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Molecular Biology of Polyketide Biosynthesis

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Dedicated to the memory of Professor Vera Johanides

Summary

Streptomyces species and related genera synthesize a large number of secondary metabolites, many of which are biologically active. Amongst them, polyketides is the largest class. Polyketides are a structurally diverse family of natural products with a broad range of biological activities. The formation of polyketides is very similar to the biosynthesis of long chain fatty acids – both in the enzymatic reactions that take place and the enzyme proteins that are involved. During the last decade many polyketide gene-clusters have been cloned and sequenced. DNA sequencing has shown that the clusters have substantial homology suggesting that they originated from a common ancestor. This similarity has resulted in the development of combinatorial biology techniques to create novel chemical entities. Two approaches have been used: targeted manipulation, *e.g.* disruption and, often, replacement of certain genes involved in the biosynthetic pathway, and the random approach, *e.g.* »gene shuffling«. A targeted approach has been used to generate several novel scaffolds by manipulation of the *S. rimosus* oxytetracycline gene-cluster. Genes encoding ketosynthase, α and β, ketoreductase, cyclase/aromatase and C-6 hydroxylase were disrupted to construct four recombinant strains. Thin layer chromatography and high pressure liquid chromatography of extracts from fermentation broths of all four recombinants showed that they produce about 20 potentially novel metabolites, 12 of which have been characterised chemically. In this review, data on the disruption and replacement of the otcC gene will be described in more detail.

Key words: polyketides, biosynthetic pathway engineering, *Streptomyces rimosus*, oxytetracycline

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Introduction

Streptomyces species and related actinomycete genera are the group of organisms that synthesize the largest number of »secondary metabolites«, many of which are biologically active. Out of the 19 400 antibiotically active substances isolated from organisms ranging from bacteria to mammals, actinomycetes synthesize 7 900, polyketides being by far the largest class (40 % of this total). Polyketides are structurally a very diverse family of natural products with an extremely broad range of biological activities and pharmacological properties. Polyketide antibiotics, antifungals, cytostatics, immunosuppressants, anticholesterolemics, antiparasitics, coccidiostatics, animal growth promotants and natural insecticides are in commercial use (*1*). In general, polyketides have been divided into two groups: the aromatic polyketides containing one to six aromatic rings and the complex polyketides, subdivided into macrolides and ansamycins (having lactone and lactam rings), polyenes and polyethers (*2*).

Biosynthesis of Polyketides

Despite their noteworthy structural diversity, polyketides are synthesized by a uniform (bio)chemistry that is likely to have evolved from fatty acid biosynthesis (Fig. 1) (*3*). The enzymes that make these polyketide backbones can be categorised into type I or type II polyketide synthase complexes (PKSs). Type I enzymes are multidomain mega-polypeptides (often >300 kDa), in which each domain is usually used only once in the biosynthetic process. Type II complexes are aggregates of small polypetides, each having a single catalytic activity that is used iteratively during the biosynthetic process. Recently, an iteratively used type I enzyme has been described (*4*).

The growth of the polyketide carbon chain is initiated by the condensation of a starter unit with an extender unit. The starter and extender units are present in the host as Coenzyme A thioesters. PKSs mainly use acetyl-CoA or propionyl-CoA as starter units with malonyl-CoA and methylmalonyl-CoA as the common extender units. However, CoA derivatives of other carboxylic acids can also be used as starters and extenders.

Condensation is driven by decarboxylation of the extender unit. The fate of the resulting diketide depends on whether functional ketoreductase, dehydratase or enoylreductase are present. In their absence, a diketide will immediately undergo another round of condensation employing a new extender to become an unreduced triketide. However, if the necessary enzyme activities are present, one or more of the sequential reactions of β-ketoreduction, dehydratation and enoylreduction will take place to form compounds containing a β-hydroxyl group, unsaturated double bond or fully reduced β-carbon. The choice of a given extender for a given condensation is determined by the specificity of the acyltransferase function that delivers the extender unit to the PKS complex for that condensation cycle only. The triketide may then be subjected to new rounds of reduction dependent upon the presence of the reductive enzyme activities that can act on triketides. The reductive fate of that triketide, whether full, partial or no reduction, does not depend upon the reductive fate of diketide formed in the prior round of condensation, or on what will hap-

