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Biogenesis of antibiotics—viewing its history and glimpses of the future

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Abstract This review aims at comparing some historical data with the current situation in the study of biogenesis of natural compounds, antibiotics in the first place. Biogenesis of tetracyclines and cycloheximide and related compounds serves as example. Examples of molecular biological and bioinformatics methods used in the study of antibiotic biogenesis are described both in terms of its historical aspects and the current knowledge.

Introduction

All living organisms produce a plethora of biologically active compounds. In this review, we will concentrate on so-called secondary metabolites, i.e., compounds that are not absolutely essential for their producers; nonproducing mutants exist that live and multiply without producing such compounds. Antibiotics belong to this group of compounds. Although the numbers are apparently only rough estimates, among eubacteria, actinobacteria seem to be the most efficient antibiotic producers followed by fungi. In total, microorganisms produce more than 40,000 antibiotics, whereas "higher" organisms including plants and even animals produce some 25,000 antibiotics (see Table 1).

The total estimated number of natural antibiotics varies from about 66,060 to 70,560. The number of semisynthetic and synthetic compounds derived from them can be estimated as 100,000 or even more. Out of this high number of compounds, only about hundreds are used in clinical practice.

The numbers are highly approximate and the actual list of antibiotics produced by microorganisms would be probably much higher. In any case, the numbers of natural antibiotics are estimated to be of the order of 10^4, total antibiotics including semisynthetic compounds amount to 10^5, whereas only roughly 10^2 are used in clinical practice. Most applicable antibiotics were discovered and brought to market during the so-called Golden Age as demonstrated by Hopwood (2007) (Fig. 1).

According to Bibb and Hesketh (2009) actinomycetes, the family to which the streptomycetes belong is responsible for the production of over two-thirds of known antibiotics. The list presented here is only intended to reflect proportions of the compounds produced by different microorganisms. Each secondary metabolite including antibiotics is formed from products of primary metabolism. The chemical structure of a

Dedicated to the late Zdenko Vaněk, an eminent scientist and dear friend.

Motto: Sir David A. Hopwood: Zdenko passed away: "It is indeed the end of an era."

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Table 1 Main antibiotic producers

Microorganisms	Number of produced antibiotics
Producing organism	
Actinobacteria	14,500
Streptomyces sp.	11,000
Other actinobacteria	3400
Eubacteria	
Myxobacteria	450
Cyanobacteria	400
Other bacteria	800
Fungi	
Microscopic fungi (*Aspergillus*, *Penicillium*)	9000
Basidiomycetes	2900
Other fungi including yeasts, slime molds, etc.	110
Total microorganisms	42,560
Higher organisms	
Higher plants	15,000–16,000
Lower plants including algae, mosses, and lichens	1500–2000
Products of terrestrial animals	1000–2000
Marine products (plants and animals)	6000–8000
Total	23,500–28,000
Total estimated number of natural antibiotics	66,060–70,560

From Berdy 2015

secondary metabolite usually indicates which biosynthetic pathway is involved in its production. The diversity of secondary metabolites is enormous, but in spite of this, their biosynthesis is in principle based on variations of only several essential pathways, viz. synthesis of polyketides, peptides or polypeptides, terpenes or sterols, etc., or their combinations. Tetracyclines, cycloheximides, and erythromycins may serve as examples of polyketide antibiotics.

A number of review articles on biogenesis of antibiotics and antibiotic production have relatively recently been published including papers by Demain and Sanchez (2009), Bibb and Hesketh (2009), Lee et al. (2009) and Demain and Spizek (2012). Other valuable, indeed historical reviews were published in the nineties (Hopwood et al. 1995; Doull and Vining 1995; Champness and Chater 1994; Chater and Bibb 1997).

The current situation has recently been described in excellent review articles published in *Nature Chemical Biology* (Focus on Biosynthesis, Volume 11, No. 9, Sept 2015). It is clear that the improved tools and procedures make it possible to obtain new information on the promising applications of natural products biosynthesis (Spížek et al. 2010). These compounds, especially antibiotics, found important applications in human and veterinary medicine. The enzymes catalyzing their biosynthesis apparently play a crucial role. Specificity of individual enzymes determines what building blocks leading to the formation of secondary metabolites will be incorporated and how they will be modified. The information obtained can be used to predict the resultant compounds. Gene clusters coding for a particular molecule can now be quickly identified from comparisons with thousands of genomes identified so far. At present, informatic tools can be used to predict what molecules will be produced. The massive information on genomic data indicates what additional clusters may

