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Biotechnological Production of Antibiotics

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Abstract. The biotechnological production of antibiotics is based on the isolation of highly productive microbial strains and the optimization of their cultivation and metabolic pathways. Antibiotics are biologically active secondary metabolites synthesized by microorganisms that inhibit the growth of competing microbes or cause their destruction. In natural ecosystems, actinomycetes—particularly species of the genus *Streptomyces*—are the most significant producers and are responsible for the majority of clinically important antibiotics.

The rapid global spread of antibiotic-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Enterobacteriaceae*, has intensified the demand for novel antimicrobial compounds and more efficient production technologies. The biotechnological production process involves several key stages, including the isolation and screening of environmental microorganisms for antagonistic activity, the selection of optimal nutrient media, and the evaluation of the physicochemical and biological properties of the produced antibiotics.

Industrial antibiotic fermentation typically follows a two-phase growth pattern. During the trophophase, microorganisms undergo balanced growth and accumulate biomass, whereas during the idiophase, growth slows and secondary metabolism is activated, leading to intensive antibiotic biosynthesis. To enhance productivity, modern biotechnology employs strain improvement strategies such as random mutagenesis, adaptive evolution, and genetic engineering, often combined with statistical optimization of fermentation media.

Recent advances in genomics, metabolomics, and genome-mining approaches have further expanded the potential for discovering new bioactive compounds by revealing previously silent biosynthetic gene clusters. This review synthesizes current knowledge on microbial antibiotic producers, fermentation strategies, and biotechnological innovations, offering a comprehensive overview of contemporary approaches to antibiotic production.

Keywords: *Antibiotics; Actinomycetes; Secondary metabolites; Fermentation; Strain improvement; Biotechnological production*

1. Introduction

Antibiotics are naturally derived bioactive compounds that have fundamentally transformed modern medicine by enabling the effective treatment of infectious diseases. Since the discovery of penicillin by Alexander Fleming in 1928, antibiotics have significantly reduced mortality and morbidity

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associated with bacterial infections and have saved millions of lives worldwide (Ventola, 2015). Their introduction marked a turning point in clinical practice, making previously fatal infections manageable and enabling advances in surgery, transplantation, and intensive care.

Despite their remarkable success, the widespread and often inappropriate use of antibiotics in human medicine, veterinary practice, and agriculture has contributed to the rapid emergence of antibiotic-resistant microorganisms. Resistant pathogens such as methicillin-resistant *Staphylococcus aureus* and carbapenem-resistant members of the *Enterobacteriaceae* family now represent serious global health threats (World Health Organization, 2015). The accelerating spread of resistance has prompted international initiatives and policy responses aimed at promoting responsible antibiotic use and stimulating the development of novel antimicrobial agents (Ventola, 2015).

Historically, the majority of clinically important antibiotics have originated from microbial secondary metabolism, particularly from soil-dwelling microorganisms. Among these, actinomycetes have played a dominant role in antibiotic discovery. It is estimated that approximately 70% of naturally derived antibiotics have been isolated from species belonging to the genus *Streptomyces*, which is part of the order *Actinomycetales* (Mast & Stegmann, 2019). More broadly, actinomycetes are responsible for producing nearly half of all known bioactive microbial metabolites, underscoring their exceptional biosynthetic potential (De Simeis & Serra, 2021).

A defining feature of actinomycetes is their extensive capacity for secondary metabolite biosynthesis, often encoded by a substantial proportion of their genomes. Genomic analyses have revealed that more than 10% of the actinomycete genome may be dedicated to secondary metabolite pathways, many of which remain silent or poorly expressed under standard laboratory conditions (Mast & Stegmann, 2019). Recent advances in high-throughput screening, genome mining, and metabolomics have revitalized interest in these microorganisms by enabling the identification and activation of previously unexpressed biosynthetic gene clusters (Andreu & del Olmo, 2023; Baltz, 2008). These developments highlight the continued relevance of microbial biotechnology in addressing the urgent need for new antibiotics.

2. Antibiotic-Producing Microorganisms

Actinomycetes, particularly soil-inhabiting species of the genus *Streptomyces*, are widely recognized as the most prolific producers of antibiotics. These filamentous bacteria synthesize a diverse array of secondary metabolites with antibacterial activity, including β-lactams, macrolides, aminoglycosides, tetracyclines, and glycopeptides (Mast & Stegmann, 2019). Many of these compounds have become indispensable in clinical practice. For example, *Saccharopolyspora erythraea* is the natural producer of erythromycin, a macrolide antibiotic widely used to treat respiratory and soft tissue infections.

