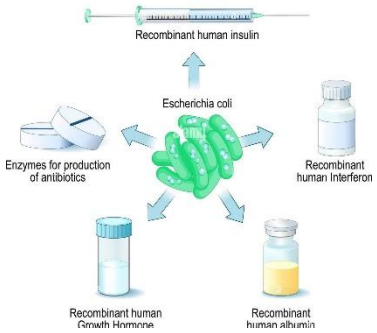
CHAPTER 22

Synthetic Biology and Metabolic Engineering for Natural Product Biosynthesis



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ABSTRACT: Natural products (NPs) have been at the epicenter of drug discovery because of their structural diversity and bioactivity. However, natural extraction and chemical synthesis are some of the traditional methods that are usually hampered by low yields, insufficient sustainability or lack of scalability. The concept of synthetic biology and metabolic engineering presents a highly effective solution through the rational design, construction and optimization of biosynthetic pathways in a microbial host. Major directions involve the design of appropriate chassis organisms, improving precursor provision, co-factor regeneration, as well as the use of current tools for genome editing and pathway construction. Studies have highlighted the successful microbial biosynthesis of complex and pharmacologically important molecules, including antibiotics, antioxidants, anti-inflammatory agents, and anti-carcinogenic agents. These integrated technologies are collectively reshaping the biosynthesis of NPs, yielding efficiency, sustainability, and viability in industries. This chapter will present an overall perspective on the intersection of synthetic biology and metabolic engineering towards the improved microbial synthesis of NPs, with a focus on genome editing, pathway design, and cutting-edge technologies like artificial intelligence and cell-free systems.

Keywords: Synthetic biology; Metabolic engineering; Natural products; Genome editing

Natural products (NPs) are isolated and extracted from microbes, plants, animals, and marine species. These substances are diverse and exhibit a distinct structural skeleton. Their stereochemistry enables the production of efficient drug combinations with diverse medicinal targets, control of physiological functions, and therapeutic approaches against a wide range of illnesses (Zhao et al., 2023). Researchers have been investigating NP-derived medical solutions to heal illnesses for millennia. About 150,000-300,000 NPs with distinct structures have been found so far owing to the improvements in analytical and identification techniques. Of the 407,270 naturally occurring compounds present in the open-access COCONUT database, about 65% are sourced from plants, while only 0.5% are from animals or marine species (Sorokina et al., 2021). As a result, NPs have emerged as one of the most valuable sources of novel medications and have made a substantial contribution to medications (Newman & Cragg, 2020). Some of diversly used NP based medicines include paclitaxel for tumors (Zhu and Chen, 2019), morphine for anesthesia, aspirin for inflammation (Desborough & Keelings, 2017), and artemisinin for malaria (Ma et al., 2020). Unfortunately, a sizable

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fraction of NPs, roughly 90%, must be omitted from novel medication screening procedures due to their poor stability, restricted bioavailability, and insufficient water solubility when used in clinical settings (Chopra & Dhingra, 2021).

In the current era of the Fourth Industrial Revolution and next-generation genomics, there is an increasing demand for translational research. This type of research could lead to the commercialization of R&D-based products and support significant further research. The advancement of synthetic biology and metabolic engineering has led to a promising and novel biotechnological platform for the creation of bioproducts, particularly with the use of microbial chassis (Ramzi, 2018). A considerable addition has been achieved on the relatively novel field of synthetic biology due to the progress and elucidation of route of secondary plant metabolite biosynthesis and engineering procedures of metabolic reconstitution in microbial hosts. Significant progress in metabolic pathway engineering has demonstrated that microbial production systems are a valuable tool for the formation of secondary metabolites in plants (Lv et al., 2019). Many new and emerging tools are currently available to help engineers build and optimize pathways for the accurate biosynthesis of plant secondary metabolites. These tools include a variety of constantly growing-omics databases, computational pathway simulations combined with specific enzyme designs and directed evolution approaches that aim towards maximizing the output of the desired product (Chae et al., 2017; Ahmed et al., 2025). This chapter examines the redesigning of NP biosynthesis that synthetic biology and metabolic engineering are accomplishing to present them as new methods of sustainable drug discovery and manufacturing.

