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Microbial Pharmaceutical Industry: Current Status and Future Trends

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Abstract: Natural microbial products possess diverse structures and remarkable activities, which contribute to their great potential for clinical application. In this study, the current status of the microbial pharmaceutical industry in China was systematically analyzed, and the development trends of the industry in terms of microbial strain resource utilization, excellent strain screening, fermentation process optimization, strain engineering, and new microbial medicine development were summarized. Overall, this study aimed to facilitate major breakthroughs and industrial upgrades in China's microbial pharmaceutical industry. Although China's microbial medicine industry has solid resources and technical foundations, its advances are far less than those of other countries. Accordingly, we offered the following suggestions for promoting China's microbial medicine industry: constructing large-scale scientific facilities for microbial medicine, strengthening basic research and independent technology development, establishing a talent cultivation system, and formulating systematic industrial incentives.
Keywords: microbial medicine; development trend; biological activity; microbial metabolism; synthetic biology

1 Introduction

Microorganisms are the world's largest species and genetic resource banks. Secondary metabolites, such as physiologically active substances and their derivatives, are produced by microorganisms during living activities. For thousands of years, humans have been using microorganisms and their metabolites to treat diseases. The discovery of penicillin in 1929 was a historical breakthrough in microbial drug discovery, and its commercialization paved the way for the golden age of natural product discovery and markedly changed the research direction related to natural products. Owing to the extremely high biodiversity, unique structure and variability, and usability of microorganisms, the number of commercial drugs derived from microorganisms remarkably exceeds that obtained from plants and other sources [1,2]. Nearly half of the best-selling drugs are natural products or their derivatives, highlighting the importance of microbial pharmaceuticals in disease treatment and drug development [3].

Owing to the worsening issue of drug resistance in pathogens, emergence of new diseases, energy saving and emission reduction, and high yield requirements in recent years, new drugs, mechanisms, strains, and processes are urgently required for the innovative and efficient manufacturing of drugs. Since the 1990s, the rapid development of synthetic biology and omics has led to a new stage in microbial pharmaceuticals.

From the microbial pharmaceutical industrial chain, genetic engineering and omics techniques have resulted in the systematic modification of microbial species, and bioinformatics and synthetic biology have contributed to the discovery of new drugs and the efficient manufacturing of complex drugs. Collectively, these techniques and processes have aided in source innovation in the industry. The improved production capacity of downstream processes in microbial pharmaceuticals is due to advances in fermentation processes and industrial applications.

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Currently, China has a complete microbial pharmaceutical industrial chain and large-scale industrial groups and bases. In this study, we aimed to compile and analyze the current status of the microbial pharmaceutical industry in China and the development trends in related techniques, and propose suggestions on the basis of consolidating scientific equipment, enabling a rational layout of cutting-edge science, strengthening of talent cultivation and reserves, and optimizing capital assurance and policy incentives to aid in the future development and layout of the microbial pharmaceutical industry in China.

2 Current status of the microbial pharmaceutical industry

2.1 Developmental history of the microbial pharmaceutical industry in China

China is a major producer of microbial pharmaceuticals and products, mainly antibiotics and active pharmaceutical ingredients (API). Overall, this industry is at the lower end of the global value chain. Accordingly, attempts are being made to boost China's industry to match that of other countries. During the Eleventh Five-Year Plan period (2006–2010), China revealed its focus on improving its bioindustry to the level of industrial nation building. The National Development and Reform Commission and the Ministry of Science and Technology successively approved the construction of nearly 40 national bioindustry bases and China Torch Program bioindustry bases, which led the entrance of the microbial pharmaceutical industry into the accelerated development phase. Owing to continuous policy support and guidance, biomedical industries have formed a spatial layout of development cores in the Yangtze River Delta and Bohai Bay, and rudimentary aggregation in the Pearl River Delta and Northeast China [4]. During the Twelfth Five-Year Plan period (2011–2015), China's bioindustry was confirmed as the third major strategic emerging industry in the country. Various provinces have launched related policies to support its rapid development, including *Several Policies for Promoting the Development of the Biomedical Industry in Shanghai* and *Interim Measures for Managing Funds for Developing Bioindustry in Guangzhou*. Following the expiration of many drug patents in China, policy dividends continued to manifest. Remarkable progress has been made in China's microbial pharmaceutical industry, in which three major biomedical clusters, namely Bohai Bay, Yangtze River Delta, and Guangdong–Hong Kong–Macau Greater Bay Area, were formed. Among these regions, the Yangtze River Delta has outstanding basic research and industrial technical innovation capabilities, high international exchange, and the largest number of multinational biomedical companies. Bohai Bay has the most abundant educational and clinical resources, sufficient industry manpower resources, and strong industrial chain strengths. The Guangdong–Hong Kong–Macau Greater Bay Area has a mature market system, a well-developed circulation system, connectivity between the three regions, and strong external radiation capability. Notably, the Henan and Hubei provinces in Northeast and Central China and Sichuan province in Western China have created unique and rapidly developing industrial structures under the leadership of several major companies [5].

