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Do we need new antibiotics? The search for new targets and new compounds

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Abstract Resistance to antibiotics and other antimicrobial compounds continues to increase. There are several possibilities for protection against pathogenic microorganisms, for instance, preparation of new vaccines against resistant bacterial strains, use of specific bacteriophages, and searching for new antibiotics. The antibiotic search includes: (1) looking for new antibiotics from nontraditional or less traditional sources, (2) sequencing microbial genomes with the aim of finding genes specifying biosynthesis of antibiotics, (3) analyzing DNA from the environment (metagenomics), (4) reexamining forgotten natural compounds and products of their transformations, and (5) investigating new antibiotic targets in pathogenic bacteria.

Keywords Antibiotics · Infectious diseases · Antibiotic resistance · Biosynthesis · Search for new compounds

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Introduction

This article is devoted to Professor Ivan Málek (1909–1994) on the occasion of the 100th anniversary of his birth. He was an eminent scientist in the fields of medical microbiology and continuous cultivation of microorganisms, and the founder and for many years director of the Institute of Microbiology of the Czechoslovak Academy of Sciences. Ivan Málek was closely connected with antibiotic research and production. As early as 1942, he was named a consultant to the chemical and pharmaceutical factory B. Frágner, in Dolní Měcholupy (later Zentiva and now a part of the multinational pharmaceutical company Sanofi-Aventis), where he and a team of researchers succeeded in manufacturing a small quantity of penicillin. The penicillin manufactured at B. Frágner was successfully tested in 1943–1944 on civilian patients suffering from neisserial meningitis, staphylococcal osteomyelitis, and pneumococcal pneumonia. In 1947–1948, Czechoslovakia received equipment for industrial production of penicillin, courtesy of the United Nations Relief and Rehabilitation Administration (UNRRA). Málek founded Czechoslovakia's first penicillin manufacturing plant in Roztoky near Prague, in October 1949. In 1962, he was named the first director of the Institute of Microbiology of the Czechoslovak Academy of Sciences. In 1964, the institute moved from its former quarters in Dejvice to a new campus in the Prague suburb of Krč. Málek's research focused on continuous cultivation; he and his coworkers devised multistage chemostats to facilitate study of trophophase and idiophase in antibiotic fermentations. Ivan Málek was thus always very close to both basic and applied aspects of antibiotic biosynthesis and production. This is the main reason why we decided to devote this contribution to some aspects of antibiotics.

The infectious disease problem

Infectious diseases are still one of the most important causes of death in humans, and apparently the most significant cause of death in children [27].

They are the second most important killer in the world, number three in developed nations, and fourth in the USA. Worldwide, 17 million people die each year from bacterial infections. Americans are infected with bacteria at the rate of over 2.5 million per year [49], resulting in 100,000 deaths. Bacteria causing serious health problems include *Enterococcus faecium*, *Staphylococcus aureus* (mainly the methicillin-resistant strains), *Acinetobacter baumanni*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, group A streptococci, *Salmonella typhimurium*, *Escherichia coli* 0157-H7, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Borrelia burgdorferi*, *Helicobacter pylori*, etc. Methicillin-resistant *Staphylococcus aureus* (MRSA) kills 19,000 people in the USA each year. MRSA incidence in US intensive care units among *S. aureus* isolates was 2% in 1974, 22% in 1995, and 64% in 2008. *S. pneumoniae* causes bacterial pneumonia resulting in 40,000 deaths in the USA each year. By 1999, 25% of US isolates of this organism were penicillin resistant. A major problem today is tuberculosis (TB), which is infecting 2 billion people. Each year, 9 million new cases are diagnosed and 2.6 million people die. Resistance is developing to the combination treatment of isoniazide and rifamycin. No new drug against TB has been commercialized since 1964. TB is the second largest infectious killer (2 million deaths/year). Only acquired immune deficiency syndrome (AIDS; 3 million deaths/year) is more dangerous. Multiple drug resistance has developed against two of the most important TB drugs, rifampicin and isoniazide. This problem is being ignored by most pharmaceutical companies. Of the 74 new therapeutic agents approved by the Food and Drug Administration (FDA) in 2007, only 2 were antibiotics. Of the 2,700 compounds recently in development, only about 50 were antibacterials; of these 50, only 10 were from large pharmaceutical companies.