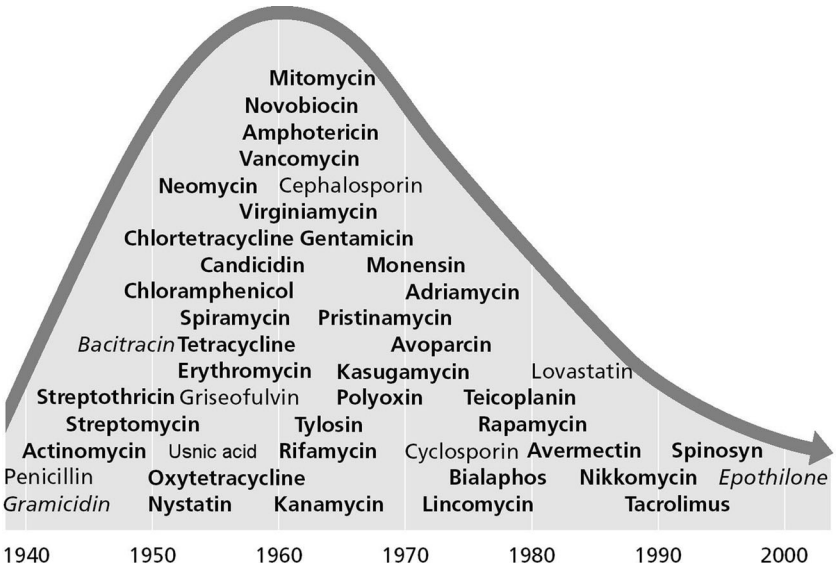


Fig. 1 Discovery of important antibiotic and other natural products over the years. *Bold type* indicates actinomycete products, *normal type* indicates fungal products, and *italic type* indicates products from nonactinomycete bacteria (according to Hopwood 2007, p. 21).

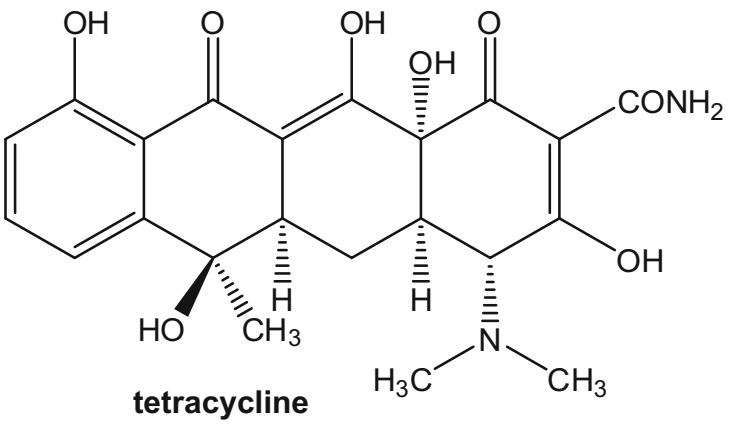
be used for the search of enzymes that can create unexpected chemical diversity which might arise from modifications such as, for instance, glycosylations of different natural compounds. Combinatorial biosynthesis as well as tools of synthetic biology can be used for the production of compounds with improved properties.

Biogenesis—a little history and some recent findings

Tetracyclines

In a stimulative hypothesis entitled "How many genes are needed for the biosynthesis of chlortetracycline" (Vaněk et al. 1971; Hošťálek and Vaněk 1985), the biosynthetic process leading to tetracyclines was divided into several parts of metabolic pathways, one part leading from glucose to pyruvate, another part of its transformation to acetyl-CoA and formation of a hypothetical tricyclic nonaketide, and the part of enzymatic reactions leading to the final product, i.e., chlortetracycline. This last part of biosynthesis was proposed to represent a chain of 11 enzymatic steps, compiled on the principles of logical chemical reactions. A hypothetical biosynthetic map was constructed leading to both known tetracycline structures and a number of additional tetracyclines which could occur among the metabolites of *Streptomyces aureofaciens*. In addition to confirmed 27 tetracycline structures, 45 other were proposed, which could occur among the metabolites of *S. aureofaciens*. It was proposed that 200–400 genes are involved in the biosynthesis of tetracycline and a cluster of genes was also postulated participating in final steps of tetracycline biosynthesis. It was also suggested that a similar theoretical approach could certainly be extended to secondary metabolites other than oligoketides and further improve our knowledge in the field which, in spite of its extraordinary importance in practical applications, remained only little elaborated in its theoretical aspects (Vaněk et al. 1971).

