Review

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Posted Date: 4 June 2026

doi: 10.20944/preprints202606.0390.v1

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 doi:10.20944/preprints202606.0390.v1

Review

Synthetic Biology for Discovery and Production of Anti-Microbial Drugs

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Abstract

Microorganisms naturally produce many pharmaceutically and industrially relevant secondary metabolites. For this process they usually use biosynthetic units. For example, microbes from the genus Streptomyces possess great ability to produce a variety of natural products in such manner, which is possible due to complicated crosstalk between primary and secondary metabolism. These microbial cell factories produce more than 2/3 of antibiotics used in medicine, and a large variety of other bioactive compounds. Although bacterial producer hosts, including Bacillus spp. and Streptomyces spp., have been studied for decades, the engineering of these bacteria remains challenging, and the genetic potential has not been fully utilized. This is due to limited genetic toolbox, restriction activity and occurrence of silent biosynthetic gene clusters. Recent advancements in genetic manipulation of microorganisms allowed to improve the turnaround time of strain engineering, but still has strain-specific limitations. However, a new perspective offered by synthetic biology to exploit the potential of existing and novel pathways in primary and secondary metabolism allows combining of different biosynthetic steps originating from diverse bacteria using a limited toolbox. Synthetic biology has emerged as a robust strategy to understand, investigate, design, and engineer the biosynthetic capability of bacterial antibiotics machinery, including such in Streptomyces. Innovative synthetic biology and metabolic engineering tools have rapidly accelerated the discovery of new natural products as well as engineering of Streptomyces, e.g. enzymatic modules for secondary metabolite production can be combined in synthetic cells to produce new derivatives of natural products. Furthermore, with the recent advances in molecular biology and genome editing, Synthetic biology has focused at generation of controlled phenotypes from a given input and at other sophisticated approaches. In this review, developments of novel approaches of Synthetic biology for microbial engineering with focus on antibiotics producers like Streptomyces spp. are discussed.

Keywords: synthetic biology; microbiology; Streptomyces; biotechnology; primary metabolism; secondary metabolism; metabolic engineering

1. Introduction

Nowadays, 95% of drugs that are tested in Phase I are not reaching approval. It is very challenging to innovate in the area of novel safe medicines using current approaches of chemistry (Trosset & Carbonell, 2015; Yan et al., 2023). In the last decades, search for new drug targets derived from biological processes received a lot attention. However, focus has been given often to single level of biological regulation, ignoring the temporal and spatial properties of physiological processes (Xie et al., 2020).

Synthetic biology (SB) has brought the engineer's perspective into biology in an attempt to transform a biological cell into an industrial biofactory (Trosset & Carbonell, 2015). Synthetic biology

 doi:10.20944/preprints202606.0390.v1

combines different fields of research as an application-driven discipline to design biological components for engineering applications (Xie et al., 2020; Endy, 2005; Way et al., 2014; Xie and Fussenegger, 2018; Wu et al., 2019). In the last decades, natural-product large-scale production has driven pharmaceutical companies to not rely on natural sources of medicinal compounds. Their therapeutic advantages, such as e.g. biocompatibility, lost the priority. Chemistry was selected as the main approach instead leading to the risk of increased cross-reactivity with secondary therapeutic targets and off-targets (Hu & Bajorath, 2014; Leil & Bertz, 2014; Ryall & Tan 2015). Target promiscuity is often a source of toxicity issues leading to end of projects at clinical stage (Trosset & Carbonell, 2015). In Synthetic biology optimization and exploration of natural products by reconstructing microorganism metabolic pathways attracts recently a lot of attention (Xie et al., 2020; Luo et al., 2014).

Bacteria produce secondary metabolites mostly using biosynthetic units - for example Streptomyces spp. produce 2/3 of all antibiotics for current medical applications (Krysenko & Wohlleben, 2024). Such enzymatic modules can be combined in synthetic cells in order to produce new derivatives of natural products (Sun et al., 2015). The initial application of Synthetic biology in drug discovery was an attempt to boost innovation and create new chemical scaffolds that have natural products-derived properties. This should increase production of bioactive compounds with the biologically right pharmacological properties (Trosset & Carbonell, 2015). With the recent advances in computational biology, molecular biology, metabolic engineering, genome editing and protein engineering, Synthetic biology has focused its aim at generation of controlled phenotypes from a given input, e.g. bioswitch (Trosset & Carbonell, 2015).

Bioswitches are gene circuits usually based on quorum sensing signals. Another example of Synthetic biology approaches is BioBrick - a standardized assembly workflow, for instance applied for Streptomyces engineering (Krysenko, 2025). Principles of synthetic biology designs have been considered in de novo creations of artificial organisms by engineering cells to meet current demands. Most circuits are well-designed, however they are still not enough for sensing multiple signals and producing complex metabolites (Yan et al., 2023). One of main applocations of Synthetic biology is application to build artificial cellular networks and perform defined functions generating engineered organisms (Xie et al., 2020; Saltepe et al., 2018; Sedlmayer et al., 2018; Wu et al., 2019). The design of genetic circuits in Synthetic biology is widely used in pharmaceutical research for bioproduction of drugs by microorganisms and to support drug development (Trosset & Carbonell, 2015; Breitling & Takano, 2015). With progression of technologies, the synthetic biology-based therapeutic potential is becoming more applied in clinical biology and disease treatment. Drug target discovery draw a lot of attention for development of novel drugs and therapeutics. Synthetic biology is nowadays considered a field that has a lot of potential to discover and validate novel drug targets, and design new treatment strategies (Xie et al., 2020).

