Recent Research in Science and Technology 2013, 5(2): 49-51
ISSN: 2076-5061
Available Online: http://recent-science.com/

Influence of media constitutions on the biosynthesis of cephalosporin C.

Tanushree Chakraborty

Bioprocess laboratory, Department of Biotechnology, St. Thomas College, Ruabandha, Bhilai-490006, Chhattisgarh, India

Abstract
Cephalosporin acremonium was used for the production of cephalosporin C in batch mode of cultivation. The Cephalosporin is a β lactam antibiotic that has the ability to kill gram positive as well as gram negative bacteria by destroying their peptidoglycan layer of cell wall. There are four generations of cephalosporin depending upon the ability to kill gram positive, gram negative bacteria or both the type of bacteria. The effect of different medium constituents for better yield of cephalosporin C was thoroughly studied. From the results of fermentation it was found that sucrose as carbon source, soya bean meal as nitrogen source, 6.8 pH, RT and 96 hrs of incubation are most suitable for higher yield of antibiotic.

Keywords: *Cephalosporin acremonium,* cephalosporin, sucrose, soya bean meal.

INTRODUCTION

Cephalosporin along with penicillin belongs to the class of β-lactam antibiotics that inhibits and abolishes the growth of micro organism. Cephalosporins are broad spectrum antibiotics in the international market and are more resistant to β-lactamase than penicillin. It was developed to overcome the allergic problems associated with penicillin.Cephalosporin is produced by different strains of *Cephalosporin acremonium.* The β-lactam antibiotics particularly penicillin and cephalosporin share approximately 65% of worldwide market (Nigam *et al*. 2007). A number of reports are available on the various aspect of production of cephalosporin in batch as well as in the continous mode. The development of improved strategies for CPC fermentation requires proper control of specific growth rates of cells using certain key nutrients (Srivastava *et al*. 2006). The optimisation of media components for cephalosporin biosynthesis has been reported and different hypothesis has been proposed. I have studied influence of medium constituents on the production of cephalosporin. In batch process, sucrose is rapidly consumed to form biomass at the beginning of the process. Such kinds of studies, nonetheless, very important to secure good production performance. The present paper deals with the optimization of different medium constituents for the increase in biosynthesis of cephalosporin.

MATERIALS AND METHODS

Organism

The production of CPC was done from *C. acremonium.* The culture was maintained on potatodextrose-agar (PDA) after incubation for 7 days at pH 7.2 and temperature 28°C.

Culture medium and conditions

The culture was propagated in the seed medium containing (gL-1) sucrose, 30; potassium dihydrogen orthophosphate, 1.5; magnesium sulphate, 0.3; and trace metal solution 15mL which was sterilized separately (Nigam *et al*. 2007). The trace metal solution contained (gL-1) ferrous ammonium sulphate, 0.15; magnesium sulphate, 0.367; calcium chloride, 0.075; zinc sulphate, 0.03; manganese sulphate, 0.03; and copper sulphate 0.008. The culture was grown in the seed medium for 6-7 days at 28ºC. The production medium contained (gL-1) sucrose, 1.6; soyabean meal 1.2; DL-methionine0.03. The pH of the medium was adjusted to 6.8 before sterilization (Nigam *et al*. 2007). Five different carbon sources (sucrose, maltose, lactose, glucose, and fructose) were selected. Five different nitrogen sources (soyabean meal, soya peptone, corn steep liquor, meat extract, malt extract) were selected. Five pH ranges (3.8, 4.8, 5.8, 6.8, and 7.8) were selected. Five incubation temperatures (4°C, RT, 37°C, 42°C) were selected. Four incubation times (24h, 48, 72, 96) were selected.

Inoculum

The surface growth from a 7-days old slant was suspended in 5 mL of sterile distilled water and was used to inoculate the seed medium.

Cultivation

After optimization of various media constituents, CPC production was performed at optimal conditions.

Analysis

After 7-10 days antibiotic sensitivity test was performed for the growth and CPC production.

RESULTS

Effects of different carbon source on the growth and biosynthesis of CPC

Fig. 1 shows the findings of different carbon source on the production of CPC. It has been observed that, sucrose when used as a carbon source increases the production of CPC in comparison to

*Corresponding Author

Tanushree Chakraborty
Bioprocess laboratory, Department of Biotechnology, St. Thomas College, Ruabandha, Bhilai-490006, Chhattisgarh, India

Email: tchakraborty@gmail.com

other carbon sources. Maltose also gives good yield followed by sucrose. The minimum concentration of CPC was found with glucose as carbon source.

