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REVIEW ARTICLE

Penicillin and cephalosporin production: A historical perspective

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ABSTRACT. Antibiosis history began with the observations made by Sanderson and Roberts on the inhibition of bacterial growth by other organisms, at the end of the XIX Century. Biomedical research in this field advanced importantly during World War II, after the discovery of penicillin by Fleming. Brotzu´s finding of cephalosporin and the massive production of these two compounds started a new era, not only for important breakthroughs in treating diseases, but also for the exploitation of living organisms to produce substances of great benefit for mankind (known today as biotechnology). In this review, we summarize the historic evolution of the knowledge about penicillin and cephalosporin, from the first observations on producer microorganisms and the chemistry of these antibiotics up to the modern ways of genetic engineering focused on developing superproducer strains at an industrial level.

Key words: β-lactam antibiotics, cephalosporin, historical development, microorganisms, penicillin

RESUMEN. La historia de la antibiosis dio comienzo con las observaciones realizadas por Sanderson y Roberts acerca de la inhibición del crecimiento de bacterias por otros organismos a finales del siglo XIX. Pero, sobre todo, la investigación biomédica tuvo un gran avance en este campo durante la Segunda Guerra Mundial, después del descubrimiento de la penicilina por Fleming. El hallazgo de la cefalosporina por Brotzu, y la posterior producción masiva de estos dos tipos de compuestos, hicieron surgir una nueva era, no solamente de avances importantes en el tratamiento de las enfermedades sino también en el aprovechamiento de los seres vivos para la producción de sustancias de alta utilidad para el hombre (lo que hoy en día se conoce como biotecnología). En esta revisión se resume la evolución histórica de los conocimientos sobre penicilina y cefalosporina, desde las primeras observaciones sobre los microorganismos productores y la química de estos antibióticos hasta las modernas formas de ingeniería genética dirigidas a la creación de cepas superproductoras a nivel industrial.

Palabras clave: antibióticos β-lactámicos, cefalosporina, desarrollo histórico, microorganismos, penicilina

HISTORICAL BACKGROUND

The essence of the antibiosis concept was born with John Burdon Sanderson and William Roberts, who independently observed that fungi were capable of inhibiting bacterial growth in certain culture media. In 1876, John Tyndall made a similar observation, concluding that fungi consumed the bacteria's oxygen and inhibited their growth (Tyndall, 1876).

Later on, Ernest Duchesne (a French physician) concluded in his Ph.D. dissertation that there was an antagonism between the fungus *Penicillium glaucum* and bacteria, because when bacteria of the *Bacillus* genus were inoculated in animals together with this fungus, bacterial virulence diminished markedly (Bustinza, 1946).

During the second half of the XIX Century, research did not focus only on the *Penicillium* genus but also on the phenomenon of growth interference exerted by one organism on another. Pasteur and Joubert, as well as Cornill and Babes, performed microbial antagonism tests *in vitro,* suggesting that a chemical substance produced by one of the microorganisms inhibited the growth of the other. In 1887, Garre showed that a substance produced by *Bacillus fluorescens* inhibited *Staphylococcus pyogenes* growth. In 1941, Selman Waksman, a microbiologist, named this phenomenon antibiosis, meaning "life versus life" (Waksman, 1941). Such a broad concept involves several mechanisms, from competing for nutrients to specific activity mediated by a chemical compound (the antibiotic). In this review we will focus only on those compounds inhibiting growth of microorganisms, specifically, on penicillin and cephalosporin.

DISCOVERY OF PENICILLIN

Many discoveries have been fortuitous, and penicillin is a good example. In 1928, Alexander Fleming was studying growth and characteristics of staphylococci at the Saint Mary's Hospital in London. In September of that year, he

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found that, in one of his Petri dishes, a bluish-greenish mould coming from his contaminated material had developed and staphylococci had practically disappeared near the fungal mycelium. Another researcher might have thrown the Petri dish away, but Fleming gave an excellent interpretation of the phenomenon: "the fungus probably produces a bacteriolytic substance, which spreads through the agar and inhibits bacterial growth", which proved to be a capital explanation for the future production of the compound and its therapeutic application (Fleming, 1929). In that same year, Fleming isolated and cultivated the fungus in liquid medium. After subsequent elimination of the fungal mycelium by filtration, an active substance was found in the broth. He called the contaminated fungus *Penicillium rubrum* and this is the reason for naming penicillin the active substance (Fleming, 1946).

These results stimulated Fleming's interest, leading him to study the sensitivity of several bacterial strains to this substance; he found that several human pathogenic species (staphylococcus, streptococcus, pneumococcus, and gonococcus) were sensitive to penicillin (Fleming, 1946).

At the beginning of the 30's, Paine (at the Royal Infirmary of Sheffield) used Fleming's filtrate on patients with eye and skin diseases produced by pneumococcus and gonococcus, getting a positive response in patients with eye infections (Wainwright & Swan, 1986).