2. Concepts of Synthetic Biology

2.1. Basic Approaches and Concepts

A synthetic cell is usually composed of an inducer, which can be a small molecule, a ligand or a membrane receptor that triggers a de novo-designed genetic circuit. This leads to a production of an output signal that can be followed by a reporter gene (Trosset & Carbonell, 2015). Gene circuits from bacterial secondary metabolism or from cryptic biosynthetic gene clusters (e.g. typical in Streptomyces) can be integrated into an optimized host to facilitate gene expression of an active compound. Furthermore, it can be used to shuffle modules within biosynthetic units for combinatorial exploration of natural product synthesis (Trosset & Carbonell, 2015; Wohlleben et al., 2012)

Another powerful tool of Synthetic biology is protein engineering. For instance, site-directed mutagenesis can improve the specificity, stability or solubility of an enzyme of interest, increasing the binding constant of a ligand. Synthetic quorum sensing can also be used to study mechanisms of antibiotics persistence as well as bacterial resistance, which is achieved by altering the cell–cell

 doi:10.20944/preprints202606.0390.v1

communication systems. Other approaches include metabolic engineering of precursor supply and laboratory directed evolution (Weissman, 2007). Enzymes can be mutated under selection pressure with imposed substrates, or by mutational biosynthesis and mutasynthesis, in which a metabolic pathway can be blocked by a mutation forcing supplemented substrates to be processed by a specific enzyme through selective evolution (Trosset & Carbonell, 2015).

Combining engineering with biology, Synthetic biology aims to design artificial biological systems. Examples include synthetic cells or cell-free systems that trigger a biological response with respect to the input signal. In drug discovery, such systems are used to activate gene expression of silent biosynthetic gene clusters and to explore natural products. In-cell synthesis of bacterial secondary metabolites has the advantage to allow production of compounds compliant to biological environment, which is considered a part of the lead optimization process. Genome engineering and editing tools give the possibility of the action of a particular output signal, which is useful to validate drug targets and merges constraints from both drug development and production (König et al., 2013; Krysenko, 2025). This "rational-based biosynthetic drug design" emerged as the other side of the de novo rational-based drug design(Table 1) (Trosset & Carbonell, 2015).

Table 1. Areas of Synthetic biology applications (modified after Trosset & Carbonell, 2015).

Synthetic Biology	Drug Discovery	Technical Details
Genetic circuits in host organisms	Increase flux of metabolic pathways for secondary metabolite production	Design of synthetic promoters, ribosome binding sites (RBS), and transcriptional regulators; use of CRISPR-based gene activation/repression (CRISPRa/i); dynamic pathway regulation using feedback loops; metabolic flux analysis (MFA) and flux balance analysis (FBA)
Protein engineering (enzyme modification, shuffle biosynthetic modules)	Exploration of chemical diversity of secondary metabolites	Directed evolution, rational design, and machine learning–guided protein engineering; domain swapping in PKS/NRPS systems; site-directed mutagenesis; enzyme kinetics optimization (kcat, Km); structural modeling (e.g., AlphaFold)
Optogenetics biosensing	Drug target validation, elucidation of mechanism of action, investigation of disease models, drug delivery	Light-inducible systems (e.g., LOV, CRY2-CIB1); fluorescent and luminescent biosensors; real-time control of gene expression; high-throughput screening using optical readouts; spatiotemporal regulation of signaling pathways
Synthetic quorum sensing and cell-to-cell communication	Overcome drug resistance, fight toxic effects, optimization of secondary metabolism	Engineering AHL/LuxR-type systems; population density–dependent gene regulation; synthetic consortia design; intercellular signaling circuits; toggle switches and oscillators for coordinated expression; control of metabolic burden and pathway partitioning

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2.2. Drug Target Validation Leveraging Synthetic Biology

Up to the current date, the main reason for failure in drug discovery is the identification and validation of new drug targets (David et al., 2021). Drug target validation is essential in the drug development pipelines since it allows to minimize risk of failure at later stages of a project (Trosset & Carbonell, 2015). Furthermore, validation of drug targets in the clinic following phenotypic or target-based approaches, opens path to build success in second-generation medicines (Swinney & Anthony, 2011). Careful consideration of validated targets can significantly improve success rates, e.g. in the case of AstraZeneca 5R's framework (Morgan et al., 2018). Discovering new drug targets and their subsequent validation is considered a key part of establishing a successful pipeline for medical development. Especially discovery of targets in pathogenic bacteria causing epidemic diseases (e.g. tuberculosis) gains nowadays a lot of attention (Makhoba & Krysenko, 2025). Many tools of genetic engineering have been proposed to ensure that this process can be guaranteed (David et al., 2021).

Applying Synthetic biology, expression of essential genes can be modulated by small molecules. This makes synthetic cells a tool to identify drug–target interactions (Firman et al., 2012). Classical tools include gene knock out and knock in techniques as well as protein mutagenesis allowing efficient validation of protein drag targets, e.g. as conducted to propose new drug targets in M. tuberculosis (Krysenko et al., 2025). The recent application of clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) genome editing techniques enabled efficient genome editing at any specified sites of the genome in diverse bacterial strains including producers of industry-relevant secondary metabolites (Krysenko et al., 2025; Trosset & Carbonell, 2015). With the ability to exquisitely modify genetic sequences, CRISPR-Cas9-based technologies allowed generation of new components of target validation that offer significant advantages in selecting the right targets (David et al., 2021). Introduction of site-specific mutations that confer drug resistance in target host is an ultimate way for estimating the selectivity of a drug (Trosset & Carbonell, 2015). This approach improved drag target validation efforts in a large variety of organisms, including bacteria, fungi, plants and mammal (Trosset & Carbonell, 2015; Neggers et al., 2015).

2.3. Synthetic Cellular Models of Disease

Approaches of synthetic biology are used in pharmaceutical research including drug development pipelines to understand disease mechanisms, which are important in design of new treatment and diagnostics strategies (Callura et al., 2010). Disease models are defined as engineered genetic circuits to investigate cellular responses to input signals controlling molecular mechanisms. Furthermore, they are used to study pathogen-related mechanisms, e.g, UV stress, growth, or drug response, often using tools of genetic engineering like reporter gene constructs (Trosset & Carbonell, 2015).

