Synthetic Biology Approaches to Combat Antibiotic Resistant Bacteria

Lei Pei[1,*]

[1]Biofaction KG, Kundmanngasse 39/12, 1030 Vienna, Austria

Synthetic biology (SB) is an innovative and emerging field, representing a confluence of science and engineering. It aims to (re)-design biological parts, devices and systems while applying engineering principles for useful applications. Valuable insight gained on the functions of biological systems will further improve the design capacity. The advance of SB is expected to provide solutions to address some of the urgent issues in healthcare, particularly to combat infections caused by multidrug resistant bacteria. Using SB approaches can help to gain new insight into the resistant mechanisms to antibiotics, discover new mechanisms to develop antibiotics, and develop new infectious control strategies. Here, some of these applications of SB in developing novel approaches to combat antibiotic resistant bacteria will be reviewed.

Keywords synthetic biology, antibiotics, antibiotic resistance, anti-infective strategy

1. Introduction

Synthetic biology (SB) is an innovative and emerging field, representing a confluence of science and engineering. It aims to (re)-design biological parts, devices and systems while applying engineering principles for useful applications. Research activities of SB are currently performed in several sub-fields: the research on DNA synthesis (or synthetic genomics), DNA-based biological circuits, the minimal genome (or minimal cell), protocells and xenobiology. DNA synthesis provides the technical basis of SB. The advances in the field make it possible to chemically synthesize longer DNA molecules in a cheaper price. The synthesis DNA has moved from the size of viruses [1] to the *de novo* synthesis of a whole bacterial genome *Mycoplasma mycoides* in over 1 million base pairs (bp) [2]. DNA-based biological circuits are aimed to build advanced genetic constructs in metabolic engineering to redesign meaningfully metabolic pathways, or fine-tuned genetic circuits and systems. The so-called standard biological parts (biobricks) have been developed to create a toolbox of well-characterized, prefabricated, standardized, and modularized genetic compounds (such as sequences of DNA) to be used as basic elements to build larger "devices" with defined functions. A couple of circuits have been built, e.g., tunable genetic oscillators [3, 4]. Minimal genome research aims to define a minimal set of essential genes to sustain life [5-7]. It will help to create a cellular platform, or chassis, to understand the origin of life and as a chassis for engineered biological circuits [8, 9]. Research on protocells, or synthetic cells, is a different approach to construct minimal versions of life assembled from chemical components [10, 11]. Aiming to develop orthogonal biological systems, research on xenobiology tries to create biological systems entirely different from the natural forms of life both in metabolism and on the genetic information level [12]. Due to the infancy of this research topic, most of the research is focused on the elementary chemical components [12-18].

Antibiotics developed by screening antibacterial compounds from microorganisms have contributed greatly to improve human health. Yet the increasing prevalence of antibiotic resistant bacteria poses a great threat to primary antibacterial therapy [19]. The emergence and spread of multidrug-resistant bacteria poses a great burden for human health: increased mortality, morbidity, and expenditure [20]. To combat these antibiotic resistant bacteria, a better understanding of the biological physiology of these strains is needed. SB helps to improve our understanding of the essential functions of the bacterial genome through research on the minimal genome. Novel antibiotics can be designed based on these essential cellular functions. It has been known that antibiotic resistance can be raised by selection of spontaneous mutations and by horizontal gene transfer. The basis of how bacteria develop responses and defenses against antibiotics can be studied by the genetic circuits. The SB derived platforms can be used to identify the actions of antibiotics and discover plausible resistance mechanisms. Using SB inspired metabolic engineering, antibiotic production can be optimized and scaled up to meet demand. Persistent resistant cells within the bacterial community play a key role in the conferring of antibiotic resistance. SB approaches could build experimental models to dissect the complex interactions of the bacterial community. Fine-tuned genetic circuits will help to improve the productivity of the industrial strains for antibiotic production. Using synthetic genome approaches may help to develop novel vaccines and treatments to combat antibiotic resistant bacteria.