Biochemical studies with penicillin and extraction of the active substance

Due to the biomedical importance of this compound, Clutterbuck *et al*., at the Royal School of Hygiene and Tropical Medicine in London started to study the penicillin-producing strain described by Fleming. They showed that penicillin was synthesized by the fungus when grown in a synthetic medium for 16-20 days, and the active substance found in the broth was stable at a pH of 5-6 for three weeks (Clutterbuck *et al.,* 1932). The producing fungus (Fig. 1A Y 1B) was characterized again by the mycologist Charles Thom, and renamed as *Penicillium notatum* (Thom, 1930).

In an environment of scarce resources due to World War II, in 1939, Professor Howard Florey (from the William Dunn School of Pathology, member of the Medical Research Council) studied the characteristics and properties of penicillin, as well as its interference on the growth of sensitive microorganisms. He invited Ernst Chain, a German chemist who had recently arrived in England to join his laboratory. Later on, Norman Heatley, a Cambridge biochemist interested in the study of the recently discovered substance, joined the group too. It was Heatley who accomplished production of the compound, developed a simpler method for its analysis and improved the methods for extraction of the active ingredients from the raw culture (Florey *et al.*, 1949).

In May 1940, the knowledge acquired on penicillin allowed this group of researchers to justify experiments with mice, thus demonstrating that these could survive inoculation with, normally lethal, doses of streptococci (Florey *et al.*, 1949).

Florey and his group, convinced of the medical importance of their findings, decided to continue with the next step: experiments with humans. Along with a young physician, Charles Fletcher, they treated five cases between February and June, 1941, at the Radcliff Infirmary. They transformed many rooms that had been left empty due to lack of research funds at the William Dunn School of Pathology into penicillin factories (Florey, 1945). Patients got better; some recovered completely, and others would have improved if they had had adequate penicillin doses. The amounts produced were still low, so one of the tasks of the group was to re-isolate penicillin from the urine of patients (Ratcliff, 1945).

The following step was to get funds from pharmaceutical companies to increase production. However, many British companies contacted by Florey refused to get in-

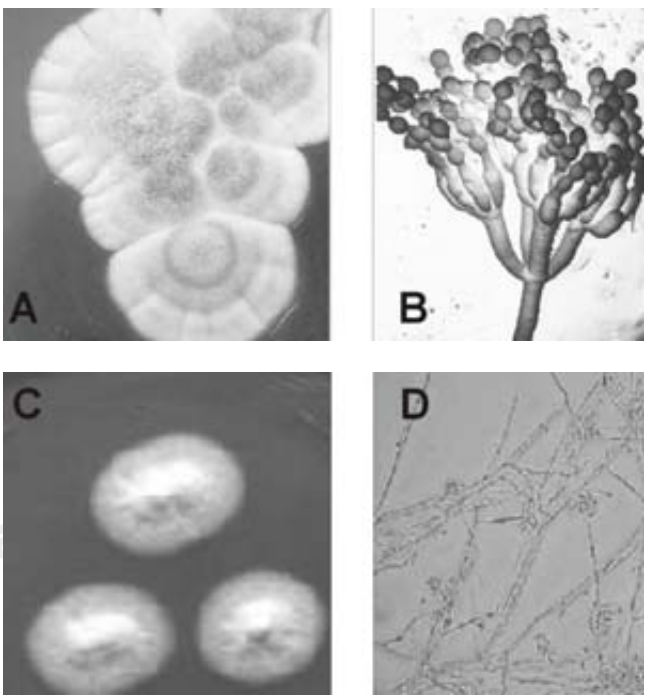

Figure 1. *A and B. Colonial and microscopic appearance, respectively, of* Penicillium chrysogenum*, a penicillin-producer. C and D. Colonial and microscopic appearance, respectively, of* Acremonium chrysogenum*, a cephalosporin-producer.*

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volved in the penicillin project, because they were committed to produce other supplies for a country at war. Under these conditions, Florey and Heatley decided to travel to the United States in 1941, in search of the necessary funds and technology to produce penicillin at a large scale. For this reason, and although the British were the ones to discover this antibiotic and to perform the first fermentations, American companies were the ones to get the patents for the penicillin production process (Oxford researchers were never mentioned in these patents). Upon Florey´s and Heatley's return to England, the interest of the press was awaken and grew when information on clinical treatments were first reported in scientific publications (Abraham, 1971). Florey avoided the press, whereas Fleming seemed to enjoy the publicity: thus, it was his name that became known as the discoverer of penicillin.