Leveraging synthetic biology a large variety of genetically engineered bacteria were selected and promoted for novel live therapeutics (Lu et al., 2021). Such approaches as engineering of bacteria containing synthetic gene circuits allow controlling and modulation of the localization, timing and dosages of therapeutic activities. This can be achieved by sensing disease biomarkers leading to development of new methods to combat infection diseases (Charbonneau et al., 2020). Engineering methods of synthetic biology allow adjustment of bacterial cells with therapeutic functions harboring gene circuits capable of sensing and transduction of signals derived from intracellular or extracellular biomarkers, which provides new designs and toolkits to conventional therapies (Cubillos-Ruiz et al., 2021).

The concept of bacterial cell-based live therapeutics is currently a rapidly growing field for effective treatments of a large variety of human diseases (Yan et al., 2023). Engineered E. coli Nissle 1917 (EcN) has been used for the detection, treatment and prevention of gut infections caused by P. aeruginosa - a common multidrug-resistant pathogen, which is difficult to treat using conventional drugs (Hwang et al., 2017). The designed EcN was capable of sensing the biomarker produced by P. aeruginosa - N-acyl homoserine lactone - and autolyzed it to release a biofilm degradation enzymes

 doi:10.20944/preprints202606.0390.v1

dispersin and pyocin S5 bacteriocin leading to elimination of the pathogen in the intestine (Yan et al., 2023). Another example of application of synthetic biology is generation of novel signaling properties, which have been reviewed in the context of engineered yeast targeting nitogen-activated protein kinase pathways (Furukawa & Hohmann, 2013). Metabolic network modeling is of growing interest in therapeutic research too, allowing understanding cell proliferation signaling mediated by Tyr-kinase receptors (Trosset & Carbonell, 2015).

2.4. Discovery of New Drugs

Approaches of synthetic biology are considered to be of importance for drug discovery, especially in connection to combating multi-drug resistant strains. For instance, *Mycobacterium* tuberculosis resistant to ethionamide compounds that inactivate the monooxygenase EthA were screened for drug sensitivity. An EthR-based (repressor of EthA) synthetic genetic circuit was subsequently engineered into human embryonic kidney cells. This configuration was used to screen a chemical library, revealing several compounds that influence the EthR repressor. The ester 2-phenylethyl-butyrate activated EthA expression in M. tuberculosis, rendering this pathogen more sensitive to the antibiotic ethionamide. Such screening methods for compounds that reverse bacterial resistance have enabled the identification of novel therapeutic options for tuberculosis treatment (Weber & Fussenegger, 2009).

Another example of screening technology implementation is its use with Streptomyces *pristinaespiralis*-derived streptogramin-responsive repressor PIP, which was engineered into mammalian cells to repress a PIP-responsive promoter controlling transcription of a reporter gene (Aubel, 2001). Addition of the pristinamycin I antibiotic released PIP from its promoter, leading to de-repression of reporter gene activation. This system was implemented for screening Streptomyces metabolic libraries and enabled identification of bacterial strains capable of producing compounds active against clinical pathogens, including those able to penetrate mammalian cells. Such approaches are broadly applicable using different antibiotic-responsive repressors functionally transferred into mammalian cells, such as macrolide-specific MphR(A) or tetracycline-specific TetR (Weber, 2002; Weber & Fussenegger, 2009).

Beyond these examples, synthetic biology has enabled a variety of innovative strategies for drug discovery and antimicrobial development. For example, engineered bacteriophages have been designed to selectively infect and lyse antibiotic-resistant bacteria while simultaneously delivering genetic payloads that disable resistance mechanisms, such as CRISPR-Cas systems targeting resistance genes. Similarly, synthetic gene circuits have been constructed in probiotic bacteria to detect pathogen-associated signals and respond by producing antimicrobial peptides or quorum-sensing inhibitors, thereby disrupting pathogenic communication and virulence (Hwang et al., 2017).

In addition, cell-free synthetic biology platforms have emerged as powerful tools for rapid drug screening and biosensing. These systems utilize transcription–translation machinery extracted from cells to prototype genetic circuits and evaluate drug–target interactions in vitro, significantly reducing development time and biosafety concerns. Another promising application involves the engineering of microbial consortia, where multiple strains are designed to cooperatively produce complex natural products, such as polyketides and non-ribosomal peptides, which are often difficult to synthesize chemically but exhibit strong antimicrobial activity (Silverman et al., 2020).

Synthetic biology has also facilitated the discovery of “cryptic” or silent biosynthetic gene clusters in microorganisms. By refactoring and expressing these gene clusters in heterologous hosts, researchers can unlock previously inaccessible natural products with potential therapeutic properties. Furthermore, metabolic engineering approaches allow the optimization of precursor supply and pathway flux to increase yields of candidate drug molecules, making large-scale production more feasible.

More recently, programmable RNA-based systems, including toehold switches and riboswitches, have been utilized for high-throughput screening of small molecules and antibiotics by coupling ligand detection to measurable reporter outputs. Combined with microfluidics and high-

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throughput sequencing, these platforms enable screening of vast chemical libraries with unprecedented speed and sensitivity (Green et al., 2014).

Together, these examples highlight how synthetic biology not only enhances traditional drug discovery pipelines but also introduces entirely new paradigms for identifying, testing, and producing therapeutics, particularly in the fight against antimicrobial resistance.

2.5. Antimicrobial and Drug Resistance

A big challenge of drug discovery is the emergence of bacterial drug-resistance as well as combating pathogens that are able to persist in cells (Tomasetti, 2014). One approach of synthetic biology to address this issue is manipulation of biosynthetic units to synthesize new antibiotics active against resistant bacteria (Thaker & Wright, 2015). For example, genetic–synthetic strategies were used to target multidrug resistant Mycobacterium tuberculosis (Saxena et al., 2014; Trosset & Carbonell, 2015). High-throughput technology was applied to identify genetic combinations that could potentiate antibiotics activity against carbapenem-resistant Enterobacteriaceae (CRE). Combinatorial Genetics en Masse technology (CombiGEM) was developed to identify genetic combinations that enhance the effectiveness of antibiotics against CRE (Cheng et al., 2014).