For a long time, it appeared that antibiotics were omnipotent and that the treatment of pathogenic bacteria with them would wipe out infectious diseases. However, bacteria developed antibiotic resistance, quite often immediately after the antibiotic had been introduced into clinical practice.

Bacteria have lived on Earth for several billion years. During this time, they encountered in nature a wide range of naturally occurring antibiotics. To survive, bacteria developed antibiotic resistance mechanisms. Therefore, it is not surprising that they have become resistant to most of the natural antimicrobial agents developed [21].

Today, the structures of around 140,000 secondary metabolites have been elucidated [17]. It is estimated that a total of 10^5 antibiotics have been described, out of which only about 10^2 are in clinical use. This is mainly due to the fact that some antibiotics are toxic, poorly water soluble, or have similar effects as antibiotics already used in clinical practice.

Resistance of bacteria to antibiotics is continually increasing. The human population is aging, and many patients undergo surgery or have implanted joint replacements, or are subjected to immunosuppressive therapy. Some bacteria produce biofilms that make them extremely antibiotic resistant and thus very dangerous for these patients. Biofilm infections of some medical devices by common pathogens such as staphylococci are not only associated with increased morbidity and mortality but also significantly contribute to the emergence and dissemination of antibiotic resistance in the nosocomial setting [31]. Microbial pathogens are thus extremely dangerous for these patients. Bacterial sepsis had already become one of the main causes of death of the elderly. Extensive use of antibiotics has selected antibiotic-resistant strains, some of them resistant to several antibiotics simultaneously. They were originally detected only in hospitals; however, their occurrence is now more widely distributed and not limited to hospitals alone. The large amounts of antibiotics used in human therapy, as well as for farm animals and even for fish in aquaculture, have resulted in selection of pathogenic bacteria resistant to multiple drugs [35].

In hospitals, resistant bacteria can survive for a prolonged time and cause epidemics, e.g., in intensive care units. The risk of infection increases with time spent in hospital. Vancomycin had long been considered the last-choice antibiotic against methicillin-resistant *S. aureus*. However, strains resistant even to vancomycin have occurred. The occurrence of antibiotic-resistant strains is apparently inevitable. Some pathogenic bacteria in intensive care units, e.g., *A. baumanii*, have been described as pan-resistant. The increasing resistance of these bacteria raises fear after failure of antibiotic treatment.

Many excellent reviews on antibiotic resistance have been published in which different aspects of this phenomenon are discussed [1–3], including the volume on antibiotic resistance edited by Walsh and Wright [48] and the review entitled "Antibiotic Resistance: Ecological Perspective on an Old Problem" [41]. It had been previously suggested that, due to the high cost of antibiotic resistance, sensitive bacteria would rapidly predominate in bacterial communities when the antibiotic is absent or removed. It is now apparent that reversibility in the clinical setting is slow or does not exist at all.

There are several ways to protect against infectious diseases. Vaccines against resistant bacterial strains could be prepared, specific bacteriophages could be used, or new therapeutics including antibiotics could be discovered. All approaches have their advantages and shortcomings.

Vaccines have been developed or are being developed against infectious diarrheal diseases, pulmonary diseases caused by pneumococci, bacterial meningitis, certain sexually transmitted diseases including human immune-deficiency virus (HIV)/AIDS, human papillomavirus (HPV), gastrointestinal diseases, against infectious agents transferred by various vectors, and against some hospital infections [26]. Use of vaccines has been facilitated by knowledge of the genomes of many pathogens together with increased knowledge of immune responses to infections. This has allowed the rational development of new recombinant vaccines.

