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Microbial Biosurfactants: Genetic, Metabolic, And Systems Biology Perspectives for Sustainable Production

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ABSTRACT: Microbial biosurfactants are biomolecules that alter surface tension. They are very important for many industries like food industries, cosmetics and personal cares, oil and gas because they are biodegradable, less toxic, and multifunctional in comparison to synthetic surfactants. This review gives a detailed summary of the genetic, metabolic, and systems biology factors that characterize biosurfactant production. It turns around the great diversity of the chemical structure of biosurfactants, such as glycolipids, lipopeptides, and polymeric bioemulsifiers along with their microbial producers like bacteria, yeasts, fungi, and extremophiles. Production of the above-mentioned compounds is very tightly connected to central metabolic pathways including glycolysis, the tricarboxylic acid cycle, and fatty acid biosynthesis, which provide essential precursors like Acetyl-CoA and NADPH. Biosurfactant synthesis is based on the DNA material, i.e. biosynthetic gene clusters, regulatory networks, and the coordinated gene expression systems at different levels: transcriptional, post-transcriptional, and translational. The regulatory mechanisms like quorum sensing, carbon catabolite repression as well as stress response pathways are leading factors in the optimization of biosurfactant production in different environmental conditions. In support of this, the development of omics technologies and systems biology has enabled the study of biosurfactant production on a global scale; such studies have uncovered metabolic bottlenecks and identified regulatory points. The paper also discusses the contribution of the use of metabolic and genetic engineering tools such as gene overexpression, promoter engineering, pathway knockouts, and heterologous expression to biosurfactant production enhancement and cost reduction. A thorough understanding of the biological system coupled with synthetic biology holds a lot of potential for the sustainable production of biosurfactants and their wide-scale industrial as well as environmental applications.

1. INTRODUCTION

In the era of global industrialisation, the exploration of natural resources has served as a source of experimentation for science and advanced technologies, giving rise to the manufacturing of products with high aggregate value in the world market, such as biosurfactants (Santos et al 2016). Biosurfactants can be defined as the surface-active biomolecules produced

by microorganisms with wide range of applications (Vijayakumar and Saravanan, 2015). Biosurfactants have become attractive microbial products in the emerging biotechnology industry due to their advantages over synthetic surfactants in terms of environmental sustainability, global public health, and the concerns of industries to produce environment friendly goods (Lang et al, 2017).

The amphipathic structure of biosurfactants with hydrophilic and hydrophobic moieties enables these molecules to play a key role in emulsification, foam formation, detergency, and oil dispersion activities, which are desirable traits in different industries like food, cosmetics, agriculture (Sarubbo et al, 2022). The interfacial surface tension reducing ability of biosurfactants play important role in oil recovery and bioremediation of heavy crude oil (Vijayakumar and Saravanan, 2015). Surfactants are amphipathic compounds with both hydrophilic and hydrophobic moieties. The apolar moiety is often a hydrocarbon chain, whereas the polar moiety may be ionic (cationic or anionic), non-ionic or amphoteric (Mao et al, 2015; Silva et al, 2014). Surfactants increase the solubility of hydrophilic molecules, thereby reducing both surface and interfacial tensions at the oil/water interface (Banat et al, 2010). The critical micelle concentration (CMC) is the concentration of surfactant at which organised molecular assemblies, known as micelles, are formed and corresponds to the point at which the tensioactive agent achieves the lowest stable surface tension (Campos et al, 2013).

Currently, most of the surfactants are chemically derived from petroleum. However, such synthetic tensioactive agents are generally toxic and difficult to break down through the action of microorganisms. In recent years, such problems have motivated the scientific community to seek surfactants that are more environmental friendly, such as those produced through microbes, known as biosurfactants (Vijayakumar and Saravanan, 2015). Moreover, concerns regarding the environment on the part of consumers and new environmental control legislation have led to the development of natural surfactants as an alternative to existing products (Santos et al, 2016).

There are many different types of biosurfactants, such as glycolipids and lipopeptides. They stay stable even at high pH, temperature, and salinity (Khairunnisa et al, 2024). They are better than synthetic surfactants for the environment because they break down more easily, are less harmful, and work better with the environment (Simões et al, 2024; Garg et al, 2025). They can also be made from materials that can be used repeatedly, and they often work better than other materials. They offer better emulsification and biological activities like antimicrobial effects. Biosurfactants are great for food, medicine, cleaning up the environment, and industrial processes and therefore are good for the environment (Bjerk et al, 2021).

Limited biosurfactant production has widely hampered the potential for industrial scale-up of the product. One of the biggest obstacles that continuously needs to be overcome is the high production cost, especially that of substrates (Gaur et al, 2022). In fact, substrate costs alone may account for more than 50% of the total production costs. Moreover, biomass yields from non-pathogenic strains are generally very low. Employing pathogenic strains like *Pseudomonas* leads to a problem of bio-safety that must be solved (Ng et al, 2025). Changes in fermentation modes may lead to sensitivities with even slightest changes in any operational parameter, rendering the process unreliable and very difficult to scale-up (Wang et al, 2025). Aside from that, isolation and purification of biosurfactants are not only complex but also require a lot of energy and are quite costly. Lack of adequate performance data for comparison and increasing regulatory barriers are still the main reasons what trade biosurfactants from pilot scale to commercial scale. Therefore, there is a need to use advanced bioprocesses as well as metabolic engineering strategies to enhance the production (Jahan et al, 2020; Sarubbo et al, 2022).

Understanding the genetic factors that influence biosurfactant production is majorly helpful in not only increasing the yield of the biosurfactants but also in cutting down on the production costs (Revathi et al, 2022). Understanding the gene clusters, transcriptional regulators and the steps in the metabolism which dictate the flow of different metabolites can be used for the rational designing of the strains to get better productivity and substrate conversion efficiency (Chabhadiya et al, 2024). Therefore, in addition to ensuring that industrial production is feasible, a thorough understanding of a microorganism's genetics is primarily used to minimize waste production and optimize fermentation conditions, which in turn lower operating and downstream processing costs (Xia and Wen, 2023). Various advanced biotechnology techniques including systems biology, synthetic biology and genome-scale metabolic modelling have made a big contribution to the world of biosurfactants helping it in its production ramp up by a wide margin. Some of these techniques which are based on genome editing through the CRISPR system and pathway reconstruction are not only giving us the ability to have quite a precise control of the metabolism of the microorganisms but also make the generation of the strains that perform well a much easier task (Chabhadiya et al, 2024). When combined with the data that is being driven by omics, these strategies aid in the production of

biosurfactants on a large scale at an affordable cost, and in a sustainable manner (Matosinhos et al, 2026).

This review mainly intends to thoroughly discuss the production of microbial biosurfactants from genetic, metabolic, and systems biology angles. It seeks to explain the different structures of biosurfactants, their biosynthetic routes, and the main enzymes that play a role in their synthesis. Besides, it also deals with the layout and governance of biosynthetic gene clusters and how gene expression controls at different levels. There is also a great focus on newly developed omics techniques, metabolic and genetic engineering, and the use of synthetic biology methods for making biosurfactants more production-efficient and large-scale sustainable in large scale industrial applications.

2. CLASSIFICATION AND STRUCTURAL DIVERSITY OF BIOSURFACTANTS

Biosurfactants occur naturally in certain microorganisms. Along with their size, biosurfactants are divided into LMW (low molecular weight) and HMW (high molecular weight) biosurfactants. Biosurfactants produced from LMW are more effective in lowering surface and interfacial tension, while those originating from HMW are better than their LMW counterparts in emulsification, because of their structural heterogeneity. The division in weight is good in harmonizing the structural variety and the functionally relevant roles of these biosurfactants in industry (Adetunji et al, 2021; Saranraj, 2022).

One of the most thoroughly researched classes of biosurfactant is the glycolipids, comprising carbohydrate moieties coupled to long chain fatty acids. Notable examples are the rhamnolipids produced by *Pseudomonas aeruginosa* which insignificantly reduce surface tension and additionally possess antimicrobial and anticancer activities (Thakur et al, 2021). Primarily produced by *Starmerella bombicola*, sophorolipids are commercially important in biodegradable, mild cosmetic and detergent products in their lactonic and acidic forms. Produced by several yeasts, including *Pseudozyma* species, mannosylerythritol lipids (MELs) are highly surface active with self-assembly, making them ideal for biomedical and nanotechnology applications. The presence of different sugar head groups and fatty acid chains is a major contributor to the structural and functional diversity of glycolipids (Salek et al, 2022; Mnif and Ghribi, 2016; Paulino et al, 2016).