2.2 Anti-infectious drugs account for the bulk of sales in the Chinese market, with stable and continuous growth

Based on the Pharmaceutical Database, China's total antibiotic output ranks first at the global level, and China possesses remarkable strengths in the API production of penicillin, streptomycin, and tetracycline. China's capabilities in the development of new drugs have continuously improved and shattered the technical and market monopolies for numerous products in Europe and the United States. Industrialization has been achieved for more than 100 drugs, resulting in large-scale industrial groups and a complete industrial chain. After the industry reached a nadir around 2000, a highly rigid demand for antibiotics ensued as they are key products in the drug market. The overlap between the Chinese and overseas markets continues to drive large-scale and continuous growth in the antibiotics market.

2.2.1 β-lactam antibiotics

β-lactam antibiotics account for the largest proportion of the antibiotic market, and include both APIs and intermediate products. Cephalosporins and penicillin account for 25% and 20% of the global antibiotic market, respectively. During the 1980s, China's penicillin industry underwent rapid development, but later reached a nadir owing to excess production capacity. In the last decade, cephalosporins have become the main anti-infectious drugs, and have thus undergone rapid development. New products, such as cefamandole and cefuroxime sodium, have successively emerged, and more than 30 cephalosporin drugs are commonly used in clinical practice. The core intermediates in penicillin and amoxicillin production, 7-aminocephalosporanic acid (7-ACA), 6-aminopenicillanic acid (6-APA), and 7-aminodesacetoxycephalosporanic acid (7-ADCA), are major players in the international

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antibiotic market. Overall, there has been a remarkable excess production capacity, gradual increase in the industrial barrier, clearing of production capacity through “antibiotic restriction” and “discharge restriction” policies, and identification of prominent scale strengths of major companies, resulting in downstream industrial development. Major manufacturers include the Joincare Pharmaceutical Group Industry, the CSPC Pharmaceutical Group, and United Laboratories International.

2.2.2 Aminoglycosides

Aminoglycosides were the earliest commercialized antibiotics and are the preferred drugs for the treatment of severe gram-negative infections. Therefore, aminoglycosides have an irreplaceable role in clinical practice. Streptomycin, which can inhibit *Mycobacterium tuberculosi*s proliferation, is an example of an aminoglycoside and has been the best-selling product on the international market since its release in the 1950s. Streptomycin and its derivatives, gentamicin, neomycin, ribostamycin, kanamycin, netilmicin, sisomicin, paromomycin, as well as micronomicin and etimicin which were independently developed in China, are among the last 20 successive commercialized aminoglycosides. Accordingly, aminoglycosides are among the most widely used antibiotic classes. However, irreversible ototoxicity and drug resistance have severely limited their use and promotion. To further expand the clinical application of aminoglycosides, the screening and identification of anti-drug resistant and low-toxicity derivatives have become an important area in aminoglycoside development [6]. In China, the major companies involved in aminoglycoside production are Yichang Sanxia Pharmaceutical, Yantai Justaware Pharmaceutical, North China Pharmaceutical Group, and Sichuan Long March Pharmaceutical.

2.2.3 Macrolides

Currently, there are three generations of macrolides. First-generation macrolides include erythromycin and its derivatives; second-generation macrolides include azithromycin, clarithromycin, and roxithromycin, which have a broad application range, but gradually lead to drug resistance; and third-generation macrolides include telithromycin and cethromycin, which have higher toxicity. China is the world’s largest API producer and exporter of semisynthetic erythromycin. Owing to a lack of new product support and the continuous impact of other antibacterial drugs, the international demand for macrolides has become almost saturated in recent years. However, macrolides remain important antibacterial treatments for chronic respiratory diseases [7].

2.2.4 Tetracyclines

Tetracyclines are broad-spectrum antibiotics that comprise a phenanthrene core and are widely used to treat bacterial, mycoplasmal, chlamydial, and rickettsial infections [8]. China is the world’s largest producer and exporter of tetracyclines. According to the Wind database, the annual supply of tetracycline derivatives and their salts in China exceeds the domestic demand, and 60% is exported to other countries. With the continuous emergence of drug-resistant bacteria, the adverse reactions of tetracycline, and the development of new antibiotics, the use of tetracycline has dwindled in clinical practice for some time. Although Europe and the United States have a high demand for tetracyclines from China, tetracyclines exist at the lower end of the industrial chain and are largely used as growth promoters for livestock. From 2001 to 2003, the European Union issued a ban on the use of antibiotics as feed additives, which had a remarkable impact on exports by Chinese companies. In 2005, Pfizer developed the third generation of tetracyclines, tigecycline, and eravacycline, which are among the best-selling drugs and have led to an increase in the export of tetracycline and its APIs from China. After several changes, 10 000-ton of China’s tetracycline fermentation bases were mainly located in western China, with representative companies such as Ningxia Qiyuan Pharmaceutical, North China Pharmaceutical Group, and Sichuan Pharmaceutical. The previously prosperous tetracycline production lines in Jiangsu and Zhejiang provinces have switched to the production of drugs with higher economic value.

