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Modification and Screening of Antibiotic-producing Strains

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Abstract: Antibiotics play an important role in the field of medicine and healthcare, and with increasing usage, there is an urgent need to improve the quality and yield of antibiotic products to achieve cost reduction and efficiency. This has forced scientists to modify and screen antibiotic-producing strains to improve the yield and quality of antibiotic products. This paper briefly describes the strain modification methods as well as strain screening methods, discusses the limitations of each method, and looks forward to the development trend of strain modification and screening.

Keywords: Antibiotics; Strain modification; Strain screening.

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1. Introduction

Antibiotics are a class of chemicals produced by microorganisms that can inhibit or even destroy bacteria and other microorganisms [1]. Since Alexander Fleming first discovered the bactericidal properties of penicillin, antibiotics have revolutionized medicine and healthcare, and the next golden age of antibiotic discovery, from the 1940s to the 1960s, saw the successful screening of tens of millions of soil microbes [2] that provide the vast majority of microbial metabolites known today [3]. These substances include widely used antimicrobial therapeutics and chemotherapeutic agents such as erythromycin, streptomycin, tetracycline, vancomycin, and Adriamycin [2], which have led to effective treatments for previously life-threatening bacterial infections [4]. Because of their relatively low manufacturing costs and wide accessibility, antibiotics have rapidly and dramatically increased the average life expectancy of many people. At the same time, antibiotics are not limited to the treatment of human diseases but are also widely used in animal husbandry, where they are used to treat disease in animals and in concentrated animal feeds at subtherapeutic levels to promote growth, improve feed conversion efficiency, and prevent disease [5,6].

However, with the massive use of antibiotics, there are still problems such as complicated production processes, low production efficiency and unstable product quality in the process of antibiotic production. These problems have prompted scientists to modify antibiotic-producing strains by methods such as induced mutation or genetic engineering to obtain high-yielding strains through appropriate screening programmes, thereby improving the yield and quality of antibiotics. This review focuses on various schemes of strain modification, including induced mutation and genetic engineering to modify strains. Moreover, the current strain screening methods commonly used in the laboratory are introduced, and a brief overview and discussion of these methods are provided, as well as ideas and a theoretical basis for improving the performance of antibiotic-producing strains in the later stage of antibiotic production.

2. Modification of Antibiotic-producing Strains

2.1. Significance of research strain modification

Rapid growth of the world population, rapid depletion of fossil fuels and environmental pollution, all of which are of great concern globally, require continuous research efforts to improve the production and quality of different antibiotic products in limited facilities [7]. However, the use of natural microbial strains to increase product yields is often hampered by inefficient substrate conversion, high byproducts, and low tolerance to stress effects [8]. One way to overcome these problems is to optimize the fermentation process, which may include screening for the best conditions for the parameters associated with this essential step in the biotransformation process [9]. Despite the effectiveness of the optimization process, it is difficult to find optimal conditions for wild strains in practice, especially when there is a simultaneous need to increase product yield and improve product quality. Strain modification is likely the most important solution to these problems and has received much attention in recent decades [10].

2.2. Current status of strain modification research

Classical strain modification is mainly used to generate a large number of mutants through various techniques, including strain-induced mutagenesis, bioengineering modification, and laboratory evolution [11,12].

2.2.1 Mutagenesis breeding

Strain mutagenesis refers to the process of using physical, chemical or biological mutagens to change the genetic material of microorganisms or other organisms to produce new strains with excellent traits. This method is an important means of microbial breeding that can effectively increase the yield of strains, improve product quality, enhance resistance and so on.

