Future Antibiotic Agents: 
Turning to Nature for Inspiration

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1. Introduction

Few drugs have made such a profound impact on modern medicine as antibiotics. With the discovery of sulfonamides, β-lactams, and subsequent antibiotic classes after World War II, bacterial infections with often fatal outcomes literally became curable overnight. These “magic bullets,” however, suffer from a serious drawback; the use (and misuse) of antibiotics induces selection pressure resulting in the development of resistance traits in bacterial populations. The process is augmented by short generation times of bacteria enabling rapid mutation and selection of resistant strains, and a horizontal transfer of resistance genes. Bacterial pathogens resistant to more than one, or even most clinically used antibiotics, have become common (Fischbach & Walsh, 2009). Faced with the fact that a renewed pre-antibiotic era might be just around the corner, the World Health Day 2011 campaign “Antimicrobial resistance and its global spread” launched by the WHO offers a strategy to safeguard existing antibiotics for future generations, and contain the spread of antimicrobial resistance (World Health Organization, 2011). However, taking a more sensible approach in prescribing and using available antibiotic drugs will only help to put off the inevitable. In the battle against the ever-increasing multidrug resistance of pathogenic bacteria, we urgently need new alternatives to the currently available broad-spectrum antibiotics. Here we review some current trends in antibiotic discovery focusing on the screening of natural products.

This chapter is composed of four parts. We start by briefly reviewing the history of antibiotic discovery, gradually moving from its most fruitful era in the 1940’s and 50’s to the unexpected outcome decline in the genomic era. Next, we address two fundamental questions of antibiotic research in the post-genomic age; namely, where to look for novel antibiotics and how. Finally, we conclude with a concise discussion on the modification of natural scaffolds to be translated into functional drugs.

2. History of antibiotic discovery

2.1 The golden age of antibiotic discovery

Before the discovery of prontosil, the forerunner of sulfonamide chemotherapeutics, the only available measures in combating bacterial infections, apart from practicing proper
hygiene, were vaccination and passive immunization. Although these approaches are still invaluable today, the advent of broad-acting antibacterial agents enabled rapid treatment of patients with infections even when the exact causative bacterial pathogen was unknown. Prontosil, later found to be a prodrug releasing folate antimetabolite sulfanilamide upon reduction in vivo, was a result of a screening campaign at Bayer, Germany in the early 1930’s, aimed at finding synthetic dyes for the potential effect on hemolytic streptococcal infection (Greenwood, 2003). Although the premise that dyes in general should exert antibacterial activity turned out to be incorrect, prontosil paved the way for antimicrobial drugs, becoming the first commercially available antibacterial agent and remained in clinical use for 30 years. Moreover, it inspired new generations of sulfonamide chemotherapeutics, some of which remain on the market today. All major antibiotic classes that form pillars of antibacterial therapy were derived from natural sources — mostly microbial secondary metabolites (Molinari, 2009) — with the exception of sulfonamides and quinolones, inhibitors of bacterial DNA gyrase that were discovered in the 1960’s.

The groundbreaking work of Rene Dubois, who studied antibiosis in pairs of soil microorganisms, eventually leading to the discovery of a mixture of peptidic antibiotics collectively termed tyrothricin (its component gramicidin is still in limited use), inspired Selman Waksman and Boyd Woodruff to adopt the principle in systematic search for novel antibiotics (Kresge et al., 2004). It is now well recognized that many microbes produce structurally extremely diverse, small molecules that are involved in complex intra- and interspecies signaling (Shank & Kolter, 2009). Antibiosis is only one of the many possible outcomes of such interaction, but is most readily detected: Waksman and Woodruff looked for growth inhibition zones surrounding single colonies of soil microorganisms cultured under different conditions and then isolated the active substance from pure cultures by activity-guided fractionation (Waksman & Woodruff, 1940). The same route led to the earlier serendipitous discovery of penicillin by Alexander Fleming in 1929 (Fleming, 1929). It took almost 15 years to scale up penicillin production and demonstrate its efficacy and safety. By that time numerous other antibiotic types were emerging (Fig. 1). The major source of antimicrobials turned out to be soil actinomycetes, such as the Streptomyces species, and various fungi. Considering the abundance of soil microbes (there are estimates of $10^9$-$10^{10}$ bacteria in a single gram of soil belonging to some $10^4$ operational taxonomic units (Curtis et al., 2006; Gans et al., 2005)) the explosion in antibiotic discovery that began in the early 1940’s is not surprising from current perspective. However, not all species are equally represented. Moreover, many related bacteria or fungi produce the same or similar secondary metabolites. For example, streptothricin was found in ~10%, streptomycin in ~0.1% of randomly collected soil actinomycetes (Baltz, 2007). So when the most abundant antibiotics were identified the pace of natural antibiotic discovery gradually slowed down (Baltz, 2007, 2008), finally culminating in a 30-year-gap in launching an antibiotic with a novel scaffold to the market (Fig. 1). The situation was exacerbated by ever stricter regulatory demands on safety and efficacy of drugs, the wrongful perception that bacterial infections no longer posed a severe threat to human health, as well as the unfavorable economics of antibacterial development (revenue from antibiotics is significantly lower compared to drugs indicated for chronic diseases), leading to a withdrawal of Big Pharma from the antibiotic business (Brötz-Oesterhelt & Sass, 2010; Fischbach & Walsh, 2009; Projan, 2003). Meanwhile, the development and spread of bacterial resistance was steadily incising into our antibacterial arsenal (Fig. 1).
Fig. 1. Timeline for the introduction of major, broad-spectrum antibiotic classes for systemic application in the clinic, and documented occurrence of bacterial resistance (adapted from (Brötz-Oesterhelt & Sass, 2010)). The asterisk denotes two new antibiotic classes with single representatives (i.e., lipopeptide daptomycin, and pleuromutulin retapamulin), both of which are intended for topical application. However, analogues for systemic application (oral or $i.v.$) are being developed (also see section 3.1.4). ESBL – extended spectrum $\beta$-lactamase, VISA – vancomycin intermediately resistant *Staphylococcus aureus*, VRE – vancomycin-resistant *Enterococcus*, VRSA – vancomycin highly resistant *S. aureus*.