BASICS OF SYNTHETIC BIOLOGY AND METABOLIC ENGINEERING

A new understanding of the many methods and resources that have been established over a long period of time about the development of metabolic engineering and synthetic biology has been brought about by the omics era. These specialties are interdependent and cannot exist without each other. The goal of synthetic biology is to create genetic component libraries, including devices, terminators, genetic circuits, transcriptional factors and their binding sequences, coding sequences, as well as promoters. Additionally, quantitative data is gathered in order to create models that can forecast how biological systems will behave in a specific condition (Cameron et al., 2014). Metabolic engineering aims at the optimization of cellular pathways that are pathway-specific to a particular organism and produce a desirable molecule out of a starting material, preferably cheap and simple. It has a wide range of databases, component and condition libraries that generate the optimum production rate of a selected chemical component and avoid inhibitory components as well as the conditions to enable this feat; the large-scale manipulation of metabolic fluxes is an important alternative (Stephanopoulos, 2012).

Nevertheless, these two fields rely on the development of genuine DNA alteration methodologies, techniques, and instruments. The rationale is that both fields are aiming to accomplish similar main goals, like logically altering DNA sequences, creating desired mutations, building modular or component parts of a genetic circuit or biosynthetic pathway, knocking out genes, and incorporating DNA segments into a plasmid or the genome of an organism of interest (Boyle & Silver, 2012). While PCR and its derivatives are among the most effective methods for generating essential changes, particularly for isolating fragments from stipulated regions, they tend to be ineffective for other applications. The "-omics" era saw the expansion of the catalog of enzymes and processes, the achievement of some biological phenomena (such as viruses or phage infections), and the sequencing of both known and undiscovered species (metagenomics), which allowed for the extraction of additional and improved data from them (Goodwin et al., 2016). The progress of both fields has hinged heavily on the development of complementing new techniques and approaches to accomplish other goals. As examples, consider Gibson Assembly, MAGE, Biobricks, Gap-repair, Lambda-red, Recombinase technologies and CRISPR-Cas9 (Stephanopoulos, 2012).

Some fungal and bacterial kingdom specimens continue to be useful in hosting secondary metabolite expressions, with the primary three being *E. coli*, *Saccharomyces cerevisiae*, and more

recently, *C. glutamicum* and *Y. lipolytica*. Compared to other crops, they are comparatively simple to grow and there has been extensive research describing genotypes of genetic manipulation and methods of modifying their metabolism (Pandey et al., 2016).

ENGINEERING MICROORGANISMS FOR NATURAL PRODUCT BIOSYNTHESIS

Genetic modification is also referred to as genetic engineering and is actually the practice of intervening with laboratory devices and altering the nucleic acid series of a living being. This is achieved through the removal or addition of base-pairs, the insertion or disabling of an unused virulence gene by the innovation of a new character in genetically modified organisms (GMOs). In the past, people manipulated microbes to make food like bread and wine whereas in modern times, microbe manipulation has been utilized in industry and clinical purposes through genetic engineering. Most microbes that change after a chemical reaction are yeast and bacteria, lactic acid bacteria (LAB), and *S. cerevisiae* (Mukai et al., 2020). With the advancement of genome sequencing and genetic methods as well as the current status of these methods as the mightiest genomic tools, one can make alterations in the gene sequence of phages and a broad variety of bacterial strains to create new strains. These may be used as methods to not only prevent or treat an infection but to diagnose the infection itself (Waksman et al., 2019). Genetic engineering often uses various mutations, protoplast fusion, transformation, recombination technology, electroporation and molecular genetics. However, former research and prior to the application of molecular genetics techniques, mutations were brought about by UV light and chemicals (Hashimoto et al., 2015).