1. Physical mutagenesis

The earliest appearance of physical mutagenesis is through UV irradiation, and the maximum absorption peak of UV light appears at 254 nm, which is the most effective wavelength for mutagenesis of DNA and RNA. UV irradiation causes the hydrogen bond to break and the DNA to cross-link, which causes the DNA of the strain to be mutated and thus results in high-throughput mutagenesis. UV mutagenesis is mainly focused on the fermentation process of common microorganisms such as Aspergillus, and highly stable mutant strains are obtained by irradiation with UV light. Chen [13] exposed *Aspergillus niger* spore suspensions to 40 W UV light for 3 min and obtained a xylanase-producing strain with a xylanase activity of 58.305 U/mL. The enzyme-producing activity of this mutagenic strain increased as much as 5 times compared to the enzyme-producing activity of the initial strain. Zhang [14] carried out four consecutive ultraviolet mutants of the engineered strain producing epidaunorubicin, and the final mutant strain was obtained with an increase of 93.7% in the fermentation unit compared with the initial strain.

The use of lasers to irradiate production-engineered strains can also lead to the generation of new mutations. This mutation method has been studied for more than 30 years, and a relatively complete system of laser mutation microbial breeding was developed by 2003. Engineered strains produce short-term responses within a short period of time (usually seconds or minutes) after the end of laser irradiation and long-lasting responses after a few hours or days, with the prevalent responses being accelerated respiration of the strains and accelerated production of ATP by the strains. Peng [15] irradiated the protoplasmic fluid of *Aspergillus niger* with a laser irradiation power of 15 W and an irradiation time between 20 and 60 s and screened a large number of mutant colonies to obtain a mutant colony, which increased the activity of phytase by 3.75-fold compared with that of the initial strain, with an enzyme activity of 14,850 U. Zhang [16] irradiated a spiramycin-producing strain with a He-Ne laser at a wavelength of 632.8 nm and a power density of 0.4 mW/cm^2 for eight hours and obtained a mutant strain with an increase in potency of 38.6% and a superior composition.

X-rays usually have a certain radiation energy, which can cause biological cell mutations. Many researchers use X-rays, γ-rays, α-rays, β-rays and other rays to prepare mutant strains. X-rays can cause base damage and intramolecular mutations in biological cell DNA through radiation energy [17]. Chen [18] used X-rays to radiate the spores of Aspergillus oryzae, a high-yield strain obtained by postscreening, and the acid production of the mutant strain increased by 56% compared with that of the original strain. Many studies have used γ-rays to mutagenize microorganisms to generate high-yield mutant strains, and various microorganisms, such as bacteria and fungi, have been studied for the use of γ-rays to produce mutant strains. Liang [17] used 60Co-γ to mutate Streptomyces viridoehrongenes, and the ability of the mutant strains to produce avilamycin was significantly improved [19]. When Rhizopus oryzae was mutagenized with ^{60}Co-γ, the amount of reducing sugars utilized by the mutagenized strain was greater than that utilized by the other strains. Mao [20] used ^{60}Co-γ to mutate Thermomonospora fusca, and the enzyme production capacity of the mutant strain was increased by 1.8 times compared with that of the starting strain.

Microwaves are also among the most frequently used low-power radiation sources in the biological field and are used for sterilization or mutation breeding of microorganisms. It is generally believed that the main influence mechanism of microwave treatment includes two categories: the thermal effect of microwave energy absorbed by organisms via internal conversion and nonthermal effects that lead to various physiological and biochemical changes in organisms [21]. In the second category, which involves microwave radiation, the intracellular DNA molecule double helix structure of the hydrogen bond breaks for reorganisation, resulting in mutation [22]. Han [23] used microwave mutagenesis on Phaffia rhodozyma TY-1 and screened it to obtain a mutant strain with improved genetic stability after 100 s of treatment, and the astaxanthin yield increased by 102.51%. Pan [24] used microwave treatment on Rhodosporidium toruloides and screened a mutant strain with an oil-producing capacity 2.56 times greater than that of the initial strain.

In addition to the classical physical mutagenesis methods mentioned above, researchers have been exploring novel physical mutagenesis methods, and the novel physical mutagenesis methods developed and used by researchers and the corresponding results are listed in **Table 1**.

