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Advances in Microbial Strain Engineering and Its Applications in Synthetic Biology

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Abstract. Microbial strain engineering has progressed from traditional random mutagenesis and recombinant DNA methods to precise genome editing and system-level design powered by synthetic biology. Recent breakthroughs, including CRISPR–Cas technologies, high-throughput automation, and integration with systems biology and artificial intelligence, have greatly enhanced the precision, efficiency, and scalability of strain development. These advances have enabled engineered microbes to serve as versatile "cell factories" in diverse domains. In industry and energy, strains are used for biofuel and green chemical production, supporting carbon neutrality and sustainable biomanufacturing. In medicine, strain engineering facilitates the biosynthesis of complex pharmaceuticals, the development of engineered probiotics, and the creation of vaccines and diagnostics. In agriculture and the environment, engineered microbes contribute to pollutant degradation, nitrogen fixation, crop microbiome modulation, and carbon capture. Despite remarkable progress, challenges remain in ensuring genetic stability, biosafety, and regulatory acceptance. Future directions point toward intelligent design guided by multi-omics data and AI, as well as ecosystem-level engineering using synthetic microbial consortia. Overall, microbial strain engineering is becoming a cornerstone of synthetic biology, offering transformative solutions for energy, health, agriculture, and environmental sustainability.

Keywords: Microbial strain engineering, Synthetic biology, CRISPR–Cas, Metabolic engineering

1. Introduction

Synthetic biology applies engineering principles to redesign biological systems for applications in drug synthesis, energy production, and environmental remediation. Microorganisms—effective "cell factories"—offer ease of genetic manipulation, short cultivation cycles, and high scalability, making them central to strain engineering [1]. Traditional approaches (e.g., mutagenesis breeding) enabled early metabolic engineering but suffer from randomness, low efficiency, and limited precision. Recent advances in genome editing, especially the CRISPR–Cas system (CRISPR, clustered regularly interspaced short palindromic repeats), together with automated high-throughput platforms and the integration of systems biology with machine learning, have markedly improved the efficiency and accuracy of strain design [2]. These technologies overcome key limitations of legacy methods, expand the scope of microbial "reprogramming," and are driving the rapid emergence of

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synthetic biology products across sectors, supported by progress in metabolic engineering, directed evolution, automated strain engineering, metagenomic mining, gene circuit design, and genome editing. Looking forward, microbial strain engineering will increasingly couple with advanced computational and systems biology frameworks to move from stochastic tinkering to precise, system-level optimization, accelerating transformative impacts in medicine, energy, materials, and environmental remediation.

2. Major techniques in microbial strain engineering

The development of microbial strain engineering techniques has broadly progressed from traditional random mutagenesis to targeted gene editing, and then to whole-genome and systems-level high-throughput engineering. This section will follow that trajectory by introducing the main content and advances of traditional modification methods, targeted modification techniques, and system-level and high-throughput engineering strategies.

2.1. Traditional strain improvement methods

Before modern genetic engineering, microbial strain improvement relied mainly on physical and chemical mutagenesis. Physical mutagens such as UV or ionizing radiation and chemical agents like NTG (N-nitroguanidine) or EMS (ethyl methanesulfonate) generated random mutations, followed by phenotypic screening to isolate strains with enhanced traits. These methods, widely applied in fermentation industries, required no prior genetic knowledge but suffered from low efficiency, high randomness, and poor genetic stability. The advent of recombinant DNA technology in the 1970s enabled targeted modifications: plasmid vectors, restriction enzymes, and DNA ligases allowed insertion, deletion, or replacement of genes. Early applications in Escherichia coli and Saccharomyces cerevisiae introduced foreign metabolic pathways, producing proteins like insulin and boosting fermentation yields [3]. These microbes became model engineered strains, inaugurating metabolic engineering. However, limitations such as random chromosomal integration, unstable expression, and difficulty in regulating multi-gene networks underscored the need for more precise and efficient tools. In summary, mutagenesis and recombinant DNA laid the foundation for microbial engineering but also revealed the importance of controllable, targeted modification, motivating the development of advanced strategies for precision strain design.

2.2. Targeted modification techniques

In the 21st century, targeted genetic modification has largely replaced random mutagenesis as the mainstream of microbial strain engineering. The most transformative advance has been the CRISPR–Cas system (CRISPR, clustered regularly interspaced short palindromic repeats), originally a bacterial immune mechanism and repurposed as a universal genome editing tool in 2012–2013. In 2013, researchers first demonstrated CRISPR–Cas9–mediated genome editing in Streptococcus pneumoniae and Escherichia coli, achieving high-efficiency targeted mutagenesis when combined with the λ-Red system [2]. Since then, CRISPR systems are applicable to a wide range of scenarios: Cas9 from Streptococcus pyogenes remains widely used, while Cas12a (Cpf1) recognizes distinct PAM sites and produces sticky ends, offering advantages in certain hosts (Figure 1). These tools now support knockouts, knock-ins, transcriptional regulation, and multiplex editing [4].