Another large class of LMW biosurfactants is made up of lipopeptides and lipoproteins that consist of cyclic and linear peptides and associated fatty acid tails. *Bacillus* species are the primary producers of these biosurfactants through their unique pathways of nonribosomal peptide synthesis (Júnior et al, 2022). An example is surfactin. It is considered the strongest biosurfactant because of its prominent surface tension lowering effects and its broad-spectrum antimicrobial activity (Begum et al, 2023). The other known lipopeptides, fengycin and iturin, are also important because of their strong antifungal activity. They are being considered for use in the biopharmaceutical and agricultural industries. Also, due to their structural diversity and environmental stability, they are of interest for use in bioremediation as well as other processes like enhanced oil recovery (Ali et al, 2022). Polymeric biosurfactants are classified as HMW and are mostly used for emulsification and not for lowering surface tension. One of the most studied bioemulsifiers, emulsan, biosynthesized by *Acinetobacter calcoaceticus*, can stabilize hydrocarbon-in-water emulsions at very low concentrations (Adetunji et al, 2021). Liposan, biosynthesized by *Candida lipolytica*, is glycoprotein complex with an overall carbohydrate composition of 83%. Because of its lipids emulsifying properties, it is used in the food and cosmetic industry (Santos et al, 2016; Saranraj et al, 2022).

Particulate biosurfactants are formed of membrane vesicles and cell-bound structures that can emulsify and take up hydrocarbons. One example of such biosurfactant vesicle producers at the microbe level is the *Acinetobacter* species. These vesicles range from 20 to 50 nm and are comprised of lipopolysaccharides, proteins, and phospholipids. These vesicles are important for microemulsion formation and enhance microbial metabolism of adjacent substrates. The biosurfactants that are particulate act via structural organization as opposed to molecular dispersion of the typical biosurfactants (Adetunji et al, 2021; Vijayakumar and Saravanan, 2025; Santos et al, 2016).

Table 1: Classification and structural diversity of microbial biosurfactants

CLASS OF BIOSURFACTANTS	SUBCLASS	SOURCES	APPLICATIONS	REFERENCES
GLYCOLIPIDS	Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Bioremediation, pharmaceuticals	Guptha and Sharma, 2024; Sarubbo et al, 2022
	Sophorolipids	<i>Starmerella</i>	Cosmetics, detergents	Dini et al, 2024

		bombicola		
	MELs	<i>Pseudozyma</i> spp.	Biomedical, nanotechnology	Hausmann et al, 2024
LIPOPEPTIDES	Surfactin	<i>Bacillus subtilis</i>	Antimicrobial, oil recovery	Hsu et al, 2025
	Fengycin / Iturin	<i>Bacillus</i> spp	Antifungal	Puyol et al, 2024
POLYMERIC	Emulsan	<i>Acinetobacter</i> spp.	Oil emulsification	Sarubbo et al, 2022
	Liposan <i>Candida lipolytica</i>	<i>Candida lipolytica</i>	Food, cosmetics	Vega et al, 2025
PARTICULATE	Vesicles	<i>Acinetobacter</i> spp.	Hydrocarbon uptake	Saranraj et al, 2022

3. MICROBIAL PRODUCERS OF BIOSURFACTANTS

Microbial biosurfactants can be produced by a wide variety of microorganisms, such as bacteria yeasts filamentous fungi, and extremophiles. Each group of organisms synthesizes different classes of molecules that have functionally different properties.

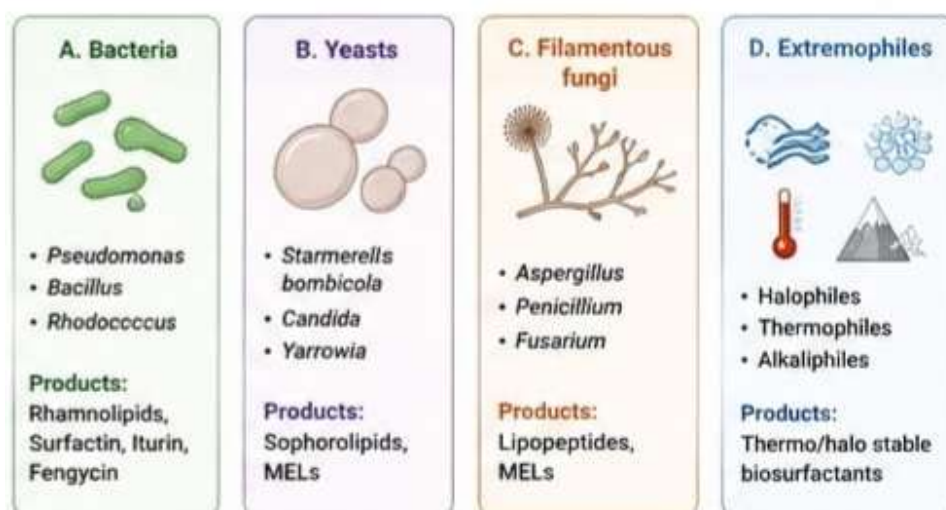


Figure 1: Major microbial producers of biosurfactants

3.1 Bacteria

Bacterial biosurfactant producers, for instance, have been studied extensively in some genera like *Pseudomonas*, *Bacillus*, *Acinetobacter* and *Rhodococcus*, which can be isolated with varying frequency (Das et al, 2017). *Pseudomonas*, especially *P. aeruginosa*, is known for producing biosurfactants called rhamnolipids, which have very strong properties as emulsifiers and antimicrobials. They have even been used widely for cleanup of the environment and in pharmaceuticals (Kaskatepe and Yildiz, 2016; Araújo et al, 2024). *Bacillus* strains, such as *B. subtilis*, make quite different biosurfactants effective lipopeptides like surfactin, fengycin, and iturin which along with their surface activity have also been demonstrated to exhibit very potent bioactivity even at extremely low concentrations (Heryani and Putra, 2017; Liu et al, 2015; Wu et al, 2022). *Acinetobacter* and *Rhodococcus* however produce high-molecular-weight bioemulsifiers and glycolipids that are employed in hydrocarbon biodegradation and oil recovery because they are capable of effectively stabilizing emulsions even under very harsh conditions (Mujumdar et al, 2019; Ohadi et al, 2020; Negrete et al, 2024).

3.2 Yeasts

Yeasts are another significant producer of biosurfactants, particularly in industrial biotechnology, because they are generally

recognized as safe (GRAS) status (Fernandes et al, 2023). *Candida*, *Starmerella*, and *Pseudozyma* are yeasts that produce glycolipid biosurfactants (Holguín-Salas et al, 2025; Fernandes et al, 2023; Kaur et al, 2017). *Starmerella bombicola* is a significant producer of sophorolipids and under optimised fermentation conditions, can be synthesized with yields greater than 300 g/L, making it commercially viable (Van Renterghem et al, 2018). Species of *Candida* synthesize a wide range of biosurfactants including sophorolipids and mannosylerythritol lipids (MELs) (Rufino et al, 2014; Khanna et al, 2023). In addition, *Pseudozyma*, produce MELs, which have excellent moisturizing and emulsifying properties and are used in the cosmetic and food industries (Fernandes et al, 2023).

3.3 Filamentous fungi

Not many researchers look closely at filamentous fungi still these microbes show real potential for making biosurfactants. Unlike simpler organisms like bacteria or yeasts, they craft complex types of active compounds some even built around proteins plus sugar parts. What stands out is how well they adapt to cheap materials left over from farming industries while growing (Cavalcanti et al, 2017; da Silva et al, 2021). Molecules made by strains such as *Aspergillus* tend to fight off microbes while also handling damaging particles in cells (Ishaq et al, 2015). Even though output stays modest next to traditional microbial sources, tweaks in gene control and growth setups slowly close that gap. Progress in lab techniques could soon make large-scale use more practical than before (Asgher et al, 2020).

3.4 Extremophiles

Extremophilic microorganisms are a very special category of biosurfactant producers that has been getting more and more attention. Such organisms can live in harsh conditions like very high temperatures, salinity, or pH levels, and as a result biosurfactants that they produce are highly stable even under those environments (Schultz et al, 2020). For example, thermophilic bacteria are able to make biosurfactants that can still be effective at temperatures higher than 80° C, whereas halophilic microorganisms produce compounds that are still functional even in very high salt concentrations (Mehetre et al, 2019; Fariq and Yasmin, 2020). That kind of stability is exactly what makes them so useful in industries like petroleum recovery, where it is quite common to encounter reservoirs with extreme conditions (Schultz et al, 2020). Moreover, biosurfactants that come from extremophiles are also more resistant to being broken down by enzymes or chemically denatured, thus they can be used in a whole range of industrial processes that involve harsh conditions (Rivero et al, 2022).

