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Research review paper

Genetic engineering of antibiotic biosynthesis for the generation of new aminocoumarins

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ABSTRACT

The aminocoumarin antibiotics novobiocin, clorobiocin and coumermycin A_1 are inhibitors of gyrase and highly effective antibacterial agents. Their biosynthetic gene clusters have been cloned from the respective *Streptomyces* producer strains, and the function of nearly all genes contained therein has been elucidated by genetic and biochemical methods. Efficient methods have been developed for the genetic manipulation and the heterologous expression of the clusters, and more than 100 new derivatives of these antibiotics have been generated by metabolic engineering, mutasynthesis and chemoenzymatic synthesis, providing a model for the power of genetic and genomic methods for the generation of new bioactive compounds.

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1. Introduction

The aminocoumarin antibiotics comprise three "classical" compounds, which are similar in structure: novobiocin, clorobiocin and coumermycin A_1. They are produced by different *Streptomyces* strains (Fig. 1). The aminocoumarins are inhibitors of gyrase, i.e. the bacterial enzyme which introduces negative supercoils into DNA in an ATP-dependent reaction and thereby is essential for DNA replication and transcription in bacteria (Maxwell and Lawson, 2003). Gyrase is a heterotetrameric enzyme, consisting of two GyrA and two GyrB subunits. While fluoroquinolones like ciprofloxacin (Ciprobay®) attack at the GyrA subunit, the aminocoumarins attack at the GyrB subunit and therefore are active even against fluoroquinolone-resistant pathogen strains.

The interaction of novobiocin and clorobiocin with gyrase has been investigated by X-ray crystallography (Tsai et al., 1997; Kampranis et al., 1999). The binding site of the aminocoumarins overlaps with the binding site of ATP on the GyrB subunit. The aminocoumarin moiety, and the deoxysugar moiety with its substituents, form hydrogen bonds to the protein and are therefore essential for the inhibitory activity. The prenylated benzoic acid moiety interacts hydrophobically with the protein.

The therapeutic potential of the aminocoumarins lies in their very high affinity to their target, with dissociation constants in the nanomolar range (Maxwell and Lawson, 2003). Novobiocin has been licensed as a drug for anti-infective therapy in humans in the USA (Albamycin®), and is active against methicillin-resistant *Staphylococcus aureus* strains (MRSA) (Arathoon et al., 1990).

In the past years, considerable progress has been made in the generation of new aminocoumarin antibiotics by genetic methods. For this purpose, it was first necessary to clone the biosynthetic gene

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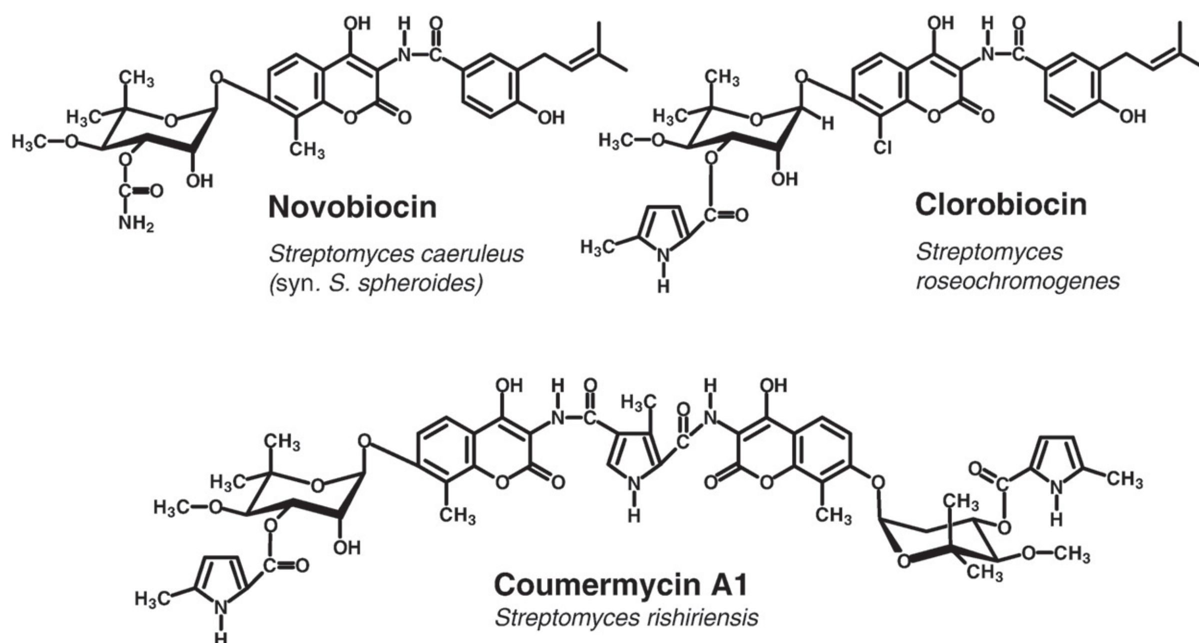

Fig. 1. The aminocoumarin antibiotics novobiocin, clorobiocin and coumermycin A_1 and their wild-type producer strains.

clusters of the respective antibiotics. Subsequently, the function of the genes contained in these gene clusters had to be elucidated, and methods needed to be developed for the genetic manipulation of the producer strains. Based on these foundations, new antibiotics could then be generated by metabolic engineering, mutasynthesis and chemoenzymatic synthesis. In this article, examples of the research work at these different steps will be presented.