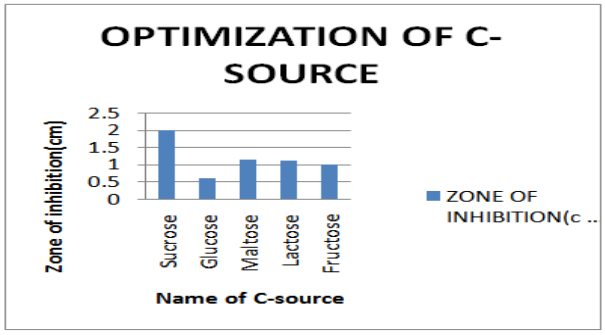

Fig 1. Optimization of C-source

Effects of different Nitrogen source on the growth and biosynthesis of CPC

Fig. 2 shows the findings of different nitrogen source on the production of CPC. It has been observed that, soyabean meal when used as a nitrogen source increases the production of CPC in comparison to other nitrogen sources. Meat extract also give good yield followed by soyabean meal. The minimum concentration of CPC was found with corn steep liquor and malt extract as nitrogen source.

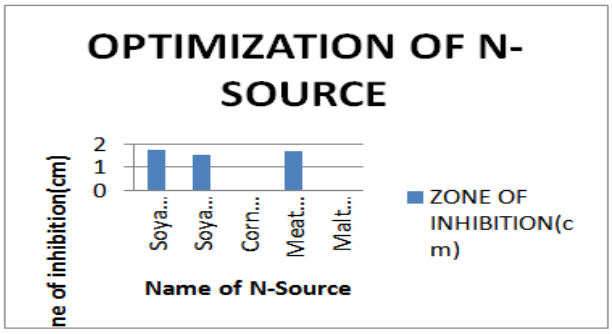

Fig 2. Optimization of N-source

Effects of different pH on the growth and biosynthesis of CPC

Fig. 3 shows the findings of different pH on the production of CPC. It has been observed that, at pH 6.8 the production of CPC increases in comparison to other pH ranges.

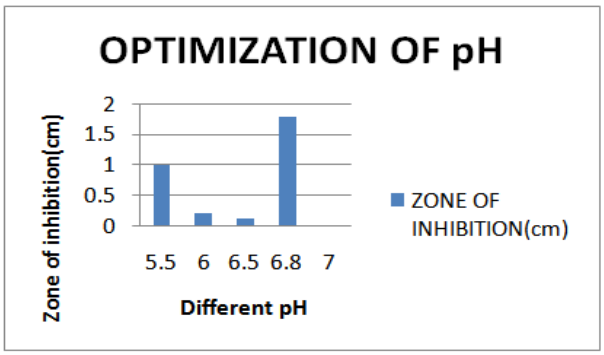

Fig 3. Optimization of pH

Effects of different temperature on the growth and biosynthesis of CPC

Fig. 4 shows the findings of different temperature on the production of CPC. It has been observed that, at room temperature the production of CPC increases in comparison to other temperature ranges.

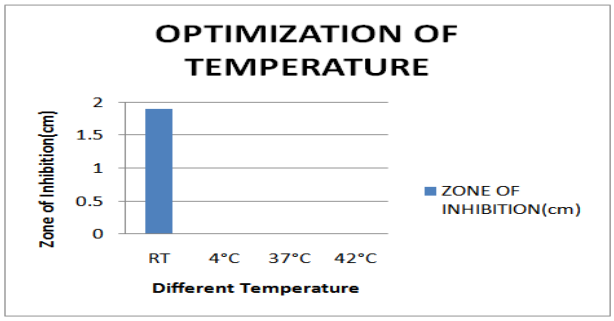

Fig 4. Optimization of Temperature

Effects of different incubation time on the growth and biosynthesis of CPC

Fig. 5 shows the findings of different incubation time on the production of CPC. It has been observed that, at 96hrs of incubation the production of CPC increases in comparison to other incubation period.

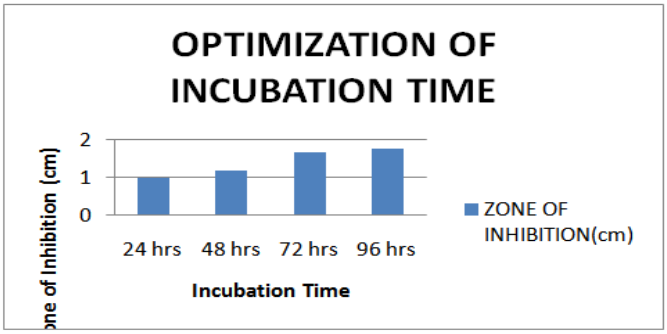

Fig 5. Optimization of Incubation time

DISCUSSION

Antibiotics are the secondary metabolites produced by the microbes. I also found that during the production of secondary metabolites, the cell biomass of microbes increases initially and after certain time, it becomes constant, and also observed that production varies with the constituents of the media and the stage of the culture development as stated by Nigam *et al* 2007. The secondary metabolites accumulate only after the growth phase (tropophase) *i.e.* when the culture attains a specific growth rate. Secondary metabolites are often called "idiolites" since they are produced in the "idiophase" (Nigam *et al.* 2007). This investigation on the biosynthesis of CPC was attempted with an aim to increase its production as it is a very important beta lactam antibiotic with a broad-spectrum activity. A thorough literature review was performed and it was noticed that though a number of highly productive strains and a number of different strategies have been used for better yield,