Table 1. Novel physical mutagenesis methods and results.

strains	mutagenic method	results-based
brewer's yeast [25]	ultrahigh pressure	Mutant brewer's yeast Gy3 reaches 68% fermentability
brewer's yeast [26]	high energy electron flow	Positive mutant yeast strain increases alcohol yield by 25.5%
Micromonospora cabonacea [27]	thermal inversion	Antibiotic production capacity increased by 98.53%
Monascus purpureus [28]	magnetic fields	γ-aminobutyric acid production increased by 35.7%
Aspergillus erythropolis M50-2 [29]	ion beam	Lovastatin yield reached 4.42 mg/g, a 70% improvement

2. Chemical mutagenesis

Chemical mutagenesis refers to the use of appropriate concentrations of chemical mutagens on a large number of initial microbial strains, inducing mutations in the genetic material of microorganisms and subsequently causing the initial morphological characteristics of a kind of mutation. Then, a large number of mutant strains were obtained for identification and screening, and new varieties with stable genetic traits were ultimately selected and bred. Chemical mutagenesis induces a greater mutation rate than natural mutagenesis. Chemical mutagenesis can not only increase the mutation rate to 1/30 but

also produce rare mutant strains that are cherished in nature. The chemical mutagens used in chemical mutagenesis can act on only a few or specific bases, which can produce mutations at individual loci. At the same time, when chemical reagents are used to induce mutation, microorganisms have a lower probability of generating chromosomal mutations and aberrations and seldom mutate to a lethal phenotype.

Chemical mutagens, when applied to microorganisms, can alter the structure of their DNA or cause DNA base mutations [30]. Some of the more commonly used mutagens are listed in **Table 2** [31].

Table 2. Commonly used chemical mutagens.

base analog	alkylating agent	shifting code mutagen	comutagen
5-Bromouracil	nitrogen mustard derivatives	Acridine Orange	Nitrous acid and its salts
5-Fluorouracil	Epoxy derivatives	proflavine	Some metal compounds
6-Azauracil	Sulfonic acid esters esters		
2-Aminopurine	Diazoparaffins		
6-Mercaptopurine	Nitroso derivatives		

The principles of chemical inducers for inducing microbial mutations are complex and varied, and the principles of two commonly used chemical inducers are described below. Ethyl methane sulfonate (EMS) is a representative alkylating agent because it is simple and inexpensive to use, has a low probability of chromosomal aberrations [32], and is highly effective [33]; therefore, it has become one of the most commonly used reagents for constructing mutant libraries [34]. EMS induces mutations by directly reacting with purines and pyrimidines in nucleotides, and it can induce three types of point mutations: silent mutations, nonsense mutations, and missense mutations [35]. 5-Bromouracil (5-BU), a base analog, is structurally similar to dTMP and can generate keto and enol isomers, which are complementary to adenine and guanine, respectively, leading to base transition mutations during DNA replication, which in turn can lead to mutations in the organism.

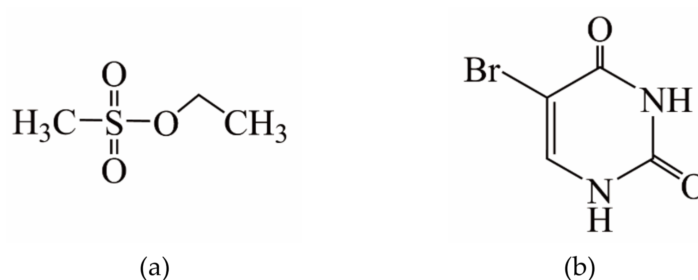

Figure 1. Chemical structures of EMS (a) and 5-BU (b).

Cheng [36] used 5-bromopyrimidine as a mutagenage agent, and the original carotene-producing engineered bacteria were used to induce mutations in the protoplasts of this strain. The results of the study showed that the yield of the mutant strain increased by 22.6% compared with that of the original strain. Chen [37] used diethyl sulfate to treat D-ribose-producing *Bacillus subtilis* and successfully obtained a high-yielding strain with an increase of 81.7% in yield [38]. *Bacillus subtilis* G3 was mutagenized with acridine orange, and the antifungal activity of the mutant strain significantly improved.

