PRODUCTION OF ANTIBIOTICS

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Summary

Since the discovery of penicillin in 1929 by Alexander Fleming the importance of antibiotics as chemotherapeutic agents has been increasing year after year. More than 8000 antibiotics are known, although only a few of them have therapeutic importance. The study of the biosynthetic pathway of many antibiotics has served as a way to design new pathways and products.

Penicillin production can be studied as an example for the antibiotic world because it was the first antibiotic produced on a large scale, and also because today it is the one most commonly used in the treatment of human infectious diseases around the world. In addition, many of the techniques used for the industrial production of penicillin have served as a model for the industrial production of other antibiotics or secondary metabolites.

Traditionally, the increase in the production of antibiotics has been obtained using classical improvement methods which have given good results, and the use of these methods has allowed researchers to improve the strains and the production processes. However, over the last few years molecular biology techniques have been implemented in order to increase final antibiotic production, and also to obtain products that are not naturally synthesized.

After approximately 20 years of gene manipulation there is still one open question. Can the DNA recombinant technology improve natural evolution or simply make it to go faster? Since industry still uses classical mutation and screening methods to select for better producing strains, molecular biology can probably serve as an additional tool to improve strains, which must be combined with the classical improvement techniques to get the best results.

The article presents an overview about i) the production of penicillin as an historical example, ii) the strain improvements techniques used since the discovery of penicillin, and iii) examples of the application of DNA-recombinant technology to increase the β-lactam antibiotic production in filamentous fungi.

1. Introduction

Antibiotics and other secondary metabolites are synthesized in response to physiological stress due to nutrient limitation (e.g. in response to limitation of phosphate or easily

assimilable carbon and nitrogen sources). Secondary metabolites, accumulated in response to nutrient starvation, may serve as biochemical signals that trigger differentiation or as microbial antagonists that inhibit the growth of competing microorganisms.

The role in nature of antibiotics and other secondary metabolites has been a subject of intense discussion for many years. Antibiotics may be antagonistic agents to combat bacteria and other microorganisms or effector molecules that trigger physiological or morphological differentiation .

Antibiotics are chemical substances produced by microorganisms that kill or inhibit the growth of other microorganisms. The development of antibiotics as agents for treatment of infectious diseases has probably been more important in the practice of medicine than any other single development.

Antibiotics are products of secondary metabolism that can be produced commercially by microbial fermentation. Commercially useful antibiotics are produced mainly by filamentous fungi and by bacteria of the actinomycete group. As secondary metabolites, each antibiotic is produced by a relatively limited number of species and is encoded by sets of dispensable genes. These compounds are synthesized at the end of the exponential growth phase and during the stationary phase, and their formation is highly influenced by the growth conditions, especially by the composition of the culture medium.

The most famous example has been the growth inhibition which was observed by Alexander Fleming in 1929, when *Staphylococcus aureus* growth was inhibited by a contaminant *Penicillium notatum* culture. The antibiotic produced by this fungus was called penicillin, and was the first antibiotic produced at large scale by submerged fermentation procedures. The World War II increase in demand for chemotherapeutic substances came at a time when processes to produce penicillin at industrial level were being developed. This was also the beginning of the era of antibiotic research and industrial production. Even today it is one of the more dynamic fields in biology research, and all the industrial countries continue to increase the number of described antibiotics: 513 antibiotics were known in 1961, 4076 in 1972, 7650 in 1985, and currently around 8000.

Despite the high number of known antibiotics, only a few are produced by fermentation. In addition, several other semisynthetic antibiotics are produced from the initial microbial product, and finally some of them are produced in a totally synthetic way, e.g. chloramphenycol, phosphomycin and pyrrolmitrin.

The significance of antibiotic production for the produced strain still remains unclear. Antibiotic production could have ecological significance for the life of such organisms in nature: it could confer upon them some advantage over other microorganisms in the competition for nutrients and habitat, but solid research to support this hypothesis is very limited. As secondary metabolites, antibiotics could play some regulatory role during differentiation, perhaps acting as temporary inhibitory agents. At this point it is important to remark that most of the new antibiotics have been detected by empirical

screening methods, which do not have any similarity with “in vivo” conditions; so it could be that the antibiotics being detected “in vitro” are produced in low amounts or even not produced at all “in vivo.”

From the industrial point of view, the improvements of the antibiotic producing strains have been traditionally carried out by classical procedures, such as the random mutagenesis or protoplast fusion. However, since the development of the DNA recombinant technology, many approaches have been made to increase the efficiency of the antibiotic biosynthetic pathways. The aim is not only to get increases in production but also to obtain new final products—and even to manipulate the pathways to direct the biosynthetic fluxes in one particular direction (normally to increase the production of one particular antibiotic).

