OPTIMIZATION OF CEPHALOSPORIN C PRODUCTION BY CEPHALOSPORIUM ACREMONIUM C-10 USING STATIONARY CULTURE

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Abstract: The present study is concerned with the production of cephalosporin C by *Cephalosporium acremonium* C-10. Two culture media were tested for the production of antibiotic. Among these culture media M₁ containing sucrose, ammonium acetate, cornstarch, sugar cane molasses, soybean meal, calcium carbonate, calcium sulphate and methyl oleate gave optimum production of cephalosporin C. The cultural conditions such as pH 6.5 and temperature 30°C were optimized. The production of antibiotic was found optimal 48 h after conidial inoculation. The effect of size of inoculum was studied and the best results were obtained at 1ml of inoculum size.

Keywords: Cephalosporin C, *Cephalosporium acremonium*, cultural conditions, stationary culture.

INTRODUCTION

*Cephalosporium acremonium*, an imperfect fungus, is an organism of interest on account of its potential use as a producer of cephalosporin and related enzymes (Jung *et al*., 2001). The beta-lactam family of antibiotics is the most important group among pharmaceutical products and cephalosporin C (C₁₆H₂₃O₁₈N₃S) is one of the important antibiotics in this group. Cephalosporin C shows a wide range of activity and is effective against many penicillin resistant strains of *Staphylococcus aureus* (Abraham, 1962). It is also effective against Gram-negative rod bacterial meningitis (Jewetz, 1982). The cephalosporin ring system (Fig., 1) consists of a four membered beta-lactam ring fused with an unsaturated six-membered dihydrothiazine ring (Unger *et al*., 1996)

Cephalosporin C can be produced by surface culture technique, submerge culture technique and solid-state culture technique (Prescott & Dunn’s 1987). The production and stability of the cephalosporin C is very sensitive to pH and temperature. Shen *et al*., (1986) reported that the complex fermentation medium with pH 6.4 gave maximum production of cephalosporin C. The optimum pH 7.0 for the cephalosporin C biosynthesis was reported by Araujo *et al*. (1999) and Silva *et al*. (1998). The temperature has great influence on the biosynthesis of cephalosporin C from *C. acremonium* C-10. Adinarayana *et al*. (2001) reported that the maximum antibiotic production was observed at 30°C. Inoculum size is of prime importance in determining the characteristics of the fermentation culture (Lee *et al*., 2001). Adinarayana *et al*. (2001) reported that higher antibiotic production was obtained at 10 % v/w inoculum level gave maximum production of cephalosporin C and higher inoculum concentration were not beneficial.
The present investigation deals with the effect of different cultural conditions (pH, temperature, inoculum and rate) on cephalosporin C by *C. acremonium* C-10 using stationary culture in 250 ml Erlenmeyer flasks.

**MATERIALS AND METHODS**

**Organism**

Cephalosporin C producing strain *C. acremonium* C-10 was obtained from Biotechnology Research Centre, Government College Lahore. The culture was maintained on nutrient agar medium (pH 6.5) and was stored at 4°C in a refrigerator. All the culture media were autoclaved at 15lbs/in² pressure at 121°C temperature for 15 minutes, unless otherwise stated.

**Preparation of Vegetative Inoculum**

Vegetative inoculum was prepared from a 3-4 day old slant culture of *C. acremonium* C-10. Ten milliliter of sterilized 0.05% (w/v) Monoxal O.T. was added to a slant having optimal growth. Hyphae were then scratched with sterilized inoculating needle and the tube was shaken gently. One milliliter of mycelial suspension was aseptically transferred to the conical flask containing 100 ml of sterilized nutrient broth medium (pH 6.5). The flask was incubated at 30°C in an incubator shaker (Gallenkamp, UK) at 200 rpm for 24 hours.

**Pretreatment of Molasses**

Cane molasses obtained from Kamalia Sugar Mills (Kamalia Pakistan) was used for the present study. The molasses was clarified according to the method of Panda *et al.* (1984). After pretreatment, the sugar content was maintained at 15 % (w/v) and stored at 15°C in an amber coloured bottle.

**Fermentation Technique**

Production of cephalosporin C was studied by surface culture technique using 250ml of Erlenmeyer flasks. Fermentation medium consisting of glv: sucrose 2.0, ammonium acetate 0.8, corn starch 3.0, sugar cane molasses 5.0 ml (sugar 15%), soy bean meal 6.0, CaCO₃ 0.5, CaSO₄ 1.25, methyl oleate 3.0 and 100 ml distilled water were transferred to the individual cotton wool plugged flask. The flasks were sterilized in an autoclave at 15 lbs/in² pressures (121°C) for 15 min. One millilitre of vegetative inoculum was aseptically transferred to each flask. Flasks were then incubated at 30°C for 48 hr. After 48 hr, the fermented broth was centrifuged and the supernatant was used for the estimation of cephalosporin C. All the experiments were performed in triplicates.

**Cephalosporin C Activity**

Three millilitre of supernatant was transferred in the individual test tubes and then 2.0 ml of nickel reagent was added. The test tubes were placed at 25°C for 20 min. Then 5.0 ml of iron reagent was transferred to each test tube. The test tubes were placed in an incubator at 30°C for 2 hr. The optical density was measured at 470 nm using a colorimeter (AE-11, Erma, Japan) against the blank. The optical density was converted into mg/ml from the standard curve of cephalosporin C.

