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STUDIES ON THE NUTRITIONAL PARAMETERS FOR CEPHALOSPORIN BIOSYNTHESIS FROM *ACREMONIUM CHRYSOGENUM* BY SUBMERGED FERMENTATION

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Abstract

In the present study, cephalosporin was produced through submerged fermentation in 250 ml Erlenmeyer flask. About one hundred fungal strains were isolated and screened for maximum productivity of the antibiotic cephalosporin. Among these, the strain IIB-10 identified as *Acremonium chrysogenum* was found to have maximum production of cephalosporin when cultivated on M-4 medium containing (%, w/v): Corn meal, 2; Baker's flour, 1.5; Ammonium sulphate, 0.1; Calcium carbonate, 0.3 and Methyl oleate, 1.6. The nutritional study for maximum cephalosporin productivity was undertaken. Sucrose as a sole carbon source at 2.5% level, peptone at a concentration of 1.5% as an organic nitrogen source and Ammonium sulphate at a concentration of 0.4% as an inorganic nitrogen source supported maximum production of cephalosporin i.e., 721.89 mg/l.

Introduction

*Cephalosporium acremonium*was first isolated by Brotzu (Campos *et al*.,_2007) from sea water in 1945. Later Abraham and coworker in 1955-56 isolated cephalosporin C, cephalosporin P and penicillin N from culture of *C. acremonium* (Bhat *et al.,* 2005). Cephalosporin C resembles the penicillin in having an acyl side chain linked to an amino group of a double ring nucleus. Cephalosporin C is a low potency antibiotic which is its main disadvantage. However, more potent semisynthetic derivatives are also produced e.g., cephalothin, cephaloridine and cephaloglycin. The modification of cephalosporin nucleus gives various compounds having different characteristics. The classification of cephalosporin into different generations depends upon their antimicrobial spectrum. Gram –ve antimicrobial properties of cephalosporins increases than the preceding generations, whereas activity against Gram +ve bacteria is decreased. Cephalosporins are most frequently used in the treatment of infections caused by bacteria in the respiratory tract, infections of skin and infections of urinary tract (Dasari *et al.*, 2008).

Different strains of *Cephalosporium* species i.e., *Acremonium chrysogenum, Emericellopsis* species; *Paecilomyces* species i.e., *P. carnius* and *Streptomycees* species like *S. clavuligerus* are used in the fermentation to produce the starting material for the synthesis of cephalosporin (Higgens *et al.,* 1974). *Acremonium chrysogenum* is naturally found in soil, plant debris and organic matter. It over grows in moist environment at 25°C-28°C forming white loose cottony hyphae giving rise to white and gray colonies.During fermentation, *A. chrysogenum* shows four different morphological forms i.e., conidia, hyphae, wide swollen hyphal fragments named yeast like forms and metabolically inactive arthrospores (Bartoshevic *et al.,* 1990). The conidia are predominantly formed by the wild strain of *A. chrysogenum.* The strains which tend to differentiate into yeast like forms e.g. swollen hyphal fragments show medium to high productivity of cephalosporin (Bartoshevic *et al.,* 1990). The highest amount of cephalosporin is produced when hyphal filaments convert into swollen hyphal fragments (Huber & Nash 1971). Yeast like morphological form of the fungus has high tendency to produce cephalosporin as compared to any other morphological form (Bartoshevic *et al.,* 1990). The morphological differentiation of the hyphae into yeast like forms (highly swollen fragments) must takes place before the onset of production of cephalosporin and these fragments will gradually transfer into arthrospores (Matsumura *et al.,* 1970).

The cephalosporin production is carried out by submerged and solid state fermentation (Kim *et al.,* 2006, Cuadra *et al*., 2008) by using free or immobilized microorganisms (Hasan *et al.,* 2009). The maximum productivity of cephalosporin has been obtained by using wheat rawa and sugarcane bagasse. In addition to the substrate optimization, other conditions are also very important such as addition of yeast, soluble starch and nutrient materials, pH control, temperature control, incubation period and inoculum and these can affect the production of the cephalosporin by solid state fermentation (Cuadra *et al.,* 2008). Many workers have reported submerged fermentation in shake flask for the production of cephalosporin (Kim *et al.,* 2007, Cruz *et al.,* 2004).