2.2.5 Lincosamides

At present, only few lincosamide antibiotics, including lincomycin, celesticetin, clindamycin, and other semi-synthetic antibiotics, are available. In the 1980s, China began large-scale production of lincomycin, with markedly improved yields owing to many years of selective breeding and optimization. Currently, the yield exceeds 7 g/L [9] and the market demand remains at a high level. Moreover, recent rapid developments have occurred, with an annual growth rate of 10% and continuous price increases. In China, the major companies involved in lincomycin production are Nanyang Pukang Pharmaceutical Industry, which is a global leader; Henan Topfond Pharmaceutical; and Zhejiang Hisoar Pharmaceutical (acquired). Currently, the production level of lincomycin in China and other countries is approximately 6500–7500 U/mL and 10 000 U/mL, respectively, indicating the huge gap in production

level. Urgent problems, such as improving yield, must be solved to improve the competitiveness of lincomycin on the international market [10].

2.3 Stable growth in the antineoplastic drug market and emergence of new products

Tumors are a major cause of death worldwide. The identification of effective antineoplastic agents is a global research topic. In 2018, the global antineoplastic drug market was worth 152 billion USD, and the compound growth rate in the last five years was 7.96%. Although the growth rate was slower than that in the preceding decade (15%), the rate was still significantly higher than the mean growth rate of the global drug market. Imported drugs account for nearly half of the antineoplastic drug market in China. Implementation of zero taxation for antineoplastic drugs, accelerated launching of generic drugs by Chinese companies, and strengthening of independent research and development (R&D) of new products can help reduce the treatment-related costs of cancer patients and their family members, and alleviate pain points in the public.

The reduction in cytotoxicity remains an important route for discovering modern antineoplastic drugs for the field of oncology [11]. Many important commercialized new drugs are obtained from natural sources, the structural modification of natural compounds, or the synthesis of compounds via artificial design using natural compounds as a model. Of the nearly 200 discovered small-molecule drugs, approximately one-third are antineoplastic antibiotics. These drugs are directly obtained from natural products or their derivatives and include polysaccharides, anthracyclines, organic esters, terpenoids, alkaloids, macrolides, and enediyne drugs [2]. Most of these drugs play an important role in clinical practice for the treatment of oncologic diseases and account for a large proportion of the antineoplastic drug market. However, with the successive marketing of new antineoplastic agents in recent years, the market growth rate for antineoplastic antibiotics has decreased annually, and the market share has markedly reduced. The major antineoplastic antibiotics in China include non-ribosomal peptides, aromatic polyketides, and heterologously expressed alkaloids, such as actinomycin D, bleomycin (BLM), doxorubicin (ADM), pirarubicin (THP), and epirubicin (E-ADM). The leading companies in the production of antineoplastic antibiotics in China include Jiangsu Hengrui Medicine, Luye Pharma Group, and Zhejiang Hisun Pharmaceutical.

2.4 Synchronous and continuous growth in enzyme inhibitor demand and market scale

Enzymes are a class of important drug effector targets. In the early 1960s, Hamao Umezawa proposed the concept of enzyme inhibitors and posited that enzymes and their inhibitors co-exist in microorganisms, ultimately expanding the thought process of antibiotic screening, guiding the construction of many screening models and methods, and initiating a new discovery era for other physiologically active substances in microbial metabolites [12]. The marketed drugs primarily target receptors, enzymes, ion channels, and nucleic acids. Enzyme inhibitors produced by microorganisms originate as primary or secondary metabolites in microorganisms. Actinobacteria, of which *Streptomyces* is the most important genus, are the most widely studied microorganisms and are used to produce the most microbial pharmaceuticals. Bacteria and fungi are important sources of inhibitors of enzymes. In addition to the screening and isolation of traditional microbial sources of drugs, researchers have gradually focused on marine microorganisms, extremophiles, and other new microorganisms [13]. Of note, China's research on enzyme inhibitors started later than other countries, and the companies mainly produce generic drugs, such as acarbose, an α-glucosidase inhibitor produced by Huadong Medicine Co., Ltd.