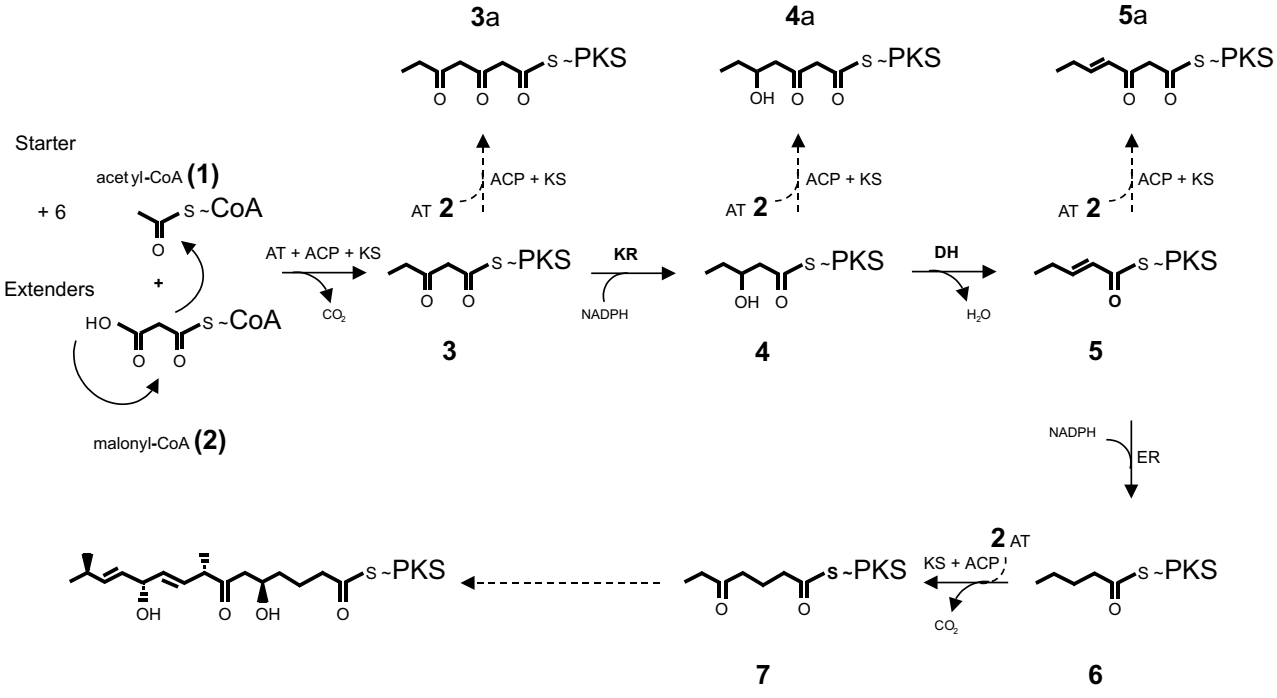

Fig. 1. Schematic representation of polyketide biosynthesis; PKS represents the polyketide synthase, carrying two thiol groups, one on the ketosynthase (condensing enzyme; KS) and the other on the acyl carrier protein (ACP). The reaction steps are labelled: AT, acyl transferase; KS, ketosynthase; KR, ketoreductase; DH, dehydrase; ER, enoylreductase (modified according to *8*)

pen to tetra-, penta-, and hexaketides formed in subsequent condensations (Fig. 1). Therefore, PKSs must be able to distinguish one condensation/reduction cycle from another and to determine the correct number of cycles to take place, acting faithfully and uniquely at each cycle of synthesis. The choice of starter and extender units, the degree of reduction of β-carbons, the stereochemistry at centres carrying hydroxyl or alkyl substituents and the length of polyketide carbon chain are degrees of freedom that are genetically programmed within polyketide biosynthetic enzymes (*3*).

Genetics of Polyketides

During the last decade more then 50 polyketide gene-clusters have been cloned and sequenced (*1*). The type I PKS gene-clusters, such as those responsible for the biosynthesis of complex polyketides like the macrolide erythromycin, are multi-functional enzymes with a modular organisation. Each module is responsible for a single cycle of polyketide carbon chain extension and contains domains for necessary reduction activities. Therefore, there is a one-to-one correspondence between the product structure and the active domains in modular PKSs (Fig. 2) (*4*,*5*). Modular proteins, that is deoxyerythronolide B synthases (DEBS1, DEBS2 and DEBS3; of about 300 kDa each) were isolated and purified as multi-enzyme complexes. The results of analytical centrifugation, limited proteolysis and chemical cross-linking of purified DEBS multi-enzymes suggested a dimeric, possibly helical, structure that might be common to all modular PKSs (*6*).

On the other hand, sequencing of gene-clusters of the type II PKSs, such as those responsible for the biosynthesis of aromatic polyketides like actinorhodin, showed that they consist of three to six separate mono- or bifunctional proteins (*7*). Their active sites are used iteratively for the assembly of the polyketide chain (Fig. 3A). In contrast to the one-to-one correspondence between active sites and product structure of type I PKS's, the type II PKS's are structurally all very similar so it is not possible to predict the nature of the polyketide structure made from the structure and architecture of the genes. For that reason, an elegant host-vector system for the expression of engineered aromatic PKS clusters was designed. The entire actinorhodin expressed in the »naive« CH999 host, a polyketide carbon chain of the predicted length was produced. Gene-cluster was deleted from the host *S. coelicolor* strain CH999 to prevent any cross-talk with the introduced cloned genes. The vector is a specially-constructed bifunctional expression plasmid (pRM5) containing two actinorhodin promoters and the actinorhodin activator gene. When a »minimal« PKS, consisting of the ketosynthase α and β, as well as the acyl carrier protein from a particular gene cluster, was cloned into the pRM5 vector and expressed in the

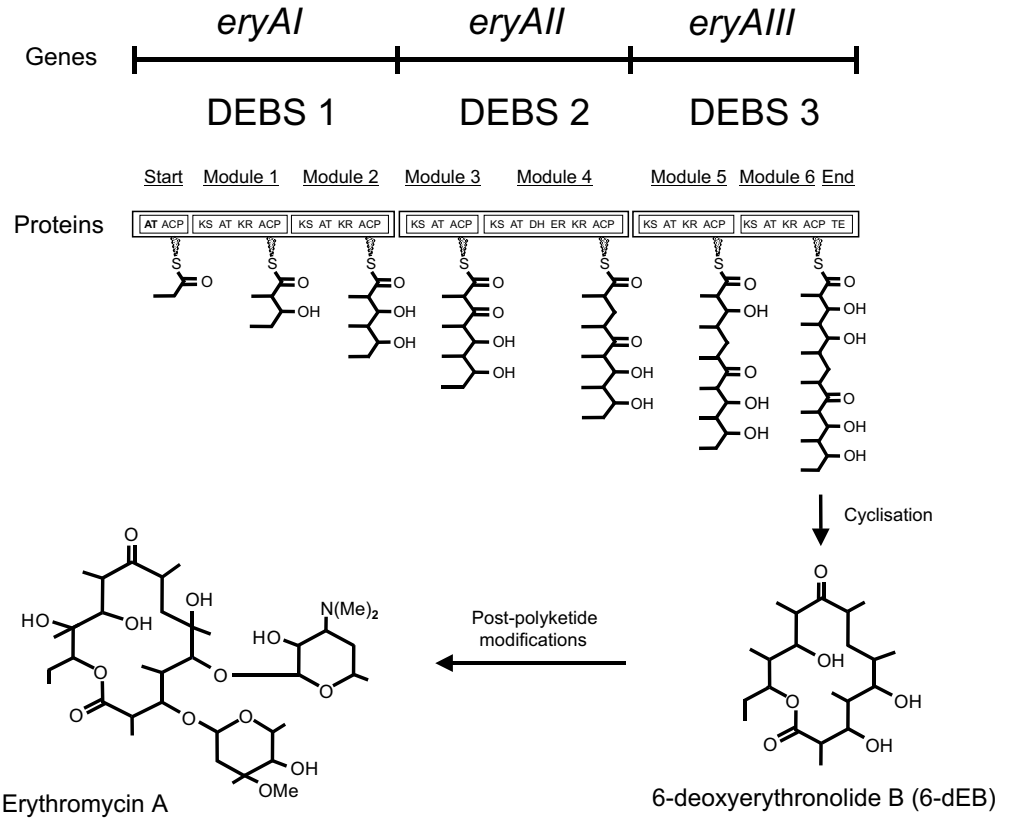

Fig. 2. Schematic representation of genes encoding the type I polyketide synthase complex responsible for the production of erythromycin A; the linear arrangement of genes (*ery*AI, *ery*AII and *ery*AIII), proteins (DEBS1, DEBS2 and DEBS3) and their products is followed by the polyketide aglycone 6-deoxyerythronolide B (6-dEB) and macrolide antibiotic erythromycin A. Abbreviations of reaction steps are as in Fig. 1 (modified according to *4*)

