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Innovation The Research Concept

Streptomyces Clavuligerus Ability for Production of Clavulanic Acid

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Abstract

Clavulanic acid (CA) has been reported as potent inhibitor of β-lactamase, produced by several species of bacteria such as *Streptomyces clavuligerus* or other microorganisms. *S. clavuligerus* ATCC 27064 is a gram-positive bacterium, showing capability to produce clavulanic acid and reported to isolate from a South American soil. Report has shown that genes directing CA biosynthesis are clustered forms along with the genes responsible for the biosynthesis of the β-lactam antibiotic, cephamycin C. And it is co-regulated in this organism showing unique properties with the production of an antibiotic and production of a small molecule to protect the antibiotic from its enzymatic degradation. Fed-batch mode has been applied to produce the clavulanic acid by *S. clavuligerus* and glycerol as carbon source is used in production media to enhance the its yield.

Keywords: β-Lactamase, Antibiotics, Clavulanic Acid (CA), *Streptomyces Clavuligerus,* Biosynthesis.

Introduction

Clavulanic acid (CA) has been reported as potent inhibitor of β-lactamases, enzymes found in many bacterial species and they are responsible for the hydrolysis of beta-lactam antibiotics. It is a produced as secondary metabolite by the filamentous aerobic bacterium *Streptomyces clavuligerus* in submerged cultivations It is active against a wide range of gram-positive and gram-negative bacteria. Genome sequence of *S. clavuligerus* studies has shown its capability to build metabolic models and ability to engineer the organism by directed approaches and it can create exciting opportunities to improve strain productivity more efficiently (Paradkar, 2013).

Clavulanic acid (CA) has been used together with β-lactam antibiotics to create drug mixtures, possessing potent antimicrobial activity and shown its clinical and industrial importance. Identification of the clavulanic acid biosynthetic pathway and the associated gene cluster(s) in the main producer species such as *Streptomyces clavuligerus* has been reported as effective producer organisms of CA by improvement through genetic manipulation (Song et al., 2010). *Streptomyces clavuligerus* has capability to produce cephem antibiotics and the N-acylated dithiolopyrrolone antibiotic holomycin, (a reported inhibitor of RNA synthesis) at industrial level. *S. clavuligerus* ATCC 27064 has been examined for a potential biosynthetic gene cluster, with the conclusion of holomycin arises from some derivative of an L-CysL-Cys dipeptide. Holomycin has undergone eight-electron oxidation, fused five-five ring formation, and decarboxylation (Li, and Walsh, 2010). *Streptomyces clavuligerus* ATCC 20764 is found to grow from spore-inocuia on a glycerol, malt extract, bacteriological peptone medium and showing shape of its spore-bearing hyphal branches. Its organism, morphological parameters such as main hyphal length, total hyphal length, number of tips, and hyphal growth unit has been measured to detect the capability of CA in fermentation medium. Growth and productivity are reported as hardly dependent on stirrer speed and after early growth fragmentation of long, highly branched mycelia to shorter, less branched fragments has been occurred in broth (Belmar-Beiny and Thomas, 1991).

Clavulanic Acid Production

Clavulanic acid has been first identified from *Streptomyces clavuligerus* and later from other Streptomyces species, *S. jumonjinensis and S. katsurahamanus.* In addition, several species of *Streptomyces* have been identified as producing other clavam metabolites structurally similar to clavulanic acid, such as clavaminic acid, 2-hydroxyethylclavam,valclavam, and clavamycins. Interestingly, all *Streptomyces* species has been described to date to produce clavulanic acid with conventional β-lactam

antibiotics. Furthermore, the number of species described as producing non-clavulanic acid clavams exceeds those producing clavulanic acid, implying that the ability to biosynthesize clavulanic acid is more restricted in distribution (Howarth et al., 1976).

Clavulanic acid productions by S. clavuligerus have been actively pursued due to the commercial and clinical importance of clavulanic acid. Several strains of *Streptomyces clavuligerus* can be used for the production of clavulanic acid. The industrial production of clavulanic acid is carried out by large-scale fermentation of *S.clavuligerus*. The wild type strain *of S.clavuligerus* which is derived through a conventional strain program is used for the industrial production because of its better productivity (Nilsson-Ehle et al., 1985).

There are more than 20 secondary metabolites, including many β-lactam antibiotics such as clavulanic acid, cephamycin C, deacetoxy cephalosporin C, penicillin N (an intermediate in cephamycin C pathway) and at least four other clavams. All the mentioned antibiotics has been produced by various strains of *S. clavuligerus.*

Different carbon sources such as palm oil, olive oil, and glycerol can be used for the production of clavulanic acid. Use of olive oil and palm gives better concentration of clavulanic acid than glycerol. But the cost of olive oil is much higher than glycerol. So, considering all necessary parameters for the production glycerol is used as the best suited carbon source (Viana Marques et al., 2011).

Selection of Effective Producer

Selection of microorganism is the most significant and crucial step for the production of the clavulanic acid. The requirements of different microorganisms vary from each other. Selection of microorganism strain and its improvement is very necessary for good yield of clavulanic acid.