3. Compound mutagenesis

In the process of mutation selection, because a single mutation selection method will make a strain resistant, even with long-term application, it is difficult to obtain new mutations; therefore, people often use two or more mutation methods to treat the same strain at the same time in the process of mutation treatment, alternating or rotating the application of various methods to reduce the problem of mutagen fatigue.

Zhang [39] combined two less toxic chemical mutagens, pingyangmycin hydrochloride and 5-BU, and determined an optimal ratio of 300:5 (μmol/L) to treat *Saccharomyces cerevisiae* HY01. A large number of mutant strains were obtained, and a mutant strain with a 10% increase in alginate yield compared with that of the departure strain was obtained after screening. Li [40] used Clostridium butyricum 209 as the original strain. After two rounds of chemical mutagenesis using diethyl sulfate, a positive mutant strain was screened to obtain a 113% increase in 1,3-propanediol production capacity compared with that of the starting strain. This mutant was then subjected to another round of UV mutagenesis and another round of combined UV and nitrosoguanidine mutagenesis to obtain a mutant strain with a 6.13-fold increase in 1,3-propanediol production compared to that of the original strain.

2.2.2. Bioengineered breeding

1. Protoplasmic fusion

Protoplasmic fusion is a process in which the protoplasts of two parental strains with different genetic characteristics are fused and chromosomes are interchanged and recombined through the application of physical, chemical and biological techniques. The fusion of microbial protoplasts through this technology can greatly increase the frequency of recombination between parents, and at the same time, it also expands the range of parent selection for genetic recombination. Therefore, it is easier to obtain strains with excellent new traits, thus improving the quality and yield of the strain's fermentation products [41]. However, at the same time, the DNA recombination between parents in this method is completely random, resulting in greater difficulties and challenges for the isolation and screening of positive mutant strains at a later stage.

2. Genetic engineering and breeding

In the 1970s, with the theoretical support of molecular genetics and biology, a new biological field of genetic engineering technology was established. The birth of this technology allowed people to isolate specific gene fragments very simply and quickly, followed by the cloning of gene fragments in vitro into plasmids and other vectors. The resulting DNA was artificially recombined and ultimately imported into recipient cells for normal replication and expression. This technology has a number of advantages in that it can completely break through interspecies barriers, allowing recombination of genetic material between plants and animals as well as microorganisms and achieving truly distant hybridization. This provides a new approach for the modification of antibiotic-producing strains, which is highly important for improving fermentation product quality and yield, as well as for the discovery of new antibiotics. Subsequently, researchers completed the deciphering of antibiotic biosynthetic pathways and the complete cloning of antibiotic biosynthetic clusters, which further accelerated the progress of genetically engineered strain breeding. Reeves [42] reported that the erythromycin biosynthesis gene cluster is located on the chromosome of Polyspora rubra, with a total length of 56 kb. Zheng [43] successfully introduced the erythromycin biosynthesis gene cluster into *Escherichia coli* and completed the heterologous expression of the antibiotic. Although the antibiotic yield did not significantly increase, the growth cycle of the strain greatly decreased. Zhou [44] cloned the PGA gene in a strain that could produce penicillin G acylase, integrated the gene into the expression plasmid pET24a, and finally introduced the recombinant plasmid into *E. coli*. When the fermentation conditions were optimized to achieve the best fermentation state, the PGA enzyme activity reached 993.4 U/L; the enzyme activity of the *E. coli* production strain increased up to 66-fold compared with that of the wild-type strain for the production of PGA.

In addition, researchers will also add, knock out, replace and perform other operations on key genes in the gene cluster to complete the targeted modification of genes to increase antibiotic yield or modify antibiotics. For example, increasing the number of PKS-

encoding genes in the erythromycin synthesis gene cluster can increase the yield of erythromycin A by 50% [45]. Researchers have found that adjusting the copy numbers of ery K and ery G to 3:2 can effectively reduce the production of B and C, respectively, while increasing the production of erythromycin A by 35% [46]. The erythromycin synthesis pathway is shown in **Figure 2**.