4.0 METABOLIC STRUCTURE AND BIOSYNTHESIS PATHWAYS

Recent research has shed light on how the metabolic framework and biosynthesis mechanisms of biosurfactants are closely linked to the cellular metabolism. Vital pathways like glycolysis, gluconeogenesis, and the tricarboxylic acid cycle (TCA) supply the biosurfactant formation with major precursors, such as acetyl-CoA, NADPH, and sugar nucleotides (Wang et al, 2025). According to the latest studies, glycolipid biosurfactants appear to be predominantly formed as a result of the integration of fatty acids metabolism with sugar biosynthesis pathways. Indeed, fatty acid synthase systems undergo the production of -hydroxy fatty acids, whereas sugars that have been activated and are derived from glucose metabolism are associated using specific glycosyltransferases to generate molecules such as rhamnolipids and sophorolipids (Chabhadiya et al, 2024). On the other hand, the lipopeptide surfactants are synthesized by means of non-ribosomal peptide synthetase (NAPS) complexes, which, besides assembling peptide chains without help from ribosomes, also ATP-dependently link peptides to lipids (Ahluwalia et al, 2026).

New research uncovers a tight link that exists between biosurfactant production, metabolic flux distribution, and substrate utilization. For instance, hydrophobic substrates like oils may be used directly for the synthesis of lipid precursors. On the other hand, hydrophilic substrates need to be changed through central metabolism pathways before their incorporation (Songdech et al, 2025). Disciplines like systems biology and metabolic engineering CRISPR-based gene regulation and pathway optimization among them are being harnessed to improve the supply of precursors, the efficiency of enzymes, and the overall production yield (Thakur et al, 2024). It is worth mentioning that biosurfactant biosynthesis is a very complex, multi-pathway process which is characterized by the integration of basic metabolism with specialized biosynthetic gene clusters. Such metabolic versatility is one of the reasons why microorganisms can adjust their biosurfactant production in response to the conditions of the environment and the requirements of the industry (Silva et al, 2026).

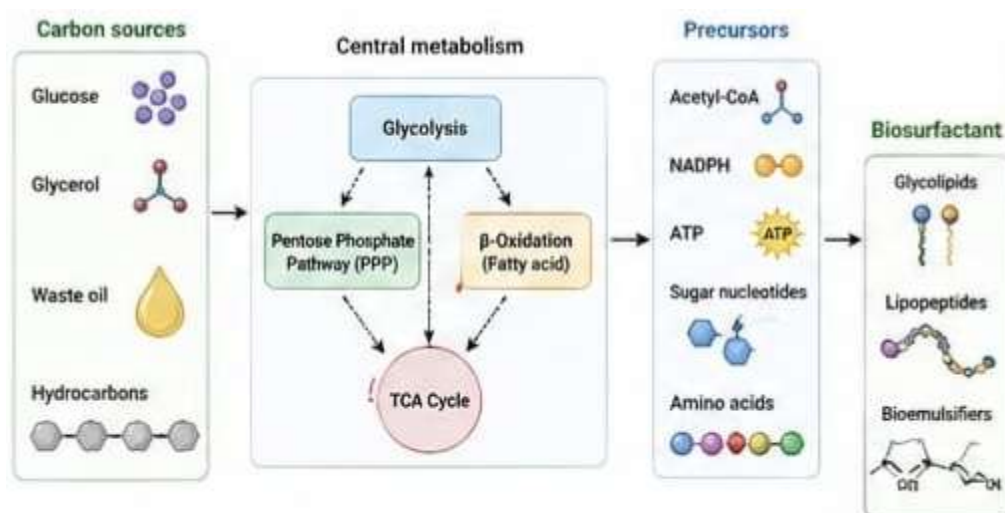


Figure 2: Central metabolic pathways involved in biosurfactant biosynthesis

4.1 Precursor supply pathways

The difference between hydrophobic and hydrophilic components is that they repel and attract water respectively which is the reason biosurfactant synthesis depends on precursor supply pathways to generate hydrophobic (fatty acid) and hydrophilic (carbohydrate) moieties for the amphiphilic structure formation. These pathways are so closely linked mainly through central carbon metabolism where fatty acid biosynthesis and carbohydrate metabolism share many steps, and therefore are heavily interconnected (Zhuang et al, 2022). A major function of fatty acid biosynthesis is to provide lipids for biosurfactants such as rhamnolipids, sophorolipids, and lipopeptides (Garg et al, 2025). First, Acetyl-CoA is formed as a main product of carbohydrate metabolism. Then Acetyl-CoA is converted to malonyl-CoA by Acetyl-CoA carboxylase and fatty acid synthase carries out several consecutive elongation cycles, resulting in long-chain fatty acids or hydroxyacyl intermediates (Zhuang et al, 2022). These intermediates serve as main hydrophobic components, like 3-hydroxyalkanoyl-ACP, which become part of biosurfactant molecules. Research has found that by increasing the production of branched-chain fatty acids, surfactin production can be significantly enhanced, which underscores the importance of the availability of lipid precursors (Jing et al, 2023). Furthermore, fatty acid biosynthesis can be integrated with oxidation and recycling steps allowing a steady supply of lipid intermediates based on the substrates available (Zhuang et al, 2022).

Carbohydrate metabolism supplies energy and carbon frameworks for the synthesis of biosurfactants. Glycolysis is a pathway that breaks down sugars or glycerol into pyruvate, producing ATP and reducing equivalents, as well as important intermediates like dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (Kloska et al, 2023). Pyruvate serves as a key molecule in cell metabolism since it can enter various biosynthetic pathways, including fatty acid synthesis and amino acid metabolism. In fact, pyruvate is converted to Acetyl-CoA that subsequently goes into the TCA cycle (Luo et al, 2023). Apart from generating energy, the TCA cycle also produces citrate that can be transported to the cytosol and cleaved to form Acetyl-CoA for lipid biosynthesis (Adetunji et al, 2021). On top of that, intermediates of glycolysis can be rerouted into sugar nucleotide biosynthesis pathways, leading to the formation of activated sugars like dTDP-L-rhamnose, which form the hydrophilic component of glycolipid biosurfactants. Biosurfactant synthesis is a result of the well-coordinated flux between glycolysis, the TCA cycle, and fatty acid biosynthesis (Garg et al, 2025). The type of carbon source greatly affects pathway regulation, resulting in the diversion of carbon either towards lipid or carbohydrate precursor biosynthesis. Hence, fine-tuning these precursor generation pathways via metabolic engineering is a key step in biosurfactant production enhancement and cost minimization (Sarubbo et al, 2022).

4.2 Biosynthesis routes

Among different types of biosurfactant production, glycolipid and lipopeptide are the main ones that research has most intensively focused on because of their industrial and biomedical applications (Wang et al, 2025). Glycolipid synthesis pathway is basically a hybridization of carbohydrate and lipid precursors mainly derived from central metabolism. For one

thing, the hydrophobic part is produced via fatty acid biosynthesis; for another, hydrophilic sugar units originate from carbohydrate metabolism intermediates like glucose-6-phosphate (Ashby and Solaiman, 2020). These elements are chemically linked by glycosyltransferases resulting in a variety of glycolipids such as rhamnolipids, sophorolipids, and mannosylerythritol lipids (Pandey et al, 2024). For instance, during the synthesis of rhamnolipids, enzymes of the *rhlAB* gene products facilitate the combination of α -hydroxy fatty acid dimers and the following incorporation of rhamnose units is carried out by rhamnosyltransferases (Raheja et al, 2026). Biosurfactant production through glycolipid formation is mostly reliant on controlling carbon flux and there is evidence that biosurfactant producing strains can reduce surface tension to about ~ 3035 mN/m, which is a sign of high biosurfactant activity. Besides that, such biosynthetic pathways are tightly controlled by quorum sensing and environmental factors (Wang et al, 2025).

NRPS (non-ribosomal peptide synthetase) systems operate as the equipment of a different biosynthetic approach for producing lipopeptides. Lipopeptides produced by *Bacillus* species as secondary metabolites are surfactin, iturin, and fengycin (Antonioli Júnior et al, 2023). Unlike ribosomal protein synthesis, NRPS systems are made up of huge modular multi-enzyme complexes capable of synthesizing peptides even without the need for mRNA templates (Hu et al, 2019). A module in an NRPS is made up of three domains adenylation (A), thiolation (T), and condensation (C) which carry out the functions of selection, activation, and the linking of amino acids respectively in a sequential manner (Wang et al, 2025). Besides, the starting module allows the attachment of a fatty acid to the first amino acid, thus forming a structure that has both hydrophilic and lipophilic components. The colinearity principle of modules is such that the sequence of NRPS modules corresponds with the amino acid sequence of the peptide (Caulier et al, 2019). Diversification of structures arises from variations in the types of amino acids used, the length of the fatty acid chain, and the patterns of cyclization, with cyclic lipopeptides being the predominant form. Recent studies indicate that over 90 lipopeptides belonging to 26 homologous families have been discovered, which highlights the metabolic versatility of the NRPS systems (Wang et al, 2025).

