Nanotechnology Perceptions
ISSN 1660-6795
www.nano-*ntp.com*

Biosurfactants- A Review: Bridging The Gap Between Microbial Production And Industrial Application

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Biosurfactants, surface-active compounds produced by microorganisms, have garnered significant attention due to their diverse applications in environmental remediation, pharmaceuticals, and industrial processes. This review comprehensively examines the current state of biosurfactant research, focusing on their classification, production methods, and characterization techniques. We explore the mechanisms by which biosurfactants enhance the biodegradation of hydrocarbons and heavy metals, highlighting their role in bioremediation strategies. Additionally, the review discusses the optimization of biosurfactant production through various biotechnological approaches, including the use of agro-industrial waste as substrates. The potential of biosurfactants in medical applications, such as drug delivery and antimicrobial agents, is also addressed. Furthermore, we evaluate the challenges and future prospects for the commercialization of biosurfactants, emphasizing the need for sustainable production methods. This review aims to provide a comprehensive overview of the advancements in biosurfactant research and their promising applications in addressing environmental and industrial challenges.

Key Words: Biosurfactants, Bioremediation, Microbial production, Environmental applications, Optimization techniques.

INTRODUCTION:

An estimated 40% of the world's energy supply is contributed by crude oil (BP, 2024). Oils with different levels of refinement have replaced most other fuels for motorized transport. The 19th century saw an unprecedented expansion of the automobile industry. Ships driven by oil could move up to twice as fast as compared to those using coal-powered fuel, providing a significant advantage for both military and civilian purposes. Gasoline engine development formed a crucial milestone for designing successful aircraft. The growing economy, along with modernization and industrialization, has resulted in increased fossil fuel consumption. Petrochemicals derived from petroleum or natural gas play an essential role in the chemical industry in recent years. With increasing consumption, concerns are growing not only towards the steady depletion of biofuels but also the challenges posed by rising environmental impacts, ranging from local pollution to global climate effects. Presently, oil and gas are produced in

almost every part of the world, from smaller quantities of 100 barrels per day in private wells to over 4,000 barrels per day, with explorations ranging from shallow wells to deep reservoirs over 2,000 to 3,000 meters deep, both in onshore and offshore installations (BP, 2024).

Petroleum hydrocarbons and other products are released into the environment, especially resulting in a major threat to the environment. Oil spills are frequently observed in the marine environment due to human activity and have disastrous consequences for society—economically, environmentally, and socially (Van Hamme et al., 2003). Petro-products are released from tankers, offshore, and onshore installations. Spills of refined petroleum products (such as gasoline, diesel) and their by-products, as well as heavier fuels like bunker fuel used by large ships, drastically affect terrestrial and marine flora and fauna (Van Hamme et al., 2003). Cleanup and recovery from an oil spill is a complex process influenced by several factors, including the type and quantity of spillage, environmental factors, salinity, etc., and can take several weeks, months, or even years to clean up (Atlas and Utermann, 1999; Van Hamme et al., 2003). Ballast water accounts for 3–4 billion tons per year and contributes to the second-highest pollution of water with fossil fuels, in addition to oil spills (Ballast Water News, 2003).

The strategies to contain oil spills range from natural cleanup to conventional methods using booms, skimmers to collect the oil from the surface, and the use of chemical dispersants to break the slick into droplets and speed up its natural removal. Removing oil spills by conventional methods can lead to harmful effects on the environment and those personally handling them (Atlas and Utermann, 1999). Bioremediation stands as an alternative solution where microorganisms or their products, like biosurfactants, are used in cleaning up the environment. Microorganisms produce a wide range of amphipathic compounds having both hydrophilic and hydrophobic moieties within the same molecule. These molecules are heterogeneous, complex, and exhibit surface activity at the interface and are called biosurfactants or bioemulsifiers (Cameotra and Makkar, 2004).