2. Cloning and functional analysis of the biosynthetic gene clusters of the aminocoumarin antibiotics

The complete DNA sequences of the biosynthetic gene clusters of novobiocin, clorobiocin and coumermycin A_1 were published between the years 2000 and 2002 (Steffensky et al., 2000b; Wang et al., 2000; Pojer et al., 2002). The clusters span a region of 23, 36 and 38 kb and comprise

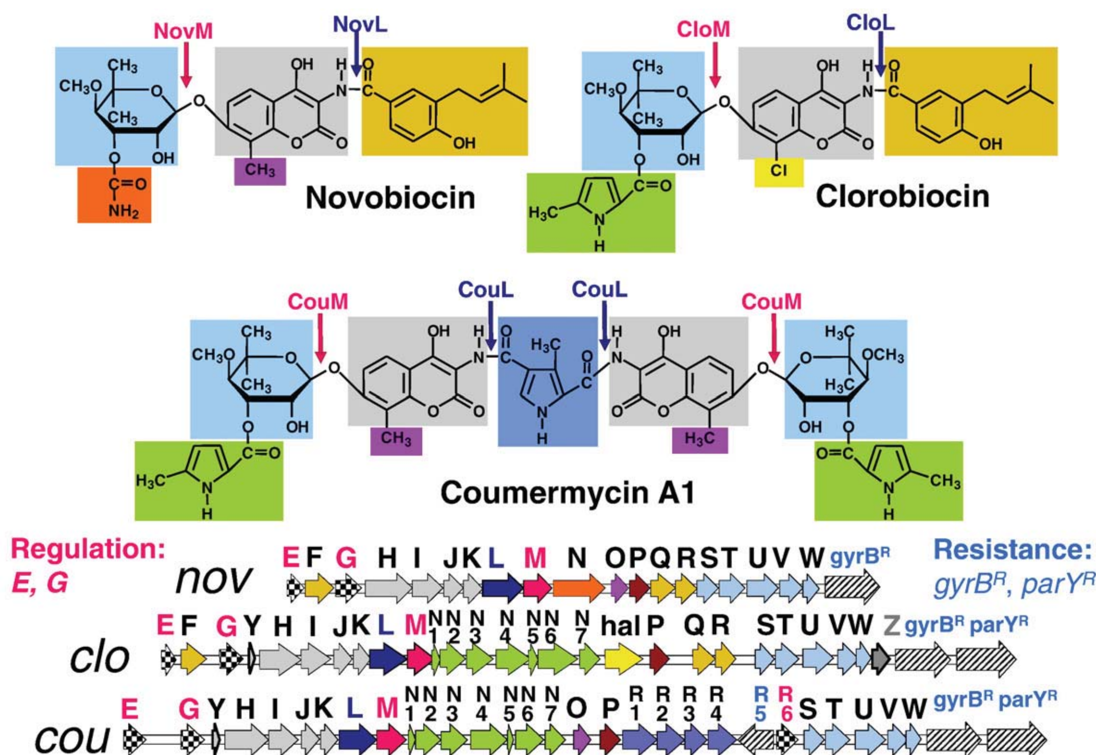

Fig. 2. The biosynthetic gene clusters of the aminocoumarin antibiotics. The involvement of individual genes in the biosynthesis of different structural moieties of the antibiotics is shown by colour coding, and their involvement in the linkage of these moieties by amide and glycosidic bonds by arrows pointing to the respective bonds.

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20, 29 and 31 coding sequences, respectively (Fig. 2). The function of nearly all genes contained in these clusters has been elucidated by gene inactivations and by biochemical experiments (Li and Heide, 2004). The biosynthetic pathway starts with the generation of the two aromatic moieties. The aminocoumarin moiety is formed from L-tyrosine, which is activated in novobiocin biosynthesis by NovH in an ATP-dependent reaction and attached via a thioester bond to the thiol group of the 4′-phosphopantetheinyl cofactor of the NovH holoenzyme (Chen and Walsh, 2001). The cytochrome P_{450} enzyme NovI then hydroxylates tyrosine at the *ß* position, and the oxidoreductases NovJ and NovK oxidize the product to enzyme-bound *ß*-keto-tyrosine (Pacholec et al., 2005a). Orthologs of *novHIJK*, named *cloHIJK* and *couHIJK*, are found in the clusters of clorobiocin and coumermycin A_1 and are expected to carry out the same reactions in clorobiocin and coumermycin biosynthesis. The mechanism of the cyclization of *ß*-keto-tyrosyl-*S*-NovH to the aminocoumarin has not yet been elucidated.

The prenylated 4-hydroxybenzoyl moiety of novobiocin and clorobiocin is generated from 4-hydroxyphenylpyruvate. This compound is prenylated under catalysis of CloQ in position 3 of the aromatic ring, using dimethylallyl diphosphate as prenyl donor. CloQ is an unusual prenyltransferase, not showing the (N/D)DxxD motif for substrate binding which is typical for many other prenyltransferases, and being independent from magnesium or other divalent metal ions in its catalytic activity (Pojer et al., 2003b). CloQ turned out to be the first member of a new family of prenyltransferase with a unique three-dimensional structure, named ABBA prenyltransferases due to their *α*–*ß*–*ß*–*α* architecture. Meanwhile, several other members of this enzyme family have been identified (Tello et al., 2008). 3-Dimethylallyl-4-hydroxyphenylpyruvate is converted to 3-dimethylallyl-4-hydroxybenzoate in two successive oxidative decarboxylation reactions, both catalyzed by the bifunctional non-heme Fe(II)-dependent dioxygenase CloR (Pojer et al., 2003a). The clorobiocin cluster further contains a gene, *cloF*, with sequence similarity to prephenate dehydrogenases, likely to supply 4-hydroxyphenylpyruvate as substrate for the subsequent CloQ reaction. Orthologs of *cloF* and *cloQR* are found in the novobiocin cluster (*novF* and *novQ* and *novR*).

Coumermycin A_1 does not contain a prenylated benzoyl moiety, but rather a 3-methyl-pyrrole-2,4-dicarboxlic acid unit. Consistently, the biosynthetic gene cluster of coumermycin does not contain orthologs of *cloF* and *cloQR*, but a group of genes (designated *couR1* to *couR4*) which have no orthologs in the novobiocin and clorobiocin cluster and which are likely to direct the biosynthesis of the central pyrrole moiety of coumermycin A_1.

The amino group of the aminocoumarin moiety of novobiocin and clorobiocin is linked to the prenylated benzoyl moiety in an amide synthetase reaction catalyzed by NovL and CloL (Steffensky et al., 2000a; Galm et al., 2004a). In coumermycin biosynthesis, two aminocoumarin moieties are linked to the central 3-methyl-pyrrole-2,4-dicarboxlic acid moiety, and the formation of both these amide bonds is catalyzed by the same enzyme, CouL (Schmutz et al., 2003b).