2. Insights about antibiotic killing and resistance mechanisms

It is known that some of the antibiotic resistances to a defined antibiotic are due to the presence of intrinsic resistance genes [21]. To define systematically the genes conferring resistance to a targeted antibiotic, a genomic library of bacterial strains can be constructed, and enriched in a selective medium containing a lethal concentration of the targeted antibiotic. A SB approach to identify these genes was reported in the study of resistance to triclosan in *E. coli* [22]. There were 47 out of 62 genes relevant to cell growth in the presence of triclosan (identified by a DNA microarray) that

were confirmed to enhance resistance to triclosan. These resistance genes were involved in inner or outer membrane synthesis, cell wall synthesis, transcriptional activation, sugar phosphotransferase system (PTS), various transporter systems, cell division, and ATPase and reductase/dehydrogenase reactions. The study on triclosan resistance in *E. coli* indicates that undefined novel mechanisms for the development of resistance to a targeted antibiotic can be also investigated using similar an approach. Some of the antibiotic resistant infections are due to pathogens that produce a small portion of persister cells, which can cause recurrence of the infection once the antibiotic pressure is released [23]. Understanding the mechanism of persister cells can provide important insights to fight infections. A SB inspired system was developed to study the persister cells in *E. coli* by investigating the control circuit responsible for generation of a persister subpopulation. A genetic regulation model was built to study the phenotypic heterogeneity [24]. Such models may be applied to study the persister cells of other microbial populations, and may provide opportunities to discover novel antibiotic compounds targeting the persister cells. The study of antibiotic resistance in a biofilm represents a challenge due to the complex composition and construction of the microbial ecosystem. But recent progress by SB in studying the artificial ecosystem may provide insights about the function of ecosystems [25]. By using two quorum-sensing signal transduction circuits, a synthetic ecosystem was constructed where various population dynamics were formed by changing environmental factors. It showed that different antibiotic levels and initial cell densities could result in correlated population dynamics such as extinction, obligatory mutualism, facultative mutualism and commensalism. Such a synthetic ecosystem, combined with settings mimicking the naturally occurring situations, such as dental plaque or biofilm on implants, may act as a chassis for construction of more complex microbial ecosystems.

Most of the antibiotics that have been developed aim to inhibit a defined bacterial cellular function, ranging from DNA replication and repair, protein synthesis, to cell wall synthesis. The bacterial responses to antibiotics and the causes of antibiotic resistance are not well understood. It is believed that the multiple-layer effects of many genetic and biochemical pathways may be involved [26]. To study the bacterial responses to an antibiotic, an engineered circuit was used to study effects on the DNA damage system (SOS response) in *E. coli* by bactericidal antibiotics. The results suggested a common killing mechanism induced by antibiotics is the formation of harmful hydroxyl radicals resulting from metabolism-related NADH depletion, leaching of iron from iron sulfur clusters, and stimulation of the Fenton reaction. Besides using designed genetic circuits to study antibiotic killing and resistance mechanisms, protocells may serve as a useful platform to investigate the complex physiological changes of bacteria in response to antibiotics. Constructing a protocell containing a cellular pathway of interest may help to dissect the molecular changes that occur in the presence of the antibiotics, such as the protocell applications proposed for toxicological studies [27]. The new insights obtained from these studies will be used to design new drugs and infection control strategies, which will be discussed in the following sections.

3. Novel antibiotics and their production

To address the challenges raised by the increase in antibiotic-resistant bacteria, there is an urgent need to understand the genetic composition of these microbes. The growing microbial sequence database provides excellent starting materials to be investigated. However, there is no efficient approach to explore these data and produce lists of essential genes for future compound screening. The research on minimal genome may provide such an approach. Currently, the research on minimal genomes focuses on identifying the essential genes that are indispensable to sustain the cellular functions for a living cell. Determination of the universal minimal set of essential genes, particularly those of the antibiotic resistant bacteria, will contribute to our understanding of plausible modes of pathogenesis. These essential genes can serve as targets to develop novel antibiotics. Several bacterial minimal genomes have been studied, for example, those of *E. coli* [28], *Pseudomonas putida* [29], and some species of mycoplasma [30-33]. Generally speaking, the essential genes of bacteria are estimated to be approximately 300 genes, ranging from 150 to 500 [7, 34, 35], and most of them are the genes encoding cellular functions. Two main approaches are currently applied for the studies on minimal genomes using SB [5-7]. One is by the top-down approach to further minimize the existing genome by removing all non-essential genes. And the other is by bottom-up approach to create a minimal cell by synthesizing the genome designed *in silico* containing all of its essential genes.

