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ORIGINAL ARTICLE

Single step Bioconversion of Cephalosporin C by strain of *Achromobacter* species isolated from rhizosphere soil

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ABSTRACT
The isolated organisms, Achromobacter species detected to convert cephalosporin C (CPC) into 7- aminocephalosporanic acid (7-ACA) in single step which suggested isolate has potential to produce enzyme cephalosporin C acylase. The organism, Achromobacter species was screened out from rhizosphere soil of Pomegranate plant. The bioconversion of cephalosporin C was initially detected by thin layer chromatography and further confirmed by HPLC. By HPLC assay, Achromobacter species showed higher cephalosporin C acylase activity 36.57 U ml^{-1} after 72 hours. Cephalosporin C acylase (CCA) converts cephalosporin C into 7-ACA which is an important intermediate for synthesis of broad spectrum derivatives of cephalosporin antibiotic.
Key Words: *Cephalosporin C, Bioconversion, Cephalosporin C acylase, Achromobacter sp.*

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INTRODUCTION

Cephalosporins are clinically active against Gram positive as well as Gram negative organisms. Cephalosporins are less toxic and resistant to degradation as compared to penicillins. Cephalosporin C produced by fungus *Cephalosporiumacremonium* [4]is not very much potent for clinical use. Its molecule can be transformed by removal of an aminoadipic acid side chain to form 7 - amino Cephalosporanic acid (7 ACA) [5] which can be further modified simply by adding side chains by chemical reaction into clinically useful broad spectrum antimicrobials. Enzymatic transformation of CPC to 7-ACA is best alternative and has industrial significance. The chemical method has been replaced by an enzymatic method [12]. An advantage of this method is avoiding most problems associated with the routine chemical process. Although the two step production of 7-ACA has potential rate of bioconversion but still single step production of 7-ACA from CPC is possible for desirable outputs and cost effectiveness. Several research reports are available and on one step biotransformation of CPC to 7-ACA.Bacterial strain belonging to *Arthrobacter* species has been found to produce cephalosporin acylases which convert both CPC and GL-7-ACA to 7-ACA. This may be an interesting feature of enzyme cephalosporin acylase [13]. A cephalosporanic acid acylase from *Pseudomonas* strain N 176 catalyzes hydrolysis of both glutarylcephalosporanic acid and cephalosporanic acid to 7 amino cephalosporanicacid[8]. *Pseudomonas diminuta* is producer of cephalosporin acylase which directly converts cephalosporin C to 7- ACA [3]. *Alcaligenesxylosoxidans* MTCC *491 revealed to produce enzyme cephalosporin acylase which converts CPC into 7-ACA[2]. Attempts are underway to find out cost effective and product effectiveone step bioconversion of CPC[7]. The present study was initiated with aim to isolate suitable bacteria for bioconversion of cephalosporin C in single step into 7-ACA. The isolate *Achromobacter* species was detected to transform CPC. Isolated organism was extensively studied for its characterization and enzyme activity assay.

MATERIALS AND METHOD

Chemicals and Media

7-aminocephalosporanic acid (7-ACA) was purchased from Sigma-Aldrich, India Cephalosporin C (CPC) was supplied by Pharmaceutical Industry, India. Yeast extract, minimal broth and all other chemical reagents and media were purchased from Hi Media Laboratories, India.

Qualitative detection of bioconversion by Thin Layer Chromatography:

Achromobacter species tested for bioconversion of cephalosporin C by thin layer chromatography[9]using TLC plates (Merck Silica Gel 60 F_{254}). The cells of *Achromobacter* species were suspended in 2 ml of sterile phosphate buffer pH 7 and subjected for disruption by sonication. The supernatant was collected after centrifugation at 4^{o}C (8000 rpm for 10 min.) and preserved at low temperature for further useas enzyme.The enzyme preparation inoculated in phosphate buffer pH 7containing 20 mM cephalosporinC and incubated for 1 hour at 37^{o}C with intermittent shaking. The supernatant obtained after centrifugation was used for TLC.

Detection of7- ACA by HPLC:

After extensive review of reports available on biotransformation it is marked that most of bacteria secret intracellular CPC acylase. The isolated organism *Achromobacter* species was grown in Minimal Broth (Himedia) at 25 ^{o}C for 24 hrs. The cells were harvested by centrifugation at 4 ^{o}C (8000 rpm for 8 min.). The cell pellet was washed 3 times with sterile phosphate buffer pH 7. Cells were suspended in 2 ml of sterile phosphate buffer pH 7 and subjected for disruption by sonication.The supernatant was collected after centrifugation at 4 ^{o}C (10,000 rpm for 10 min.) and used as enzyme preparation for biotransformation of CPC. The enzyme assay as per Deshpande et al., 1996 with slight modifications was performed in triplicates. An amount of 0.1 ml of enzyme preparation (cell free extract) was incubated with 10 mM of cephalosporin C in 50 mM Tris buffer pH 8.0. The reaction was terminated by the addition of equal volume of 1M acetate buffer pH 4.0. The amount of 7- ACA formed in reaction mixture was detected by HPLC. Reverse phase Enable H C-18 column (250mm × 4.6mm × 5µm particle size) was employed for the assay of CPC and 7-ACA using method reported by Qiang et al. [10]. The peaks were monitored at 254 nm. Previous report used the biological and colorimetric method for determination of cephalosporin acylase activity which found simple and useful for the study.Enzyme activity was calculated as the amount of enzyme required to form 1 µmole of product per minute under standard assay conditions.