The aminocoumarin moiety shows two different substitution patterns: in novobiocin and coumermycin A_1, a methyl group is attached to position 8, but in clorobiocin a chlorine atom is attached here. Both genetic and biochemical studies have proven that NovO and CouO carry out the methylation reaction (Eustáquio et al., 2004; Freel Meyers et al., 2004b), while the halogenation reaction is directed by *clo-hal*, coding for a FAD-dependent halogenase (Eustáquio et al., 2003). The methylation reactions catalyzed by NovO and CouO have been proven to occur after the linkage of the aminocoumarin to the respective acyl moiety (Pacholec et al., 2005b). The halogenation reaction has not yet been demonstrated *in vitro*, but feeding studies *in vivo* suggest that also in this case the amide bond formation precedes the substitution of the aminocoumarin moiety in position 8 (Anderle et al., 2007b).

All three aminocoumarin antibiotics contain the same deoxysugar, a 5-methyl L-rhamnose, gycosidically linked to the 7-hydroxy group of the aminocoumarin moiety. A group of five genes, *novSTUVW*, with closely related orthologs in the clorobiocin and the coumermycin cluster, directs the biosynthesis of this deoxysugar, and biochemical and genetic evidence has been provided for the function of several of these genes (Thuy et al., 2005; Freitag et al., 2006a,b; Tello et al., 2006). The dTDP-activated deoxysugar is attached to the aglycon by the action of the glycosyl transferase NovM (or CloM or CouM, respectively) (Albermann et al., 2003; Freel Meyers et al., 2003). Subsequently, a methyl group is attached to the 4-OH group of the deoxy sugar; biochemical and genetic experiments have proven that this reaction is catalyzed by NovP and its orthologs CloP and CouP (Freel Meyers et al., 2004b; Freitag et al., 2005a). The last reaction in the pathway is the acylation of the 3-OH group of the deoxysugar moiety. In novobiocin biosynthesis, a carbamoyl group is attached to this position under catalysis of NovN (Freel Meyers et al., 2004b; Xu et al., 2004). In clorobiocin and coumermycin, however, the acyl group is a 5-methyl-pyrrole-2-carboxyl moiety which is formed in clorobiocin biosynthesis from proline under catalysis of *cloN3*, *cloN4* and *cloN5* (Xu et al., 2002; Garneau et al., 2005), and subsequently transferred to the deoxysugar moiety in an unusually complicated two-step mechanism, involving two acyl carrier proteins (CloN5 and CloN1) and two acyl transferases (CloN2 and CloN7) (Xu et al., 2003; Freitag et al., 2005b; Garneau-Tsodikova et al., 2006; Balibar et al., 2007). In the course of this transfer process, the 5-methyl group is attached to the pyrrole moiety under catalysis of the methyl transferase CloN6 (Westrich et al., 2003; Anderle et al., 2007a).

Therefore, nearly all steps of the biosynthetic pathway to the aminocoumarin antibiotics have been elucidated, and the function of the responsible enzymes is known. In a microorganism, this process needs to be regulated, and two regulatory genes have been identified in all three clusters, i.e. *novE* and *novG* and their orthologs, respectively (Fig. 2). NovG shows sequence similarity to StrR of streptomycin biosynthesis and has been shown to be a DNA binding protein, binding to the promoter region of *novH* and acting as a positive regulator of novobiocin biosynthesis (Eustáquio et al., 2005b). *novE* is also a positive regulator, but the mechanism of its action is not yet understood (Dangel et al., 2008).

Obviously, the producer strains need to protect themselves against the toxic effects of the aminocoumarin antibiotics. The novobiocin cluster contains the resistance gene $gyrB^R$ which codes for an aminocoumarin-resistant GyrB subunit, replacing the aminocoumarin-sensitive $GyrB^S$ subunit, which is encoded elsewhere in the genome, when the biosynthesis of aminocoumarins is initiated (Thiara and Cundliffe, 1993). The biosynthetic gene clusters of clorobiocin and coumermycin contain an additional resistance gene, $parY^R$. This encodes an aminocoumarin-resistant subunit of topoisomerase IV, an enzyme related to gyrase which represents the second target of the aminocoumarin antibiotics (Schmutz et al., 2003a, 2004).

The coumermycin cluster further contains a putative transporter gene, *couR5*, linked to a putative regulator gene, *couR6*. The clusters of clorobiocin and coumermycin, but not that of novobiocin, contain a small open reading frame, *cloY* and *couY*, which shows similarity to the *mbtH* gene of mycobactin biosynthesis (Wolpert et al., 2007). Orthologs of *mbtH* are found in many biosynthetic gene clusters, especially of non-ribosomal peptides, but their function is yet unknown. *cloY* has been shown to be required for the biosynthesis of the aminocoumarin moiety of clorobiocin, but its precise function is yet to be elucidated (Wolpert et al., 2007).

The gene *cloZ* of the clorobiocin cluster, which has no orthologs in the two other clusters, could be inactivated without any resulting phenotypical change and may not have a function in clorobiocin formation (Eustáquio et al., 2003).

