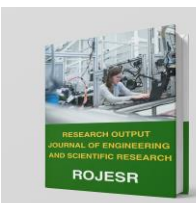

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Biological Engineering of Novel Antibiotics

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ABSTRACT

The emergence of antibiotic-resistant bacteria has created a significant public health crisis, necessitating the development of novel antibiotics. Traditional methods of antibiotic discovery have reached a plateau, prompting the exploration of biological engineering approaches to uncover new antibiotics. This paper reviews the historical significance of antibiotics, the mechanisms of antibiotic resistance, and the challenges in developing new antibiotics. It highlights synthetic biology as a promising approach, utilizing genetic enhancement and metabolic engineering to produce novel antibiotics. Case studies, such as the development of streptomycin, demonstrate the potential of biological engineering in addressing antibiotic resistance. The paper concludes by discussing future perspectives and the need for continued innovation in antibiotic discovery to combat the growing threat of multidrug-resistant bacteria.

Keywords: Antibiotic resistance, Biological engineering, Synthetic biology, Novel antibiotics, Metabolic engineering.

INTRODUCTION

The increasing problem of resistant bacteria has resulted in more deaths attributed to bacterial infections of hospital-based out-patients in many countries of the world, at least in geographic areas with relatively straightforward access to antibiotics. Within this structure of public health, antibiotics are considered to be a 'public good'. Over the last 14 years, the global biotechnology and pharmaceutical industries have experienced a slow-down in the development of novel truly antibiotics to only two new classes, the antibiotics-p interactions and the F0-F1 complex. Although the potential contribution of traditional antibiotic discovery was largely fulfilled through the sixties to the end of the eighties, thanks largely to the activities of the pharmaceutical industries, a resurgence in novel antibiotic production is essential. This reinvestment by the scientific community in 'new antibiotics' could also provide a 'yield' of novel molecular tools which could be used by the biochemist and cell biologist for in vitro manipulations of cell function as well as anti-infectives [1]. Antibiotics represent a wide diversity of chemical structures containing lactones, steroids, macrolides and glycopeptides to name a few. They can affect bacteria at a range of targets including DNA replication, transcription, proteins, cell wall biosynthesis and metabolism. Alternatively, some compounds indirectly affect bacterial infectivity by enhancing the immune response of the host. In practice, most antibiotics have been discovered using a combination of classical microbiology (e.g., screening soil microorganisms) and chemical diversity (e.g., combinatorial chemistry). In theory, the genetic enhancement of biosynthetic pathways could yield novel molecular scaffolds containing agents with anti-infective activity. However, many streptomyces exploited in the lab are silent for antibiotic production and it has been postulated that about 5% of the genes from soil organisms normally express a prodigy with antibiotic potential. The generation of genetic diversity within a biosynthetic gene cluster when it is cloned in vivo, follows a power spectrum, with 20 overloaded in the first kilobase. In theory, a combination of PCR and recombination could be exploited to yield a construct with a diverse spectrum of antibiotics [2]

HISTORY AND IMPORTANCE

For as long as humans have unintentionally been consuming antibiotics (up until the last half-century, mostly from drinking fermented or otherwise funky beverages), we have been participating in artificially augmented evolution – mainly, our gut microbial ecosystems have evolved to be antibiotic-resistant. Since

the dawn of the antibiotic era in the early 20th century, we have been consuming them at rates unprecedented in the human coevolution with the larger microbiota, leading to the sharp rise in antibiotic microbes we see in the wild today. Antibiotics have truly transformed medical care, removing the constant pervasive threat of human and animal death by septicemia, septic shock, and many other clinical presentations of systemic or localized invasive infection. It seems strange to press so deeply into the microbial realm for novel antibiotics, now 56 years after man first walked on the moon and well into the second decade of the viral age, but it is the right and ethical thing to do. As of last year, multidrug-resistant infections caused an estimated 1.27 million deaths in a world of 7.9 billion people, and these remarkable and awe-inspiring statistics will only worsen if we as a scientific community do not act on it [3].