The capacity of genetically modified microbes to create new food ingredients or improve existing ones is one of the main advantages of using them. Altering yeast strains, for instance, can result in the manufacturing of different proteins, which are becoming increasingly essential in managing global food availability and satisfying the needs of an expanding population. These proteins offer vital nutrients that can enhance nutritional health in addition to acting as alternatives to foods originating from animals. Genetically modified microorganisms play a significant part in the synthesis of enzymes, which are crucial for a number of food processing applications. Food products' texture, flavor, and nutritional value are enhanced by the biochemical interactions with enzymes (Boyle & Silver, 2012).

Saccharomyces. cerevisiae (baker's yeast) is an increasingly crucial chassis organism for the fermentation of NP production. Although both *E. coli* and *S. cerevisiae* possess a comprehensive synthetic biology toolbox, with which exogeneous genes can be easily expressed under autonomously replicating vectors, or genomic integrative systems to quickly prototype NPs biosynthesis pathway (Guirimand et al., 2021), but yeast is considered a particularly relevant chassis to refactor NPs biosynthesis pathways owing to endomembrane functionality and organelle compartmentalization. This promotes the intracellular routing of NPs pathway modules and the production of membrane-anchored enzymes (like P450s). However, to properly modify the NPs biosynthesis route in yeast, iterative cycles of strain engineering, pathway discovery in plants, and reconfiguration in yeast are often necessary, a process common to any heterologous host (Avalos et al., 2013).

Microbial chassis has been extensively surveyed as means of developing economically feasible and sustainable methods for producing industrially valuable compounds, such as pharmaceuticals, biofuels, enzymes and polymers in a sustainable and economically attractive method. Of these microbe hosts, baker’s yeast or *S. cerevisiae* has become the most popular eukaryotic synthetic biology chassis (Malcı et al., 2022). Among the pros of *S. cerevisiae* are high productivity in low-cost medium, easy-to-manage fermentation, and fermentation scale-up (Teworte et al., 2022). Furthermore, the development of robust engineered yeast strains with feasible execution and improved reaction has been accelerated by the creation of numerous synthetic biology tools and advanced genetic components (Jensen & Keasling, 2015). Recombinant therapeutic proteins and plant-derived NPs are among the high-value biopharmaceuticals that have

been produced using *S. cerevisiae* as a cell-based platform (Cao et al., 2023).

Paclitaxel is a powerful and broad anti-cancer drug first isolated from bark of *Taxus brevifolia* and it is extensively employed in the clinical practice (Long, 1994). Extraction yield in the yew plants barks was very low, and it would take at least three mature yew trees to produce 1 g paclitaxel which is not favorable to the environment (Nadeem et al., 2002). However, chemists have achieved significant breakthroughs in producing paclitaxel from simple building blocks. While these synthetic methods are impressive, they still have disadvantages, such as low yields and the need for numerous steps (Hu et al., 2021). With the rapid advancement of metabolic engineering and synthetic biology, the creation of rapidly growing microbial cell factories provides a viable and sustainable technique to easily express intricate biosynthetic pathways of natural chemicals (Hussain et al., 2021). The development of microbial cell factories to produce paclitaxel and paclitaxel-related intermediates has emerged as a new area of study (Xiong et al., 2021).

Major paclitaxel intermediate, taxadien-5a-yl-acetate (T5OAc) was *de novo* synthesized by the *Escherichia coli*. Supply of the precursor was increased by improvement of endogenous methylerythritol phosphate pathway. Problems in metabolic burden were eliminated to allow cell growth and in order to enhance production by streamlining the plasmid production system. The biosynthetic pathway was accurately adjusted using multivariate-modular metabolic engineering to establish a stable metabolic balance (Ajikumar et al., 2010). This increases output by roughly 272 times compared to the original strain. This was the highest yield of T5OAc ever recorded in *E. coli*. Therefore, *E. coli* is believed to improve the manufacture of paclitaxel (Xie et al., 2024).