The remarkable biosynthetic versatility of actinomycetes is attributed to their complex enzymatic machinery, which enables the generation of structurally diverse and pharmacologically potent molecules. According to De Simeis and Serra (2021), actinomycetes possess unique metabolic pathways that facilitate the production of antibiotics with varied mechanisms of action. In addition to *Streptomyces*, other actinomycete genera such as *Amycolatopsis* and *Streptomyces griseus* have yielded clinically important antibiotics, including vancomycin and streptomycin, respectively (Baltz, 2008).

Filamentous fungi also represent an important group of antibiotic producers. The discovery of penicillin from *Penicillium chrysogenum* and cephalosporins from *Acremonium* (formerly *Cephalosporium*)

species laid the foundation for the development of β-lactam antibiotics. Through decades of strain improvement and fermentation optimization, multiple generations of penicillins and cephalosporins have been produced, including advanced semi-synthetic cephalosporins capable of overcoming certain resistant bacterial strains.

In recent years, antibiotic discovery efforts have expanded beyond traditional soil microorganisms to include previously underexplored ecological niches. Marine environments, endophytic microorganisms, and extremophilic bacteria are increasingly investigated as potential sources of novel antimicrobial compounds. Studies indicate that extremophilic and thermophilic microorganisms, in particular, may produce structurally unique metabolites with promising antibacterial activity (Andreu & del Olmo, 2023; Pardo-Este et al., 2024). This expanded search strategy is driven by the recognition that relatively few new antibiotic classes have been introduced in recent decades, despite the growing burden of antibiotic resistance (Ventola, 2015).

Although the pace of new antibiotic approvals has slowed considerably since the early 2000s, advances in microbial biotechnology, genomics, and fermentation science offer renewed opportunities to expand the antibiotic pipeline. By combining traditional microbiological approaches with modern molecular and bioinformatic tools, researchers aim to unlock the full biosynthetic potential of antibiotic-producing microorganisms and address one of the most pressing challenges in global health.

3. Screening and Strain Selection

The discovery of new antibiotics traditionally begins with the isolation of microorganisms capable of inhibiting the growth of pathogenic bacteria. Soil remains one of the most important reservoirs of antibiotic-producing microorganisms due to its high microbial diversity. Soil samples are typically diluted and plated onto selective media designed to enrich actinomycetes and filamentous fungi while suppressing fast-growing bacteria and molds. This selectivity is often achieved through the use of specific antibiotics, acidic pH conditions, or nutrient limitations.

Each isolated microorganism, commonly referred to as an antagonist, is subsequently cultivated, and its culture broth or extracted metabolites are tested for antimicrobial activity against indicator organisms. Classical screening methods, such as agar diffusion assays and overlay techniques, were pioneered by Waksman and remain foundational in antibiotic discovery. Modern screening approaches continue to rely on these principles but increasingly incorporate high-throughput liquid assays and automated detection systems.

Once biologically active strains are identified, their taxonomic affiliation, physiological properties, and growth characteristics are evaluated. Because primary isolates frequently display unstable or low antibiotic yields, further strain selection is required. Classical strain improvement strategies, particularly random mutagenesis using ultraviolet radiation, X-rays, or chemical mutagens, are widely applied to enhance productivity. Repeated cycles of mutation and selection often result in strains with significantly increased antibiotic output. Despite advances in molecular biotechnology, random mutagenesis remains a cost-effective and efficient approach for short-term strain development (Jeyachandran et al., 2024).

In parallel, modern screening increasingly integrates genomic and molecular tools. Genome sequencing and biosynthetic gene cluster analysis allow researchers to identify strains harboring

cryptic or silent antibiotic pathways that may be activated through specific cultivation conditions or genetic interventions. Reporter assays and transcriptional profiling further aid in selecting strains with high biosynthetic potential. Consequently, contemporary strain selection relies on a combination of traditional antagonistic screening and advanced molecular diagnostics to identify promising antibiotic producers for industrial development.

4. Fermentation and Cultivation Strategies

Following the selection of a productive strain, antibiotic production is carried out through controlled fermentation processes, most commonly in bioreactors. The design of fermentation media and operational parameters plays a critical role in determining antibiotic yield. Microbial metabolites are generally classified as primary metabolites, which are essential for growth, and secondary metabolites, such as antibiotics, which are synthesized during later stages of cultivation.