Use of genetic circuits has provided new insights into natural gene-network dynamics and they have served as new screening platforms, leading to the identification of new drug candidates. It is known that the compounds that prevent *Mycobacterium tuberculosis* EthR from binding to a specific operator (OethR) could enhance drug killing. A synthetic mammalian gene circuit was constructed to study the EthR–OethR interaction in human cells by providing a quantitative reporter gene expression readout [36]. This platform was then challenged by the compounds of a chemical library, and a novel drug candidate, 2-phenylethyl-butyrate (an inhibitor of EthR) was identified. The design of this platform can be applied to screen other antibiotic compounds that cannot be screened by current methods. Aided by SB approaches to target cellular responses, new insights could be gained about the effects of antibiotic drugs on those cellular processes. This would have important implications for the development of novel treatments with known antibiotics. The study on SOS response induction in *E. coli* by bactericidal antibiotics suggested that they could be combined with small molecules to block activation of the DNA damage response (e.g., RecA inhibitors), and this would

result in novel antibiotics [37]. Thus, novel antibiotic agents can be screened in combination with the drugs of interest to enhance the cellular killing to develop new antibacterial therapies [38-40].

As the use of SB approaches to combat antibiotic resistant bacteria attracts more interest, more research has been focused on the construction of biological systems for antibiotic production. SB approaches will enable complex design of microorganisms to maximize their productivity. In many cases, the production of antibiotics involves the cooperation of multiple genetic circuits. Multiple protein complexes and the metabolic network for antibiotic production frequently relies on the efficient over-expression of all metabolites and enzymes in the same cell [41]. A plug-and-play strategy was proposed to insert the re-engineered versions of secondary metabolite biosynthetic pathways first into the screening hosts and then into the production hosts (Medema *et al.*, 2011). Several research projects have been focused on streptomyces species, well-known sources of antibiotics, in an effort to improve their antibiotic productivities [42, 43]. The physiological and genetic characterization of an orphan histidine kinase (OhkA) in *Streptomyces coelicolor* and its homolog in *Streptomyces avermitilis* were identified, showing that the *ohkA* mutant of *S. coelicolor* could overproduce multiple antibiotics, such as actinorhodin and calcium-dependent antibiotic, due to increased production of precursor malonyl coenzyme A (Lu *et al.,* 2011). Methods to modify metabolic pathways to convert fast growth and thermophilic strains into production strains were also developed to address the slow growth of the industrial strains, which often took a long time to grow in a large scale and consumed more energy and materials than some other bacterial industrial strains (such as *E. coli*) [44]. These findings will make possible future genetic designs of better antibiotic producing strains of *S. coelicolor* and *S. avermitilis*. Furthermore, a 22-kb *mmy-mmf* gene cluster within a 356-kb linear plasmid SCP1 of *S. coelicolor* A3 was discovered to encode the genetic elements necessary for the production of the antibiotic methylenomycin (Mm). A putative operon mmfLHP was believed to direct the biosynthesis of an A-factor-like signaling molecule to regulate the Mm biosynthesis [45]. Five new possible signaling compounds produced by the *mmfLHP* genes were identified as 2-alkyl-4-hydroxymethylfuran-3-carboxylic acids. Further experiments are needed to study the roles of these molecules as new class of antibiotic biosynthesis inducers to produce novel antibiotics in *Streptomyces* species.