Over time, biosurfactants have gained significant importance as an alternative to chemical surfactants due to their safety and biodegradability. These compounds are excreted extracellularly and adhere to the cell surface of both prokaryotes and eukaryotes inhabiting different environmental niches (Rosenberg and Ron, 1999; Mishra and Jha, 2009; Kavitha et al., 2011; Shing et al., 2011). Biosurfactants produced by microorganisms are emerging as viable alternatives to synthetic surfactants. The interfacial surface tension-reducing ability of biosurfactants makes them play an important role in oil recovery and bioremediation of heavy crude oil. They are used both as emulsifiers and demulsifiers, wetting agents, foaming agents, spreading agents, functional food ingredients, and detergents (Rodrigues et al., 2006; Sivachidambaram, 2008; Singh et al., 2006). In addition, biosurfactants find applications in several industries, including organic chemicals, mining, metallurgy (mainly bioleaching), agrochemicals, fertilizers, foods, beverages, cosmetics, pharmaceuticals, and many others (Calvo et al., 2009).

In food industries, biosurfactants mediate the formation and stabilization of emulsions, improve the consistency and texture of fat-based products by controlling the aggregation of fat globules, and enhance the texture. Biosurfactant usage has improved the volume, flavor, texture, and shelf-life of bakery products, ice cream, frozen confections, and other starch-containing products (Kamal Alahmad, 2015). They also control the consistency, prolong staling, and solubilize oils in foods (Kosaricet al., 1987; Fazilet Vardar Sukan, 2014).

Biosurfactants possess properties not observed in conventional chemical surfactants. The three major functions played by biosurfactants include increasing the surface area and bioavailability of hydrophobic substrates through solubilization/desorption. Other features include tolerance to pH, temperature, and ionic strength, biodegradability, low toxicity, emulsifying and demulsifying ability. They also regulate the attachment and removal of microorganisms from surfaces and exhibit antimicrobial activity (Vijayakumar and Saravanan, 2015). In addition to these characteristic features, the production efficiency of biosurfactants from microorganisms can be improved along with progress in modern biotechnology methods. Biosurfactants play an important role in lowering the surface tension of water at the air/water and oil/water interfaces, which can be measured by the change in surface and interfacial tensions using a tensiometer (Desai and Banat, 1997).

An emulsion is formed when one liquid phase is dispersed as microscopic droplets in another liquid continuous phase. The characteristic hydrophilic-lipophilic balance of biosurfactants makes them attractive to be used as detergents, foaming agents, emulsifiers, and dispersing agents (Subramanya Karanth et al., 2005; Sarafin et al., 2014).

STRUCTURE AND CLASSIFICATION OF BIOSURFACTANTS:

Surfactants mediate the lowering of surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants function as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Surfactants contain both a water-insoluble (or oil-soluble) hydrophobic component, sometimes called tails, and a water-soluble or hydrophilic group, also called a head, and are thus amphiphilic. They are mainly organic compounds that adsorb at interfaces between air and water or oil and water, in mixtures with a hydrophobic group that extends into the air or oil-water phase, while the hydrophilic group remains in the..water phase (Rosenberg & Ron, 1999; Vijayakumar & Saravanan, 2015).

Surfactants are primarily classified based on their origin and the nature of their head groups. Synthetic surfactants are typically chemically synthesized and categorized into four groups depending on the charge of the head group: cationic (positively charged), anionic (negatively charged), non-ionic (neutral), and zwitterionic (containing both positive and negative charges). For example, quaternary ammonium compounds are commonly found in cationic surfactants, sulfonates or carboxylates in anionic surfactants, and sugars or alcohols in non-ionic surfactants. Zwitterionic surfactants often include betaines or sulfobetaines as their functional groups. The hydrophobic tail portion may vary significantly in chain length and structure, including straight or branched alkyl chains, aromatic rings, or more complex groups like perfluoroalkyl or polysiloxane units (Zhou et al., 2019; Singh &Cameotra, 2020).