The fermentation medium composition is very important as nutrient materials such as carbon, nitrogen, phosphorous, trace element and amino acids can influence the cephalosporin production. The carbon source such as glucose, sucrose, fructose, molasses, lactose etc can also affect the production of cephalosporin. As the carbon in fermentation medium can influence morphological differentiation of the fungus so the cephalosporin production is altered (Sohn *et al*., 1994). The inorganic and organic nitrogen sources i.e., peptone, meat extract, urea, CSL, yeast extract, casein, beef extract, meat extract, ammonium sulphate, ammonium chloride, ammonium phosphate, ammonium nitrate and potassium nitrate can also affect the synthesis of cephalosporin. It was found that presence of the excess of nitrogen in the culture medium can reduce the cephalosporin biosynthesis. This is probably due to the interference of nitrogen in the differentiation of mycelium to swollen hyphae and arthrospores (Nigam *et al.,* 2007).

The main objective of the present studies was the isolation of highly productive strain of *Acremonium chrysogenum* from different habitats and optimization of the nutritional parameters for fermentation process.

Materials and Methods

Isolation and screening of microorganisms: The fungal strains were isolated from the soils of different areas of Lahore. One gram of each soil sample was suspended in 100ml of distilled water and diluted up to 10^{-6} by serial dilution. One milliliter of the dilutions (10^{-5} and 10^{-6}) was spread on the isolation plates containing PDA and incubated at 28ºC for 5-7 days for the appearance of mold colonies. The white or grey colored fungal colonies resembling *Acremonium chrysogenum* in appearance were picked up and transferred to the slants containing PDA medium and were stored at 4°C after growth. All the fungal isolates were screened for cephalosporin production using shake flask fermentation. The potent fungal strains were selected on the basis of their ability to form zone of inhibition on the test plates.

Fermentation experiments

Preparation of inoculum: Vegetative inoculum was prepared in a medium consisting of Peptone, 20; malt extract, 20; corn steep liquor, 5; Magnesium sulphate, 0.25; dipotassium hydrogen phosphate, 0.5; potassium dihydrogen phosphate, 1.0 and calcium chloride, 0.1 (pH 6.5). The flask was inoculated with fungal spores from the slant and incubated at 28ºC for 4 days in incubator shaker rotating at a speed of 200 rpm. One ml of this inoculum was used to inoculate the fermentation flasks.

Fermentation batch: Submerged fermentation carried out in 250ml conical flasks containing 50ml culture medium for the production of cephalosporin from selected fungal cultures. The culture medium was consisted of (g/l): peanut meal, 60; starch, 40; dextrose, 7; sucrose,5; calcium carbonate, 10; D.L. Methionine, 10; methyl oleate, 25 (Trilli *et al*., 1977). The flasks were sterilized in autoclave at 15 psi (121ºC) for 15 min. One ml of the vegetative inoculum was transferred to each flask when cooled to room temperature and the fermentation was carried out in an incubator shaker at 28ºC at 200 rpm for 7 days. After that, the fermentation broth was centrifuged at 6000 rpm for 10 min and the antibacterial activity of the cell free culture supernatant was determined.

Analytical procedures: Agar plate disc diffusion method (Masuda & Tomioka, 1978) was used to test the antimicrobial activity of the fermentation broth while spectrophtometeric method, using hydroxyl amine nickel reagent and iron III reagent was used for the assay of cephalosporin (Mays *et al.,* 1975). The absorbance of each solution was measured at 470 nm. A working curve of absorbance and concentration of reference cephalosporin was obtained.

Dry mycelial mass: The fermentation broth was filtered using pre-weighed filter paper (Whatman-44) to obtain the mycelial mass on the filter paper. The cell mass of the fungus was dried in a hot air oven at 80°C for 24 hrs and weighed again. The dry mass was calculated by subtracting preweight from after weight.

Results and Discussion

Isolation and screening of microorganisms: One hundred fungal colonies, which were white or grey in colure resembling *Acremonium chrysogenum,* were isolated from soil as our soils are rich in the antibiotics producing microflora (Naz & Rasool, 2013). These colonies were screened for the antibiotic production by allowing them to grow in the test plates containing nutrient agar as medium and *Bacillus* sp. as a test organism. The fungal strains which were capable of producing antibiotic inhibited the growth of the test organism and formed an inhibition zones on the test plate. On the basis of formation of zone of inhibition, 16 isolates were selected as shown in the Table 1. Among those 16 strains, IIB-10, IIB-41, IIB-65 and IIB-67 showed significant antibiotic activities. These strains produced zones of inhibition with diameters of 20, 15, 14 and 18mm, respectively on the test plates. The concentration of antibiotic which was produced by these strains was 240.41, 131.1, 109.37 and 196.93mg/l, respectively as analyzed by disc diffusion method. It is clear from the results that the strain IIB-10 produced the maximum zone of inhibition on the test plate and correspondingly showed the maximum antibiotic production. All the other fungal isolates showed antibiotic production ranging from 0.043 to 240.41 mg/l. Similarly, the maximum biomass was also produced by the strain IIB-10 i.e. 48.06 mg/ml. So these four fungal isolates were selected for further screening using different fermentation media for the production of cephalosporin under submerged fermentation conditions.