2.2 The disappointing investment in combinatorial chemistry and high-throughput screening in antibiotic discovery

Rediscovery of known antibiotics from screening microbial extracts and the development of highly effective synthetic (fluoro)quinolones caused a shift in antimicrobial drug R&D strategy in the industry. The search for antibiotics occurring in the environment was mostly abandoned (although semisynthetic modification of natural scaffolds resulted in numerous improved antibiotics (Fischbach & Walsh, 2009)) and the screening efforts were once again invested in synthetic compounds. Since the early 1990’s combinatorial chemistry was employed to produce large libraries of compounds that demanded high-throughput assaying for activity. The advent of genomics and bioinformatics raised the hopes for identification of entirely new antibacterial targets by mining bacterial genomes. Genes conserved among bacteria but sharing no homology to eukaryotic counterparts were considered as potential targets, and their activity or expression were manipulated by mutation studies or knockout technology to examine whether their products were indispensable for bacterial survival in vitro. Following a functional analysis, selected targets were expressed using recombinant technology and purified. This allowed for setting up miniaturized inhibitory activity assays for screening vast
chemical libraries against isolated targets, as well as elucidation of targets’ structures to guide the subsequent optimization of leads. Despite enormous efforts in the last 20 years, however, the target-oriented drug discovery approach has not resulted in a single new antimicrobial chemotherapeutic. The reasons for that are multitude (reviewed in detail in (Baltz, 2006; Brötz-Oesterhelt & Sass, 2010; Projan, 2003)).

Firstly, the abandonment of whole-cell assays meant that cell penetrating capabilities were not a selection criterion for hits early in the discovery process. Therefore, most compounds that were highly active against an isolated target possessed no antimicrobial activity. Secondly, it appears that the properties of antibiotics in general do not conform to Lipinski’s rule (Lipinski et al., 2001); they are more polar and have a higher molecular weight than drugs for other indications (O’Shea & Moser, 2008). Chemical libraries, on the other hand, had mostly been designed to meet Lipinski’s criteria, and were thus likely biased against antibiotic compounds (Payne et al., 2007). Thirdly, inhibiting targets that were validated to be indispensable for bacterial survival \textit{in vitro} does not necessarily lead to antibacterial effect \textit{in vivo}. A prominent example is that of the type II fatty acid synthesis (FASII) pathway, intrinsic to bacteria: bacterial pathogens susceptible to FASII inhibitors \textit{in vitro} were shown to be resistant to them when cultured in the presence of unsaturated fatty acids, or \textit{in vivo} upon infection of rodents (Brinster et al., 2009). This indicates that bacteria can thrive in the nutrient-rich environment of the host by acquiring exogenous fatty acids, fully bypassing FASII pathway inhibition. Similarly, there is no guaranty that a target essential for viability of one bacterial strain will also be indispensable in others – as alternative biochemical pathways may be present that allow the targeted pathway to be circumvented (Gentry et al., 2003).

2.3 Reappraising the natural products

Historically, most drugs were derived from natural products. This trend continues today with ~50% of new small molecule drugs approved between the years 1981 and 2006 being either (semi)synthetic derivatives of compounds isolated from natural sources or synthetic mimetics of pharmacophores found in natural products (Newman & Cragg, 2007). In the field of antibacterial drugs, the trend is even more pronounced. Of 98 new molecular entities that were approved for human therapy in the same time period, only 23 are of totally synthetic origin, most of them (20) belonging to the quinolone group (Newman & Cragg, 2007). A notable exception is linezolid, the first and, to date, the only representative of oxazolidinone chemotherapeutics developed from initial hits of cell-based screening efforts for antibacterial activity from a chemical library (Barbachyn & Ford, 2003; Slee et al., 1987). Among the 40 antibacterial compounds currently undergoing clinical trials, 20 are natural product-derived, 18 are synthetic, and 2 are of unknown origin (Butler & Cooper, 2011). Interestingly, while the ratio of natural product-derived vs. synthetic entities is roughly 1:1 in phases I and II, the former predominate in phase III (i.e., 4:1). Moreover, there are more novel antibacterial classes among natural-product derived antibiotics compared to synthetic ones (a total of 7 new chemical scaffolds vs. 4) in the pipeline.

Disappointment from antibacterial drug discovery in the genomic era brought a renewed interest in screening natural products (Baltz, 2008; Davies, 2011; Li & Vederas, 2009; Molinari, 2009). Chemists have been isolating and analyzing secondary metabolites from plants, fungi, and bacteria for over 200 years, yet only a small percentage of species has been addressed (Li & Vederas, 2009). Undoubtedly, the natural supply of small molecules (sometimes referred to as parvome (Davies, 2011), from the Latin \textit{parvus} meaning small) remains vast; however, there
is a problem accessing it. A majority (maybe up to 99%) of microbes, renowned for their rich and diverse metabolism, cannot be cultured in a laboratory, at least not under standard conditions (Amann et al., 1995; Li & Vederas, 2009). There are species of microbes that thrive in geographical or ecological niches, such as deep sea and thermal springs, or as symbionts of plants and animals, respectively, that still await to be explored. Besides rediscovery, a major obstacle that can impede natural product research is that some compounds are found in the environment in rather low concentrations, complicating their detection and isolation in quantities allowing structural and functional studies.

Nevertheless, the thesis that the laborious screening for natural products with antibiotic activity is still worth the effort is supported by several facts. The parvome displays structural diversity unmatched by synthetic compounds; secondary metabolites often possess numerous chiral centers and display astonishing steric complexity. Furthermore, many natural antibiotics display complex and multilayer mechanisms of action that might not have been devised by rational design. Last but not least, millions of years of evolution have optimized antibiotics with respect to affinity and specificity for their targets, as well as physicochemical properties to penetrate bacterial envelopes (Butler & Buss, 2006; Pelaez, 2006; Swinney & Anthony, 2011). Encouragingly, owing to the revival of screening for natural antimicrobials or reinspection of collections of old antibiotics in the last decade, we have witnessed attempts to develop antibiotics based on novel chemicals templates, such as lipopeptides, pleuromutilins, ramoplanins, and actinonins (Butler & Buss, 2006; Butler & Cooper, 2011). Drugs based on new scaffolds exerting novel mechanisms of action should be superior to existing antibiotic classes in the fight against multi-drug resistant pathogens (Butler & Buss, 2006). Of note, two such antibiotics have recently been approved for use in humans. Daptomycin, the first member of lipopeptide antibiotics, acts through a complex mechanism involving the disruption of the bacterial membrane leading to inhibition of DNA, RNA, and protein synthesis, and is indicated for the treatment of skin and skin structure infections caused by Gram-positive pathogens (Baltz et al., 2005). Retapamulin, a pleuromutilin type antibiotic with indications similar to those of daptomycin, selectively inhibits the P site of peptidyl transferase centre on the bacterial 50S ribosomal subunit, exhibiting a mechanism that differs from other protein synthesis-inhibiting antibiotics (Dubois & Cohen, 2010; Schlunzen et al., 2004).