4.3 Key enzymes

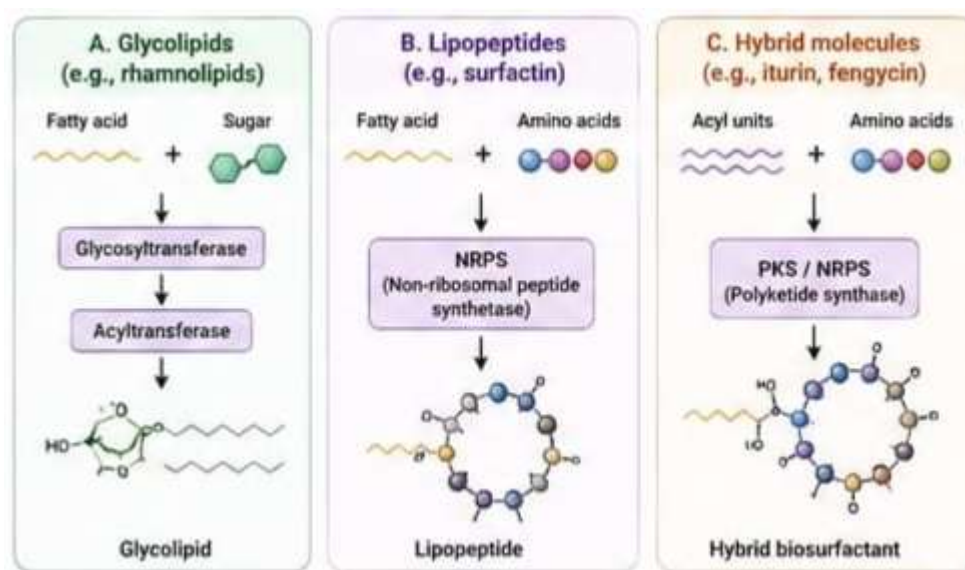


Figure 3: Biosynthetic routes and key enzymatic systems

Biosurfactant production is a co-operative enzymatic pathway, which builds amphiphilic components from sugars, fats, proteins, etc. Three major types of enzymes are glycosyltransferases, acyltransferases and complex multi-enzyme assemblies such as non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS), which are responsible for structural formation and diversity of the biosurfactant (Kabeil et al, 2025; Wang et al, 2025). Glycosyltransferases are the enzymes that carry out the transfer of activated sugar donors (e.g. UDP-glucose, dTDP-rhamnose) to lipid molecules. This results in the formation of the hydrophilic head groups of glycolipid biosurfactants (Kim et al, 2021). In the case of rhamnolipid-producing *Pseudomonas aeruginosa*, *RhlB* and *RhlC* are enzymes that by the addition of rhamnose units to α -hydroxy fatty acid dimers lead to the production of mono- and di-rhamnolipids with different properties (Kabeil et al, 2025). It has been found that altering

the expression of the glycosyltransferase gene has a powerful influence on production and product distribution, and this is now facilitating the option of generating customized biosurfactant profiles for the industry (Santos et al, 2016). The yeast *Starmerella bombicola* that produce sophorolipids also depend on glycosyltransferases which bind sophorose to hydroxylated fatty acids. These enzymes have recently been genetically modified to change their substrate specificity (Castelein et al, 2023).

Acyltransferases play a pivotal role in the establishment of hydrophobic segments of biosurfactants by facilitating ester or amide bond creation between fatty acids and sugar or peptide elements (Musiol-Kroll and Wohlleben, 2018). In the case of glycolipid biosynthesis, corresponding enzymes such as RhlA produce -hydroxyalkanoyl--hydroxyalkanoic acid (HAA), which is the lipid source for rhamnolipids. Indicators like the fatty acid chain length, saturation, and branching that are modulated by acyltransferase functions in turn influence the extent to which surface tension can be lowered as well as the capability of the system to emulsify (Buyachuihan et al, 2024). Latest metabolite-based results indicate that aligning acyltransferase task level can increase carbon allocation into lipid sources which in fact, leads to better production of biosurfactants as a whole (Musiol-Kroll and Wohlleben, 2018).

Nonribosomal peptide synthetases (NRPS) are huge, modular enzyme assemblies accountable for the production of lipopeptide biosurfactants like surfactin, iturin, and fengycin in *Bacillus subtilis* (Waongo et al, 2025). Every NRPS segment comprises adenylation (A), thiolation (T), and condensation (C) sections that individually activate and add amino acids to peptide chains without relying on ribosomal machinery (Roongsawang et al, 2010). Thanks to progress in structural biology, and genome database scraping, various new NRPS forms and domain configurations have been discovered, further enabling identification of novel lipopeptides with stronger antimicrobial and surface-active activities. Besides, modifying NRPS sections has now become a highly effective way to develop tailor-made biosurfactants with superior characteristics (Nakkeeran et al, 2021).

Polyketide synthases (PKS) are enzymes that help in elongating lipid chains and creating new molecules of complex structures by adding acyl-CoA units one after the other. When PKS systems are integrated with NRPS pathways, they produce hybrid lipopeptide-polyketide biosurfactants, and these combinations are also the sources of structural diversities (Zhang et al, 2022). During the last few years, through the use of omics-based methods, not only the PKS gene clusters responsible for biosurfactant production have been uncovered, but also those that were regarded as uncharacterized have been identified; this finding has significantly highlighted their function in the production of novel compounds for the industry (Dhakal et al, 2019; Courtial et al, 2022). These enzymatic systems are elements of a biosynthetic network that among other functions regulates the making of biosurfactants. Understanding their working modes and the ways they are regulated will lead to metabolic engineering approaches that can enhance production, lower cost, and obtain biosurfactants with the most desirable properties (Wang et al, 2025; Wang et al, 2014).

Table 2: Key enzymes involved in biosurfactant biosynthesis

ENZYME	FUNCTION	ASSOCIATED BIOSURFACTANT	REFERENCES
GLYCOSYLTRANSFERASES	Transfer sugar moieties	Glycolipids	Wang et al, 2025
ACYLTRANSFERASES	Attach fatty acid chains	Glycolipids, lipopeptides	Nayariseri et al, 2025
NRPS	Peptide assembly	Lipopeptides	Wang et al, 2025
PKS	Lipid chain elongation	Hybrid biosurfactants	Matosinhos et al, 2026

5.0 GENETIC BASIS OF BIOSURFACTANT PRODUCTION

5.1 Biosynthetic Gene Clusters (BGCs)

Biosynthetic gene clusters (BGCs) that are behind microbial biosurfactant production are most often in the form of tightly regulated operons encoding enzymes, transporters, and regulatory proteins necessary for the orchestrated synthesis. Such

clusters can have a modular setup where primary biosynthetic genes are accompanied by accessory genes to make sure the production and secretion of the metabolite are efficient (Al-Siyabi et al, 2025). In bacteria, operon configuration allows for the co-transcription of multiple genes under a single promoter, making it possible to have the genes be expressed in a synchronized fashion after an environmental change like nutrient scarcity or quorum sensing (Ullah et al, 2026). As an example, the clusters that produce lipopeptides are commonly associated with very large non-ribosomal peptide synthetase (NRPS) operons which are acting as assembly-line enzymatic systems that are sequentially adding substrates into complex molecules (Qi et al, 2023).

Another key trait that affects BGC functionality and biosurfactant production is gene copy number variation. Microbial genomes may contain single or multiple copies of particular biosynthetic genes or whole clusters, thus being a source of metabolic diversity as well as a way to self-adapt (Wang et al, 2024). Research on some genomes revealing multiple NRPS or PKS clusters whereas others have only a few ones indicate the strain-specific biosynthetic capacity (Xu et al, 2023). On the one hand, latest genome-wide studies have discovered gene copy number to be frequently correlated within operons, which implies coordinated regulation and functional interdependence (Wang et al, 2024). On the other hand, for ecological comparisons, gene presence/absence is sometimes given more importance than the exact copy number due to redundancy and functional equivalence. This kind of diversity allows microbes to alter biosurfactant production depending on the environmental situation (Ji et al, 2024).

These organizational principles are well illustrated by several well-studied BGCs. For example, in rhamnolipid-producing *Pseudomonas aeruginosa*, the *rhlAB* operon codes for the enzymes that make mono-rhamnolipids, while *rhlC* is the enzyme responsible for producing dirhamnolipids by adding a second rhamnose unit, and their expression is mostly regulated by quorum sensing systems (Put et al, 2024). These genes usually are found together with genes encoding for precursors such as *rml* and *fab* within a larger metabolic module. On the other hand, in *Bacillus subtilis* the *srfA* operon (*srfAAAD*) codes for a big NRPS complex that biosynthesizes surfactin with additional genes like *sfp* for enzyme activation (Al-Siyabi et al, 2025; Kwon and Hovde, 2024).

