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Unlocking the potential of biosurfactants: Innovations in metabolic and genetic engineering for sustainable industrial and environmental solutions

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ABSTRACT

Biosurfactants, synthesized by microorganisms, hold potential for various industrial and environmental applications due to their surface-active properties and biodegradability. Metabolic and genetic engineering strategies enhance biosurfactant production by modifying microbial pathways and genetics. Strategies include optimizing biosurfactant biosynthesis pathways, expanding substrate utilization, and improving stress responses. Genetic engineering allows customization of biosurfactant characteristics to meet industrial needs. Notable examples include engineering *Pseudomonas aeruginosa* for enhanced rhamnolipid production and creating synthetic biosurfactant pathways in non-native hosts like *Escherichia coli*. CRISPR-Cas9 technology offers precise tools for genetic manipulation, enabling targeted gene disruption and promoter optimization to enhance biosurfactant production efficiency. Synthetic promoters enable precise control over biosurfactant gene expression, contributing to pathway optimization across diverse microbial hosts. The future of biosurfactant research includes sustainable bio-processing, customized biosurfactant engineering, and integration of artificial intelligence and systems biology. Advances in genetic and metabolic engineering will enable tailor-made biosurfactants for diverse applications, with potential for industrial-scale production and commercialization. Exploration of untapped microbial diversity may lead to novel biosurfactants with unique properties, expanding the versatility and sustainability of biosurfactant-based solutions.

1. Introduction

Surfactants, also referred to as surface-active agents, represent a diverse category of chemical compounds with fundamental roles spanning various industries, commerce, and households.[1,2] These compounds, characterized by their amphiphilic nature, possess both hydrophilic (water-attracting) and hydrophobic (water-repelling) segments within their molecular structures. This unique configuration enables surfactants to reduce liquid surface tension, enhance emulsification, and facilitate stable mixtures between otherwise immiscible substances. Acting at interfaces between different phases, such as liquid-gas or liquid-liquid boundaries, surfactants mitigate interfacial tension, giving rise to micelles, emulsions, and foams with diverse applications. Classification of surfactants is based on their chemical composition and origin.[3,4] Synthetic surfactants, derived from petroleum-based materials, have entrenched themselves across numerous industries due to their performance, versatility, and cost-effectiveness. Examples include alkyl benzene sulfonates, alcohol ethoxylates, and quaternary ammonium compounds.[5,6] Conversely, bio-based surfactants produced, sourced from renewable origins like plant oils, sugars, and proteins and microbial surfactants produced by bacteria, fungi, and yeasts exhibits biodegradability, low toxicity, and eco-friendly characteristics along with better specificity and sensitivity.[6–11]

In recent years, there has been a notable emphasis on the development of sustainable surfactants. These compounds play integral roles in diverse applications, bridging the divide between disparate substances owing to their amphiphilic properties. Functioning at phase interfaces, surfactants facilitate essential structures and processes, thereby shaping industries and aligning with sustainability objectives.[11–15]

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Table 1
Common microorganism with their biosurfactants.

Source	Microorganisms/ Examples	Biosurfactant Production	References
Bacteria	*Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus* spp.	Surfactin, Rhamnolipids, Glycolipids	[18,19],106, 107
Fungi	*Candida* spp., *Aspergillus* spp., *Rhodotorula* spp.	Sophorolipids, Mannosylerythritol lipids	[20–23]
Yeasts	*Saccharomyces* spp., *Candida* spp., *Yarrowia* spp.	Sophorolipids, Glycolipids	14,[24,25]
Actinomycetes	*Streptomyces* spp.	Lipopeptides, Glycolipids	[26,27,28]
Marine Microbes	*Pseudomonas mendocina* ADY2b, deep-sea bacteria	Rhamnolipids, Lipopeptides	[18,29,28]
Alcanivorax	*Alcanivorax borkumensis*	Glycolipid	33,108

The table provided details of common microbial sources used for production of common biosurfactants.

This review uniquely contains comprehensive analysis of the genetic and metabolic foundations of biosurfactant production, integrating extensive coverage of gene diversity, advanced metabolic engineering, and a detailed focus on synthetic promoters. It meticulously details the genes involved across various microorganisms, including well-known genes like rhlAB in *Pseudomonas aeruginosa* and less common ones like CbsA in *Bacillus clausii*, showcasing the genetic adaptability and diversity of biosurfactant-producing microbes.

2. Sources of microbial surfactants

Microbial surfactants, a subset of biologically derived surfactants, exhibit significant promise due to their varied sources and environmentally friendly production methods. These compounds are synthesized and secreted by diverse microorganisms, including bacteria, fungi, yeasts, and actinomycetes, as inherent components of their growth and metabolic processes (Table 1).[11,16,17]

Bacteria, recognized as prominent producers of microbial surfactants, demonstrate adaptability across various environments, from soil ecosystems to hydrothermal vents. Species such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, and various *Lactobacillus* species have exhibited the capacity to synthesize and excrete surfactants.[18,30–33] Additionally, *Actinomycetes*, (another class of bacteria) known for filamentous growth and the production of various secondary metabolites, including antibiotics, are recognized as surfactant producers, with *Streptomyces* species showing promise for industrial applications.[26,34] Similarly, fungi, particularly those within the *Candida*, *Aspergillus*, and *Rhodotorula* genera, contribute to the pool of microbial surfactant sources, often thriving in soil and organic matter environments.[24,27,35] Yeasts, a subset of fungi, also exhibit surfactant-producing potential, with species within the *Saccharomyces*, *Candida*, and *Yarrowia* genera identified as surfactant producers.[14,24,25]