NATURAL PRODUCTS OPTIMIZATION STRATEGIES

The growing demand for energy and the environmental pressures exerted by traditional NPs and petrochemicals have led to a constantly increasing need for microbial production systems. These systems can either significantly reduce or completely replace conventional chemical methods that rely on their native state (Liu, 2010). Recently, several researchers have demonstrated that balanced expression of genes involved in metabolic pathways can result in a notable improvement in yield, titter, and productivity (Ajikumar et al., 2010). The main aim of balancing a metabolic pathway is the creation of additional target products by lessening the possible influence of flux imbalance in the host organism. This is achieved primarily by preventing the accumulation of excess intermediate metabolites and precursors, which allows for the efficient conversion of substrates and co-factors into the final products (Lee et al., 2013). Pathway optimization and balancing methods tend to be combinatorial and iterative, and many thousands of screenings can be required in order to find an optimal solution. Fig. 1 demonstrates some approaches for balancing metabolic pathways.

The generation of molecules using microbial systems with high productivity, titter, and yield remains compromised (Wu et al., 2019). One of the main reasons for this is the potential for metabolic imbalance, which can negatively affect both cell growth and the production of target products during the biosynthetic pathway engineering of microbial systems (Dabirian et al., 2019). Therefore, it is essential to optimize the metabolic flow into the target products and balance the cellular resources. Furthermore, metabolites that are generated are predominantly characterized using analysis techniques like gas and liquid chromatography that take a lot of time and have moderate rate of throughput (Jang et al., 2017). These obstacles make it more difficult to use microbial engineering to produce high-quality NPs. The first world-wide drawback is that configurations that must be taken into consideration before they can come up with a global optimal solution are numerous. Despite considerable advances in the high-throughput screening technologies, high levels of amenability of products to high-throughput screening remain elusive (Lee et al., 2013). To cope with this issue, computational optimization strategies, several mechanistic or non-mechanistic models, and algorithm-aided mapping methodologies have been utilized to design a landscape of production under each possible combination of genetic components (Farasat et al., 2014). The literature has repeatedly shown that optimizing the scale-up process is just as