CEPHALOSPORIN DISCOVERY

Once penicillin's properties had been discovered; in 1945, Giuseppe Brotzu (professor of Hygiene at the University of Cagliari, Italy) questioned why typhoid fever was less virulent in his city than elsewhere. He formulated several, unfortunately useless hypotheses and devoted this efforts to establishing why, despite the habit many young people had of going swimming at "Su Siccu" Bay, precisely at the site where the city sewer system drained into the sea, there were no outbreaks of thyphoid fever cases related to bathing. Then he decided to take a water sample and test its effect on a *Salmonella typhi* culture. As a result, he isolated a fungus (Fig. 1C y 1D) that produced an effective substance against gram-negative bacteria (all enterobacteria, including *Salmonella,* are gram-negative) (Paracchini, 1992).

Biochemical studies on cephalosporin and extraction of an active substance

Although Brotzu was able to isolate *Cephalosporium acremonium* extracts (nowadays this fungus is known as *Acremonium chysogenum* (Thirum. & Sukapure) W. Gams 1971), he lacked the resources for further studies. He tried, in vane, to convince an Italian pharmaceutical company to invest in his research. Therefore, after consulting a friend and discussing it with the Secretary of the Medical Research Council in London, he sent a culture of this organism, along with a copy of his findings, to the research group at Oxford University (Brotzu, 1948; Abraham, 1991). Despite his great accomplishments (he was proposed for the Nobel Prize for the discovery of the *Acremonium* fungus, the cephalosporin producer), Brotzu is barely mentioned in Italian texts, although he is recognized in pharmacological texts from the rest of the world (Paracchini, 1992).

Brotzu´s studies on *Acremonium*, then being performed at the William Dunn School of Pathology in Oxford, indicated that this fungus could produce at least five different antibiotics. At the beginning, an antibiotic that could be extracted just like penicillin (in an organic solvent) was found, and was called cephalosporin P. However, the compound was not the one originally described by Brotzu, because it only showed activity against certain gram-positive bacteria. In 1949, a second antibiotic was found in the liquid cultures of *Acremonium*. This new substance remained in the aqueous phase after cephalosporin P extraction and was active against gram-negative and gram-positive bacteria (Burton and Abraham, 1951). It was called cephalosporin N and its activity was confirmed to be eliminated after treatment with a penicillinase obtained from *Bacillus subtilis.*

In 1953, during studies on the chemical nature of cephalosporin, Guy Newton and Edward Abraham found a second hydrophilic antibiotic in the culture broths of *Acremonium* that was named cephalosporin C (Newton and Abraham, 1954). The new antibiotic was easily obtained in its sodium salt form and showed antibiotic activity against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. Although its specific activity against these microorganisms corresponded only to 10% of that measured for cephalosporin N (nowadays known as penicillin N), some properties of the new antibiotic were interesting. Its stability in a diluted acid medium and its resistance to penicillinase action from *B. subtilis* suggested that the chemical structure of cephalosporin C differed from that postulated for penicillin. Later, in 1961, nuclear magnetic resonance spectroscopy studies and X-rays crystallographic analyses (Hodgkin and Maslen, 1961) confirmed the proposed cephalosporin structure as a heterocyclic β-lactamic-dihydrotiazinic system (Abraham and Newton, 1961), whereas penicillin possesses heterocyclic β-lactamic and thiazolidinic rings.

In 1955, it was shown that cephalosporin C presented a wide spectrum of activity, including many *Staphylococcus aureus* strains, both sensitive and resistant to penicillin. The antibiotic showed to be innocuous when administered intravenously in large doses, and was shown experimentally to protect mice from infections caused by streptococci. Afterwards, it was shown that it could also protect mice from penicillin-resistant staphylococci infections (Florey *et al.,* 1956).

Research and development for cephalosporin's industrial production differed from that of penicillin: patents had been issued regarding its nucleus, and several pharmaceutical companies became interested in them at an early stage of the research. Glaxo, from the United Kingdom, and Ely Lilly, from the United States, were the first to ne-

gotiate with the NRDC (National Research Development Corporation) and became involved in the production process almost from its beginning.

PENICILLIN AND CEPHALOSPORIN BIOSYNTHESIS

Once some physiological characteristics of both *P. chrysogenum* and *A. chrysogenum* were known, the next studies were focused on the biosynthesis of penicillin and cephalosporin C. It was observed that these and some other microorganisms use the same pathway for the production of diverse β-lactam antibiotics (Fig. 2). For penicillin and cephalosporin, the pathway begins with non-ribosomal condensation of three leading amino acids: L-α-aminoadipic acid, L-cysteine, and L-valine to produce the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (or its abbreviation, ACV) (Arnstein and Morris, 1960). During the second step of the pathway, tripeptide cycling takes place to form isopenicillin N (Fawcett *et al*., 1976), the first intermediary of the pathway with antibiotic activity. At this point, the pathway diverges for the two microorganisms: in the case of *Penicillium* (and the other penicillin-producing fungi), the