»naive« CH999 host, a polyketide carbon chain of the predicted length was produced. The nascent polyketide chain cyclised and aromatised spontaneously. However, if ketoreductase, cyclase/aromatase and cyclase genes were added to the »minimal« PKS construct, normal reduction, cyclisation and aromatisation took place. By »mixing and matching« of PKS genes from different polyketide producers, this system was used to study the role of different type II polyketide genes during biosynthesis. From these experiments, the catalytically inactive ketosynthase β seemed to be the factor that determined the chain length (*8*). However, recent work has shown that the ketosynthase β encodes a decarboxylase function and the view that this subunit is wholly responsible for determining chain length has had to be revised (*9*).

Until now, nobody has succeeded in the isolation and purification of the entire type II multi-enzyme complex. However, some individual proteins have been purified and used in reconstitution experiments (*11,12*). It was demonstrated that actinorhodin ketosynthase α and β (of 40 to 45 kDa each) can be purified as a heterotetramer of 168 kDa, presumably α_2 β_2, and together with acyl carrier protein can be used *in vitro* to produce a car-

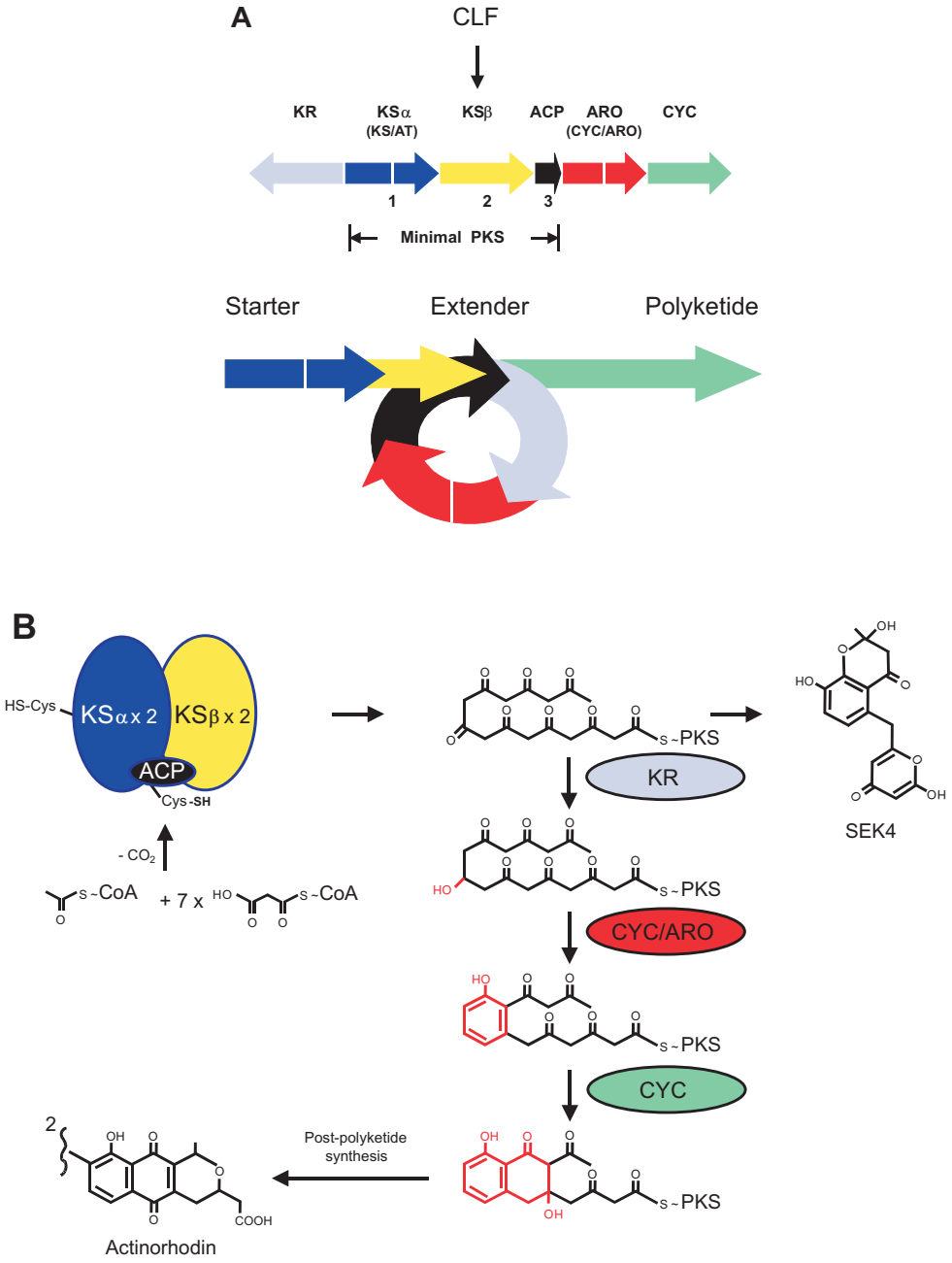

Fig. 3. Schematic representation of genes encoding the type II polyketide synthase complex responsible for the production of actinorhodin; the linear arrangement of the three adjacent genes, including the β-ketoacyl synthase (KS) α and β, as well as the acyl carrier protein (ACP) (»minimal« PKS) is conserved for all type II PKS gene clusters so far characterized (top part of figure). The iterative use of mono- or bifunctional proteins that are used for the assembly of polyketide backbone during pre-polyketide biosynthesis is shown (lower part of figure) (modified according to *10* and *11*)

