International Research Journal of Education and Technology
Peer Reviewed Journal
ISSN 2581-7795

Microbial Synthesis of Bioactive Compounds: Biotechnology's Pharmaceuticals Revolution

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Abstract

Various organisms, including bacteria, fungi, and plants, are known for their ability to synthesize secondary metabolites, often referred to as natural products. Natural products have emerged as a prolific source of inspiration for the creation of diverse medical agents. These agents exhibit a wide range of chemical structures and biological activities, encompassing attributes like antimicrobial, immunosuppressive, anticancer, and anti-inflammatory properties. Many of these compounds have not only been developed into treatments but also hold promising therapeutic potential for addressing various human diseases. In addition to natural products, recent advancements in recombinant DNA technology have ushered in a new era of biopharmaceutical products, including recombinant proteins. These innovations have significantly advanced the treatment landscape for an extensive array of medical conditions. This article aims to introduce the chemical structures and the diverse biological activities associated with natural products and recombinant proteins, both of which have become invaluable assets in the realms of medicine, agriculture, and insect control. Furthermore, the piece will delve into past and ongoing endeavors and accomplishments in harnessing robust microorganisms as cell factories for the production of biologically active compounds. It will also explore multidisciplinary engineering approaches that are geared towards enhancing the yields of microbial production of natural products and proteins, as well as the creation of novel molecules. Throughout the narrative, we will propose potential avenues through which biologically active compounds derived from microorganisms and their analogs can continue to serve as wellsprings of inspiration for the development of novel therapeutic agents in both academic and industrial settings.

Keywords: *Microbial synthesis, Bioactive compounds, Biotechnology, Pharmaceuticals, Revolution, Pharmaceutical industry, Microorganism*

Introduction

Natural products are a diverse and valuable source of therapeutics, originating from a wide range of organisms, including plants, animals, and microorganisms. The history of natural product usage in medicine dates back to ancient Mesopotamia, where sophisticated

medicinal systems were developed. In the early 1900s, plant-derived medicines dominated the field, but the discovery of penicillin from Penicillium notatum by Alexander Fleming in 1928 marked a shift toward microorganisms as a source of natural products. Streptomycin, chloramphenicol, and other antibiotics followed, revolutionizing medicine, agriculture, and scientific research. Today, natural products continue to play a significant role in drug discovery. About 60% of approved small molecule medicines have roots in natural products, with a remarkable 69% of antibacterial agents originating from these sources. However, the low concentrations of many potential drug candidates in nature make their discovery and development challenging. To overcome this, researchers are turning to engineered microbes, primarily bacteria and fungi, as hosts for biosynthetic genes from original producers. This approach enables the production of larger quantities of rare natural compounds, facilitating the development of novel drugs and derivatives [1].

The field of natural products has seen substantial growth and therapeutic products derived from living organisms are on the rise. Microbial cells, both prokaryotic and eukaryotic, coupled with advances in recombinant DNA techniques, have led to the proliferation of biologics. Biologics encompass a diverse group of molecules whose active pharmaceutical ingredients are sourced from living organisms, including animals, plants, microorganisms, human blood products, and complex tissue transplants that cannot be synthesized through traditional organic methods [2]. These biologics fall into five main categories: (1) monoclonal antibodies, such as trastuzumab and rituximab; (2) blood factor derivatives, like coagulation factor VIIa and epoiten alfa; (3) vaccines; (4) enzymes; and (5) recombinant proteins, including immunomodulatory cytokines and thrombolytic agents [3]. With the approval of early biopharmaceuticals like recombinant human insulin and recombinant human growth hormone, microbial expression systems gained prominence in the 1980s and remain a key player in biopharmaceutical development. Microbial cells are the predominant hosts used for producing approved recombinant pharmaceuticals due to their advantages, including predictable post-translational modifications, proteolytic stability, solubility, and minimal activation of cell stress responses [4]. This underscores the convenience and reliability of microbial platforms for efficient recombinant protein production, despite some inherent challenges.

In this review, we will highlight the biological activities and applications of natural products and biologics, examining the microbial systems utilized for their pharmaceutical production. We'll delve into efforts to enhance microbial production and engineer novel compounds, addressing challenges and showcasing advances for drug discovery. Additionally, we'll explore future prospects and technological breakthroughs in microbial production for bioactive natural products and recombinant proteins as crucial sources of therapeutics.

BIOLOGICAL ACTIVITIES OF NATURAL PRODUCTS AND BIOLOGICS

In this section, we explore the diverse biological activities of natural products, including their antibiotic, antifungal, anticancer, immunosuppressive, anti-inflammatory, and biofilm inhibitory

properties. We also delve into the biological activities of microbial recombinant proteins.

Antibiotics

Natural products are a valuable resource for developing antibiotics, with prominent classes including polyketides, non-ribosomal peptides, and aminoglycosides. Polyketides, synthesized by polyketide synthases (PKS), form a diverse group of natural products widely recognized for their applications in medicine, agriculture, and industry. For instance, pikromycin, an early-discovered polyketide antibiotic produced by S. venezuelae, shows potent activity against multi-drug resistant respiratory pathogens. Another essential polyketide antibiotic, erythromycin A, is a broad-spectrum antibiotic prescribed to treat various bacterial infections, especially in individuals with penicillin allergies. Additionally, tetracyclines are notable for their ability to combat both Gram-positive and Gram-negative bacteria. These natural products play a critical role in antibiotic drug development [5, 6, 7].

Penicillin, derived from Penicillium notatum, is a renowned antibiotic secondary metabolite primarily effective against Gram-positive bacteria, causing illnesses such as scarlet fever, pneumonia, gonorrhea, meningitis, and diphtheria [8]. Classified as a non-ribosomal peptide antibiotic, penicillin belongs to the same group as vancomycin [9]. Non-ribosomal peptides, synthesized by non-ribosomal peptide synthetases (NRPS), encompass a diverse array of secondary metabolites known for their therapeutic potential. Vancomycin (Figure 1 and Table 1) is a potent non-ribosomal peptide antibiotic effective against pathogenic bacteria such as Clostridium difficile, Listeria monocytogenes, Streptococcus pneumoniae, Staphylococcus epidermidis, and methicillin-resistant Staphylococcus aureus (MRSA). Aminoglycosides constitute another class of antibiotics that exert their action by binding to the rRNA subunit of the bacterial ribosome's 30S subunit, thereby inhibiting protein synthesis. The first discovered aminoglycoside, streptomycin (Figure 1 and Table 1), was isolated from Streptomyces griseus in 1944 and demonstrated efficacy against pulmonary tuberculosis. Subsequently, additional aminoglycoside antibiotics, such as kanamycin, gentamicin, sisomicin, and lividomycin, have been developed to combat infectious organisms that developed resistance to streptomycin over time [10]. Despite their remarkable antibacterial activity, aminoglycosides have encountered resistance issues. To address this challenge, semi-synthetic aminoglycoside antibiotics were specifically tailored to evade resistance mechanisms [11]. These semi-synthetic derivatives, including amikacin, netilmicin, dibekacin, and isepamicin, were developed to enhance their efficacy and overcome bacterial resistance [12].

