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Synthetic biology platform technologies for antimicrobial applications☆

Dana Braff [a,1], David Shis [b,1], James J. Collins [b,c,d,e,f,g,*]

[a] Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA
[b] Institute for Medical Engineering & Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[c] Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[d] Synthetic Biology Center, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[e] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA
[f] Harvard-MIT Program in Health Sciences and Technology, Cambridge, MA 02139, USA
[g] Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

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ABSTRACT

The growing prevalence of antibiotic resistance calls for new approaches in the development of antimicrobial therapeutics. Likewise, improved diagnostic measures are essential in guiding the application of targeted therapies and preventing the evolution of therapeutic resistance. Discovery platforms are also needed to form new treatment strategies and identify novel antimicrobial agents. By applying engineering principles to molecular biology, synthetic biologists have developed platforms that improve upon, supplement, and will perhaps supplant traditional broad-spectrum antibiotics. Efforts in engineering bacteriophages and synthetic probiotics demonstrate targeted antimicrobial approaches that can be fine-tuned using synthetic biology-derived principles. Further, the development of paper-based, cell-free expression systems holds promise in promoting the clinical translation of molecular biology tools for diagnostic purposes. In this review, we highlight emerging synthetic biology platform technologies that are geared toward the generation of new antimicrobial therapies, diagnostics, and discovery channels.

Contents

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* Corresponding author at: Institute of Medical Engineering & Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
E-mail address: jimjc@mit.edu (J.J. Collins).

[1] These authors contributed equally to this work.

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1. Introduction

The growing prevalence of antibiotic-resistant pathogens calls for the development of a new generation of antimicrobial therapies, diagnostics, and discovery platforms. The Centers for Disease Control and Prevention (CDC) reports that the rise of antibiotic resistance has become a public health crisis, leading to over 2 million infections and 23,000 deaths per year in the United States alone [1]. Limited diagnostic capabilities leave healthcare providers unable to precisely diagnose clinical infections and administer effective treatments in a timely manner. Furthermore, the use of broad-spectrum antibiotics clears out microbial niches and enables microbiome colonization by opportunistic pathogens [2]. This threat has motivated efforts to reinvigorate antibiotic research and streamline funding and approval processes for new therapies [3]. However, the rapid transmission of antibiotic resistance challenges our present ability to develop additional small-molecule therapeutics, broad spectrum or otherwise [4]. Amidst this antibiotic crisis, new research approaches are needed to progress the discovery of novel antimicrobial treatments.

Synthetic biology is well positioned to address the challenges in developing next-generation antimicrobial agents. By studying the design principles underlying native gene networks, synthetic biologists have developed tools that enable the rational engineering of biological systems [5,6]. Early efforts focused on the development of model transcriptional networks to recapitulate and understand native gene regulation [7–10]. These works advanced our ability to engineer complex behavior such as memory encryption and oscillatory gene expression, and catalyzed advancements in the rapid design and implementation of synthetic gene networks [11–20]. The field has since moved towards repurposing natural biological processes for tunable and targetable synthetic gene regulation [21–25]. The innate biochemistry of microorganisms has been harnessed in the biosynthesis of organic compounds, such as the antimalarial drug artemisinin [26] and various opioids [27]. Strides have also been made in engineering genetic networks for direct clinical applications such as customized cancer treatments and nonconventional cell therapies [28,29]. The extensive knowledge base that has emerged from synthetic biology and its union with other scientific fields, combined with the pressing need for next-generation antimicrobials, has led to the creation of new methods to combat pathogen resistance. In this review, we highlight synthetic biology platform technologies that can be utilized to produce novel antimicrobial therapeutics and diagnostics.

2. Therapeutics

Current broad-spectrum antibiotic treatments often result in the elimination of both pathogenic and commensal microorganisms. This clearing out of microbial niches leads to iatrogenic infections such as *Clostridium difficile* (*C. difficile*) and contributes to the increasing prevalence of antibiotic-resistant microbes [30,31]. Synthetic biology approaches have demonstrated potential in the development of targeted therapies that improve on existing treatment schemes by narrowing the antimicrobial target spectrum and providing spatiotemporal control over the delivery of therapeutic agents.