Therefore, nearly all genes contained in the biosynthetic gene clusters (Fig. 2) have been assigned to their functions in catalysis, regulation and resistance, using genetic and biochemical investigations. The similarities and differences in the structures of the aminocoumarin antibiotics are closely reflected by similarities and differences in the gene clusters. The genes required for the biosynthesis of each structural

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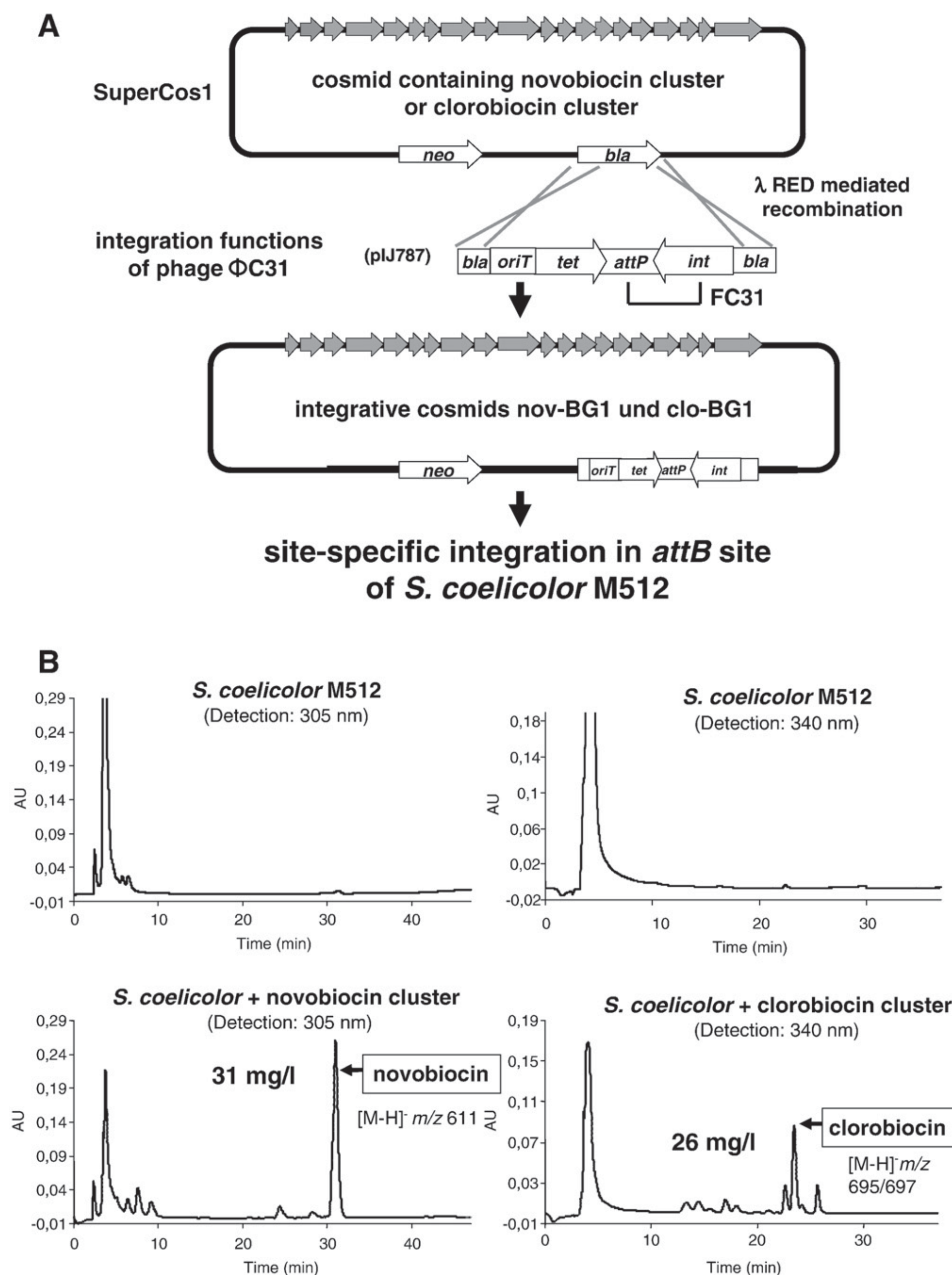

Fig. 3. A. Heterologous expression of biosynthetic gene clusters, cloned in a SuperCos 1-derived cosmid vector, using the integration functions of phage ΦC31. B. HPLC analysis of the heterologous production of novobiocin and clorobiocin after integration of their gene clusters into the genome of the heterologous host *Streptomyces coelicolor* M512.

moiety of the antibiotics are mostly grouped together within the clusters. Obviously, this forms an excellent basis for the generation of new aminocoumarin antibiotics by genetic engineering.

3. Method development for genetic manipulation

Two technical advances have greatly facilitated the generation of new aminocoumarin antibiotics by genetic engineering: the manipulation of large DNA fragments in *Escherichia coli* by λ RED-mediated recombination, and the heterologous expression of biosynthetic gene clusters after their integration into the genome of a heterologous host using the integration functions of the ΦC31 phage. λ RED-mediated recombination ("recombineering") allows to carry out the replacements of genes, or of any DNA region irrespective of its size, at any desired position without the need for suitable restriction sites (Gust et al., 2003, 2004). This method is especially useful for the manipulation of large DNA fragments like cosmid inserts, and even allows to rapidly combine ("stitch") the inserts from different cosmids in order to assemble entire large gene clusters which could not be obtained in a single cosmid clone (Wolpert et al., 2008).

The integrase of the ΦC31 phage allows to site-specifically integrate a circular DNA fragment, containing the *attP* recognition site, into the *attB* site found in the genome of most streptomycetes (Smith et al., 2004). As shown in Fig. 3A, a gene cassette containing the integrase gene, the *attP* site, an *oriT* for conjugal transfer and a resistance marker, can be used to replace the *ß*-lactamase gene in the backbone of a SuperCos 1-derived cosmid clone using λ RED-mediated recombination (Eustáquio et al., 2005a). The resulting cosmid can then be introduced into the genome of a suitable heterologous host, e.g. the fully sequenced strain *Streptomyces coelicolor* A3(2). For the heterologous expression of aminocoumarins, a derivative of *S. coelicolor* A3(2) was used, i.e. strain M512 which does not contain the SCP1 plasmid and carries defects in the *act* and *red* clusters