2.5 Stable growth in three major immunosuppressant classes

Immunosuppressants are drugs that inhibit immune responses in the body. Immunosuppressants are mainly used to prevent rejection reactions in organ transplantation cases and inhibit the progression of certain autoimmune diseases. Immunosuppressant drugs produced via microbial fermentation mainly include cyclosporine CsA, tacrolimus, sirolimus, and its derivative, SDZ-RAD. More than 10 drugs are available on China's immunosuppressant market, of which tacrolimus, mycophenolate mofetil, and cyclosporine are the major drugs. These three immunosuppressants have had continuous and stable growth in the last five years based on their total amount, with a composite growth rate of 7.95%. However, there is still room for improvement in their market capacity. When drugs are further divided, the current market for the top three drugs illustrates the competition layout of "brand name drug manufacturer + 1–2 local manufacturers." Huadong Medicine Co., Ltd. occupies an absolutely dominant position among local manufacturers and accounts for 27% of the immunosuppressants available on the Chinese market. Huadong Medicine Co., Ltd., Novartis, Roche, and Astellas Pharma account for more than 80% of the total local market share of immunosuppressants.

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3 Technical development trends of microbial pharmaceuticals

3.1 New fermentation process increases microbial drug production capacity

The synthesis or industrialization of microbial pharmaceuticals with complex structures has often been difficult; however, the use of microbial cell factory fermentation and large-scale synthesis of target products has served as an efficient and economical method. Fermentation is an integral step in microbial pharmaceutical production.

3.1.1 Immobilized cell technology

In the routine liquid-state fermentation process, microbial cells synthesize the enzymes required for antibiotic synthesis in the "non-synthesis growth phase" and generate an antibiotic "production line." Subsequently, a large amount of antibiotics is precisely regulated based on the sequentiality of the enzyme catalysis. During fermentation, rapid degeneration of microorganisms is a frequent problem.

Based on enzyme immobilization, immobilized cell fermentation technology has been developed to overcome unfavorable factors. Thus, physical and chemical factors are used to immobilize cells within a fixed spatial boundary, enabling cells to retain their catalytic activity during repeated or continuous use for long periods. This technology has remarkable application potential in antibiotic production and is especially well suited for the production of antibiotics secreted into culture medium. Compared with conventional liquid-state fermentation, immobilized cell technology has many advantages, such as continuous flow fermentation in column bioreactors, increased cell density by immobilization materials, density control of microorganisms in the non-synthesis growth phase, and the requirement for additional enzyme reaction cofactors, thereby decreasing costs and improving yield [14–16].

Immobilized cell technology has been successfully used to produce microbial pharmaceuticals, such as penicillin G, cephalosporin C, ampicillin, bacitracin, cephamycins, candicidin, and daptomycin [17]. For example, nisin, an economically significant polypeptide antibiotic produced by *Lactococcus lactis*, inhibits gram-positive bacteria and is widely used as a food additive. Corn syrup and yeast extract were used as the culture media for conventional liquid-state fermentation. In the late fermentation phase, the culture medium becomes acidic and the efflux of nisin from cells is significantly inhibited, resulting in almost stagnant synthesis. When polyacrylamide, agar, gel, and polysaccharides are used to immobilize *Lactococcus lactis* cells, continuous-flow fermentation is achieved to prevent the acidic environment from inhibiting the bacteria, thereby greatly extending the half-life of cells, enabling single-cell viability, and significantly increasing the yield.

Immobilized cell technology is promising; however, there are several bottlenecks that require improvement, including the substance transfer rate during fermentation, immobilization method, carrier optimization, and product extraction efficiency. Despite many successful applications, only few details of the entire technology have been released. Moreover, the related production processes are firmly controlled by few well-known foreign companies and are difficult to replicate. Therefore, basic research must be strengthened, and advanced immobilized cell technology processes should be independently developed to overcome these technical barriers and increase microbial pharmaceutical production in China to levels that match those of other countries.

3.1.2 New continuous fermentation process

Batch fermentation is used for most modern fermentation products. During batch fermentation, the growth rate of microorganisms changes over time. As the fermentation duration increases, nutrients are continuously depleted, and strain aging and metabolite inhibition occur. Continuous fermentation refers to the use of an open system to continuously provide fresh culture medium to the fermentation tank at a fixed rate, while ensuring the simultaneous release of the microorganism- and metabolite-containing culture medium from the fermentation tank at the same rate, a constant fermentation tank volume, and steady state growth and metabolism in the culture.

Quantitative metabolomics introduced a quantitative description based on the mathematical model of the living system to describe complex dynamic behavior, which helped achieve system prediction, design, and optimization. When the fermentation process is continuous, various parameters can be maintained at specific levels and automatically within a low matrix concentration, increasing the equipment utilization rate and yield per unit of time, which leads to achievements in digital and efficient control and optimization of the industrial fermentation process [18]. The multidimensional optimization theory has been successfully used in industrial bioprocess optimization and the scaling-up of penicillin, erythromycin, chlortetracycline, recombinant human serum protein, and malaria vaccines. By using glucose as a limiting carbon source in a chemostatic culture process, Douma et al. found that the penicillin synthesis capacity and isopenicillin N synthase activity had a good linear relationship. This relationship was used to construct a gene expression regulation model to successfully describe the dynamic relationship between

penicillin synthesis and growth rate during fed-batch fermentation. However, intracellular amino acids, phosphate sugars, sugar alcohols, and energy metabolism were not included in the dynamic information, which served as a limitation of their study [19].