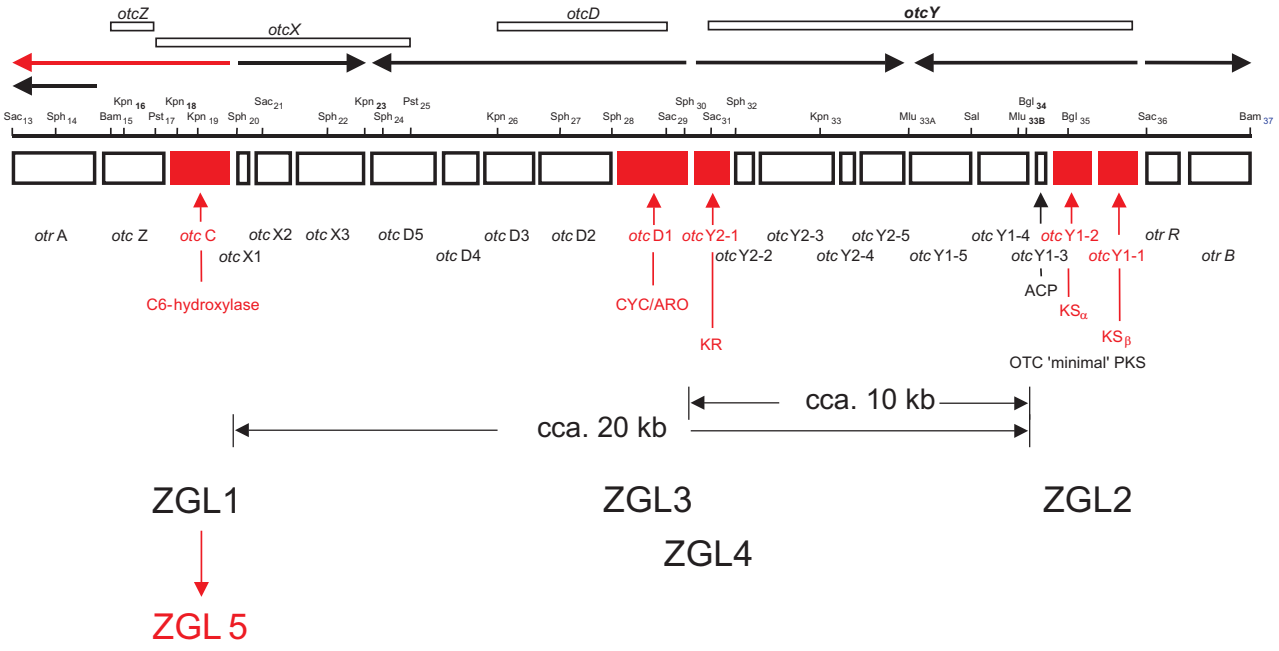

Fig. 4. Architecture of the oxytetracycline gene-cluster; bars indicate regions where mutants belonging to the same cosynthesis group map, arrows indicate direction of transcription while open boxes are ORFs corresponding to presumed biosynthetic genes. Shaded boxes are disrupted genes with products indicated by arrows. Their disruption (ZGL1 – ZGL4) and replacement (ZGL5) lead to these respective recombinants. Distances of *otc*D1 and *otc*C genes from the OTC 'minimal' PKS are shown (modified according to *14*)

bon chain of the same length as that of actinorhodin. The reconstitution of this »minimal« actinorhodin PKS activity with other purified protein components indicated that the set of proteins required to synthesise, fold and correctly cyclise the actinorhodin backbone consisted of at least six proteins: ketosynthase α and β, acyl carrier protein, ketoreductase, cyclase/aromatase and cyclase (Fig. 3B). This confirmed the previous »mixing and matching« genetic experiments.

In PLIVA, Croatian pharmaceutical company, the task set was to develop a technology platform for the generation of novel chemical entities, based on the backbone of the polyketide, oxytetracycline (OTC). OTC is an aromatic polyketide antibiotic synthesized by the condensation of a malonamide starter unit with 8 malonyl extenders. Thus, there are 19 carbon atoms in the polyketide backbone of OTC (*13*). The entire oxytetracycline gene cluster from PLIVA's *Streptomyces rimosus* OTC production strain was cloned and restriction mapped (Fig. 4) (*14*). Its restriction map was indistinguishable from the map of OTC gene cluster of the *S. rimosus* strain M4018, which has been completely sequenced at the University of Strathclyde (*13*).

If one considers a gene cluster for the biosynthesis of aromatic polyketide as a wall consisting of individual bricks, the majority of work done so far can be depicted as taking the bricks from different walls, and »mixing and matching« them to synthesize aromatic polyketide intermediates (*8*). However, a somewhat different approach could be also taken. The whole wall could be taken, the OTC gene cluster, and bricks pulled out of it with the intention of replacing them with the same (but biologically inactivated) bricks, or with bricks from different walls (*i.e.* from different clusters). The first step for this approach was to disrupt individual *otc* genes, by inserting selectable genetic markers within their chromosomal structures or by precise deletion of genes from the chromosome. This targeted approach allowed the disruption of four OTC biosynthetic loci. Genes encoding C-6 hydroxylase, the ketosynthase α and β pair, cyclase/aromatase, and ketoreductase were disrupted to construct four recombinant strains (Fig. 4). Thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC) of extracts from fermentation broths of all four recombinants constructed showed that they were producing about 20 potentially novel chemical entities (see example in Fig. 6), 12 of which have been characterised chemically.

The major problem during this work was the genetic instability of constructs, manifested by the appearance of amplifications and deletions of targeted DNA in almost all of the transformants analysed (Fig. 5) (*15,16*). This is most probably due to the location of the OTC gene-cluster near one end of the *S. rimosus* linear chromosome and within a 250 kb amplifiable unit of DNA (*17*).

Nevertheless, it was shown that the recombinant ZGL3, disrupted in the genomic copy of cyclase/aromatase gene (*otc*D1) located about 10 kb from the OTC »minimal« PKS on a separate transcription unit (Fig 4), synthesizes four novel polyketides. Like OTC, they all contain the malonamide starter unit, but have chain lengths that are shorter [from 9 to 17 carbon atoms, Fig. 6 (previously called LH1 to LH4; *18*)] than the 19 carbons present in OTC. Therefore, the loss of the *otc*D1 gene product has a profound influence on the length of