Morphological characteristics of microorganism: The macroscopic and microscopic characteristics of the strain IIB-10 were studied to identify it. Strain IIB-10 showed moderate growth rate with a white colony color on PDA plates. The diameter of the colonies ranged from 2 to 3 cm. The colonies were compact, centrally raised and velvety in texture. Under the microscope, hyphae appeared septate and hyaline. The phialides were solitary, unbranched and erect which were directly attached to the tips of the hyphae. The conidia were unicellular and hyaline (Srinivasan 2004). Moreover, four different morphological forms i.e., filamentous hyphae, swollen hyphal fragments, arthrospores and conidia were also observed during fermentation (Huber & Nash, 1971). On the basis of these characteristics, the strain IIB 10 was identified as *Acremonium chrysogenum.* The strain was further confirmed as *Acremonium chrysogenum* by the culture bank of Institute of Mycology and Plant Pathology, University of the Punjab, Lahore.

Screening of culture media: Five different culture media were screened for the production of cephalosporin by four selected fungal strains i.e., IIB-10, IIB-41, IIB-65 and IIB-67 (Fig. 1). The highest amount of cephalosporin (131.1 mg/l) was produced by the strain IIB-10 in the medium M-4 while the lowest amount of cephalosporin production was observed by the strain IIB-41 i.e. 21.89 mg/l in the medium M-1. The other strains showed the intermediate amounts of cephalosporin production in all the media. The highest amount of the biomass i.e., 30.84 mg/ml was also produced by the strain IIB-10 in the medium M-4 whereas minimum amount of the biomass (12.70 mg/ml) was produced by the strain IIB-41 in the medium 3 as shown in Fig. 2. Therefore, the strain IIB-10 with highest yield of cephalosporin in all the media was finely selected for further studies.

Table 1. Screening of fungal strains for the production of Cephalosporin in shake flasks*.

Serial #	Strain code	Diameter of zone of inhibition (mm)	Concentration of cephalosporin (mg/l)	Dry mycelial mass (mg/ml)
1.	IIB-3	10 ± 2	21.89 ± 2	16.9 ± 1
2.	IIB-5	9 ± 1	0.437 ± 2	30.76 ± 2
3.	IIB-10	20 ± 1	240.41 ± 1	48.06 ± 1
4.	IIB-13	8 ± 2	0.437 ± 2	21.80 ± 2
5.	IIB-26	3 ± 1	0.043 ± 1	19.60 ± 1
6.	IIB-30	9 ± 2	0.473 ± 1	18.54 ± 1
7.	IIB-39	13 ± 2	87.48 ± 2	38.16 ± 2
8.	IIB-40	10 ± 2	21.89 ± 1	28.90 ± 2
9.	IIB-41	15 ± 1	131.1 ± 1	44.68 ± 2
10.	IIB-65	14 ± 1	109.37 ± 2	37.80 ± 1
11.	IIB-66	11 ± 2	43.70 ± 2	34.56 ± 1
12.	IIB-67	18 ± 2	196.93 ± 1	41.34 ± 2
13.	IIB-70	11 ± 2	43.70 ± 2	39.56 ± 1
14.	IIB-85	10 ± 2	21.89 ± 1	19.96 ± 2
15.	IIB-87	10 ± 1	21.89 ± 2	38.16 ± 1
16.	IIB-95	10 ± 2	21.89 ± 1	33.08 ± 1

The values are the means of three replicate, ± Indicates the standard error from the mean
*Fermentation conditions: - Medium: M 5; Incubation temperature: 28°C; Incubation period: 144 hrs

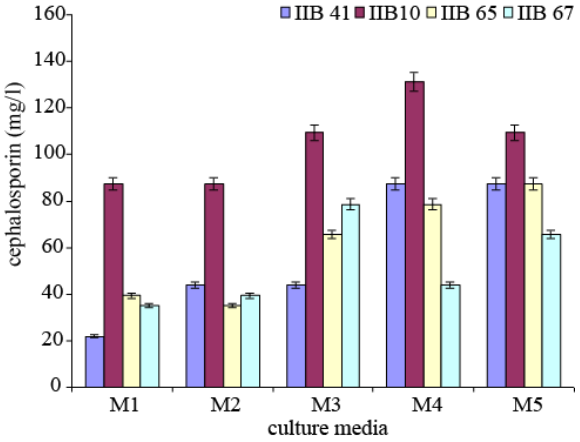

Fig. 1. Selection of the Culture Medium for Cephalosporin Production by *Acremonium chrysogenum* in shake flasks*.
All the values are means of three parallel replicates. Y-error bars indicate the standard error from mean
*Fermentation conditions:- Incubation temperature: 28°C; Incubation period: 144 hrs