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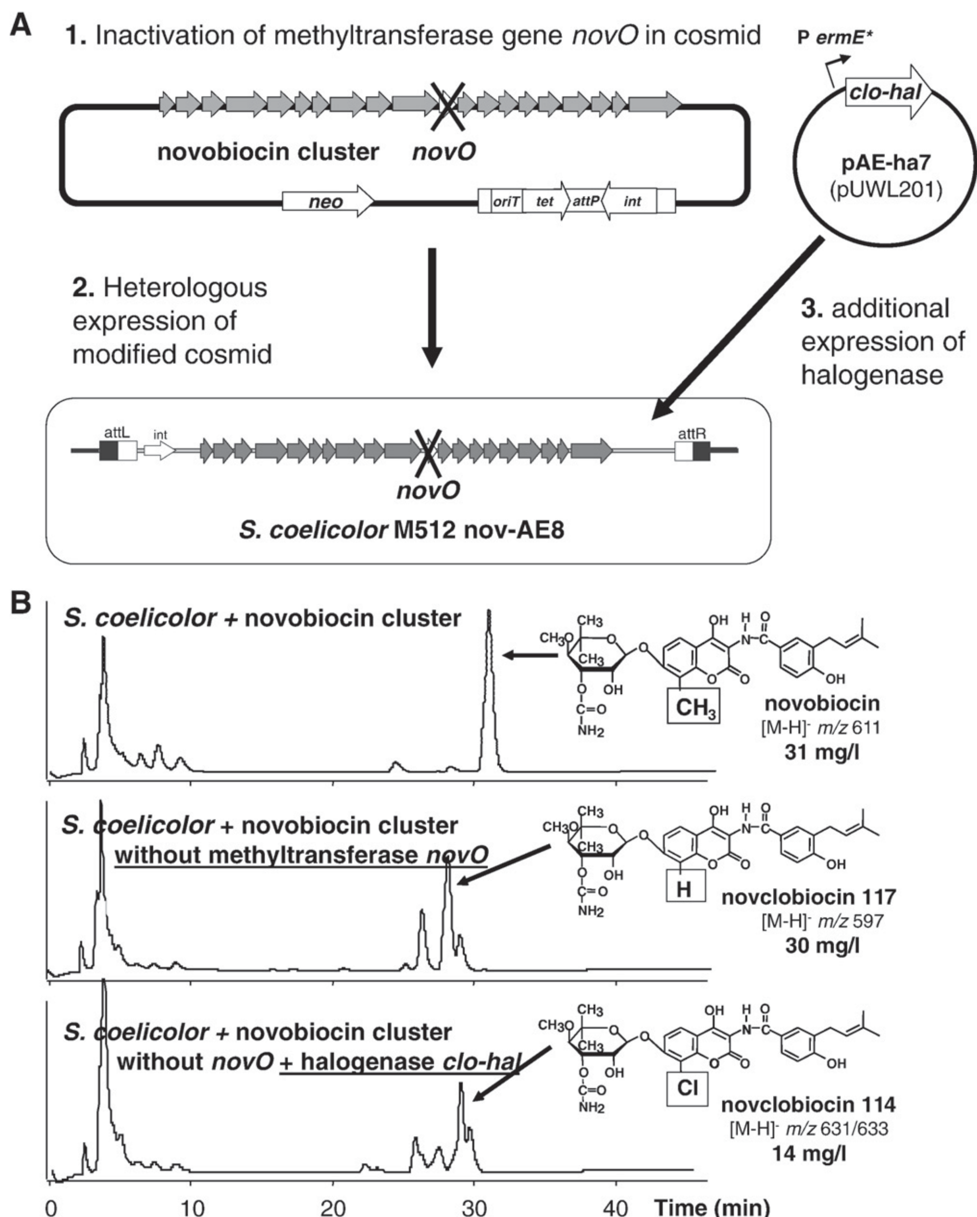

Fig. 4. A. Generation of structurally modified aminocoumarin antibiotics by genetic manipulation of biosynthetic gene clusters followed by their heterologous expression in a suitable host. B. HPLC detection of structurally modified aminocoumarin antibiotics derived from genetic engineering.

(Floriano and Bibb, 1996). Therefore this strain is unable to produce three of the genuine antibiotics of *S. coelicolor*, i.e. methylenomycin, actinorhodin and prodigiosin, making the analysis of the heterologously produced antibiotic easier.

Fig. 3B shows that the heterologous host, *S. coelicolor* M512, does not produce any aminocoumarin antibiotics prior to the introduction of the clusters. Once the clusters of novobiocin or clorobiocin have been integrated into the genome, however, the formation the respective antibiotic can be readily observed in HPLC analysis. The production levels, even without any optimization of the media or the producer strain, are comparable to the production of the wild-type producer strains (Eustáquio et al., 2005a).

Heterologous expression of the aminocoumarin clusters offers two principal advantages for the metabolic engineering process: it allows a fast genetic manipulation of clusters by λ RED-mediated recombination, while the cosmids are still present as cosmids in *E. coli*, and it allows antibiotic production in a completely sequenced host, with all the implied possibilities for influencing primary metabolism and regulatory processes.

4. Metabolic engineering for the generation of new aminocoumarin antibiotics

A considerable number of new aminocoumarins has meanwhile been generated by metabolic engineering experiments (Li and Heide, 2005). One example is the generation of novobiocin and clorobiocin analogs ("novclobiocins") for structure–activity investigations carried out by Flatman et al. (2006). As shown in Fig. 1, novobiocin and clorobiocin differ only in two points of their chemical structure: clorobiocin has a chlorine rather than a methyl group as substituent of the aminocoumarin ring, and it contains a methyl-pyrrole-2-carboxyl rather than a carbamoyl moiety attached to the 3-OH group of the deoxysugar. Clorobiocin is known to be 10 times more active as gyrase inhibitor and antibacterial agent than novobiocin (Maxwell and Lawson, 2003). However, it was not known how much each of the two structural differences contributed to this higher activity.

To investigate these structure–activity relationships, which may guide the future development of antibiotics with improved properties, it would be most useful to compare the activities of a set of nine different

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compounds: each would contain the principal skeleton of novobiocin and clorobiocin, consisting of the prenylated benzoyl moiety, the aminocoumarin moiety and the deoxysugar moiety. But they would differ by carrying either a methyl group or a chlorine atom or no substituent (i.e. just a hydrogen atom) at C-8 of the aminocoumarin moiety; and further they would differ by carrying either a carbamoyl or a methyl-pyrrole-2-carboxyl moiety or no substituent at 3-OH of the deoxysugar. Only two of these nine compounds, i.e. novobiocin and clorobiocin, were readily available. Therefore, metabolic engineering was used to generate the other compounds (Flatman et al., 2006).