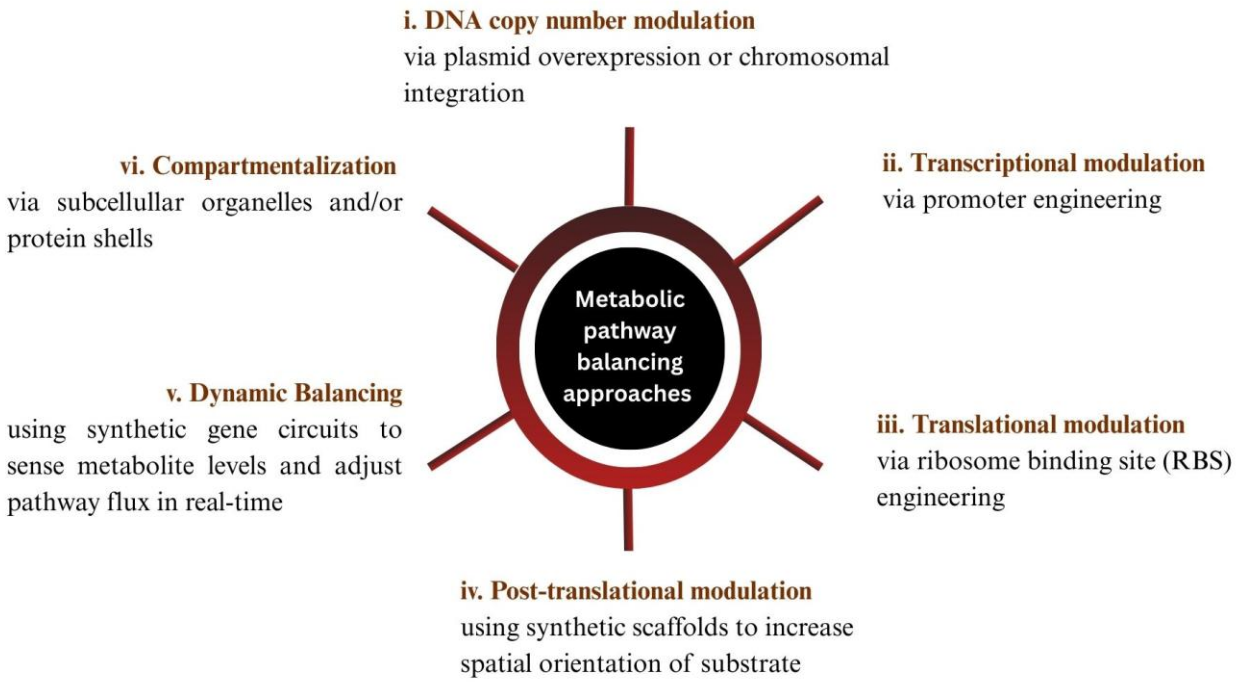

Fig 1. Approaches for balancing metabolic pathways

significant as having a stable, overproducing, and genetically optimized strain. In order to do this, there is still much to be done to create broad standards for the scalability of paths that are properly balanced.

ADVANCES IN GENOME EDITING AND SYNTHETIC PATHWAY DESIGN

Genome Editing

Genome editing is a method used *in vivo* to alter particular genomic DNA sequences. The use of genome editing techniques in metabolic engineering greatly facilitates the identification and assessment of pertinent genes and pathways as well as the construction of strains. In addition to restriction enzymes, other DNA editing tools are now in use for genome modification. These include transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), recombinases, and more recently, CRISPR-Cas systems (Bhatia and Yadav, 2023). The first endonuclease to cut through the DNA called ZFN was the most popular but the ZFN had a single disadvantaging nature that they acted offsite when it came to the mutations in the genome. In contrast to ZFN, the TALENs were better in terms of specificity, and they were quick. As TALENs have a large size and thus they contain a lot of repetitive sequences, their injection with the help of lentiviruses or adenoviruses is not so effective (Shamshirgaran et al., 2022). CRISPR-Cas system of prokaryotes is also among numerous of these defense mechanisms and has acquired the most popularity in the past few decades (Butiuc-Keul et al., 2022).

The clustered regularly interspaced palindromic repeats (CRISPR)-associated (Cas) systems have today become the top-of-mind modality to cope with genome engineering in various organisms, even the industrially significant organisms. CRISPR systems are mostly used as adaptive immunity systems against foreign invading DNA in bacteria and archaea. CRISPR-Cas-mediated DNA cleavage offers many genome-editing possibilities, including indels (insertions and deletions), replacements, large deletions, knock-ins, and chromosomal rearrangements. However, it is crucial to consider the differences in DNA repair pathways across different host organisms. Because of the CRISPR system's adaptability, nuclease-based editing which is particularly harmful to microorganisms has been

complemented by derivative technologies (Nishida & Kondo, 2021). To a much lower extent, base editing via deaminase installation produces much less toxicity while making targeted point mutations. CRISPRi and CRISPRa are capable of regulating gene expression non-permanently without altering the sequence of the genetic makeup (Akinci et al., 2021). The efficient design and production of gRNA libraries facilitate multiplex, combinatorial, and large-scale editing, which further expedites the metabolic pathways' thorough identification, assessment, and development (Dong & Chen, 2024).

Synthetic Pathway Construction

Mining (meta)genomic Biosynthetic Gene Clusters (BGCs) are appealing candidates for (meta)genomic mining, which looks for novel, specialized metabolites with possible uses in medicine and biotechnology. A number of accurate platforms have been developed to foresee (or predict) BGCs, which are responsible for producing novel NPs from the genomes of culturable bacterial strains. Some of these platforms have also been shown to be applicable to the genomic data of bacteria that have not been grown (Fig. 2). Curating unique BGCs databases and developing new machine learning and deep learning methods to mine BGCs are noteworthy advancements in the field (Kang & Brady, 2014).