It thus appears that antibiotics are still of extreme therapeutic significance, and thus, it is still desirable to continue to look for compounds that would be effective against bacteria resistant to currently used antibiotics. The reasons for developing new antibiotics were summarized by Demain and Sanchez [17]. The so-called "golden age" of antibiotics was relatively short, roughly from 1940 to 1960, during which most of the major classes of natural antibiotics were isolated [48]. During this short period, the pharmaceutical industry was extremely interested in the discovery of new antibiotics, but its involvement substantially decreased later. Although pharmaceutical companies still invested in the search for new antibiotics and some antibiotics were discovered, some of the compounds exhibited similar or identical mechanisms of action to previously described and clinically used compounds. In addition, the expense of research and development continually increased, and investments often did not pay off. The value of antibiotics to pharmaceutical companies may decrease further due to the increasing requirements of regulatory institutions and the increasing number of individuals required for clinical trials. Therefore, in the last decade, many pharmaceutical companies terminated their support of the search for new antimicrobial compounds and redirected themselves towards projects they considered to be much more profitable. In the 1990s, many pharmaceutical companies invested significantly in projects of bacterial genomics with the aim of finding new targets in pathogens. However, few antibiotics were developed and approved.

It now appears however that the antibiotic era is still not over. Investment in newer anti-infective platforms is essential and urgent and apparently requires collaboration among industry, academia, and government. Such cooperation could result in a revolution in our understanding of bacterial resistance and development of new approaches to control it [1].

Why natural products are more likely to become drugs than synthetic compounds

From 1988 to 2008, 877 pharmaceuticals were commercialized. Of these, 60% are from natural sources or derived from them [30]. Quality appears to be more important than quantity when it comes to new drug discovery. Whereas only 0.001% of the total synthetic compounds have become drugs, 0.2–0.3% of microbial metabolites have become drugs, and another 0.2–0.3% have become lead compounds for chemical modification. This is more than two orders of magnitude difference. Natural product collections have a much higher hit rate in high-throughput screens than do combinational libraries. Breinbauer et al. [11] point out that the number of compounds in a chemical library is not the important point; it is the biological relevance, design, and diversity of the library, and that a scaffold from nature provides viable, biologically validated starting points for the design of chemical libraries.

Products from nature are unsurpassed in their ability to provide novelty and complexity. With respect to the number of chirality centers, rings, bridges, and functional groups in the molecule, natural products are spatially more complex than synthetic compounds. Synthetic compounds highlighted via combinatorial chemistry and in vitro high-throughput assays are based on small chemical changes to existing drugs, and of the thousands, perhaps millions, of chemical "shapes" available to pharmaceutical researchers, only a few hundred are being explored. Many compounds are probably being missed. Natural products differ from synthetic compounds by having more oxygen atoms and stereochemical elements such as chiral centers and polycyclic (often bridged) carbon skeletons. Most drugs in use today are chiral. In a survey comparing about 670,000 chemical combinatorial compounds, about 11,000 drugs, and over 3,000 natural products, it was found that 82% of natural products were chiral, 55% of drugs were chiral, but only 29% of combinatorial products were chiral [18].

According to Sam Danishefsky, prominent chemist at Memorial Sloan-Kettering Cancer Center, New York City, it is appropriate "to critically examine the prevailing supposition that synthesizing zillions of compounds at a time is necessarily going to cut the costs of drug discovery or fill pharma pipelines with new drugs any time soon" [9]. Danishefsky continues: "At the risk of sounding Neanderthal, I would even put in a pitch for industry getting back to the screening of natural products. Some of the most valuable products and promising leads in oncology are naturally derived or naturally inspired. For instance, paclitaxel, a chemically established drug, came from natural product sources, as did doxorubicin, the etoposides and the latter-day camptothecins. In fact, even tamoxifen arose from natural product leads, steroid hormones. Moreover, several of today's most promising pipeline candidates in oncology—such as ecteinascidin, halichondrin, bryostatin, and of course, the epothilones—all arose from natural product screening followed by synthetic modifications. A small collection of smart compounds may be more valuable

than a much larger hodgepodge collection mindlessly assembled. Thus, the decision on the part of several pharma companies to get out of the natural products business is gross foolishness. There are major teachings in these natural products that we would do well to consider. They may be reflecting eons of wisdom and refinement. The much maligned natural product collections did, after all, bring us statin, β-lactam, aminoglycoside, and macrolide blockbuster drugs. In fact, one of the most promising approaches in diversity chemistry is to produce diversity-chemistry-derived collections that benefit from or partake of the "wisdom" of natural products."