Natural antimicrobials play a crucial role in ensuring food safety within the food industry by safeguarding against foodborne pathogens. Microorganisms like lactic acid bacteria produce a wide array of compounds known to inhibit the growth and development of various other microbes. For instance, Nisin A, a bacteriocin derived from Lactococcus lactis, is authorized for food preservation in more than 50 countries. It exhibits significant activity against Gram-positive bacteria that are resistant to conventional antibiotics. Similarly, Reuterin, sourced from Lactobacillus reuteri, has demonstrated antimicrobial efficacy against foodborne pathogens and spoilage

microorganisms when assessed in dairy, meat, and milk products. These natural antimicrobials are pivotal in enhancing food safety and quality [13].

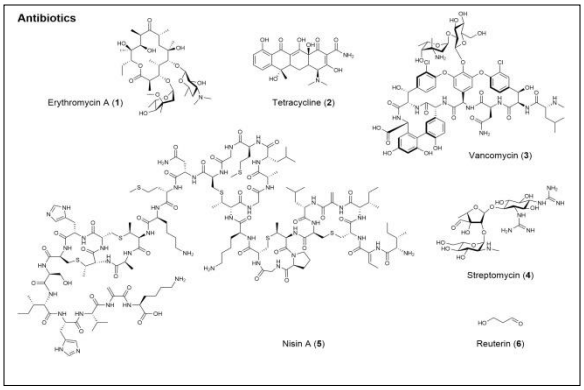


TABLE 1: Biological Activities of Microbial-Derived Natural Products and Biologics

Name	Origin	Biological Activity
Antibiotic		
Erythromycin A (1)	*Saccharopolyspora erythraea*	Antibacterial
Tetracycline (2)	*Streptomyces rimosus*	Antibacterial
Vancomycin (3)	*Amycolatopsis orientalis*	Antibacterial
Streptomycin (4)	*Streptomyces griseus*	Antibacterial
Nisin A (5)	*Lactococcus lactis*	Antimicrobial
Reuterin (6)	*Lactobacillus reuteri*	Antimicrobial
Antifungal Agents		
Amphotericin B (7)	*Streptomyces nodosus*	Antifungal
Ieodoglucomide C (8)	*Bacillus licheniformis*	Antifungal

Antifungal Agents

Nystatin, an early and effective polyene antifungal agent derived from Streptomyces noursei in 1950, has demonstrated efficacy

against Aspergillus species. Clinically, nystatin serves a significant role as a topical antifungal treatment for oral, gastrointestinal, and genital candidosis. Amphotericin B (Figure 2 and Table 1) is another traditional polyene antifungal product produced by Streptomyces nodosus, primarily used to combat life-threatening fungal infections, especially those resulting from Aspergillus species, and notably effective in patients who have undergone organ transplantation, received aggressive chemotherapy, or have acquired immunodeficiency syndrome [14]. Recently, in a review of natural products with anti-Candida albicans activity, 71 substances of the 142 evaluated were determined to have antifungal activity under the criteria of having minimum inhibitory concentration (MIC) values below 8 mg/mL. The glycolipids ieodoglucomide C (8; Figure 2 and Table 1) and ieodoglycolipid were isolated from the marine bacterium Bacillus licheniformis and exhibited antifungal activities with a 21 mg/L MIC against Aspergillus niger, Rhizoctonia solani, Botrytis cinerea, Colletotrichum acutatum, and C. albicans (Tareq et al., 2015). Both ieodoglucomide C and ieodoglycolipid also exhibit good antibiotic properties against S. aureus, Bacillus subtilis, Bacillus cereus, Salmonella typhi, E. coli and Pseudomonas aeruginosa with MICs ranging from 0.01 to 0.05 mM, establishing these compounds as strong potential candidates for the development of new fungicides [15].

Anticancer Agents

Numerous microbe-derived anticancer agents have undergone rigorous evaluation in clinical trials. For instance, actinomycin, a polyketide initially isolated from Streptomyces parvulus in 1940, marked a significant milestone as the first antibiotic with proven anticancer activity. One of its variants, actinomycin D (or dactinomycin), received FDA approval and has been widely used in clinical practice for combating various cancers, including Wilms' tumor, childhood rhabdomyosarcoma, Ewing's sarcoma, and metastatic non-seminomatous testicular cancer. Another noteworthy success story involves the therapeutic trio of microbial product bleomycin, plant compound etoposide, and synthetic agent cisplatin. This combination contributed substantially to elevating the cure rate for disseminated testicular cancer from a mere 5% in 1974 to an impressive 90% in 2011. Bleomycin, derived from Streptoalloteichus hindustanus, has found utility in the treatment of diverse cancers, including squamous cell carcinomas, melanomas, sarcomas, and testicular, ovarian, Hodgkin's, and non-Hodgkin's lymphomas. Its derivative, blenoxane, is also employed in clinical settings, often in conjunction with other compounds, to combat lymphomas, skin carcinomas, and tumors affecting the head, neck, and testicles. The family of anthracyclines, encompassing daunorubicin, epirubicin, and doxorubicin, has emerged as vital polyketide compounds produced by Streptomyces species through iterative polyketide synthase (PKS) pathways. The FDA granted approval for the use of daunorubicin and doxorubicin in cancer therapy back in the 1960s. Daunorubicin is applied in the treatment of acute lymphoblastic or myeloblastic lymphoma, while doxorubicin is an effective agent in the management of breast cancer, pediatric solid tumors, soft tissue sarcomas, and aggressive lymphomas [16].

Several recent examples, including rapamycin, wortmannin, and geldanamycin, have demonstrated their antiproliferative properties when used clinically as novel chemotherapeutic agents. Rapamycin,

derived from Streptomyces rapamycinicus, boasts anticancer effects alongside its immunosuppressive, anti-inflammatory, and antifungal properties. It combats tumor cells by inhibiting proliferation, promoting apoptosis, and blocking angiogenesis. Wortmannin, a fungal furanosteroid from Penicillium funiculosum, serves as an effective inhibitor of phosphoinositide 3-kinases (PI3Ks) and related enzymes, which are crucial in cellular signaling pathways. Studies on human breast MCF-7 cells treated with wortmannin reveal its antitumor activity by triggering apoptosis, hindering proliferation, and suppressing PI3K/Akt signaling. Geldanamycin, derived from Streptomyces hygroscopicus var. geldanus, disrupts ATPase activity by binding to heat shock proteins, destabilizing oncogenic protein kinases involved in signal amplification cascades controlling proliferation and apoptosis. Geldanamycin and its analogs are used as anticancer agents in multiple myeloma, breast, and prostate cancer. Epothilone, produced from mycobacterium Sorangium cellulosum, disrupts microtubule depolymerization, leading to G2-M cell cycle arrest. Additionally, marine microbial natural products, like dolastatin from cyanobacteria genera Symploca and Lyngbya, exhibit anticancer activities [17].