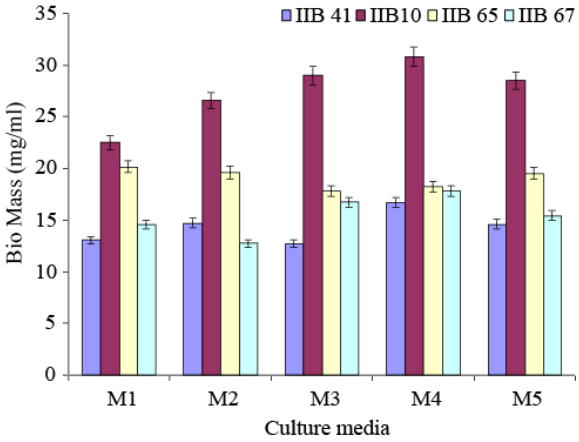

Fig. 2. Production of the fungal biomass of *Acremonium chrysogenum* in different culture media used for the production of the cephalosporin in shake flasks*.
All the values are means of three parallel replicates. Y-error bars indicate the standard error from mean
*Fermentation conditions:- Incubation temperature: 28°C; Incubation period: 144 hrs

The composition of the fermentation medium plays an important role in the production of cephalosporin by the microorganism. The medium provides basic components required for the growth of the fungus and the production of the secondary metabolites is associated with this growth (Amato & Pisano, 1976). Cephalosporin biosynthesis requires the morphological differentiation of the fungus i.e., conversion of hyphal filament to swollen hyphal fragment (Huber & Nash, 1971). The biosynthesis of cephalosporin is also affected by the inorganic salts present in the medium therefore, a decrease or increase in the concentration of the inorganic salts in the fermentation medium can result in increased or decreased production of cephalosporin. Lipids may serve as carbon source in the production of the cephalosporin (Amato & Pisano 1976). The medium 4 consisted of Corn meal, Baker flour, Ammonium sulphate, Calcium carbonate and Methyl oleate (Ott *et al.,* 1977). The medium 4 contained inorganic salts, lipids and other important ingredients which favored the productivity of cephalosporin. Moreover, corn flour and baker flour were the complex agricultural products which had provided the important nutrients required for the growth of fungus and production of the cephalosporin (Ott *et al.,* 1977). Some other workers have also used the same medium for the production of cephalosporin by *A. chrysogenum* (Ott *et al.,* 1977). So the medium 4 was selected as most suitable medium for the production of cephalosporin by *A. chrysogenum* in shake flasks.

Screening of carbon sources: Figure 3 shows the effect of addition of various carbon sources to the fermentation

medium, on the production of cephalosporin by *A. chrysogenum* during submerged fermentation. The results showed that maximum amount of the cephalosporin (480.7 mg/l) was produced when sucrose was used as a carbon source in the medium while the glucose and molasses resulted in the minimum production of cephalosporin (65.59 mg/l) when used as carbon sources. The other carbon sources did not show any significant production of cephalosporin (Fig. 3). The maximum biomass (36.96 mg/ml) was also produced when sucrose was used as a carbon source in the culture medium while minimum amount of the biomass (21.80 mg/ml) was observed when molasses was used.

The concentration of the sucrose in the culture medium was also optimized for the production of cephalosporin by *A. chrysogenum*. Figure 4 shows the effect of different concentrations of the sucrose used and the amount of cephalosporin produced. As the concentration of sucrose was increased from 1%, the production of cephalosporin was also increased. The maximum amount of cephalosporin produced by *A. chrysogenum* was 525.28 mg/l at 2.5% sucrose concentration. As the amount of the sucrose was increased above 2.5%, the production was decreased and reached the minimum value of 87.48 mg/l at 4% sucrose concentration. The dry mycelial mass was also increased with the increasing concentration of the sucrose up to 2.5%, but later on the amount of dry mycelial mass was also decreased. Therefore, sucrose at the concentration of 2.5% was selected as best carbon source for the production of cephalosporin.