Approaches used in the search for new compounds

Several approaches were proposed by Baltz [6]. They include high-throughput miniaturized fermentation and screening, enrichments, selections, special niches for uncommon actinomycetes, mining actinomycete genomes for cryptic antibiotic pathways, use of genomics as a guide for where to focus resources, evolution of secondary metabolic genes and gene clusters, and accelerated evolution of new secondary metabolic pathways. We shall discuss the following approaches:

1. Search for new antibiotics from nontraditional or less traditional sources
2. Use of microbial genome sequences to search for genes specifying biosynthesis of antibiotics
3. Use of metagenomics to analyze DNA from the environment
4. Forgotten natural compounds and their transformation
5. New antibiotic targets in pathogenic bacteria

Search for new antibiotics from nontraditional or less traditional sources

Antibiotic producers are searched for in extreme locations, such as hot springs, deep sea bottom vents, or water reservoirs with high salt content. Among microorganisms, actinomycetes found primarily in the soil are the most significant antibiotic producers. Relatively recently, however, a number of biologically active compounds produced by marine streptomycetes have been described. The oceans cover 70% of the Earth's surface and harbor most of the planet's biodiversity. However, the microbiological component of this diversity remains relatively unexplored. The marine actinomycete genus *Salinospora* was found to be a particularly rich source of new chemical structures with promising biological activity [19]. The importance of the results obtained was emphasized by Hopwood [24]. Bacterial symbionts of invertebrates have been examined with respect to production of new compounds [39].

Microbial genome sequences

Genome sequences of microbes can be used for identification of potential novel chemical entities that could be used as pharmaceuticals. With the continually decreasing costs of genome sequencing, genome mining of microbial genera and species with high potential for biosynthesis of biologically active compounds represents a great potential and opportunity for drug discovery. The surprising technical innovations in sequencing procedures supported by steadily increasing computing power and constantly improving software [4, 28] are overwhelming. A bacterial genome can now be completely sequenced, assembled, and annotated in less than 24 h [20, 40]. There are now more than 1,000 sequenced prokaryotic genomes deposited in public databases and available for analysis [29]. Biological diversity is far greater than we had thought. The genetic information obtained can be used for combinatorial biosynthetic strategies. Many genes coding for biosynthesis of antibiotics are not expressed (silent genes), and it may thus be useful to decipher molecular signals that trigger production of previously undetected compounds. New strategies to activate cryptic gene clusters had been described by Bergmann et al. [8], who reported a new strategy for successful induction of a silent metabolic pathway in *Aspergillus nidulans* which led to the discovery of new polyketide synthase-non-ribosomal peptide synthetase (PKS-NRPS) hybrid metabolites. Genome sequences of *Streptomyces coelicolor* A3(2) and *Streptomyces avermitilis* revealed more than 20 cryptic silent biosynthetic gene clusters specifying secondary metabolites. The most significant antibiotic producers, i.e., the streptomycetes, are being investigated in several genome projects. So far, three *Streptomyces* genomes, viz. those of *S. coelicolor* [7], *S. avermitilis* [25], and *S. griseus* [36], have been completely sequenced. An additional 19 *Streptomyces* genomes deposited by the Broad Institute are finished for most uses, and a total of 25 genomes have been listed in GenBank. The properties of the *Streptomyces* chromosome have been reviewed by Hopwood [22]. The chromosome is linear, with a "core" containing essential genes and "arms" carrying conditionally adaptive genes that can sustain large deletions in the laboratory. Before the sequencing of the *S. coelicolor* chromosome, only three antibiotics and one spore pigment had been described in this streptomycete. After sequencing, 25 closely linked clusters coding for biosynthesis of pigments, complex lipids, and signal molecules were identified. Prior to sequencing of the producer's genome, only the veterinary antibiotic avermectin was known in *S. avermitilis*, whereas after