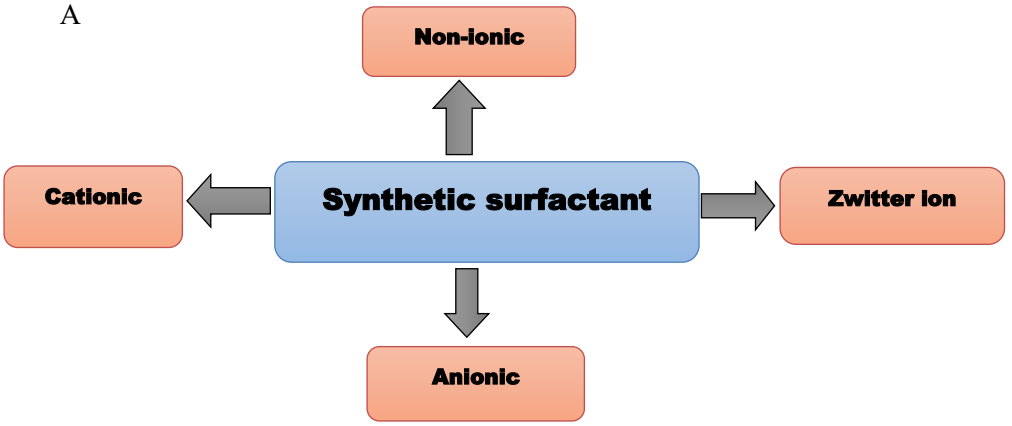
Biosurfactants, the biologically derived counterparts, are classified based on chemical structure, nature of hydrophobic and hydrophilic moieties, and the microbial source of production. Hydrophobic components usually consist of fatty acids or hydrocarbon chains, while hydrophilic parts are composed of peptides, amino acids, or sugars. Based on molecular weight, biosurfactants are divided into:

Low molecular weight biosurfactants: These include glycolipids, lipopeptides, phospholipids, and fatty acid derivatives.

High molecular weight biosurfactants (polymeric or particulate): These include lipopolysaccharides, lipoproteins, and polysaccharide-protein complexes (Gudiñaet al., 2016; Sharma et al., 2021).

Low molecular weight biosurfactants, such as rhamnolipids, sophorolipids, and surfactin, are particularly efficient at reducing surface and interfacial tension, making them highly suitable for applications like oil spill remediation, enhanced oil recovery, and industrial emulsification processes (Gaur et al., 2022). On the other hand, polymeric biosurfactants, while less effective in reducing surface tension, are excellent stabilizers of emulsions and find applications in biocontrol, food stabilization, and pharmaceuticals (Mishra et al., 2020).