Antibiotic production typically follows a biphasic growth pattern. During the trophophase, microorganisms undergo rapid, balanced growth characterized by intense nutrient consumption and biomass accumulation, while antibiotic synthesis remains minimal. In contrast, the idiophase, corresponding to the stationary or late growth phase, is marked by reduced growth rates and the activation of secondary metabolic pathways responsible for antibiotic biosynthesis (Singh et al., 2017). Antibiotic production often reaches its maximum during the late trophophase or early stationary phase.

The composition of the fermentation medium is carefully optimized to support this metabolic transition. Carbon and nitrogen sources, trace elements, and oxygen availability must be precisely balanced. A well-known example is glucose catabolite repression in *Penicillium chrysogenum*, where high glucose concentrations inhibit penicillin synthesis. Replacing glucose with slowly metabolized carbon sources such as lactose alleviates this repression and enhances antibiotic production. Similar strategies are applied across multiple antibiotic-producing systems, with glycerol, galactose, or starch frequently used to promote secondary metabolism.

Fermentation processes may be conducted in batch, fed-batch, or continuous modes. Fed-batch fermentation is particularly advantageous for antibiotic production, as it prevents substrate inhibition and prolongs the productive idiophase. Key environmental parameters—including pH, dissolved oxygen, temperature, and agitation—are continuously monitored and adjusted to maintain optimal biosynthetic conditions. Statistical optimization methods, such as Plackett–Burman designs and response surface methodology, are widely employed to refine medium composition and process parameters. Integrated approaches combining strain improvement with fermentation optimization have been shown to substantially increase antibiotic yields (Singh et al., 2017).

In industrial practice, antibiotic fermentation is often performed using a two-stage protocol. An initial biomass production stage generates a large and healthy inoculum, followed by a production stage under conditions optimized for antibiotic synthesis. Harvesting is timed according to metabolite accumulation, typically when antibiotic concentration reaches a plateau.

5. Strain Improvement and Genetic Strategies

To further enhance antibiotic production, selected strains are subjected to genetic and biotechnological improvement. Traditional strain improvement relies on induced mutagenesis followed by rigorous screening for overproducing mutants. Although labor-intensive, this approach

has historically led to dramatic increases in antibiotic titers, with some industrial strains achieving yields tens or even hundreds of times greater than their wild-type ancestors. Random mutagenesis remains widely used due to its simplicity and effectiveness, particularly for short-term productivity gains (Jeyachandran et al., 2024).

Modern strain improvement increasingly incorporates targeted genetic engineering techniques. Genes involved in precursor biosynthesis, pathway regulation, and self-resistance can be manipulated to enhance antibiotic production. Overexpression of pathway-specific regulatory genes or deletion of transcriptional repressors can activate otherwise silent biosynthetic gene clusters. Additionally, engineering primary metabolic pathways to increase precursor availability indirectly boosts secondary metabolite synthesis.

Advances in genomics and systems biology have significantly expanded the scope of strain engineering. Whole-genome sequencing of actinomycetes has revealed numerous silent biosynthetic gene clusters with the potential to encode novel antibiotics. Activation of these clusters through promoter engineering, heterologous expression, or regulatory rewiring has led to the discovery of new compounds. Combinatorial biosynthesis, which involves recombining genes from different biosynthetic pathways, further enables the generation of novel antibiotic analogues (Baltz, 2008).

Other innovative approaches include adaptive laboratory evolution and co-cultivation strategies. By exposing producer strains to selective pressures or competitive microbial environments, antibiotic production can be naturally induced or enhanced. Importantly, improvements achieved through genetic modification must be validated under industrial fermentation conditions to ensure their effectiveness at scale.

6. Conclusion

The biotechnological production of antibiotics is founded on the remarkable biosynthetic capabilities of microorganisms and is optimized through an integrated combination of microbiological, biochemical, and engineering approaches. Actinomycetes and filamentous fungi remain the most prolific sources of antibiotic compounds, providing the foundation for both natural and semi-synthetic antimicrobial agents. As antibiotic resistance continues to threaten global health, the efficient production of existing antibiotics and the discovery of new compounds have become increasingly urgent.

Advances in strain selection, fermentation technology, and genetic engineering have significantly improved antibiotic yields and process efficiency. Key innovations include statistical optimization of culture media, fed-batch and two-stage fermentation strategies, and targeted manipulation of biosynthetic pathways. The biological principles underlying trophophase and idiophase metabolism are now routinely exploited to maximize secondary metabolite production.

Future progress in antibiotic biotechnology will depend on the successful integration of classical fermentation expertise with modern genomic and molecular tools. By unlocking the full biosynthetic potential of known producers and exploring novel microbial niches, biotechnology continues to play a central role in sustaining the development of life-saving antibiotics and addressing the global challenge of antimicrobial resistance.

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