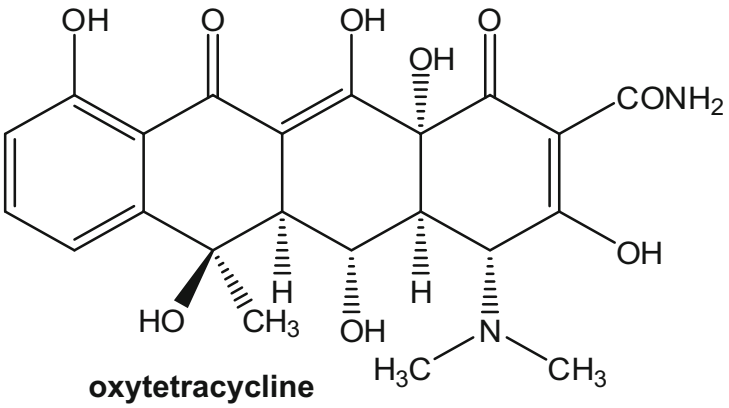
As far as Vaněk's predictions are concerned, it is very difficult to compare them with the present knowledge. Twenty-five structural genes were described in the *otc* cluster of *Streptomyces aureofaciens*, whereas 24 genes were identified in the *oxy* cluster of *Streptomyces rimosus* (Yin et al. 2015). According to Pethick et al. (2013), *Streptomyces rimosus* genome includes 9.5 Mbp and some 48 clusters coding for secondary metabolites. Out of these, three clusters are known as to their products, while the 45 remaining are putative secondary metabolite biosynthetic clusters whose functions remain to be elucidated. Thus, as compared with the original Vanek's proposal, it appears that the number of clusters involved in the secondary metabolite biosynthesis in streptomycetes might be higher by an order of magnitude. It would be very difficult to find the real numbers since an overwhelming majority of the genes code for unknown proteins (Pethick et al. 2013).



tetracycline

Since the above findings, a number of results on the biosynthesis of tetracyclines were obtained, parts of the respective biosynthetic pathways were elucidated, and genes specifying them were identified. The unique

structural features of tetracyclines provide exciting opportunities for both synthetic chemists and biomolecular engineers. The recent knowledge gained from sequencing of the tetracycline producing gene clusters has made it possible to construct biosynthetic plans of these pathways. The systematic investigation and rational engineering of the oxytetracycline biosynthesis have provided the first insight into the combinatorial potential of these polyketide pathways.



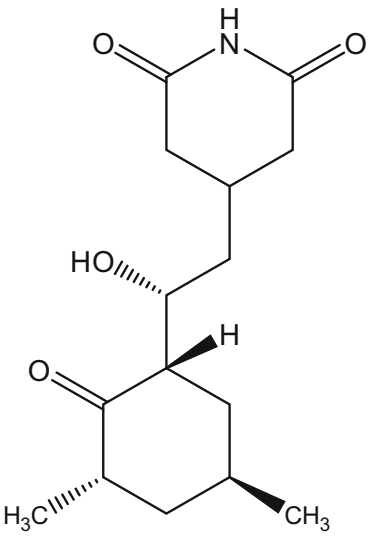
oxytetracycline

In their highly interesting paper, the excellent Slovenian group headed by Petković, with international coauthors from Germany, Spain, and Scotland (Lešnik et al. 2015), indicated that several existing natural product scaffolds (including chelocardins; CHD) have not been developed because their suboptimal pharmacological properties could not be addressed at the time. The authors proposed that reviving such compounds through the application of biosynthetic engineering can provide novel drug candidates. They introduced the carboxamido moiety of tetracyclines, which is responsible for their activity, into the atypical tetracyclines chelocardins, whose mechanism of action remains unresolved, and generated a broad-spectrum antibiotic lead with an improved activity against all Gram-negative pathogens of the ESKAPE group. Since the lead structure is also accessible to further chemical modification, according to the authors, it may serve as a platform for further development through medicinal chemistry and genetic engineering. Enzymatic mechanisms leading to carboxamido moiety formation in tetracyclines are apparently of medicinal interest. The authors were the first to successfully perform the heterologous expression of acyltransferase (AT) and amidotransferase (AMT) from the *otc* cluster to derive a novel malonamate-primed molecule. The addition of the carboxamido moiety significantly increased the biological activity of CHD. The acetate-primed impurity of oxytetracycline (OTC), 2-acetyl-2-decarboxamido-oxytetracycline (ADOTS), exhibited a tenfold reduction of the antibacterial activity. Their findings for CHD are thus in agreement with the structure-activity relationships (SAR) found in typical tetracycline (TC) antibiotics. The authors showed clearly that the finding of incorporation of the carboxamido moiety into chelocardins provides a unique diversification of the TC backbone and that the resulting derivatives show a potent antibacterial activity and could thus prove to be a promising drug lead. They demonstrated efficient incorporation of the carboxamido starter moiety, important for tetracycline activity, into CHD and could obtain a high yield of this novel compound 2-carboxamido-2-deacetyl-chelocardin (CDCHD). The authors directly transferred the developed process to an industrial scale. The usefulness of this bioengineering approach was thus not only demonstrated but also developed into a unique tetracycline lead with potent