The gene clusters in MEL-production yeasts have *emt1*, which codes for a glycosyltransferase, and *mac1/mac2*, which are responsible for acylation steps. They often have transport and regulatory genes as well forming a clustered arrangement (Xu et al, 2023). Sophorolipid biosynthesis in *Starmerella bombicola* is another example. Genes are clustered here which includes those coding for hydroxylation (*cyp52m1*), glycosylation (*ugtA1*), and the export processes (Wang et al, 2024).

Table 3: Major genes and biosynthetic gene clusters (BGCs)

Gene/Cluster	Organism	Function	References
rhlAB	<i>Pseudomonas aeruginosa</i>	Rhamnolipid synthesis	Hausmann et al, 2024
rhlC	<i>Pseudomonas aeruginosa</i>	Dirhamnolipid formation	Puyol McKenna et al, 2024
srfA operon	<i>Bacillus subtilis</i>	Surfactin synthesis	Amjad et al,
sfp	<i>Bacillus subtilis</i>	NRPS activation	Th��atre et al, 2021
emt1, mac1/mac2	Yeasts	MEL biosynthesis	Qin et al, 2025
ugtA1, cyp52m1	<i>Starmerella bombicola</i>	Sophorolipid synthesis	Zhi et al, 2017

5.2 Functional Gene Categories

Biosurfactant production related functional gene categories cover coordinated genetic modules which are responsible for production, precursor supply, transport, and cell protection. These categories are normally arranged in biosynthetic gene clusters and are co-regulated to increase production under stress (Sharma et al, 2021). Genes of biosynthesis characterizing the structural part encode the main enzymes that are directly responsible for the production of biosurfactant molecules. Examples are *rhlAB/rhlC* genes for rhamnolipid production and *srfA* operon genes for lipopeptides. They code for enzymes

such as rhamnosyltransferases, non-ribosomal peptide synthetases (NRPS), and polyketide synthases (PKS), which accelerate the building of complex amphiphilic structures (Ullah et al, 2026). Genome-wide analyses have pointed out such gene clusters as well preserved and usually showing co-occurrence and operon-based organization which mirror coordinated expression during biosurfactant production (Wang et al, 2024). Precursor-supplying genes are responsible for providing the biosynthesis with the necessary metabolic intermediates. These genes participate in fundamental metabolic pathways like fatty acid biosynthesis and carbohydrate metabolism (Claus et al, 2022). As an example, rhamnolipid biosynthesis requires the synthesis of dTDP-rhamnose by the action of rmlABCD genes which transform glucose-1-phosphate to sugar moieties in the active form (Ullah et al, 2026). In a similar manner, fatty acid biosynthetic routes provide ω -hydroxyacyl chains. Connecting primary metabolism and biosurfactant synthesis results in a targeted carbon flow towards product synthesis (Claus et al, 2022).

Transport and secretion systems, especially the ATP-binding cassette (ABC) transporters, are very important in the process of releasing biosurfactants from the cell to the exterior. By means of ATP hydrolysis, these transporters can transfer amphipathic molecules through membranes thus avoiding the accumulation and toxicity of molecules in the cell (Ullah et al, 2026). ABC transporters are present in all organisms and capable of transporting various molecules which are structurally different such as lipids and secondary metabolites. In microbes producing biosurfactants, it is a common thing that these systems are placed on the same DNA segment as biosynthetic clusters so they can produce and secrete in a coordinated way (Claus et al, 2022). Self-resistance mechanisms are the ways through which producers of biosurfactants can protect themselves from being harmed by the biosurfactants they produce. These mechanisms may include, among others, the use of efflux pumps which often contain ABC transporters, systems for membrane modification, and stress response genes that help maintain membrane integrity (Wang et al, 2024). For instance, multidrug resistance transporters can export toxic amphiphiles from cells which therefore can survive in the presence of high levels of biosurfactants. Besides, regulatory networks can adjust gene expression according to environmental changes (Claus et al, 2022).

5.3 Genetic organisation

The genetic setup of biosurfactant production is very well arranged. It mainly mirrors the necessity for the coordinated expression of various enzymes, transporters, and regulatory elements together (Blin et al, 2023). The gene organization responsible for the biosurfactant production is usually identified as modular gene clusters that are well preserved across various microbial taxa including bacteria, yeasts, and filamentous fungi. Modular gene clusters (also called biosynthetic gene clusters, BGCs) are made up of genes that are localized together and they encode enzymes that are engaged sequentially in the biosurfactant synthesis (Terlouw et al, 2023). Usually, these clusters consist of the main biosynthetic genes, modifying enzymes, regulatory genes, and transport-related components. Modular systems such as NRPS and PKS become very significant as they display domain-based architectures that help in the stepwise assembly of complex molecules such as lipopeptides and glycolipids (Evdokias et al, 2021). Modularity allows substrate specificity and structural diversity, as each module is responsible for adding the specific chemical unit to the final biosurfactant molecule. The clustered kind of organization makes sure that the transcription and efficient metabolic flux are coordinated. (Terlouw et al, 2023).

Studying the genomes of bacteria, yeasts and fungi has disclosed a mixture of conserved evolution paths and lineage-specific motifs. For example, in bacteria, the genes that produce biosurfactants are generally very close to each other and form an operon-like cluster (Ullah et al, 2026). On the other hand, fungal and yeast genomes frequently show more scattering of genes and less tight clusters, although the genes that work together are still located close to one another. Comparative studies have shown that biosurfactant gene clusters can be found all over ecosystems and different species, and that there is great variation in gene composition, gene copy number, and gene regulation among these clusters (De la Cruz-Rodríguez et al, 2023). The genome-wide studies reveal that biosurfactant genes have co-occurred in quite a different environment, which is a sign of evolutionary conservation together with ecological adaptation (Ullah et al, 2026). Moreover, fungal biosynthetic gene clusters are generally bigger and contain more genes than bacterial ones. This is because fungi have the potential to generate chemically diversified secondary metabolites (Terlouw et al, 2023).

Further improvements in genome mining and gene cluster prediction methods have opened up new possibilities to discover and study biosurfactant-related gene clusters. antiSMASH, for instance, is a tool that has become synonymous with BGCs detection (De la Cruz-Rodríguez ET AL, 2023). It works by recognizing biosynthetic domains that are conserved across bacteria and fungi and then annotates these domains by comparing them to known clusters (Terlouw et al, 2023). Resources like MIBiG serve as a place to store data on experimentally verified gene clusters that have been functionally annotated and

allow researchers to do comparative analyses of the authors' newly identified BGCs (Kautsar et al, 2020).

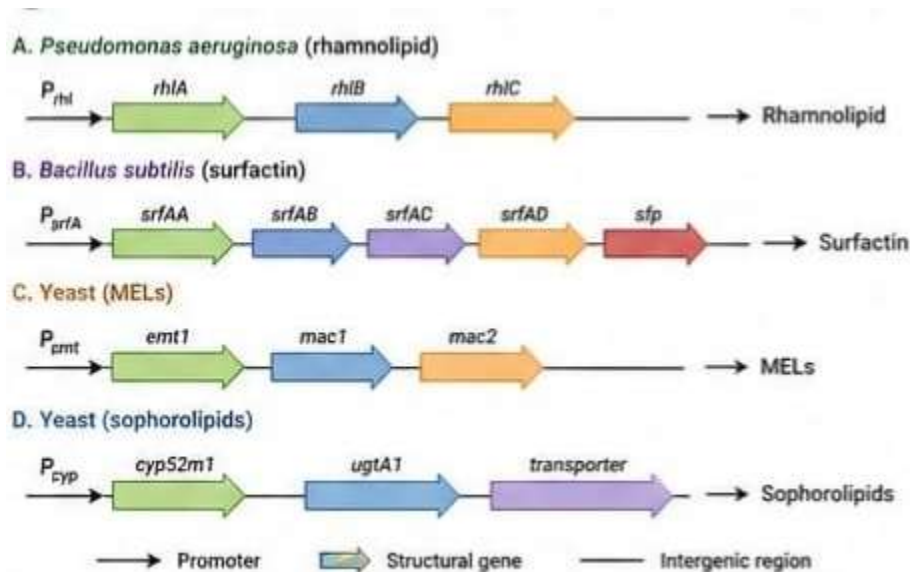


Figure 4: Genetic organisation of biosynthetic gene clusters (BGCs)

6.0 REGULATORY NETWORKS IN BIOSURFACTANT BIOSYNTHESIS

Biosurfactants synthesis regulatory mechanisms are very complex. They are capable of integrating information from the environment, the physiological condition of the cells, as well as their density, so as to achieve maximum production. Such mechanisms entail quorum sensing (QS), transcriptional regulators, global regulatory systems, carbon catabolite repression (CCR), and stress response pathways (Ruiz-Villafán et al, 2022; Mukherjee and Bassler, 2019). Quorum sensing is the primary communication mode between cells, as well as gene regulation dependent on the population size among bacteria. It is extremely important especially in Gram-negative bacteria such as *Pseudomonas aeruginosa* (Mukherjee and Bassler, 2019). The Las and Rhl systems together constitute a hierarchical QS network which controls the production of rhamnolipid. The Las system (LasI/LasR) not only produces but also recognizes N-3-oxo-dodecanoyl homoserine lactone, a molecule which triggers the activation of the Rhl system (RhlI/RhlR). Then, the RhlR transcriptional regulator directly induces the transcription of the *rhlAB* operon, coding for the enzymes that produce rhamnolipids (Papenfort and Bassler, 2016). Besides this, the PQS system of *Pseudomonas epidermidis* engages with the Las and Rhl systems, thus forming a complex regulatory network that is consistent with cell density and environmental factors (Sharma et al, 2020).