The advance of SB offers possible methods to screen novel antibacterial compounds from natural products [46, 47]. Natural products, particularly those from the Chinese traditional medicine, have been the key components in some antibacterial remedies. Due to the complexity of these natural products, SB approaches that combine multiple design, assembly and integration of biosynthetic pathway involved are particularly useful, and a couple of strategies were applied for the medicinal natural products [48]. The current SB approaches have been focused on the methods to screen novel compounds, building biosynthetic element modules database, rational biosynthesis pathway design, and upgrading to large-scale production with further optimization (Lu *et al*, 2011). A method was developed for affinity screening and analysis of bioactive components that interacted with cells [49]. This method was based on three-dimensional cell bioreactor coupled with high performance liquid chromatography–mass spectrometry to screen novel anti-tumor components from herbal medicines by affinity chromatography. The compounds that could interact with fixed cancer cells in the bioreactor were subjected to HPLC fingerprinting chromatography. Selected compounds from herbal medicine extract were compared with known compounds to evaluate their binding properties. The method was used to screen bioactive components from *Polygonum cillinerve* (Nakai) Ohwi (PCO) extract. Such approaches can be applied to screen antibiotic compounds from the traditional Chinese medicinal remedies against bacterial infections. These remedies have been proved to be efficient in curing infections in empirical practice over thousands of years. The safety profile of the remedies has been built, unintentionally, on the large scale of practice. What is needed is to identify the potent components in these mixed remedies. An efficient screen method, combined with rational design for mimicking the underlying biosynthesis pathways, is critical to gain new insight about Chinese traditional medicine, and to develop better antibiotics from traditional ones. Optimization of production is demonstrated by the production of Paclitaxel, a mitotic inhibitor used in cancer chemotherapy, discovered by screening compounds extracted from *Taxus brevifolia*. Traditionally, Paclitaxel is extracted from the plant, which is costly and scarce. It is an attractive natural product to be developed in plant cell culture using SB approaches. Yet, loss of secondary metabolite production is a common obstacle in a large-scale plant cell culture. A study has been conducted on cell morphology, biosynthetic ability, and genetic and epigenetic variations in the culture of *Taxus media* cv Hicksii cells over a 5-year period. The study revealed that the gradual loss of Paclitaxel yield, and a decreased level of Paclitaxel biosynthesis key genes transcription in long-term culture might be related to a higher level of DNA methylation. Overcoming the gradual loss of Paclitaxel biosynthesis capacity in cell culture may improve productivity further [50]. The approach can be applied to optimize the production of other natural antibiotic compounds.

Peptide-based antibiotics are small molecules produced by the host organisms that provide frontline defense against a large spectrum of invading microbes [51]. Compared to classical antibiotics, peptide-based antibiotics can kill target bacteria by destroying their membranes, as well as by other mechanisms, ranging from inhibiting cell wall synthesis, binding to DNA, inhibiting DNA, RNA and protein synthesis, inhibiting enzymatic activity, and activating autolysin [52]. The toxic nature of this group of antibiotics to the host cells, as well as the post-translational modification during their biosynthesis, make them difficult to produce in large scale in common bacteria-based industrial production strains [53]. In recent years, these types of antibiotics have become attractive to SB research for their potential therapeutic applications. One example is our current project, SYNMOD (Synthetic biology to obtain novel antibiotics and

optimized production systems funded by Austria Science Fund) [54]. SB approaches are applied to design and produce novel lantibiotics. First, modules are designed for the biosynthesis of a group of post-translationally modified peptide antibiotics, the lantibiotics. Then, a context-insensitive post-translational machinery will be built by exploiting modification enzymes and enabling a fine-tuning of the composition of the modification pathway. The engineered pathway will then be implemented in a novel production chassis of reduced complexity, for example in *Staphylococcus carnosus*, and the resulting strain will be used to produce a variety of novel lantibiotics.