To further back the *in silico* analyzing of new BGCs, repositories of characterized BGCs are available: IMG-ABC v.5.0 (Integrated Microbial Genomes Atlas of Biosynthetic Gene Clusters) and MIBiG 2.0 (Minimum Information about a Biosynthetic Gene cluster) (Kautsar et al., 2020). Finally, the processes of host genetic engineering, refactoring, and BGC cloning have been greatly accelerated by new metabolic engineering and synthetic biology (Abbasi et al., 2020). Various advanced computation tools, e.g., antiSMASH (Blin et al., 2019) and PRISM (Skinnider et al., 2017) have already been designed to predict NPs biosynthetic gene clusters (BGCs) and encoded chemical structures at large scale (Ziemert et al., 2016).

The use of machine learning (ML) techniques in synthetic biology and the creation of designer cell factories using ML have become more and more attractive due to the quick development of omics and other high throughput techniques like Next-Generation Sequencing (NGS), and Chromatin Immunoprecipitation Sequencing (ChIP-Seq), in

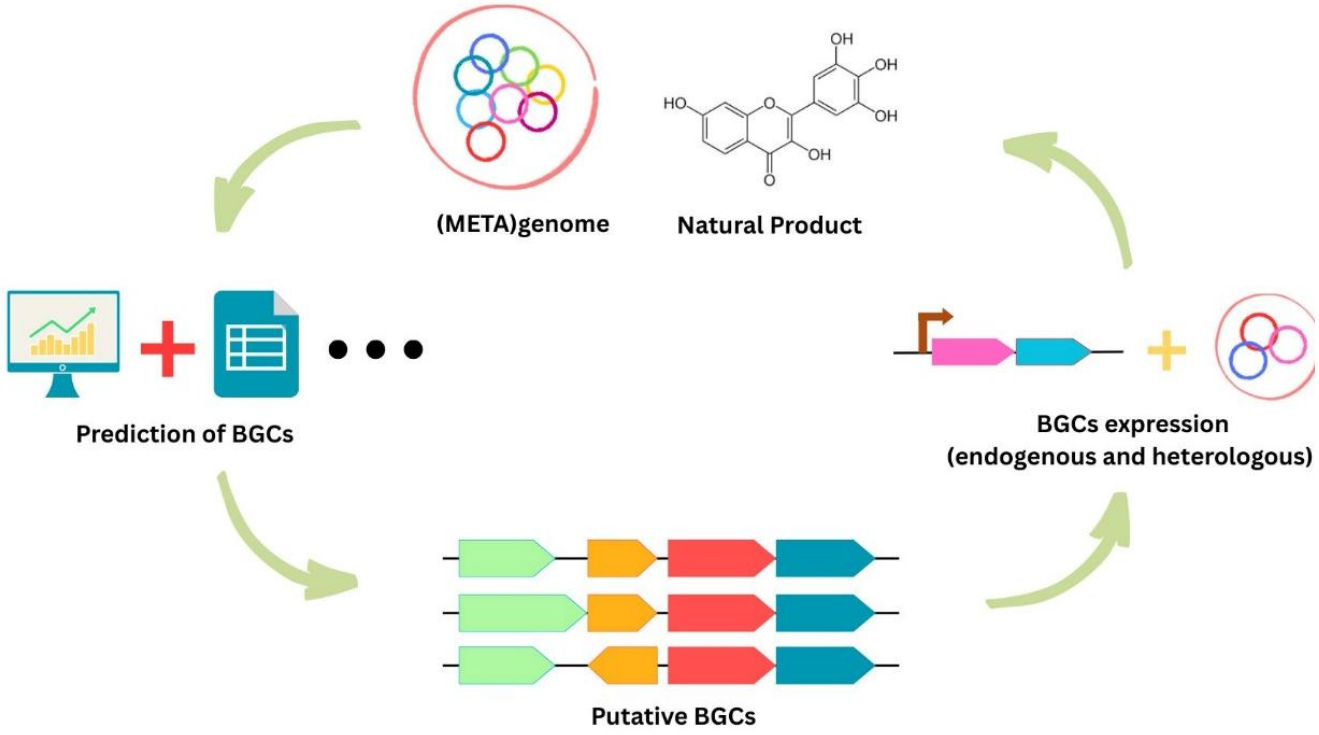

Fig 2. Biosynthetic Gene Cluster (BGC) Analysis and Natural Product Discovery based on (Meta) genomic Data