It is important to realize that all small molecular weight microbial products are active even though they might not induce antibiosis at concentrations found in the environment, suggesting their role as signaling molecules (Dufour & Rao, 2011; Miao & Davies, 2010; Shank & Kolter, 2009; Wyatt et al., 2010). Remarkably, this holds true even for well established antibiotics; a number of recent studies reported specific modulation of gene expression in different bacteria when exposed to subinhibitory concentrations of various antibiotics (Davies et al., 2006; Fajardo & Martinez, 2008; Linares et al., 2006). Reevaluation of known natural products for traits other than antibiosis might thus present another route leading to antibacterial drug discovery; inhibiting the production of metabolites that provide the producing microbe with an advantage in colonizing a certain niche could prove to be a fruitful approach in designing antimicrobials (Wyatt et al., 2010).

3. Where do we search for natural antibiotics?

The search for new antibiotics compounds goes hand in hand with the discovery of new (micro)organisms producing them. For this purpose the search has continued on land and at
sea with great expectations. Soil microorganism exploitation has not subsided and continuous effort is put into the expanding the diversity of actinomycetes and fungi, taking advantage of little explored ecological niches and developing new ways of growing previously uncultivable strains (Harvey, 2000).

Almost all kinds of living things have the ability to produce secondary metabolites with antibiotic properties (Berdy, 2005), although this ability is not equally distributed among different species. Overall, it is clear that unicellular bacteria, eukaryotic fungi, and first of all filamentous actinomycetes are the most frequent and most versatile producers. The filamentous actinomycetales species produce over 10,000 bioactive compounds, of which 7600 derived from *Streptomyces* represent the largest group (45%) of bioactive microbial metabolites. Streptomyces are demonstrably a rich source of compounds, but no more so than other members of the actinobacteria. In 2001 Watve et al. set about to produce a mathematical model that would estimate the number of undiscovered antimicrobials from the genus *Streptomyces* (Watve et al., 2001). They found that there are still around 150,000 antimicrobials to be discovered. Theoretically speaking, this number does sound encouraging and one might expect the antibiotic pipeline to be pouring with new drugs. The reality is quite different. According to Butler and Cooper, in 2011, there were five compounds undergoing phase-III clinical trials, one compound was under NDA/MAA evaluation, 22 compounds were in phase-II, and 12 compounds in phase-I clinical trials (Butler & Cooper, 2011). Twenty of those compounds are derived from natural products. Clinical development of a drug requires certain *in vitro* activity, stability, and pharmacokinetic criteria to be met. It seems that not many of the lead secondary metabolites make it all the way to clinical trials and development and, eventually, drug approval. Nevertheless, finding an effective lead substance remains the most important starting point in antibiotic development. In the following text we give some examples on where some of these lead compounds can and have been found.

### 3.1 The producing organisms

Natural product resources, including the microbial world, are mainly unexplored both in its dimension and in the respect of geographic, ecological, and environmental points of view. There surely exist, besides the presumed numbers of microorganisms, millions of microbes living in remote and exotic parts of the world, or even the ones living in other organisms as endophytes or symbionts that await discovery and thorough study.

#### 3.1.1 Endophytes

Endophytes reside in tissues between living plant cells. The relationship that they establish with the plant varies from symbiotic to bordering on pathogenic. Of all of the world’s plants, it seems that only a few grass species have had their complete complement of endophytes studied, although endophytic fungi have been found in each plant species examined. The estimated number of endophytic fungal species existing in nature is over one million (Petrini, 1991). As a result, the opportunity to find new and interesting endophytes among the myriad of plants is great.

Plant endophytic fungi have a special ability to produce a great number of diverse bioactive compounds, which have been implicated in protection of its host against pathogens and herbivores (Wicklow et al., 2005). These structurally diverse molecules have potential
therapeutic value, which is why interest in screening endophytic fungi for discovery of novel metabolites, and more specifically novel antibiotics, has increased. The initial step in discovering secondary metabolites of endophytes is their successful isolation from plant materials. Then, the isolation and characterization of bioactive substances from culture filtrates is done using bioassay guided fractionation and spectroscopic methods (Strobel, 2002). For a detailed explanation on how these endophytic microorganisms are isolated the reader is referred to other publications (Hallmann et al., 2006; Strobel, 2002).

A short selection of substances with antibiotic properties that have been found in endophytic fungi and reported so far is included in Table 1 to provide the reader with an idea of how many potential lead compounds there are presently at our disposal.

