agarose (2%) + Gelatin (1%), Alginate (2%) + Gelatin (1), Agar (2%) + Gelatin (1%), PVA (2%) + Alginate (1%) and experimental conditions like fermentation temperature, 27^0C; fermentation time, 72 hr; shaking condition, 250 rpm, pH,6.0 were optimized. In order to evaluate the best support material for cephalosporin production, *C. acremonium* cells were immobilized with different support materials. A set of free cells was also carried with same experimental conditions as control. During fermentation, the cephalosporin production by these immobilized & free cells was estimated by bioassay at every 24 hrs time intervals and zone of inhibition was measured. Corresponding cephalosporin concentrations were obtained from standard cephalosporin calibration curve.

From fig: 1 we can see that agar is the best matrix for cephalosporin production & next to agar is chitosan + Gelatin. Agar beads show the maximum cephalosporin production of 620.24 µg/ml where as in case of free cells it is 180.88 µg/ml. Agar beads are almost 3-4 times more productive than free cells. The decrease in cephalosporin production in case of free cells may be due to the limitation of O_2 diffusion caused by the densely packed mycellial layer or due to antolysis of free suspended mycelia, which have higher growth rates [18]. If the growth is diminished this mycellial layer does not seem to limit diffusion. This could be observed by the stability of the specific cephalosporin production.

If the concentrations of support materials is increased more than 2% the decrease in antibiotic production was noticed which could be due to the resistance to the diffusion of nutrients into the beads, and diffusion of the product from the beads [19] – [22]. based on the results, 2% agar was selected for the subsequent experiments. When the agar immobilized beads were used for repeated batch fermentations, the production of cephalosporin was sustained for more than 5 cycles. The lower the leakage the better is the immobilization process and better support. The reduced cell leakage in agar beads facilitates enhanced separation of product, reduced diffusion and mass transfer limitations, clogging problems during the fermentation. Hence, very low cell leakage was observed in case of agar.

Immobilized cell systems with various supports can be an alternative to the established antibiotic fermentations, only when they offer distinct advantages [23] over existing processes such as low leakage of cells and high biocatalytic activity. Thus the present investigation suggests that agar as the support material was better than other support material for cell immobilization for the production of cephalosporin.

Hence, agar was selected as support material for further studies. The free cells produced maximum amount of antibiotic by 120 hrs while the immobilized cells were able to produce maximum amount of antibiotic by 72 hrs in the first cycle and by 24 hrs from the second cycle onwards.

When the 2% agar immobilized cells were used for repeated batch fermentations, the production of cephalosporin was carried for six cycles. The cells immobilized in agar exhibited high productivity when compared to free cells. Thus, the present investigation suggests that the agar was found to be a good support material for cell immobilization for the production of cephalosporin. Thus less expensive agar may be very attractive support material for large quantities of immobilized cells. Agar seems to afford an ideal microenvironment for immobilized spores. Thus agar gels meet the demand for lower costs.

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