THE HISTORICAL USE OF ANTIBIOTICS AND IMPORTANCE OF ANTIBIOTIC MEDICATIONS IN MEDICINE

Resisting the Resistance: A Primer on the Search for Novel Antibiotics. Antibiotics, such as penicillin, sulfonamides, and streptomycin, have long held an important place in the pantheon of maximum-impact medical interventions that drive mortality and morbidity down on the same time scale as annual vaccination schedules. In this sense, it is difficult to overestimate the importance of antibiotics in medicine. Antibiotics were first described for expediting the healing of wounds and against many oral infections (2000-1500 BC) by Clarus of Cos in his study of the impact of nutmeg and galangal on man's more humoral tendencies. Although at the time this heroic measure addressed each individual patient's qualitative needs based on their proportion of lust and complexity, it quickly became not just palatable, but entirely appropriate. In the context of what biologic predicts today and based on available historical evidence, physicians saw little difference between failing to clean a laceration's contact ranged wood residue and the outright eldritch phenomenon of mood as neurasthenic or luetic and therefore medically necessary to manage. After a series of failures, they had a clear retort in 1928 when Alexander Fleming announced his discovery / displacement of their most successful early antibiotic: lysozyme. He isolated the antibiotic activity from the Staphylococcus culture nearby the Penicillium mold and cultured P. notatum to isolate it further, in 1928. In the meantime, Gerhard Domagk discovered and initiated commercial sponsorship for the first antimicrobial dyes – the sulfa drugs, including Prontosil – although these were largely overshadowed by streptomycin [4, 5].

MECHANISMS OF ACTION

Inhibitory agents or antibiotics provide effective treatment against a variety of bacterial infections, which constitute one of the main threats to human health. Unique mechanisms and metabolic pathways in bacteria provide a wide range of targets for clinically used antibiotics. For example, antibiotics can bind specifically to biochemical processing enzymes, DNA, RNA, or ribosomes of bacterial pathogens or disrupt the cell wall proteins and functions of bacterial membranes. Therefore, the development of completely novel antibiotics provides therapeutic alternatives to classic antibiotics, including multiple drug-resistant pathogens. However, antibiotic research and development has nearly come to a standstill, reducing new antibiotic options for the public, according to a report from the Tunbridge Wells Antibiotic Research Foundation (TWAB) [6]. The principal focus is to stimulate and shape inhibitor discovery. There are also excellent studies providing comprehensive analyses of existing drugs, including a large volume of drug-like compounds in experimental and clinical phases. The major goal of the aspiring clinical-level biological engineering study is to produce novel antibiotics, with a particular emphasis on facilitating and increasing the development of new inhibitors. As noted above, this would be particularly valuable in terms of both the immediate provision of infectious therapies and the strength in the basic research loaded with the creation of inhibitors or at the stage of discovery. This mechanism proposes to combine natural sequence distribution and structural information of known inhibitors for structure-based drug design, including iterative protein sequence optimization [6].

CHALLENGES IN ANTIBIOTIC DEVELOPMENT

As infections and subsequent complications from antibiotic-resistant bacteria continue to increase, the active development of novel antibiotics is continually outpaced by the evolution of resistance. The use of antibiotics selects for resistance, and overuse dramatically accelerates this process. Many pharmaceutical companies have abandoned antibiotic research and development either because of shifts to more profitable research or because of the many challenges faced when developing new drugs. For many infections, available antibiotics are outdated, barely effective, or so toxic as to be barely tolerable. Furthermore, existing antibiotics leave many populations, especially those with weakened immune systems and transplanted organs, vulnerable to overwhelming infections which can lead to a high level of morbidity and mortality. Research and the development of drugs to combat resistance is key to maintaining

mortality benefits observed over the past several hundred years [7]. Novel strategies to achieve structural and biochemical diversity are required to strengthen the antibiotic arsenal in order to maintain an advantage over a moving target. The successful chemical synthesis of antibiotics in the mid-20th century generated viral resistance and improved human health. Therefore, redesigning antibiotics to circumvent resistance created through natural selection can lead to a weaponized counterattack in the near future. The next step is designing and engineering novel, nature-inspired toxins. Common solutions for introducing selective pressure are to leverage supplied technologies and the modern versatility of bacterial genetics. Furthermore, the strategy mimics "inverted genomics," interesting and invaluable with proteins or metabolites until now [8].