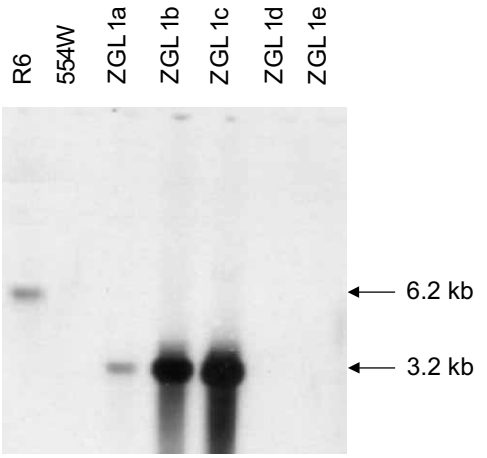

Fig. 5. DNA hybridisation of *Streptomyces rimosus* strain R6 and its derivatives 554W, ZGL1a, ZGL1b, ZGL1c, ZGL1d and ZGL1e; the total DNA was isolated from selected strains, digested with *Sac*I, subjected to Southern blotting and hybridized with a probe consisting of the *otc*C gene

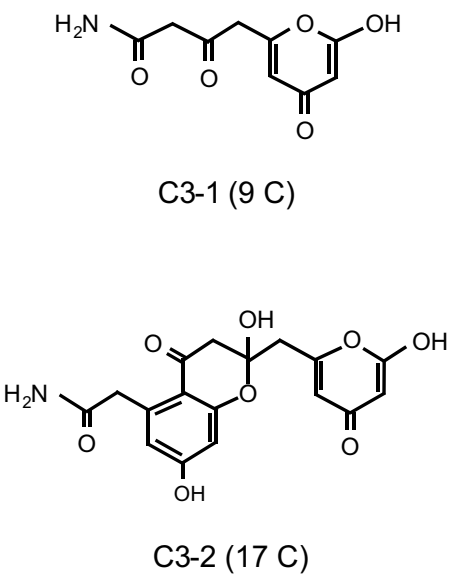

Fig. 6. Structures of compounds C3-1 and C3-2 isolated and purified from the fermentation broth of the strain ZGL3 (modified according to *18*)

polyketide chain assembled, implying that OtcD1 plays a greater role in the overall integrity of the OTC PKS quaternary structure than hitherto imagined. However, since the ZGL3 strain contained an amplified 250 kb AUD, gene replacement experiments in this strain were unable to proceed.

During disruption of the *otc*C gene, a transformant carrying only one copy of the disrupted gene (Fig. 5, ZGL1a) was successfully isolated. The *otc*C gene lies outwith the transcription units of all genes involved in biosynthesis and folding of the OTC backbone and is located some 20 kb from the OTC »minimal« PKS (Fig 4). This strain has yielded valuable information on PKS quaternary structure and thus the results will be described in more details.

The role of the *otc*C gene product in OTC biosynthesis is to add the hydroxyl group to the sixth carbon atom of the OTC polyketide backbone. It is therefore a post-polyketide biosynthetic enzyme (*13*). The original hypothesis was that the absence of this gene from the cluster would generate the known semi-synthetic antibiotic, doxycycline (DOTC). TLC profiles of extracts from fermentation broths of the *otc*C-disrupted strain, ZGL1, revealed only two major novel products (Fig. 7A). However, HPLC showed at least five potentially novel compounds (Fig. 7C), and there are likely many more. These major metabolites were isolated and purified (Fig. 7B). Their structural elucidation was undertaken largely on the basis of 2D homonuclear and heteronuclear NMR (*e.g* Fig. 9; compound C1-1). The structures of the major metabolites of the strain ZGL1 were anthrone derivatives, with carbon chain lengths of 17 – two carbons shorter than OTC (*16*). Thus the disruption of the *otc*C gene results in metabolites having structures not predictable from the deduced and proven function of the gene-product.

The *otc*C gene is localized at the 5′ end of a poly-cistronic mRNA that also encodes the distal *otc*Z and *otr*A genes (Fig. 4) (*13*). Therefore, disruption of *otc*C could, in principle, cause polar effects on the translation of these two downstream genes. To circumvent this potential problem a cloned copy of the *otc*C gene was mutated by site-directed mutagenesis so that a catalytically-inactive gene product was made. The chromosomal copy of the disrupted *otc*C gene in strain ZGL1 was replaced by this inactive form, to generate recombinant ZGL5 (Fig. 4). Site-directed mutagenesis was undertaken by preparing and combining two PCR products in which three guanines were replaced by three cytosines so that three glycine codons present in the enzyme active site were changed into three alanine codons (Fig. 8) (*16*). Enzyme assays (data not shown) confirmed that the inactivated *otc*C gene-product could not hydroxylate its substrate anhydro-tetracycline, whereas the parental, biologically active, *otc*C gene-product could (N. Perić, unpublished results).

TLC of extracts from fermentation broths of three independent transformants (ZGL5.1, ZGL5.2 and ZGL5.3) containing the inactivated *otc*C gene synthesized oxytetracycline instead of doxycycline (Fig. 9). The data showed that the elimination of a chromosomal pre-polyketide gene (in ZGL3) or even a post-polyketide gene (in ZGL1) results in the loss of the ability of the OTC PKS to form a carbon chain with the correct number of carbon atoms. This is despite the OTC »minimal« PKS (which others have deduced is solely responsible for determining chain length) remaining untouched and intact in all of the ZGL strains discussed here. Therefore, when all proteins from the multi-complex type II PKS are present, the *otc*D1 and *otc*C gene-products must obviously interact with the PKS complex and influence its quaternary structure (*16,18*). Returning an *otc*C gene-product which is enzymatically inactive to the OTC PKS multi-enzyme complex restores production of a polyketide of the correct carbon chain length. Hydroxylation of this structure at the 6 position has, nevertheless, taken place in the absence of the biologically active *otc*C gene-product. This may be explained by relaxed substrate