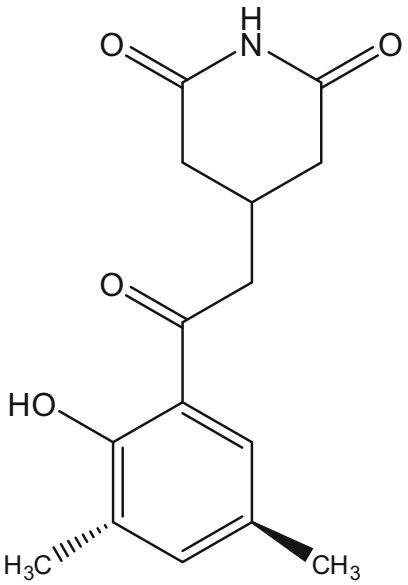
antibacterial activity. This class of compounds can apparently be further diversified by semisynthesis or additional biosynthetic engineering to develop urgently needed novel broad-spectrum antibacterial drugs. Metabolic engineering and engineered biosynthesis, combined with chemical synthesis, will apparently play valuable roles in the discovery and production of future generations of tetracyclines.

Cycloheximide and related compounds

The study of biosynthesis of cycloheximide and its derivatives in Zdenko Vaněk's laboratory pointed out (Vaněk et al. 1962) that cycloheximide is formed by condensation of five acetate units and one malonamide unit and that the methyl groups of a dimethylcyclohexanone ring are formed by transmethylation reactions. It was also found that the producing organism protects itself from the toxic concentration of cycloheximide by aromatization of dimethylhexanone ring into quite biologically inert actiphenol, which was one of the first examples of resistance mechanisms protecting the producer against suicide. It was also shown that cycloheximide added to the culture medium of *Streptomyces noursei* interfered with a further accumulation of the antibiotic indicating that a control mechanism resembling repression or feedback inhibition operates in this system. In addition, fungicidin added to the same culture decreased its own production and increased production of cycloheximide (Spížek et al. 1965). As compared with the biosynthesis of tetracyclines, more recent data on the biosynthesis of cycloheximide have long been rather scarce, due perhaps to the fact that medicinal and other uses of tetracyclines are much more significant.



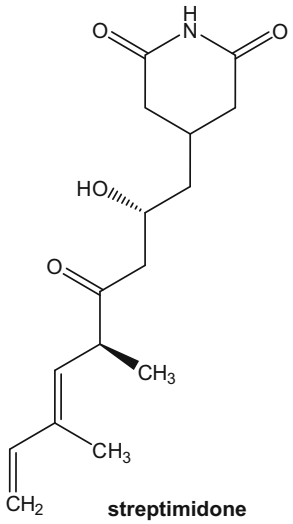
cycloheximide



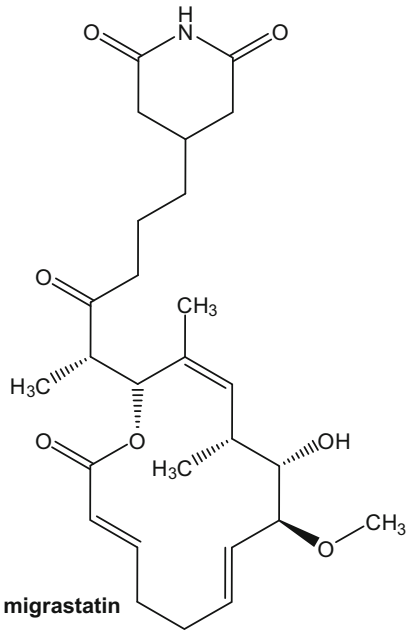
actiphenol

Cycloheximide is the best known member of the glutarimide-containing polyketide family of natural products and has been used for many years as an inhibitor of eukaryotic translation. Actiphenol has the

same carbon skeleton as cycloheximide but contains a phenol in place of the cyclohexanone moiety and only exhibits a low translation inhibition activity. Other members of this family include streptimidone, 9-methylstreptimidone, iso-migrastatin, migrastatin, and lactimidomycin. Whereas cycloheximide inhibits translation globally, lactimidomycin inhibits preferentially translation initiation but not elongation (Yin et al. 2014). The authors discovered the production of cycloheximide in two *Streptomyces* species and found that both of them also produced actiphenol. This property was reported previously in *Streptomyces griseus*, *Streptomyces albulus*, and also in *S. noursei* in Vaněk's laboratory.

