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Cephalosporin C Production from *Acremonium chrysogenum*

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Abstract

Cephalosporin C demand is increasing worldwide because of its enhanced antibacterial spectrum that it can be used to treat diseases and infections caused by Gram positive/or Gram negative bacterial strains. Cephalosporin C is easily produced from the fungus *Acremonium chrysogenum* by the process of fermentation in a bioreactor under optimum reaction conditions to obtain maximum yield of the antibiotic. Additional chemical and structural modifications in the existing cephalosporin C could further enhance its antimicrobial spectrum.

Keywords: *Acremonium chrysogenum*; Cephalosporin C; Fermentation

Introduction

Acremonium chrysogenum also known as *Cephalosporium acremonium* is the industrial producer of the antibiotic cephalosporin C, a beta-lactam antibiotic. Today cephalosporin and its derivatives are widely use in the treatment of number of infectious diseases caused by bacteria in the respiratory tract, infections of skin and infections of urinary tract [1]. Cephalosporin C is one of the major biotechnological products with a total world market of about $10 billion. The genus Acremonium is a polyphyletic taxon containing distantly related fungi including species associated with atleast three or four ascomycete orders. Different strains of *Cephalosporium* species such as Emericellopsis, Paecilomyces, *chrysogenum*, *S. clavuligerus* etc is used for the production of cephalosporin antibiotic. *Acremonium chrysogenum* is naturally found in soil, organic matter and plant debris and overgrows in humid environment forming grey and white colonies of loose interwoven hyphae [2].

Literature Review

Morphology

Reproduction in *Acremonium chrysogenum* is strictly asexual by means of conidiophores and arthrospores. However, no associated teleomorphic state showing sexual propagation has been found. Microscopic study revealed that its hyphae is separated holding unbranched erect phialides that are positioned directly at its tips. Its conidia appeared to be unicellular and hyaline. Four different morphological forms of the fungi *Acremonium chrysogenum* has been reported during fermentation such as swollen hyphal fragments, Conidia, hyphae and arthrospores which are metabolically inactive. The classification of *Acremonium chrysogenum* is explained in Table 1.

Yeast like morphological form of the fungi (wide swollen hyphal fragments) has high tendency to produce cephalosporin in greater concentration [3].

Kingdom	Mycota
Sub-kingdom	Diakarya
Phylum	Ascomycota
Sub-phylum	Pezizomycotina
Class	Sordariomycetes
	Pyrenomycetes
Sub-class	Sordariomycetidae
Order	Hypocreales
Family	Hypocreaceae
Genus	*Acremonium*
Species	*A. chrysogenum*

Table 1: Classification of *Acremonium chrysogenum*.

Cephalosporin C: Cephalosporin c is a popular antibiotic because of its excellent characteristics such as broad spectrum, low toxicity and resistance to beta lactamase than penicillin.

Chemical structure: The molecular formula of cephalosporin c shows that it consists of 16 C-atoms, 21 H-atoms, 3 N-atoms, 8 O-atoms and 1 S-atoms. It consists of a beta lactam ring, six membered sulphur containing dihydrothiazine ring and an acyl side chain with a R group (Figure 1). This substitute group is responsible for giving cephalosporin different level of spectrum activity. Molecular weight of cephalosporin C is 415.42 g/mol with UV absorption of 260 nm [4].

Generations of cephalosporins

Cephalosporin C is classified in to various generations on the basis of their antimicrobial potency against a number of Gram negative and Gram positive bacterial strains. Synthesis of its various generations is accomplished either by using microorganism as bio factories or by means of enzymatic conversion of cephalosporin c (Figure 2).

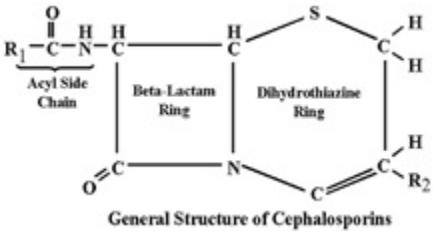

Figure 1: Cephalosporin C chemical structure [5] Molecular formula: $C_{16}H_{21}N_3O_8S$.

First generation cephalosporin (Narrow spectrum): This class of cephalosporins is known as narrow spectrum antibiotics with high beta lactamase sensitivity and activity against Gram positive bacteria e.g. *Streptococcus, Staphylococcus* etc.

Second generation cephalosporin (Intermediate spectrum): This class of cephalosporins is known as intermediate spectrum antibiotics because their activity lies between first and third generation cephalosporin with decreasing activity against Gram positive bacteria and increased potential against Gram negative bacteria e.g. *E. coli, Haemophilus influenzae* etc [6].