not much attention has been given in the constituents of synthetic medium used for its biosynthesis (Nigam *et al*. 2007). In addition, a number of other reports are available for the production of CPC using complex medium. When different nitrogen sources and carbon sources were added to the production medium, it has been found that only a few are used by mold for maximum synthesis CPC. Others are not utilized by mold at the same extent. Soyabean meal was observed to be the best source of nitrogen for the higher production of CPC but according to Nigam *et al*. ammonium sulphate was observed to be the best source of inorganic nitrogen for the production of CPC. High content of nitrogen is found to decrease the production might be due to the reason that it interferes to the process of differentiation of mycelium to swollen hyphal fragments and anthrospores during production stage (Nigam *et al*. 2007). When different carbon source was optimized sucrose was found to be the best carbon source for the production of CPC and according to other literature obtained it was observed that sucrose always found to be the best carbon source for the production of CPC (Nigam *et al*. 2007) (Srivastava *et al*. 2006). Production of secondary metabolites is often subjected to the regulation by the readily metabolizable carbon sources. After performing this optimization procedures it was found that if suitable carbon and nitrogen sources were added to the medium and optimized temperature and pH were maintained the we can get the sufficient production of antibiotics.

CONCLUDING REMARKS

The above studies conclude that for better yield of CPC, the nutritional requirement of mold should be at their optimal concentrations. It was found that soyabean meal as nitrogen source and sucrose as a carbon source are most suitable for higher yield of antibiotic.

REFERENCES

[1] Brakhage, Axel A. 1998. Molecular regulation of â-lactam biosynthesis in filamentous fungi. *Microbiology and Molecular Biology Reviews*, September, vol. 62, no. 3, p. 547-585.

[2] Cruz, Antonio J.G.; Almeida, Renate M.; Araujo, Maria Lucia G.C.; Giordano, Roberto C. and Hokka, Carlos O. 2001. Modeling and simulation of cephalosporin C production in a fed batch tower type bioreactor. *Applied Biochemistry and Biotechnology*, vol. 93, no. 1-3, p. 537-549.

[3] Cruz, Antonio J.G.; Pan, Tai; 2004. Giordano, Roberto Araujo, Maria Lucia G.C. and Hokka, Carlos Cephalosporin C production by immobilized *Cephalosporium Acremonium* cells in a repeated batch tower bioreactor. *Biotechnology and Bioengineering*, vol. 85, no. 1, p. 96-102.

[4] Ellaiah, P.; Premkumar, J.; Kanthachari, P.V. and Adinarayana K. 2002. Production and optimization studies of cephalosporin C by solid state fermentation. *Hindustan Antibiotics Bulletin*, vol. 44, no. 1-4, p. 1-7.

[5] Shen, Y.-Q.; Heim, J.; Solomon, N.A. and Demain, A.L. 1984.Repression of â-lactam production in *Cephalosporium acremonium* by nitrogen sources. *The Journal of Antibiotics*, vol. 37, no. 5, p. 503-511.

[6] Srivastava pradeep, Mishra Punita, Kundu Subir. 2006. Process strategies for Cephalosporin C production. School of biochemical engineering, Institute of technology, Banaras Hindu University. *Journal of Scientific and Industrial research*, Vol 65, pp 599-602.

[7] Tabaraie Bahman, Ghasemian Ehsan, Tabaraie Termeh, Parvizi Elnaz, Rezazarandi Mahdi. 2012. comparative evalution of cephalosporin-c production in solid state fermentation and submerged liquid culture. *Journal of Microbiology, Biotechnology and Food Sciences,* 2 (1) 83-94

[8] Tollnick, C.; Seidel G.; Beyer M. and Schugerl, K. 2004. Investigations of the production of cephalosporin C by *Acremonium chrysogenum*. *Advances in Biochemical Engineering/Biotechnology*, vol. 86, p. 1-45.

[9] Verma, Ruchi; Nigam, Vinod Kumar; Kundu, Subir and Ghosh, Purnendu. 2006. Morphological differentiation and effect of seed inoculum on the production of CPC by *Acremonium chrysogenum*. *Asian Journal of Experimental Sciences*, vol. 20, no. 2, p. 275-280.

[10] Vinod Kumar Nigam*,Ruchi Verma, Abhishek Kumar, Subir Kundu, Purnendu Ghosh. 2007. Influence of medium constituents on the biosynthesis of cephalosporin-C**,** Vol.10 No.2.