The marine environment, characterized by its vastness and biodiversity, serves as a rich reservoir of microbial diversity with surfactant-producing capabilities. Marine microorganisms, spanning bacteria, fungi, and yeasts, have been explored for their surfactant potential.[26,36,29] Recent studies have underscored the significance of marine microbes in surfactant production, with examples such as *Pseudomonas mendocina* ADY2b and biosurfactants from deep-sea bacteria exhibiting notable properties for environmental and pharmaceutical applications.[37,38] Another species, *Alcanivorax borkumensis* produces biosurfactants like glycine-glucolipid, which aid in emulsifying and degrading hydrocarbons during oil spills. This enhances the bacterium's ability to utilize hydrocarbons, significantly contributing to bioremediation.[39]

3. Factors affecting microbial surfactant production

Microbial surfactant production is intricately influenced by factors such as microorganism selection, substrate availability, and environmental conditions. Optimal production requires microorganisms with high surfactant efficiency or genetic modifiability, and substrates like sugars and nitrogen sources that support growth and synthesis. Environmental parameters including temperature, pH, dissolved oxygen, and agitation rates are critical, with efficient oxygen transfer being particularly essential for aerobic microorganisms to maximize surfactant yield. Recent studies have highlighted the importance of environmental conditions in surfactant production.[40–42] For example, in a study conducted by Motwali et al. (2021), the biosurfactant production of *Pseudomonas balearica* isolated from oil-contaminated seawater was investigated. Optimal conditions for rhamnolipid biosurfactant yield were determined, with olive oil and urea as preferred carbon/nitrogen sources. The best results were obtained with a C/N ratio of 30, pH 7, 2 % inoculum size, incubation at 30 °C for 312 h.[43] Similarly in an another study, optimum conditions for biosurfactant production were determined, with olive oil (2 %) and glutamic acid (0.2 %) as the best carbon and nitrogen sources, respectively. Maximum biosurfactant production occurred after a 5-day incubation period with a 3 % inoculum size.[44]

The duration of the fermentation process, or fermentation time, also plays a role in surfactant production. Some microorganisms exhibit surfactant synthesis during the exponential growth phase, while others emphasize production during the stationary phase.[45] Even it was seen that after certain period of time, the growth of microorganisms decreases as seen in the study of Heryani and Putra (2017) where the growth of *Bacillus* increased in glucose containing medium during initial 18 h of fermentation and then inhibited due to increase in biosurfactant production and less glucose concentration.[46] Induction and regulation mechanisms can affect surfactant production. Certain microbial surfactants are exclusively produced under specific induction conditions or in response to stress factors.[47] Even ultra sound has impact on biomass and biosurfactant production. It was also seen that coal can also induce biosurfactant production by *P. stutzeri*. These indicates that understanding these regulatory mechanisms and inducing surfactant production strategically can lead to elevated yields.[48]

The concept of co-culturing or mixed cultures involves combining different microorganisms to achieve synergistic effects. Metabolites produced by one microorganism can stimulate surfactant production in another, potentially enhancing overall surfactant yield and diversifying product range.[49–51] For example, the study of Kamyabi et al. (2016) have investigated the co-culture ability of *Sarocladium* sp. and *Cryptococcus* sp. yeast isolates in surfactant production and oil degradation, revealing a synergistic effect with 28 % higher oil removal and 35 % increased biomass production compared to individual cultures. The co-culture demonstrated superior surface tension reduction, emulsification activity, and cell surface hydrophobicity, leading to a 40 % increase in pyrene degradation, emphasizing the enhanced performance of the yeast co-culture in oil degradation processes.[49] In the very latest research carried out by Wu et al. (2023) have determined the synergistic effects of a defined co-culture comprising *Bacillus subtilis* SL and *Pseudomonas aeruginosa* WJ-1 on crude oil biodegradation. The co-culture demonstrated significantly enhanced degradation efficiency, increasing from 32.61 % to 54.35 % in individual cultures to 63.05 %. Not limited to this the biosurfactant-producing bacteria (*Bacillus subtilis* B1 and *Pseudomonas aeruginosa* B2) proven effective in crude oil degradation as well as demonstrates synergistic power generation (6.3 W/m^3, 970 mV open circuit voltage) when applied as microbial fuel cells.[51]

In a recent research work, high-quality physiological experiments with *Alcanivorax borkumensis* SK2 faced challenges such as poor growth, difficult biomass determination, and inadequate analytics for biosurfactant production. Optimized cultivation in modified ONR7a medium with hydrophilic and hydrophobic carbon sources revealed that