Third generation cephalosporin (Broad spectrum): This class of cephalosporins is known as broad spectrum antibiotics with broad activity against Gram negative bacteria and beta lactamase resistance e.g. *Pseudomonas, Staphylococcus aureus, Streptococcus pneumonia, Enterobacteriaceae* etc.

Fourth generation cephalosporin (Extended spectrum): This class of cephalosporins is an extended version of third generation antibiotics with increased activity against Gram positive & Gram-negative bacteria with high resistance to beta lactamase [7].

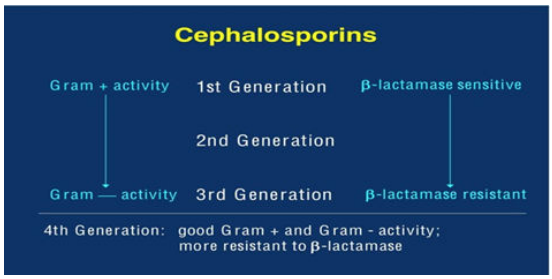

Figure 2: Activity spectrum of different cephalosporin generations [8].

Mode of action

Cephalosporin c inhibits bacterial synthesis by inhibiting cell wall formation by inferring with peptidoglycan synthesis which is an important constituent of bacterial cell wall. Final synthesis of peptidoglycan is achieved by PBP (penicillin binding protein) a trans peptidase which binds to D-Ala-D-Ala site to cross link. Cephalosporins mimic this site and there by irreversibly bind to PBP to inhibit its activity by ceasing cross linking of peptidoglycan [9].

Methods of production

Cephalosporin C production is an aerobic process, carried out either by conventional or non-conventional fermentation methods using free or immobilized fungi.

Conventional mode of production: Cephalosporin c uses either surface liquid or solid-state fermentation in a batch bioreactor or continuous stirred tank reactor. But this method is not much favorable for cephalosporin c production because of high probability of oxygen limitation. However, improvements in the bioreactor construct by attachment of sparging rings and agitators to ensure a continuous supply and equal distribution of oxygen throughout fermentation process is done in order to increase cephalosporin c yield.

Non-conventional mode of production: Cephalosporin c uses either packed bed or airlift bioreactor with an advantage of being operated in batch or continuous mode with proper oxygenation and agitation resulting in high yield of cephalosporin [2,3].

Requirements of the fermentation process for cephalosporin C production

Fermentation medium composition provides basic components required for the fungus growth and for secondary metabolites production associated with their growth it consisted of the following components.

Carbon source i.e., glucose, sucrose, fructose, molasses, and lactose etc. It is required for morphological differentiation of the fungus. So, cephalosporin yield is altered.

Organic and inorganic nitrogen sources i.e. peptone. urea, meat extract, yeast extract, casein, beef extract, ammonium sulphate, ammonium chloride, ammonium nitrate, ammonium phosphate and potassium nitrate influence mycelium differentiation to swollen hyphae or metabolically inactive arthrospores. Thus, affecting biosynthesis of cephalosporin.

Trace elements

- **Essential vitamins:**
- Aerobic conditions i.e., enough dissolved oxygen supply around 40% or above. Sparging rings ensure continuous oxygen supply and its distribution throughout fermentation process.
- Appropriate buffer for maintaining optimum pH. Small changes in the pH can easily be detected by the pH meter.
- Optimum temperature maintained by flowing water through the jacket to promote fast growth of the fungus. A slight change in the temperature can easily be detected using temperature probes that are sensitive to small temperature variations.
- Silicone oil to control foaming where necessary.
- Moisture.

Chemical inhibitors and blockers to prevent or inhibit the interaction between crude cephalosporin c and other chemical compounds which may be structurally similar or dissimilar to crude cephalosporin c and accumulates in the liquid broth at the end of the fermentation reaction [10,11].

Culture medium conditions

The culture was propagated in the sterilized seed medium containing 30 g/l sucrose, 1.5 g/l potassium dihydrogen

orthophosphate, 0.3 g/l magnesium sulphate and 15 ml trace metal solutions. The culture was grown for about 6 days to 7 days at 28°C in a rotatory shaker set at rpm of 200 [12].