Immunosuppressive Agents

Rapamycin (also known as sirolimus) and FK506 (tacrolimus) are microbial natural products with immunosuppressive properties. Rapamycin blocks the proliferation of most cell types in response to activation by IL-2, IL-3, platelet-derived growth factor, epidermal growth factor, and insulin. Rapamycin also exhibits synergism with other immunosuppressants, such as cyclosporin, to significantly reduce kidney toxicity and acute renal allograft rejection. This compound has been developed to coat coronary stents and prevent organ transplant rejection and lymphangioleiomyomatosis; it was approved by the FDA for wider use in 1999. In addition to its immunosuppressive activity, rapamycin possesses several other biological activities, including antitumor, neuroprotective/neuroregenerative, antineoplastic, and lifespan extension activities. FK506 is also an immunosuppressive drug and was first discovered in soil samples containing Streptomyces tsukubaensis and several other Streptomyces species. FK506 is used to minimize organ rejection and to induce immunosuppression via calcineurin inhibition and interruption of T cell activation pathway. It has been demonstrated to be more effective than cyclosporin and non-toxic in low doses. The discovery of its immunosuppressive activity led to its use in heart, liver, and kidney transplants with overwhelming success. Like rapamycin, FK506 possesses various biological activities, including antifungal, anti-inflammatory, neuroprotective, and neuroregenerative activities [18].

Anti-inflammatory Agents

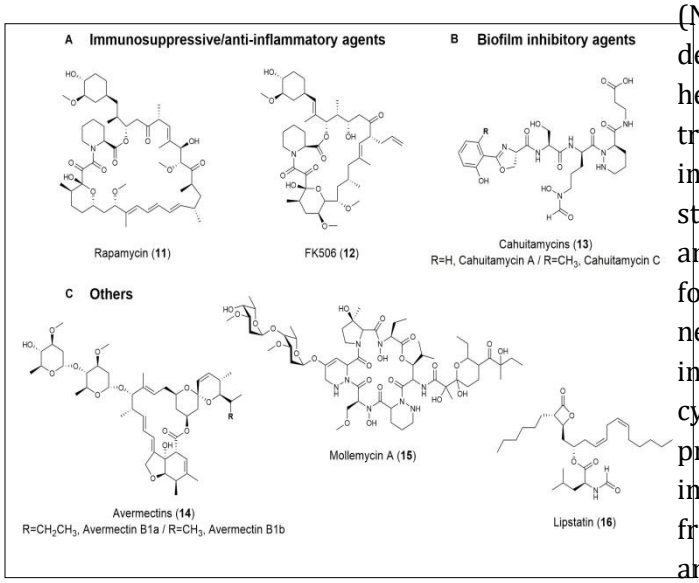
Some natural products also have anti-inflammatory activities. FK506 has shown efficacy in the treatment of refractory rheumatoid arthritis, a chronic inflammatory disease. Rapamycin also inhibits the inflammatory response after spinal cord injury by diminishing the activation and proliferation of inflammatory cells and the expression of inflammatory cytokines, thereby reducing secondary injury in the spinal cord and providing a neuroprotective effect. Recently, strepsesquitriol, isolated

from Streptomyces sp. SCSIO 10355, has been found to have anti-inflammatory activity through the inhibition of tumor necrosis factor-a production in lipopolysaccharide-activated macrophages. Salinamides A and B from marine Streptomyces sp. CNB-091 also displayed potent topical anti-inflammatory activity through a phorbol ester-induced mouse ear edema assay. One study evaluated 7 peptides found in the Faecalibacterium prausnitzii supernatant, all belonging to a protein named microbial anti-inflammatory molecule . These peptides were able to inhibit the NF-kB pathway in vitro and showed anti-inflammatory properties in vivo in a dinitrobenzene sulfate-induced colitis model. Biofilm Inhibitory Agents Parasitic microorganisms adhere to solid surfaces and form layers of a complex polysaccharide matrix called a biofilm that confers resistance against antibiotics as wells as inflicts significant chronic bacterial infections. Analogs of 5-benzylidene-4-oxazolidinones are small molecules derived from marine natural products. These molecules inhibit 89% of biofilm formed by MRSA at 0.78 mM and disperses pre-formed biofilms at 4.7 mM. A synthetic library of 2-aminoimidazole triazoles was able to successfully inhibit 94% of biofilm formation in Acinetobacter baumannii and MRSA at 100 mM. Another recent example is cahuitamycins A-C derived from the marine bacterium Streptomyces gandocaensis. Cahuitamycins have been evaluated as inhibitors of A. baumannii biofilms and it has been found that cahuitamycin C shows half maximal inhibitory concentration (IC50) at 14.5 mM. Modifications of cahuitamycins through selective mutasynthesis have yielded cahuitamycins D and E with an increased the potency of antibiofilm activity against A. baumannii. The FDA-approved antitumor agent actinomycin D has also significant biofilm inhibitory activity against methicillin resistant- and sensitive-strains of S. aureus. In addition to bacterial biofilm, fungal biofilm associated with Candida pathogens is responsible for serious C. albicans infections linked to biofilm formation on medical devices. One study showed that Lactobacillus biosurfactants displayed high anti-adhesive biofilm formation properties against C. albicans and also prevented biofilm formation of L. monocytogenes, Salmonella arizonae, E. coli, and S. aureus [19].

Others

Natural products can also act as antiparasitic agents. The avermectins and the derivative ivermectin have shown antiparasitic activity by significantly lowering the incidence of onchocerciasis and lymphatic filariasis. Spinosad and milbemycin also have insecticidal activity. Spinosad is a combination of spinosyn A and D, which are both produced by Saccharopolyspora spinosa and have insecticidal activity against livestock external parasites via the disruption of nicotinic acetylcholine receptors. Milbemycin is an isolated fermentation product of S. hygroscopicus subsp. aureolacrimosus that acts as an insecticide and acaricide with GABAergic activity on the post-synaptic membranes of the inhibitory motor neurons of mites and arthropods through hyperpolarization and impeding neuronal signal transduction mechanisms. Mollemycin A 20 is a first-inclass glycol-hexadepsipeptide-polyketide from a Streptomyces sp. and has antibacterial properties against certain Grampositive and Gram-negative bacteria, as well as extremely potent antimalarial activity against drug sensitive and MDR Plasmodium falciparum clones. Microbial natural products also

function as enzyme inhibitors. Lipstatin (Figure 3) is a pancreatic lipase inhibitor produced by Streptomyces toxytricini that is used to combat obesity and diabetes by interfering with the gastrointestinal absorption of fat. Lipstatin contains a beta-lactone structure that is likely responsible for irreversibly binding to the active site of lipase [20].