4. Novel approaches to combat antibiotic resistant bacteria

A number of SB strategies have been proposed, other than antibiotics, aiming to treat infections caused by antibiotic resistant bacteria. Bacteriophages have been of research interest to combat bacterial infections for decades, although with limited successes and clinical practices [55]. The development of SB makes it possible to synthesize the phage entirely, and adds more freedom to incorporate the circuits of interest into the engineered phage. It helps to turn the phages into attractive agents to develop antibacterial treatments. Some progress has been achieved in using engineered bacteriophages to treat bacterial infections [56]. Lytic bacteriophages (T7 phage) were constructed to express dispersin B that could degrade the extracellular polymeric substances of bacterial biofilm [57]. Engineered bacteriophages could act as antibiotic adjuvants to enhance the efficacy of existing antibiotics, for example, phages to target the SOS networks (over-expressing SoxR or LexA3), phages to target the non-SOS networks and multiple factors (CrsA to represses biofilm formation and antibiotic tolerance and/or OmpF to enhance antibiotic penetration) for ofloxacin treatment [58]. Lacking effective approaches to deliver drugs into target microbes reduces the efficacy of the antibiotics. To tackle these issues, phages were engineered to deliver the antibacterial agents into the target cells. A nonlytic phage was engineered to deliver lethal agents Gef and ChpBK to the offending bacterium *E. coli* [59]. Besides their use as therapeutic treatments, engineered phages might serve as nosocomial infectious control strategies. A proof of principle using bacteriophages to deliver genetic constructs into bacteria was demonstrated in a system to restore antibiotic efficiency by reversing pathogen resistance. Temperate phages were engineered to introduce the expression of RpsL and GyrA to confer sensitivities to two antibiotics, streptomycin and nalidixic acid, respectively [60]. It is believed that these engineered phages, as alternative infectious control agents, would gradually change the composition of nosocomial microbial population toward being antibiotic susceptible rather than resistant.

Another novel approach to combat antibiotic resistant bacteria is by engineering microbes to sense and destroy a targeted pathogen [61]. An engineered *E. coli* was constructed and equipped with a synthetic genetic system comprising the quorum sensing, killing, and lysing devices. This engineered *E. coli* could detect and destroy pathogenic *P. aeruginosa* through the production and release of pyocin. Since the production of pyocin occurs only after sensing the targeted pathogen in this approach, it would serve as an infection control strategy to prevent the growth of pathogenic species in microflora. This suggested that non-pathogenic bacteria with a specific sense-and- destroy genetic system may provide a novel antimicrobial strategy to combat pathogens, particularly those with antibiotic resistance. To enhance the safety profile of engineered organisms, similar sense-and-destroy genetic systems can be incorporated into the genome of a chassis cell that is designed based on minimal cells or protocells. Eventually, this type of sense-and-destroy system could be executed via an implantable device to deploy the anti-infectious agent automatically once a target pathogen is present.

The use of SB approaches will have great impacts on the design of vaccines against infectious disease [62]. Conventional vaccines are developed mainly by the live-attenuated approach, by inactivating microorganisms by chemical or physical approaches, or by producing subunit formulas composed of purified components or recombinant proteins. It is believed that vaccines can be a promising strategy to mitigate health care problems raised by the exponential growth of antibiotic resistant bacteria, by directly reducing the use of antibiotics, and/or indirectly through the establishment of herd immunity to halt the levels of transmission of pathogenic bacteria to the susceptible individuals [63]. Although conventional vaccines have contributed significantly to combating bacterial infections, novel vaccines are needed for those suboptimal formulas and for those caused by antibiotic resistant strains. The contributions of SB to vaccine development depend mainly on a better understanding of the genetic underpinning of pathogens, *in silico* designed immunogens with improved immunogenicity and breadth, and *de novo* synthesized live-attenuated vaccines [64]. For example, a flu vaccine was developed by the SB inspired approach, using DNA, the “software of life”. A process was developed to make influenza vaccines in less time, and yet with improved yield by employing synthetically-derived virus stock seeds [65, 66]. Currently, most the SB vaccine research is focused on the virus; while less has been done for bacteria. Applying SB approaches to develop vaccines to antibiotic resistant pathogens will be a future research topic to be explored (e.g., vaccines to methicillin-resistant *Staphylococcus aureus*, multidrug resistant *Mycobacterium tuberculosis*, *Clostridium difficile* and Shiga toxin-producing *E. coli*).