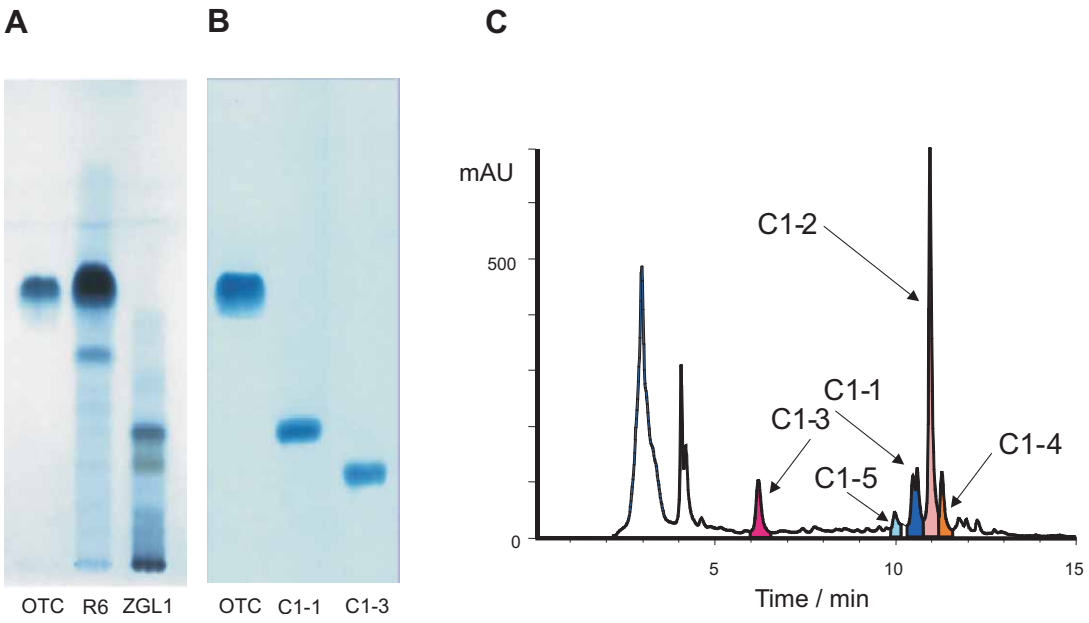

Fig. 7. Thin-layer chromatography (TLC) of extracts from fermentation broths of *Streptomyces rimosus* strains R6 and ZGL1 (A), TLC of compounds C1-1 and C1-3 isolated and purified from ZGL1 extract (**B**) and high-pressure liquid chromatography (HPLC) of extract from fermentation broth of the strain ZGL1(**C**). In TLCs, authentic OTC was used as control

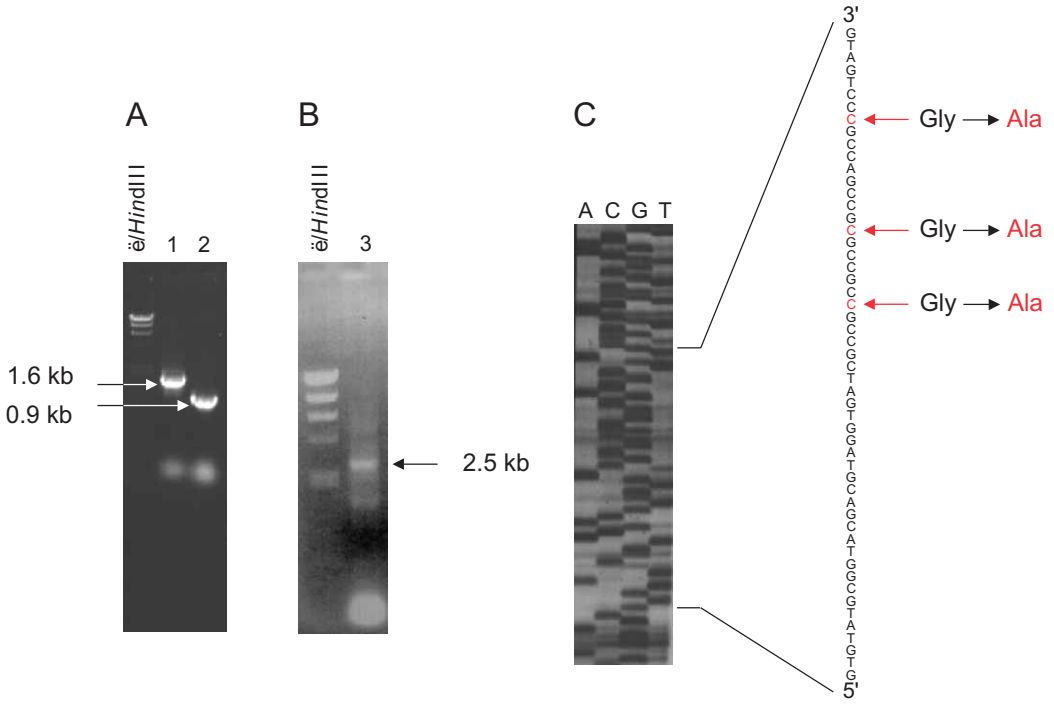

Fig. 8. Site-directed mutagenesis of the cloned otcC gene; two PCR products (**A**), their combination (**B**) and partial DNA sequence (**C**) are shown replacing three guanines by three cytosines in order to change three glycines, in the enzyme active site, into three alanines

specificity of other two hydroxylases present within the oxytetracycline gene-cluster (*16*), or indeed some other hydroxylase activity that is expressed in this recombinant strain under these conditions.

Thus, it has been shown that the disruption of genomic copies of pre-polyketide and post-polyketide genes can generate novel chemical entities – opening further opportunities to create biodiversity of polyketide structures.

Combinatorial biology

During the last decade, the cloning and sequencing of polyketide gene clusters has opened the possibility of using combinatorial biology to create novel polyketide compounds.

Combinatorial biology aims to create biodiversity by engineering of polyketide biosynthetic pathways in such a way to create »natural« substances that Nature