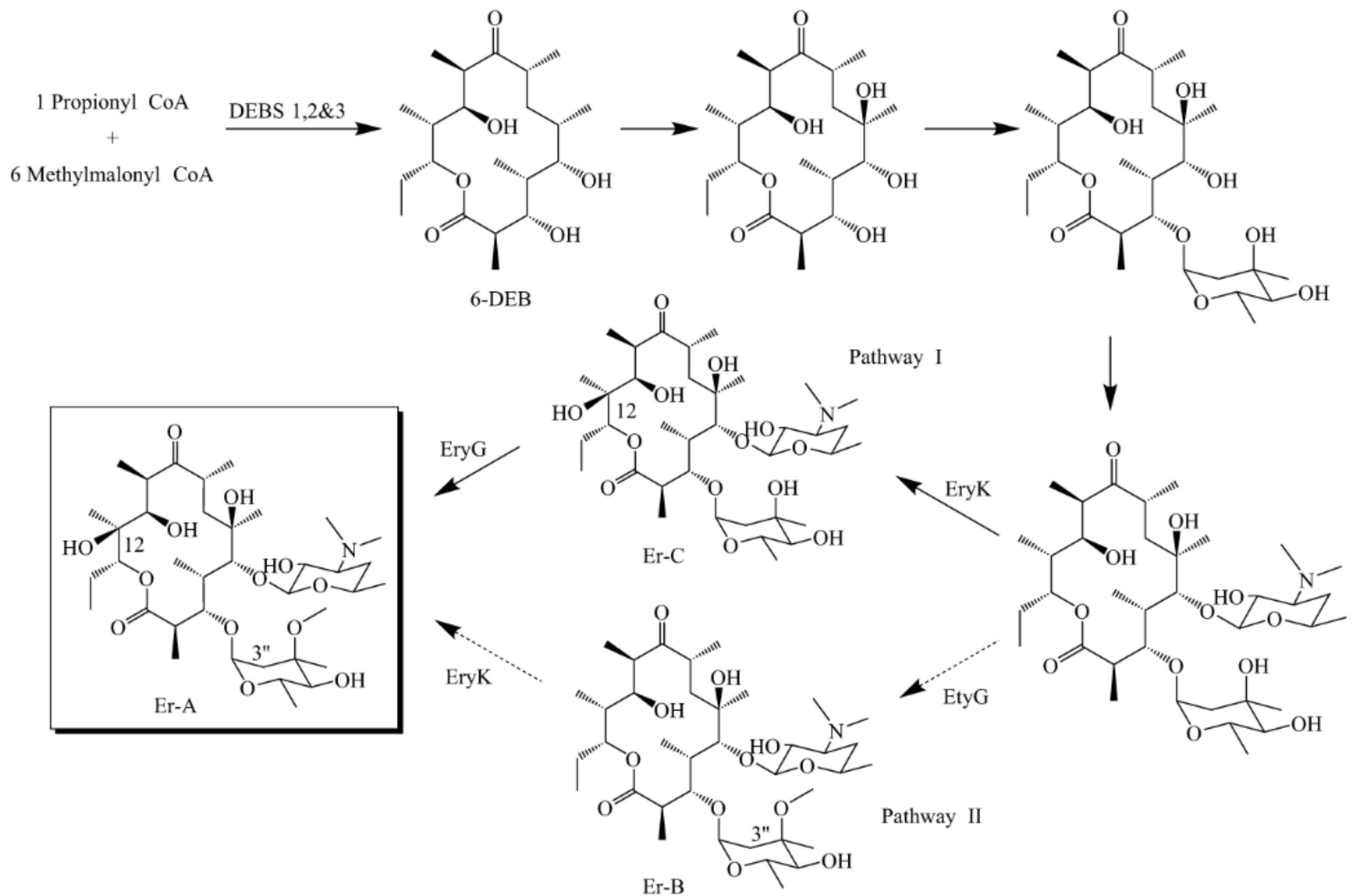

Figure 2. Erythromycin synthesis pathway.