recent years (Perakakis et al., 2018). ML is an algorithm-based mechanism that uses data to simulate human brain behavior, allowing it to interact with the environment with an increasing degree of accuracy. An optimized ML algorithm can assist in several key areas. It can help in determining the optimal target compound to be produced and identifying potential metabolic pathways for its production. The algorithm can then select the most suitable routes to achieve the highest desired titter, rate, and yield (TRY). Finally, it can analyze experimental data, which will lead to the subsequent refinement of metabolic engineering actions. More specifically, ML models can be useful in defining patterns of detailed biological data at various levels of analysis. They can also be employed to assist in the design of cell factories by predicting new targets for optimal performance (Zhang et al., 2021). A wide variety of ML approaches and algorithms are being employed for extensive uses in other areas, from tumor detection in biology to the field of self-driving cars. This demonstrates the capacity of ML to drive innovation in the metabolic engineering sector (Volk et al., 2020).

APPLICATIONS FOR NATURAL PRODUCT DRUG DEVELOPMENT

NPs have a great number of pharmacological activities like antiviral, antioxidant, anticancer, anti-inflammatory, antibacterial, anti-radiation, and immunomodulatory impacts. They are also widely used as food, health supplements, pharmaceuticals, and various other areas (Roy et al., 2022). However, NPs also provide challenges for drug discovery, such as technological barriers to separation, characterization, screening, and optimization. Therefore, starting in the 1990s, the pharmaceutical industry started to pursue NPs less frequently (Atanasov et al., 2021). Besides the use of NPs as medicine, the natural resources have also played a great role in nutraceuticals and in coloring foodstuffs (Table 1). All of these compounds exhibit various activities, such as an immune-modulating effect and the ability to treat a wide variety of diseases, including cancers and neurological conditions like depression. NPs have succeeded in drug discovery because of their vast structural complexity, diverse pharmacological properties, and innate ability to bind with other biomolecules (Newman & Cragg, 2020).

NPs have undergone additional processing and manufacturing to become active pharmaceutical ingredients on an industrial scale. Some of the most well-known NPs and their related uses are Wormwood sesquiterpene lactones (antimalarial drugs), Mayapple lignans (anticancer and immunosuppressive drugs), Yew taxane-type terpenoids (anticancer drugs), and monoterpene indole alkaloids of Apocynaceae (antihypertensive and anticancer drugs) (Newman & Cragg, 2016). NPs are used as biopharmaceuticals, agrochemicals, as well as other valuable chemicals. It is, however, difficult to purify NPs away from their origins (i.e. bacteria, fungi, plants). Access to many valuable molecules is impossible without relying on synthetic chemistry or heterologous expression. Previously, cell-free synthetic biology has surfaced as a bottom-up technology to be used in prototyping pathways and in the actual production of molecules (Rice et al., 2025). Recently, this technology was implemented in the field of NPs to identify, characterize, and synthesize new biosynthetic pathways, as well as to generate new metabolites. Among them, there are bioactivity-guided drug discovery, antimicrobial resistance (AMR), and an increased use of cell-free innovation in industry (Meyer et al., 2021). In the past, the pharmaceutical

Table 1. Application of NPs in drug development

Natural Product Class	Natural Resource	Applications	Citations
Glucosinolates	Cole crops	Nutraceutical – anti-carcinogenic, antioxidant, detoxifying agents	Ciska et al., 2000
Limonoids	Citrus fruits	Nutraceutical – anticancer, cholesterol-lowering, antiviral	Manners et al., 2003
Isoflavones	Soybeans and soy products	Nutraceutical – phytoestrogens, cardiovascular health, bone health	Sajilata et al., 2008
Lycopene	Tomatoes and red fruits	Natural food colorant (red pigment), antioxidant, anticancer	Upadhyay, 2018

industry used the Selman Waksman method of high-throughput screening of extracts of many organic commodities, such as fungus, soil bacteria, and plants, to find the majority of antibiotic chemical scaffolds (Woodruff, 2014). Specifically, the genus *Streptomyces* was a strong source of antibiotics, e.g. griseomycin, streptomycin, and kanamycin. The Golden Age of Antibiotics gradually came to an end due to the rediscovery of existing compounds and a shift in the pharmaceutical business toward synthetic chemistry (Hutchings et al., 2019).