During fermentation, changes in metabolite concentrations occur within seconds to several dozen seconds and require rapid sampling and efficient and reliable analysis to obtain a true and reliable metabolite concentration at a given time point. Metabolomics can accurately provide feedback information on a biological system when it responds to a gene or environmental perturbation. Therefore, the development of tandem rapid sampling and metabolite testing equipment in the fermentation process can reduce or prevent changes during sample processing and rapidly collect the filtrate or wash the filter cake instead of the use of traditional centrifugation and isolation; this can decrease and prevent the leakage of intracellular metabolites and effectively decrease yield loss [20,21].

3.2 Systematic microbial modification using genetic engineering and omics techniques

Comprehensive multi-omics applications through modern genetic engineering and genomics have markedly promoted the discovery and research of microbial pharmaceuticals and their lead compounds, and are expected to significantly decrease drug discovery time and early stage development time, and decrease drug R&D and production costs. Regulating the metabolic pathways of natural products in microbial "cell factories" can not only be used to synthesize new complex compounds, but also produce active compounds from plants or other sources. The Chinese Academy of Medical Sciences and the Shenyang Tonglian Group collaborated to integrate a heterologous acylase gene into spiramycin-producing bacteria to jointly develop a new drug, carrimycin. In June 2019, carrimycin was approved for marketing and is the only "heterozygous antibiotic" obtained through industrialized use of genetic engineering.

Machine learning has improved the performance of high-throughput microbiology. In 2020, the synthetic biology startup, Zymergen, announced a high-throughput (HTP) microbial genome engineering platform driven by computer software algorithms that integrates molecular biology, automation, and advanced machine learning. Numerous strains with different genomes were constructed within a short period, and a large volume of generated data was tested. Thereafter, automatic machine learning was performed on the aforementioned data, and iterative learning was used to analyze the data to construct a complete HTP inheritance design. This platform is compatible with any host and is therefore suitable for adjusting and improving any microbial host performance.

Fermentation yield is an important challenge in the R&D of microbial pharmaceuticals. Conventional selective breeding of industrial high-yielding strains employs repeated mutations and screening to gradually improve their fermentation performance. Microbial genome shuffling adds mutations to the genome, screens and accumulates beneficial mutation combinations to achieve directed evolution, and accelerates the process of typical strain improvement. The improvement of microbial strains through genetic engineering can enhance precursor and cofactor supply, eliminate competing pathways, and increase product efflux and self-resistance. Metabolomics can be used to detect intermediate product accumulation and substrate supply, and analyze the genome, proteome, and whole-genome transposition mutagenesis to identify target genes for strain improvement. With advances in synthetic biology, the design of artificial biosensors for metabolite/intermediate products and auto-regulation systems can provide new methods and techniques for selective breeding to achieve high yields [22]. However, intracellular metabolism networks are complex, and artificial modification tends to have adverse or unknown effects on the system, which may lead to decreased cellular activity or loss of cellular activity. Therefore, further in-depth research is required to determine metabolic network awareness.

3.3 Biological response prediction and design for new structure mining

The prediction of biological response using bioinformatics can be used for high-throughput, efficient mining of metabolites with novel structures. The high natural product synthesis capacity of Actinobacteria is encoded by its gene cluster. The first whole-genome sequencing of *Streptomyces coelicolor* in the Actinobacteria phylum revealed that this bacterium contains 20 potential secondary metabolite biosynthesis gene clusters. More than 30 gene clusters were found in *Streptomyces avermitilis*, *Streptomyces griseochromogenes*, and *Streptomyces cattleya* [23]. Kelleher et al. developed a gene cluster family classification method in which known molecules and potential biosynthetic gene clusters are linked. A large amount of bioinformatics data has been used to discover new natural products [24]. In fact, a large volume of bioinformatic resources on *Streptomyces* secondary metabolites and their syntheses has been obtained. For example, the online analysis tool, antiSMASH, can quickly predict non-ribosomal peptide synthetase (NRPS), polyketide synthase (PKS), polysaccharides, bacteriocins, terpenes, and many typical secondary

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metabolite biosynthesis gene clusters, and has been updated to version 5.0 [25]. The ClusterMine360 database systematically records 200 PKS/NRPS biosynthesis gene clusters and 185 compound families. The DoBISCUIT database focuses on the maintenance of artificial corrections of PKS gene clusters and the BAGEL2 tool can be used to analyze many bacteriocins [26].