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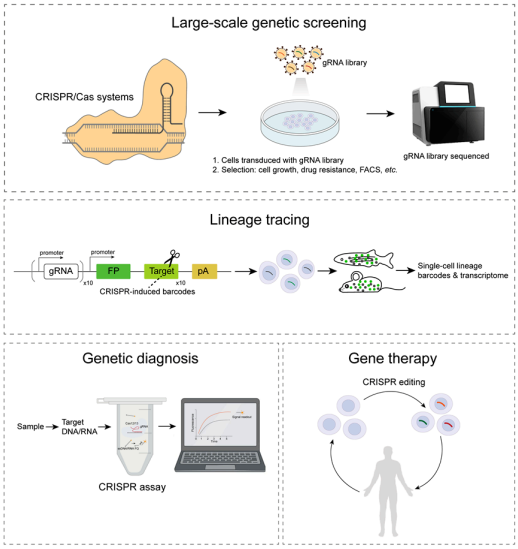

Figure 1. CRISPR-Cas technology applications across various fields [5]

CRISPR-based engineering provides efficiency, programmability, and marker-free selection: altering only the sgRNA sequence enables new targets, while unedited cells are eliminated by nuclease cleavage. Recent innovations include: (1) Scarless editing—precisely introducing mutations without leaving foreign DNA, as in one-plasmid CRISPR systems for E. coli; (2) Base and prime editing—fusing Cas variants with deaminases or reverse transcriptases to achieve single-nucleotide conversions without double-strand breaks; (3) Multiplex editing—using sgRNA arrays or multi-component editors for simultaneous modification of multiple loci. Notably, the INTEGRATE platform, based on a type I-F CRISPR–associated transposase, enables kilobase-scale DNA insertions at defined sites with near 100% efficiency, supporting multi-locus integration and modular genome editing. CRISPR has also proven efficient for "genome sanitation," such as eliminating unnecessary plasmids or prophages.

With expanding editing toolkits—including Cas nuclease variants, base and prime editors—and improved protocols for non-model microorganisms, strain customization is becoming faster and more precise [6]. Many previously intractable microbes, such as natural product–producing actinomycetes, can now be engineered. In summary, targeted modification techniques have established the foundation for rational microbial design, enabling researchers to "program" cell factories with new and optimized functions.

2.3. System-level and high-throughput modifications

With the rise of systems biology, strain engineering has shifted from single-gene manipulation to genome- and system-level optimization. A landmark in this direction was the construction of minimal and synthetic genomes. In 2016, the J. Craig Venter Institute reported JCVI-syn3.0—an artificial bacterium with only 473 genes (531 kb), the smallest genome of any autonomously replicating cell (Figure 2). Despite ~150 genes of unknown function, JCVI-syn3.0 sustained essential life processes and became a model for exploring cellular "minimal requirements". Parallel efforts, such as building synthetic yeast chromosomes, further highlight our growing capacity to redesign entire genomes.

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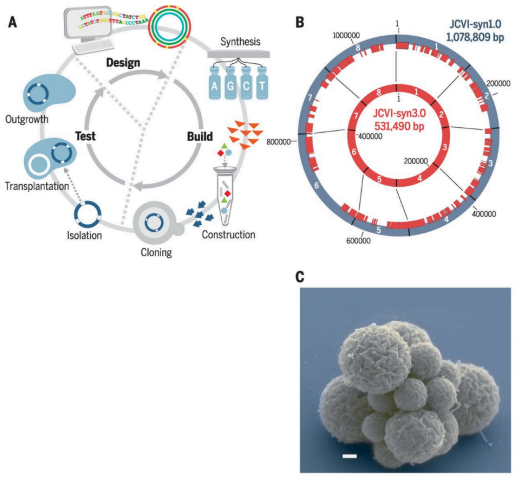

Figure 2. Four design-build-test cycles produced JCVI-syn3.0 [7]

Another system-level strategy is modular metabolic engineering, which divides complex pathways into functional modules (e.g., precursor supply, catalytic reactions, product export). These modules can be recombined to reconstruct natural or synthetic circuits. Examples include transferring plant secondary metabolic modules into yeast to produce natural products, or reconstituting photosynthesis and nitrogen fixation in bacteria. Advances in computational pathway design, enzyme engineering, standardized part libraries, and large-scale genome integration now enable assembly of multi-step pathways in a single step, alongside flux optimization strategies such as dynamic sensor–regulator systems. For instance, quorum-sensing–based dynamic control circuits can automatically balance metabolic fluxes, avoiding intermediate toxicity and boosting titers across products.