2. β -lactam Antibiotics as a Model System

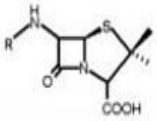
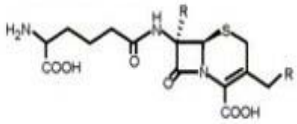
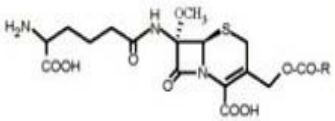
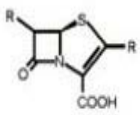
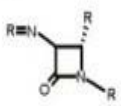
BASIC STRUCTURE	ANTIBIOTIC	MOST IMPORTANT PRODUCING MICROORGANISMS
PENICILLIN 	Penicillins	*Penicillium chrysogenum* *Aspergillus nidulans* *Acremonium chrysogenum* *Streptomyces clavuligerus*
CEPHALOSPORINS 	Cephalosporins 7-Methoxycephalosporins	*Acremonium chrysogenum* *Nocardia lactamdurans* *Streptomyces clavuligerus*
CEPHAMYCINS 	Cephamycins	*Nocardia lactamdurans*
CARBAPENEMS 	Thienamycins Olivanic acids Epitheinamycins	*S. cattleya* *S. olivaceus* *S. flavogriseus*
MONOLACTAMS 	Monobactams	*Gluconobacter sp.* *Chromobacter violaceum* *Agrobacterium radiobacter* *Pseudomonas acidophila*
	Nocardins	*Nocardia uniformis subsp. tsuyamenensis*

Figure 1. Classes of ß-lactam antibiotics.

β-lactam antibiotics can be divided into five distinct classes (Figure 1). Penicillin was discovered by Fleming in 1929. A research group at Oxford under the direction of Florey and Chain isolated it from surface cultures of *Penicillium notatum* in 1940 and the first clinical application of penicillin occurred in 1941.

Penicillins and cephalosporins, as β-lactam antibiotics, belong to the most effective of all traditional therapeutic agents for the control of infectious diseases. In addition to the development of numerous semisynthetic β-lactams, antibiotics with completely new β-lactam ring systems have been isolated in the past few years using new specific and sensitive screening methods.

Penicillins and cephalosporins can be considered as model systems within the antibiotic world for several reasons: They were the first antibiotics produced on a large scale in submerged fermentations, and in addition, penicillin was the first antibiotic used worldwide. They were the antibiotics which instigated the development of fermentation technology, and many of the techniques used to produce penicillins have been used as the basis for the production of other microbial metabolites, especially antibiotics.

3. Penicillin, Cephalosporin and Cephamycin Biosynthesis: An Overview

The biosynthesis of penicillins, cephalosporins, and cephamycins has been reviewed by several authors. A concise overview of the β-lactam biosynthetic pathways is presented here.

The *penam* nucleus of penicillins and the *cephem* nucleus of both cephamycins and cephalosporins are formed by the condensation of three precursor amino acids: L-α-aminoadipic acid, L-cysteine, and L-valine, by a mechanism designated as “non-ribosomal peptide synthesis” that involves activation and condensation of the three component amino acids and epimerization of the L- to D-valine to form the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (LLD-ACV).

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Biographical Sketches

Santiago Gutiérrez graduated in Biology from the University of León (Spain) in 1988—where he also received his Ph.D. He held a post-doctoral position as Visiting Associate Scientist in Biology at the California Institute of Technology (CALTECH) from 1995 to 1996. He returned to Spain in 1996, holding positions of Assistant and Associate Professor in the University of León. In 1999 he got a tenured position as Associate Professor of Microbiology at the University of León.

Javier Casqueiro graduated in Pharmacy from the University of Santiago de Compostela in 1991. He received his doctorate in Biology Science from the University of León (1997). He held several positions as Research Associated and Full Scientist at the Institute of Biotechnology (INBIOTEC) in León from 1998 to 2000. In March 2000, he got a position as Assistant Professor of Microbiology at the University of León.

Juan F. Martín graduated in chemistry from the University of Salamanca (Spain) from which he also received his Ph.D. He held a post-doctoral position at the Institute of Microbiology, Rutgers from 1971-1974, and then was an Invited Scientist at MIT. He returned to Spain in 1975, holding positions at the High Research Council and the University of Salamanca. In 1980, he was appointed Professor of Microbiology at the University of León, where he is also Director of the Institute of Biotechnology.