A



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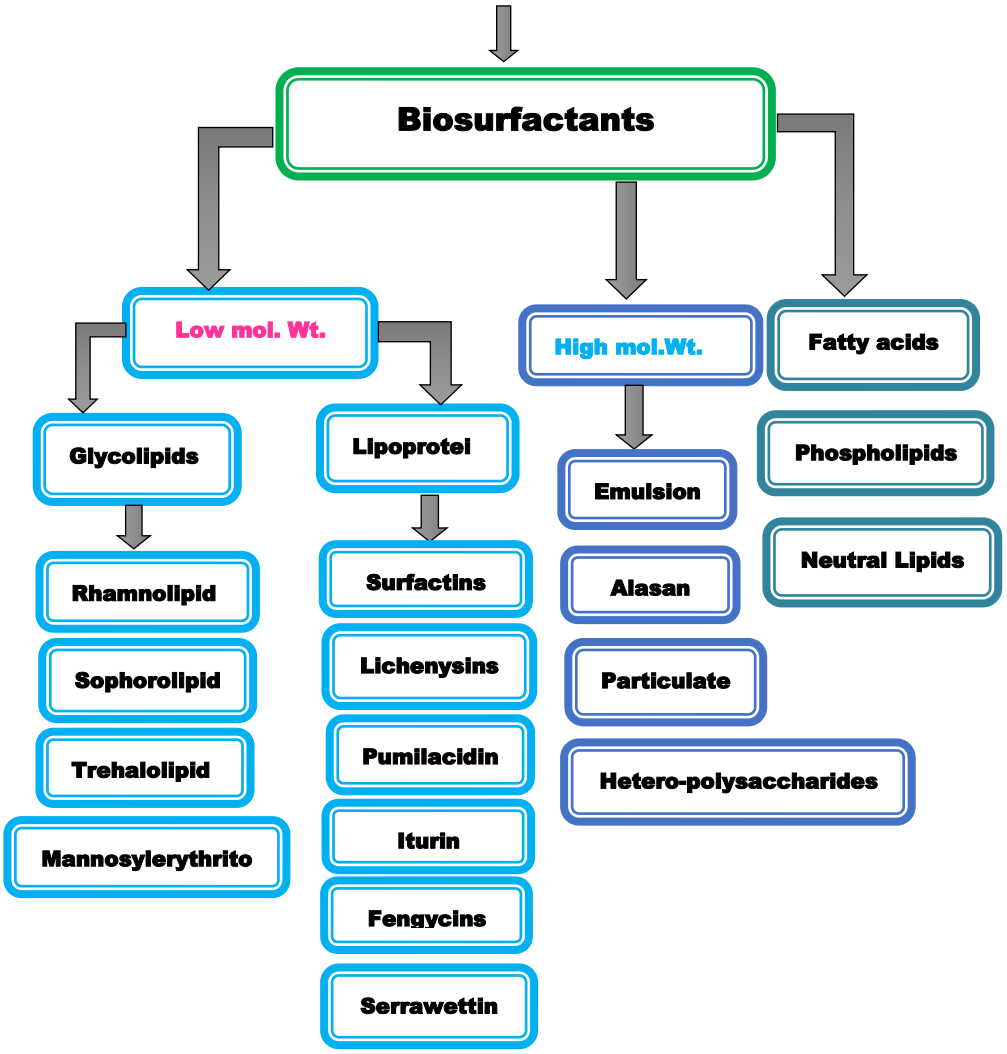


Fig. 2.2 Categorization of surfactants (A) Synthetic surfactants

Glycolipids: Glycolipids are among the most well-known biosurfactants, formed by the conjugation of carbohydrates and fatty acids linked by ether or ester bonds. These glycolipids are typically mono- or disaccharide compounds acylated with long-chain fatty acids or hydroxyl fatty acids (Khopadeet al., 2018). The major subclasses of glycolipids include rhamnolipids, mannosylerythritol lipids (MELs), sophorolipids, and trehalolipids. These subclasses are distinguished by their unique structures and properties (Makkar &Cameotra, 2015).

Rhamnolipids: Rhamnolipids are composed of one or two molecules of rhamnose linked to

one or two molecules of β-hydroxydecanoic acid. These glycolipids are primarily produced by Pseudomonas aeruginosa and Burkholderia species (Raza et al., 2020). Rhamnolipids exhibit excellent surface activity and have been utilized in the preparation of microemulsions and nanoparticles. Additionally, they display antimicrobial, antifungal, antiviral, and anti-adhesive activities, making them valuable in biomedical applications (Li et al., 2018). Rhamnolipids also play a significant role in the dewatering efficiency of waste crude oil, achieving up to 90% dewatering efficiency, thereby aiding in the industrial demulsification of waste crude oil by degrading and dispersing different classes of hydrocarbon groups (Raza et al., 2020).