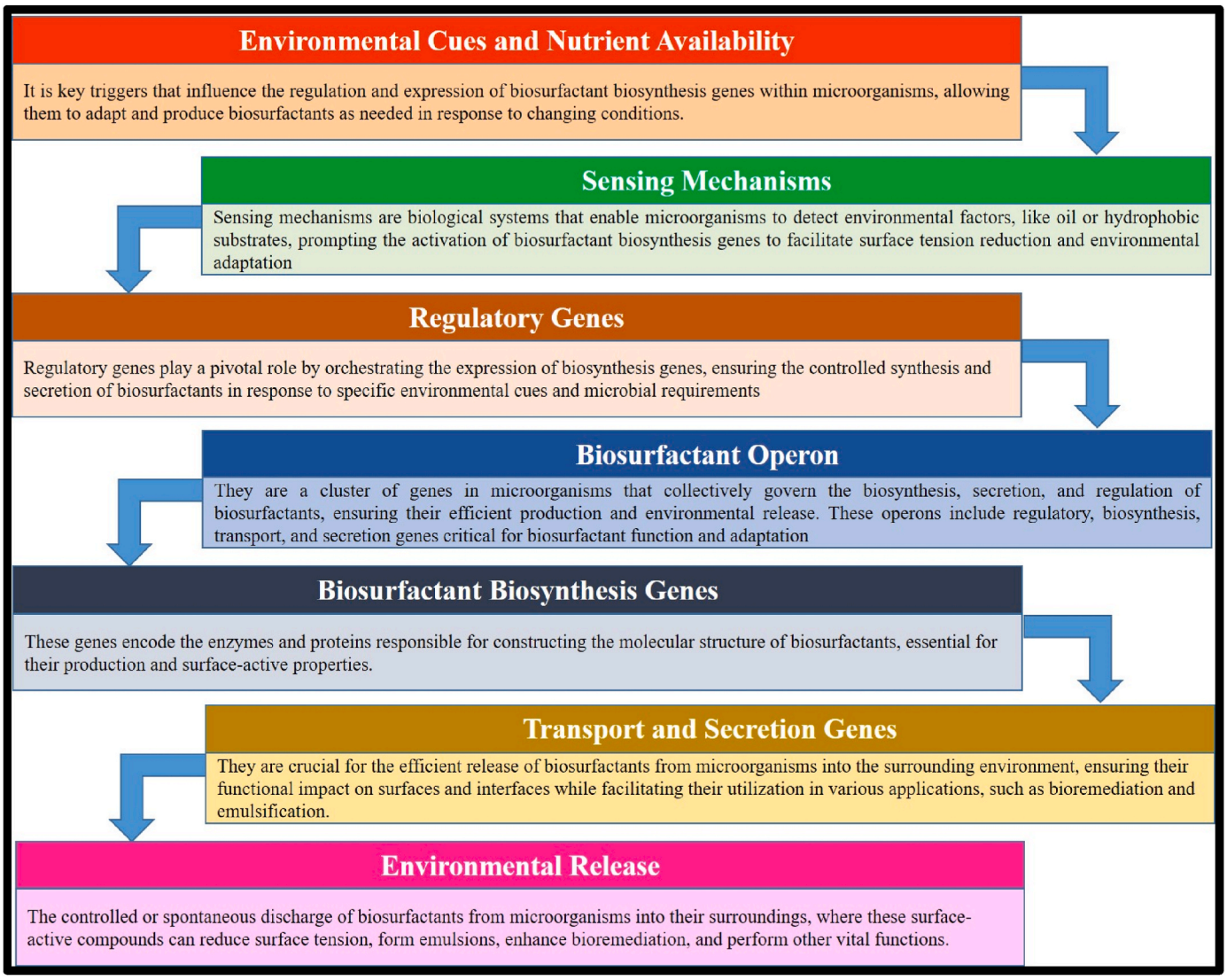

Fig. 1. General Mechanism of gene expression and biosurfactant production (Figure illustrate the activation of sensing mechanisms and genes involved in biosurfactant production).

hydrophobic substrates like n-tetradecane significantly enhance glycolipid production. Stirred-tank bioreactors improved growth and product formation kinetics compared to shake flasks. Acetate proved suitable for controlled conditions, facilitating better physiological studies. Bubble-free membrane aeration mitigated foam formation without anti-foaming agents but resulted in lower biomass and glycolipid yield due to biofilm formation.[39] In another study on *Aureobasidium pullulans*, researchers optimized a minimal medium for producing polyol lipids (liamocins) by achieving a 56 % increase in titer to 48 g/L and improved space-time yield in microtiter plate cultivations. The process was successfully scaled to a 1 L bioreactor, enabling further exploration of carbon sources and metabolic pathways for industrial applications.[52]

By genetically modifying microorganisms to enhance the expression of crucial enzymes involved in surfactant biosynthesis, researchers can achieve increased yields. Recent studies have explored genetic modifications to improve surfactant production, further expanding the potential applications of microbial surfactants across various industries.[53–56] Details of genetic engineering are discussed in the later part of this review.

4. Genes involved in biosurfactant

Biosurfactants are synthesized through the activity of specific enzymes and/or genes within microorganisms. Fig. 1 represents the common mechanism for production of biosurfactant. This may vary depending on the type of microorganism and gene involved. The production of Rhamnolipids, classified as glycolipid biosurfactants, is primarily attributed to the *rhlAB* gene in *Pseudomonas aeruginosa*. These microorganisms are frequently isolated from natural habitats, including soil and aquatic environments.[57–59] Similarly, Surfactin is synthesized via the *srfA* gene within *Bacillus subtilis*, commonly found in soil and the rhizosphere, as well as other environments.[60,61] *Bacillus subtilis* also contributes to Iturin production through the *ituD* gene, with such biosurfactant-producing microorganisms being inherent to soil ecosystems and plant-associated niches.[3,54] Lichenysin, another lipopeptide biosurfactant, is associated with the *lichenysinA* gene within *Bacillus licheniformis*, commonly found in soil and fermented food products.[18,62] Mannosylerythritol Lipids, another class of biosurfactants, are attributed to various microbial species such as *Pseudozyma* spp., inhabiting a range of environmental niches including plants and related ecosystems.[3,17,63] Emulsan biosurfactants, synthesized by *Acinetobacter calcoaceticus* via the *emulsanB* gene, are predominantly found in aquatic environments,

Table 2
Common genes involved in Biosurfactant Production.