5. Concluding remarks

SB can have a significant impact on antibiotics development and the potential to generate novel anti-infection strategies. The prerequisites for these developments are based on continuous technological advances from the field. Progress in synthesizing and sequencing DNA, fine-tuned genetic circuits, and sophisticated platforms derived by protocell research, will enhance our understanding of cellular systems of the bacteria as well as those of the hosts. Based on the knowledge of the cellular systems, biologically active compounds can be screened for their potential to kill bacteria and to discover novel antibiotics. The improved capacity of SB to design complex cellular systems will help enhance antibiotic production further. Besides antibiotic-based treatment, inspiring by SB, new ideals will be developed for infectious control strategies. The complete *de novo* synthesis of genome has become technically and economically feasible. The bacteriophage-based anti-infection strategies have already shown their potential. Engineered nonpathogenic bacterial cells, with a sense-and-kill biological system, would provide an attractive method for infection control and treatment. SB concepts can also be applied to develop novel vaccines, although not much has been done for vaccines against bacterial pathogens. It is highly expected that the advance of SB will lead to potential applications to improve human health. Being able to develop novel approaches to combat antibiotic resistant bacteria is the one that most likely to be achieved in the coming years.

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References

[1] Cello, J., *et al.* (2002) Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. *Science* 297, 1016-1018
[2] Gibson, D.G., *et al.* (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329, 52-56
[3] Tigges, M., *et al.* (2009) A tunable synthetic mammalian oscillator. *Nature* 457, 309-312
[4] Stricker, J., *et al.* (2008) A fast, robust and tunable synthetic gene oscillator. *Nature* 456, 516-519
[5] Jewett, M.C. and Forster, A.C. (2010) Update on designing and building minimal cells. *Curr Opin Biotechnol* 21, 697-703
[6] Moya, A., *et al.* (2009) Toward minimal bacterial cells: evolution vs. design. *FEMS Microbiol Rev* 33, 225-235
[7] Juhas, M., *et al.* (2011) Essence of life: essential genes of minimal genomes. *Trends in cell biology* 21, 562-568
[8] Danchin, A. (2009) Information of the chassis and information of the program in synthetic cells. *Syst Synth Biol* 3, 125-134
[9] Deplazes, A. and Huppenbauer, M. (2009) Synthetic organisms and living machines : Positioning the products of synthetic biology at the borderline between living and non-living matter. *Syst Synth Biol* 3, 55-63
[10] Rasmussen, S. (2009) *Protocells: bridging nonliving and living matter*. MIT Press
[11] Walde, P. (2010) Building artificial cells and protocell models: experimental approaches with lipid vesicles. *BioEssays* 32, 296-303
[12] Schmidt, M. (2010) Xenobiology: A new form of life as the ultimate biosafety tool. *BioEssays* 32, 322-331
[13] Pinheiro, V.B., *et al.* (2012) Synthetic Genetic Polymers Capable of Heredity and Evolution. *Science* 336
[14] Kwok, R. (2012) Chemical biology: DNA's new alphabet. *Nature* 491, 516-518
[15] Marliere, P., *et al.* (2011) Chemical evolution of a bacterium's genome. *Angew Chem Int Ed Engl* 50, 7109-7114
[16] Ramaswamy, A., *et al.* (2010) Helical structure of xylose-DNA. *J Am Chem Soc* 132, 587-595
[17] Herdewijn, P. and Marliere, P. (2009) Toward safe genetically modified organisms through the chemical diversification of nucleic acids. *Chem Biodivers* 6, 791-808
[18] Adelfinskaya, O., *et al.* (2007) Polymerase-catalyzed synthesis of DNA from phosphoramidate conjugates of deoxynucleotides and amino acids. *Nucleic Acids Res* 35, 5060-5072
[19] Davies, J. and Davies, D. (2010) Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 74, 417-433
[20] Wold Health Organization 2012) Antimicrobial resistance *Fact sheet.* N°194, http://www.who.int/mediacentre/factsheets/fs194/en/
[21] Courvalin, P. (1996) The Garrod Lecture. Evasion of antibiotic action by bacteria. *The Journal of antimicrobial chemotherapy* 37, 855-869
[22] Yu, B.J., *et al.* (2012) Genome-wide enrichment screening reveals multiple targets and resistance genes for triclosan in Escherichia coli. *J Microbiol* 50, 785-791
[23] Lewis, K. (2012) Recover the lost art of drug discovery. *Nature* 485, 439-440
[24] Lou, C., *et al.* (2008) A molecular model for persister in E. coli. *J Theor Biol* 255, 205-209
[25] Hu, B., *et al.* (2010) An environment-sensitive synthetic microbial ecosystem. *PLoS One* 5, e10619
[26] Kohanski, M.A., *et al.* (2010) How antibiotics kill bacteria: from targets to networks. *Nat Rev Microbiol* 8, 423-435
[27] Schmidt, M. and Pei, L. (2011) Synthetic toxicology: where engineering meets biology and toxicology. *Toxicol Sci* 120 Suppl 1, S204-224
[28] Zhang, R., *et al.* (2004) DEG: a database of essential genes. *Nucleic Acids Res* 32, D271-272
[29] Molina-Henares, M.A., *et al.* (2010) Identification of conditionally essential genes for growth of Pseudomonas putida KT2440 on minimal medium through the screening of a genome-wide mutant library. *Environ Microbiol* 12, 1468-1485
[30] Lin, Y. and Zhang, R.R. (2011) Putative essential and core-essential genes in Mycoplasma genomes. *Sci Rep* 1, 53