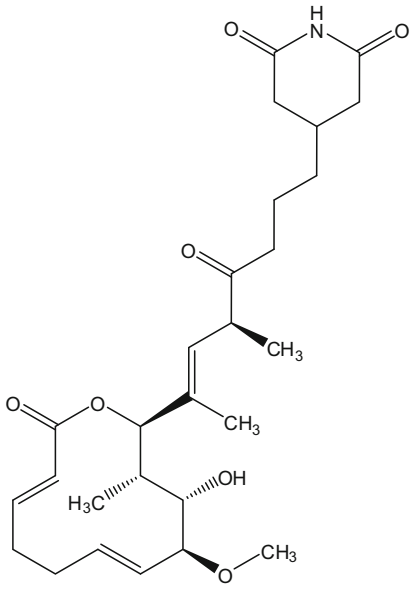


streptimidone

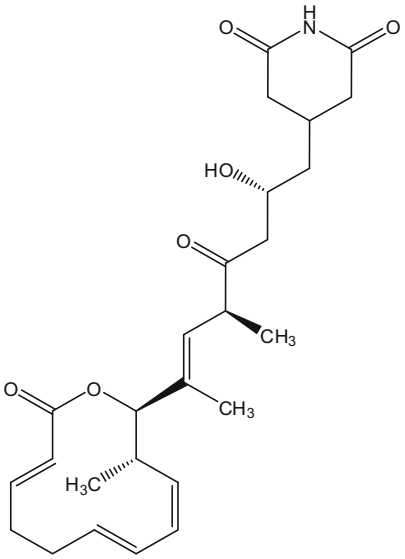


migrastatin

Cloning, sequencing, and characterization of a gene cluster from *Streptomyces* sp. YIM65141 showed that production of cycloheximide and actiphenol is governed by a single biosynthetic machinery. As summarized by the authors, the biosynthesis of cycloheximide is catalyzed by an acyltransferase-less type I polyketide synthase giving rise to its carbon backbone, but it may also proceed via actiphenol as a key intermediate, leading to hydrogenation of a phenol to a cyclohexanone moiety in natural product biosynthesis. The glutarimide-containing polyketide backbone of cycloheximide is apparently assembled similarly to that of other members of this family of natural products, such as iso-migrastatin, migrastatin, and lactimidomycin, catalyzed by an AT-less type I polyketide synthase (PKS). Yin et al. (2014) showed clearly that comparative studies among these machineries provide an outstanding opportunity to study glutarimide biosynthesis and many of the common features unique to AT-less type I PKSs. They conclusively demonstrated that ChxG and ChxH proteins are necessary and sufficient to catalyze the conversion of actiphenol to cycloheximide as the last two steps of cycloheximide biosynthesis, leading to a highly interesting phenol-to-cyclohexanone reduction that is apparently unparalleled in natural product biosynthesis.



iso-migrastatin



lactimidomycin

We feel that it would be worth the effort to investigate whether regulatory phenomena such as feedback or allosteric inhibition are involved in this highly interesting system.

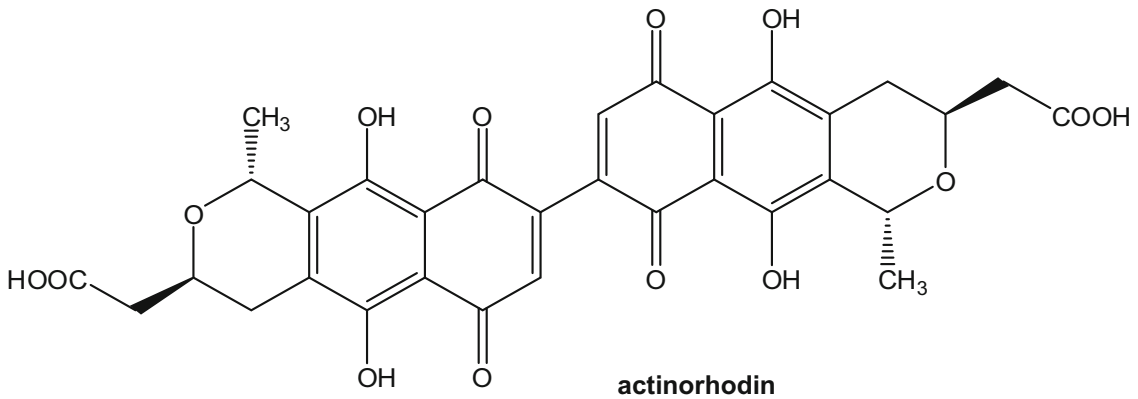
Molecular biology

History

The experimental data collected during the years of aromatic polyketide studies showed that it is possible to really consider the preparation of newly engineered substances possessing new properties. By choosing a combination of a β-ketoacyl:acyl carrier protein synthase and chain length factor pair, an acyl carrier protein, one or more polyketide synthases to define the length of the polyketide and subsequent cyclization, and a β-ketoacyl reductase or genes specific for the starter unit, it is possible to engineer the formation of novel compounds rationally (Shen et al. 1995). A specially designed host-vector system was developed consisting of *Streptomyces coelicolor* strain CH999 from which the *act* aromatic PKS gene cluster and other *act* genes have been deleted, together with the expression plasmid pRM5. The concept of rational design was illustrated by the preparation of *Streptomyces* strains that produce two new polyketides via expression of combinations of appropriate enzymatic subunits from naturally occurring polyketide synthases.