Expression of biosurfactants genes can also be modulated further by transcriptional regulators coming from LuxR, TetR and GntR families. LuxR type regulators not only function as QS receptors but also induce the transcription by directly binding to the promoters of biosynthetic gene clusters (Bylino et al, 2020). In TetR family, members are mostly repressors, and sometimes, after the ligand binding, they change to activators. For example, they affect glycolipid synthesis. Similarly, GntR have a role in metabolism regulation and by controlling genes involved in precursor supply and biosynthesis of secondary metabolites, they integrate carbon flux with biosurfactant production (Bhukya and Anand, 2017; Yeo et al, 2017). Global regulatory systems are best known in Gram-positive bacteria such as *B. subtilis* whose biosynthesis of lipopeptides is very tightly controlled. The ComP ComA two-component system that detects extracellular competence signals is responsible for inducing transcription of the *srfA* operon (Suvorova et al, 2015). DegU is a further response regulator influencing surfactin synthesis depending on its phosphorylation state while Spo0A serves as the main regulator of sporulation and starvation, which also has an impact on biosurfactant production. Basically, all these regulators cooperate to biosynthesize biosurfactants only when the environmental conditions are favourable for the survival and social behaviour of the bacteria (Subramoni et al, 2015).

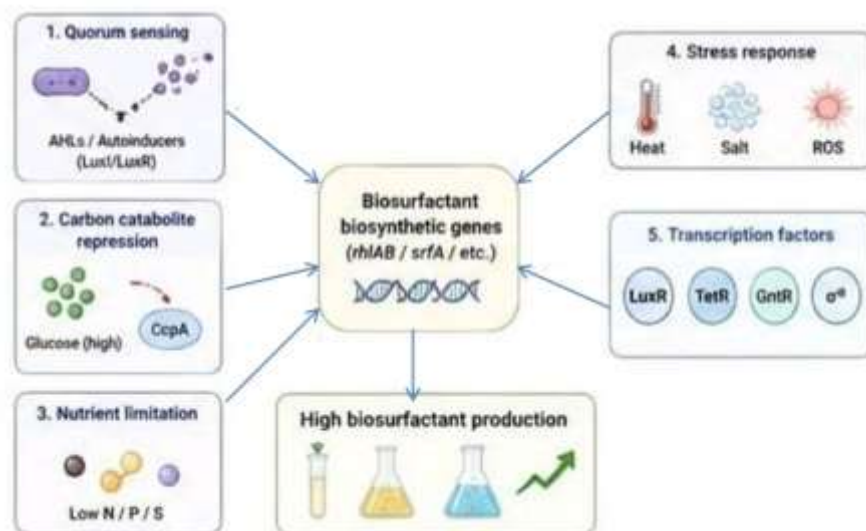


Figure 5: Regulatory networks controlling biosurfactants synthesis

Carbon catabolite repression (CCR) is a crucial metabolic regulatory mechanism that can completely stop biosurfactants production, for example, when cells are provided with a preferred carbon source, e.g. glucose (Kremling et al, 2015). The *Bacillus CcpA* protein makes it possible for the CCR to take place by interacting with catabolite-responsive elements (cre sites) located in the promoter regions and suppressing the transcription of genes that lead to secondary metabolism including biosurfactant production (Ruiz-Villafán et al, 2022). The removal of CCR during the scarcity of carbon therefore results in increase of production, hence linking the production level to metabolic condition (Park, 2025).

Furthermore, signalling pathways for stress response dramatically influence biosurfactants production. Stress inducers in the environment such as changes in osmotic pressure, oxidative stress and lack of nutrients lead to activation of sigma factors (e.g. σ^B in *Bacillus*) as well as other stress-responsive regulators that may up-regulate biosurfactants genes (Harm et al, 2024). Besides, these molecules are in fact adaptive agents that aid in cell survival by stimulating surface activity, formation of biofilms, and antimicrobial defences. As an illustration, the generation of rhamnolipid is upregulated as a protective response when oxidative stress is inflicted (Harm et al, 2024).

Table 4: Regulatory mechanisms controlling biosurfactant production

Regulatory Mechanism	Key Components	Role	References
Quorum sensing	Las, Rhl systems	Cell density-dependent regulation	Li et al, 2025; Zhou et al, 2020
Transcription factors	LuxR, TetR, GntR	Gene regulation	Dagenais Roy et al, 2025
Carbon catabolite repression	CcpA	Represses production under glucose	Sarubbo et al, 202
Stress response	Sigma factors	Enhances production under stress	Li et al, 2025

7.0 GENE EXPRESSION AND MOLECULAR REGULATION OF BIOSURFACTANT PRODUCTION

7.1 Transcriptional control

Transcriptional control is the major regulatory level through which biosurfactant synthesis is controlled, making it possible for microorganisms to produce the biosurfactants in coordination with environmental factors and their own physiological

states. Generally, in bacteria, the genes for biosurfactant biosynthesis are formed into operons (e.g. *rhlAB* *srfA*), which are tightly controlled by promoter activity and transcription factors (Dagenais et al, 2025). Quorum sensing (QS) is a major mechanism here: it is a cell-density dependent system in which the transcription of biosurfactant genes is triggered only when the population reaches a threshold (Gu et al, 2022). The *RhlI* enzyme is capable of making acyl-homoserine lactone signals (e.g. C4-HSL) which then bind to the transcriptional regulator *RhlR*, and the *RhlR*-C4-HSL complex can directly initiate transcription of the *rhlAB* operon (Dagenais et al, 2025). In bacilli, a type of Gram-positive bacteria, biosurfactant transcription is regulated by a network of interacting cascades. The *srfA* gene cluster is the target of the ComQXPA quorum sensing system with *ComA* being the response regulator, whereas global transcription factors such as *Spo0A*, *CodY*, and *AbrB* can work both ways to regulate the biosurfactant gene transcription depending on the nutrient levels and the phase of the cell cycle (Qi et al, 2023). Inspired by respective changes, phosphorylated *Spo0A* represses *AbrB*, which has the effect of lifting the inhibition on *srfA* transcription and thus enabling biosurfactant synthesis (Kok et al, 2024).

7.2 Post-transcriptional regulation

Post-transcriptional regulation is one of the very important ways cells work to biosurfactant production because the final piece of this machinery is, among other things, the stability of mRNA molecules and the translation efficiency of these molecules into proteins. The post-transcriptional processes of RNA are also regulated in this way (Kim and Kim, 2024). This level of regulation equips cells with the ability to rapidly adjust the quantity of biosurfactants to changing environmental and metabolic conditions. Small non-coding RNAs such as microRNAs (miRNAs) and small interfering RNAs (siRNAs) are the main players of this type of regulation (Li et al, 2024). Through the formation of complementary pairs with the target mRNAs, sRNAs decide gene expression resulting in mRNA degradation or translational repression by means of RNA-induced silencing complexes (RISC) (Kim and Kim, 2024). For example, sRNAs in biosurfactant-producing bacteria like *Pseudomonas* and *Bacillus*, could post-transcriptionally influence biosurfactant production by interacting with the mRNAs of major biosynthetic enzymes. Besides, RNA-binding proteins (RBPs) present another potential layer of regulation by altering mRNA stability, re-folding, and translation processes. These proteins bind to the untranslated regions (UTRs) of mRNAs for biosurfactants. Such an interaction affects translation initiation and mRNA turnover. Besides, RBPs also impact the production and activity of small RNAs thus providing an additional layer of gene expression regulation (Wang et al, 2025; Hsu et al, 2025).

Also, the mRNA lifecycle and breakdown pathways are the key. The deliberate breakdown by ribonucleases can remove the biosurfactant transcripts when they are not required, thus no unnecessary metabolic burden is exerted. RNA maturation steps such as splicing, polyadenylation, and RNA changes can affect the stability of transcripts and the efficiency of translation (Georg et al, 2025). New research points to the role played by long non-coding RNAs (lncRNAs), circular RNAs, and epitranscriptomic modifications (e.g. methylation) in the precise control of metabolic processes. These components affect the RNA conformation and the networks of their interactions as well as the translation changes thereby enabling the cell to respond effectively to stress or shortage of nutrients (Vigil et al, 2024).