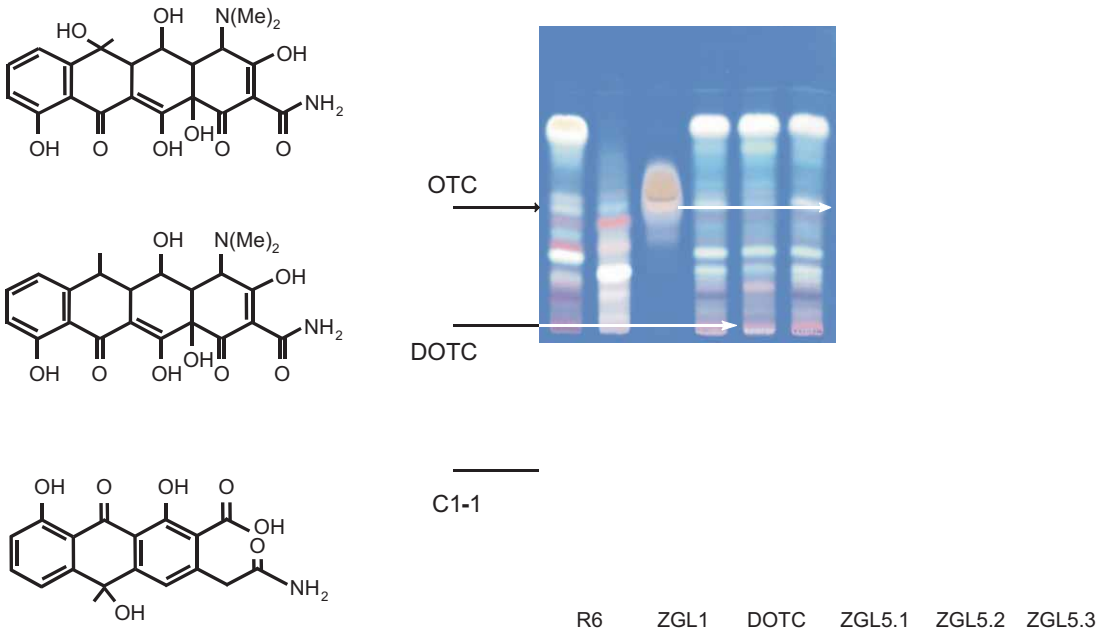

Fig. 9. Thin-layer chromatography (TLC) of extracts from fermentation broths of *Streptomyces rimosus* strains R6, ZGL1 and three independent transformants (ZGL5.1, ZGL5.2 and ZGL5.3) containing the *otc*C gene-product inactivated by the site-directed mutagenesis; authentic DOTC was used as control. Chemical structures of OTC, DOTC and C1-1 are shown

never imagined. In theory, the number of products available by this approach is given by the formula R^N, where R is the number of different genes that can be used in each construct, while N is the number of different allelic forms of these genes that can be obtained. Thus, if two polyketide pathways, each containing four homologous polyketide biosynthetic genes with different molecular recognition features, are combined in all possible ways, 16 different polyketides could result. Likewise, four pathways with four genes could be combined to generate 256 unique polyketides – hence the term combinatorial biology (*19*).

Who is using combinatorial biology to create biodiversity worldwide today? There are a dozen small biotechnology companies that are potential competitors (*20*). However, only four companies: Biotica Technology Ltd., Kosan Biosciences Inc., TerraGen Diversity Inc. and Maxygen Inc. will be considered here to illustrate the different approaches that are being used. For complex polyketides like macrolides, Kosan and Biotica are disrupting specific enzyme active sites, deleting whole modules, or replacing parts of modules with genes from other clusters for multifunctional PKSs. These strategies are generating novel macrolides with differently reduced oxo groups with different stereochemistry, or novel macrolide structures with shorter carbon chain lengths (Fig. 10A). For aromatic polyketides, Kosan and TerraGen are using a targeted approach by mixing and matching specific polyketide synthase genes from different gene-clusters using the elegant host/vector system described above to generate novel aromatic polyketides (Fig. 10B) (*8*). The difference between these two companies is that Kosan is using genes from already cloned and sequenced clusters, while TerraGen is cloning new aromatic polyketide genes from uncultured micro-organisms present in environment (*21*).

Recently, a new structural class of polyketides (possessing a 2,4-dioxoadamantane ring-system; Fig. 11) has been isolated from an engineered *Streptomyces* strain, thus supporting the claim that combinatorial biology is capable of producing novel chemotypes (*22*).

In the continuing search for novel antibiotics to combat the emergence of antibiotic-resistant pathogenic bacteria, a novel group of macrolides, the ketolides, have been derived recently. These semi-synthetic erythromycin derivatives are also amenable to biosynthesis by engineered PKS's – opening up the possibility of using the combinatorial technology to derive second generation ketolides with improved performance (*23*).

However, up till now these approaches enabled the production of only several polyketides per experiment, or group of experiments.

Nature has itself exploited combinatorial biology to produce about 7 000 polyketides that are currently known. However, the natural polyketides characterized thus far represent only a small fraction of the combinatorial potential that might be realized from permutations of PKS's. For example, if the seven acyltransferases and five β-carbon modifier building blocks, used by the erythromycin PKS, could be permutated into the 6-module DEBS, the theoretical number of polyketides would reach 10^7. Complete permutation of the 14-module rapamycin PKS with the same building blocks could theoretically yield a remarkable 10^{14} polyketides (*24*).

At Kosan, gene cassettes for modular complex polyketide PKSs (containing ATs and β-carbon modifier building blocks) were constructed that allow relatively simple replacement, or loss or gain of functions. Using two possible AT domains and the four possible β-carbon modifications, a modest library of less than 60 single, double and triple »mutants« produced a polyketide library of more than 50 polyketides (*24*). To come closer