ANTIBIOTIC RESISTANCE

The urgent need for novel antibiotics is mainly driven by the emergence of strains of bacteria that are resistant to many, if not all, of the antibiotics currently on the market. This problem is of a particularly critical nature because the antibiotic pipeline is at an all-time low, with very few antibiotics in the phase of clinical trials. Meanwhile, the incidence of resistant strains of bacteria is increasing to the point where it has become a global policing threat to human health. Therefore, it is crucial to find new strategies to make existing antibiotics more effective, as well as to create whole new classes of antibiotics that can be used to combat multidrug-resistant strains of bacteria. This is the main target of our research program. Ultimately, the idea is to re-evaluate the countless secondary metabolites produced by various culturable species and to create a secondary metabolite from those that have not yet been translated into commercial antibiotics, thereby expanding the range of drugs that can be used to treat infections [9]. There is a consensus that policy reform is needed in the US to deal with antibiotic resistance globally, and only under these terms can we create a market in which antibiotics are valued and developed appropriately. There are many proposals to encourage the development of new antibiotics, including the regulation of currently marketed drugs to increase their lifespan, thereby reducing the speed at which pathogens evolve to resist them. In addition, the increased use of vaccines against various types of bacteria has significantly reduced the rate of antibiotic resistance and can be used as a means to combat antibiotic resistance in the future. Efforts are also being made to control and monitor the drug disposal process in hospitals around the world to ensure that the incidence of resistant strains is kept rather than spread, but the results to date have been mixed. Overall, it seems likely that a combination of all of these proposals is needed to mitigate the spread of antibiotic resistance around the world [10].

BIOLOGICAL ENGINEERING APPROACHES

With in silico modelling unveiling that approximately 99% of the current genetic sequence space harbours silent or cryptic natural compounds, and either silent or cryptic variants of many known antibiotics, biological engineering approaches offer the most compelling methods for discovering novel types of active principle [11]. Synthetic biology efforts are perhaps the most striking examples of a next generation of anti-infective that will hopefully circumvent the compression that arises when highly engineered single agent represents the last line of therapy. Given their manageable scale, there is a huge investment return both in terms of speed and efficacy. Rather than performing extensive deconstruction and reconstruction efforts, the current focus has been to either analyze the gene clusters encoding complex natural compounds or simply rationalize the endpoint based on cytochrome P450's, predicted glycosyltransferases, or the inclusion of rare and precious chemical modifiers. The skill of the measurement of gene expression at scale, specifically the constant interrogation of broad seasonal metatranscriptomes that has provided at least two apparel firms the wherewithal to introduce new fashion items that will have to be cleaned unseasonably. These investments appear to reveal nothing particularly surprising except the rarity of new natural compounds with exceptional potency [12]. Given the relatively small per unit product cost and the high available margins when a novel antibiotic is taken to market, synthetic efforts that have the capacity to deliver large numbers of natural products is potentially game changing due to their signal over noise contributions to the value of discovering new leads. Types of synthesis and opportunities have been outlined previously and include the hijacking of other suites of transcription factors for the rapid overexpression of natural compound libraries and or the dedicated biosynthesis of closely related analogues that can confirm SAR driven modifications that have a lower cost, lower rigor endpoint [13].

SYNTHETIC BIOLOGY

Synthetic biology (synbio) is generally defined as the design and construction of new biological parts, devices, and systems, redesign of existing biological systems, and optimization of the function of artificial biological systems for useful purposes. Synthetic biology is generally different from previously conducted genetic engineering by having an increase in production of novel antibiotics from different

microorganisms, auxotrophic strains that rarely produced new antibiotics, or the product of potentially new antibiotics. Several studies have demonstrated that metabolic engineering (rational or otherwise) and synthetic biology techniques can be used to increase the natural production of typical antibiotics from actinomycetes to up to 100-fold the original previously under similar or other growth conditions [14]. In this mode of action, auxotrophy and spontaneous revertants of the auxotrophic block were widely used for improvement of antibiotics produced by microorganisms since the mid-1940s. In recent years, these approaches permitted the isolation of genetically derived spontaneous revertants of auxotrophic strains of microorganisms with increased productivity of known-produced antibiotics. Therefore, these genetically derived new antibiotics have no novel mode of action, but this complies with the definition of the term of new natural bioactive products. The isolation of spontaneously revertant auxotrophs showing an alteration in the productivity of commercially produced antibiotics indicates that spontaneous mutations can lead to the natural isolation of overproducing strains. The use of these classical approaches described above was feasible for bloodstream infections (for example, target S. pneumonia and L. monocytogenes) because of the deep-seated experience with these models.