3. Genome shuffling technology

However, although the classical strain modification approach has been extensively studied for decades to improve the abundances of different industrially important microorganisms, it is a time-consuming and labor-intensive approach. More importantly, obtaining transgenic strains by classical strain modification techniques requires comprehensive information about the genome of the parental microorganisms [47]. In addition, although strain modification actually implies mutations in microbial genetic patterns, the main goal is phenotypic improvement of the strains. Reports on improving the phenotypic expression of strains have been increasing annually in the last few years, and one of the more commonly used techniques is genome shuffling [48].

Genome shuffling is one of the newest and most promising techniques for rapid phenotypic improvement and has received significant attention for the phenotypic improvement of industrially important strains. This technique allows combinatorial recombination by recursive recombination in the genotypes of parental strains related to the desired phenotype [49]. Genome shuffling has some similar attributes to classical strain improvement, as both provide genome diversification and screening for improved strains. The main difference between the two techniques is that the genome shuffling process is sexual

and involves the evolution of the entire population of the improved strain, which is not found in the classical approach. In addition, genomic shuffling is a faster and more efficient technique for generating the desired phenotype than the classical approach. In addition, genome shuffling can sporadically induce mutations in complex phenotypes at different points throughout the genome without requiring genome sequencing data [12].

Figure 3 summarizes the general genome shuffling workflow, encompassing a variety of possible pathways to introduce genetic diversity into the starting microbial population and recursive recombination to rapidly generate new and potentially beneficial combinations of mutations. Interventional screening or selection steps can be applied at different points in the process to isolate improved mutants that can be further recombined. The process can be repeated, and good mutants can be obtained. Each time a mutant is isolated, it can be characterized.

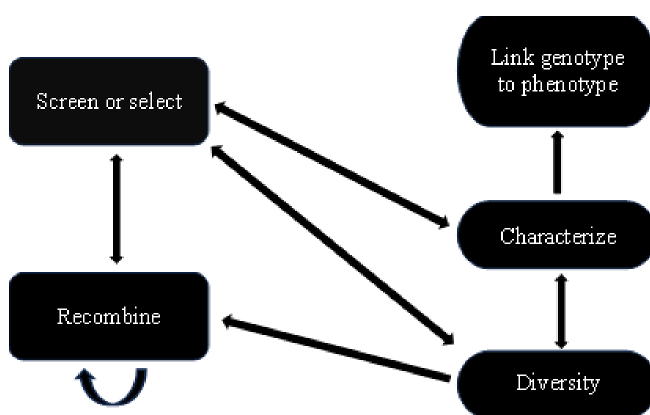

Figure 3. Schematic diagram of the general genome shuffling workflow.

Genome shuffling techniques are designed to improve the efficiency of microbial strains used for the production of various biotechnological products. The objectives of strain improvement activities can be broadly classified into several types, such as increasing substrate utilization and improving product yield and tolerance to stress (**Table 3**). The history of the use of genome shuffling is limited, and the technique was first reported in 2002 for improving the yield of the antibiotic tylosin in Streptomyces fradiae [50] and the acid tolerance of Lactobacillus [49]. Since the development of genome shuffling technology, successful strains have been developed for the production of a wide range of bioproducts, including lactic acid, riboflavin, lipase, bioethanol, antibiotics, biopesticides, adriamycin, avilamycin, alkaline lipase, and many others of a similar nature [51,52].

Table 3. Some applications of genome shuffling in facilitating bioproduction of bacterial strains.

strains	goal	Number of cycles	outcome
Aspergillus niger [53]	Expand the range of carbon sources	3	Modified strains utilize four more carbon sources
brewer's yeast [54]	Increased glutathione production	2	3.2-3.3-fold increase in glutathione production
Propionibacterium F2-3 [55]	Increased production of vitamin B12	2	54-61% increase in vitamin B12 production
Lactobacillus rhamnosus [56]	Increased glucose tolerance	2	62.2% increase in glucose consumption
Clostridium [8]	Improved isopropanol tolerance and butanol yield	2	75% increase in butanol production

brewer's yeast [57]	Improving ethanol production by increasing tolerance to multiple stresses	3	Alcohol concentration increased by approximately 10.96% in ultrahigh density fermentation

In addition to genome shuffling techniques, several new genetic engineering techniques, such as ribosome engineering [58] and artificial transcription factor engineering [59], have emerged in recent years, and all of these technologies have shown good performance in enhancing microbial production capacity.