High-throughput automated platforms have further accelerated strain optimization. Modern "biofoundries" integrate DNA synthesis, robotics, and microfluidics to parallelize the Design–Build–Test–Learn (DBTL) cycle. Such platforms can construct and screen millions of variants annually, while machine learning extracts patterns from large datasets to guide subsequent design. For example, Zymergen's high-throughput system generated millions of microbial variants to enhance yields of bio-based materials. Multi-omics datasets (genomic, transcriptomic, proteomic, metabolomic) and genome-scale metabolic models (GEMs) are also widely applied to predict beneficial knockouts or overexpressions [8]. Artificial intelligence further enhances efficiency: the MutCompute platform predicted key mutations in PET hydrolase, yielding FAST-PETase with dramatically improved plastic degradation.

In summary, microbial strain engineering is progressing from single-gene edits to genome-scale design, and from low-throughput experimentation to automated, intelligent search. By integrating systems biology, high-throughput automation, and AI-driven design, strain engineering is evolving into an industrial-scale, system-level discipline, enabling the rapid creation of customized microbial cell factories for medicine, energy, materials, and environmental applications.

3. Applications of strain engineering in synthetic biology

Modern microbial strain engineering techniques have already shown enormous application potential across multiple domains. In this section, we review recent advances in engineered strains in three areas—industry and energy, medicine and health, and environment and agriculture. These case

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studies demonstrate how strain engineering is driving the development of biomanufacturing and providing innovative solutions to societal challenges.

3.1. Industry and energy

Industrial biotechnology was the earliest beneficiary of microbial strain engineering, enabling "biology to replace chemistry" by redirecting microbial metabolism. In biofuels, engineered yeasts and bacteria now produce ethanol, biodiesel, and advanced fuels with higher energy density (Figure 3). For example, optimizing the isopentanol pathway in E. coli markedly increased titers, while engineering efforts for butanol and isoprene are approaching industrial requirements. Reviews note that although ethanol and fatty-acid ethyl esters are partially commercialized, further improvements in yield, cost reduction, and host tolerance remain critical. Strategies include rewiring central metabolism for efficient carbon flux, evolving tolerance to harsh conditions, and applying machine learning to identify stress-related genes for strain optimization.

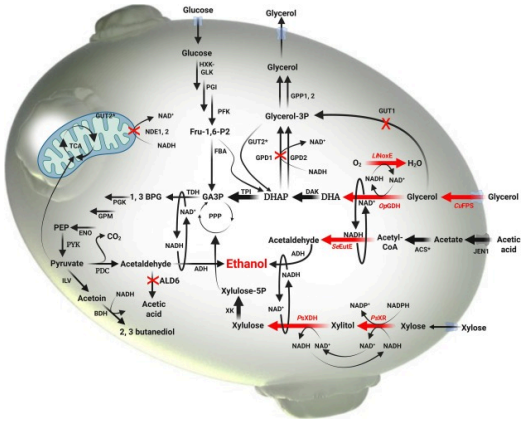

Figure 3. A schematic diagram of ethanol production using yeast [9]

Beyond fuels, engineered microbes produce bulk chemicals and monomers. E. coli and lactic acid bacteria have been optimized for lactic acid, a precursor of biodegradable PLA; strategies such as mitochondrial pathway relocation or quorum-sensing–based dynamic regulation significantly boosted titers [10]. Succinic acid has reached titers above 100 g/L via pathway optimization, while metabolic engineering of 1,4-butanediol, 1,3-propanediol, and terephthalic acid employs precursor enhancement, heterologous pathway introduction, and co-culture systems. Biodegradable polymers are also advancing: PLA and polyhydroxyalkanoates (PHA) have been produced in microbes, with systems metabolic engineering enabling E. coli to synthesize lactate–glycolate copolymers (PLGA) directly from glucose. Further optimization—such as β-oxidation knockouts and PHA synthase overexpression—has raised PHA content to >50% of cell dry weight [11,12].

Strain engineering is also contributing to carbon neutrality and circular economy goals [13]. For instance, Pichia pastoris was engineered for autotrophic growth on CO_2 alone, while E. coli was reprogrammed with Rubisco and phosphoribulokinase to utilize CO_2 through a synthetic Calvin cycle. Although efficiencies remain below those of natural photosynthetic organisms, these pioneering cases point toward using industrial CO_2 emissions as feedstock for chemical synthesis [14].