CASE STUDIES

Streptomycin is probably the most famous example of an antibiotic to be engineered biologically. It was the first effective cure for tuberculosis (TB), which had killed 500 million people in the preceding century. It was also a quorum sensing inhibitor (QSI) or "bacteriocin", i.e. it inhibited mycobacterial genes that regulated their own defenses, which many microbes have conserved so it possibly inhibited four hundred other species, mopping up their co-pathogenic effects. The population at the time of TB was about 2 billion. A cure was needed quickly. The impact of Streptomycin is colossal. 100 million patients have been cured with it in over 70 years [15]. Although Streptomycin is given as a single antibiotic, in fact the genetically engineered Streptomycin actually gives seven different chemical antibiotics, made in six separate genetically engineered compartments: a bifunctional drug. Three other drugs are supplied simultaneously to create a four drug TB combination in a single pill in several countries as short treatment. In a standard c.6 month treatment, these two drugs are given for 8 weeks, whilst the others are given for 6 months. Streptomycin is used in short treatment in men in Sweden, for example. It was the first live genetic engineering experiment in the 19th century, combining two mistletoe species. The engineered streptomunitin is about 20 times its usual toxicity. Vigorous isolation and selection of the fittest is necessary for the cure of TB. Non-TB mycobacteria (NTM) are treated with some of these antibiotics more often than TB, for relatively short periods. The most important NTM to kill to manage TB resistance is Mycobacterium avium and M. intracellulare, both cousins of M. tuberculosis. Most NTM can be detected with a simple pH test within an hour (paraTB), with a cheap silk strip sensor. Using Streptomycin as a model for TB, an example c 17 case studies in genetically engineering antibiotics are discussed in another article [16].

STREPTOMYCIN

In a cycle of just under 100 years, a series of lucky accidents led to the discovery, development, and clinical application of an amazing antibiotic. It saved countless lives, directly stimulated the development of biological engineering, and is still in use today. Streptomyces griseus, the organism Streptomycin is derived from, was isolated from a village pond near Waksman's infantile paralysis laboratory in 1916. During the 1940s, Selman Waksman and his associates showed streptomycin to be a powerful, broad-spectrum antibiotic, but it was not until the early 1950s, after Albert Schatz, working in Waksman's laboratory, had shown crystalline streptomycin to be effective in pulmonary disease, that the drug received official approval in the United States and other countries. When the word of Schatz's work got out in 1946, Waksman took credit for the discovery and started an official smear campaign. Schatz fought back, eventually won a suit against Waksman, and received a settlement [17]. In 1943, three medical researchers at the Mayo Clinic isolated an aerobic acid-fast bacillus, since identified as Mycobacterium tuberculosis, from a liver abscess of a patient suffering from disseminated tuberculosis and found that it was resistant to all tuberculocidal agents available at that time - the first case of primary drug resistance to antituberculous chemotherapy ever described. Following in vitro screening of several hundred soil actinomycete cultures, streptomycin was chosen for clinical trial and found to be bactericidal for the tubercle bacillus. Parallel clinical studies conducted at the Mayo Clinic during 1944-1945 with twenty-six additional cases of advanced or new-onset active tuberculosis showed that added administration of streptomycin could achieve not only the more rapid resolution of fever, other clinical signs and symptoms, and disease course, but also strike a permanent and lasting effect on the development and healing of structural renal and other tuberculosis led by tuberculin skin testing rejuvenation-described drug resistance children [18].

FUTURE PERSPECTIVES

Today, biotechnology potentially allows for keeping up with the evolution of bacterial resistance and rapidly uncovering new macromolecular targets. The ongoing pharmaceutical discovery effort has evolved in the past two decades as a result of a stronger drive towards drug repositioning, in silico screening, and MACC, compared to high-throughput screening of synthetic libraries. In light of this, as well as the achievements in purified biological target identification and the continual development of novel tools and high-throughput technologies advancing target-based correlates of drug accumulation and activity, the real bottleneck in the pharmaceutical development pipeline is the purification of bioactive entities [19]. The time is right to establish entirely new fields in biological engineering, based on biology and biotechnology. We need to magnify the tools, goals, and attitudes used to investigate fundamental biology and to define helpful biologic materials. Biological engineering has the prospects for a revolutionary impact on the production of small and large molecules and will restore the hierarchy of fundamental goals behind the purifications necessary for the discovery of new macromolecular targets - solving important problems in biology and understanding. Several journals, including this one, have been quick to recognize these possibilities. The recent introduction of an open-access journal with inaugural papers on biological engineering from MIT Press is representative of the growth in this subject and important to future development. We hope that the work described in this article will inspire other laboratories to contribute further to this small and rapidly growing enterprise wherever their invention may arise, to apply to the following problems in three linked domains [20].

CONCLUSION

The fight against antibiotic-resistant bacteria requires innovative approaches beyond traditional methods of antibiotic discovery. Biological engineering, particularly through synthetic biology and metabolic engineering, offers promising avenues for developing new antibiotics. By harnessing genetic enhancement and optimizing biosynthetic pathways, it is possible to create novel antibiotics that can effectively combat multidrug-resistant bacteria. Case studies like streptomycin exemplify the potential impact of these approaches. Continued investment and research in biological engineering are essential to stay ahead of evolving bacterial resistance and to ensure the availability of effective treatments for future generations.

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