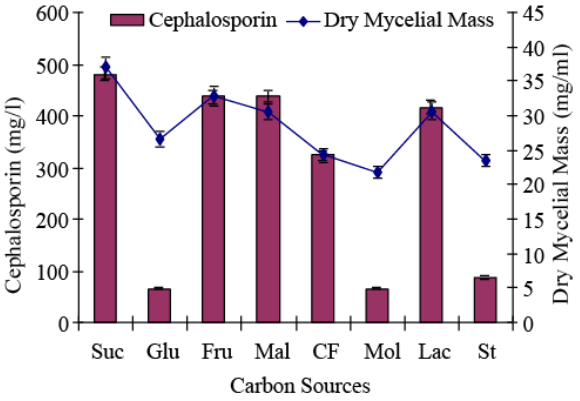

Fig. 3. Effect of the different carbon sources on the production of Cephalosporin by *Acremonium chrysogenum* in shake flasks*. All the values are means of three parallel replicates. Y-error bars indicate the standard error from mean.
*Fermentation conditions: Medium: M 4; Incubation period: 144 hrs; Incubation temperature: 30°C; pH: 6.5; Amount of carbon sources: 2%.
Abbreviations: Suc: sucrose; Glu: glucose; Fru: fructose; Mal: maltose; CF: corn flour; Mol: molasses; Lac: lactose; St: starch.

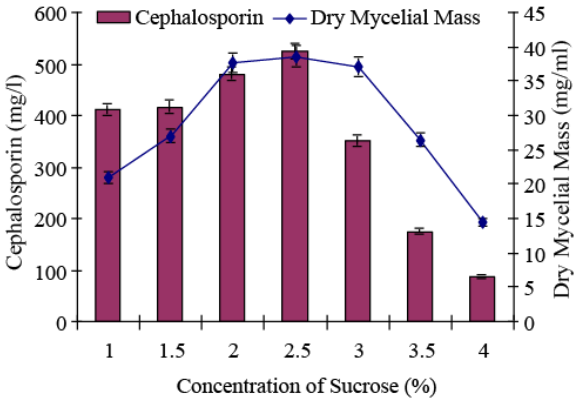

Fig. 4. Effect of various concentrations of sucrose on the production of Cephalosporin by *Acremonium chrysogenum* in shake flasks*.
All the values are means of three parallel replicates. Y-error bars indicate the standard error from mean
*Fermentation conditions: Medium: M 4; Incubation period: 144 hrs; Incubation temperature: 30°C; pH: 6.5; Carbon source: sucrose

The production of secondary metabolites such as antibiotics is greatly affected by carbon source in the culture medium. Early studies showed that the cephalosporin production was highly dependent upon the carbon sources used (Demain, 1963). Sucrose is reported to stimulate the cephalosporin production more as compared to other carbon sources (Adinarayna *et al.*, 2003), while some carbon sources such as glucose does not favor the production of cephalosporin (Seidel *et al.*, 2002). This decrease in cephalosporin production might be due to the stimulation of growth by the glucose at the expense of secondary metabolism. It was also reported that the readily metabolized carbohydrate suppressed the production of cephalosporin while the carbon sources which were metabolized slowly could increase the production of cephalosporin (Pan *et al.*, 1982). The concentration of the carbon source is also equally important for the production of cephalosporin. The maximum production of cephalosporin is reported at 2.7-3% sucrose concentration by different workers (Sabbagh *et al.*, 2007, Karaffa *et al.*, 1997) which is in agreement with our finding. But by increasing the amount of sucrose in the medium, the cephalosporin production was suppressed. That might be due to the inhibitory effect of sucrose on the growth of the fungus. Moreover sucrose causes the autolysis of the cell due to its accumulation in the cells (Nigam *et al.*, 2007).

Screening of nitrogen sources: The effect of addition of different organic nitrogen sources on the amount of cephalosporin produced by *A. chrysogenum* is shown in the Fig. 5. The minimum amount of cephalosporin (21.89 mg/l) was produced in the presence of meat extract while the maximum amount of cephalosporin (559.36 mg/l) was produced when peptone was used as an organic nitrogen source in the medium. Corn steep liquor also showed the favorable effect on cephalosporin production showing 541.88 mg/l of cephalosporin in the broth. The maximum biomass (36.30 mg/ml) was produced by peptone while the minimum amount of the biomass (13.80 mg/ml) was produced when beef extract was used. Figure 6 further shows the effect of various concentrations of peptone on the production of cephalosporin. It was observed that maximum cephalosporin production (576.84 mg/l) occurred at

peptone concentration of 1.5% in the culture medium. The amount of cephalosporin produced at 0.5% of peptone was 437 mg/l which gradually increased and reached maximum at 1.5% of peptone. There was sharp decline in the cephalosporin production as the concentration of peptone was increased above 1.5%. The maximum growth of the fungus as indicated by dry mycelial mass was also observed at 1.5% concentration of peptone i.e., 37.86 mg/ml.Therefore, peptone at a concentration of 1.5% was optimized for the production of cephalosporin.