Biological Activity of Microbial Biologics

Since Humulin R (Figure 4A) became the first recombinant biopharmaceutical as a treatment for diabetes, additional FDA-approved microbial biologics have been produced by E. coli. Somatrem (Protropin R) and somatropin (Humatrope R) are used to treat children with growth hormone deficiency. Another biopharmaceutical produced from E. coli is pegloticase (Krystexxa R) for the treatment of chronic gout and interferon (IFN) a-2b (Intron R A; Figure 4B) for the treatment of certain types of genital warts, malignant melanoma, hairy cell leukemia, follicular lymphoma, Kaposi's sarcoma, and chronic Hepatitis B or C). Top selling biopharmaceuticals of 2015 from microorganisms include insulin glargine (Lantus R) derived from E. coli, which functions as an insulin analog, and the pneumococcal vaccines (Prevnar R family) derived from S. pneumoniae and Corynebacterium diphtheriae. Biopharmaceuticals are also utilized for their antitumoral properties, such as the cytokines filgrastim (Neupogen R) and granulocyte colony stimulating factor pegfilgrastim (Neupeg R ; Figure 4C), which are both derived from E. coli. Flgrastim stimulates hematopoiesis for bone marrow transplantation and cancer chemotherapy-induced neutropenia, whereas pegfilgrastim stimulates the differentiation, proliferation and activation of neutrophilic granulocytes for cancer chemotherapy-induced neutropenia. Recombinant human interleukin-3 (hIL-3; Figure 4D) protein is a cytokine that regulates the differentiation and proliferation of the various cells of the immune system. The hIL-3 protein is derived from B. subtilis, B. licheniformis, and E. coli and has utility as a laboratory reagent in hematology for cell cultures, differentiation studies and functional assays. It has shown that hIL-3 has potential in treating bone marrow failure, hematological malignancies, and can support engraftment after bone marrow transplantation. In addition, recombinant Pfs48/45 is a disulfide-rich malaria transmission-blocking vaccine produced by L. lactis that provides immunization against malaria from P. falciparum. Recombivax is produced by S. cerevisiae and can prevent infection of all known subtypes of the Hepatitis B virus. Some examples of currently approved protein therapeutics derived from yeast include human serum albumin (Recombumin R and Albucult R; Figure 4E), human insulin (Actrapid R) and primary immunization for infants born of Hepatitis B virus (HBV)

surface antigen (Pediarix R), all of which are obtained exclusively from S. cerevisiae. Recombinant human serum albumin is utilized to increase the shelf life of protein drugs by preventing physical and chemical degradation. Actrapid R is used to treat diabetes, and subcutaneous injections of Pediarix is designed for immunization against diphtheria, tetanus, pertussis, poliomyelitis, and infection caused by all known subtypes of HBV. Ecallantide (Kalbitor R) is an FDA-approved recombinant peptide produced by Pichia pastoris for the treatment of hereditary angioedema. Additionally, anakinra (Kineret R) was approved in 2001 in the United States for rheumatoid arthritis. Anakinra is expressed in E. coli and functions as an IL-1 receptor antagonist that is effective and safe for patients with systemic-onset juvenile idiopathic arthritis, adult-onset Still's disease, hereditary autoinflammatory syndromes, and Schnitzler's syndrome [21, 22].

MICROBIAL CELL FACTORIES

In our upcoming discussion, we will emphasize the critical role of choosing an appropriate host strain in the development of processes for natural product and recombinant protein production. This section will provide insights into the traits of microbial strains employed in the synthesis of natural products and biologics. Additionally, we will introduce various techniques and approaches that streamline the engineering of these microbial hosts, transforming them into efficient cell factories for the manufacturing of biopharmaceutical compounds (as summarized in Table 2).

Gram-Negative Bacteria

Escherichia coli

Escherichia coli has been seen as one of the optimal systems for the production of natural products because it is easily manipulated, highly productive, there is an availability of genetic tools to use with it and there is a deep knowledge of its physiology. Artemisinin, a sesquiterpene lactone endoperoxide from Artemisia annua L. plants, has strong antimalarial activity against the multi-drug resistant parasite P. falciparum. Yet the synthesis of artemisinin is costly and low yields are isolated from the natural plant source. Researchers reported the production of approximately 24 mg/L of amorpha-4,11-diene (amorphadiene), an artemisinin precursor, by the expression of a codon-optimized synthetic amorphadiene synthase gene and the mevalonate pathway from S. cerevisiae in E. coli. Additionally, after further processing modifications and optimal conditions, they were able to produce 105 mg/L of artemisinic acid [23]. However, there are some obstacles and limitations with E. coli as a dominant host in natural product biosynthesis. E. coli requires extensive genetic manipulation and lacks native natural product biosynthetic machinery and/or precursors. An example is phosphopantetheinyl transferase, which is responsible for the activation of the carrier protein domains of the PKSs and NRPSs. This enzyme must be introduced into E. coli to support of natural product biosynthesis [24]. There have been efforts to overcome these hurdles, such as the production of erythromycin A and its derivatives in the engineered E. coli strain. The study generated two analogs through directed manipulation of polyketide biosynthesis in which variations were made to the deoxyerythronolide B synthase (DEBS) 1 and DEBS3 enzymes in order to utilize the multi-catalytic capability of the modular polyketide synthase.

Escherichia coli has also been the pioneering host for recombinant protein production. To date, E. coli continues to be the first-choice microorganism for manufacturing recombinant proteins at laboratory and industrial scales. Its success is mostly due to its fast growth, simple culture procedures, cost-effectiveness, unusually high versatility, and the associated systems that make it adaptable to varying production demands [25].