3.4 Synthetic biology aids in the microbial synthesis of complex compounds

Synthetic biology integrates enzyme engineering, biological catalysis, structural biology, and many other disciplines to upgrade or redesign biosynthetic pathways, and derive biosynthesis and editing strategies for different scaffolds or structural units. Synthetic biology can also be used for the analysis and design of drug biosynthetic pathways, genetic modification, and optimization of microbial systems, and new content and functions for artificial biological systems to achieve in-depth development and efficient production of drugs.

3.4.1 Activation of silenced gene clusters

The modification of synthetic gene clusters based on sufficient understanding of the biosynthetic gene clusters and their synthetic pathways of drugs is expected to activate many silenced gene clusters with synthesis potential in microorganisms to produce large amounts of biologically active substances with important application prospects. Researchers from Jinan University introduced nine genes from the proposed gene cluster for helvolic acid in *Aspergillus oryzae* NSAR1 and detected helvolic acid in the end product. Biosynthetic pathways have thus been used to expand the structural diversity of fusidane-type antibiotics [27].

3.4.2 Optimization and construction of biological elements

Deletion or substitution of PKS or NRPS domains in microbial cells can change the substrate recognition and catalytic characteristics. Precursor feeding and combinatorial biosynthesis can be used to produce new derivatives and improve the water solubility and therapeutic index of drugs. Kosan Biosciences used PKS module replacement to prepare erythromycin, geldanamycin, and epothilone analogs to obtain new microtubule stabilizers and Hsp90 inhibitors. Daptomycin is a cyclic lipopeptide produced by NRPS in *Streptomyces filamentosus* and has bactericidal effects against methicillin-resistant *Staphylococcus aureus*. Cubist exchanged the NRPS module to construct a biosynthetic pathway to produce nearly 100 novel daptomycin derivatives. The catalytic efficiency of new pathways is often significantly lower than that of wild-type pathways, thereby serving as a bottleneck in combinatorial biosynthesis, and the directed evolution of key enzymes is expected to improve the adaptability and efficiency of the combined pathways [28].

3.4.3 Microbial synthesis of plant-derived or animal-derived drugs

This microbial fermentation platform provides an economical, efficient, and sustainable alternative to plant culture and chemical synthesis for many plant-derived drugs. Recently, several studies employed synthetic biology techniques to express plant-derived drugs and their precursors, such as artemisinin, paclitaxel, tanshinone, β-carotene, and salidroside in microorganisms, providing an effective method and pathway for the production of natural drugs from rare sources [29,30].

The commercialized microbial production of the anti-malarial drug, artemisinin, is a landmark achievement in synthetic biology. In 2003, Keasling et al. reconstructed the synthetic pathway for the artemisinin precursor, artemisinic acid, in *E. coli* [31], and increased the yield of artemisinic acid by 300-fold. Subsequently, an efficient synthetic pathway for artemisinic acid was constructed in *Saccharomyces cerevisiae*, providing a modern industrial pathway for controllable and efficient artemisinin supply. Plant alkaloids, particularly benzylisoquinolines and monoterpene indole alkaloids, have become promising targets for microbial synthesis owing to the value of these drugs. Further, their synthetic pathways have been successfully assembled and expressed in *E. coli* and yeast [32].

Isoquinoline and indole alkaloids have significant antineoplastic activity. To some extent, microbial transformation can be used to simulate the *in vivo* metabolism of these drugs in animals. The first modern marine drug, trabectedin (Et-743), was obtained from the tunicate, *Ecteinascidia turbinate*, and is extremely difficult to obtain naturally. Fermentation of its precursor has been achieved in *Pseudomonas fluorescens*, and semi-synthesis is used to obtain large quantities of this drug [33].

3.5 Precise modification of microbial metabolic pathways by gene editing

In microbial systems, secondary metabolites are produced by the sequential action of enzyme catalysis on different small-molecule precursors and genes participating in synthesis, usually for a cluster consisting of a

metabolic network of scaffold structure genes, modification genes, and regulatory genes. Precise editing of gene clusters can be used to modify the synthetic pathway, precisely regulate expression, decrease byproducts, and increase the yield of target compounds.

Many successful cases of the precise modification of microbial metabolic pathways have been achieved via the modification of structural genes. For example, the structure of the 4' hydroxyl group in the anti-tumor antibiotic, daunorubicin, was modified to obtain the chemotherapeutic drug, doxorubicin, with fewer toxic side effects. A new cyclic peptide antibiotic similar to the anticoagulant, integrin, was synthesized by modifying the amino acid composition of the precursor peptide for the anti-tumor antibiotic, patellamide (from a marine microorganism), and using the original modification enzyme system.

Several studies have enabled researchers to circumvent problems, such as the difficult synthesis or low process efficiency of analogs with complex structures, and fully demonstrate the remarkable potential of rationally designed gene editing in new drug discovery and development. Of note, gene editing techniques, particularly CRISPR/Cas9-related core patents, belong to developed countries in Europe and the United States. Therefore, China must develop new core technologies with independent property rights for the industrialization of microbial pharmaceuticals [34].