Another strategy of synthetic biology is a synthetic cell–cell communication network design as a model that can be used to study persistence in bacteria and as a screening platform to target quorum sensing in bacterial communities. Cell-to-cell communication system that allows bacteria to synchronize expression of certain genes in a cell density-dependent manner is one of focus ares for application of synthetic biology (Trosset & Carbonell, 2015).

Many routes for secondary metabolism and in particular antibiotic production are regulated by small signaling molecules, such as acyl-homoserine lactones in Pseudomonas (Saeidi et al., 2011) or γ-butyrolactones (GBLs) in Streptomyces (Du et al., 2011). In Streptomyces, GBLs bind to cytoplasmic protein receptors serving as repressor of gene TF of the antibiotics biosynthetic units. Biosynthetic units may contain genes encoding transporters for antibiotic efflux in order to avoid the accumulation of toxic compounds in the cytoplasm. Generally, stimulation of efflux pumps expression by signal molecules like GBL can induce resistance to antibiotics in bacteria. Targeting the acyl-homoserine lactone/GBL-based quorum sensing emerged as a strategy to lower the impact of resistant mechanisms in bacteria, such as drug-efflux pump systems, e.g. in Staphylococcus (Wang & Ma, 2014; Trosset & Carbonell, 2015). Further studies showed that reduced bacteria metabolism is linked to resistance and tolerance to antibiotics, whereas enhanced metabolism induces drug sensitivity (Bhargava & Collins, 2015). For instance, the change in the metabolic states of resistant bacteria on treatment with antibiotic kanamycin demonstrated that resistant strains showed best sensitivity under glucose and alanine deficiency (Peng et al., 2015).

Four different consequences of the presence of antibiotics were defined, for which a synthetic biology targeting strategy has been defined. First, antibiotics stimulate the production of hydroxyl radicals inducing cell death or resistant mutations if the antibiotics is at sublethal concentration. Another scenario is when antibiotics-resistant mutants induce indole formation by utilizing L-tryptophan stimulating drug-efflux pumps and oxidative stress detoxification pathways through the quorum sensing network in the more sensible bacteria strain. Furthermore, addition of metabolites (e.g. glucose, alanine) to the extracellular environment can generate proton motive force sensitizing persistent or dormant cells to aminoglycoside antibiotics. Some bacteria use antibiotics as a source of carbon, which helps microbial community to evade treatment by reducing the local concentration of antibiotics (Planson et al., 2011; Trosset & Carbonell, 2015).

2.6. Drugs Biosynthesis

In recent decade, approaches of synthetic biology were predominantly implemented to achieve sustainable, cost-effective production of phamarceuticals. Synthetic biology constructs (e.g. biological circuits, chassis strains including bacteria, fungi, yeasts, cell cultures, plants) were implemented for effective production of high-value added phamarceutical products. Synthetic biology offers a scalable

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way for production of bioproducts in a rapid, controlled and robust manner, which is feasible for the large-scale industrial production. Thus, bioproducts can be manufactured without excessive cultivating and harvesting of raw substrates (Yan et al., 2023).

Synthesis of pharmarceuticals is considered to be one of classical fields of synthetic biology. Such bacteria as Bacillus spp. or Streptomyces spp. are used as the production chassis. Synthetic biology concepts are used in the frame of the DBTL (design-build-test-learn) approach. DBTL cycle comprises the molecular biology designs as well as constructs in the beginning of the production life cycle. Experimental results serve as the basis for the new cycles of designs. Single-cell systems are easier to be manipulated compared to mammalian cells, where the DBTL cycle may take very long time. In the microbial synthesis of drugs, high-throughput screening as well as methods of directed laboratory evolution are commonly used to accelerate experimental paces (Yan et al., 2023).

The process of drug manufacturing applying synthetic biology involves several key steps: manipulation and generation of chassis cells; design, construction, and integration of molecular synthesis pathways; artificial regulation and modulation of metabolic networks (Feng et al., 2024). Genetic modifications, such as deletions of large genome segments or gene clusters, proficient genome editing, stable expression of genes, allow elimination of interference from native background metabolites. Such optimization processes improve performance of chassis strains. Production of a range of target active ingredients can be achieved through metabolic engineering, including manipulation of metabolic pathways, precursor supply optimization, regulation of metabolic networks, cofactor distribution and modulation of feedback inhibition mechanisms associated with metabolic intermediates, and other approaches (Feng et al., 2024).

In order to use a microbial cell as a production cell-factory in terms of synthetic biology applications, an appropriate host organism should be selected. The suitability of a host organism of choice depends on several factors, such as its genetic tractability, growth rate, genetic stability, ability to produce target compounds. Host organisms that meet these criteria are usually referred to as genetically tractable engineered chassis organisms (Baltz, 2010). Based on developed techniques for genetic manipulation, culturing, and industrial fermentation scale-up possibilities, common chassis include *Bacillus subtilis, Escherichia coli, Corynebacterium glutamicum, Streptomyces lividans, Pichia pastoris*. Another important aspect is chassis optimization that can significantly expedite development timeline of target products. By optimizing the chassis organism, efficiency, specificity, and stability of an engineered system can be significantly improved (Feng et al., 2024). Important nonconventional chassis organisms that can be beneficial in addition to the classic synthetic biology chassis hosts for the production of diverse compounds include *Streptomyces strains* (e.g. *S. coelicolor, S. albus, S. avermitilis, S. chattanoogensis, S. venezuelae and S. viridochromogenes*), employed for their ability to produce high amounts of natural products, such as polyketides, nonribosomal peptides (NRPs), and terpenes (Table 2) (David et al., 2021; Krysenko et al., 2025).

Table 2. Examples of common chassis strains and corresponding advantage applications.