<table>
<thead>
<tr>
<th>Endophytic fungal strain</th>
<th>Host plant (family)</th>
<th>Habitat of the host plant</th>
<th>Isolated metabolite(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colletotrichum gloeosporioides (Penz.) Penz. &amp; Sacc.</td>
<td>Artemisia mongolica (Fisch. ex Bess.) Nakai (Asteraceae)</td>
<td>Zijin Mountain, the suburb of Nanjing, China</td>
<td>colletotric acid</td>
</tr>
<tr>
<td>Colletotrichum sp.</td>
<td>Artemisia annua L. (Asteraceae)</td>
<td>ns</td>
<td>6-isoprenylindole-3-carboxylic acid 3β,5α-dihydroxy-6β-acetoxy-ergosta-7,22-diene 3β,5α-dihydroxy-6β-phenylacetoxy-ergosta-7,22-diene 3β-hydroxy-ergosta-5,7-diene; 3-oxo-ergosta-4,6,8(14),22-tetraene 3β-hydroxy-5α,8α-epidioxy-ergosta-6,22-diene</td>
</tr>
<tr>
<td>Phomopsis isolate MF6031</td>
<td>Salix gracilistyla var. Melanostachys (Salicaceae)</td>
<td>acquisition number 237-71-5282, Wakehurst Place, UK</td>
<td>phomopsichalasin</td>
</tr>
<tr>
<td>Phomopsis sp. strain E02018</td>
<td>Erythrina cristagalli L. (Fabaceae)</td>
<td>Boraso Stream-Delta del Parana, Argentina.</td>
<td>phomol</td>
</tr>
<tr>
<td>unidentified endophytic fungus CR115</td>
<td>Daphnopsis americana (Thymelaeaceae)</td>
<td>Guanacaste Conservation Area in Costa Rica</td>
<td>guanacastepenes A-O</td>
</tr>
<tr>
<td>Periconia sp. OBW-15</td>
<td>Taxus cuspidate Siebold &amp; Zucc (Taxaceae)</td>
<td>Kangwon region, Korea</td>
<td>periconicin A periconicin B</td>
</tr>
<tr>
<td>Guignardia sp. IFB-E028</td>
<td>Hopea hainanensis Merrill &amp; Chun (Dipterocarpaceae)</td>
<td>Hainan Island, China</td>
<td>monomethylsulochrin rhizoctonic acid guignasulfide</td>
</tr>
<tr>
<td>Rhizoctonia sp. strain Cy064</td>
<td>Cynodon dactylon (L.) Pers. (Poaceae)</td>
<td>Jiangsu Province, China</td>
<td>rhizoctonic acid monomethylsulochrin ergosterol 3β,5α,6α-trihydroxyergosta-7,22-diene</td>
</tr>
<tr>
<td>Aspergillus sp. strain CY725</td>
<td>Cynodon dactylon (L.) Pers. (Poaceae)</td>
<td>Sheyang Port on the Yellow Sea</td>
<td>helvolic acid monomethylsulochrin ergosterol 3β-hydroxy-5α,8α-epidioxy-ergosta-6,22-diene</td>
</tr>
<tr>
<td>Endophytic fungal strain</td>
<td>Host plant (family)</td>
<td>Habitat of the host plant</td>
<td>Isolated metabolite(s)</td>
</tr>
<tr>
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</tr>
<tr>
<td>Pichia guilliermondii</td>
<td><em>Paris polyphylla</em> var. <em>yunnanensis</em> (Franch) Hand.-Mazz (Trilliaceae)</td>
<td>Kunming, China</td>
<td>helvolic acid</td>
</tr>
<tr>
<td>Xylaria sp.</td>
<td><em>Ginkgo biloba</em> L. (Ginkgoaceae)</td>
<td>Jiangsu and Shandong provinces, China</td>
<td>7-amino-4-methylcoumarin</td>
</tr>
<tr>
<td>Thielavia subthermophila</td>
<td><em>Hypericum perforatum</em> L. (Hypericaceae)</td>
<td>Harwan, Jammu and Kashmir, India</td>
<td>hypericin, emodin</td>
</tr>
<tr>
<td>Twenty-nine unidentified endophytic fungal strains</td>
<td><em>Eucommia ulmoides</em> Oliver (Eucommiaceae)</td>
<td>Sichuan University, Chengdu, Sichuan Province, China</td>
<td>crude ethanol extract of fermentation broth, chlorogenic acid</td>
</tr>
<tr>
<td>Ampelomyces sp.</td>
<td><em>Urospermum picroides</em> (L.) F.W. Schmidt (Asteraceae)</td>
<td>Alexandria, Egypt</td>
<td>3-O-methylalterosolanin, altersolanol A, phomodione, usnic acid, cincosporamide</td>
</tr>
<tr>
<td>Phoma sp.</td>
<td><em>Saurauia scaberrinae</em> (Actinidiaceae)</td>
<td>central highlands of Papua New Guinea</td>
<td></td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td><em>Quercus variabilis</em> Blume (Fagaceae)</td>
<td>southern hillside of the Zijin Mountain in the eastern suburb of Nanjing, China</td>
<td>cebroside 1, cebroside 2</td>
</tr>
<tr>
<td>Trichoderma ovalisporum</td>
<td><em>Panax notoginseng</em> (Burkill) F.H.Chen ex C.Y.Wu &amp; K.M.Feng (Araliaceae)</td>
<td>Yunnan Province, China</td>
<td>koninginin A, (E)-2,3-dihydroxypropyl octadec-9-enoate, shikimic acid, cytosine ribonucleoside, a compound considered to be adenine ribonucleoside</td>
</tr>
<tr>
<td>Unidentified Ascomycete endophytic fungus strain 6650</td>
<td><em>Melilotus dentatus</em> (Waldst. &amp; Kit.) Pers. (Fabaceae)</td>
<td>coastal area of the Baltic Sea, Ahrens hoop, Germany</td>
<td>4-hydroxyphthalide, 5-methoxy-7-hydroxyphthalide, (3R,4R)-cis-4-hydroxymellein</td>
</tr>
<tr>
<td>Microsphaeropsis sp.</td>
<td><em>Lycium intricatum</em> Boiss. (Solanaceae)</td>
<td>Playa del Ingles, Gomera, Spain</td>
<td>microsphaeropsone A, microsphaeropsone C, citreorosein, enone (oxidized microsphaeropsone A)</td>
</tr>
<tr>
<td>Microsphaeropsis sp.</td>
<td><em>Zygophyllum fortanensis</em> (Zygophyllaceae)</td>
<td>Gomera, Spain</td>
<td>fusidienol A, 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid methyl ester</td>
</tr>
<tr>
<td>Microdiplodia sp.</td>
<td><em>Erica arborea</em> L. (Ericaceae)</td>
<td>Gomera, Spain</td>
<td>3,4-dihydroglobosuxanthone A</td>
</tr>
<tr>
<td>Alternaria sp.</td>
<td><em>Sonnerata alba</em> J.E. Smith (Sonneratiaceae)</td>
<td>Dong Zhai Gang Mangrove Garden on Hainan Island, China</td>
<td>xanalteric acid I, xanalteric acid II, altenusin</td>
</tr>
<tr>
<td>Chloridium sp.</td>
<td>(J.F.H. Beyma) W. Gams &amp; Holubova-Jchova</td>
<td><em>Azadiractha indica</em> A. Juss. (Meliaceae)</td>
<td>Varanasi district, India</td>
</tr>
</tbody>
</table>