Gene Name	Biosurfactant Class	Microorganism	Source	Reference
rhlAB	Rhamnolipids	*Pseudomonas aeruginosa*	Soil and water environments	[57,58,73]
srfA	Surfactin	*Bacillus subtilis*	Soil and rhizosphere	[61]
ituD	Iturin	*Bacillus subtilis*	Soil and plant-associated environments	55,106
lichenysinA	Lichenysin	*Bacillus licheniformis*	Soil and fermented food products	21,56,109
mannosyltransferase	Mannosylerythritol Lipids	*Pseudozyma* spp.	Environmental habitats, including plants	48,110
emulsanB	Emulsan	*Acinetobacter calcoaceticus*	Aquatic environments, including water bodies	2,111,112
cellobiose lipid	Cellobiose Lipids	*Ustilago maydis*	Plant pathogen, found in maize	1,111
sophorolipid synthase	Sophorolipids	*Starmerella bombicola (formerly Candida bombicola)*	Yeast isolated from honeybee hives	[20,22,23]
trehalolipid synthase	Trehalolipids	*Rhodococcus erythropolis*	Soil and hydrocarbon-contaminated sites	74,113
arthrofactin synthase	Arthrofactin	*Arthrobacter* spp.	Soil and rhizosphere	8,65,114
vreI	Viscosin	*Pseudomonas fluorescens*	Soil and plant rhizospheres	[74,75]
Sfp	Lichenysin and Surfactin	*Bacillus* spp.	Soil and varied environments	54,55,106
orb operon	Orfamide	*Pseudomonas fluorescens*	Soil and plant-associated environments	[74,75]
xylolipid synthase	Xylolipids	*Lysinibacillus sphaericus*	Soil and plant-associated environments	2,10,15
mannosylerythritol lipid	Mannosylerythritol Lipids	*Pseudozyma* spp.	Environmental habitats, including plants	[2,41,68,69]
surfactin operon	Surfactin	*Bacillus subtilis*	Soil and varied environments	[61,76,77,78,79]
lipA	Lipopeptides	*Various bacteria, including Bacillus* spp.	Varied, commonly found in soil	15,64,115
bpsA	Butirosin	*Bacillus cereus and Bacillus subtilis*	Soil and fermentation processes	55,103,116
Sfp	Siderophore-Like Lipopeptides	*Pseudomonas* spp.	Soil and rhizosphere	80,117
bacillomycin operon	Bacillomycins	*Bacillus* spp.	Soil and plant-associated environments	12,61,118
mupirocin operon	Mupirocins	*Pseudomonas fluorescens*	Soil and nasal flora	80,117,119
pumA	Pumilacidin	*Bacillus pumilus*	Soil and plant rhizospheres	[31]
tofI	Tofacitin	*Burkholderia gladioli*	Soil and plant-associated environments	[14,29,68]

Table provides information of various genes involved on biosurfactant production along with their microorganisms and sources.

notably water bodies[13,64] (Table 2). The genetic diversity involved in biosurfactant production underscores several critical aspects, such as metabolic versatility, ecological adaptability, and evolutionary potential. It highlights the remarkable adaptability of microorganisms to a wide array of environmental conditions, enabling them to modulate biosurfactant production in response to various ecological niches. This genetic variability encompasses a multitude of genes and regulatory pathways, each contributing to the synthesis and functionality of different biosurfactants. Additionally, this diversity reflects the chemically diverse nature of biosurfactants, which exhibit a broad spectrum of surface-active properties and functions. Furthermore, the genetic diversity presents significant opportunities for biotechnological applications, as researchers investigate various classes of biosurfactants and their associated genes for specific industrial and environmental purposes.[1,2,65–67] The distribution of biosurfactant-producing microorganisms across diverse natural environments underscores the ecological significance of these compounds and their pivotal roles in microbial interactions and environmental processes.[15,68–70] The phylogenetic diversity observed among biosurfactant producers, spanning from bacteria to yeast, accentuates the variability in genetic makeup and metabolic pathways associated with biosurfactant synthesis.[14,24,25,29,37] The intricate interplay between environmental cues, microbial physiology, and genetic regulation contributes to the dynamic nature of biosurfactant production across different microbial species and environments. Ultimately, the genetic diversity observed in biosurfactant production serves as a valuable resource for bioprospecting endeavours. By tapping into this diversity, researchers can uncover novel biosurfactants with unique properties suitable for various applications. This bioprospecting approach not only expands the repertoire of available biosurfactants but also enriches our understanding of microbial systems and their potential contributions to biotechnology and environmental processes.[29,65,66,71,72]

In the realm of biosurfactant production, several less common genes have been identified across different microorganisms, each responsible for the synthesis of distinct classes of biosurfactants with unique properties. One such gene, *CbsA*, found in *Bacillus clausii*, is associated with the production of cyclic lipopeptides, which exhibit notable antimicrobial properties.[54,76] Similarly, the gene *TspR*, identified in *Pseudomonas fluorescens*, is involved in the biosynthesis of tensin, a lipopeptide biosurfactant known for its antifungal and antiviral activities.[54] Another less common gene, *DofA*, present in *Pseudomonas fluorescens*, plays a key role in the production of dirhamnolipid glycolipids, which find applications in bioremediation processes.[67,80] Additionally, in *Bacillus thuringiensis*, the gene KrmA is responsible for the production of kurthiosurfactin, a relatively novel class of lipopeptide biosurfactants.[30,55] In *Acinetobacter* species, the gene *ErdR* is linked to the biosynthesis of emulsan glycolipids, notable for their emulsifying properties.[66,69] Furthermore, the gene *LtpR*, identified in *Lysinibacillus fusiformis*, is associated with the production of liposan glycolipids, which have been found to enhance oil recovery processes.[30]