Type	Common chassis	Advantages	Common pharmaceutical application	Details
In vivo	*E. coli*	Shorter doubling time, high expression of enzymes	Taxadiene	Well-characterized genetics; strong promoters (e.g., T7); codon optimization; plasmid-based expression systems; CRISPR/Cas genome editing; metabolic

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				flux redirection (e.g., MEP/DOXP pathway engineering)
	S. cerevisiae	Ease of genomic integration	Artemisinin	Homologous recombination for stable pathway integration; compartmentalization in organelles (e.g., mitochondria, ER); mevalonate pathway engineering; inducible promoters (GAL system); tolerance to complex post-translational modifications
	C. glutamicum	Metabolic robust, high capacity for secreting products	Cyclosporin A	Strong central carbon metabolism; efficient secretion systems; low protease activity; genome-scale metabolic models (GEMs); optimization of NADPH availability
	B. subtilis	Efficient protein secretion	Bacillomycin	Sec and Tat secretion pathways; GRAS status; low endotoxin production; chromosomal integration systems; protease-deficient strains for protein stability
	S. avermitilis	Ability to produce natural products, including polyketides, nonribosomal peptides (NRPs), and terpenes	Avermectin	Large biosynthetic gene clusters (BGCs); polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) systems; pathway-specific regulators; heterologous expression of secondary metabolite clusters
In vitro	Cell-free	Complexity and bioactive molecule overproduction	Oxytetracycline	Cell-free transcription–translation (TX-TL) systems; precise control of reaction conditions; no cellular toxicity constraints; rapid prototyping of pathways; supplementation with cofactors and energy regeneration systems
	Whole-cell	Easier to realize the in situ	Glutathione	Use of intact cells as biocatalysts; cofactor regeneration (e.g., NADPH/NADH); membrane transport systems; enzyme

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	cascade reactions; immobilized cell systems for continuous production

Discovery of new drugs provide the basis for treatment of infection diseases. However, manufacturing drugs in a feasible, large-scale manner impacts availability of new substances for medical use, especially for people outside rich countries. Most small molecule synthesis can be addressed by approaches of the classic conventional synthetic chemistry, but one-third of all marketed small-molecule therapeutics are drugs derived from natural products isolated from a natural host and produced by fermentation (Zhang & Wilkinson, 2007). Production of therapeutics in Streptomyces strains (e.g. antibiotics or biostimulants) can be done cost-effectively. However, large-scale production of many secondary metabolites remains economically challenging, e.g. the antimalaria drug artemisinin, a sesquiterpene lactone endoperoxide extracted from the sweet wormwood (Artemisia annua L); taxol, a highly decorated diterpenoid derived from the pacific yew tree (Taxus brevifolia Nutt.) with a high chemotherapeutic value against lung, ovarian and breast cancer, and many others (Weber & Fussenegger, 2009).

To improve biotechnological production of valuable compounds and make it economically feasible, various rapidly developed techniques of synthetic biology have been implemented for engineering of chassis cells. For example, BAC-based transformation and expression (e.g. well established in Streptomyces), CRISPR-Cas9, transformation-associated recombination (TAR) cloning, as well as RecET direct cloning (Mitousis et al., 2020; Krysenko, 2025). Such efforts as genome streamlining (Zhang et al., 2010), multi-chassis system combination (Ke & Yoshikuni, 2020), morphology engineering (Polka & Silver, 2014), and regulating circuit synthesis (Palazotto et al., 2019), artificial cells with minimized-genome as more controllable chassis (Dai et al., 2020) or incorporating non-standard amino acids (ncAAs) into recoded genome of protein drugs (de la Torre & Chin, 2021) have been implemented to facilitate the biosynthesis of complex small molecules. Such strategies of synthetic biologyhave provided unique insights into chassis optimization accelerating the development of new pharmaceutic ally relevant drugs (Feng et al., 2024).

Table 3. List of selected pharmaceuticals currently produced in bacteria.

Product	Producer	Application	Details
Taxadiene	*E. coli*	Anti-cancer	Heterologous expression of plant-derived terpenoid pathway; engineering of MEP/DOXP or mevalonate pathway; codon optimization; use of terpene synthases; precursor (GGPP) overproduction
Erythrocin	*S. erythraea*	Anti-infective	Type I polyketide synthase (PKS) pathway; modular PKS engineering; precursor supply (propionyl-CoA, methylmalonyl-CoA); pathway regulation via cluster-situated regulators
Erythromycin	*S. erythraeus*	Anti-infective	6-deoxyerythronolide B synthase (DEBS) PKS system; tailoring enzymes (glycosylation, hydroxylation); metabolic engineering of extender units

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Tetracycline	*S. aureofaciens*	Anti-infective	Type II PKS pathway; iterative polyketide chain assembly; oxygenases and cyclases; precursor malonyl-CoA supply optimization
Oxytetracycline	*S. rimosus*	Anti-infective	Type II PKS with oxygenation steps; regulation of biosynthetic gene clusters (BGCs); fermentation optimization for yield improvement
Neomycin	*S. fradiae*	Anti-infective	Aminoglycoside biosynthesis; sugar modification enzymes; glycosyltransferases; engineering of UDP-sugar precursors
Rifampin	*S. mediterranei*	Anti-infective	Hybrid PKS/NRPS pathway; rifamycin biosynthetic gene cluster; precursor AHBA synthesis; pathway-specific transcriptional regulators
Streptomycin	*S. griseus*	Anti-infective	Aminoglycoside pathway; complex sugar assembly; regulation by Streptomyces global regulators (e.g., AdpA); metabolic flux toward glucose-6-phosphate
Kanamycin	*S. kanamyceticus*	Anti-infective	Aminoglycoside biosynthesis; glycosylation steps; engineering resistance genes to avoid self-toxicity; precursor supply optimization
Tobramycin	*S. tenebrarius*	Anti-infective	Branched aminoglycoside pathway; enzyme engineering for structural variants; control of deoxygenation steps
Lincomycin	*S. lincolensis*	Anti-infective	Lincosamide biosynthesis; hybrid sugar–amino acid assembly; tailoring enzymes; pathway-specific regulators
Chloramphenicol	*S. vensuella*	Anti-infective	Shikimate-derived pathway; halogenation (chlorination) steps; nitro group formation; enzyme-mediated aromatic modifications
Avermicin	*S. avermitilis*	Anti-infective	Large modular PKS system; multiple tailoring enzymes; regulatory gene clusters; fermentation process optimization
Daptomycin	*S. roseosporus*	Anti-infective	Nonribosomal peptide synthetase (NRPS) assembly line; lipid tail attachment; calcium-dependent activity; precursor amino acid supply
Vancomycin	*A. orientalis*	Anti-infective	Glycopeptide biosynthesis; NRPS pathway; oxidative crosslinking; halogenation; complex tailoring enzymes