7.3 Translational and protein-level regulation

The regulation of translation and protein synthesis is an important aspect of controlling the production of biosurfactants at a level beyond the transcriptional regulation. This kind of regulation enables the cells to adapt quickly to environmental and metabolic changes (Kim et al, 2023). Regulation at the translation level is predominately done through control of initiation, elongation, and termination stages of protein synthesis (Zhang et al, 2022; Qiao et al, 2024). Elements like the structure of mRNA, the changes in riboswitches, the extent of binding site accessibility of ribosomes on mRNAs, etc. determine the efficiency of translation of biosynthetic genes (Dougherty et al, 2020). Besides that, there are cases where programmed ribosome pausing and frameshifting influence the production of different enzymes in biosynthetic pathways, leading to changes in biosurfactant levels and varieties. Environmental factors such as nutrient shortage can lead to activation of a global response system like the stringent response which changes the rates of translation and also leads to prioritization of the secondary metabolite biosurfactant (Zhang et al, 2022; Qi et al, 2023).

7.4 Feedback inhibition and pathway control

Feedback inhibition alongside pathway control is pretty much the mechanisms by which biosurfactant production gets regulated through biosurfactant-producing microorganisms (Qiao et al, 2024). They act in consonance with each other to

ensure metabolic balance, and optimum utilization of resources, among other things. Generally, the accumulation of the end products, say surfactin or rhamnolipids among others, in the case of such bacteria as *Bacillus* and *Pseudomonas*, can lead to the inhibition of key enzymes or regulatory proteins within their biosynthetic pathways (Das and Rao, 2024). Such a mechanism, called feedback inhibition, acts as a deterrent against the excessive accumulation of metabolites that might have the opposite effect of being a metabolic burden or even the cause of toxicity to the cell. Biosurfactant synthesis at the pathway level is quite reliant on central metabolism (Thakur et al, 2024). For example, biosurfactant production shares precursor molecules such as fatty acids and amino acids with certain fundamental cell processes. An increase in the amount of biosurfactants can accompany, among other things, signals for feedback that might result in the downregulation of precursor-feeding pathways or in the inhibition of nonribosomal peptide synthetases (NRPS) and rhamnosyltransferases, hence leading to a reduction in production rates (Das and Rao, 2024; Chabhadiya et al, 2024). In *Bacillus subtilis*, master regulators such as Spo0A and AbrB assist in the coordination of pathway control by being responsive to situations of nutrient assumptions and hence they act as the links between biosurfactant biosynthesis and stationary-phase physiology (Chabhadiy et al, 2024). Likewise, quorum sensing systems exert their effect on gene expression as a function of cell density. Indirectly, they even help in feedback control by modulating the biosurfactant synthesis on the condition that the concentration of biosurfactants is sufficient (Thakur et al, 2024). Pathway control is also a way of regulating carbon flux and catabolite repression. If there is an abundance of the most preferred carbon sources, the genes that encode biosurfactant production might be repressed whereas under nutrient deprivation, secondary metabolite production is activated (Das and Rao, 2024).

8.0 OMICS AND SYSTEMS BIOLOGY APPROACHES

Omics and systems biology approaches have changed the game of studying biosurfactant production totally. They allow us to understand microbial metabolism and regulation from a very broad viewpoint (Gaur et al, 2022). Genomics and comparative genomics give us the basic information like biosynthetic gene clusters, regulatory elements, and evolutionary conservation of biosurfactant pathways in various microorganisms like *Pseudomonas* and *Bacillus* (Jimoh et al, 2021). The progress in high-throughput sequencing allows comparisons that expose gene organization, horizontal gene transfer events, and variations at the strain level that impact biosurfactant yield and diversity (Gutierrez Reyes et al, 2024). On the other hand, transcriptomics (RNA-Seq, microarrays) documents the gene expression fluctuations in response to different environmental or nutritional conditions, thus paving the way for the discovery of important regulatory genes, quorum sensing circuits, and stress-responsive pathways that govern biosurfactant production. Altogether, these methods greatly help in finding transcriptional bottlenecks and regulatory switches that can be manipulated for metabolic engineering (Veenstra, 2021; Gutierrez Reyes et al, 2024).

On a functional level, proteomics uncovers the amount, changes, and functioning of enzymes that are by and large responsible for the production of biosurfactants, such as non-ribosomal peptide synthetases and lipid-modifying enzymes (Veenstra et al, 2021). Proteomics based on mass spectrometry can help correlate gene expression with the resulting phenotype by making visible the post-translational modifications and protein-protein interactions (Go et al, 2024). The metabolomics and flux analysis (fluxomics) provide quantitative data on intracellular metabolites and carbon flux through biosynthetic pathways (Occhipinti et al, 2018). Furthermore, the levels of metabolites are highly indicative of the cell's condition and reaction to the environment which makes it possible to identify bottlenecks and imbalances in the biosynthetic pathways (Veenstra et al, 2021). However, metabolomics sometimes by itself can lead to incomplete interpretations because of the limitations of the analytical techniques. This is why it becomes very important to complement it with other omics levels (Jena and Sahoo, 2024).

Coupling these different datasets with the application of multi-omics and network modeling essentially constitutes the core of systems biology. By integrating data from genomics transcriptomics proteomics, and metabolomics, researchers can reconstruct genome-scale metabolic networks and pinpoint the regulatory circuits that govern biosurfactant synthesis (Abimbola et al, 2023). Such integrative approaches are instrumental in mitigating the occurrence of false positives/negatives that are generally a byproduct of single-omics investigations also enable the formulation of models that can predict metabolic flows and cell behaviour (Occhipinti et al, 2018). Studies at the systems-level, in fact, facilitate the identification of the main components, regulatory centers, and external factors responsible for biosurfactant production, which in turn is beneficial for the targeted improvement of strains and process optimization (Abimbola et al, 2023). Simply put, systems biology provides a comprehensive, predictive understanding of biosurfactant production, thereby enabling the design of new microbial cell

factories with high production yields and the development of industrial bioprocesses that are environmentally sustainable (Occhipinti et al, 2018).

9.0 METABOLIC AND GENETIC ENGINEERING STRATEGIES

Metabolic and genetic engineering have been used to greatly increase biosurfactant production by modifying microbial pathways and gene regulatory networks. One of the main strategies is to increase the expression of biosynthetic gene clusters (Chabhadiya et al, 2024). Other strategies include removing the cellular pathways that consume the substrate, and diverting carbon to the product. By engineering foreign organisms like *Escherichia coli* it becomes possible to have regulated and large-scale production (Thakur et al, 2024). Synthetic biology also helps in pathway reconstruction and promoter tuning. Besides that, biosurfactant production can also be improved by precursor supply, stress tolerance, and substrate utilization. Balancing metabolism at the systems-level and genome editing by CRISPR are further ways to increase production, making industrial-scale biosurfactant synthesis with desired properties (She et al, 2024).

Table 5: Metabolic and genetic engineering strategies

Strategy	Approach	Outcome	References
Gene overexpression	Increase biosynthetic genes	Higher yield	Sundaram et al, 2024
Promoter engineering	Modify promoter strength	Controlled expression	Markande et al, 2021
Knockout pathways	Remove competing pathways	Increased carbon flux	Thakur et al, 2024
Heterologous expression	Use alternative hosts	Scalable production	Anburajan et al, 2015

9.1 Metabolic engineering

Metabolic engineering has been one of the most efficient ways to improve biosurfactants production. It works by altering cellular metabolism so that it produces more biosurfactants. The major methods to achieve this are changing carbon flux and increasing precursor supply (Zhang et al, 2025). Changing carbon flux means targeting the biosurfactants production pathways inside the cell and trying to keep carbon away from other competing pathways. This can be done by blocking or reducing the activity of certain pathways, usually through gene knockouts. At the same time, the enzymes of desired pathways are usually overexpressed (Wu et al, 2023). For example, a study has shown that carbon flux can be directed to biosurfactant production by blocking triacylglycerol synthesis in *Rhodococcus erythropolis*, which greatly improves biosurfactant yield. Likewise, metabolic engineering strategies often target central carbon metabolism (Zhang et al, 2025).

Enhancing the supply of precursors is geared towards boosting cellular pools of major metabolic intermediates that are directly implicated in the biosurfactant building blocks, e.g. fatty acids, amino acids, and sugar moieties (Zhao et al, 2025). This may be done by elevating the expression level of the genes encoding enzymes for precursor synthesis, by the introduction of heterologous biosynthetic pathways, or by improving the uptake and assimilation of substrates (Chabhadiya et al, 2024). For instance, the overexpression of biosynthetic genes (like *rhlAB* in *Pseudomonas aeruginosa*) lead to increased production of rhamnolipids by increasing the precursor pools (Zhao et al, 2025). Also, the regulated control of precursor flux and cofactor engineering have been effectively used to substantially increase metabolite production in engineered strains. Moreover, the development of substrate utilization capabilities, such as the usage of alternative carbon sources, also aids in increased precursor availability and general enhanced productivity (Zhang et al, 2025).