sequencing, 30 gene clusters coding for biosynthesis of biologically active secondary metabolites were detected. *Salinospora tropica* is another actinomycete whose genome has been sequenced. The sequencing revealed a high number of new biosynthetic gene clusters that will have to be confirmed by fermentation studies and identification of the metabolites. As compared with the sequenced *Streptomyces* genomes, the *S. tropica* chromosome is circular, no particular region seems to be unstable, and secondary metabolite clusters are dispersed along the chromosome. It thus appears that *Salinospora* species acquired many of their secondary metabolic systems by horizontal transfer from other species [46].

It is estimated that only 1–2% of microorganisms can be cultivated. Only about 0.001–1% of microorganisms have been cultivated from seawater, 0.25% from freshwater, and apparently about 1–15% from activated sludge. It is thus apparent that the known antimicrobial compounds have been isolated from only a limited reservoir of biodiversity. Many additional groups of producers are still awaiting identification and utilization. According to the mathematical model of Watve et al. [50], it should be possible to identify 294,300 compounds that have not yet been described, and even according to much more conservative estimates, 150,000 new compounds could still be isolated. Even if the mathematical model is doubted by some mathematicians, it may still be reasonably estimated that only 3% of the antibiotics produced by streptomycetes have so far been isolated. Quite clearly, new groups of chemical compounds can be discovered with the aid of ecological, taxonomic, and metagenomic approaches.

Metagenomics: analysis of DNA directly from the environment

Metagenomics has been utilized only relatively recently, i.e., as a means for analysis of microbial communities irrespective of their cultivation under laboratory conditions. Here, genomics is applied to uncultured microorganisms. The procedure includes genomic analysis of microorganisms in DNA samples isolated from the environment that are suitably treated and cloned in a cultivable microbial producer. Then, the genes encoding biosynthesis of antibiotics and other biologically active secondary metabolites are further used. The development of metagenomics was supported by the evidence that uncultured microorganisms predominate in most of the Earth's environment. The evidence is based on analysis of ribosomal RNA directly from the environment. Soil microorganisms have long been the most valuable source of natural products, including antibiotics. Gene mining based on construction and screening of complex libraries derived from the soil metagenome provides new opportunities to explore and utilize the enormous genetic and metabolic diversity of soil microorganisms [15]. The metagenomic approach has not been very successful in antibiotic discovery. This has been mainly due to technical limits. The problem also lies in the notion that many uncultivable microbes will produce novel antibiotics [5]. However, from the academic point of view, metagenomics can contribute to our knowledge of microbial communities, in the soil in particular, and lead to advancement in different areas such as human health, agriculture, and environmental remediation.

Genes coding for biosynthesis of secondary metabolites and their manipulation

Manipulation of genes encoding modular polyketide synthases has been studied in most detail. Polyketides are a group of varied secondary metabolites with complex chemical structures and a wide range of biological activities. The group includes antibacterial compounds (erythromycin, clarithromycin, azithromycin, tetracycline), compounds decreasing the level of cholesterol in blood (lovastatin, simvastatin, pravastatin), antitumor compounds (adriamycin), antiparasitic drugs (avermectin), and immunosuppressive agents (FK506, rapamycin). All of them share a similar biosynthetic mechanism. At present, much is known about polyketide and nonribosomal peptide biosynthetic pathways, the two biosynthetic classes to which many useful antibiotics belong. The knowledge in this area, including that of genes and enzymes, may help medicinal chemists manipulate natural product structures to generate new compounds with better properties [38]. By means of genetic manipulation of polyketide synthases it is possible to prepare new derivatives of known polyketides. A classical example is that of erythromycin biosynthesis. Genetic manipulation of type I polyketide synthase (PKS) catalyzing biosynthesis of 6-deoxyerythronolide B, the erythromycin aglycone, was studied in detail by Menzella et al. [33]. Alteration or replacement of individual PKS modules led to biosynthesis of novel natural products. The authors synthesized 14 modules from eight PKS clusters and associated them in 154 bimodular combinations. Nearly half of the combinations successfully mediated biosynthesis of a new polyketide in *E. coli*, and all individual modules participated in productive bimodular combinations. This work can serve as an example of the combinatorial biosynthetic approach for production of polyketides. The actinomycete family, including streptomycetes, produces more than two-thirds of the known antibiotics. Knowledge of the regulation of antibiotic production provides new opportunities for strain improvement, complementing the classical strategy of mutation and screening for improved productivity [12].