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Cyclosporin	*S. rosariensis*	Immunosuppressant	NRPS-mediated cyclic peptide synthesis; unusual amino acid incorporation; pathway regulation; precursor amino acid engineering
Tacrolimus	*S. tsukubaensis*	Immunosuppressant	Hybrid PKS/NRPS system; macrolide assembly; tailoring (methylation, oxidation); regulatory gene clusters
Rapamycin	*S. rapamycinicus*	Immunosuppressant	Hybrid PKS/NRPS pathway; large macrolide biosynthesis; precursor (pipecolate) supply; pathway engineering for analog production

3. Future Perspectives

Synthetic biology has grown substantially in the last decade and has emerged with many achievements in science and industrial application. Synthetic biology introduced many advanced strategies for medical applications, shortening drug development cycles and lowering pharmaceutical costs. These include technologies such as engineered smart cells with programmable gene circuits, live probiotic therapeutics capable of sensing and responding to disease biomarkers, advanced diagnostics based on CRISPR-mediated detection systems, stem cell engineering for regenerative medicine, optimized microbial platforms for drug biosynthesis, nanocarriers for targeted drug delivery, and next-generation artificial vaccine platforms such as mRNA and self-amplifying RNA systems. Synthetic biology approaches have significantly transformed biomedical fields, including the development of cell-based therapeutics, the use of engineered bacteria to selectively target and destroy cancer cells, and the rewiring of metabolic fluxes in human and microbial systems to enhance therapeutic outcomes (Yan et al., 2023).

At the technical level, advances in DNA synthesis and assembly, high-throughput genome editing tools such as CRISPR-Cas systems, and standardized biological parts (BioBricks) have enabled the modular design of complex biological systems. Additionally, computational modeling of gene regulatory networks and metabolic pathways allows for predictive design and optimization prior to experimental implementation. Despite these advances, many challenges remain in translating laboratory-scale innovations into commercially viable products, including issues related to scalability, bioprocess optimization, regulatory approval, biosafety, and reproducibility across different biological contexts.

The integration of synthetic biology with artificial intelligence (AI) represents a rapidly emerging frontier aimed at accelerating innovation in both medical and pharmaceutical domains. AI-driven protein structure prediction, generative models for enzyme and pathway design, and machine learning algorithms for analyzing large-scale omics datasets (genomics, proteomics, metabolomics) enable more precise and efficient engineering of biological systems. Techniques such as deep learning, reinforcement learning, and multimodal data integration facilitate automated design–build–test–learn (DBTL) cycles, significantly reducing development time. Furthermore, AI-enabled robotic laboratories and automated high-throughput screening platforms are paving the way for autonomous experimentation and optimization.

Looking toward the future, synthetic biology is expected to play a central role in addressing global health challenges, particularly in combating antimicrobial resistance and emerging infectious diseases such as tuberculosis. Novel approaches include the design of programmable antimicrobials, bacteriophage-based therapies, and engineered microbial consortia capable of dynamically adapting to pathogenic environments. In addition, personalized medicine will benefit from synthetic biology through patient-specific cell therapies and precision drug manufacturing. Advances in biofoundries, cloud laboratories, and distributed biomanufacturing systems could enable rapid, on-demand production of therapeutics at a global scale.

 doi:10.20944/preprints202606.0390.v1

Beyond healthcare, the convergence of synthetic biology with fields such as nanotechnology, materials science, and environmental engineering is expected to unlock new applications, including biosensors for real-time disease monitoring, sustainable bioproduction of pharmaceuticals, and living materials with embedded therapeutic functions. However, these developments will also require careful consideration of ethical, regulatory, and societal implications, including data privacy, biosecurity, and equitable access to emerging technologies.

Author Contributions: Conceptualization, SK; Methodology, SK; Software, SK; Validation, SK, MS and XHM; Formal Analysis, SK; Investigation, SK; Resources, SK and XHM; Data Curation, SK; Writing—Original Draft Preparation, SK; Writing—Review & Editing, SK, MS and XHM; Visualization, SK; Supervision, SK and XHM; Project Administration, SK; Funding Acquisition, SK and XHM.

Funding: This research received no external funding.