Table 1. Plant endophytic fungi producing metabolites with antibacterial activity. ns – not specified.
Another great source of antibiotic producers among endophytes is bacteria. Munumbicins are an example of antibacterial compounds found in these microorganisms. Gary Strobel’s research group has isolated and studied the *Streptomyces* NRRL 30562 strain, which is endophytic in the medicinal plant snakevine (*Kennedia nigriscans*), native to the Northern Territory of Australia (Castillo et al., 2002). Bioassay-guided HPLC purification of the culture broth of this endophytic bacterium led to the discovery of four major components. They were characterized as four functionalized peptides named munumbicins A, B, C, and D. The munumbicins possessed widely differing biological activities depending upon the target organism. For instance, munumbicin B had a minimum inhibitory concentration (MIC) of 2.5 µg/ml against a methicillin-resistant strain of *Staphylococcus aureus* (MRSA), whereas munumbicin A was not active against this organism. In general, the munumbicins demonstrated activity against Gram-positive bacteria such as *Bacillus anthracis* and multidrug-resistant *Mycobacterium tuberculosis*. The most impressive biological activity of any of the munumbicins was that of munumbicin D against the parasite *Plasmodium falciparum*. However, in 2006, they reported that some of the munumbicins are identical to the better known antibiotics, the actinomycins (Castillo et al. 2006). Further effort resulted in the isolation of several novel antibiotics from *Streptomyces* NRRL 30562 with wide-spectrum biological activity that were termed munumbicin E-4 and E-5 (Castillo et al. 2006). Both compounds were tested alongside vancomycin against *Escherichia coli* and MRSA. The MIC of munumbicin E-5 against *E. coli* was 16 µg/ml, while the MIC for vancomycin was 128 µg/ml. The MICs were 16 and 2 µg/ml against MRSA, respectively.

Other antibiotic compounds of different chemical structures such as the bafilomycins (Yu et al., 2011), kakadumycins (Castillo et al., 2003) and many others are also produced by endophytic *Streptomyces* strains, making these bacteria worth investigating. The ability to make bioactive small molecules is not exclusive to microbes. Plants are rich sources of a great variety of compounds, but the original producer of those might be questionable. Opinions on this subject are divided and nobody can say for certain how many microbial metabolites/antibiotics considered today as marine animal or plant products, are produced, in fact, by symbiotic microbes in marine invertebrates and by endophytic fungi or bacteria living in the vascular plants. It has become generally accepted that at least for some compounds isolated from marine invertebrates, and in several cases from higher plants, the actual producers are the symbiotic microbes; bacteria, cyanobacteria, algae, or endophytic fungi. Indeed, several bioactive metabolites (e.g. taxol, bryostatin, theopalauamide, caphalomannin, etc.) have been proven to originate from symbiotic or endophytic microbes and not the “higher” (host) organisms (Berdy, 2005; Newman & Cragg, 2004).

### 3.1.2 Insects

Insects represent 80% of all fauna and are the most widespread group within the animal kingdom. Furthermore, some of these organisms such as cockroaches live in the filthiest places known to man and thrive in such conditions (Lee et al., 2011).

An investigation of the potential antibacterial activity in various tissues of the desert locust (*Schistocerca gregaria*) and American cockroach (*Periplaneta americana*) was undertaken at the School of Veterinary Medicine and Science, University of Nottingham. Brain lysates of locust and cockroach exhibited powerful broad-spectrum antibiotic
properties (>90% bactericidal effects) against MRSA and neuropathogenic *E. coli* K1, strain E44 (a cerebrospinal fluid isolate from a meningitis patient, O18:K1:H7), a spontaneous rifampicin-resistant mutant (Lee et al., 2011). A preliminary test suggested that the active substance is proteinaceous in nature. Brain lysates had no cytotoxic effects on human brain microvascular endothelial cells, suggesting that the putative target(s) is not present in eukaryotic cells. By combining size-exclusion spin columns and fast-performance liquid chromatography, eight different molecules (3–10 kDa in molecular mass) in brain lysates were identified that were toxic both to MRSA and neuropathogenic *E. coli* K1.

Higher insects protect themselves against bacterial infection by rapid synthesis of a battery of potent antibacterial peptides. Antimicrobial peptides (AMPs) have become recognized as important components of the nonspecific host defense or innate immune system in a variety of organisms including bacteria, fungi, plants, insects, birds, crustaceans, amphibians, and mammals (Zasloff, 2002). In order to overcome the problem of multi-resistant pathogenic bacteria, it is imperative to discover and clinically develop agents selectively toxic to bacteria that act on new targets which have not yet experienced selective pressure in the clinical setting. The research group of Laszlo Otvos has prepared derivatives of native proline-rich antibacterial peptides which exhibit these required features. As a lead they used pyrrhocoricin (Fig. 2), a peptide their group originally isolated from the European sap-sucking bug *Pyrrhocoris apterus* (Cociancich et al., 1994). In a couple of publications they report that pyrrhocoricin is non-toxic to eukaryotic cells and healthy mice, has good activity against model bacterial strains *in vitro* and when administered intravenously *in vivo*, and can protect mice from systemic *E. coli* challenge (Cudic et al., 2002; Cudic et al., 2003; Otvos et al., 2000a). Although pyrrhocoricin is toxic to infected animals at a high dose (50 mg/kg), its derivative in which the peptide is protected from exopeptidase cleavage by replacement of the N-terminal Val1 with 1-amino-cyclohexane-carboxylic acid and the C-terminal Asn20 with acetylated 2,3-diamino-propionic acid lacks this high dose toxicity and shows improved protease resistance, while maintaining the *in vitro* and *in vivo* efficacy over a broad concentration and dose range. Even more importantly, pyrrhocoricin and the derivative seem to have a completely new mechanism of action which makes the likelihood of fast accumulation of resistant strains insignificant. Native pyrrhocoricin kills the sensitive species by binding to DnaK, the 70-kDa bacterial heat shock protein (Otvos et al., 2000b). Remarkably, pyrrhocoricin does not bind to the human equivalent protein Hsp70, indicating the potential of this peptide as a drug lead to treat human or animal infections.

A list of antimicrobial peptides in clinical trials was published in 2004 (Andres & Dimarcq, 2004), and to date none of the peptides described has obtained FDA approval for any of the various clinical indications.