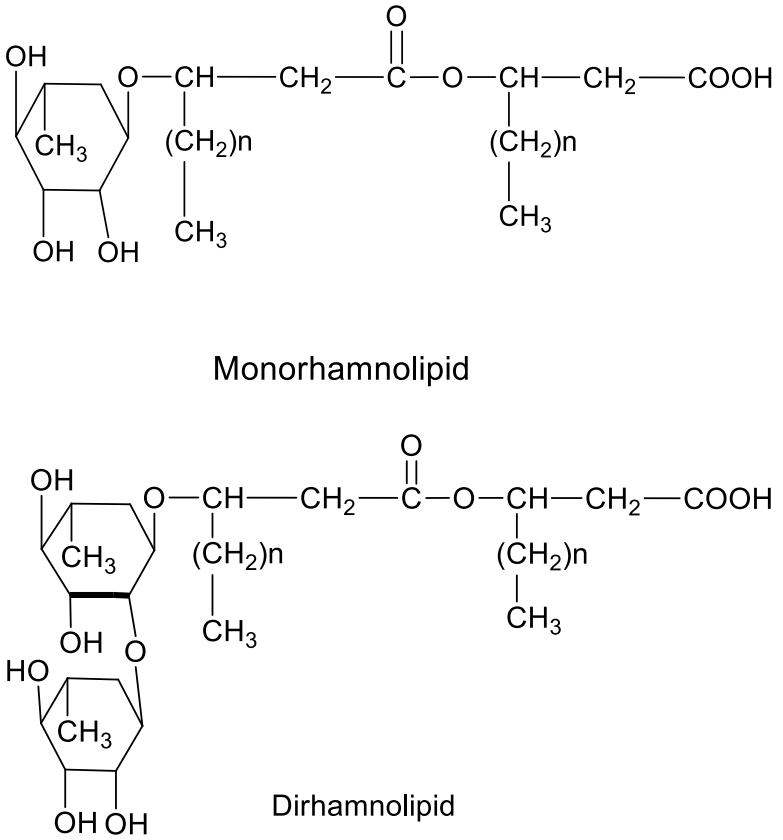


Fig. 2.3: Structures of Rhamnolipids

Mannosylerythritol Glycolipids (MELs): MELs are a mixture of partially acylated derivatives of 4-O-β-D-mannopyranosyl-D-erythritol. They contain fatty acids such as C2:0, C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, and C18:1 as the hydrophobic groups (de Carvalho & Fernandes, 2018). Depending on the degree of acetylation at C4 and C6 positions, MELs are classified into MEL-A, MEL-B, MEL-C, and MEL-D. MEL-A are diacetylated compounds produced by Pseudozyma species and Ustilago species when grown on soybean oil or n-alkane. MEL-B and MEL-C are monoacetylated at C6 and C4, respectively, whereas MEL-D is

deacetylated (Makkar &Cameotra, 2015). MELs have demonstrated antimicrobial, antitumor, and immunomodulatory activities, finding applications in gene and drug delivery as well as in cosmetics as skin moisturizers (de Carvalho & Fernandes, 2018).

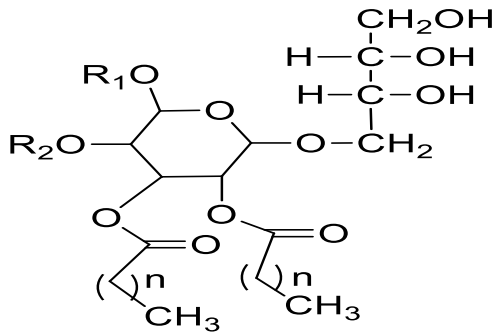


Fig. 2.4: Structures of Mannosylerythritol glycolipids

Trehalolipids: Trehalolipids are composed of disaccharide trehalose linked at C-6 and C-6′ to mycolic acid, which is an α-branched-β-hydroxy fatty acid. These biosurfactants are primarily produced by species of Mycobacterium, Nocardia, and Corynebacterium (Zhou et al., 2016). The structural differences in various trehalolipids are due to variations in the number of carbon atoms and the degree of unsaturation present in the mycolic acids. Succinoyl trehalose lipids have been found to induce differentiation in leukemia cell lines and inhibit protein kinase activity, thereby exhibiting antitumor activity (Zhou et al., 2016). They also aid in permeability through phospholipid membranes and stimulate hemolysis of human red blood cells. Trehalose lipids from Rhodococcus species have been shown to play a significant role in interacting with antimicrobial compounds (Singh et al., 2020).