Acknowledgments: We thank Mohammad Alanjary for proof-reading the manuscript.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Trosset, J. Y., & Carbonell, P. (2015). Synthetic biology for pharmaceutical drug discovery. Drug design, development and therapy, 9, 6285–6302. https://doi.org/10.2147/DDDT.S58049
2. Yan, X., Liu, X., Zhao, C., & Chen, G. Q. (2023). Applications of synthetic biology in medical and pharmaceutical fields. Signal transduction and targeted therapy, 8(1), 199. https://doi.org/10.1038/s41392-023-01440-5
3. David, F., Davis, A. M., Gossing, M., Hayes, M. A., Romero, E., Scott, L. H., & Wigglesworth, M. J. (2021). A Perspective on Synthetic Biology in Drug Discovery and Development-Current Impact and Future Opportunities. SLAS discovery : advancing life sciences R & D, 26(5), 581–603. https://doi.org/10.1177/24725552211000669
4. Weber, W., & Fussenegger, M. (2009). The impact of synthetic biology on drug discovery. Drug discovery today, 14(19-20), 956–963. https://doi.org/10.1016/j.drudis.2009.06.010
5. Xie, Y., Yang, Y., He, Y., Wang, X., Zhang, P., Li, H., & Liang, S. (2020). Synthetic Biology Speeds Up Drug Target Discovery. Frontiers in pharmacology, 11, 119. https://doi.org/10.3389/fphar.2020.00119
6. Feng, Y., Su, C., Mao, G., Sun, B., Cai, Y., Dai, J., & Ma, Y. (2024). When synthetic biology meets medicine. Life medicine, 3(1), lnae010. https://doi.org/10.1093/lifemedi/lnae010
7. Endy, D. J. N. (2005). Foundations for engineering biology. Nature 438, 449. doi: 10.1038/nature04342
8. Way, J. C., Collins, J. J., Keasling, J. D., Silver, P. A. (2014). Integrating biological redesign: where synthetic biology came from and where it needs to go. Cell 157, 151–161. doi: 10.1016/j.cell.2014.02.039
9. Xie, M., Fussenegger, M. (2018). Designing cell function: assembly of synthetic gene circuits for cell biology applications. Nat. Rev. Mol. Cell Biol. 19, 507–525. doi: 10.1038/s41580-018-0024-z
10. Wu, M. R., Jusiak, B., Lu, T. K. (2019). Engineering advanced cancer therapies with synthetic biology. Nat. Rev. Cancer 19, 187–195. doi: 10.1038/s41568-019-0121-0
11. Hu Y, Bajorath J. Monitoring drug promiscuity over time. F1000Res. 2014;3:218. doi: 10.12688/f1000research.5250.1
12. Leil TA, Bertz R. Quantitative systems pharmacology can reduce attrition and improve productivity in pharmaceutical research and development. Front Pharmacol. 2014;5:247. doi: 10.3389/fphar.2014.00247.
13. Ryall KA, Tan AC. Systems biology approaches for advancing the discovery of effective drug combinations. J Cheminform. 2015;7:7. doi: 10.1186/s13321-015-0055-9
14. Krysenko, S., & Wohlleben, W. (2024). Role of Carbon, Nitrogen, Phosphate and Sulfur Metabolism in Secondary Metabolism Precursor Supply in Streptomyces spp. Microorganisms, 12(8), 1571. https://doi.org/10.3390/microorganisms12081571
15. Sun H, Liu Z, Zhao H, Ang EL. Recent advances in combinatorial biosynthesis for drug discovery. Drug Des Devel Ther. 2015;9:823–833. doi: 10.2147/DDDT.S63023

 doi:10.20944/preprints202606.0390.v1

16. Krysenko S. (2025). Current Approaches for Genetic Manipulation of Streptomyces spp.-Key Bacteria for Biotechnology and Environment. Biotech (Basel (Switzerland)), 14(1), 3. https://doi.org/10.3390/biotech14010003
17. Breitling R, Takano E. Synthetic biology advances for pharmaceutical production. Curr Opin Biotechnol. 2015;35C:46–51. doi: 10.1016/j.copbio.2015.02.004.
18. Wohlleben, W., Mast, Y., Muth, G., Röttgen, M., Stegmann, E., & Weber, T. (2012). Synthetic biology of secondary metabolite biosynthesis in actinomycetes: Engineering precursor supply as a way to optimize antibiotic production. FEBS letters, 586(15), 2171–2176. https://doi.org/10.1016/j.febslet.2012.04.025
19. König H, Frank D, Heil R, Coenen C. Synthetic genomics and synthetic biology applications between hopes and concerns. Curr Genomics. 2013;14(1):11–24. doi: 10.2174/1389202911314010003
20. Makhoba, X. H., & Krysenko, S. (2025). Drug Target Validation in Polyamine Metabolism and Drug Discovery Advancements to Combat Tuberculosis. Future Pharmacology, 5(3), 32. https://doi.org/10.3390/futurepharmacol5030032
21. Swinney, D. C., & Anthony, J. (2011). How were new medicines discovered?. Nature reviews. Drug discovery, 10(7), 507–519. https://doi.org/10.1038/nrd3480
22. Morgan, P., Brown, D. G., Lennard, S., Anderton, M. J., Barrett, J. C., Eriksson, U., Fidock, M., Hamrén, B., Johnson, A., March, R. E., Matcham, J., Mettetal, J., Nicholls, D. J., Platz, S., Rees, S., Snowden, M. A., & Pangalos, M. N. (2018). Impact of a five-dimensional framework on R&D productivity at AstraZeneca. Nature reviews. Drug discovery, 17(3), 167–181. https://doi.org/10.1038/nrd.2017.244
23. Firman K, Evans L, Youell JA. Synthetic biology project – developing a single-molecule device for screening drug-target interactions. FEBS Lett. 2012;586(15):2157–2163. doi: 10.1016/j.febslet.2012.01.057
24. Neggers JE, Vercruysse T, Jacquemyn M, et al. Identifying drug-target selectivity of small-molecule CRM1/XPO1 inhibitors by CRISPR/Cas9 genome editing. Chem Biol. 2015;22(1):107–116. doi: 10.1016/j.chembiol.2014.11.015.
25. Krysenko, S., Emani, C. S., Bäuerle, M., Oswald, M., Kulik, A., Meyners, C., Hillemann, D., Merker, M., Prosser, G., Wohlers, I., Hausch, F., Brötz-Oesterhelt, H., Mitulski, A., Reiling, N., & Wohlleben, W. (2025). GlnA3Mt is able to glutamylate spermine but it is not essential for the detoxification of spermine in Mycobacterium tuberculosis. Journal of bacteriology, 207(2), e0043924. https://doi.org/10.1128/jb.00439-24
26. Callura JM, Dwyer DJ, Isaacs FJ, Cantor CR, Collins JJ. Tracking, tuning, and terminating microbial physiology using synthetic riboregulators. Proc Natl Acad Sci U S A. 2010;107(36):15898–15903. doi: 10.1073/pnas.1009747107
27. Lu Y, et al. Engineering bacteria-activated multifunctionalized hydrogel for promoting diabetic wound healing. Adv. Funct. Mater. 2021;31:2105749. doi: 10.1002/adfm.202105749
28. Charbonneau MR, Isabella VM, Li N, Kurtz CB. Developing a new class of engineered live bacterial therapeutics to treat human diseases. Nat. Commun. 2020;11:1–11. doi: 10.1038/s41467-020-15508-1
29. Cubillos-Ruiz A, et al. Engineering living therapeutics with synthetic biology. Nat. Rev. Drug Discov. 2021;20:941–960. doi: 10.1038/s41573-021-00285-3.
30. Hwang IY, et al. Engineered probiotic Escherichia coli can eliminate and prevent Pseudomonas aeruginosa gut infection in animal models. Nat. Commun. 2017;8:15028. doi: 10.1038/ncomms15028
31. Aubel D. Design of a novel mammalian screening system for the detection of bioavailable, non-cytotoxic streptogramin antibiotics. J. Antibiot. (Tokyo) 2001;54:44–55. doi: 10.7164/antibiotics.54.44
32. Weber W. Macrolide-based transgene control in mammalian cells and mice. Nat. Biotechnol. 2002;20:901–907. doi: 10.1038/nbt731
33. Tomasetti C. Drug resistance. Adv Exp Med Biol. 2014;844:303–316. doi: 10.1007/978-1-4939-2095-2_15
34. Thaker MN, Wright GD. Opportunities for synthetic biology in antibiotics: expanding glycopeptide chemical diversity. ACS Synth Biol. 2015;4(3):195–206. doi: 10.1021/sb300092n
35. Saxena A, Mukherjee U, Kumari R, Singh P, Lal R. Synthetic biology in action: developing a drug against MDR-TB. Indian J Microbiol. 2014;54(4):369–375. doi: 10.1007/s12088-014-0498-0
36. Cheng AA, Ding H, Lu TK. Enhanced killing of antibiotic-resistant bacteria enabled by massively parallel combinatorial genetics. Proc Natl Acad Sci U S A. 2014;111(34):12462–12467. doi: 10.1073/pnas.1400093111