3. Current Status of Strain Screening Research

It can be found through the above description that, whether it is the discovery of wild strains, mutation breeding or genetic engineering breeding, the modified strains ultimately obtained need to undergo certain screening work to obtain the target strains or positive mutant strains, and researchers have developed a variety of screening methods according to the actual needs.

3.1. Random screening

Random screening is the most traditional way to discover microorganisms in nature, mainly through the isolation and purification of soil, seawater, sand and gravel samples to obtain all kinds of strains, isolation and purification of the target strains, fermentation culture, and determination of active ingredients in the fermentation broth for biological activity. To ensure the accuracy of the results, after the initial screening generally requires rescreening, random screenings of indicator bacteria, such as generally gram-positive bacteria, gram-negative bacteria, viruses, and parasites.

Random screening, as the most traditional screening method for wild bacterial strains, is still widely used due to its low technical requirements and simple method, although the screening process is time-consuming and labor-intensive, and the screening efficiency is low. To identify natural anticancer drugs, a researcher isolated 29 strains of marine bacteria from sponge samples collected at low tide, and a strain with dominant cytotoxic activity was obtained by screening, and it was found that the fermentation product of this strain, Norharman, was cytotoxic to HeLa cervical cancer cells and BGC-823 gastric cancer cells [43].

3.2. Model selection

Model screening involves targeted screening rather than random screening, and corresponding screening models can be constructed for different target substances to achieve screening. Due to the massive use of antibiotics, strains can easily acquire resistance to antibiotics, and researchers have established vancomycin-resistant bacterial models based on the resistance of the strains and screened them to obtain vancomycin-resistant strains from which highly effective resistant compounds were extracted [60].

3.3. Genetic screening

With advances in biotechnology, a large number of biosynthesis-related gene clusters for antibiotics have been sequenced and analyzed, such as the biosynthetic polyketide synthase (PKS) gene cluster. Designing corresponding primers based on the conserved sequences of these gene clusters and scanning the genome or gene libraries can rapidly determine whether a strain has the potential to synthesize a particular class of antibiotic [61]. Some researchers amplified the adenylation domain (A domain) of nonribosomal peptide synthetase (NRPS) by PCR and selected strains with the potential to synthesize nonribosomal peptides from a large number of candidate strains [62]. In addition, biological analyses of the genomes of the screened strains allowed the prediction of possible secondary metabolites.

3.4. High-throughput screening (HTS)

The rapid development of genetic engineering and DNA assembly techniques has revolutionized the field of biotechnology, and the ability to construct bioproductive strains with different phenotypes has been further enhanced, resulting in a large number of strains that need to be screened. It is therefore crucial to screen the most suitable candidate strains from the vast population of modified strains, where high-throughput screening (HTS) techniques have been shown to play a vital role in identifying and isolating effective strains for a range of biotechnological purposes.

The use of HTS at the molecular and cellular level has been well established, and automated microquantitative experiments combined with big data analytics [63] have been used. The automated steps include sampling, dilution of the sample to the appropriate detection range, transfer of the sample and color developer, mixing of the sample, washing of the cells, onset of the chromogenic or enzyme-linked reaction, color or fluorescence detection, data analysis, and collection of improved strains [64]. Since the first appearance of HTS in the early 1990s, new equipment, such as colony pickers, liquid handling systems, fluorescence-activated cell sorting (FACS) and droplet microfluidics, has been developed [65], and to date, HTS has been widely used in different areas of biotechnology [66,67].