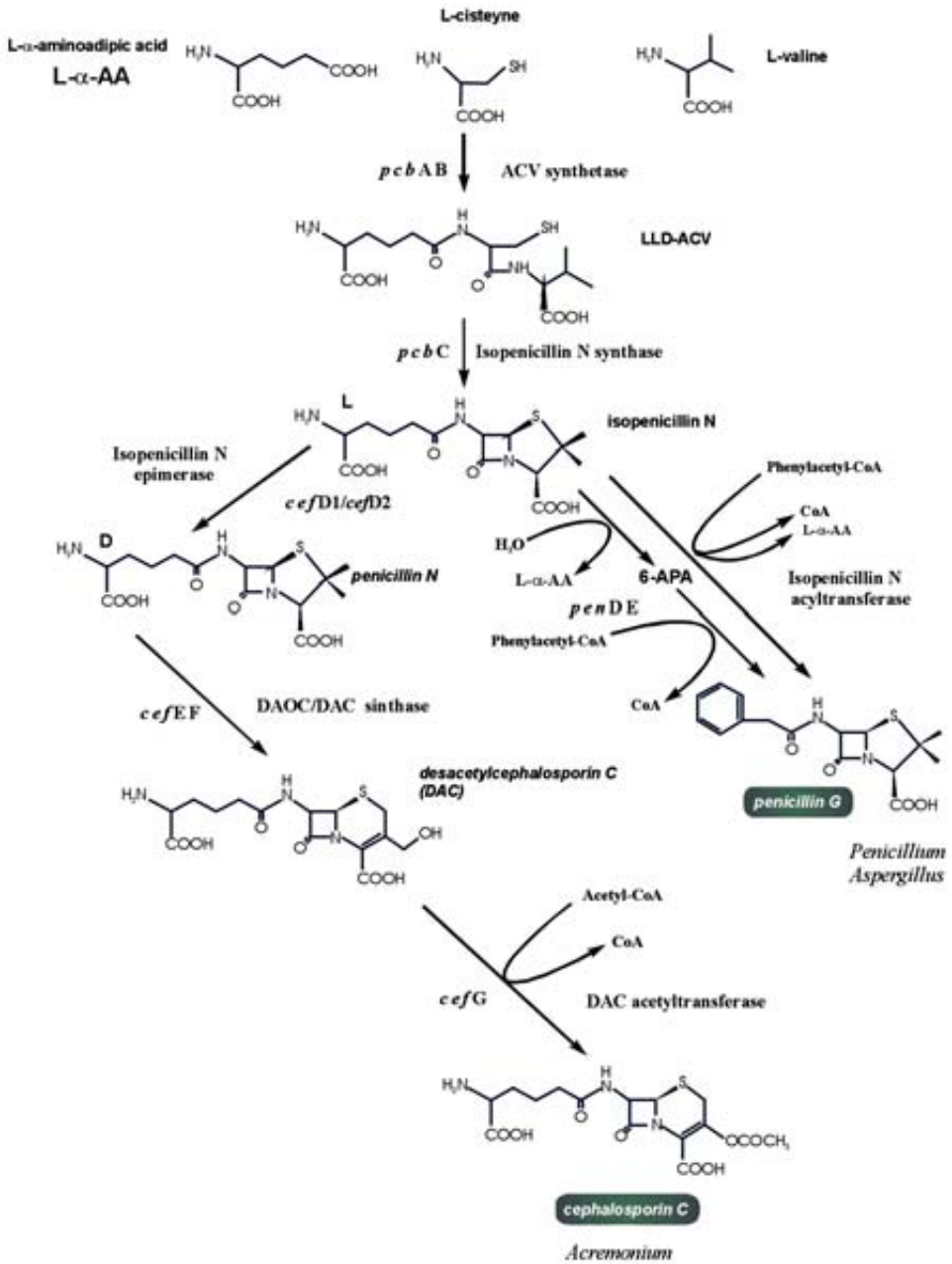

Figure 2. *Biosynthetic pathways for penicillin and cephalosporin.*

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lateral chain of the α-aminoadipyl is exchanged by a hydrophobic lateral chain (Jayatilake *et al*., 1981), whereas in *Acremonium,* isopenicillin N is converted into penicillin N by means of a two-enzymes system: an acyl CoA synthetase and an acyl CoA racemase (Ullán *et al*., 2002a); although the final transformation into penicillin N also requires hydrolysis of the CoA thioester, by means of different thioesterases (Knihinicki *et al*., 1991). In the case of cephalosporin's biosynthetic pathway, penicillin N is transformed subsequently into deacetoxycephalosporin C, expanding the thiazolidinic ring of five atoms into a dihydrothiazonic ring of six (typical of cephalosporins and cephamicins). The enzyme responsible for this step, called DAOC synthase/DAC hydroxylase, is also in charge of hydroxylating deacetoxicephalosporin C leading to deacetylcephalosporin C formation (Samson *et al*., 1987). The last step in cephalosporin biosynthesis is the acetylation of deacetylcephalosporin C into cephalosporin C (Fujisawa *et al*., 1973 and 1975).