Antibiotic producers must necessarily be resistant to their own products in order to produce molecules with antibiotic activity and excrete them into the environment, enhancing perhaps their ability to compete with their neighbors. Since these compounds are often toxic to their producers, mechanisms exist to ensure that the export apparatus accompanies or precedes biosynthesis. *S. coelicolor* produces the polyketide antibiotic actinorhodin in a multistep pathway involving enzymes encoded by a gene cluster. The cluster also includes genes for actinorhodin export, two of which, *actR* and *actA*, resemble the classic *tetR* and *tetA* repressor/efflux pump-encoding gene pairs conferring resistance to tetracycline. Similarly to TetR, which represses *tetA*, ActR is a repressor of *actA*. Tahlan et al. [45] identified several molecules that can relieve repression by ActR. The three-ringed 4-dihydro-9-hydroxy-1-methyl-10-oxo-3-*H*-naphto-[2,3-*c*]-pyran-3-(*S*)-acetic acid [(*S*)-DNPA] (an intermediate in the actinorhodin biosynthetic pathway) and kalafungin (a molecule related to the intermediate dihydrokalafungin), are particularly potent ActR ligands. It may thus be suggested that not only the end products but also the intermediates in the biosynthetic pathway activate expression of the export genes, thereby coupling export to biosynthesis. The authors propose that this could be a common feature in the production of many bioactive natural products. This pioneering study has been discussed by Hopwood [23]. Relatively recently, the first complete signaling cascade from nutrient sensing to cell development and to antibiotic biosynthesis was proposed, which probably mimics the accumulation of *N*-acetylglucosamine after autolytic degradation of the vegetative mycelium [42].

A new enzymatic technique called glycorandomization is being used to prepare glycoside libraries and to make optimized or novel glycoside antibiotics. Sugars in natural products such as antibiotics are usually members of the 6-deoxyhexose family. Over 70 different variants were found in products of bacteria, fungi, and plants. Novel deoxy sugars can be placed on macrolide antibiotics by combinatorial biosynthesis [37]. The presence of glycosidic residues in antibiotics is very important for their activity.

Forgotten natural compounds and their transformation

Modern chemical methods as well as methods of genomics and combinatorial biosynthesis can be utilized for modification of known compounds with biological activity. During the "golden age" of antibiotics, thousands of biologically active compounds were discovered. However, many of them were not developed further, either because better compounds were available at that time or because they could not be developed into drugs due to their unsuitable pharmacological properties. The possibilities to modify natural compounds by chemical and genetic methods are much better developed at present, and new procedures can now be combined with the aim of revitalizing precious biologically active compounds and using them, particularly in cases where the pathogenic microorganism has become resistant to currently used antibiotics.