HTS has significant advantages over traditional screening methods [68], including (1) more efficient automated operation and advanced equipment improving the automation of HTS, thus preventing potential contamination and human error; (2) less human resources being needed, and the use of microtiter plates and FACS to establish an automated operating system and incubation and analysis modes greatly reduces labor costs; (3) more sensitive and accurate, the new assay achieves rapid and accurate screening by detecting changes in the levels of the target metabolites that are changes associated with the target metabolite content and enables rapid and accurate screening; and (4) requiring less sample volume, experimental quantification requires only a few microlitres (in microtiter plates) or even nanolitres (in droplets), thus significantly reducing the cost of culture media and reagents. **Table 4** lists several typical high-throughput screening methods and their applications in the screening of antibiotics for microbial production.

Table 4. Application of high-throughput screening in the selection of high-performance industrial microorganisms.

strains	offerings	HTS methodology	results-based
ARTP mutagenesis of Streptomyces avermitilis [69]	avermectin	Screening followed by absorbance detection	20.6% increase in effectiveness
Streptomyces albacans [70]	salinomycin	Bacterial indicators of HTS	200% increase in effectiveness
ARTP mutagenesis of actinomycetes [71]	acarbose	Specific identification of antibiotics	62.5%increase in effectiveness
Streptomyces avermitilis S-233 [72]	avermectin	Direct detection of absorbance	23.8% increase in effectiveness
Complex mutagenesis of Xanthomonas [73]	Cephalosporin C	Direct detection of absorbance	200% increase in effectiveness
Streptomyces erythraea T-13 [74]	erythromycin	Absorbance detection using bacterial indicators	Establishment of HTS screening methodology

Overall, the sustainable production of biosynthetic antibiotics requires the development of efficient microbial strains, which, when combined with HTS or molecular biosensors, can be evaluated more effortlessly and efficiently with modified strains.

4. Conclusions and prospects

Since the discovery and widespread use of antibiotics, research on how to improve the yield of antibiotics has continued, and the modification and screening of antibiotic-producing strains is an important part of improving the yield and quality of antibiotics. In the course of strain modification, people have explored a variety of strain modification methods. One of the classic and traditional methods is mutagenesis breeding technology, which is limited by blindness, randomness and other shortcomings; however, this technique is simple and does not require expensive modern high-grade equipment. With the development of life sciences, genetic engineering technology has been gradually used for strain modification. This technology can completely overcome interspecies barriers, but many mutation breeding methods are not advantageous and have been widely used for strain modification. Until genome shuffling technology was first described in 2002, this technology, as a gene-level transfer method, became a favorable means to overcome the limitations of reverse metabolic engineering. After obtaining a large number of mutant strains, how to efficiently and rapidly isolate forward mutant strains is another challenge that people face, from time-consuming and laborious random screening to model screening, which can be directed to isolate strains, and then to genetic screening up to high-throughput screening. The throughput of these methods is becoming increasingly large, and the efficiency is increasing. In combination with highly efficient strain modification technology, researchers can obtain specific high-yield antibiotic strains.

With the advancement of science and technology, the current research trend in strain modification has focused on the use of genetic engineering combined with novel breeding techniques, making it possible to rapidly and efficiently modify strains. At the same time, breeding techniques are combined with metabolic engineering and histological analyses to expand the scope of application for the rapid improvement of unconventional microorganisms. Although all kinds of strain modification programmes have made breakthrough progress, as of now, the technology of novel strain modification has limited application in enhancing the yield and quality of antibiotics, which requires people to introduce more cutting-edge technologies of strain modification in the field of biosynthetic antibiotics to make the process of modifying strains more explicit, rapid and efficient. After obtaining a large number of mutant strains, finding a high-throughput screening method to match the strains is also a key research direction, especially in the case of modifying unconventional strains. The currently reported high-throughput screening protocols for modified strains are not only complicated and expensive in terms of equipment but also difficult in terms of technical operation. Therefore, future research needs to identify a screening technique that is technically convenient, rapid and cost-effective. More importantly, most of the recent studies on strain modification have been limited to the laboratory scale, which requires an attempt to scale up production experiments in future studies.

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