GENETICS OF PENICILLIN AND CEPHALOSPORINS BIOSYNTHESIS

In *P. chrysogenum*, the genes encoding the three enzymes involved in the biosynthesis of penicillin are linked in a group (Fig. 3A) or cluster (Díez *et al.,* 1990; Fierro *et al*., 1993). The *pcbAB* gene codes for the ACV synthetase, in charge of ACV tripeptide formation, and is unusually large (11,336 bp). The *pcb*C gene encodes isopenicillin N synthetase; it is transcribed into an mRNA of 1,100 pb and encodes a polypeptide with a molecular mass of 37,900 Da. It is normally located upstream the *pcb*AB gene; the promoter regions of both genes are located in a region of 1,503 bp in length (Carr *et al.,* 1986). The *pen*DE gene codes for isopenicillin N acyltransferase, and is located downstream the *pcb*C gene. It is expressed in the same direction as the latter, and possesses a region encoding a protein of 357 amino acids, with a relative molecular mass of 39,943 Da (Barredo *et al.,* 1989).

Most of the genes implicated in cephalosporin biosynthesis in *A. chrysogenum* have been identified and characterized biochemically (reviewed by Schmitt *et al.,* 2004). As observed in *Penicillium,* formation of the ACV tripeptide in *Acremonium* is also achieved through the ACV synthetase enzyme, encoded by the *pcb*AB gene (Gutiérrez *et al.,* 1991). The tripeptide is cycled by the action of the isopenicillin N synthetase, which is encoded by the *pcb*C gene (Samson *et al.,* 1985), and from this point on, the pathways diverge, as mentioned previously. In *Acremonium,* isopenicillin N is converted into penicillin N through proteins encoded by the genes *cef*D1 and *cef*D2 genes (Ullán *et al.,* 2002a). The next step in the pathway is accomplished by the product of the gene *cef*EG (Samson *et al.,* 1987), and the final formation of cephalosporin C is catalyzed by the protein encoded by gene *cef*G, the acetyl CoA DAC acetyltransferase (Gutiérrez *et al.,* 1992). In *Acremonium*, a protein involved in transporting CPC has also been identified, which is encoded by the *cef*T gene (Ullán *et al.,* 2002b). All these genes, analogously to the genes responsible for penicillin biosynthesis, are arranged in two groups or clusters (Fig. 3B), located in two different chromosomes of *A. chrysogenum* (Skatrud & Queener, 1989).

IMPROVEMENT OF β-LACTAM ANTIBIOTICS INDUSTRIAL PRODUCTION

Application of classical and recombinant DNA techniques has been very important in the increased production of penicillin and cephalosporin as well as the production of suitable intermediates to obtain more potent and low-cost semi-synthetic antibiotics.

Classical improvement of penicillin and cephalosporin production

The low production of the original *P. chrysogenum* and *A. chrysogenum* strains forced the potential genetic exploitation of both microorganisms, aimed at generating commercial superproducer strains of β-lactam antibiotics. From a classical improvement approach, where mutant agents are used at random and high producing mutants are subsequently selected,

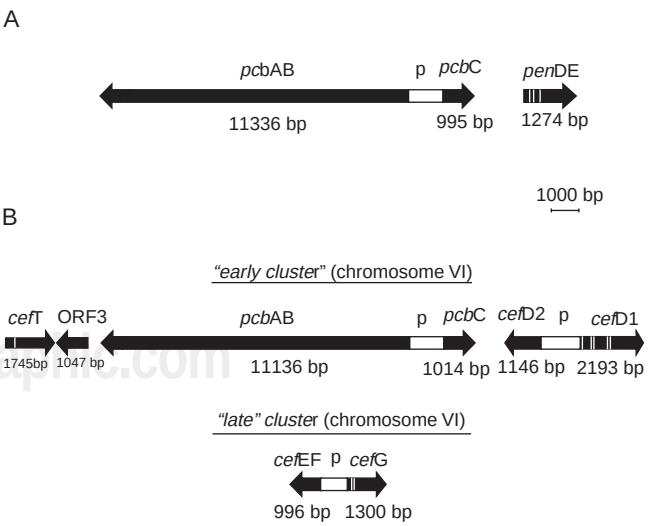

Figure 3. *A. Gene organization of penicillin biosynthesis genes in* Penicillium chrysogenum*, sizes estimated from Fierro* et al. *(2006). B. Gene organization of cephalosporin biosynthesis genes, modified from Schmitt* et al. *(2004).*

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two types of mutant agents have been traditionally employed: physical (ultraviolet radiation, gamma rays or X rays), and chemical (ethyl methanosulphonate, N-methyl-N'-nitro-N-nitrosoguanidine, nitrogenated mustards, or nitrous acid). For application examples, see figs. 4 and 5.

