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Strain improvement studies for cephalosporin C production by *Cephalosporium acremonium*

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Treatment of spore suspension of *Cephalosporium acremonium* ATCC 48272 with UV rays and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (NTG) induced mutants capable of producing higher yields of cephalosporin C. Antibiotic yield was improved from 630 μg/ml to 1995 μg/ml of the broth resulting in a high yielding mutant.

1. Introduction

One of the most important methods for improving the antibiotic yields is the development of improved strains by natural or induced mutation and selection. Strain improvement programs have given rise to extremely high yielding strains of *Penicillium chrysogenum* [1, 2]. A mutant of *Streptomyces marinensis* has produced higher yields of neomycin by this method [3]. Mutants of *Streptomyces* species giving higher antibiotic yields than the parent culture have been obtained using various mutagens [4–9]. *Cephalosporium acremonium* was treated by mutagens to produce high yielding mutant *Cephalosporium acremonium* M 8650 [10].

Cephalosporin C production has been studied with various species and strains of *Cephalosporium* and *Acremonium chrysogenum*. There are a few reports on the development of improved strains for cephalosporin C production [10]. The present study involves the use of UV irradiation and N-methyl-N-Nitro-Nitrosoguanidine (NTG) treatment for the development of high yielding mutants.

2. Investigations, results and discussion

The parent culture, *Cephalosporium acremonium* ATCC 48272 was subcultured on potato dextrose agar slants. These slants were incubated for 5 days at 28 °C and scrapped off into sterile water containing Tween-80 (0.02%). The suspension was thoroughly shaken for 30 min on a rotary shaker (220 rpm) to break the spore chains, filtered through a sterile cotton wad and diluted to 1×10^9 cells/ml. The suspension of *Cephalosporium acremonium* spores was exposed to UV light (2550 °A) in a petri dish or treated with NTG solution (3 mg/ml) at 30 °C. Mutants were selected and tested for cephalosporin C production.

The results of the parent strains and the mutant strains are shown in the Table. The parent strain *C. acremonium* ATCC 48272 yielded only 630 μg/ml. This strain was exposed to UV rays and strain *C. acremonium* CUS 8 was selected. The UV mutant yielded (1000 μg/ml) 370 μg/ml (58.73%) more than the parent strain. In the next stage the strain, *C. acremonium* CUS 8 was subjected to NTG treatment and a superior strain *C. acremonium* CNT 15 was obtained. NTG treatment gave an improvement of 995 μg/ml than the *C. acremonium* CUS 8 strain. It accounts to 216% more than the parent strain.

From the results, it is evident that UV and NTG were effective mutagens for *C. acremonium*. By employing these techniques a superior mutant (CNT 15) with a productivity of 3 times more (improvement from 630 μg/ml to 1995 μg/ml) than that of starting culture was obtained. The mutants were stable and retained their activity when stored as soil stock.

3. Experimental

3.1. UV irradiation

Spore suspension (4 ml) of *Cephalosporium acremonium* in phosphate buffer at pH 6.5 was taken in a petridish (80 mm dia) and exposed to UV light (2550 °A) in dark at a distance of 265 mm. Exposure times varied from 1 to 10 min and the percentage survival was determined by dilution plating technique using potato dextrose agar medium. Those survived at 99% kill were subcultured and kept for the production of Cephalosporin C. For the quantitative estimation of Cephalosporin C, the standard microbiological assay procedure was employed using *Alcaligenes faecalis* MTCC 126 as test organism [11, 12]. This assay procedure was used throughout the studies. From the production data, one of the mutant, *C. acremonium* CUS 8 (Table) was found to be superior and was used for further treatment.

3.2. NTG treatment

Spore suspension of *C. acremonium* CUS 8 in phosphate buffer at pH 6.5 was treated with NTG (3 mg/ml) at 30 °C. The survival data was determined using various exposure times (30, 60, 90, 120 & 150 min) and

Table: Productivity of Cephalosporin C by UV and NTG mutants

UV mutants		NTG mutants	
Strain No.	Yield (μg/ml)	Strain No.	Yield (μg/ml)
C.A ATCC 48272	630.09	CUS-8	1000
CUS-1	501.18	CNT-1	Nil
CUS-2	501.18	CNT-2	Nil
CUS-3	251.18	CNT-3	794.32
CUS-4	199.52	CNT-4	794.32
CUS-5	Nil	CNT-5	1000
CUS-6	Nil	CNT-6	794.32
CUS-7	Nil	CNT-7	501.18
CUS-8	1000	CNT-8	398.1007
CUS-9	316.22	CNT-9	501.18
CUS-10	316.22	CNT-10	501.18
CUS-11	316.22	CNT-11	630.09
CUS-12	251.18	CNT-12	501.18
CUS-13	501.18	CNT-13	316.22
CUS-14	316.22	CNT-14	794.32
CUS-15	316.22	CNT-15	1995.26
CUS-16	398.107	CNT-16	795.32
CUS-17	398.107	CNT-17	501.18
CUS-18	398.107	CNT-18	316.22
CUS-19	Nil	CNT-19	316.22
CUS-20	Nil	CNT-20	316.22
CUS-21	316.22	CNT-21	Nil
CUS-22	316.22	CNT-22	Nil
CUS-23	316.22	CNT-23	795.32
		CNT-24	630.09
		CNT-25	630.09

those survived were plated on potato dextrose agar medium and selected mutants (Table) were tested for Cephalosporin C production as described earlier. Among the selected mutants, *C. acremonium* CNT 15 was found to be the best strain.

3.3. Fermentation

All the fermentations were conducted in shake flasks as detailed below. The strains were grown on potato dextrose agar slants for 5 days at 28 °C and spore suspension was prepared and transferred into inoculum medium [13]. The flasks were incubated on rotary shaker (220 rpm) at 28 °C for 48 h. Five ml of inoculum was transferred into 45 ml of production medium [14] with the following composition: (g/l) Glucose 27.0; sucrose 36.0; oleic acid 1.5; DL-methionine 3.0; ammonium acetate 8.8; KH_2PO_4 1.8; K_2HPO_4 2.97; Ferric ammonium sulphate 0.16; Concentrated salt solution 50 ml; distilled water to make up 1 litre and pH 7.0 ± 0.1. The composition of salt solution is: (g/100 ml) Na_2SO_4 16.2; $MgSO_4 \cdot 7\,H_2O$ 7.68; $CaCl_2 \cdot 2\,H_2O$ 7.68; $MnSO_4 \cdot H_2O$ 0.64; $ZnSO_4 \cdot 7\,H_2O$. 0.64; $CuSO_4 \cdot 5\,H_2O$ 0.004; distilled water up to 100 ml and pH 7.0 ± 0.1. The flasks were incubated for 96 hrs at 28 °C on a rotary shaker (220 rpm). Samples were withdrawn and centrifuged at 3000 rpm and Cephalosporin C content was estimated by microbiological assay.

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