### 3.1.3 Marine (micro)organisms

The annual Marine natural products report has been published continuously since 1984 (until 2002 by John Faulkner (Faulkner, 2002) and later by Blunt et al. (Blunt et al., 2011)). The 2011 report states that 1011 new compounds of marine origin were described in literature in 2009 alone, proving that the oceans are a vast resource of diverse natural products, primarily from invertebrates such as sponges, tunicates, bryozoans, and molluscs, and from marine bacteria and cyanobacteria (Donia & Hamann, 2003).
Research conducted with a marine fungus of the genus *Pestalotia* isolated from the surface of the brown alga *Rosenvingea* sp. collected in the Bahamas, lead to the discovery of pestalone (Fig. 2), a new chlorinated benzophenone antibiotic, which has potent antibiotic activity against MRSA, with a MIC of 37 ng/mL, and vancomycin-resistant *Enterococcus faecium*, with a MIC of 78 ng/mL (Cueto et al., 2001). The potency of this agent toward drug-resistant pathogens suggests that pestalone should be assessed in more advanced infectious diseases animal models. Interestingly, pestalone is produced in the mixed fermentation of a marine fungus, *Pestalotia* sp. (strain CNL-365) and an unidentified, antibiotic-resistant marine bacterium (CNJ-328), highlighting the complex dependence of metabolite biosynthesis on culture conditions and the potential for enhanced antibiotic production through cross-species induction. This observation clearly demonstrates that pestalone is a product of fungal biosynthesis in response to an external trigger, suggesting that this method may have use for drug discovery in the future (Cueto et al., 2001).
Sponges alone produce more than 3300 antibiotics and other bioactive compounds. It is noteworthy to mention that these isolated “animal” compounds very frequently show surprising analogy to microbial or algal products. As with the secondary metabolites produced by plants and their endophytes, it is not surprising that in numerous occasions the active compounds isolated from sponges proved to be derived from the microorganisms living in symbiosis with their host (Berdy, 2005). Marine microbes are particularly attractive because of the high potency required for bioactive compounds to be effective in the marine environment, due to the diluting effect of seawater (Zhang et al., 2005).

Psammaplin A (Fig. 2) is a symmetrical bromotyrosine-derived disulfide natural product isolated from the *Psammaplysilla* sponge (Arabshahi & Schmitz, 1987), with *in vitro* antibacterial activity against MRSA. Based on the structure of psammaplins, Nicolaou et al. produced a library of 3,828 compounds. Six of these optimized antibacterial agents possessed more than 50-fold higher activities than the natural product, demonstrating MIC levels in methicillin-resistant/intermediate vancomycin-resistant strains of *S. aureus* at less than 1µg/ml. In order to construct these heterodimeric disulfide analogues they used a novel combinatorial disulfide exchange strategy, thus demonstrating the power of modern combinatorial techniques when applied to a base active structure from nature (Newman & Cragg, 2004; Nicolaou et al., 2001a; Nicolaou et al., 2001b). Most significantly, a number of these agents exhibited increased selectivity against bacterial cells over fibroblasts and lymphocytes as compared to the natural product.

In similar efforts of the marine natural products community, many antibacterial agents have been identified from sponges (Laport et al., 2009). Despite their high number, none of them has yet been involved in clinical trial as an antibacterial agent.

### 3.1.4 Higher fungi

Among the eukaryotes, fungal genomes are rich in biosynthetic gene clusters for encoding small molecule production (Miao & Davies, 2010). Fungi are the second largest group of eukaryotes next to insects and exceed not only the bacteria and actinomycetes, but also the higher plants in terms of the number of potential existing species. It looks like the world of fungi is one of the largest reservoirs for isolating further bioactive metabolites (Berdy, 2005).

Besides the discovery of new compounds, the re-evaluation of “old” substances, including microbial metabolites formerly believed to be inactive, have proven to be just as important. On numerous occasions such compounds have been shown to be active in later investigations, or were rediscovered by screening a different stock of microbes, or with specific screening methods. It is unpredictable how many “new” bioactive metabolites will be discovered in this way (Berdy, 2005).

An excellent example of this is pleuromutilin (Fig. 2). It was initially discovered in 1951 in a study of the culture broth of the edible basidiomycete mushroom *Pleurotus multilus* (Kavanagh et al., 1951). After more than 50 years, a derivative of pleuromutilin, named retapamulin, was approved in 2007 by the FDA for the treatment of bacterial skin infections. The low oral bioavailability of retapamulin seems to have been improved in the new derivative named BC-3205, which is being investigated in phase-I clinical trials by Nabriva (Butler & Cooper, 2011). Another pleuromutilin derivative, BC-7013 ([14-O-[(3-hydroxymethyl-phenylsulfanyl)-acetyl]-mutilin]), is in phase-I clinical trials as a topical
antibiotic, while BC-3781 successfully completed a phase-II clinical trial for the treatment of acute bacterial skin and skin structure infections (ABSSSI) (US National Institutes of Health, 2011). Nabriva’s lead product BC-3781 is the first of a new class of systemically available pleuromutilin antibiotics for the treatment of serious skin infections and pneumonia. BC-3781 is being developed for both oral and intravenous formulations.

4. How do we search for natural antibiotics?

Although the number of antibiotics present in nature may truly be huge, many of them are already known or will not be usable (i.e., will not display selective toxicity to bacteria, will be too weak, or will lack the desired pharmacokinetic properties) (Pelaez, 2006). Yet historically, the development of antibiotics from natural templates has seen an unprecedented gain compared to the de novo synthesis. The conventional discovery process of antibiotics from the pool of microbial natural products requires having a given microorganism grown in conditions appropriate to induce the production of (the desired) metabolite, which is then extracted and tested in a screen able to detect it as a hit. Finally, the compound has to be isolated from the original mixture and identified.

Identification of novel antibiotic types that occur in relatively low frequency in nature clearly requires innovative detection and characterization techniques. Numerous promising microbiological approaches supplemented with bioinformatic, genetic, and structural methods have been developed over the last decade to address the issue (Fig. 3).

![Diagram of antibiotic discovery process](image-url)

Fig. 3. Postgenomic approaches in antibiotic discovery (adapted from (Davies, 2011)).
These allow for laboratory culturing of previously inaccessible microorganisms as potential antibiotic producers, extracting genomes of uncultivable species from environmental samples or mining for and inducing expression of cryptic biosynthetic clusters to yield yet untapped secondary metabolites, direct solvent extraction and subsequent characterization of low molecular weight compounds from natural samples, and high-throughput fermentation of underexplored bacterial strains. In addition, intelligent strategies to avoid antibiotic rediscovery have been devised. In the following sections, we critically review the recent methodology of antibiotic discovery.