Great progress was achieved by using molecular biology techniques. It was proved that the so-called biosynthetic genes encoding secondary metabolites are organized in clusters. Parts of them are regulatory genes and genes involved in resistance, not only for final products (antibiotics) but also for intermediates which are also toxic to producing microorganisms (Martín and Liras 1989). The appearance of resistance is an extraordinarily important discipline, not only from the point of view of the so-called highly productive strains (Davies 1992) but also mainly because of unusually easy occurrence of microbial strains in hospitals resistant to the different chemotherapeutics (e.g., multidrug resistance, etc.). To understand the differences between the synthesis of aromatic polyketides and the synthesis of polyketides of the macrolide type, the recognition of two types of fatty acid synthases was of great importance. Type I synthase resembles the vertebrate FAS and type II which occurs in bacteria and plants. For several aromatic polyketides, sequencing revealed type II architecture, with a cluster of genes coding for the components of each PKS. The building units for chain assembly are transferred from CoA directly to a cysteine thiol on the synthase, but this thiol is not part of a motif that resembles the active site

of other FAS- or PKS-condensing enzyme (for review, see Hopwood et al. 1995). Comparison of the polyketide and fatty acid synthases has led to the conclusion that these systems must share a common mechanism of carbon chain assembly from similar, if not identical, precursors. The principal challenges to understanding the catalytic mechanism of the type II polyketide synthases were to determine how the starter unit is specified, how the length of the growing polyketide chain is controlled, how the nascent polyketide chain is folded into the correct conformation for cyclization, and how the carbonyl and methylene groups are directed to form the six-membered rings of the final cyclic compounds (Shen et al. 1995). Two papers have shown that malonyl coenzyme A:acyl carrier protein acyltransferase might be shared by both pathways—fatty acid and polyketide synthases (Revill et al. 1995; Summers et al. 1995). Moreover, Shen et al. (1995) have brought evidence that in *S. noursei* producing tetracenomycins, *tcmKL* polyketide synthase and *tcmN* polyketide cyclase genes govern the size and shape of aromatic polyketides. The possibility of "cross talk" or interference with fatty acid biosynthesis might be shared by both pathways—fatty acid and polyketide synthases (Revill et al. 1995; Summers et al. 1995). The possibility of cross talk or interference with fatty acid biosynthesis was also discussed in the work of Yu and Hopwood (1995), following the ectopic expression of the *S. coelicolor whiE* genes for polyketide spore pigment synthesis and their interaction with *act* genes for actinorhodin biosynthesis. Most of the research and methods used in *Streptomyces* genetics were described in an excellent monograph by Hopwood (2007).



actinorhodin

The present time

In an excellent recent review article, Wohlleben et al. (2012) proposed that actinomycetes are a rich source for the synthesis of medically and technically useful natural products and that the genes encoding the enzymes for their biosynthesis are normally organized in gene clusters, which include also the information for resistance (in the case of antibacterial compounds), regulation, and transport. According to the authors, this facilitates the manipulation of such pathways by molecular genetic techniques. Recent advances in DNA sequencing and analytical chemistry revealed that not only new strains isolated from as yet unexplored habitats but also already known strains possess a large potential for the synthesis of novel compounds. Synthetic biology now offers a new perspective to exploit this potential further by generating novel pathways and thereby novel products through combining different biosynthetic steps originating from different bacteria. As specified by the authors, the procedures involved include production of precursors of biosynthesis and their potential for generating diversity, engineering precursor biosynthesis for optimizing antibiotic production, including increasing the product yield, and finally the impact of lateral gene transfer on the evolution and diversity of biosynthetic gene clusters.

Many previously undetected microbial biosynthetic pathways exist, thus much new chemistry could still be found and many kinds of biosynthetic enzymatic transformations are still to be characterized. The enzymes for the production of secondary metabolites, or natural products, are generally encoded by biosynthetic gene clusters (Walsh 2015). The importance of genome mining, including the research programs capable of sequencing whole culture collections of bacterial and fungal strains and identification of biosynthetic gene clusters in genome sequences, was accentuated. Strategies for identification of biosynthetic gene clusters in genome sequences were explained, with the assumption that all secondary metabolic

enzymes are distant analogs of primary metabolic enzymes. Two main approaches to identifying biosynthetic gene clusters in metagenomes were proposed, viz. the PCR-based sequence-tag approach and the shotgun assembly approach. Metagenome sequences were discussed as a special challenge. Thousands of gene clusters can be handled effectively, with an approach "from genes to chemistry," thus predicting an entire chemical structure from scratch, matching genes to molecules using mass spectrometry, connecting multiple data types with networking approaches, and the question of how computation will change natural product workflows. Examples of enzymes that are most important in natural products chemistry, viz. oxygenases catalyzing key chemical steps in biosynthetic pathways, various glycosylases, enzymes catalyzing complex cyclizations, polycyclic fungal alkaloid biosynthetic pathways, and so-called hybrid assembly lines, were described. In a highly stimulating conclusion, the author indicated future perspectives in this field (Walsh 2015).