From 2004 to 2013, 24% of the biopharmaceuticals approved by the FDA and the European Medicines Agency were derived from E. coli. Currently, biopharmaceuticals produced from E. coli are used in the treatment of diabetes, growth hormone-deficiency in children, leukemia, gout, and many other therapeutic indications as previously discussed in Section "Biological Activity of Microbial Biologics". A major concern when using E. coli as a production platform is the lack of post-translational modifications (PTMs) present in most eukaryotic proteins; lacking PTMs can lead to protein products being insoluble, unstable, or inactive. However, it is possible to add synthetic PTMs to generate versions of the protein that are more stable than the original naked product. Examples of this include pegylated products, like human growth hormone, stimulating factor, IFNs a-2a and a-2b. Additionally, there is a risk of translational errors due to the presence of a large number of rare codons that appear in human genes that are different from those occurring in E. coli genes. Even at low levels, these errors may cause an impact on the tertiary structure, premature termination of protein synthesis or amino acid misincorporation which results in low protein expression [26]. One strategy to bypass the issue with codon bias is to synthesize the whole human gene based on codon usage in E. coli through site-directed mutagenesis, which is currently a preferred method; however, it is limited by the high cost of production and time consumption. An alternative method that is less time consuming utilizes the co-transformation of E. coli strains with a plasmid(s) containing a gene encoding the tRNA cognate to the rare codons. Increasing the copy number allows for E. coli to be manipulated to match the codon usage frequency in heterologous genes. Currently, there are numerous commercial E. coli strains available that harbor plasmids containing gene sequences encoding the tRNA for rare codons, such as BL21(DE3) CodonPlus-RIL, BL21(DE3) CodonPlus-RP and Rosetta (DE3). Another common problem associated with recombinant protein expression in E. coli involve inclusion body formation, which refers to insoluble and inactive protein aggregates. E. coli producing recombinant proteins have the ability to assemble in cells and form conglomerates of inclusion bodies as well as result in erroneous protein folding which hinder the extraction of proteins directly from the cell leading to costly purification of proteins. Inclusion bodies formed from lack of proportion between protein solubilization and aggregation can be resolved by combining the desired protein with a solubility enhancer fusion partner acting as an intrinsic chaperone in order to ensure the production of soluble recombinant proteins. The fusion of maltose-binding protein to polypeptides such as human tissue inhibitor of metalloproteinase and p16 improved their solubility significantly in E. coli [27].

Table 2: Comparison of Microbial Host Systems for Recombinant Proteins and Natural Products Productio

Microbial Hosts	Advantages	Disadvantages	Compounds
Gram-negative			
Escherichia coli	- Fast growth	- Lack of post-translational modifications (PTMs)	Recombinant human insulin
	- Simple culture procedures	- Risk of translational errors (rare codons)	Artemisinin
	- Cost-effective	- Expensive and challenging purification process	Erythromycin A
	- High versatility		Somatrem
	- of the enterobacterium		Somatropin
	and its associated systems		Pegloticase
Gram-positive			Insulin glargine
Lactococcus lactis	- Simplified downstream	- Lack of secreted heterologous proteins degradation	Pneumococcal vaccines
	purification processes	- Per liter secretion generally less robust than Bacillus sp.	Filgrastim
	- Absence of endotoxins or	- AT-rich codon usage and/or the distribution of rare codons	Pegfilgrastim
	unwanted glycosylation of		Human serum albumin

	Proteins		Hepatitis B virus immunization
	- Generally recognized as safe		IFN a-2b
Streptomyces sp.			IL-6
	- Rapid growth		
	- Abundant supply of	- Forms pellets or clumps	
	secondary metabolite	- Low protein yield	
	Precursors		
	- Ability to produce natural		
	Products		
	- Efficient protein secretion		
	System		
Bacillus sp.			
	- Outstanding fermentation	- Plasmid instability	
	properties and protein	- Presence of proteases (difficulty in the production of recombinant proteins)	
	production yield (20–25 g		
	per liter)		

	- Completely free toxin		
	Production		
	- Flexibility for genetic		
	Engineering		
	- Presence of proteome		
	secretory pathway		
	- Primarily used in Enzyme		
	production.		
Fungi/Yeast			
**Saccharomyces	- Fast growth rate	- N-linked glycosylation patterns differ from higher eukaryotes	
cerevisiae**	- Technically practical	- Lack some required precursor pathways	
	- Cost-effective	- Codon usage is biased toward ACT	
	- Ability to generate post-		
	translational modification		
	(O-linked glycosylation,		
	phosphorylation,		

	acetylation, and acylation)		
Aspergillus sp.			
	- GRAS status	- Many host proteases	
	- Tolerate extreme cultivation	- Freely dispersed filaments or highly compact pellets formed during submerged fermentations	
	Conditions		
	- Degrade and utilize diverse		
	biopolymers, allowing		
	cultivation on renewable		
	Resources		
	- Major Source of citric acid production		
	- Production of mycotoxins		
(alpha toxins)			
Hansenula polymorpha			
	- GRAS status	- The use of methanol creates hazardous conditions in lab use	
	- Combined genetic	- Hyperglycosylation of heterologous products	

	manipulations, low cost	- Can lead to production instabilities due to sequence repetition on vector	

Gram-Positive Bacteria

Lactococcus lactis

Lactococcus lactis is becoming an attractive alternative in genetic engineering for the production of various recombinant proteins. Unlike E. coli, which uses intracellular production strategies that involve expensive and often challenging purification processes, L. lactis utilizes extracellular secretion system. This is because L. lactis has a monolayer cell wall that allows direct secretion into the extracellular environment. The presence of exported proteases such as HtrA in L. lactis contributes to recombinant protein production by minimizing the destruction of heterologous proteins in the medium. Additionally, L. lactis does not generate undesired glycosylation of protein, is generally recognized as safe (GRAS), does not produce endotoxins, and has probiotic properties. Another advantage of L. lactis includes a lack of inclusion body formation. There is a diverse selection of cloning and inducible expression vectors available for use with this host that are compatible with large-scale upstream and downstream processes [28].

Lactococcus lactis has been used for centuries in the fermentation of food, especially in cheese, yogurt, and sauerkraut because of its production of nisin. Beyond the food industry, lactic acid is used as an emulsifier and moisturizing agent in the cosmetic industry and as an important raw material in the pharmaceutical industry. The L. lactis host has also been chosen after researchers had unsuccessfully attempted to obtain correct conformation using a variety of other prokaryotic and eukaryotic recombinant protein expression systems. L. lactis has multiple advantages over E. coli for expression of 5r-methylthioadenosine/S-adenosylhomocysteine nucleosidase (Pfs) recombinant proteins, including the following: (1) codon-optimization of the recombinant gene is not necessary to achieve successful expression in L. lactis; (2) the recombinant protein is secreted into the L. lactis culture supernatant, which results in easier and less expensive down-stream processing, and (3) there is no lipopolysaccharide contamination in L. lactis expression. L. lactis has been used in the successful production of recombinant Pfs48/45, a vaccine candidate against P. falciparum. GMZ2, a recombinant fusion protein expressed in L. lactis, is also a malaria vaccine candidate that has been shown to elicit high levels of active IgG antibodies with inhibitory activity against a broad range of P. falciparum strains. A recent study concluded phase 2 trial of GMZ2 adjuvanted with aluminum hydroxide in a cohort of 1,849 children revealed GMZ2 as well tolerated with modest efficacy. Not only is L. lactis being utilized for the production of recombinant proteins for vaccines, but the host is also being tested as a factory for antigen production, allowing the bacteria to function as live vaccines. Using L. lactis as a vaccine carrier is beneficial because it can induce both mucosal and systemic immune responses, has adjuvant properties and is free from the risks associated with the use of

conventional attenuated live pathogens, such as Salmonella species and Mycobacterium species. However, while L. lactis has been studied against an array of antigens from various pathogens, there is no current live vaccines under clinical trial which may be due to a lack of containment strategies. Without a plan for containment, studies on liveL. lactis vectors risk the chance of proliferation and dispersion. An additional limitation of AT rich L. lactis as a cell factory is due to codon usage as well as distribution of rare codons to express heterologous genes [29].