[31] Sirand-Pugnet, P., *et al.* (2007) Being pathogenic, plastic, and sexual while living with a nearly minimal bacterial genome. *PLoS Genet* 3, e75
[32] Ma, L., *et al.* (2007) Mycoplasma genitalium: an efficient strategy to generate genetic variation from a minimal genome. *Mol Microbiol* 66, 220-236
[33] Glass, J.I., *et al.* (2006) Essential genes of a minimal bacterium. *Proc Natl Acad Sci U S A* 103, 425-430
[34] Forster, A.C. and Church, G.M. (2007) Synthetic biology projects in vitro. *Genome Res* 17, 1-6
[35] Feher, T., *et al.* (2007) Systematic genome reductions: theoretical and experimental approaches. *Chemical reviews* 107, 3498-3513
[36] Weber, W., *et al.* (2008) A synthetic mammalian gene circuit reveals antituberculosis compounds. *Proc Natl Acad Sci U S A* 105, 9994-9998
[37] Kohanski, M.A., *et al.* (2007) A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* 130, 797-810
[38] Barreiro, C., *et al.* (2012) Proteomics shows new faces for the old penicillin producer Penicillium chrysogenum. *J Biomed Biotechnol* 2012, 105109
[39] Montalban-Lopez, M., *et al.* (2011) Are bacteriocins underexploited? Novel applications for old antimicrobials. *Curr Pharm Biotechnol* 12, 1205-1220
[40] Mouton, J.W., *et al.* (2011) Conserving antibiotics for the future: new ways to use old and new drugs from a pharmacokinetic and pharmacodynamic perspective. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* 14, 107-117
[41] Wang, T., *et al.* (2012) Available methods for assembling expression cassettes for synthetic biology. *Appl Microbiol Biotechnol* 93, 1853-1863
[42] Freitag, A., *et al.* (2006) Metabolic engineering of the heterologous production of clorobiocin derivatives and elloramycin in Streptomyces coelicolor M512. *Metab Eng* 8, 653-661
[43] Xie, P., *et al.* (2009) cmdABCDEF, a cluster of genes encoding membrane proteins for differentiation and antibiotic production in Streptomyces coelicolor A3(2). *BMC Microbiol* 9, 157
[44] Chen, W. and Qin, Z. (2011) Development of a gene cloning system in a fast-growing and moderately thermophilic Streptomyces species and heterologous expression of Streptomyces antibiotic biosynthetic gene clusters. *BMC Microbiol* 11, 243
[45] Corre, C., *et al.* (2008) 2-Alkyl-4-hydroxymethylfuran-3-carboxylic acids, antibiotic production inducers discovered by Streptomyces coelicolor genome mining. *Proc Natl Acad Sci U S A* 105, 17510-17515
[46] Walsh, C. (2003) Where will new antibiotics come from? *Nature Reviews Microbiology* 1, 65-70
[47] Herrera, S. (2005) Synthetic biology offers alternative pathways to natural products. *Nat Biotechnol* 23, 270-271
[48] Huang, W., *et al.* (2011) Synthetic biology toward medicinal natural products. *Chinese Bulletin of Life Sciences* 23, 891-899