In the article "Computational approaches to natural product discovery," the authors (Medema and Fischbach 2015) presented their idea of how to computationally identify thousands of biosynthetic gene clusters (abbreviated BGCs by the authors) in genome sequences and explore them for experimental characterization. The authors define BGC as a physically clustered group of two or more genes in a particular genome that code for a biosynthetic Gene Cluster ("Minimum Information about a Biosynthetic Gene Cluster," abbreviated MIBiG). MIBiG provides a standardized specification of BCG annotations and gene cluster-associated metadata that will make their systematic deposition in databases possible. According to the available data, the authors constructed a MIBiG-compliant seed dataset. Moreover, a large part of the research community has committed to continue submitting data on newly characterized gene clusters in the MIBiG format in the future. Together, the MIBiG standard and the resulting MIBiG-compliant data sets will allow data infrastructures to be developed that will facilitate key future developments in natural product research. In this review, the authors proposed the systems and procedures should be adopted with the aim of making the international cooperation in this field possible.

In their review article "Reinvigorating natural product combinatorial biosynthesis with synthetic biology," Kim et al. (2015) discussed possibilities of extending nature's chemistry through combinatorial biosynthesis, viz. by altering functional groups, regiochemistry, and scaffold backbones through the manipulation of biosynthetic enzymes. According to the authors, this offers unique opportunities to create natural product analogs. The authors also proposed a new perspective for the combinatorial biosynthesis of natural products that could revive drug discovery by using synthetic biology in combination with synthetic chemistry. In the paragraph entitled "Building up new machines," they presented examples illustrating a few case studies in which unnatural building blocks can be selectively incorporated into PKs or NRPs using noninvasively engineered AT or A domains. Different *Streptomyces* hosts have been used for the expression of heterologous genes, *S. coelicolor*, *S. lividans*, *S. albus*, and the avermectin producer *Streptomyces avermitilis* being the most commonly employed. Although most existing synthetic biology tools are designed for *E. coli* and may not be directly applicable to combinatorial biosynthesis in actinomycetes, advancements in synthetic biology methodology offer new approaches to address the problems associated with combinatorial biosynthesis. According to the authors, the field of combinatorial biosynthesis is now ready to tackle the current challenges in natural product drug development.

Biosynthetic genes of microorganisms are organized in clusters coding for large multidomain and multimodular enzymes, e.g., polyketide synthases, prenyltransferases, nonribosomal peptide synthases, and terpene cyclases. Multiple gene clusters encoding secondary metabolites are common in species of *Streptomyces*. *S. coelicolor* and *S. avermitilis* contain 20–30 of these clusters (Demain 2014).

According to Katz and Baltz (2016), the history of natural products discovery can be divided into three overlapping periods, viz. 1940s–1970s, 1970s–2000s, and 2000 and further. The authors chose these periods because significant progress during these periods dramatically affected the strategies used to discover new natural products including antibiotics. During the first years (1940–1970), penicillin was discovered but, even more importantly, Waksman and his group started screening soil samples leading to the discovery of actinomycin, streptothricin, and, most importantly, streptomycin. This induced extensive screening of various extracts for antibiotic activity. According to the authors, during those first 30 years, more than 1000 natural products exhibiting antibacterial or antifungal activity were discovered. These included some 35 antibiotics for uses in human medicine. During the second period (1970–2000), new sources were used for the discovery of natural products. They included marine environments and the samples collected included bacteria, algae, and marine invertebrates (tunicates, corals, sponges, etc.). However, it should be mentioned here that the samples were mainly tested for antitumor activity. During this period, combinatorial chemistry was developed giving rise to thousands of compounds. According to the authors, the last period (2000 and later) was based on genomics-based approaches. The first two *Streptomyces* genome sequences showed that many secondary metabolite gene clusters are encoded in their large genomes, followed by other streptomycetes with large genomes. It may be assumed that many secondary metabolite gene clusters can be used for production of new compounds.

In the review article entitled "The structural biology of biosynthetic megaenzymes," Weissman (2015) described the role of biosynthetic megaenzymes. The modular polyketide synthases (PKSs) and nonribosomal peptide synthetases

(NRPSs) are among the largest and most complicated enzymes in nature. Products of PKSs and NRPSs include a number of important medicines, and this has motivated researchers to understand how they operate so that they can be modified by genetic engineering. In the review, the author showed a number of examples as to how these modular multienzymes operate and what their role in the biosynthesis of natural products including antibiotics is. Although sound progress was reached, there are apparently areas in which we lack sufficient information, viz. how the modules interact with each other.