Experimental models for production, selection, and employment of *P. chrysogenum* mutant strains, with high penicillin yields, have been developed since the middle of the last century. The starting point was the isolation of the Wisconsin Q-176 strain, although the subsequent mutation experiments were not achieved directly with this strain, but rather with its descendants, obtained after analyzing thousands of spontaneous mutant strains (considering their capacity to produce the antibiotic). Reductions in sporulation and even in fungal growth are also desirable characteristics (Elander and Espenshade, 1976); it should not be forgotten that β-lactam antibiotics are secondary metabolites and, therefore, they are not linked to microbial growth. An example of this is the NRRL-1951 strain (Fig. 4), in which a decrease of up to 60% in growth and sporulation was obtained, along with a six-fold increase in antibiotic production.

Regarding *Acremonium chrysogenum,* a program to improve the low cephalosporin C titers produced by the

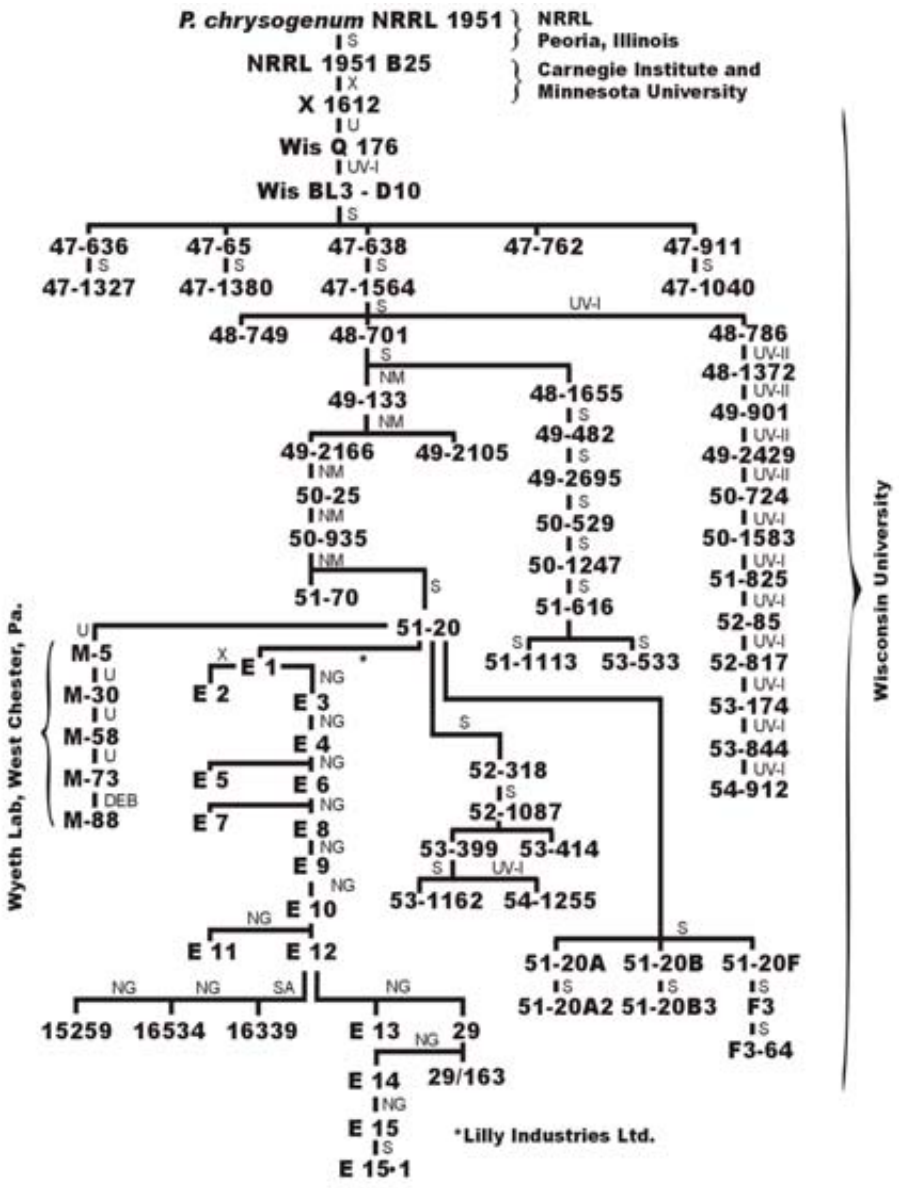

Figure 4. *Penicillin production improvement: a classical approach (from Elander, 1967).*