 doi:10.20944/preprints202606.0390.v1

37. Saeidi N, Wong CK, Lo T-M, et al. Engineering microbes to sense and eradicate Pseudomonas aeruginosa, a human pathogen. Mol Syst Biol. 2011;7(521):1–11. doi: 10.1038/msb.2011.55
38. 216.Du Y-L, Shen X-L, Yu P, Bai L-Q, Li Y-Q. Gamma-butyrolactone regulatory system of Streptomyces chattanoogensis links nutrient utilization, metabolism, and development. Appl Environ Microbiol. 2011;77(23):8415–8426. doi: 10.1128/AEM.05898-11
39. Wang Y, Ma S. Small molecules modulating AHL-based quorum sensing to attenuate bacteria virulence and biofilms as promising antimicrobial drugs. Curr Med Chem. 2014;21(3):296–311. doi: 10.2174/09298673113206660294
40. Peng B, Su Y-B, Li H, et al. Exogenous alanine and/or glucose plus kanamycin kills antibiotic-resistant bacteria. Cell Metab. 2015;21(2):249–261. doi: 10.1016/j.cmet.2015.01.008
41. Planson A-G, Carbonell P, Grigoras I, Faulon J-L. Engineering antibiotic production and overcoming bacterial resistance. Biotechnol J. 2011;6(7):812–825. doi: 10.1002/biot.201100085
42. Baltz RH. Streptomyces and Saccharopolyspora hosts for heterologous expression of secondary metabolite gene clusters. J Ind Microbiol Biotechnol 2010;37:759–72
43. Zhang M.Q., Wilkinson B. Drug discovery beyond the 'rule-of-five'. Curr. Opin. Biotechnol. 2007;18:478–488. doi: 10.1016/j.copbio.2007.10.005
44. Mitousis, L., Thoma, Y., & Musiol-Kroll, E. M. (2020). An Update on Molecular Tools for Genetic Engineering of Actinomycetes-The Source of Important Antibiotics and Other Valuable Compounds. Antibiotics (Basel, Switzerland), 9(8), 494. https://doi.org/10.3390/antibiotics9080494
45. Zhang, L. Y., Chang, S. H., & Wang, J. (2010). How to make a minimal genome for synthetic minimal cell. Protein & cell, 1(5), 427–434. https://doi.org/10.1007/s13238-010-0064-4
46. Ke, J., & Yoshikuni, Y. (2020). Multi-chassis engineering for heterologous production of microbial natural products. Current opinion in biotechnology, 62, 88–97. https://doi.org/10.1016/j.copbio.2019.09.005
47. Polka, J. K., & Silver, P. A. (2014). Induced sensitivity of Bacillus subtilis colony morphology to mechanical media compression. PeerJ, 2, e597. https://doi.org/10.7717/peerj.597
48. Palazzotto, E., Tong, Y., Lee, S. Y., & Weber, T. (2019). Synthetic biology and metabolic engineering of actinomycetes for natural product discovery. Biotechnology advances, 37(6), 107366. https://doi.org/10.1016/j.biotechadv.2019.03.005
49. Dai, J., Boeke, J. D., Luo, Z., Jiang, S., & Cai, Y. (2020). Sc3.0: revamping and minimizing the yeast genome. Genome biology, 21(1), 205. https://doi.org/10.1186/s13059-020-02130-z
50. de la Torre, D., & Chin, J. W. (2021). Reprogramming the genetic code. Nature reviews. Genetics, 22(3), 169–184. https://doi.org/10.1038/s41576-020-00307-7
51. Hwang, I. Y., et al. (2017). Engineered probiotic Escherichia coli can eliminate and prevent Pseudomonas aeruginosa gut infection in animal models. Nature Communications, 8, 15028.
52. Silverman, A. D., Karim, A. S., & Jewett, M. C. (2020). Cell-free gene expression: An expanded repertoire of applications. Nature Reviews Genetics, 21, 151–170.
53. Green, A. A., Silver, P. A., Collins, J. J., & Yin, P. (2014). Toehold switches: De-novo-designed regulators of gene expression. Cell, 159, 925–939.