RESULT AND DISCUSSION

Detection of bioconversion by Thin Layer Chromatography:

Presence of 7- ACA was visualized under short wave ultraviolet light in UV Trans-illuminator.Among bacterial isolates, organism *Achromobacter* species shown identical Rf value(Figure 1) and desirable band as similar to the standard 7-ACA[11].

Study of transformer organism:

The isolate was further confirmed as *Achromobacter* species by BIOLOG identification at CSMCRI (CSIR, Govt. of India), Bhavnagar, Gujarat and shown similarity value 0.704 with *Achromobacter xylosooxidans.* 16S rRNA sequences were analyzed by NCBI- BLAST and then phylogenetic tree was constructed using Clustal W. The isolated organism found closest relation with *Achromobacter xylosooxidans* ASU10 (Figure 2).

Detection of 7- ACA by HPLC:

The experiment was set up to test the ability of *Achromobacter* species to produce enzyme CCA and to reveal the qualitative and quantitative detection of 7-ACA by HPLC. The batch fermentation was conducted and samples withdrawn and analyzed by HPLC for detecting the amount of 7- ACA. The retention time of 7-ACA and CPC was 2.7 and 4.1 minutes similar as standards respectively. The peak area was used for calculation of conversion rate. The concentration of 7- ACA formed after enzyme reaction at an interval of every 24 hours of incubation was used for determination of CCA activity. The CCA activity was measured in *Achromobacter* species was 31.93, 35.57, 36.57 and 27.17 U ml^{-1} after 24, 48, 72, and 96 hours respectively (Figure 3).The enzyme activity was low at 24 hours which rose considerably at 72 hours. The 72 hours was thereby the optimum time period for production of Cephalosporin C acylase.

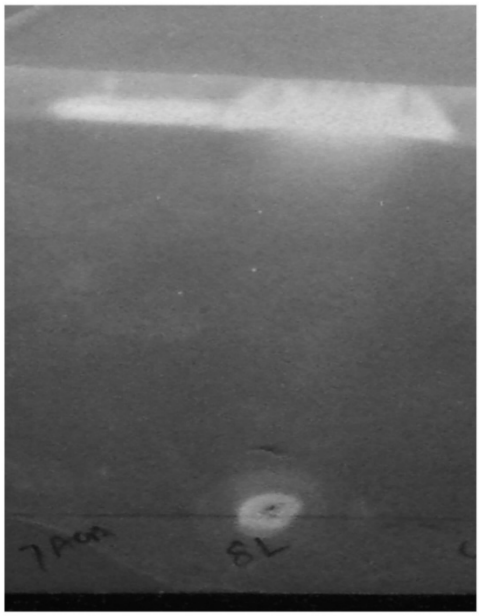

Figure 1: Chromatogram showing similar band and Rf value with standard 7-ACA.

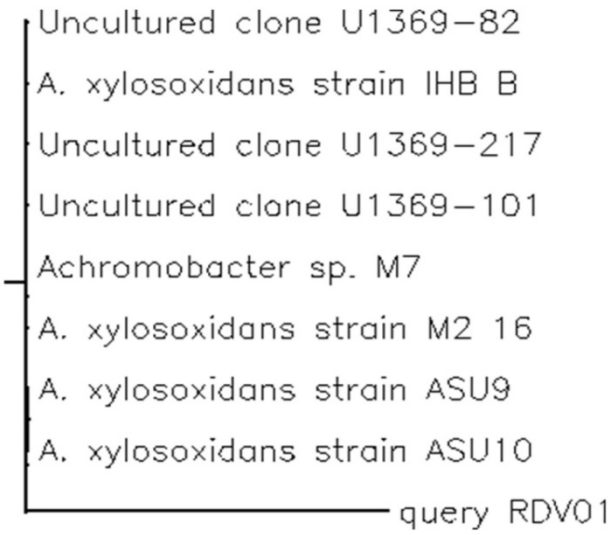

Figure 2: Phylogenetic analysis of isolate by Clustal W.

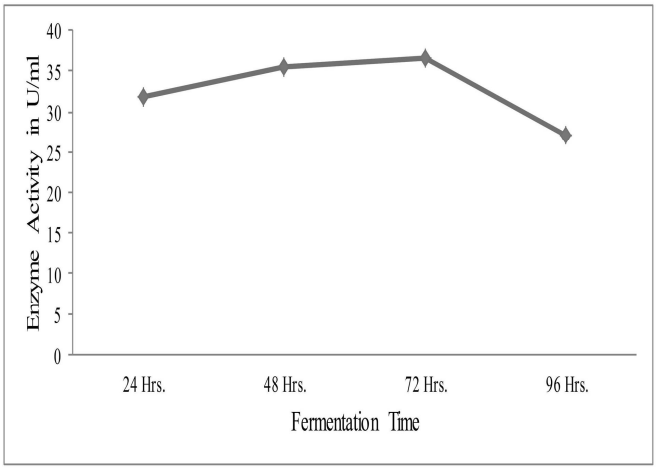

Figure 3: Cephalosporin C acylase activity determined in *Achromobacter* species by HPLC assay.

CONCLUSION

The single step bioconversion of cephalosporin C by potential cephalosporin C acyalse producer has industrial significance. In this study we have isolated organism *Achromobacter* species which showed potential of bioconversion of cephalosporin C in single step. The higher CCA activity was detected after 72 hours 36.57U ml^{-1} by HPLC studies.

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