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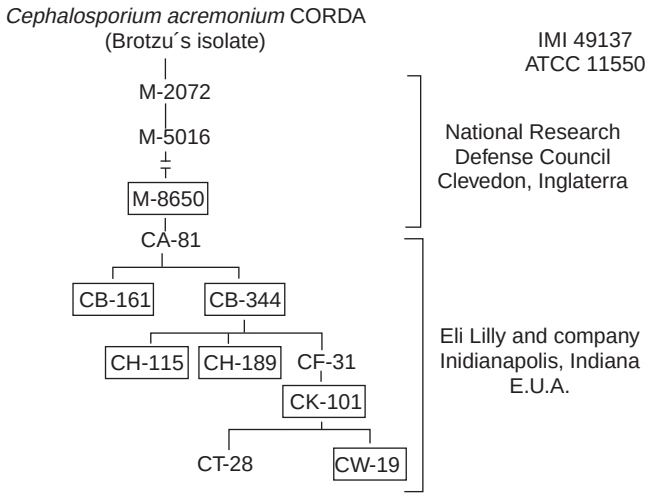

Figure 5. *First* A. chrysogenum *improved mutants production chart (from Elander* et al.*, 1976).*

Brotzu strain began in the middle of the last century. Mutagenesis of this strain was useful to isolate a mutant strain, called M8650, which became the progenitor strain for many programs of industrial improvement (Elander and Aoki, 1982). Fig. 5 shows the first *A. chrysogenum* improved mutants production chart, obtained after treatments with UV rays as mutant agent. As an example, an improved mutant strain developed by the American company Eli Lilly & Co., called CW19, produced three times more antibiotics than the Brotzu strain and, under favorable fermentation conditions; it could produce up to 15 times more antibiotic than the progenitor strain (Elander and Espenshade, 1976).

For both *Penicillium* and *Acremonium,* as important as mutagenesis *per se*, is the rational selection of mutants to find superproducer strains (mainly in large-scale strain improvement programs). To accomplish this, different strategies, chemical compounds, and even protein activities have been employed as indicators of the presence of improved mutants (Queener *et al.,* 1975; Trilli *et al.,* 1978; Chang and Elander, 1979). Perhaps, one of the most used strategies, due to its low cost, is the isolation of mutants grown in smaller and more compact colonies on well-defined chemical media. The good correlation between the presence of improved mutants and a progressive reduction in the diameter and vegetative development of colonies was confirmed later (Vialta *et al.,* 1997).

Although highly productive strains have been obtained through the above mentioned methods, further improvement is needed, specifically in the titer achieved at the end of fermentation and the stability of strains. Industrial strains are very unstable, because mutagenic events are random and do not necessarily affect only the genes involved in antibiotic synthesis. In practice, industrial strains mutate constantly, and re-isolation of the best performing strains is conducted routinely, since prolonged storage of high producing strains can occasionally result in the loss of their productivity or, at least, part of it (Barber *et al.,* 2004). Molecular biology techniques helped to know in detail the nature of changes occurring during the random mutagenic processes (either when increased production is achieved or when lower yields are obtained) and to specifically locate such changes in the microorganism's genome. In this way, it has been proven that high penicillin-producer strains posses the biosynthetic "cluster" amplified in tandem repeats (several repeated copies, one after the other) (Fierro *et al.,* 1995). Instability of industrial strains can be explained by a higher probability of recombinogenic events, implying these iterative sequences.

Molecular improvement of penicillin and cephalosporin production

Molecular biology represents an advanced tool in the genetic improvement of industrial producer strains. These techniques allow for controlled modifications of the microorganisms' genome and establishment of cause-effect relationships between any introduced alteration and the observed result (which is harder to analyze in mutations induced by the classic methods). Thanks to this discipline, the changes introduced at all levels of the biosynthetic pathway (incorporation of precursor compounds, assembly of intermediary products of the pathway, and secretion into the medium) can be followed. Likewise, the effects of introducing factors affecting the process globally can be observed and compounds that formerly could not be produced by the microorganism can be obtained.

The use of genetic engineering techniques has enabled new approaches to the development of higher antibiotic-producing strains. Once the genes responsible for the biosynthesis of antibiotics had been cloned (or, at least, some of them), the first assays to improve the productive strains were based on the idea that if production depended upon the expression of given genes, any change capable of positively affecting that expression would be translated into increased production. The simplest way to increase production at these initial stages, applying recombinant DNA techniques, consisted in increasing the genetic dose (the increase of the number of gene copies encoding a determined protein

within the microorganism, a copy "without secondary effects" of the amplifying process observed in classical improvement processes). The development of better fungal transformation techniques contributed to molecular improvement; these transformation methods were based on modifications developed for the bread yeast, *Saccharomyces cerevisiae* (Hinnen *et al.,* 1978). These methods consist of some basic steps: protoplasts production from mycelium, and their transformation and regeneration. Through these techniques, it was possible to observe the effect induced by integration of several structural genes in the fungal genome on antibiotic production.