**RESULTS AND DISCUSSION**

Selection of suitable fermentation medium is essential for the production of antibiotics (Zhou *et al.*, 2001). In the present study, two fermentation media M₁ and M₂ were tested for optimum production of cephalosporin C from *C. acremonium* C-10. The production of antibiotic was
found to be maximal (32.0 mg ml\(^{-1}\)) with \(M_1\) while \(M_2\) gave insignificant results. It might be due to the fact that \(M_1\) contained molasses that provided the optimal levels of essential nutrients such as sucrose and potassium salts. Shen et al. (1986) have also reported that cephalosporin C was found to be optimum using molasses based medium. The production of cephalosporin C reached maximum at 48 h after inoculation (32.0 mg ml\(^{-1}\)). Fig., 2 shows that further increase or decrease in the incubation period resulted in decreased productivity of cephalosporin C. It might be due to the exhaustion of available nutrients in the medium with increase in incubation period (Jermini & Demain, 1989; Ellaiah et al., 2001).

Production of cephalosporin C was found to be optimum at 48 hr and varied significantly (\(p \leq 0.05\)) than others. Incubation temperature plays a critical role for the production of antibiotics as well as for the growth of producer organism (Mastumara et al., 1970). Fig., 3 shows the effect of different incubation temperatures (25-40°C) on the production of cephalosporin C by \textit{C. acremonium C-10}. Maximal production of Cephalosporin C (32.0 mg ml\(^{-1}\)) was observed when fermentation was carried out at 30°C. Marked decrease in the yield of cephalosporin C was noticed when incubation temperature was higher or lower than the optimum. It might be due to the fact that high temperature had some adverse effects on metabolic activities of microorganism (Adinarayana et al., 2001). At low temperature the production was extremely inhibited. Similar kinds of results were reported by Mudgetti et al. (1986). Cephalosporin C production varied significantly (\(p \leq 0.05\)) at this temperature than others.

The production of antibiotics is very sensitive to pH. Fig., 5 shows the effect of different initial pH (4.5-7.0) on the production of cephalosporin C by \textit{C. acremonium C-10}. Fig., 4 shows that initial pH of fermentation medium at the level of 6.5 gave maximum production of cephalosporin C (39 mg ml\(^{-1}\)). Further increase in the pH resulted in decreased productivity of cephalosporin C. At low pH, the antibiotic production was reduced. It might be due to fact that the production of biomass was greatly inhibited due to the toxic effect of H\(^+\) ions at acidic pH of fermentation medium. Due to decrease in the biomass, the productivity of Cephalosporin C was adversely affected. Similar findings were reported by Shen et al. (1986) and Zhou et al. (1992) who also selected pH 6.5 for the production of cephalosporin C. The productivity of antibiotic was found to be best at pH 6.5 and varied significantly (\(p \leq 0.05\)). Thus, pH 6.5 for the production of cephalosporin C was selected and used for further studies.

Inoculum level is also an important factor for the production of cephalosporin C (Adinarayana et al., 2001). Effect of different size of inoculum on the production of cephalosporin C by \textit{C. acremonium C-10} was studied (Fig. 5). The size of inoculum varied from (2-12\%) per 250 ml of fermentation medium. Of all the concentrations examined, 4\% inoculum gave maximum production of cephalosporin C (39.64 mg ml\(^{-1}\)). The production of cell mass in the fermentation medium was significantly increased when number of cells was increased in fermentation medium thus productivity significantly decreased as reported by Demain et al. (1999). A lower inoculum density may give insufficient biomass and result in reduced product formation, whereas a
higher inoculum level may produce too much biomass leading to the poor product formation (Mudgetti, 1986). At low size of inoculum the results were extremely inhibited because of less number of spores in the medium. Similar results were reported by Da-Silva et al. (2000). The productivity of antibiotic was found to be best at 4% of size of inoculum and varied significantly \( p \leq 0.05 \) over other concentrations tested.

**CONCLUSION**

Based on the present study we conclude that the production of antibiotic can be improved by optimizing the cultural conditions using molasses based medium even after 48h of inoculation. The productivity can further be improved by subjecting the *C. acremonium* C-10 to the mutagenic agents like radiations or chemicals.

**REFERENCES**


Figure 1: Structure of cephalosporin C

Figure 2: Selection of fermentation medium for cephalosporin C production by *C. acremonium* C-10.

Temperature 30ºC, pH 6.5.

Y error bars indicate the standard error of means among the three parallel replicates. The value differs significantly at p≤0.05.
Figure 3: Effect of incubation temperature on production of cephalosporin C by *C. acremonium* C-10.

Temperature 30ºC, pH 6.5.

Y error bars indicate the standard error of means among the three parallel replicates. The value differs significantly at p≤0.05.

Figure 4: Effect of different initial pH on production of cephalosporin C by *C. acremonium* C-10.

Temperature 30ºC, pH 6.5.

Y error bars indicate the standard error of means among the three parallel replicates. The value differs significantly at p≤0.05.
**Figure 5:** Effect of different size of inoculum on production of cephalosporin C by *C. acremonium* C-10.

Temperature 30°C, pH 6.5.

Y error bars indicate the standard error of means among the three parallel replicates. The value differs significantly at *p*≤0.05.