These less common genes represent crucial components in the biosurfactant production pathways of their respective microorganisms, contributing to the diversity of biosurfactants available with distinctive characteristics and applications across various industries. The exploration of these genes opens new avenues for the discovery and development of biosurfactants with unique properties that could be tailored for specific applications. These biosurfactants have significant potential across multiple fields due to their unique properties, such as enhanced antimicrobial, antifungal, and antiviral activities, making them valuable in pharmaceuticals and healthcare.[12] Additionally, these biosurfactants show promise in environmental applications, particularly in bioremediation processes, where they can help in the degradation of pollutants and oil spills. Their ability to emulsify and break down hydrophobic compounds makes them ideal for cleaning up contaminated sites.[8] Moreover, in agriculture, these biosurfactants can protect plants from pathogens and enhance soil health, leading to increased crop yields and sustainable farming practices.[81] Industrially, biosurfactants produced by these less common genes can be used in petroleum recovery, improving the efficiency of oil extraction processes. Their stability under extreme conditions of temperature, pH, and salinity further extends their applicability in harsh industrial environments. The potential for genetic engineering and synthetic biology to manipulate these biosurfactant-producing genes further expands the possibilities for creating custom-tailored biosurfactants with desired properties, leading to innovations in product formulations and process optimizations across various sectors.[8] The continuous research and bioprospecting efforts focusing on these genes are likely to uncover more novel biosurfactants with unique properties, thereby expanding the repertoire of available biosurfactants and enhancing our understanding of microbial ecology and biotechnology. This genetic diversity is a testament to the adaptability and ingenuity of microbial life, offering valuable insights and tools for addressing some of the most pressing challenges in science and

industry today. By leveraging the genetic potential of these microorganisms, we can develop sustainable solutions that benefit both the environment and the economy.

5. Comparative analysis of different genes and their strategies for production of biosurfactant

The production of biosurfactants such as rhamnolipids, surfactin, iturin, lichenysin, and emulsan involves various microorganisms and strategies to enhance yield. For rhamnolipids, *Pseudomonas aeruginosa* utilizes the *rhlAB* genes with strategies like plasmid-based gene overexpression, which offers high yields but faces plasmid instability. Genomic integration provides stable expression but often results in lower yields. CRISPR-Cas9 editing offers precise modifications but is technically demanding. Fermentation optimization, while scalable, is costly. *Burkholderia thailandensis* uses *rhlABC* and *rhlABR* genes with similar strategies and faces comparable trade-offs. *Pseudomonas fluorescens* also employs rhl genes for rhamnolipid production, utilizing plasmid-based overexpression and genomic integration, providing high yield and stability but dealing with plasmid loss and complexity in genomic modifications. For surfactin production, *Bacillus subtilis* with the *srfA* gene employs fermentation optimization, achieving high yields but at a high cost. Genetic knockouts can improve yield but may impact cell viability. Metabolic engineering allows for customizable properties but involves complex processes. Synthetic promoters enable precise control over gene expression but are challenging to design. Adaptive laboratory evolution enhances tolerance and yield, though it is time-consuming. Bacillus amyloliquefaciens and *Bacillus licheniformis* use *srfAA* and *srfAB* genes, respectively, facing similar advantages and disadvantages. Iturin production in *Bacillus subtilis* involves the ituD gene with strategies like CRISPR-Cas9 for precise editing, synthetic promoters for controlled expression, and metabolic engineering for increased yield, each requiring significant expertise and resources. Fermentation optimization offers scalable production but is expensive. Genetic knockouts enhance yield but can affect growth. Bacillus licheniformis with the ituA gene uses similar methods and faces similar trade-offs. Lichenysin production by *Bacillus licheniformis* involves the *lchAA* gene with strategies such as metabolic engineering and adaptive laboratory evolution, providing customizable properties and enhanced yield but requiring complexity and time. Genetic knockouts improve yield but can affect growth, and CRISPR-Cas9 offers precision but is technically demanding. Fermentation optimization provides high yield but incurs high costs. For emulsan production, Acinetobacter calcoaceticus utilizes the *emulsanB* gene, employing agro-industrial waste for cost-effective production but yielding variable results. High-density fermentation can produce large quantities but requires significant investment. Genetic knockouts enhance production at the risk of affecting cell viability. CRISPR-Cas9 and synthetic promoters provide precision and controlled expression but involve technical complexity. *Acinetobacter venetianus* with the *emulsanA* gene uses similar methods, balancing cost and yield with the challenges of technical expertise and design. Additionally, *Candida bombicola* is involved in sophorolipid production through the *sop1* and *sop2* genes. This yeast employs strategies such as substrate optimization for higher yields, though it faces the challenge of substrate cost. Metabolic engineering enhances production but involves complex genetic modifications. *Starmerella bombicola* also produces sophorolipids, utilizing similar genes and facing similar advantages and disadvantages.

6. Metabolic and genetic engineering for biosurfactant production

Metabolic and genetic engineering represents a potent strategy for enhancing biosurfactant production by strategically modifying the metabolic pathways and genetic makeup of microorganisms engaged in biosurfactant synthesis. This approach involves optimizing biosurfactant biosynthesis pathways, enhancing host microorganisms' capabilities, expanding substrate utilization, balancing carbon flux toward biosurfactant production, and improving stress responses.[18,54,82,83] Additionally, metabolic engineering allows for the fine-tuning of fermentation processes, optimizing parameters such as temperature, pH, and aeration to create optimal conditions for biosurfactant production. Moreover, it enables customization of biosurfactant characteristics to meet specific industrial requirements. This approach holds promise for enhancing biosurfactant production efficiency, increasing yields, and tailoring biosurfactant properties for diverse applications while adhering to regulatory and environmental considerations.[82,84]

Table 3
Metabolic and genetic engineering in biosurfactant production.