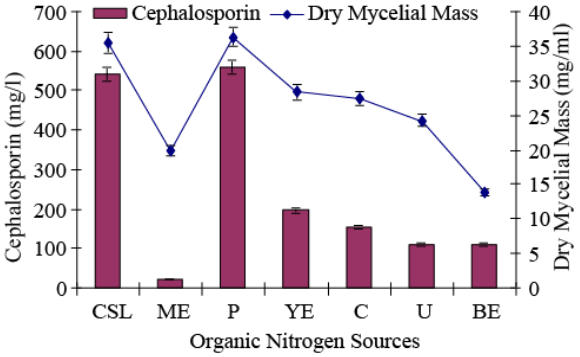

Fig. 5. Effect of the organic nitrogen source on the production of Cephalosporin by *Acremonium chrysogenum* in shake flasks*.
All the values are the mean of three parallel replicates. Y- error bars indicate the standard error from mean.
*Fermentation conditions: Medium: M 4; Incubation period: 144 hrs; Incubation temperature: 30°C; pH: 6.5; carbon source: 2.5% sucrose; amount of nitrogen source: 1%.
Abbreviations: CSL: corn steep liquor; ME: meat extract; P: peptone; YE: yeast extract; C: casein; U: urea; BE: beef extract.

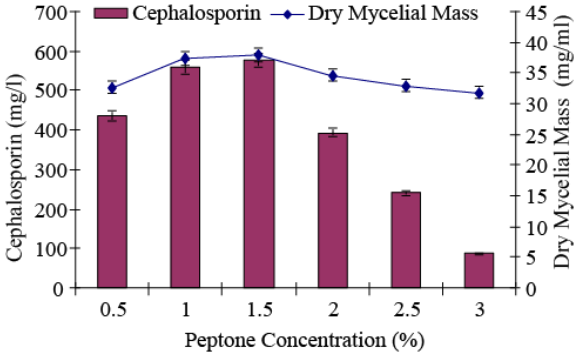

Fig. 6. Effect of the concentration of peptone on the production of Cephalosporin by *Acremonium chrysogenum* in shake flasks*.
All the values are means of three parallel replicates. Y- error bars indicate the standard error from mean
*Fermentation conditions: Medium: M 4; Incubation period: 144 hrs; Incubation temperature: 30°C; pH: 6.5; carbon source: 2.5% sucrose; organic nitrogen source: peptone.

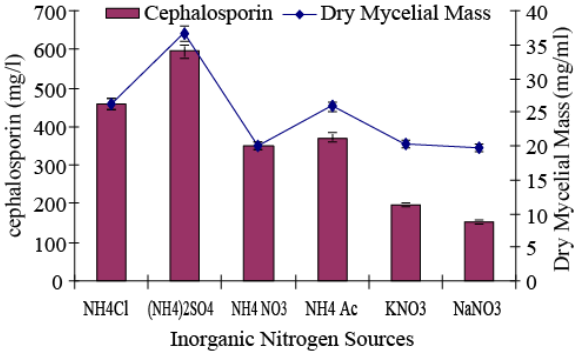

Fig. 7. Effect of the inorganic nitrogen source on the production of Cephalosporin by *Acremonium chrysogenum* in shake flasks*.
All the values are means of three parallel replicates. Y- error bars indicate the standard error from mean.
*Fermentation conditions: Medium: M 4; Incubation period: 144 hrs; Incubation temperature: 30°C; pH: 6.5; carbon source: 2.5% sucrose; organic nitrogen source: 1.5% peptone; amount of inorganic nitrogen source: 0.1%.

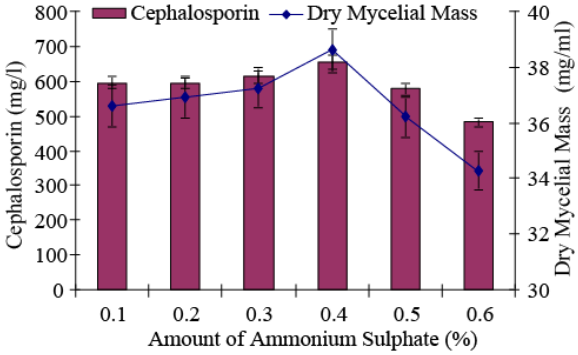

Fig. 8, Effect of the concentration of ammonium sulphate on the production of Cephalosporin by *Acremoniumchrysogenum* in shake flasks*.
All the values are means of three parallel replicates. Y- error bars indicate the standard error from mean.
*Fermentation conditions: Medium: M 4; Incubation period: 144 hrs; Incubation temperature: 30°C; pH: 6.5; Carbon source: 2.5% sucrose; Organic nitrogen source: 1.5% peptone; Inorganic nitrogen source: Ammonium sulphate.