2.1. Bacteriophage-based therapeutics

One area of research that holds promise towards such applications is that of bacteriophage-based therapeutics (Fig. 1A). Bacteriophages (phages) are bacteria-specific viruses that insert their genetic information into a host microbe upon infection, taking over essential cellular functions and in some cases inducing microbial lysis [32]. Phages naturally discriminate between microbial species within mixed populations and bear great specificity toward their bacterial targets [33]. Shortly after their discovery, phages were recognized for their potential to combat bacterial infections, with the first generation of phage-based treatments relying on the natural specificity and lytic action of wild-type viruses [32,34,35]. Though their specificity presents an advantage at face-value, a narrow targeting spectrum without equally specific diagnostics for identifying the infectious pathogen, as well as the selection of resistance to phages during phage-based treatments, fueled the development of therapeutic cocktails that consisted of multiple phage species, each with different targeting spectrums. However, with the discovery of penicillin and other potent antibiotics, efforts to develop phage-based therapies were largely abandoned.

As the efficacy of antibiotics threatens to continue its decline in the face of growing resistance, interest in developing phage-based therapeutics has regained traction. Genetic manipulation of phages

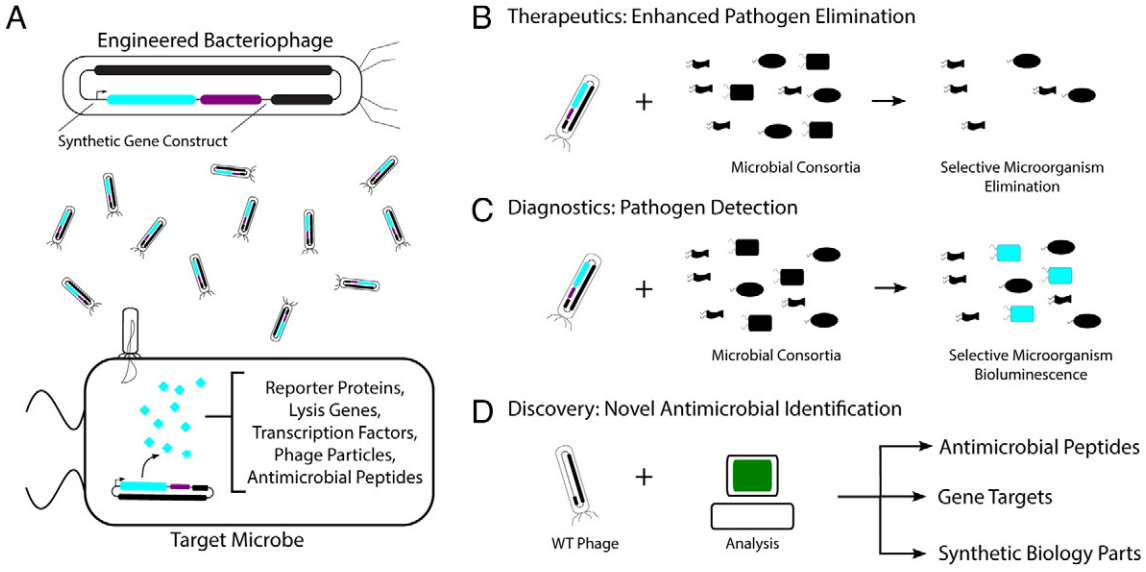

Fig. 1. Engineered phages. (A) The evolved ability of phage to infect and replicate within a host microorganism has made them a popular technology platform. An engineered phage can induce the expression of heterologous constructs in a specific population of microbes for therapeutic and diagnostic applications. (B) Modifying the phage genome to include the expression of bactericidal genes enables the utilization of phage as an antimicrobial therapeutic for specific microbial elimination. (C) By inducing microbial expression of reporter proteins, an engineered phage can facilitate bioluminescence that enables precise and sensitive detection of a microorganism. (D) Mining phage biology can aid in the identification of novel antimicrobial peptides, essential gene targets, and synthetic biology parts.