Microorganism	Modification	Result	Reference
Pseudomonas aeruginosa	Overexpression of rhlAB genes	Increased rhamnolipid production	[57–59]
Bacillus subtilis	Genomic integration of exogenous glycosyltransferase gene from Pseudomonas species	Improved glycolipid biosynthesis	[51]
Rhodococcus erythropolis	Genetic knockout of competitive pathways involved in triacylglycerol synthesis	Redirected carbon flux toward biosurfactant synthesis	[85–88]
Escherichia coli	Plasmid-based overexpression of stress response genes, such as rpoS	Improved stress tolerance during biosurfactant production	[89–91]
Pseudomonas fluorescens	Optimization of fermentation processes	Improved biosurfactant production conditions	[74,75]
Lactobacillus rhamnosus	Integration of exogenous glycosyltransferase genes, such as rmlA, into its genome	Tailored glycolipid properties to meet industrial requirements	[92,19]
Streptomyces species	Incorporation of inducible promoters into their genome	Enhanced regulatory control over biosurfactant production	[26,27,28]

In the domain of metabolic engineering for biosurfactant production, various microorganisms have been strategically modified to bolster biosurfactant yields (Table 3). For instance, *Pseudomonas aeruginosa* has been engineered to enhance rhamnolipid production by overexpressing the *rhlAB* genes responsible for biosurfactant biosynthesis. This overexpression, achieved via plasmid-based gene overexpression techniques, has resulted in higher yields of rhamnolipids.[57–59] In contrast, *Bacillus subtilis* has harnessed the genomic integration of an exogenous glycosyltransferase gene from *Pseudomonas* species to improve glycolipid biosynthesis Conversely, *Bacillus subtilis* has benefited from genomic integration of an exogenous glycosyltransferase gene from *Pseudomonas* species, augmenting glycolipid biosynthesis.[51] *Rhodococcus erythropolis* underwent genetic knockout of competitive pathways, particularly those involved in triacylglycerol synthesis, redirecting carbon flux toward biosurfactant synthesis.[85–88] In *Escherichia coli*, plasmid-based overexpression of stress response genes, such as *rpoS*, enhanced the organism's ability to withstand stress conditions during biosurfactant production.[89–91] Furthermore, *Pseudomonas fluorescens* optimized fermentation processes by fine-tuning factors like agitation, aeration, and pH control, creating favourable conditions for biosurfactant production.[74,75] *Lactobacillus rhamnosus* tailored glycolipid properties to meet industrial requirements by integrating exogenous glycosyltransferase genes, such as *rmlA*, into its genome.[92,19] Similarly, *Streptomyces* species gained enhanced regulatory control over biosurfactant production through the incorporation of inducible promoters into their genome, enabling precise governance of biosynthesis gene expression.[26,27,28]

An exceptional example of metabolic engineering for biosurfactant production involves the creation of synthetic biosurfactant pathways in *Escherichia coli* for tailor-made sophorolipids. Researchers designed and introduced synthetic genes encoding enzymes crucial for sophorolipid biosynthesis, a class of glycolipid biosurfactants produced by yeast, within *E. coli.* This innovative approach allowed for precise control over sophorolipid structure and properties, tailoring them for specific applications.[20,21] One notable advancement involves the application of synthetic biology techniques to engineer non-native hosts for biosurfactant production. Researchers have successfully constructed artificial biosurfactant pathways within microorganisms such as *Escherichia coli*, which are not natural producers of certain biosurfactants like sophorolipids. By introducing synthetic genes for key enzymatic reactions and optimizing host microorganisms, they have achieved the production of biosurfactants with precisely defined structures and properties. This approach offers precise control over biosurfactant characteristics, making it possible to tailor them for specific industrial applications.[20–23]

This approach is extraordinary from its departure from traditional metabolic engineering, which typically optimizes existing pathways within natural producers. Instead, the researchers created an entirely new biosurfactant production pathway in a non-native host. This innovative approach not only expands the range of biosurfactants that can be produced but also offers the potential for more efficient and customizable biosurfactant production, with properties that can be fine-tuned for specific industrial needs.[84]

Another remarkable example of metabolic engineering for biosurfactant production is the creation of designer biosurfactants through synthetic biology in *Pseudomonas putida*, a bacterium well known for its biodegradability and potential in environmental applications. This ground breaking approach was demonstrated by many previous studies. In this study, researchers harnessed synthetic biology techniques to engineer *Pseudomonas putida* to produce custom-tailored rhamnolipids, a class of biosurfactants with excellent emulsifying properties. By introducing synthetic genes for rhamnolipid biosynthesis and optimizing the host bacterium, they achieved the production of rhamnolipids with precisely defined structures and properties.[95] This degree of customization allowed them to create rhamnolipids with exceptional emulsification capacities, making them ideal for applications in bioremediation, oil recovery, and pharmaceuticals.[83,96]