9.2 Genetic engineering

One of the most effective approaches for producing biosurfactants through microbes is the genetic modification of the organisms, i.e. the direct alteration of microbial genomes intended for higher production, better efficiency, and higher specificity of the product (Sekhon et al, 2011). Techniques mainly employed here include gene overexpression, promoter engineering, and knockouts of competing pathways (Chabhadiya et al, 2024). Overexpressing a gene is definitely one of the main methods for ramping up the biosynthesis of a biosurfactant. The essential structural genes coding for most enzymes

such as the *rhlAB* operon in *Pseudomonas aeruginosa* which produces rhamnolipids or the *srfA* operon in *Bacillus subtilis* are artificially overexpressed by the plasmid-based or chromosomal integration systems (Hu et al, 2021). The outcome of this is an increased amount of enzyme and an accelerated biosynthetic flux towards the desired product. As an illustration, enhancing *rhlAB* expression dramatically increases the synthesis of rhamnolipid, whereas the host's greater resistance under production conditions is one of the consequences of the stress-response regulators (e.g. *rpoS*) being upregulated (Chabhadiya et al, 2024). In a similar fashion, a modification of the fatty acid biosynthesis routes has been proven to lead to a more elevated production of surfactin through the precursor supply increase (Wu et al, 2019).

Promoter engineering is a technique aimed at the level of gene expression by either modifying or substituting the original promoters (Chabhadiya et al, 2024). The use of synthetic or inducible promoters can lead to very fine temporal and quantitative control of the biosurfactant gene clusters. Such a method can diminish the metabolic load and gives the possibility of dynamic management of the production process (Sundaram et al, 2024). The development of synthetic biology tools and CRISPR-based methods have made it easier to come up with powerful and adjustable promoters that can be used for improving transcriptional effectiveness in a variety of organisms (Zhang et al, 2020). In fact, inducible promoters were the key component for *Streptomyces* and other bacterial host manipulations allowing us to precisely control the biosynthetic pathways which result in better yields and more consistent products. Besides that, they allow the expression of biosurfactant genes in bacteria that normally do not produce them such as *Escherichia coli* (Chabhadiya et al, 2024). Another important method to rechannel metabolic fluxes is the knockout of competing pathways. By removing genes responsible for side-products or cell storage compounds, the majority of carbon and energy can be used for producing biosurfactants (Hu et al, 2021). As an example, deleting the triacylglycerol synthesis pathway in *Rhodococcus erythropolis* resulted in a drastic increase in biosurfactant levels thanks to minimizing carbon diversion to the storage lipids (Zhang et al, 2020). Removal of repressors or competing metabolic branches in *Bacillus* is another instance which increases the efficiency of surfactin production (Hu et al, 2021).

9.3 Heterologous expression systems

Heterologous expression systems have become one of the innovative methods to greatly increase the production of biosurfactants (Geraldi et al, 2025). The essence of this method is to move a complete gene set into well-characterized hosts whose properties can be easily manipulated for production at a high level. Bacteria such as *Escherichia coli*, yeasts like *Saccharomyces cerevisiae* and *Komagataella phaffii*, and actinomycetes such as *Streptomyces* belong to the list of hosts usually used for this purpose (Ahluwalia et al, 2026). Apart from this, these platforms provide several other advantages such as a reduced generation time, easier genetic modifications, and better scalability than the native producers.

In the field of bacteria, *E. coli* is the main player for producing enzymes related to biosurfactants or even entire biosynthetic gene clusters as it is a rapidly growing organism and the tools for recombinant DNA are very well developed (Vega and Stampino, 2025). However, issues like the absence of post-translational modifications and protein misfolding may negatively impact the score. Normally, highly advanced methods like codon optimization, promoter engineering, and chaperones' co-expression are performed to resolve these issues (Ahluwalia et al, 2026). The combination of synthetic pathways in *E. coli* was not only successful for complicated biosurfactants like rhamnolipids but also allowed the separation of production from the native pathogenic host and made independent production possible (Geraldi et al, 2025).

Yeast-based systems are an attractive alternative because they can perform eukaryotic post-translational modifications and secretion of metabolites efficiently. One example is the engineering of *S. cerevisiae* and methylotrophic yeasts, which led to increased expression of biosurfactant enzymes via secretion pathway optimization and metabolic engineering (Raheja et al, 2026). Introducing heterologous rhamnosyltransferase genes into *Starmerella bombicola* is a good example, it gave rise to production of new sophorolipid variants with superior functional properties by bio-synthesis (Lavanya, M., & Agarwala). Besides, *Streptomyces* hosts are commonly used for the expression of large biosynthetic gene clusters, which is related to their natural ability to produce secondary metabolites and genetic stability. Heterologous engineered *Streptomyces* platforms allow efficient expression of complex pathways and biosurfactant discovery (Raheja et al, 2026).

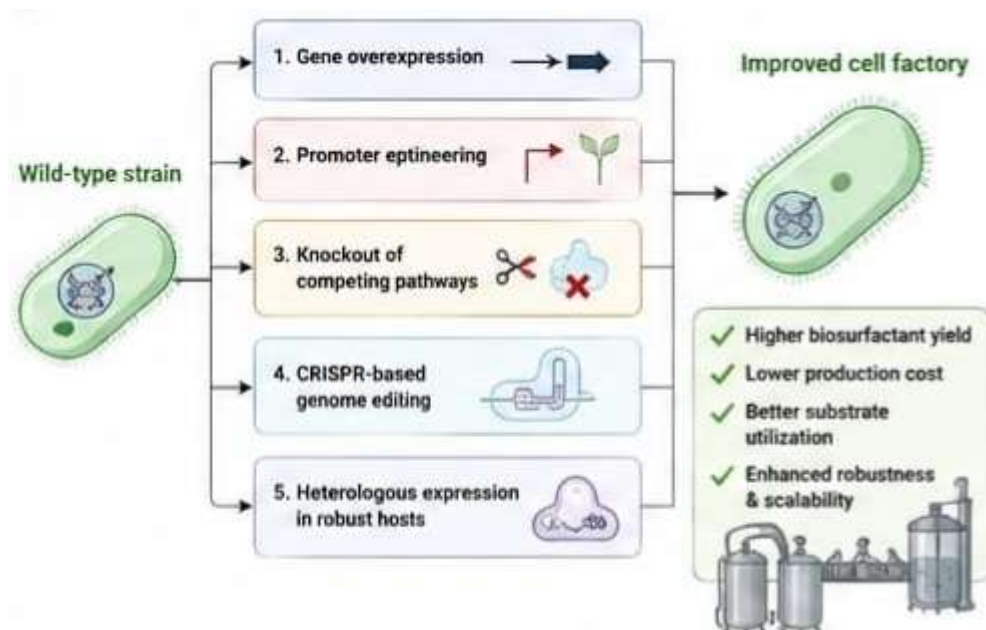


Figure 6: Metabolic engineering and synthetic biology strategies for improved biosurfactants production

CONCLUSION

Biosurfactants from microbes are a green alternative to synthetic surfactants with less environmental impact and more versatile applications in industries. This article highlights how genes arrangement, metabolic processes, and regulatory systems interact in complex ways to control biosurfactant production. The synthesis of these amphiphilic compounds is very closely connected to the central metabolism of the cell. To enhance the production of biosurfactants it is a must to have a good knowledge of the function of biosynthetic genes, expression regulators, and the control of gene expression at different levels. Omics techniques and systems biology have recently opened new avenues to the global characterization of biosurfactant-producing microbes. Such methods offer great potential to unravel metabolic fluxes, gene regulation, and enzyme characteristics, thus making it possible to implement targeted measures for higher productivity. Furthermore, metabolic and genetic engineering approaches have been demonstrated effective in changing carbon flux distribution, ensuring higher supply of precursors, and reducing by-product formation, all of these resulting in increased biosurfactant yields. However, along with these progressive steps, challenges such as high-cost production, the low yield of non-pathogenic strains, and complex downstream processing still pose as major impediments for industrial-scale commercialization. However, utilizing synthetic biology, CRISPR genome editing, and heterologous expression systems are probably necessary to address these issues. In conclusion, it is a combination of genetics, metabolic engineering, and systems biology that is necessary for developing top-quality microbial cell factories, capable of high performance. These advances will make it possible to produce biosurfactants on a large-scale at a low cost and with the minimum environmental impact, which will, in turn, lead to their wider pharmaceutical, environmental, and industrial applications.

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