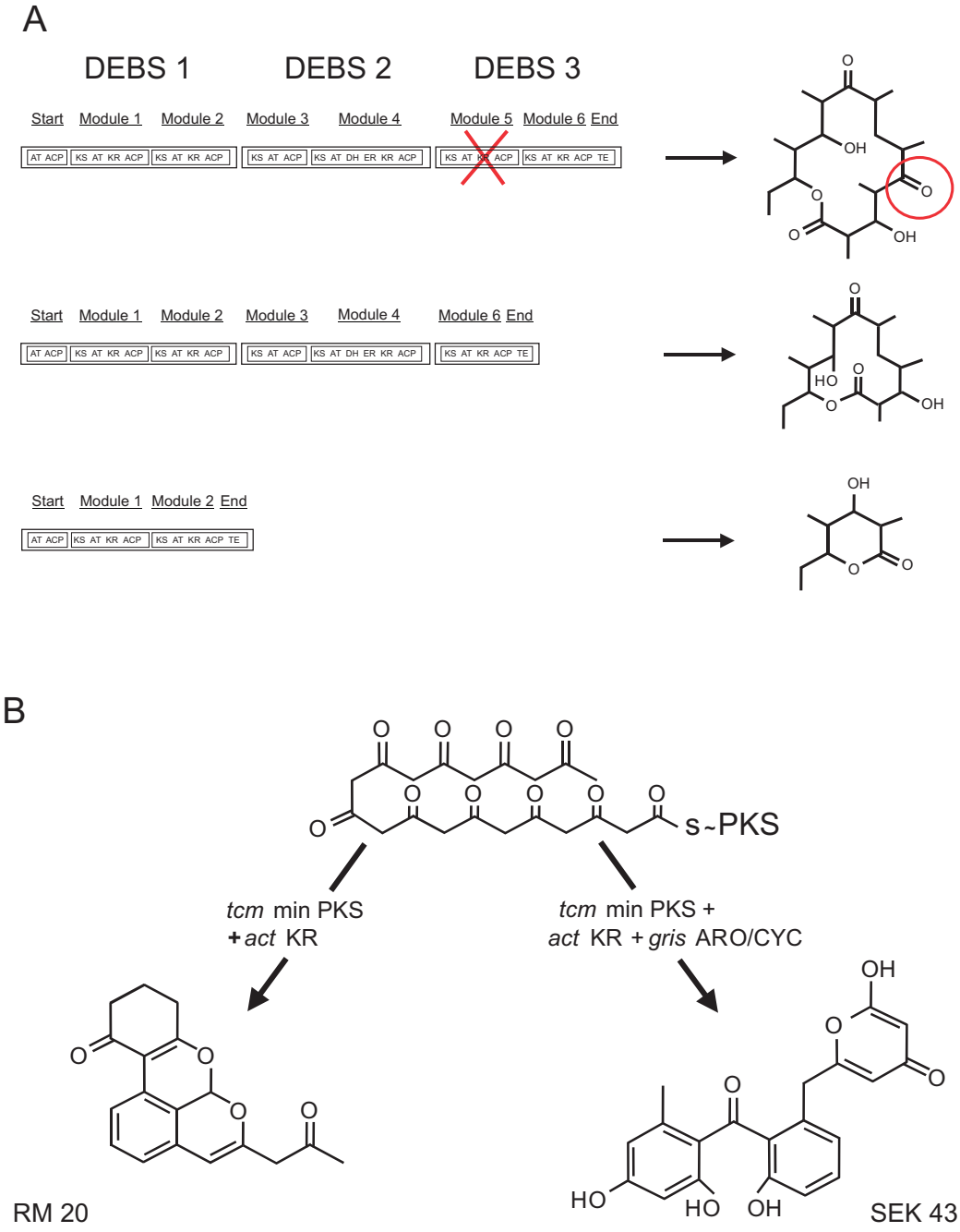

Fig. 10. Targeted manipulations of complex (A) and aromatic (B) gene-clusters for the generation of novel polyketide structures (modified according to *8*)

to theoretical numbers a multiplasmid approach to prepare large polyketide libraries has been constructed using the same AT and β-carbon modifying domains. In contrast to the single plasmid system that theoretically allows the construction of a total of 192 »mutants« producing the same number of polyketides, cotransformation of a host strain with three plasmids would generate sufficient »mutant« PKSs necessary to achieve, in theory, a library of 262 144 polyketides, as 6-dEB analogs. From only 14 vectors prepared, 64 triple transformants producing 43 different polyketides were obtained (*25*). Therefore, in the last two papers cited about 50 polyketides were derived.

The targeted approaches of Biotica, Kosan and TerraGen have been validated and generated several hundred novel aromatic and complex polyketides. However, the derivation of such recombinant strains is time and labour consuming with a relatively low output of novel compounds, giving a low chance that one will have a novel biological activity. For these reasons scientists started to look for novel strategies.

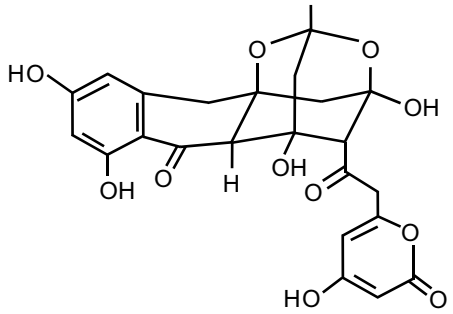

Fig. 11. Structure of the dodecaketide TW93h obtained by the expression of the *whi*E »minimal« PKS in the *Streptomyces coelicolor* strain YU105/pIJ4293 (modified according to *22*)

Maxygen have, for example, developed a rational approach called »gene shuffling« by recombining alleles of a particular gene from different sources using the PCR. The PCR products are sheared mechanically and repeated PCR reactions are run using the broken fragments. In this way, mosaic genes are created that can be used for the generation of novel products (*26*). The disadvantage of this technology is that it is limited to DNA fragments of only a few kb in size. The sizes of polyketide gene clusters range from 20 to more than 100 kb. In January 2000, Maxygen published a patent application showing that they can perform »gene shuffling« using gene-clusters and even the whole genomes (*27*). These novel approaches will be supported by the development of novel tools for combinatorial biology such as the development of a database of natural products (*28*) and a database of biosynthetic pathways (*29*). Nevertheless, once the technology for the rapid generation of much larger numbers of structures of novel chemical entities is developed and validated, this field of research will have a significant success.

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Molekularna biologija biosinteze poliketida

Sažetak

Vrste roda *Streptomyces* i taksonomski srodni rodovi sintetiziraju veliki broj sekundarnih metabolita od kojih su mnogi biološki aktivni. Među njima poliketidi su najveća skupina. Oni su strukturalno raznovrsna porodica prirodnih spojeva s raznovrsnim spektrom bioloških aktivnosti. Sinteza je poliketida vrlo slična biosintezi ravnolančastih masnih kiselina i u enzimskim reakcijama i u proteinima koji te reakcije kataliziraju. U posljednjem je desetljeću kloniran i sekvenciran veliki broj poliketidnih genskih nakupina. Sekvenciranje genskih nakupina pokazalo je da među njima postoji bitna podudarnost na razini DNA te da, prema tome, vjerojatno potječu od zajedničkoga pretka. Ti rezultati upućuju na mogućnost primjene kombinatorne biologije u kreiranju novih kemijskih supstancija. U tu se svrhu primjenjuju dva pristupa: tzv. »ciljani« pristup inaktivacijom, a često i zamjenom, određenih gena čiji produkti sudjeluju u biosintetskom putu, te tzv. »nasumičan« pristup nazvan »miješanje DNA«. Ciljani je pristup upotrijebljen za proizvodnju novih poliketidnih struktura manipulacijom genske nakupine vrste *S. rimosus*, odgovorne za sintezu antibiotika oksitetraciklina. Inaktivirani su geni koji sadržavaju genetičku uputu za ketosintaze α i β, ketoreduktazu, ciklazu/aromatazu i C-6 hidroksilazu radi konstrukcije četiriju rekombinanata. Tankoslojna kromatografija i visokotlačna tekućinska kromatografija ekstrakata prevrelih komina svih četiriju rekombinanata pokazala je da oni sintetiziraju dvadesetak potencijalnih novih metabolita od kojih je 12 kemijski okarakterizirano. U ovom će revijalnom prikazu iscrpnije biti opisana inaktivacija i zamjena *otc*C gena.