The effect of various inorganic nitrogen sources on the production of cephalosporin was also studied (Fig. 7). It is clear from the results that the highest amount of cephalosporin (594.32 mg/l) was produced when Ammonium sulphate was added to the culture medium. The other inorganic nitrogen sources had no significant effect on the cephalosporin production by *A. chrysogenum.* The maximum fungal biomass (36.60 mg/ml) was also produced by Ammonium sulphate. Figure 8 shows the effect of various concentrations of ammonium sulphate on the production of cephalosporin by *A. chrysogenum.* The highest amount of cephalosporin (655.5mg/l) was produced at 0.4% of Ammonium sulphate concentration but on further increasing the Ammonium sulphate concentration, the amount of cephalosporin was decreased. There was increasing tendency in the fungal biomass (from 36.60 mg/ml to 39.26 mg/ml) in the concentration range of 0.1% to 0.4%. But on further increase in the concentration of Ammonium sulphate, the biomass production started to decrease.

The nitrogen is essentially required for the growth and metabolism of the fungus especially for the formation of swollen hyphal fragments which contribute towards the

production of cephalosporin (Nigam *et al*., 2007). Sabbagh *et al*., (2008) have also reported that peptone is required for high yield of the cephalosporin. Moreover, the peptone on hydrolysis produces methionine and ammonia and methionine is a favorable medium constituent for cephalosporin production (Sabbagh *et al.*, 2008). It provides sulphur for the synthesis of cephalosporin (Caltrider & Niss, 1965). The presence of the inorganic nitrogen source in the medium also affects the production of cephalosporin. It has been reported that the most important inorganic nitrogen source for cephalosporin production is Ammonium sulphate (Nigam *et al.*, 2007, Sabbagh *et al.*, 2008). A particular C/N ratio is required for cephalosporin production, so cephalosporin production increases on increasing C/N ratio from 5.3 to 8.0 and on further increasing it starts to decrease (Nigam *et al*., 2007) due to the accumulation of carbohydrates in the culture media. The increased concentration of nitrogen has inhibitory effect on the production of cephalosporin in the culture broth (Nigam *et al*., 2007).

Conclusion

In the present study, the locally isolated strain of *Acremonium chrysogenum* had shown significant amount of antibiotic (cephalosporin) production, which was active against gram positive and gram negative bacteria. By optimizing the nutritional parameters such as C& N requirements, a substantial enhancement in the antibiotic production was accomplished which was highly significant. During the course of study, 721.89 mg/l cephalosporin production was observed.

References

Adinarayana, K., T. Prabhakar, V. Srinivasulu, M. Anitha Rao, P. Jhansi Lakshmi and Ellaiah. 2003. Optimization of process parameters for cephalosporin C production under solid state fermentation from *Acremonium chrysogenum*, *Process Biochemistry*, 39: 171-177.

Amato, R.F.D. and M.A. Pissano. 1976. A chemically defined medium for cephalosporin C production. *Antonie van Leeuwenhoek*, 42: 299-308.

Bartoshevic, Yu., P.L. Zasalavskaya, M.J. Novak and O.D. Yudina. 1990. *Acremonium chrysogenum* differentiation and biosynthesis of cephalosporin. *J. Basic. Micrbiol*., 30: 313-320.

Bhat, S.V., B. A. Nagasmpagi and M. Sivakumar. 2005. Chemistry of natural product. Narosa Publishing House New Delhi.

Caltrider, P.G. and H.F. Niss. 1965. Role of methionine in cephalosporin synthesis. *Appl. Microbiol*., 14: 746-753.

Campos Muñiz, C., E. C. Z. Tania, E. Gabriela Rodríguez and Francisco J. Fernández, 2007. Penicillin and cephalosporin production: A historical perspective. Rev Latinoam Microbiol; 49 (3-4): 88-98.

Cruz, A.J.G., T. Pan, R.C. Giordano, M.L.G.C. Araujo and C.O. Hokka. 2004. Cephalosporin C Production by Immobilized *Cephalosporium acremonium* Cells in a Repeated Batch Tower Bioreactor. *Biotechnology & Bioengineering*, 85(1): pp. 96-102.

Cuadra, T., F.J. Fernandez, A. Tomasini and J. Barrios-Gonzalez. 2008. Influence of pH regulation and nutrient content on cephalosporin C production in solid-state fermentation by *Acremonium chrysogenum* C10". *Letters in Applied Microbiology*, 46(2): 216-220.