4.1 Improvements in screening platforms

Parallel fermentation coupled with whole-cell assays for antibiotic activity remains the cornerstone of antibiotic discovery. Yet, introduction of certain implementations are vital to detect antibiotic compounds that occur at low concentrations or to prevent rediscovery of old antibiotic types. For example, researchers at Merck developed a highly sensitive assay for detection of inhibitors of $\beta$-ketoacyl-[acyl-carrier-protein] synthase II (FabF), a component of FASII pathway, by introducing a plasmid that encodes antisense RNA against the fabF transcript in $S. aureus$ (S.B. Singh et al., 2007). Thereby, FabF expression is knocked down to growth-limiting levels, resulting in a strain that is hypersensitive to FASII pathway inhibitors. The mutant is assayed in parallel with the control wild-type strain to monitor for differential sensitivity. This combination of target-based and whole-cell screening had a high hit rate of 0.3% and led to discovery of platensimycin, a broad-spectrum Gram-positive antibiotic, from a screen of ~250,000 natural product extracts (Wang et al., 2006). Another interesting approach was reported by a team at Cubist Pharmaceuticals. They constructed a model target organism (CM400) by using $E. coli$ engineered to harbor multiple resistance markers (conferring resistance to 16 most frequent antibiotics) (Baltz, 2006; Gullo et al., 2006). In this way, the hits are preselected to belong to new antibiotic classes. Additionally, a derivative of CM400 (termed CM435) with increased permeability was created to achieve enhanced sensitivity to antibacterial compounds. However, this strategy requires enormous input of natural products to be tested due to extremely low hit rates.

4.2 High-throughput fermentation focusing on relevant microorganisms

It is absolutely essential to screen extracts of only those organisms that have the capacity to produce complex secondary metabolites. The size of the genome provides a good indication of metabolism complexity; actinomycetes, the most important group of antibiotic producers, have large genomes relative to other bacteria with up to 10% of all genes devoted to production of secondary metabolites, such as nonribosomal peptides and polyketides (Baltz, 2008; Donadio et al., 2007). Using relatively selective antibiotics, bacterial populations can empirically be enriched for rare species (Baltz, 2006 and references cited therein) after which microbial diversity in the remaining population is conveniently assessed by 16S rRNA gene sequencing (Amann et al., 1995; Rajendran & Gunasekaran, 2011). If the population is considered interesting in terms of secondary metabolite-producing potential, thousands of strains are typically screened for antibiotic activity. This, however, is no trivial task and represents a bottleneck of screening for antibiotics.
An obvious solution to increasing the throughput of fermentation is to miniaturize initial batches (i.e., perform microfermentations), allowing accommodation of larger numbers of strains and/or growth conditions simultaneously. At Cubist Pharmaceuticals they faced the challenge by encapsulating individual environmental microbes in ~2 mm alginate macrodroplets and growing them in media favouring actinomycete growth supplemented with antibiotics against single-cell eubacteria and fungi. This technology supports the fermentation and screening of up to 10 million actinomycetes per year (Baltz, 2006; Gullo et al., 2006). Similarly, a method that couples bacterial encapsulation in gel microdroplets with flow cytometry to detect those beads that contain microcolonies was reported (Zengler et al., 2002). This enables rapid isolation of bacterial strains from environmental samples in order to prepare pure cultures for subsequent studies.

4.3 New cultivation techniques

Since the vast majority of prokaryotes are not amenable to simple cultivation (indeed, only ~0.1% of existing prokaryotes have been cultured so far (Alain & Querellou, 2009)), numerous efforts to develop strategies for efficient bacterial growth in vitro have been made. Undoubtedly, expanding the accessible pool of antibiotic producers will raise the odds of discovering novel antimicrobials.

Attempts to recover diverse microorganisms from environmental samples by manipulating growth conditions (e.g. media formulation, light, temperature, agitation) have shown some success (Köpke et al., 2005; Uphoff et al., 2001; Zengler et al., 2002). However, the approach is strictly empirical and the yield is rather unpredictable. Moreover, the projects are often endangered by overgrowth of (common) opportunistic fast-growing microorganisms, especially when using nutrient-rich artificial media (Alain & Querellou, 2009). Furthermore, in vitro culturing attempts typically disregard the importance of chemical components or physical conditions of natural growth environments. Culturing in situ or under simulated natural conditions was demonstrated to be successful in some instances. For example, new bacteria were isolated from intertidal marine sediments using diffusion chambers and growth in seawater aquarium (Kaeberlein et al., 2002). The membranes of diffusion chambers allow for exchange of chemicals between the chamber and the environment, while restricting cell movement. Interestingly, two isolates easily grown in diffusion chambers could only be maintained in petri dishes in coculture, indicating the requirement for specific signaling between the two species as a marking of a favorable environment. Other studies found specific physical requirements for culturing different strains, such as high hydrostatic pressure (Alain et al., 2002) or carriers for adhesion (Yasumoto-Hirose et al., 2006). Previously uncultured bacteria were successfully recovered from soil, marine sediments or activated sludge by these innovative methods. Unfortunately, they are rather specialized and as a result were not adopted by a wider scientific community.

4.4 Direct isolation of metabolites from environmental samples

Direct sampling of natural products from the environment represents an alternative to microbial strain isolation and fermentation for production of secondary metabolites. In theory, this grants access to the complete metabolome, which cannot be retrieved by classical means because most microbes defy cultivation (see section 4.3). On the other
hand, environmental concentrations of numerous antibiotics are too low to be readily detected by conventional analytical methods or activity screening. Modern liquid chromatography-mass spectrometry (LC-MS) instruments combine high resolution and high sensitivity with the power of structure determination, and as such hold great potential for analysis of secondary metabolites in organic extracts of various, complex environmental samples (Davies, 2011).

An exciting new field in natural product research is imaging mass spectroscopy (IMS) (Esquenazi et al., 2009). Application of IMS enables analysis of spatial distribution of compounds in a substrate, such as a plant organ or a marine sponge. This method led to identification of various (endosymbiotic microorganisms as true producers of secondary metabolites which were initially erroneously attributed to the host organism (Esquenazi et al., 2009; Simmons et al., 2008). Another promising application of IMS is the so-called thin layer agar natural product MALDI-TOF imaging. Here, microorganisms are grown on a thin agar film deposited on a MALDI plate, after which the sample is covered with a matrix and analyzed by MALDI. Thus, a complete set of metabolites produced under different culturing conditions (even in cocultures to trigger interspecies interactions) can be examined (Yang et al., 2009).