In summary, microbial strain engineering in industry and energy has expanded from yield and cost optimization to broader goals of reducing petrochemical dependence and valorizing carbon

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waste. Synthetic biology is effectively transforming the “factory” from steel-based infrastructure into living organisms, offering sustainable and environmentally friendly manufacturing routes.

3.2. Medicine and health

Microbial strain engineering is increasingly central to medicine, supporting drug biosynthesis, engineered probiotics, and vaccine and diagnostic development.

3.2.1. Pharmaceutical biosynthesis

Engineered microbes have enabled the production of complex natural products traditionally dependent on rare plants. A landmark case is yeast production of artemisinic acid, the precursor of artemisinin: by introducing plant-derived enzymes and optimizing fermentation, high-yield strains were developed, leading to a semi-synthetic artemisinin production process by Sanofi. Although later discontinued due to cost, this case demonstrated the feasibility of industrial-scale microbial drug biosynthesis. Similar strategies have been applied to the microbial reconstruction of pathways for paclitaxel precursors, opioid intermediates, and other plant-derived alkaloids. In Streptomyces, engineering of silent or weakly expressed secondary metabolite gene clusters has uncovered new antibiotic structures, showing that synthetic biology can generate “innovative antibiotics” beyond natural diversity.

3.2.2. Engineered probiotics (live biotherapeutics)

Commensal microbes can be endowed with therapeutic functions. For example, Lactococcus lactis engineered to secrete interleukin-10 alleviated colitis in mice. The probiotic E. coli Nissle 1917 (EcN), with proven safety, has been engineered to deliver therapeutic molecules such as antibody fragments (anti-PD-L1) or metabolic regulators, showing potential in inflammation, metabolic disease, and cancer [15]. Diagnostic and therapeutic EcN strains—capable of sensing gut inflammation, lowering blood glucose, or modulating the tumor microenvironment—have been reported globally, with some entering clinical trials. Safety concerns, such as EcN’s production of colibactin, are addressed by deleting relevant genes or introducing biosafety switches. Overall, live microbial therapeutics represent a paradigm shift: microbes functioning as “walking drug factories” and dynamic regulators inside the host [16].

3.2.3. Vaccines and diagnostics

Engineered microbes remain key platforms for antigen production, as in the hepatitis B and HPV vaccines. Recombinant systems, including engineered baculovirus–insect cells, accelerated vaccine development for SARS-CoV-2, while lactic acid bacteria are being explored as oral vaccine carriers. In diagnostics, synthetic biology has produced living biosensors such as EcN engineered to fluoresce in feces upon detecting intestinal inflammation, and CRISPR-integrated bacterial systems for real-time pathogen monitoring.

In summary, microbial strain engineering is driving medicine toward new paradigms: from microbial cell factories supplying drugs and vaccines, to engineered probiotics that act as live therapeutics and biosensors. These advances suggest a future where “the medicine is a microbe, and the microbe is the medicine.”

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3.3. Environment and agriculture

Microbial strain engineering holds great promise for environmental protection and sustainable agriculture, enabling pollutant degradation, ecological remediation, and greener farming practices (Figure 4).

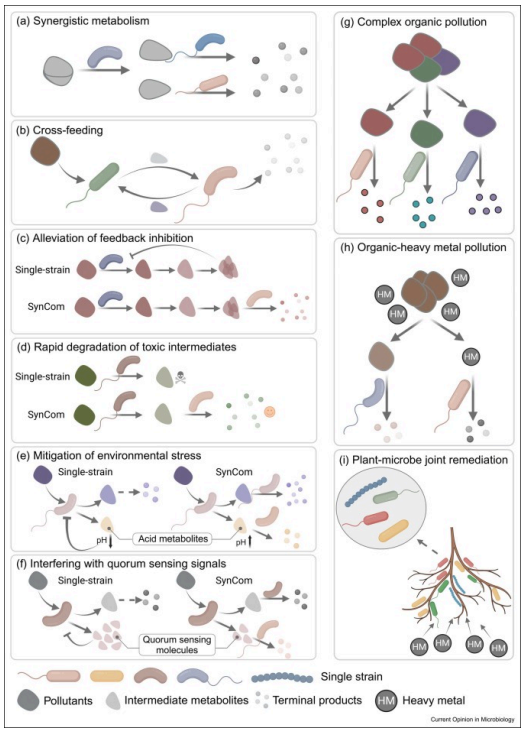

Figure 4. A schematic diagram of the synergistic mechanisms and advantages of engineered microbial strains in bioremediation [17]