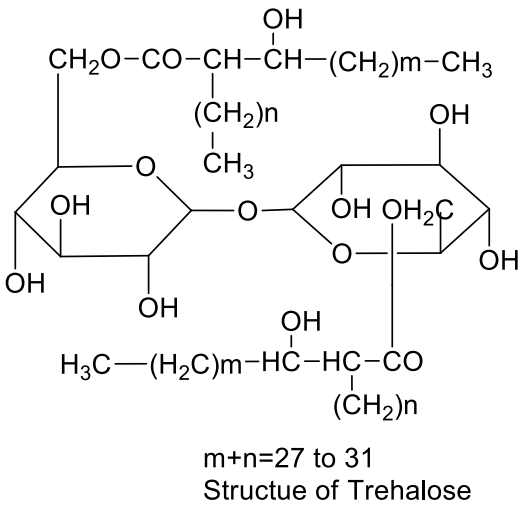
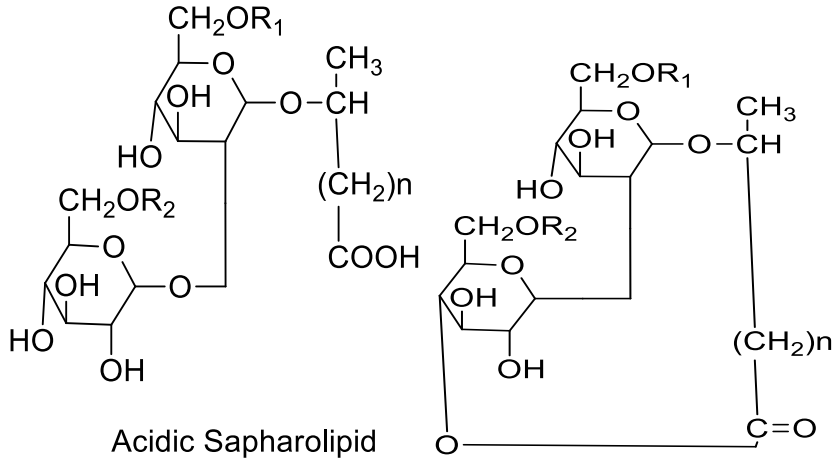


Fig. 2.5: Structures of Terhalolipids

Sophorolipids: Sophorolipids have a dimeric carbohydrate sophorose (two glucose units linked by β-1,2 linkage), which is linked to long-chain hydroxyl fatty acids by glycosidic linkage. These glycolipids are produced by yeasts such as Torulopsisbombicola, Torulopsispetrophilum, Torulopsisapicola, Candida bombicola, Candida apicola, Rhodotorulabogoriensis, Wickerhaminelladomercqiae, and Candida batistae (de Carvalho & Fernandes, 2018). Sophorolipids exist in nature in a mixture of two forms: macrolactones and free acids. The lactone form of sophorolipids is preferred due to its wider applications (Makkar &Cameotra, 2015). They have a greater solubilization ratio comparable to synthetic surfactants. Sophorolipids are recognized as low-foaming surfactants with high detergency and low cytotoxicity. They exhibit antimicrobial, antiviral, and anticancer activities, with applications in the biomedical field (Sharma et al., 2021). Sophorolipids are used in cosmetic and pharmacodermatological products in the form of metal-bound nanoparticles (Makkar &Cameotra, 2015).



