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Streptomyces clavuligerus: The Producer Organism of Clavulanic Acid

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Abstract

Clavulanic acid is a major β-lactam antibiotic and a powerful inhibitor of the beta-lactamases, enzymes produced by bacteria, which are resistant to penicillin and cephalosporin. The industrial production of clavulanic acid is carried out by large-scale fermentation of *Streptomyces clavuligerus*. Since the first clinical applications, the efficiency of the β-lactam antibiotics has been declining, due to the astonishing increasing number of bacteria capable of displaying β-lactam resistance. Such resistance is due to pathogenic microorganisms that produce β-lactamaseenzymes, the most important mechanism of bacterial β-lactam resistance. Thus, the genetic production improvement using physical and chemical mutagenic agents is an important strategy in programs of industrial production of bioactive metabolites, such as clavulanic acid. This review focused on the biosynthesis, improvement and the importance of future investigations on clavulanic acid.

Keywords: *Streptomyces clavuligerus*; clavulanic acid; β-lactamase inhibitor; mutagenesis

Introduction

β-lactam antibiotics are among the most popular classes of antibacterial agents, whose mechanism of action consists on the inhibition of bacterial cell wall synthesis [1]. Clavulanic acid was discovered from *Streptomyces clavuligerus*. Its structure was elucidated and it was first purified as a novel β-lactamase inhibitor [2]. This compound presents a similar nucleus to that of penicillin, with some notable differences such as the lacking of the anacylamino side chain; the presence of oxygen instead of sulfur, and a β-hydroxyethylidine substituent in the oxazolidine ring [3]. Three β-lactamase inhibitors are currently available: clavulanic acid, tazobactamand sulbactam. Clavulanic acid is mainly found in combination with amoxicillin, tazobactamis often combined with piperacillin and sulbactam with ampicillin [4]. Clavulanic acid effectively inhibits the activity of β-lactamases of A and D molecular classes, such as cephalosporinases, penicillinases, and broad spectrum β-lactamases. Therefore it has been widely used clinically to treat diseases caused by β-lactam resistant bacteria [5]. There is an important number of pathogens resistant to β-lactam antibiotics, including Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Proteus mirabilis and Haemophilus influenzae. All of these microorganisms produce β-lactamases that hydrolyze the β-lactamic ring from antibiotics, releasing compounds that lack the original antibiotic activity [6].

Biosynthesis of Clavulanic Acid

In general, the genes in S. clavuligerus responsible for the biosynthesis of clavulanic acid are arranged in a super-cluster. Approximately 18 genes (orf2-19) are proposed to encode proteins involved inthe biosynthesis, transport, and regulation of the clavulanic acid. The biosynthesis initiates with the condensation of L-arginine and D-glyceraldehyde-3-phosphate, followed by several steps to form clavaminic acid. Clavaminic acid serves as a branch point between the biosynthesis of clavulanic acid that has the 3S,5S stereochemistry. In the remaining steps, clavaminic acid undergoes a double epimerization followed by oxidative deamination to yield (3R,5R)-clavulanate-9-aldehyde (clavaldehyde), which has been identified as the final intermediate in clavulanic acid biosynthesis. The enzyme encoded by orf9, clavulanic acid dehydrogenase (CAD), is responsible for the final step in clavulanic acid biosynthesis [7].

Improvement of Clavulanic Acid Production by Mutation in *Streptomyces clavuligerus*

Streptomyces are mycelial bacteria that resemble filamentous fungi in their apical growth, branching, and morphogenetic development [8]. The Streptomyces chromosome is very unstable and undergoes a very large amount of spontaneous deletions at rates higher than 0.1% of spores. This genetic instability affects different phenotypical properties, often pleiotropically, including morphological differentiation, production of secondary metabolites, such as pigments and antibiotics, antibiotic resistance, secretion of extracellular enzymes and sometimes genes for primary metabolism, particularly one or more steps in the arginine biosynthetic pathway [9]. Genetic instability can be stimulated by mutagens, such as ultraviolet (UV) light [10].

UV irradiation is one of the strain improvement strategies through random mutation. UV is a very convenient and relatively safe mutage, and inthe range of 200-300 nm it produces thymidinedimmers and increases the probability of deletion during the duplication process [11]. UV irradiation can increase the concentration of clavulanic acid up to two-folds, as it also makes their production cheaper [12,13]. Clavulanic acid can be increased also by genetic engineering through mutation of the gap1 gene. Through over expression and integration of regular genes ccaR and claR in gap1 deleted mutants resulted in a 5 to 6 fold increase in clavulanic acid production [14].

Conclusions

The increased resistance to the currently recommended antibiotics is responsible for several human deaths and it has been a constant concern. The major cause of antibiotic resistance is the inappropriate use and excessive treatment of antibiotics. Thus, more guidance acts are necessary, as well as stricter regulation of antibiotics. Therefore, a better understanding of the mechanisms involved in the production of clavulanic acid as well as to increase the production is fundamental. Additionally, new research it is necessary for a greater understanding of the biosynthesis of clavulanic acid and the genome of S. clavuligerus with respect to genetic instability.

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