3.3.1. Environmental remediation

Microbes drive biogeochemical cycles and serve as frontline agents against pollution. Engineered strains can broaden degradation capacity or improve efficiency. For example, directed evolution of PET hydrolases yielded high-performance enzymes: in 2020 Carbios reported an engineered variant that depolymerized 90% of PET bottles within 10 hours, while in 2022 the machine learning–designed FAST-PETase achieved complete PET depolymerization at room temperature in 24–48 hours. Similar engineering has enhanced microbial degradation of hydrocarbons, PCBs, and dioxins, and enabled recovery of heavy metals from wastewater. Synthetic ecology approaches—assembling engineered consortia into biofilms or reactors—further expand remediation potential by enabling cooperative pollutant breakdown.

3.3.2. Agricultural applications

Excessive nitrogen fertilizer use drives costs, eutrophication, and greenhouse gas emissions. Strain engineering seeks alternatives through biological nitrogen fixation. A notable case is Pivot Bio's PROVEN product: an engineered γ-proteobacterium colonizing corn roots, re-activated for nitrogen fixation, reduced fertilizer demand by ~25 lbs/acre while maintaining yields, and was applied on millions of U.S. acres by 2021 [12]. Similar approaches are being extended to wheat and rice.

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Beyond nitrogen, engineered rhizosphere microbes can promote root development, secrete phytohormones, or provide biopesticide activity. For instance, Pseudomonas carrying a Bt toxin gene functions as a living insecticide, while other engineered strains can sense pathogen signals and release antagonistic molecules—strategies aligned with "crop microbiome engineering" to enhance resilience, yield, and health.

3.3.3. Carbon capture and synthetic ecosystems

Engineered autotrophic yeast and CO_2-fixing E. coli exemplify strains designed for carbon assimilation, with potential for coupling emissions mitigation with product synthesis. Engineered cyanobacteria and algae with optimized photosynthetic pathways have shown improved CO_2 fixation and biomass yields. At the ecosystem scale, "synthetic ecosystems" combining engineered bacteria, yeast, and algae have been proposed for wastewater treatment, soil remediation, and biomining, integrating pollutant removal with resource recovery.

In environmental and agricultural contexts, strain engineering enables solutions unattainable by conventional methods: microbes that degrade plastics and toxic chemicals, symbionts that replace fertilizers and pesticides, and photosynthetic organisms that fix carbon while producing fuels. As one commentary emphasized, "what was once pollution can, through engineered microbes, become a resource". Such advances not only provide direct ecological and economic benefits but also chart a path toward a circular, efficient, and sustainable bioeconomy [18].

4. Conclusion and outlook

Microbial strain engineering has progressed from random mutagenesis and recombinant DNA to precise genome editing and system-level optimization. These technologies are reshaping industry, medicine, agriculture, and the environment, enabling cleaner production, new therapies, and sustainable practices. Yet significant challenges remain.

Engineered strains that perform well in laboratories may lose productivity or accumulate mutations under industrial or environmental conditions [3]. Improving genetic stability—such as by integrating key genes into the genome or removing mobile elements—together with process optimization will be essential for practical applications. At the same time, biosafety measures such as kill switches or nutrient dependencies, as well as ecological risk assessments, are necessary to prevent unintended impacts. Ethical and regulatory concerns are also increasingly important. Questions about the stability of therapeutic microbes inside patients or the possibility of gene exchange in natural environments highlight the need for rigorous evaluation, transparent communication, and regulatory frameworks that safeguard public trust while enabling innovation.

Looking ahead, strain engineering is expected to move toward intelligent design and ecosystem-level approaches. AI-driven platforms that integrate multi-omics data may support "digital microbe" simulations to predict the outcomes of modifications and guide experiments more efficiently [1]. In parallel, attention is shifting from single strains to synthetic microbial consortia for applications such as wastewater treatment or gut therapies, marking the emergence of a new field of "synthetic ecology." At the same time, fundamental research questions remain. The creation of minimal cells raises questions about the essential gene set, while engineered probiotics underscore the complexity of host–microbe interactions. These insights will continue to inform and refine the discipline.

In sum, microbial strain engineering is giving us the ability to "program" life: in factories, engineered strains are producing fuels, materials, and medicines; in agriculture and healthcare, they are beginning to safeguard crops and human health. But this power must be accompanied by

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responsibility—ensuring safety, stability, and ethical oversight. With continued collaboration between science, industry, and regulation, microbial strain engineering is poised to deliver profound benefits and realize synthetic biology's vision of advancing society while protecting the environment.

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