Search for new targets

As late as 1985, many well-known scientists doubted that it was at all possible to sequence the human genome. Others assumed that it would take several tens or even 100 years. Contrary to those assumptions, the human genome was sequenced much earlier, even earlier than the main players of this project had hoped. The sequencing, particularly of microbial models, is incredibly fast and cheap. Not only bacterial species but even specific strains of bacteria are being sequenced. It appears that the information on the genome sequence of microbial species and their strains resistant to antibiotics will facilitate the search for new targets of antibiotics in pathogenic microorganisms. In an excellent review by Brown and Wright [13], the efforts to identify new antibiotic targets were described and their main features were proposed. Well-known processes were described as targets, e.g., biosynthesis of peptidoglycan, cell-wall teichoic acid, folate biosynthesis, fatty-acid biosynthesis, and protein secretion. Biosynthesis of isoprenoids in microorganisms was previously thought to be based on the mevalonate-dependent pathway, similarly to in virtually all living organisms. However, it was later found that another pathway, called 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway, involving successive action of newly discovered enzymes on DOXP to yield isopentenyl-diphosphate and its isomer dimethylallal-diphosphate, the building blocks of polyisoprenoids, functions in many microorganisms, including bacteria and protozoa. The DOXP genes were found to be conserved among significant human pathogens such as *M. tuberculosis*, *P. aeruginosa*, and *H. pylori*, but absent in humans. This pathway is thus a specific target in these and many other bacteria. An additional strategy may be to target factors that are essential to microbial virulence. This would be an extremely important approach which would only target those factors playing a role in infection and would thus be specific for pathogenic microorganisms.

Other targets include inhibitors of peptide deformylase and fatty-acid biosynthesis, lipid A biosynthesis, and transfer ribonucleic acid (tRNA) synthetases. New screening technologies are also very important. For example, a new means of discovering antifungal drugs uses an assay based on the application of the nematode *Caenorhabditis elegans* as host for *Candida albicans* and other pathogenic

Candida species [10]. The yeast is ingested by the worm, which causes an infection in the worm's intestinal tract and kills it. Known antifungal agents such as amphotericin B and caspofungin prolong the worm's survival. The test is done in liquid media contained in 96-well plates and appears to be a valuable discovery tool. Of 1,266 compounds tested, 1.2% (15 compounds) were active. The most active were caffeic acid phenethyl ester and the fluoroquinolone enoxacin. Another novel method to discover new antimicrobial agents employs the laboratory nematode *C. elegans* infected with *Enterococcus faecalis* [34]. Antibiotics rescue the infected nematode in an assay done in 96-well microtiter plates.

There are now 1,194 complete microbial genomes available, and 3,586 microbial genomes are in progress [29]. Most of them are of pathogenic microorganisms. The detection of bacterial genes that are nonhomologous to human genes and are essential for the survival of the pathogen represent a promising means of identifying novel drug targets [43]. A number of sophisticated methods used to determine antibiotic targets in bacteria have been described [14].

New antibiotics, mainly against resistant pathogenic microorganisms, are very much needed. Microbial natural products are still the most promising source of new antibiotics. Although the cost may appear very high, research and development of new compounds will be rewarding for both academia and the pharmaceutical industry. It will be important for society, possibly solving one of the most serious health threats that exist today and in the future. Undoubtedly, antibiotics have often been used excessively and sometimes even uselessly. This is apparently one, but not the only, cause of development of antibiotic resistance in pathogenic microorganisms. The lifetime of available antimicrobial compounds can be extended by their reasonable use, but for the treatment of infectious diseases we will still need new compounds. Louis Pasteur once stated that the microbes always have the last word. In spite of this, we should not give up the battle. Antibiotics have been the main component in treatment of infectious diseases, and despite all the pessimism, they remain effective under most conditions [16]. There are still possibilities to obtain new compounds, including the use of uncultivated microorganisms. The metagenomic approach should provide new data on the immense reservoir of genetic and metabolic diversity, which may lead to discovery of new antimicrobial compounds. In addition, chemical and biological modification of old compounds could still supply new useful drugs. A long time ago, we reviewed different concepts involving control of antibiotic biosynthesis [32], investigated overproduction of secondary metabolites, and proposed ideas and hypotheses on how to proceed [44, 47]. It is interesting to compare those results and hypotheses with our present knowledge to appreciate the fantastic progress that has been made since then. We predict that, with the available tools and methods, the search for new secondary metabolites exhibiting important biological activities will have a bright future.

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