Streptomyces Species

Another major species that has shown promise as a cell factory through its wide production of natural products and biologics is Streptomyces. This Gram-positive bacterium has been studied for more than 70 years and has proven to be of great use in biotechnology due to its ability to produce natural products acting as antibiotics, anticancer agents, and immunosuppressants. Some examples include tetracycline, daptomycin, and chloramphenicol. There are many species of Streptomyces currently to produce various natural products and biologics. Among the Streptomyces species, Streptomyces coelicolor, Streptomyces lividans, Streptomyces albus, and S. venezuelae are favored heterologous hosts to produce specialized metabolites due to the relative ease of their genetic manipulation, the availability of their genome sequences, and the abundant supply of their natural substrates. Streptomyces has also been used to produce a wide array of heterologous proteins of both eukaryotic and prokaryotic origin because Streptomyces has well-developed genetic manipulation and fermentation technologies as well as efficient protein secretion systems such as the secretory (Sec) pathway and the twin-arginine-translocation (Tat) pathway. The Sec-pathway catalyzes the translocation of unfolded proteins while the Tat pathway allows for the export of folded proteins across the cytoplasmic membrane. Tumor necrosis factor (TNF) α and human interleukin (hIL) 10 are able to be expressed in both the Sec- and Tat-pathways. In particular, S. lividans could be the ideal Streptomyces host due to limited restriction-modification systems and low endogenous protease activity. Streptokinase, transforming growth factor-α, IL-2 and many other proteins have been successfully expressed and secreted from S. lividans. However, aside from its efficient secretory pathways, when in culture, Streptomyces grows as mycelial networks, leading to the formation of pellets or clumps. These pellets are unappealing from an industrial standpoint because of mass-transfer problems, slow growth, and culture heterogeneity which leads to lower product yield [30].

Bacillus Species

Bacillus species are some of the most popular species used in enzyme production. It accounts for roughly 50% of enzymes market within the industrial sphere. Certain species, like B. subtilis, Bacillus amyloliquefaciens, and B. licheniformis are favored because of their outstanding fermentation properties, high protein production yield, and their completely toxin-free production. The fermentation capacity of Bacillus species in acid, neutral, and alkaline pH ranges in addition to thermophiles accounts for the prolific production of enzymes that have desirable temperature, pH, and stability, which makes them appealing for specific use in various industries. Bacillus species are known for their production of iturins and

fengycin which have antifungal activity as well as surfactin for its function as a surfactant [31].

Among these species, B. subtilis is the most widely studied due to (1) its flexibility during genetic engineering, (2) its naturally high secretory capacity, (3) its ability to produce valuable antibiotics, such as bacillomycins D-L and bacitracin, and (4) its ability to produce enzymes, such as stable alkaline cellulase, alkaline protease and alkaline α-amylase. It may also elicit better folding conditions, leading to the prevention of inclusion bodies. In addition, its ability to adapt to varying environmental conditions as well as its classification as toxic free GRAS has contributed tremendously to its success in the industrial platform. B. subtilis as an endotoxin free host amplified its utilization in the production of sterile recombinant and therapeutic proteins expression as compared to E. coli which could have potential contamination due to the lipopolysaccharide endotoxins. For instance, B. subtilis and Bacillus megaterium were the preferred hosts over E. coli in the production of bioengineered heparin in order to diminish toxin contamination. Moreover, B. subtilis is able to produce high yield in enzyme as it secretes the enzymes straight into the fermentation medium due to the absence of outer membrane which allows easy recovery of purified proteins from the medium into their active form. It has the capacity to secrete about 20–25 g/L of enzymes into the medium. Enzymes produced by B. subtilis has a wide variety of applications ranging from pharmaceutical, leather, detergent, food, and waste management industries [32].

Aside from enzyme production, cytokines like hIL-3 have been produced by B. subtilis and B. licheniformis. The production of hIL-3 has been tested in various host organisms, including E. coli. However, the production of IL-3 within other organisms has often exhibited problems, such as insolubility or the degradation of produced hIL-3. This led to the use of B. licheniformis and B. subtilis to minimize such complications. The production of hIL-3 in B. licheniformis was engineered to lack four C-terminal residues, resulting in a fully active hIL-3 protein. However, residual proteolytic degradation of some hIL-3 still occurred, leading to use B. subtilis to achieve complete folding and full biological activity of hIL-3. Among the Bacillus species, Bacillus thuringiensis is best known for being widely used within the agricultural industry due to its insecticidal properties through its production of parasporal crystals during the stationary phase of its growth cycle. Upon ingestion, the parasporal crystals are solubilized in the midgut of insects, resulting in the release of protoxin proteins known as δ-endotoxins, leading to the formation of pores throughout the cell membrane. Parasporal proteins also have selective cytotoxicity against liver and colon cancer cells while leaving normal cells unharmed [33].

However, the use of Bacillus has been restricted to mainly enzyme production and non-recombinant protein therapeutics, which may be due to the lack of associated expression vectors, plasmid instability and the presence of native proteases. Despite B. subtilis success as the industrial workhorse, it has its drawbacks in the production of heterologous proteins. Heterologous protein yield could diminish when using the Bacillus as a host due to the proteolytic destruction of foreign protein by host secreted extracellular proteases. Efforts have been made to improve the production of heterologous protein by manipulating the expression of proteins

involved in the post translocation phase resulting in amplified levels of heterologous protein secretion. In contrast to E. coli, the absence of distinguished and controllable promoters in B. subtilis interferes with successful expression of heterologous genes resulting in inefficient production of heterologous proteins [34].