Process: Cephalosporin c production by *Acremonium chrysogenum* on the fermentation medium at 28°C for 144 hours is characterized by the rapid consumption of sucrose to form biomass at the beginning of the process. pH of the medium was kept 7.2 and the temperature was maintained at 28°C by passing water through the jacket. pH of the broth was maintained by using 2 molar potassium hydroxides and 2 molar HCL. Dissolved oxygen was maintained above 30% saturation by agitation and aeration. After the depletion of carbohydrate by the slow consumption of sucrose during which most of the Cephalosporin c is produced is called the idiophase and observed cell growth is insignificant. Maximum cephalosporin c has been obtained at 120 h of fermentation while highest cell growth occurs at 42 h of fermentation [13]. Accumulation of secondary metabolites occurs in idiophase after growth phase (tropophase) therefore called as idiolites. Cephalosporin c is separated from the liquid broth by the use of a number of separation and distillation techniques in a sequential step wise manner depending on the interactive chemical nature of the obtained product [14].

Analysis: The dry cell weight was estimated by centrifugation of 10 ml of fermentation broth, washed twice with distilled water, recentrifuged and kept for drying at 80°C till the constant weight.

Downstream processing: Downstream processing of cephalosporin antibiotic involves a series of steps in a sequential manner to isolate and purify the produced antibiotic from the liquid broth by using a number of separation and distillation techniques depending on the physical and chemical nature of the product i.e., resin ion-exchange method or solvent extraction is used to separate the obtained antibiotic from the liquid broth. After that a purified form of the antibiotic is produced which is than ready for packaging and shipping.

Factors effecting biosynthesis of cephalosporin C

Effect of methionine: With increased methionine concentration, up to 4.0 g/l cephalosporin C concentration also increases. Because it supplies sulphur for the synthesis process and thereafter started decreasing. Optimum concentration of methionine stimulates fungal differentiation and increases the antibiotic yield. Hence it is reported that 0.4% of methionine concentration is optimum for the synthesis reaction [15].

Effect of ammonium sulphate: It increases cephalosporin C production because of increased dry cell weight. But its high concentration above the optimum requirement decreases the rate of synthesis by interfering in mycelial morphological differentiation.

Effect of C/N ratio: C/N ratio of 5.33 to 8.0 increases the synthesis rate of cephalosporin C. But due to sucrose accumulation in the broth which inhibits the synthesis of enzymes accountable for Cephalosporin C formation. its yield is decreased [16].

Discussion

Current trends in new generation cephalosporins

Due to extensive research, it is revealed that incorporation of methoxy group in cephalosporin led to a considerable increase in beta lactamase stability. Therefore, methoxy and formamide derivatives of cephalosporin were prepared and screened for their antibacterial activity. Research teams are also attempting to synthesize some new semi synthetic cephalosporins and chemically modifying the existing semi synthetic cephalosporin to produce antibiotic with high resistance against beta lactamase. *Cephalosporin* is poorly soluble in water therefore attempts have been made to prepare cephalosporin with enhanced solubility using cefotaxime during cephalosporin preparation. Enzymatic methods have been employed to produce the key intermediate (7-ACA) required for synthetic cephalosporin production which in turn increases the availability of the drug worldwide [17]. Availability of the drug at the target site, increased oral absorptivity and increased cephalosporin c stability can be achieved by easily cleaved blocking groups for the carboxylic acid in the cephalosporin synthetic structure which forms the basis of current researches going in the discipline of chemistry. Besides new ways have been devised to improve the bioreactor layouts by making alterations in their core structure as well addition of new internal and external features such as automated sparging rings, agitators, acid/base indicators, temperature probes etc. to improve their performance by ensuring optimum reaction condition for fermentation to produce high quantities of cephalosporin c and also maintaining stable reaction kinetics [18]. Furthermore, this well engineered bioreactor also ensures efficient utilization of all the reaction components during fermentation process and maintains the obtained antibiotic product in a chemically stable form by preventing its degradation from accumulations in the liquid broth. The optimization of media composition for cephalosporin c production is under study such as the influence of inorganic nitrogen sources, methionine etc. and optimization of carbon nitrogen ratio to secure good production performance. Attempts to increase the oxygen transfer rate to the broth by immobilizing mold with algae called *Chlorella pyrenoidosa* in a symbiotic mode. With the current developments in the chemical industrial sector easy detection of the geometric phase of fermentation during which fungus growth is at its peak and maximum cephalosporin c product is obtained, can be identified. The fermentation reactor can also be integrated with automated or semi-automated distilling equipments for the separation of the crude cephalosporin from the liquid broth much easily and efficiently in less time [19].

Conclusion

Cephalosporin C is one of the mostly used antibiotic worldwide with broad activity spectrum making it suitable to be used against numerous Gram negative and Gram positive bacterial strains for the treatment of various bacterial associated diseases.

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