The first successful application, in the field of β-lactam antibiotics, with increased doses of relevant genes was achieved with the *cef*EF gene in *Acremonium chrysogenum* (Skatrud *et al.,* 1989). Over-expression of the gene that encodes the expandase/hydroxylase enzyme produced an increase in this activity, a decrease in the amount of accumulated penicillin N, and an increase in cephalosporin C production in the modified strain. Even better results were obtained when over-expressing the *cefG* gene, which encodes the deacetylcephalosporin C acetyltransferase, the last enzyme in the biosynthesis pathway of cephalosporin C (Gutierrez *et al.,* 1997).

Regarding the genes of penicillin biosynthesis, overexpression of the *pcb*AB gene in *Aspergillus nidulans* has led to 30 times higher yields than normally obtained (Kennedy and Turner, 1996).

Introduction of additional copies of the *pcbC* gene in the *P. chrysogenum* strain Wisconsin 54-1255 did not produce any remarkable increase in penicillin G production, although it slightly accelerated its biosynthesis between 30 and 80 hours of fermentation (Barredo, 1990). This author explained these results by stating that probably the activity of isopenicillin N synthase did not constitute a "bottle neck" in penicillin production and the increase observed for this enzymatic activity would not be enough to favor biosynthesis of this compound. In any case, utilization of an integrative plasmid in this transformation suggested caution in interpreting the results. Introduction of the last gene of the penicillin pathway, i.e., *pen*DE gene of *P. chrysogenum* (Fernández, 1997) and of *A. nidulans* (Fernández-Cañón and Peñalva, 1995; Montenegro, 1996) did not lead to satisfactory results. Good results were obtained when a DNA fragment, containing *pcbC* and *pen*DE genes of *P. chrysogenum* (Veenstra *et al.,* 1991) was used in the transformation. Penicillin G production mean values obtained with 26 transformants of *P. chrysogenum* strain Wisconsin 54-1255, in which the DNA fragment had been integrated, became significantly higher than the yields of those transformants where only the plasmid without insert had been integrated. The two transformants with the highest production reached a 40% increase as compared with the control. The homogeneous amplification of both genes (and therefore their joint expression) would seem necessary to get an improvement in penicillin production (at least in the strain studied). This hypothesis was confirmed after introducing the complete cluster for penicillin biosynthesis (*pcb*AB, *pcb*C, and *pen*DE genes), although this was performed in a low-producing laboratory strain (Theilgaard *et al.,* 2001).

Molecular strategies, different from those involving an increase in biosynthesis gene doses, have also been developed. One of them, of great interest, involves semi-synthetic cephalosporins (the most useful ones from the medical point of view). These antibiotics are normally produced from 7-aminocephalosporic acid (7-ACA) or from 7-aminodeacetoxycephalosporanic acid (7-ADCA) (Demain and Elander, 1999), both coming from CPC. Traditionally, the production processes for these compounds involved complex, expensive, and environmentally unfriendly chemical processes. However, through the application of genetic engineering techniques, it has been possible to produce 7-ACA and 7-ADCA directly through fermentation processes, using new metabolic pathways created in both *Penicillium* and *Acremonium* (Velasco *et al.,* 2000).

CONCLUSION

After World War II, penicillin was the antibiotic widely used for the treatment of infections such as syphilis, pneumonia, streptococcal infections of the pharynx, scarlet fever, diphtheria, bacterial meningitis, and septicemia. In 1945, Fleming, Florey, and Chain were awarded the Nobel Prize of Medicine for this discovery. Years later, the anti-staphylococcus penicillins appeared, as well as the wide spectrum ones, to be used also orally (known as ampicillins and amoxicillins). After penicillin finding, a great step in antibiotics therapy was the discovery of cephalosporin C, another β-lactam compound produced by *Acremonium chrysogenum.*

The discovery of the active nucleus of cephalosporin C and the possibility of modifying its lateral chain allowed for the development of new semi-synthetic compounds with a greater antibacterial activity, especially efficient against β-lactam resistant microorganisms (replacing in many cases penicillin-based antibiotics). Development programs for new semi-synthetic cephalosporins continue at different pharmaceutical companies, so

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new generations of these antibiotics will be available in the future. However, and due to the high prices of these products, the new advances must be paralleled by progresses in the molecular biology knowledge regarding CPC biosynthesis.

Molecular biology and its practical counterpart (genetic engineering) have been incorporated into the strain improvement programs of most pharmaceutical companies and, together with a careful control of the fermentation processes, they are expected to become more efficient. It remains to be seen whether it is possible to obtain cephalosporin production levels similar to those of penicillin, although it has to be noted that the *Penicillium* improvement programs began before those of *Acremonium.*

At our Laboratory of the Universidad Autónoma Metropolitana (Genetics Engineering and Secondary Metabolism Group of the Biotechnology Department) we are working under this guideline, searching to increase production of penicillin G as well as cephalosporin C, by means of a strategy of a coordinated increase in the doses of those genes implicated in the biosynthesis of these antibiotics.

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