Yeast/Fungi

Saccharomyces cerevisiae

As with E. coli, S. cerevisiae has been extensively used for the production of recombinant human insulin since the early 1980s, and it currently produces half of the world's supply of insulin. S. cerevisiae is preferred because it is also cost-effective, fast growing, technically practical, and is amenable to large-scale fermentation in bioreactors. Yeast is often utilized as a cell factory when the target protein is not produced in a soluble form in prokaryotic systems or when a specific PTM cannot be produced or added to the naked product. S. cerevisiae, as with other yeast species, can perform many PTMs such as O-linked glycosylation, phosphorylation, acetylation, and acylation, which allows recombinant proteins to be expressed in a soluble, correctly folded, and functionally active form. Some examples of currently approved protein therapeutics derived from yeast include human serum albumin, insulin, and primary immunization for infants born of HBV surface antigen, all which are obtained in S. cerevisiae. However, the significant drawback to producing protein therapeutics from S. cerevisiae is that higher eukaryotes have a different pattern of N-linked glycosylation, which can decrease the half-life and cause hyper-immunogenicity, leading to less effective therapeutics. In recent years, there have been some advances in limiting S. cerevisiae hypermannosylation. These yeast glycoengineering techniques involve two main stages, (1) the removal of yeast hypermannosylation and (2) the conversion to complex glycoforms containing terminal sugars, such as N-acetylglucosamine, galactose, or sialic acid. These recent reports on yeast N-glycan humanization indicate a move from the proof of concept phase to implementation [35].

Another current area of study is the production of plant and microbe-derived secondary metabolites. Due to the structural complexity of secondary metabolites, chemical synthesis is an inefficient route for large-scale production, and fermentation is the main mode for economic commercial production of pharmaceutically useful natural products. S. cerevisiae could be an ideal candidate as a microbial host as it boasts relatively rapid growth, and it is accompanied by highly developed genetic tools and advanced fermentation science. Like E. coli, S. cerevisiae has been shown to be an outstanding production host for artemisinic acid, a precursor to the antimalarial agent artemisinin, with a high productivity (25 g/L) that led to the industrial production of semi-synthetic artemisinin beginning in 2013. Research has also produced the paclitaxel precursor taxadiene (73 mg/L) by engineering the taxol biosynthetic genes in S. cerevisiae. Besides plant secondary metabolites, S. cerevisiae has generated a remarkable titer (1.7 g/L) of microbial polyketide 6-methylsalicylic acid in un-optimized shake-flask fermentations. In addition, S. cerevisiae has been developed as a heterologous host to express fungal cryptic BGCs and their respective metabolites. In this study, 30 ADH2-like promoters in Saccharomyces species were developed as

tools for expression of 41 heterologous BGCs, 22 of which were capable of producing heterologous compounds, including novel compounds. For example, BGCs derived from basidiomycete were found to produce N-, S-bis-acylated amino acids and a leucine O-methyl ester with an additional polyketide chain amidated to the amino ester. However, barriers still exist to the heterologous production of complex molecules. This includes the production of polyketides in S. cerevisiae, as the host lacks some required polyketide precursor pathways, its codon usage is biased toward A T and it lacks the appropriate endogenous phosphopantetheinyl transferase capable of the necessary PTMs [36].

Aspergillus Species

Multicellular filamentous fungi, such as A. niger and Aspergillus oryzae, can also offer great potential in the production of a desired substance by fermentation due to the following reasons: (1) they are well-characterized GRAS organisms, (2) are amenable to scaled-up fermentation, (3) can be genetically engineered, (4) they are capable of secreting a high level of proteins and (5) can withstand adjustable cultivation conditions, including temperature (5–45 ◦), pH (2–11), salinity (as much as 34%), water activity (0.92–0.98), and both nutrient rich and poor environments. Aspergillus niger has been predominantly used for industrial- level production of citric acid through anaerobic fermentation process. As a weak acid, citric acid can serve as a natural preservative, flavoring agent in food and beverages, antioxidant, acidulant, pH-regulator, chelating agent or vegetable rinse, as well as comparable applications in the pharmaceutical and cosmetics industries. Due to its wide variety applications, its ease of production, and economical potential of citric acid, the market of citric acid has become one of the fastest- growing regions of the food additives market due to the rising demand: according to estimations, in 2007, the market value of citric acid exceeded $2 billion in 2014 and is predicted to rise to $3.6 billion by 2020. Phytase is another example that have produced by A. niger fermentation. The significance of phytase enzymes lie in its ability to interact with the nutrient rich compounds known as phytate. Phytate, or phytic acid, is a common energy source found in oilseeds, cereals, and legumes, which are used in providing nutrition to animal feeds. Combining citric acid with phytase has also been shown to enhance phytase activity on phytate, producing greater nutrient outcomes in tested animal [37].

Hansenula polymorpha

Another industrially important yeast species that has shown promise in the production of peptides is Hansenula polymorpha. H. polymorpha is a methylotrophic yeast species with the ability to use and grow on methanol, glucose, or glycerol as its primary carbon source. Like S. cerevisiae and Aspergillus species, H. polymorpha, classified as GRAS organism, does not harbor pyrogens, toxins, pathogens, or viral inclusions. It is distinguished by very high cell densities in bioreactors and characterized by simple cultivation mode in inexpensive growth media. For example, H. polymorpha has allowed for cost-effective production of phytase through cheap carbon sources. It possesses well-established genetic tools such as strong regulatory and constitutive promoters, which consequently give high product yield. It also has thermotolerance properties, making H. polymorpha successful in crystallographic studies and in the

production of recombinant proteins like IFN-2α, IL-6, recombinant human serum albumin, glucose oxidase, and catalase. A notable feature of H. polymorpha is the significant growth of peroxisomes when grown on methanol which allows for high storage capacity of soluble proteins. The lack of protein modifying enzymes in the matrix of peroxisomes also provides an advantage for the development of heterologous proteins that are susceptible to proteolytic degradation. Furthermore, the host has been used to produce L antigens found on the HBV viral envelope in attempt to produce the HBV vaccine. The L protein produced by H. polymorpha has increased stability in comparison to other yeast species, such as S. cerevisiae and P. pastoris. In addition to its use in vaccine production, H. polymorpha is also used in the production of human hemoglobin through the use of a single expression vector. However, hyperglycosylation has been observed as a main drawback of H. polymorpha to produce heterologous products [38].

EFFORTS IN PRODUCT IMPROVEMENTS AND GENERATION OF NEW ANALOGS

There are multiple approaches that have been taken to advance product improvement for microbial natural products and biologics. This section will discuss efforts to combat the challenges of production of natural products and its analogs, including strain improvement, increasing precursor supply, pathway engineering, combinatorial biosynthesis, and genome mining.

Strain Improvement

Whole-genome shuffling is a process that utilizes the advantages of the multi-parental crossing allowed by DNA shuffling with the genome recombination normally associated with conventional breeding. Genome shuffling has been successfully improved the titers of variety of microorganisms.

For example, two strains of Streptomyces fradiae generated from two rounds of genome shuffling were able to produce up to a ninefold increase in antibacterial tylosin production in comparison to the initial strain. Using genome shuffling in a combination of protoplast fusion, mutant strain of S. cellulosum GSUV3−205 generated a 130- fold increase (104 mg/L) in production of epothilone when compared to starting strain S. cellulosum So0157-2 (0.8 mg/L). Ribosome engineering is also a method useful in increasing secondary metabolite production titer and productivity. Studies demonstrate that rpoB mutations are effective in activating silent and poorly expressed secondary metabolite biosynthetic gene clusters (BGCs) at the transcriptional level in S. griseus, S. coelicolor, and S. erythraea. For example, the H437R mutant of rpoB from S. erythraea was screened for drug resistance and was found to have an increased production of erythromycin. Another study found a 37-fold increased production of avilamycin in a recombinant Streptomyces viridochromogenes strain due to a mutation in ribosome protein S12 (rps12) acquired through a combination of gene shuffling and ribosome engineering [39].