The other significant area of interest of synthetic biology is biosynthetic pathways reconstruction as well as optimizing synthesis of secondary metabolites. TALENs, ZFNs, and CRISPR-Cas are recent plant genome editing and engineering techniques that provide a new dimension for the rational design of biosynthesis pathways (Huang et al., 2022). Antimicrobial or resistance-reversal agents can be synthesized in transgenic microorganisms or transgenic plants. Transgenic plants, in particular, have been very successful in producing desired products such as artemisinin (Paddon & Keasling, 2014), vinblastine (Zhang et al., 2022), etoposide aglycone (Lau & Sattelt, 2015), vindoline, and catharanthine. Moreover, the entire pathway of biosynthesis with all the involved enzymes and associated genes has to be deciphered by the application of omics, such as proteomics, transcriptomics, metabolomics and genomics, to control and engineer the biosynthesis process and enhance its productivity (Swift et al., 2019).

CHALLENGES AND FUTURE PERSPECTIVES

Lead optimization and additional structural diversification of NP libraries now depend heavily on multimodal biosynthetic engineering. However, our ability to rationally reconstruct biosynthetic pathways is often limited by our incapacity to understand the structure, dynamics, and interactions among the many enzymes involved in complex biosynthetic pathways (Wilkinson & Micklefield, 2007). Modern genomics techniques have greatly increased our ability to access and describe NP pathways using sequence-similarity-based bioinformatics discovery tools. However, in bacterial (meta) genomic sequence data, a large number of biosynthetic enzymes catalyze previously unknown reactions. These reactions often resist functional prediction and remain hidden from us. Although their efficacy and necessity to human health are not questioned, one of the main disadvantages with most NPs remains to be the need to use plants to extract the final products or precursors (Bucar et al., 2013). In fact, the vast majority of these molecules are so structurally complex that their profitable manufacture by purely chemical means is impossible. In addition to climatic changes (natural disasters) or specific health-care situations (pandemics) which may severely affect NPs production and procurement based on natural sources, low aggregation of these compounds (or selective precursors) in the plant coupled with overexploitation of natural resources, has often been cited to cause the high cost of production of the majority of these NPs. Due to these reasons, frequent shortages in the supplies of NPs occur (Gordon et al., 2018).

Biotechnological NPs production alternatives started to develop in the early 2000s as the synthetic biology era began to emerge with the goal to address the limitations in NP research. This was initially done by introducing functionally characterized plant enzyme genes into genetically tractable organisms (e.g., the bacteria *Escherichia coli*, the yeast *Saccharomyces cerevi*siae, or transient in *Nicotiana benthamiana* leaves) for heterologous NP production (Li et al., 2018). This general strategy has been extended in detail over the past 10-15 years via the application of advanced technology in the fields of genome sequencing, genome engineering, elucidation of plant pathways, as well as metabolic engineering.

CONCLUSION

Synthetic biology and metabolic engineering have been used to radically transform the discovery and production of NPs by providing environmentally friendly, scalable and precise alternatives to traditional extraction technologies. The rational engineering of biosynthetic pathways, a good choice of microbes, and the implementation of sophisticated genome-editing techniques have created the potential to synthesize complex NPs with the same efficiency with which they are generated in nature. Enhancements in co-factors and precursor availability surpass production yields and

further enhance production. The practical importance of innovations is proven by the successes in the development of antibiotics, anticancer agents and other therapeutic products. New opportunities to address some of these obstacles, including AI-guided design, cell-free systems, and minimal genome platforms, are quickly resolving the former issues related to pathway complexity and host limitations. Further development of this sector in future will be crucial for drug development and biomanufacturing.

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