According to Keller (2015), filamentous fungi are renowned for producing a diverse array of secondary metabolites (SMs) where the genetic material required for the synthesis of a SM is typically arrayed in a BGC. SMs (in their majority, low-molar-mass compounds) can even be produced in large amounts and can play roles in a range of cellular processes, such as transcription, development, and intercellular communication. Some of them have practical applications, e.g. as antibiotics or immunosuppressants. According to the author, the capability of fungi to produce secondary metabolites has been substantially underestimated due mainly to the fact that many of the fungal secondary gene clusters are silent under standard cultivation conditions. The improved knowledge of regulatory mechanisms involved in the regulation of biosynthesis of secondary metabolites will certainly lead to a better understanding of the physiological and ecological functions of these important compounds and should also lead to drug discovery through targeted activation of silent gene clusters.

Keller also discusses self-protection mechanisms preventing producing microorganisms from suicide and indicates that a better understanding of the physiological and ecological functions should lead to the discovery of new or modified and practically important drugs.

In another review article of this series titled "Minimum Information about a Biosynthetic Gene Cluster," a coalition of about 150 scientists (Medema et al. 2015) showed that a wide variety of enzymatic pathways producing secondary metabolites in bacteria, fungi, and plants are encoded in biosynthetic gene clusters. According to the authors, the information about these clusters, pathways, and metabolites is widely distributed throughout the scientific literature which makes it difficult to exploit the available data. With the aim of facilitating solid and systematic storage and recovery of data on biosynthetic gene clusters, the authors propose that the standard MIBiG should be used. In the schematic overview proposed by the Genomic Standard Consortium, they include general parameters and compound type-specific parameters incorporating polyketides, nonribosomal peptides, post-translationally modified peptides, terpenoids, saccharides, and alkaloids. The authors propose the MIBiG data standard and submission system which should lead to a continuously growing set of data that will be loaded into several databases and web services. The research community represented in the paper in question has committed itself to submitting the MIBiG data sets and updates to existing entries, when publishing new experimental results on BGCs. The larger scientific community is encouraged to join this attempt.

Discussion and conclusions

Resistance to antibiotics continues to increase and constitutes a global threat to human health. It appears that the fight against antibiotic resistance is a war that can never be won. The strength of the immense numbers of microorganisms appears to overpower our drugs.

It is mainly due to the fact that some antibiotics are too toxic or poorly water-soluble, have other pharmacologically unsuitable properties, or have effects identical to those already used in the clinical practice. Does it thus make sense to look for new antibiotics that would help us against antibiotic resistance? According to Davies and Davies (2010), the importance of antibiotics cannot be overestimated. We are totally dependent on them for the treatment of infectious diseases, and they should never be considered mere commodities. In addition to their use in the treatment of infectious diseases, antibiotics are critical to the success of advanced surgical procedures, including organ and prosthetic transplants. For years, antibiotics have provided an effective treatment for a number of diseases caused by microorganisms. However, as mentioned above, the number of microorganisms that have adapted to the current antibiotics has increased dramatically. Many challenges face the discovery and development of new antibiotics, making it difficult to reach the market, especially since many pharmaceutical companies stopped searching for new antibiotics due mainly to the fact that the antibiotic production does not pay back. With the advent of genome sequencing, new antibiotics are being found by genome mining that offers hope for the future (Scheffler et al. 2013). In this review, also we wanted to show a few examples of how biogenesis of antibiotics can be used for the practical production purposes and could help to improve the situation concerning antibiotic resistance. We presented the biogenesis of tetracycline antibiotics and cycloheximide and related compounds as examples. It appears to us that microbial natural products still represent the most promising source of new antibiotics.

Whereas the classical approaches including mutagenesis and selection may still be useful, genomic-based methods will certainly predominate in the future. We should also keep in mind that microorganisms living in extreme environments such as high temperature, high salt concentration, and deep sea vents will continuously serve as sources of new natural compounds including antibiotics.

According to the old but still interesting mathematical model of Watve et al. (2001), it should be possible to identify some

294,300 compounds that have not yet been described. Even if the mathematical model is questioned by some mathematicians, it may still be reasonably estimated that only 3 % of the antibiotics produced by streptomycetes have so far been isolated.

We propose that it will be useful to combine the efforts of both academia and industry in the fight against antibiotic resistance. As concluded by Davies and Davies (2010), we should renew a concerted attack on antibiotic resistance; otherwise "the preantibiotic era awaits our descendants." We feel that the study of biogenesis of antibiotics with the aim of discovering new compounds is a useful field of research. Metagenomic and other studies indicate that a number of secondary metabolite gene clusters could still be discovered. With some degree of optimism, it appears that a second "Golden Age for Antibiotics" can be predicted (Katz and Baltz 2016).

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