Engineering Precursor Supply

Precursor supply is defined as the enhancement of the availability of primary metabolites or molecules derived from primary metabolism involved in the biosynthesis of natural products. Precursor supply engineering can be achieved by manipulating either the pathways or enzymes involved with the precursor supply. Malonyl-

CoA and methylmalonyl- CoA are the most commonly used and metabolically available precursors for the biosynthesis of polyketides. One study found that supplying methyl oleate enhanced the internal concentration of methylmalonyl-CoA, which is a biosynthetic precursor for FK506, and led to a 2.5-fold increase in FK506 production in Streptomyces clavuligerus CKD1119. In another study, propionyl-CoA carboxylase with supplementation of propionate was found to effectively increase methylmalonyl-CoA and rapamycin titers in the mutant strain S. rapamycinicus UV2−2 induced by ultraviolet mutagenesis in comparison to wild-type strain (78 mg/L). The mutant strain was found to have a 3.2-fold improvement (23.6 mg/L) in comparison to wild-type strain S. rapamycinicus ATCC 29253 (78 mg/L). Further, Méndez and coworkers improved precursor metabolite pools for the production of the antitumor polyketide mithramycin in Streptomyces argillaceus by increasing the precursor supply of malonyl-CoA and glucose-1-phosphate. Several classes of natural products utilize aromatic amino acids or other metabolites derived from the shikimate pathway as precursors, including flavonoids, alkaloids, polyketides, and non-ribosomal peptides (Knaggs, 2001). The production of the vancomycin analog balhimycin was increased 2.5-fold in Amycolatopsis sp. Y-89,21022. This was achieved by increasing the non-ribosomal peptide precursor 3-deoxy- D-arabino-heptulosonate7-phosphate synthase, the first enzyme in the shikimate pathway. In addition, manipulating key enzymes that direct carbon flux through core biochemical pathways involved in glucose, fatty acid, and amino acid metabolism can increase biosynthetic precursor pools. A study on the modulation of carbon flux between the pentose phosphate pathway and the glycolysis pathway found that a deletion of phosphofructokinase isoenzymes led to the enhanced production of antibiotics actinorhodin and undecylprodigiosin in S. coelicolor by increasing carbon flux through the pentose phosphate pathway [40].

FUTURE PROSPECTS

In recent years, there has been a notable surge in the introduction of natural products and compounds derived from natural sources into various industries. This trend is particularly evident in the realm of pharmaceuticals and drug discovery. Since the year 2000, an astonishing 77% of FDA-approved antibiotics have been identified as natural products, all of which trace their origins to microbial sources. Numerous comprehensive reviews have been conducted, shedding light on natural products, semi-synthetic derivatives, and nature-inspired molecules that have received FDA approval. These analyses underscore the continued significance of natural products in the field of medicine and healthcare. Within the global biologics market, microbial biologics are poised to maintain a prominent position. The biologics market was valued at 277 billion USD in 2015 and is projected to reach a substantial 400 billion USD by 2025. While many biological activities of microbial natural products and biologics are well-documented, new advances and insights continually surface. The rich chemical diversity derived from microbial natural products remains a valuable resource for the discovery of novel drugs with a wide range of pharmacological activities, including antibiotics, anticancer agents, and immunosuppressants.

However, amidst this exciting landscape, numerous challenges persist in the

production of microbial natural products and biologics. Challenges include achieving higher production titers, overcoming obstacles in product isolation and structural identification, and improving the expression of recombinant proteins in microbial systems. The accumulation of end products within microbial cells can trigger global stress responses, inhibiting cell growth. Misfolded and biologically inactive proteins can also diminish the yield of recombinant proteins, particularly in the case of membrane proteins, high-molecular-weight proteins, and multi-domain proteins. Moreover, the expression of eukaryotic proteins within prokaryotic systems can lead to products that lack the appropriate post-translational modifications necessary for functionality. Nevertheless, a wide array of engineering strategies can be employed alongside conventional recombinant DNA technologies, encompassing genome editing, ribosome engineering, precursor engineering, mutagenesis, and the overexpression of structural genes. These strategies hold the potential to facilitate efficient production in microbial systems. Cutting-edge technologies such as CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein) should also be embraced as genome editing tools to further enhance production.

Genome mining, another promising avenue, involves extracting valuable information from genome sequencing data to uncover silent cryptic biosynthetic gene clusters (BGCs). Genome mining has opened exciting prospects for the discovery of novel molecules, ultimately contributing to the expansion of the natural product repertoire. An exemplary case is the use of genome mining in conjunction with bioinformatics predictions to isolate the novel natural product orfamide A from Pseudomonas fluorescens. In addition, innovative approaches, such as gene shuffling and ribosome engineering, can be implemented to increase the production of secondary metabolites. By integrating 'omics' data, particularly metabolomics, biochemical changes and metabolic pathways can be accurately quantified, offering valuable insights into natural product drug discovery. Advances in metagenomics have furthered our understanding of complex microbial sources, including those from diverse environments such as lakes, rivers, marine ecosystems, and extreme conditions like sub-seafloor sites and ice cores. Structural characterization is vital in the field of microbial natural products. Advanced techniques such as X-ray crystallography and cryo-electron microscopy allow for the precise determination of molecular structures. Cryo-electron microscopy, for instance, offers a high-resolution method to visualize macromolecular structures. Computational strategies have evolved to identify BGCs in genome sequences and predict chemical structures of natural product compounds. To maximize the potential of genome mining, a focus on sequencing campaigns likely to yield novel natural products, combined with the exploration of well-characterized microbial clades like actinomycetes, is recommended. Advancements in computational strategies, including the mining of vast metagenomic data, will be essential for the future of genome mining.

In conclusion, microbial natural products and biologics will continue to play a pivotal role in various facets of human life. The expansion of recombinant drugs is on the horizon, thanks to emerging protein production platforms and efforts aimed at product enhancement.

Microbial cells will remain versatile and cost-effective factories for the production of proteins due to their adaptability. A combination of engineering strategies, recombinant DNA technologies, and genome editing tools will be essential to address the challenges currently faced. The quest for natural product analogs promises novel compounds with enhanced biological activities compared to their natural counterparts. Advanced technologies and computational approaches will further revolutionize the field of microbial natural products, which remains a valuable resource for innovative compounds in drug discovery.

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