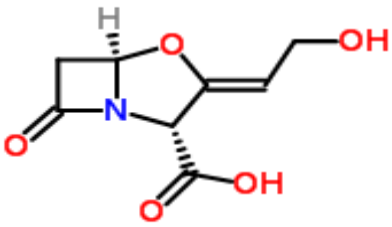
Streptomyces clavuligerus, a Gram-positive bacterium with a high G+C content, is a member of the family actinomycetes, members of which have a complex life cycle and morphological differences from mycelia, spores, and hyphae. *S. clavuligerus* is an industrially important microorganism that produces various beta-lactam antibiotics, including cephamycin, clavams, and clavulanic acid (a wide-spectrum beta-lactamase inhibitor) (Neto et al., 2005). Clavulanic acid has been used effectively in combination with other antibiotics to treat diseases caused by various pathogenic microorganisms that would otherwise be resistant to beta-lactam antibiotics. *S. clavuligerus* potentially contains genes for different secondary metabolites that could be used for pharmaceutical and industrial compounds (Hamedi et al., 2012).

Specific nutritional requirements of microorganisms used in industrial fermentation processes are as complex and varied as the microorganisms in question. Not only are the types of microorganisms diverse (bacteria, molds and yeast, normally), but also the species and strains become very specific as to their requirements. Microorganisms obtain energy for support of biosynthesis and growth from their environment in a variety of ways (Bellão et al., 2013). Besides a source of energy, organisms require a source of materials for biosynthesis of cellular matter and products in cell operation, maintenance and reproduction. These materials must supply all the elements necessary to accomplish this. Some microorganisms utilize elements in the form of simple compounds; others require more complex compounds, usually related to the form in which they ultimately will be incorporated in the cellular material. The four predominant types of polymeric cell compounds are the lipids (fats), the polysaccharides (starch, cellulose, etc.), the information-encoded polydeoxyribonucleic acid and polydeoxyribonucleic acid and polyribonucleic acids and proteins (Hamedi et al., 2012).

Physical and Chemical Properties

CA is a carboxylic acid and able to inhibit the growth of strains of *Staphylococcus aureus.* It forms a sodium salt which shows characteristic of Infra-red spectrum. It can synergies the antibacterial effect of ampicillin against the β-lactamase producing strains of *Escherichia coli, Klebsiella aerogenes and Staphylococcus aureus.* It has capability to synergize the antibacterial effect of cephaloridine against the β-lactamase producing strains of *Proteus mirabilis* and *Staphylococcus aureus*

Figure 1: Structure of Clavulanic Acid with Molecular Formula: $C_8H_9NO_5$ and weight 199.16076



Commercial Prospective

The antibacterial market is currently best described as saturated, highly segmented and increasingly flooded with generics. Furthermore, bacterial drug-resistance is threatening currently marketed drugs, although this represents an opportunity for the more audacious drug maker. Glaxo SmithKline's (GSK) former community blockbuster 'Augmentin' is a classical example of the impact of generic incursion following patent expiry. Since the first amoxicillin/CA generics entered the US market in 2002, Augmentin's US sales plummeted from a peak of $1.6 billion in 2001 to only $107 million in 2004, representing 93% sales erosion in only three years. In anticipation of Augmentin's patent expiry, GSK launched two follow-up products; Augmentin ES-600 and Augmentin XR. However, although these two products partially offset the loss in Augmentin revenues, dampening the drop in sales from 93% to 68% by generating combined sales worth $505 million in 2004, GSK still registered a significant loss in antibacterial revenues.

CA has limited market availability because of its complex production process, both concerning the technology and intellectual property rights. In addition to GSK, there are two other generic manufacturers present in the market since July 2003: the American company, Geneva, with its generic co-amoxiclav, and an Israeli company, Teva. An Indian company, Ranbaxy, has also received a marketing authorization for co-amoxiclav tablets, but their product is not yet available in the U.S. market. Lek D.D, a Slovene pharmaceutical company, has entered the world's largest pharmaceuticals market with its leading product, co-amoxiclav.

By entering the US market, Lek has, to date, achieved its most far-reaching business goal. Lek's affiliated company, Lek Pharmaceuticals, Inc., sold $27 million worth of co-amoxiclav on its first workday. Lek D.D. began its co-amoxiclav project in 1989 with its own R&D efforts, developing its own technology for the production of CA and its own finished forms of co-amoxiclav, which are in compliance with international regulatory requirements. Lek D.D. is producing the active ingredient (ReF: Finecure Pharmaceuticals Limited).

Conclusions

Clavulanic acid (CA) has properly of potent inhibitor of β-lactamase enzyme occurred in most pathogenic microorganism. Several species of bacteria such as *Streptomyces clavuligerus* or other microorganisms has produced it. Gram-positive bacterium *Streptomyces clavuligerus* strain reported and capable to produce clavulanic acid and isolated from a South American soil. Genes of CA biosynthesis is found clustered along with the genes, responsible for the biosynthesis of the β-lactam antibiotic of cephamycin C. This organism has showed unique properties to co-regulate with the production of an antibiotic and its enzymatic degradation for production of a small molecule. Fed-batch mode is used to produce the clavulanic acid by *S clavuligerus* and glycerol as carbon source is used in production media via enhancing its yield.

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