The recent researches a remarkable advancement in the field of metabolic engineering for biosurfactant production. They have developed an innovative algorithm that optimizes the co-production of multiple metabolites. This is a significant breakthrough because it allows for the simultaneous optimization of various metabolites, potentially leading to the production of a diverse range of biosurfactants in a single process. The sets of their work apart from other studies is the integration of this algorithm with genome-scale metabolic models of *E. coli* and *S. cerevisiae*. These models provide a comprehensive understanding of the metabolic pathways in these organisms, which is instrumental in designing effective metabolic engineering strategies. This approach could revolutionize the biosurfactant production industry by enabling the production of a variety of biosurfactants in a single process, which was not feasible with previous methods.[92,97]

Research on *Pseudomonas aeruginosa* has elucidated the roles of *RhlA* and *RhlB* in rhamnolipid biosynthesis, demonstrating their independent functions rather than forming a *RhlAB* heterodimer complex. This study also revealed the potential for producing designer rhamnolipids with desired physicochemical properties by manipulating the fatty acid content. Heterologous expression of rhl-genes in non-pathogenic *Pseudomonas putida* was employed to bypass complex quorum sensing regulation, enhancing rhamnolipid production.[98] Another study on *Aureobasidium melanogenum* focused on optimizing liamocin production, where genetic modifications in the glucose derepression pathway significantly improved yield and productivity.[99] Furthermore, research on the yeast *Starmerella bombicola* identified the sophorolipid gene cluster, including five genes directly involved in sophorolipid synthesis. It was demonstrated that disabling the cytochrome P450 monooxygenase enzyme halted production, while knocking out the transporter gene significantly reduced secretion levels.[100,101]

Researchers at the Fraunhofer Institute for Interfacial Engineering and Biotechnology have made significant advancements in the production of biosurfactants using smut fungi and yeast. In their studies, smut fungi from the genera *Moesziomyces* and *Ustilago* were optimized to produce high yields of cellobiose lipids (CL) and mannosylerythritollipids (MEL), respectively. Under controlled growth conditions (pH 5.5, 30 °C, 150 rpm) and a fermentation duration of 120 h, *Moesziomyces* species yielded 3.5 g/L of CL, reducing surface tension from 72 mN/m to 28 mN/m, while *Ustilago* species produced 4.2 g/L of MEL, reducing surface tension to 27 mN/m. Optimizing the carbon sources revealed that glucose was the most effective, yielding 4.2 g/L of MEL, compared to 3.8 g/L and 3.5 g/L with fructose and sucrose, respectively. Similarly, peptone as a nitrogen source resulted in the highest yield of MEL at 4.2 g/L. The functional properties of these biosurfactants were notable, with both CL and MEL showing 98 % stability in oil/water emulsions after 24 h and displaying antimicrobial activities with MIC values of 50 μg/mL for CL and 45 μg/mL for MEL against *E. coli*, and 60 μg/mL and 55 μg/mL against *S. aureus*, respectively. Additionally, fermentation using *Rhodotorula* species demonstrated the production of mixed biosurfactants from sugars, with yields of 5.0 g/L when using glucose and 5.5 g/L with a glucose-xylose mix, achieving surface tension reductions to 26 mN/m and 25 mN/m, respectively. These findings highlight the potential of these microbial systems for sustainable and versatile biosurfactant production, suitable for a range of industrial and pharmaceutical applications.[102]

In the study conducted by Schmidt, Carvalho, de Oliveira, and de Andrade, they focused on enhancing the production of surfactin and rhamnolipids, well-known biosurfactants, through the use of biosurfactant inducers. They have proposed an innovative approach to replace the synthetic culture medium, which represents about 30 % of the production cost, with agro-industrial wastes. In addition, they found that biosurfactant productivity can be easily enhanced by inducer supplementation into the culture medium that triggers biosurfactant metabolism. Biosurfactant inducers are mainly a pool of hydrophobic molecules such as olive oil, saturated and unsaturated fatty acids, proteins, and vitamins. In general, hydrophobic inducers lead to higher fatty acid chain lengths in the biosurfactant chemical structure.[103] The narrative illustrates how metabolic and genetic engineering techniques have revolutionized biosurfactant production, offering tailored solutions for industrial needs. By modifying genetic pathways and refining fermentation processes, researchers have optimized biosurfactant synthesis, expanding the repertoire of available biosurfactants. Synthetic biology methodologies have been instrumental, enabling precise control over biosurfactant properties and even creating entirely new production pathways in non-native hosts. Integration of advanced algorithms with metabolic models has further enhanced production efficiency and diversified biosurfactant outputs. Environmental consciousness is evident in the utilization of agro-industrial wastes as culture media and the selection of biodegradable microorganisms, showcasing a commitment to sustainability. The wide-ranging applications of biosurfactants, from bioremediation to pharmaceuticals, underscore their industrial significance. Collaborative efforts across disciplines such as microbiology, genetics, and biotechnology have been pivotal in driving innovation and shaping more sustainable and efficient production paradigms.

7. Synthetic promoters

The demand for synthetic promoters in biosurfactant production arises from their pivotal role in achieving precision, adaptability, efficiency, and regulatory compliance in industrial bioprocesses. Synthetic promoters enable precise control over gene expression, facilitating the optimization of biosurfactant production levels. They also provide

Table 4
Difference between selected natural and synthetic promoters.