As exemplified in Fig. 4, the methyltransferase gene *novO*, responsible for the attachment of the methyl group to position 8 of the aminocoumarin moiety, was inactivated by λ RED-mediated recombination on a cosmid containing the novobiocin biosynthetic gene cluster. Subsequently, the modified cluster was heterologously expressed in *S. coelicolor* M512 by integration into the genome via the ΦC31 attachment site. HPLC analysis (Fig. 4B) showed that the strain produced not novobiocin but a derivative thereof. Preparative isolation and structural elucidation showed that this new compound was indeed the desired compound (termed novclobiocin 117), carrying a hydrogen rather than a methyl group at position 8 of the aminocoumarin moiety (Eustáquio et al., 2004).

Subsequently, the halogenase gene *clo-hal*, which in clorobiocin biosynthesis directs the attachment of a chlorine atom to the aminocoumarin moiety, was cloned into a Streptomyces expression vector, pUWL201, placing it under control of the constitutive *ermE** promoter. When this plasmid was introduced into the *novO*-defective heterologous expression strain, again a new compound was formed, and preparative isolation and structural investigation proved that it was a "hybrid" antibiotic which carried the carbamoyl group typical of novobiocin and the chorine atom typical of clorobiocin (Fig. 4B) (Eustáquio et al., 2004).

Fig. 5 schematically summarizes the experiments leading to the other desired compounds: when the halogenase *clo-hal* was inactivated in a clorobiocin producer strain, a compound lacking the halogen (novclobiocin 101) was produced (Eustáquio et al., 2003). When the methyltransferase *novO*, under control of the constitutive *ermE** promoter, was introduced in this strain, another hybrid antibiotic was produced, now carrying the methyl-pyrrole carboxylic acid group (typical of clorobiocin) at the deoxysugar, and the methyl group (typical of novobiocin) at the aminocoumarin moiety (Eustáquio et al., 2003).

Inactivation of the acyl transferase gene *cloN2* in a clorobiocin producer strain led to novclobiocin 104, lacking the methyl-pyrrole carboxylic acid moiety at the deoxysugar (Xu et al., 2003). When both the halogenase gene *clo-hal* and the acyltransferase gene *cloN2* were inactivated in consecutive gene deletion experiments, the resulting strain produced a compound lacking both substituents in question. The last of the nine desired compounds was novclobiocin 103, with a methyl group at the aminocoumarin moiety and no acyl group at the deoxysugar (Fig. 5). Obviously, this compound might have been generated e.g. by inactivation of the carbamoyl transferase gene *novN* in the novobiocin cluster. However, it could be isolated already as a side product from one of the available strains (Fig. 5), and therefore no additional experiment was necessary for the generation of this compound (Flatman et al., 2006).

All compounds were analyzed for their inhibitory activity on both gyrase and topoisomerase IV, providing the first systematic evaluation of the action of different aminocoumarin antibiotics on these two targets. This revealed that it is primarily the methyl-pyrrole-carboxyl moiety which results in the higher activity of clorobiocin as compared to novobiocin. It further showed that clorobiocin, in contrast the earlier discovered (and clinically used) novobiocin, is an effective inhibitor of both gyrase and topoisomerase IV, i.e. of two vital targets in the bacterial cell, and can therefore be expected to produce much slower resistance development than novobiocin (Flatman et al., 2006). Therefore, future developments of new aminocoumarins may focus on the generation of clorobiocin rather than novobiocin derivatives.

5. New aminocoumarin antibiotics by mutasynthesis

As shown above, genetic engineering has become a powerful tool for the generation of new structural variants of natural products. Even more chemical diversity can be achieved when the geneticist cooperates with the synthetic chemist, combining the powers of both technologies. One strategy for such a collaboration is mutasynthesis: the biosynthesis of a precursor of a structural moiety of a natural product is blocked by an appropriate gene inactivation experiment, and synthetic analogs of this precursor are then added to the culture medium, leading to an incorporation of the foreign moiety into the antibiotic and thereby to the formation of new compounds.

As discussed above, the prenylated 4-hydroxybenzoyl moiety of clorobiocin is formed from 4-hydroxyphenylpyruvate and dimethylallyl diphosphate under catalysis of CloQ and CloR. If one of these enzymes,

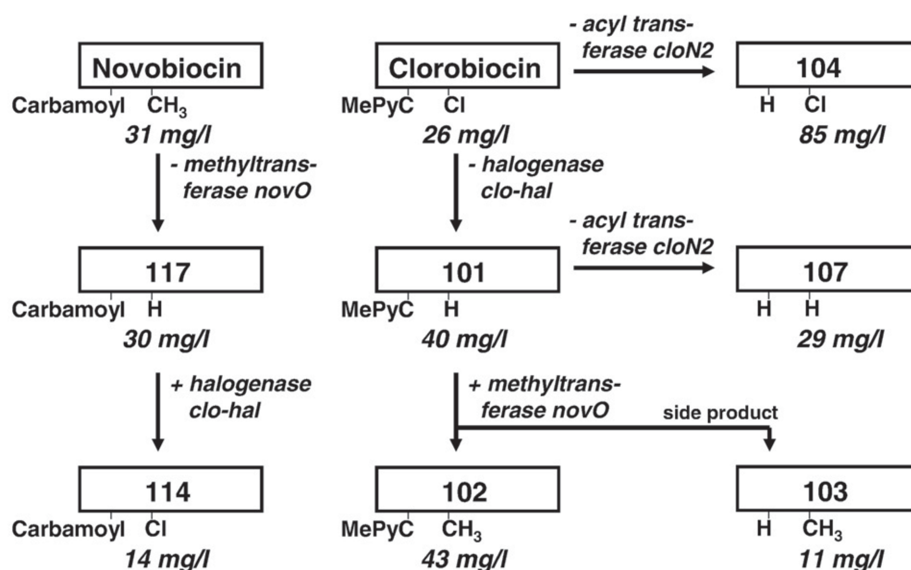

Fig. 5. Generation of a series of novobiocin and clorobiocin analogs by genetic engineering. The productivity of the mutant strains for the respective antibiotics is indicated.