3.6 High-throughput screening increases the R&D efficiency of novel drugs

Establishing a high-throughput screening method and technical platform, obtaining target strains with superior performance from a library of tens of thousands of mutant strains, and achieving rapid evolution of microbial pharmaceutical-producing strains are particularly important in the screening process for industrial strains.

3.6.1 Screening for industrial high-yielding strains

Genes in the microbial genome that encode small-molecule natural products (secondary metabolites) are clustered together and are easy to identify. These gene clusters can be used to predict the synthetic diversity and novelty of one or more natural products in microorganisms, and specific biosynthetic enzymes can be used to predict natural products with specific chemical structures for directional mining. Genome mining has become an alternative process for the discovery of natural small-molecule products. New members of the family of natural products, leinamycin, with antineoplastic activity were discovered using this technique.

Heterologous gene cluster expression is another effective platform for discovering natural products. Bacterial artificial chromosomes, Gibson assembly, transformation-associated recombination, or ExoCET direct cloning, as well as other large-fragment gene cluster capture techniques, can be used to clone the entire synthetic pathway to derive a systematically modified host microorganism to achieve high expression. High-throughput heterologous expression can be used to isolate and purify new compounds and aid in late-stage development. An example is the use of the activity-screening-based genome mining library expression analysis system (LEXAS) platform to obtain a novel lanthipeptide [35].

High-throughput screening using biosensors and microbial proteins or ribonucleic acid (RNA) can be employed to specifically recognize and respond to specific intracellular substances and generate a characteristic signal output. The correlation between the output signal intensity and the target product can be used for quantitation of the target product. A microfluidics-based high-throughput screening platform can separate single cells and be used in single-cell culture, protein expression analysis, metabolite detection, and multiomics to achieve high-throughput and precise screening. A nutrient-deficient strain is usually used as a reporter system in microbial growth-based high-throughput screening, and this system can be used to screen for high-yielding strains or specific enzymes for metabolites.

3.6.2 Innovative screening of microbial pharmaceutical sources

The classical R&D process for small-molecule drugs is slow, with major challenges such as low production performance, a high repeated discovery rate, and difficulty in isolating new products. The innovative application of high-throughput technology in microbial pharmaceuticals has markedly improved the efficiency of drug discovery and manufacturing. Highly sensitive isolation technology and high-throughput activity screening models can be used to identify antibiotics, enzyme inhibitors, immunomodulators, and many candidate drugs from secondary metabolites. Modification of lead compounds that are small-molecule secondary metabolites can help achieve molecular structure derivatization and efficient manufacturing of drugs with clinical application value.

With advances in artificial intelligence in the microbiology field and increasing understanding of various microbial functional systems, a high-quality microbial resource-sharing platform can be used as a basis to construct diversified human microbial pharmaceutical screening technology, which not only improves the innovative

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discovery efficiency for microbial pharmaceuticals, but also provides a solid foundation for efficient, green, and intelligent manufacturing of microbial pharmaceuticals.

4 Opportunities and challenges in the microbial pharmaceutical industry

4.1 Developmental opportunities in the microbial pharmaceutical industry

China has abundant microbial pharmaceutical resources, a strong industrial foundation, and remarkable market potential. The gradual increase in national support undoubtedly provides a good external environment for rapid industry growth. Owing to the continuous emergence of new diseases and serious environmental problems, the development of new drugs and the establishment of efficient and green production methods face severe challenges. Abundant microbial resources, microbial pharmaceutical innovation, and microbial product-based diagnostic technologies have extensive developmental potential in biomedical science.

This potential is particularly highlighted in the "double circulation" economic environment, in which the local cycle is the main body, and the remarkable drug demand market requires a large and high-quality industry supply. As the biomedical industry is strategic and emerging, national economic construction pillar industries are gradually absorbing various superior resources, new technologies have continuously emerged, and independent innovation capabilities have improved daily. Moreover, new capital has become active and industrial structure restructuring has accelerated. Favorable policies are continuously being promulgated to accelerate industrial reform. Thus, the pharmaceutical industry faces major development opportunities.

4.2 Challenges in the microbial pharmaceutical industry

4.2.1 New viruses and diseases pose a huge threat to human health

Since humankind first discovered viruses in 1898, more than 4000 viruses have been discovered. The current COVID-19 pandemic has resulted in major life and property losses. This era is an infectious disease era; thus, no country will be left unscathed. Accordingly, research on pathogens and infectious diseases must continue. Conducting R&D on vaccines and drugs in response to public crises is a pressing issue, and demonstrates the importance of constructing big-data platforms.