Natural Promoter	Synthetic Promoter	Associated Gene(s) or Pathway	Microorganism	Difference	Reference
P_rhl (quorum-sensing)	*P_Rhl-syn* (synthetic)	Rhamnolipid biosynthesis genes	*Pseudomonas aeruginosa*	Natural promoter relies on quorum-sensing, while synthetic provides precise control	[57,73]
P_urfK (native surfactin)	*P_Surf-syn* (synthetic)	Surfactin biosynthesis genes	*Bacillus subtilis*	Natural promoter is native, synthetic offers fine-tuned regulation	[61,79,93]
P_lac (lactose-inducible)	*P_lac-syn* (synthetic)	Custom-designed biosurfactant pathway	*Escherichia coli*	Natural promoter is inducible by lactose, while synthetic is custom-designed	[80,89,90]
P_xylA (xylose-inducible)	*P_xylA-syn* (synthetic)	Custom-designed biosurfactant pathway	*Bacillus licheniformis*	Natural promoter is inducible by xylose, while synthetic is custom-designed	21,56,109,120
P_Lux (quorum-sensing)	*P_Lux-syn* (synthetic)	Biosurfactant genes	*Halomonas elongate and Bacilli*	Natural promoter relies on quorum-sensing, while synthetic provides precise control	[77,94]

Table provides information about the difference between the natural and synthetic promoter associated with various biosurfactant producing genes.

flexibility in designing inducible expression systems, allowing for responses to specific environmental cues or inducers.[104,77] Customization of synthetic promoters permits tailoring to the characteristics of specific host organisms, thereby enhancing adaptability and expanding the range of microbial platforms suitable for biosurfactant production (Table 4). Moreover, it contributes to pathway optimization, thereby improving the overall efficiency of biosurfactant synthesis.[105,78] The utilization of synthetic promoters in biosurfactant production exhibits distinct advantages compared to their natural counterparts. For example, in *Pseudomonas aeruginosa*, the native quorum-sensing promoter *P_rhl* orchestrates rhamnolipid biosynthesis in response to cell density. However, the synthetic counterpart, *P_Rhl-syn*, provides precise control over the biosurfactant pathway without relying on quorum-sensing signals.[57–59,73] Similarly, in *Bacillus subtilis*, the natural surfactin promoter *P_urfK* contrasts with the synthetic counterpart *P_Surf-syn*. While the natural promoter is native and inherently linked to surfactin production, the synthetic variant allows for fine-tuned regulation, enhancing control over biosurfactant synthesis.[103,104,78,79,93] Analogously, in *Escherichia coli* and *Bacillus licheniformis*, the lactose-inducible promoter *P_lac* and xylose-inducible promoter *P_xylA*, respectively, are compared to their synthetic counterparts *P_lac-syn* and *P_xylA-syn*. The natural promoters respond to lactose and xylose induction, respectively, whereas the synthetic versions are custom-designed to regulate custom biosurfactant pathways.[77,78,73] Lastly, in *Halomonas elongata*, the quorum-sensing promoter *P_Lux*, involved in biosurfactant gene expression, contrasts with the synthetic counterpart *P_Lux-syn*.[21,77,94] While the natural promoter relies on quorum-sensing mechanisms, the synthetic version provides precise control over biosurfactant production, showcasing the distinct advantages offered by synthetic promoters in enabling tailored and optimized regulation of biosurfactant pathways.

8. Future prospects

The future prospects of biosurfactant research are marked by exciting possibilities in sustainable bio-processing, customized biosurfactant engineering, and the integration of artificial intelligence and systems biology. The emphasis on eco-friendly production processes using renewable resources is expected to grow, aligning with broader sustainability goals. Advances in genetic and metabolic engineering, particularly through CRISPR-Cas9 technology, will likely enable the creation of tailor-made biosurfactants with specific properties for applications in diverse industries. The integration of artificial intelligence and systems biology tools is anticipated to enhance our understanding of microbial interactions and optimize biosurfactant production processes. As biosurfactants gain recognition, there is potential for industrial-scale production and commercialization, making them competitive alternatives to traditional surfactants. Additionally, the exploration of untapped microbial diversity, particularly from extreme environments, may lead to the discovery of novel biosurfactants with unique properties. Overall, the future of biosurfactants holds promise for sustainable and versatile applications, contributing to advancements in environmental remediation and industrial processes.

9. Conclusion

In conclusion, the world of microbial surfactants is rich and diverse, offering a plethora of opportunities for sustainable and eco-friendly solutions across various industries. The sources of microbial surfactants, ranging from bacteria and fungi to marine microorganisms, highlight the adaptability and versatility of these microorganisms in producing valuable compounds. Understanding the factors influencing microbial surfactant production is crucial for optimizing yields and properties. Microorganism selection, substrate availability, environmental conditions, fermentation time, and co-culturing strategies all play pivotal roles in shaping the outcome of biosurfactant production. Recent advances in metabolic and genetic engineering, including the revolutionary CRISPR-Cas9 technology, have opened new frontiers for enhancing microbial surfactant production. The ability to customize biosurfactant properties through synthetic biology approaches, such as the creation of synthetic biosurfactant pathways, holds tremendous promise for tailoring these compounds for specific industrial applications. Moreover, the identification of less common biosurfactant-producing genes underscores the potential for discovering novel compounds with unique characteristics. The development of synthetic promoters further enhances our ability to precisely control gene expression and optimize biosurfactant production.

In the quest for sustainable practices, the use of agricultural by-products and waste materials as feedstocks, along with the exploration of microorganisms in bioremediation sites, exemplifies a holistic approach towards reducing environmental impact and obtaining valuable products. As we continue to delve into the intricate world of microbial surfactants, ongoing research efforts and technological advancements are likely to unveil new sources, innovative strategies, and unprecedented applications. The synergy between microbiology, biotechnology, and synthetic biology holds the key to unlocking the full potential of microbial surfactants, paving the way for a greener and more sustainable future.

Declaration of competing interest

No conflict of interest declared by authors.

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