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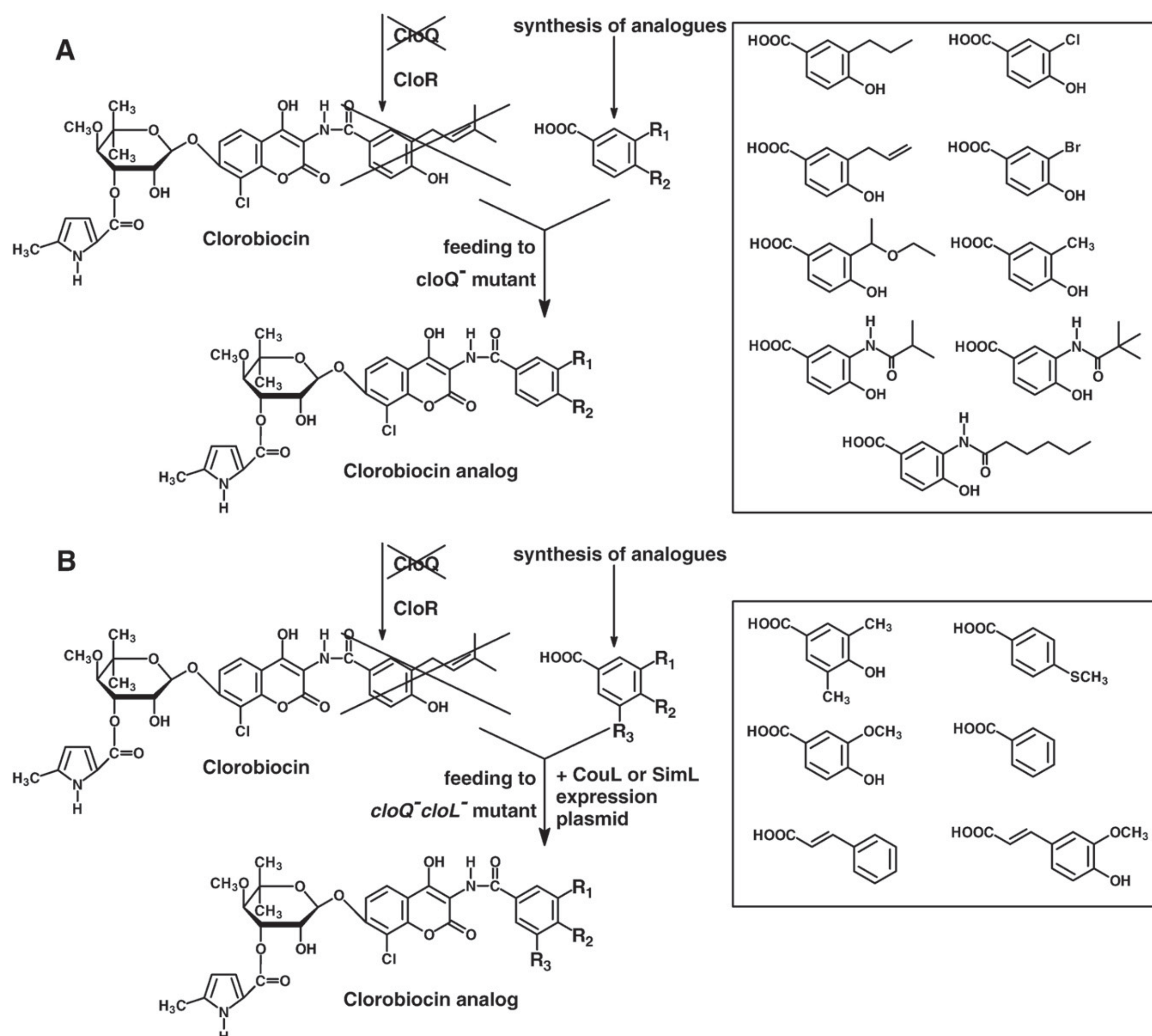

Fig. 6. Mutasynthetic generation of new aminocoumarin antibiotics. A. New aminocoumarin antibiotics generated using a CloQ-defective mutant of the genuine clorobiocin producer, *Streptomyces roseochromogenes*. B. New aminocoumarins generated using a CloQ-defective heterologous expression strain, *Streptomyces coelicolor* M512(clo-CA5), expressing the amide synthetases CouL or SimL. Structural analogs of the genuine substituted benzoyl moiety which were incorporated and gave rise to preparative amounts of new aminocoumarin antibiotics are indicated.

e.g. CloQ, is inactivated, the formation of the prenylated 4-hydroxybenzoyl moiety is abolished. Since this is the starter molecule of the biosynthetic pathway to clorobiocin, no antibiotics are formed by such a mutant. If, however, synthetic analogs of this moiety are added to this mutant, they are attached to the aminocoumarin moiety and incorporated into the clorobiocin skeleton. Fig. 6A shows examples of synthetic precursors which were successfully used in mutasynthesis experiments (Galm et al., 2004a). The respective products could be isolated in milligram amounts, their structures were elucidated by spectroscopic methods, and their gyrase inhibitory effect as well as their antibacterial activities were evaluated (Galm et al., 2004b).