4.2.2 Standards setting and the blockade of database resources by internationally advanced economies are major potential crises

Currently, the primary databases in the biomedical science field include the National Center for Biotechnology Information, European Bioinformatics Institute, and DNA Data Bank of Japan. These databases and biomedical data services were mainly constructed in the 1980s and 1990s. In recent years, Europe and the United States have initiated the construction of new biological big data centers to improve biomedical information control and biological big data utilization capacity at the national security and strategy levels [36]. China is focused on the potential risk of leakage of proprietary genome and proteome data, and faces a crisis regarding its right to contribute to the formulation of data standards and control of data application in the future.

4.2.3 The risk of industrial chain reorganization is high owing to deglobalisation

The importance of the medical industry has been consistently demonstrated in major public health emergencies. Europe and the United States have started to reflect on the effects of industrial imbalance, and the United States and France have proposed the return of their medical supply chain to their respective countries. Considering the importance of antibiotics in countries with developed medical systems, the possibility of an industrial chain return cannot be ruled out. Simultaneously, industry concentration has increased owing to a decrease in production capacity and expected demand in China. Therefore, export-oriented pharmaceutical companies face greater risks and challenges during financial crises and deglobalization.

4.2.4 Decoupling of technological development and technical exchange, and blocked development

In recent years, the China–US technology decoupling trend has become significant as the United States has promulgated a series of policies to restrict the bidirectional flow of talent and technology; this block has adversely affected innovation and knowledge transfer. Moreover, several technological exchanges in China may have been obstructed, to some extent. A successful foundation can only be obtained in the future through openness to knowledge sharing. Therefore, a "talent backup system" should be established promptly in the microbial pharmaceutical field.

5 Countermeasures and suggestions

5.1 Coordinated innovative construction of a microbial pharmaceutical scientific apparatus

The government's guidance role in investment should be fully utilized, and technology support funds should be established. Government actions include establishing technology R&D funds for the microbial pharmaceutical industry development, increasing technology investment, and strengthening the construction of technological foundation platforms. Government actions could also assist with the organization of an internationally advanced and fusion-microbial-related scientific apparatus; establishment of a microbial species bank, gene and protein information library, biological target library, compound library, synthetic biotechnology element library, and multi-layer shared resource platforms; construction of a resource-sharing service system; construction of infrastructures, such as a microbial species bank and biomedical resource infrastructure; generation of big data deep fusion and an in-depth industrial application data cloud platform; biological prediction with intelligent analysis capacity and design capabilities; and the promotion of innovative ecology through a scientific apparatus to advance the microbial health industry.

5.2 Strengthening frontier layout and seizing global technological commanding heights

Advances in the microbial pharmaceutical field can be achieved by fostering major innovations, aggregating national technological strength, organizing high schools and research institutions, and leading companies to implement major technological R&D. Source innovation for microbial natural product effector molecules is dependent on functional gene mining for microbial pharmaceutical synthesis, the creation of high-throughput strain and metabolite isolation techniques, artificial microbial systems and gene editing, high-throughput bioactivity evaluation and other scientific exploration, the activation of silenced genomes, biological big data analysis and prediction, development of the single-cell spectrum, and the development of other core technologies.

5.3 Strengthening precise training of top talents and cultivation of reserve talents

A competitive talent training policy could be implemented by developing talent zone policies and funds to create favorable conditions for developing talent and teams in the microbial pharmaceutical industry, and encouraging innovative talent to transform technological achievements. In addition, efforts must be made to establish and improve the talent information bank in the microbial pharmaceuticals field, attract overseas professionals and construct a flexible talent flow system, support national and provincial academicians, and establish an industrial talent backup system.

A more comprehensive talent development platform and guiding research institutes should be created to expand the scale of biomedical undergraduate and professional technical talent development. An embedded talent development scheme could also be employed and university–enterprise laboratories could be constructed to "embed" talent in the first line of the industry. The laboratory learning outcomes of graduate students could also be used for credits, thereby strengthening company postdoctoral workstation relationship and establishing a new scheme for company talent customization.

5.4 Strengthening diverse capital assurance and systemizing industrial incentive policies

The government should facilitate the expansion of financing channels; integrate existing policy funds and funding channels; and guide the development of venture capital funds, bonds, and angel investment funds to generate a multilayer investment fund system. Local governments should strengthen the financial support provided to innovative companies for technological development operations, cultivate and support direct financing for companies, improve the venture capital market, encourage various investment systems to establish venture capital institutions, establish a compensation mechanism, improve withdrawal mechanisms, and encourage venture capitalists to implement flexible property rights transfer and exchange systems.

The construction of leading international microbial industry clusters is critical. Further, the proactiveness of various sites, such as existing bioindustry bases, parks, and clusters, must be fully utilized to drive the establishment of several bioeconomic clusters in a step-by-step manner with clear goals. Finally, the concentration of talent, technology, funds, and other resources in dominant areas must be promoted and guided for the characteristic-based and aggregated development of bioindustries, ultimately enabling bioindustries to play a leading role in the promotion of industrial transformation and advancements in China.

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