Dasari, V.R.R.K., S.R.R. Donthirddy, M.Y. Nikku and H.R. Garapati. 2008. Optimization of medium constituent for cephalosporin C production using response surface methodology and artificial neural network. *J. Biochem Tech*., 1(2): 69-74.

Demain, A.L., 1963. L- Valine: a precursor of cephalosporin C. *Biochem. Biophys. Res. Commun*., 10: 45-48.

Hasan, F., S. Khan, A.A. Shah and A. Hameed. 2009. Production of antibacterial compounds by free and immobilized *bacillus pumilus* saf1. *Pak. J. Bot*., 41(3): 1499-1510.

Higgins, C.E. and R.L. Hamill. 1974. The occurrence of deacetoxy Cephalosporin in Fungi and Streptomycetes. *The Journal of Antibiotics*, 298: 300.

Huber, F.M. and C.H. Nash. 1971. Antibiotic synthesis and morphological differentiation of *Cephalosporium acremonium*. *Applied Microbiology*, 6-10.

Karaffa, L., E. Sandor, J. Kozma and A. Szentirmai. 1997. Cephalosporin-C production, morphology and alternative respiration of *Acremonium chrysogenum* in glucose-limited chemostat. *Biotechnology Letters*, 18(6): 701-706.

Kim, J.C., S.W. Kang, J.S. Lim, Y.S. Song and S.W. Kim. 2006. Stimulation of cephalosporin C production by *Acremonium chrysogenum* M35 with fatty acids. *Journal of Microbiology & Biotechnology*, 16(7): 1120-1124.

Kim, J.C., Y.S. Song, D.H. Lee, S.W. Kang and S.W. Kim. 2007. Fatty acids reduce the tensile strength of fungal hyphae during cephalosporinCproduction in *Acremonium chrysogenum*. *Biotechnology Letters*, 29(1): 51-55.

Masuda, G. and S. Tomioka. 1978. Quantitative Assessment of Bactericidal activities of beta Lactam antibiotics by Agar plate method. *Antimicrobial agent and Chemotherapy*, 587-595.

Matsumura, M., T. Imanaka, T. Yoshida and H. Taguchi. 1970. Morphological differentiation in relation to cephalosporin synthesis by *Cephalosporium acremonium*. *J. Ferment. Technol*., 58: 197-204.

Mays, D.L., F.K. Bangert, W.C. Cantrell and W.G. Evans. 1975. Hydroxlamine determination of cephalosporin. *Anal. Chem*. 47: 2229-2234.

Naz, S.A. and S.A. Rasool. 2013. Isolation, production and characterization of bacteriocins produced by strains from indigenous environments. *Pak. J. Bot*., 45(1): 261-267.

Nigam, V.K., R. Verma, A. Kumar, S. Kundu and P. Ghosh, 2007. Influence of medium constituents on the biosyntheses of cephalosporin C. *Electronic Journal of Biotechnology*, ISSN 0717-3458.

Ott, J.L., W. Godzeski, D. Pavey, D. Farran and D.R. Horton. 1977. Factors affecting the fermentation. Biosynthesis of Cephalosporin. *Biotechnology Progress*, 10: 515-523.

Pan, C.H., E. Mckillip, C.H. Nash and S.V. Speth. 1982. Methyle oleate based fermentation medium for cephalosporin production. *Dev. Ind. Microbiol*., 23: 315- 323.

Sabbagh, N., L.M. Harvey and B. McNeil. 2008. Effects of dissolved carbon dioxide on growth, nutrient consumption, cephalosporin C synthesis and morphology of *Acremonium chrysogenum* in batch cultures. *Enzyme & Microbial Technology*, 42(4): 315-324.

Seidel, G., C. Tollnick, M. Beyer and K. Schugerl. 2002. Process engineering aspects of the production of cephalosporin C by *Acremonium chrysogenum*. Part II. Cultivation in diluted and enriched complex media. *Process Biochemistry*, 38(2): 241-248.

Sohn, Y.S., K.C. Lee, Y.H. Koh and G.H. Gil. 1994. Changes in cellular fatty acid composition of *Cephalosporium acremonium* during cephalosporin C production. *Applied & Environmental Microbiology*, 60(3): 947-952.

Srinivasan, M.C. 2004. Practical Mycology for Industrial Biotechnologist/ Tata McGraw–Hill Publishing Company Ltd. New Dahlia

Trilli, A., V. Michelini, V. Mantovani and S.J. Pirt. 1977. Development of agar disc method for the rapid selection of cephalosporin producers with improved yield. *Anartimicrobial agent and Chemotherapy*, 7-13.

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