[49] Mou, Z.L., *et al.* (2012) Three-dimensional cell bioreactor coupled with high performance liquid chromatography-mass spectrometry for the affinity screening of bioactive components from herb medicine. *Journal of chromatography. A*
[50] Fu, C., *et al.* (2012) Assessment of genetic and epigenetic variation during long-term Taxus cell culture. *Plant Cell Rep* 31, 1321-1331
[51] Hancock, R.E. and Chapple, D.S. (1999) Peptide antibiotics. *Antimicrobial agents and chemotherapy* 43, 1317-1323
[52] Brogden, K.A. (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol* 3, 238-250
[53] Zorko, M. and Jerala, R. (2010) Production of recombinant antimicrobial peptides in bacteria. *Methods Mol Biol* 618, 61-76
[54] SYNMOD (2011) Synthetic biology to obtain novel antibiotics and optimized production systems (SYNMOD). http://www.esf.org/activities/eurocores/running-programmes/eurosynbio/projects-crps/synmod.html
[55] Deresinski, S. (2009) Bacteriophage therapy: exploiting smaller fleas. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 48, 1096-1101
[56] Ruder, W.C., *et al.* (2011) Synthetic biology moving into the clinic. *Science* 333, 1248-1252
[57] Lu, T.K. and Collins, J.J. (2007) Dispersing biofilms with engineered enzymatic bacteriophage. *Proc Natl Acad Sci U S A* 104, 11197-11202
[58] Lu, T.K. and Collins, J.J. (2009) Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. *Proc Natl Acad Sci U S A* 106, 4629-4634
[59] Westwater, C., *et al.* (2003) Use of Genetically Engineered Phage To Deliver Antimicrobial Agents to Bacteria: an Alternative Therapy for Treatment of Bacterial Infections. *Antimicrobial agents and chemotherapy* 47, 1301-1307
[60] Edgar, R., *et al.* (2012) Reversing bacterial resistance to antibiotics by phage-mediated delivery of dominant sensitive genes. *Appl Environ Microbiol* 78, 744-751
[61] Saeidi, N., *et al.* (2011) Engineering microbes to sense and eradicate Pseudomonas aeruginosa, a human pathogen. *Mol Syst Biol* 7, 521
[62] Kindsmüller, K. and Wagner, R. (2011) Synthetic biology: Impact on the design of innovative vaccines. *Human vaccines* 7, 658-662
[63] Mishra, R.P., *et al.* (2012) Vaccines and antibiotic resistance. *Curr Opin Microbiol* 15, 596-602
[64] Kindsmuller, K. and Wagner, R. (2011) Synthetic biology: impact on the design of innovative vaccines. *Human vaccines* 7, 658-662
[65] Gobel, U. (2012) Meeting report: Synthetic DNA - Writing with the Letters of Life: January 24, 2012, Frankfurt, Germany. *Biotechnol J* 7, 471-472
[66] Mueller, S., *et al.* (2010) Live attenuated influenza virus vaccines by computer-aided rational design. *Nat Biotechnol* 28, 723-726.