Finally, the soaking of potential ligands from environmental extracts into crystals of recombinant proteins was proposed as another method to enrich for and analyze the structures of compounds with desired affinity to bacterial targets (Davies, 2011). Ideally, structural information gathered on the isolated secondary metabolite should assist in identification of the biosynthetic pathway from (meta)genomic library sequences (see sections 4.5 and 4.6).

4.5 Genome mining for cryptic metabolic pathways

In prokaryotes and fungi, gene encoding enzymes involved in secondary metabolite production are often clustered together. Polyketides and nonribosomal peptides (some of which are well established antibiotics) are typically assembled by massive synthetases of modular nature, wherein the modules consist of multiple domains, each being accountable for recognizing and fastening a specific substrate or catalyzing a sequential reaction step (e.g. building block activation, condensation, or tailoring) (Walsh & Fischbach, 2010). Therefore, the products of such assembly lines are said to be templated. Genome sequencing has revealed that certain microbes, especially many actinomycetes, harbor many (20 or more) biosynthetic gene clusters, most of which are cryptic (i.e., direct the production of unknown natural products) (Davies, 2011). This indicates that there are numerous complex secondary metabolites remaining to be discovered. The fact that polyketides and nonribosomal peptides are templated can aid in bioinformatic identification of genomic loci encoding biosynthetic pathways as well as provide clues to the structure and properties of metabolic products that are essential in developing methods for their detection and isolation (Fig. 4). If the product possesses the desired antibiotic activity and has favorable physicochemical properties, it is chosen as drug lead and platforms for sufficient production must be set up in order to support preclinical development. Strategies to elicit cryptic biosynthetic gene expression have been devised but will not be covered here. Readers interested in this topic are referred to two excellent recent reviews (Baltz, 2011; Chiang et al., 2011).
Fig. 4. Strategies for identifying metabolic products of cryptic gene clusters (compiled and adapted from (Challis, 2008)). Based on homology searches, novel biosynthetic gene clusters are predicted from genome sequences. A) The modular structure of synthetases allows assumptions on putative substrates, which together define structural and physicochemical features of secondary metabolites that guide the design of isolation procedures. B) Alternatively, the organism can be grown on a medium containing putative precursors labeled with stable isotopes to facilitate subsequent identification of final products by 2D NMR. C) The predicted synthetase can be expressed using recombinant DNA techniques and used in isolated form to reconstitute the product in vitro. D) The putative biosynthetic gene cluster can be knocked out and metabolites in culture supernatants analyzed by LC-MS in comparison to the metabolome of the wild-type strain. E) Similarly, the entire biosynthetic gene cluster-containing locus can be transferred to a heterologous host. The metabolome of the transgenic strain is compared to the untransformed host. F) Attempts to force expression of cryptic biosynthetic genes using induction of various endogenous activators have also been made.

4.6 Metagenomics

The term metagenomics refers to “the application of modern genomic techniques to the study of microbial organisms directly in their natural environments, by-passing the need for
isolation and laboratory cultivation of individual species” (Miao & Davies, 2009). At the heart of metagenomics lies the recovery and sequencing of genomes of entire microbial communities occupying diverse ecological niches. Thereby, even the uncultivable microorganisms are addressed. The gathered genetic information is then scanned for potential biosynthetic genes in the hope for identification of novel natural products in a similar way as previously discussed (see section 4.5) (Banik & Brady, 2010; Miao & Davies, 2009). Alternatively, metagenomic expression libraries can also be directly assayed for functional products (Brady, 2007). However, due to methodological obstacles no complex biosynthetic gene clusters have been recovered from environmental DNA (eDNA) to date (Miao & Davies, 2009).

One of the biggest problems in metagenomics is the inefficient cloning of extremely large DNA segments needed to harbor intact gene clusters for preparation of metagenomic libraries. The transformation of vectors such as cosmids or bacterial artificial chromosomes to surrogate hosts is the main factor that limits construction of libraries with acceptable complexity. Moreover, the host might not efficiently express biosynthetic transgenes because of differences in codon usage or incompatibility of promoters (Miao & Davies, 2009; B.K. Singh & Macdonald, 2010). Finally, it is imperative to enrich microbial populations for strains with potential to produce complex secondary metabolites (see section 4.2) (Miao & Davies, 2009) or enrich isolated eDNA samples for genes of interest (Banik & Brady, 2010) before the metagenomic library is constructed to minimize background.

5. Modification of natural scaffolds

Natural resources are and will continue to provide structurally and mechanistically new molecules that serve as useful drugs or lead compounds. One should realize, however, that natural antibiotics rarely possess the appropriate characteristics to be directly considered as drugs. Instead, they typically need to undergo chemical modifications in order to be translated into functional drugs. The goal of such projects may be to improve the pharmacokinetic properties of drug leads (e.g. increase stability and bioavailability) or produce derivatives with higher activity and wider antibiotic spectrum (e.g. by incorporating moieties to evade bacterial efflux pumps or to engage into additional interactions with the bacterial target protein). Both focused rational design and combinatorial chemistry approaches backed up by structural studies of targets complexed with natural or synthetic antibacterials and their derivatives have resulted in numerous optimized antibiotic drugs (Brötz-Oesterhelt & Sass, 2010; Butler & Cooper, 2011; Newman & Cragg, 2007).

Combinatorial biosynthesis is a rapidly expanding field in natural antibiotic optimization (Baltz, 2008; Kopp & Marahiel, 2007). The modular nature of polyketide synthases and nonribosomal peptide synthetases enables generation of natural product variants by exchange or alteration of individual modules within the bioassembly line. Many polyketides and nonribosomal peptides are not amenable to chemical synthesis and semisynthetic modification due to extreme structural complexity. Here, chemoenzymatic approaches represent a viable alternative. One prominent example is the generation of a library of lipopeptides based on the daptomycin structure (Nguyen et al., 2006). The daptomycin biosynthetic pathway was engineered by module and subunit exchange, and inactivation of a tailoring enzyme. Some of the lipopeptide variants produced in fermentations were highly
active antibiotics. Another group used error-prone PCR to generate gene mutants of glycosyltransferase that catalyses glucosylation of macrolide antibiotic oleandromycin (Williams et al., 2007). Thereby, they managed to broaden the specificities of the glycosyltransferase for acceptor substrates as well as donor nucleoside diphospho-sugars. Such innovative chemoenzymatic strategies combined with semisynthetic modification of natural products (novel and old) seem to provide a powerful tool for the development of new and improved antibiotics.

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