One of the principal limitations of mutasynthesis lies in the substrate specificity of the biosynthetic enzymes, which in some cases do not allow an efficient incorporation of the synthetic substrate analog into the natural product in question. E.g., when 3,5-dimethyl-4-hydroxybenzoic acid was added to a CloQ-defective mutant strain, no significant production of a new antibiotic was observed (Anderle et al., 2007b). Investigations of the substrate specificity of the amide synthase CloL, which has to accept the synthetic benzoyl moiety and attach it to the aminocoumarin moiety, showed that indeed 3,5-dimethyl-4-hydroxybenzoic acid was poorly accepted by this enzyme. However, the current rapid advances of our understanding of the genetic basis of antibiotic biosynthesis allow to overcome such problems by improved genetic strategies. Amide synthetases, linking the aminocoumarin moiety to acyl moieties, are also found in the biosynthetic gene clusters of other aminocoumarin antibiotics, not only of novobiocin and coumermycin A_1 (Schmutz et al., 2003b), but also in the clusters for two structurally different aminocoumarin antibiotics, simocyclinone and rubradirin (Luft et al., 2005; Kim et al., 2008). When the amide synthetases from different gene clusters were overexpressed, purified and investigated for their substrate specificity, it was found that especially CouL and SimL, from the coumermycin and simocyclinone cluster, accepted many acyl substrates which were not accepted by CloL. Therefore, the genes *couL* and *simL* were cloned into a *Streptomyces* expression vector and transformed into a heterologous expression strain, containing a *cloQ*- and *cloL*-defective clorobiocin cluster. When 3,5-dimethyl-4-hydroxybenzoic acid or other analogs of the benzoyl moiety, not accepted by CloL, were added to such strains, the formation of the desired new

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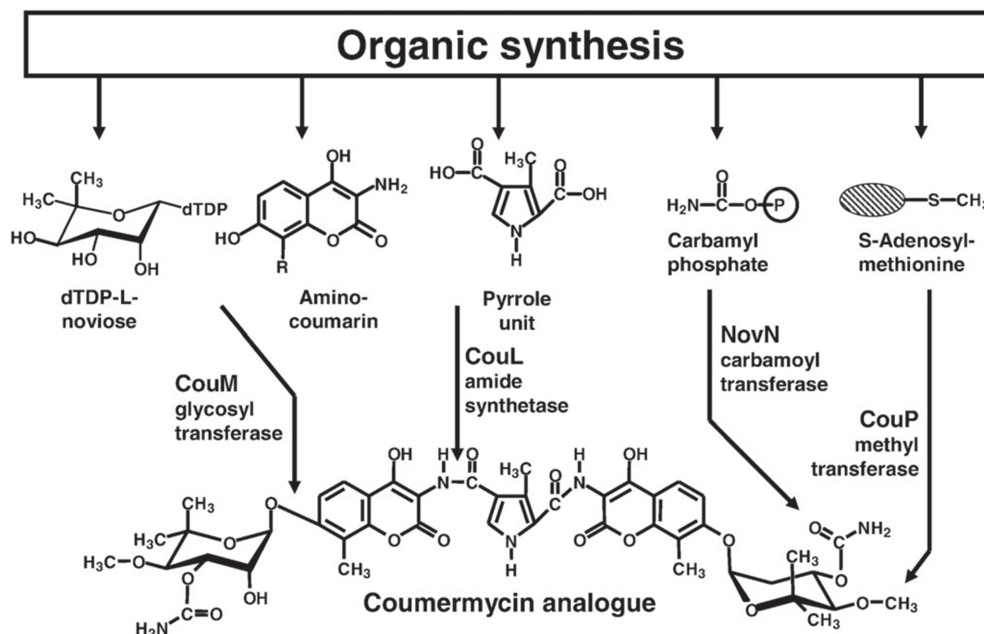

Fig. 7. Chemoenzymatic synthesis of a new aminocoumarin antibiotic.

clorobiocin analogs was readily observed. Fig. 6B shows examples of structural analogs of clorobiocin which were obtained in preparative amounts by this approach (Anderle et al., 2007b). Again, the structures of all these compounds were elucidated by NMR and mass spectrometry, and their antibacterial and gyrase inhibitory effects were determined (Anderle et al., 2008). Twelve of the new compounds obtained by mutasynthesis showed antibacterial and gyrase inhibitory activities similar to or higher than those of the clinically used compound, novobiocin.

6. New aminocoumarin antibiotics by chemoenzymatic synthesis

Chemoenzymatic synthesis offers an additional strategy which combines the powers of microbial genetics and of synthetic chemistry for the generation of new structural variants of natural products. An example is shown in Fig. 7: the organic chemist synthesizes the building blocks of the aminocoumarin antibiotic coumermycin, i.e. the central pyrrole moiety, the aminocoumarin moiety and the dTDP-activated deoxysugar, as well as S-adenosylmethionine as donor of the methyl groups and carbamoyl phosphate a donor of a carbamoyl group. The microbial geneticist clones and overexpresses the enzymes required for the successive linkage of these precursors by amide bond formation, glycosylation, methylation and carbamoylation. In a one-pot reaction, without purification of the intermediates, the complete assembly of an aminocoumarin antibiotic can be achieved in this way (Freel Meyers et al., 2004a). In the example shown, a carbamoyl moiety was thereby attached to the 3-OH group of the deoxy sugar moieties of coumermycin, instead of the genuine 5-methyl-pyrrole-2-carboxyl moiety, yielding a new aminocoumarin antibiotic as product of this multi-step chemoenzymatic synthesis procedure.

Chemoenzymatic synthesis is more expensive than mutasynthesis, since the various precursor molecules and the enzymes need to the generated first. However, in comparison to mutasynthesis it offers access to an even larger range of structural diversity. One limitation of mutasynthesis is that the synthetic substrate precursor, added to the culture of the mutant strain, must be taken up across the bacterial cell envelope. For precursor molecules which are not taken up by bacteria, or which are not stable under culture conditions, chemoenzymatic synthesis offers an attractive alternative for their incorporation into antibiotic molecules.

7. Conclusion

Microbial genetics and genomics have, over the last years, supplied an attractive range of tools for the generation of new bioactive compounds, especially of antibiotics and anticancer agents. New compounds can be generated by purely genetic approaches, but even more efficiently by combinations of genetic and organic–synthetic strategies, as exemplified by mutasynthesis or chemoenzymatic synthesis experiments. The aminocoumarins antibiotics, with their closely related structures and their well-studied gene clusters, have provided a successful model, demonstrating the potential of genetic engineering for antibiotic biosynthesis. It may be expected that in future drug discovery programmes, genetic methods will form a new principal tool for the generation of new compounds, besides natural product screening and organic synthesis.

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