(A) Acidic Sophorolipids (B) Lactone Sophorolipids

Fig. 2.6:Structures of Sophorolipids

Lipopeptides and Lipoproteins: Lipopeptides and lipoproteins are lipids attached to a polypeptide chain. A lactone ring is formed by cyclization in the polypeptide chain, and the fatty acid is present at the N-terminal. A large number of cyclic lipopeptides, which vary in the lengths of polypeptide and fatty acid chains, are produced by Bacillus species. These include antibiotics like gramicidins (decapeptides) and polymyxins (lipopeptides). Other common lipoproteins include surfactin, lichenysin, pumilacidin, iturin, and fengycins (Jiao et al., 2021).

Surfactin: Surfactin is an important biosurfactant named after its superior surface activity and belongs to a group of cyclic lipoheptapeptides containing β-hydroxy fatty acids and D−/L-amino acid residues. Four isoforms of surfactins A–D are produced in nature as mixtures, which differ in the amino acids involved in the lactone ring with the C14–C15 β-hydroxy fatty acid (Lépineet al., 2019). Alteration in the composition of amino acids,

particularly in surfactin, significantly affects its ability to form stable structures in aqueous solutions. Surfactin has an extremely low critical micelle concentration (CMC), contributing to its superior surface activity, and it has been employed in various applications such as oil recovery, biodegradation, and as a drug carrier (Yang et al., 2018). In addition, surfactin has shown potent antimicrobial, antiviral, and antitumor properties, leading to its exploration in therapeutic applications (Lépineet al., 2019). This biosurfactant can be used in bioremediation to break down environmental pollutants due to its ability to solubilize hydrophobic compounds (Yang et al., 2018). Surfactin is produced by various Bacillus species, with Bacillus subtilis being the most studied (Yang et al., 2018). It consists of a cyclic peptide containing a β-hydroxy fatty acid chain, typically C14–C15, and is known for its excellent surface activity (Lépineet al., 2019). Alteration in the composition of amino acids, particularly in surfactin, significantly affects its ability to form stable structures in aqueous solutions. Surfactin has an extremely low critical micelle concentration (CMC), contributing to its superior surface activity, and it has been employed in various applications such as oil recovery, biodegradation, and as a drug carrier (Yang et al., 2018). In addition, surfactin has shown potent antimicrobial, antiviral, and antitumor properties, leading to its exploration in therapeutic applications (Lépineet al., 2019). This biosurfactant can be used in bioremediation to break down environmental pollutants due to its ability to solubilize hydrophobic compounds (Yang et al., 2018).

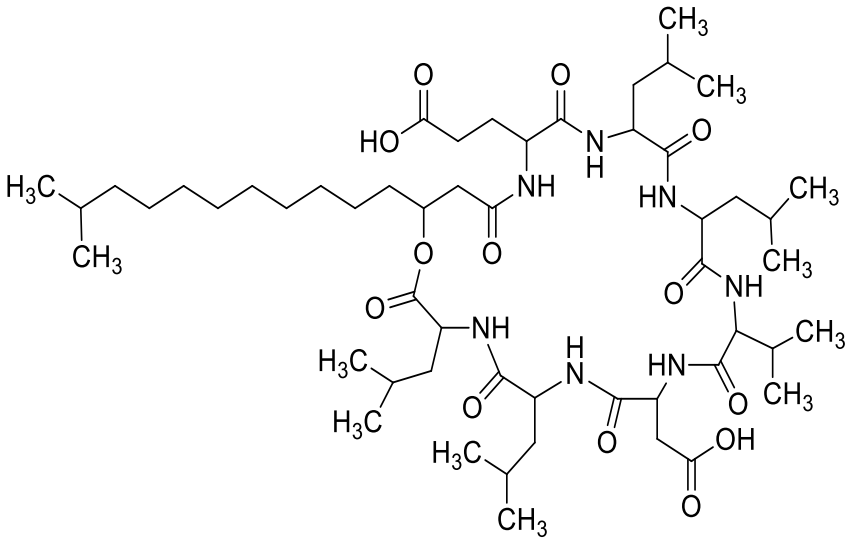


Figure 2.7: Structures of Surfactin

Lichenysin: Lichenysin is another cyclic lipopeptide that is produced by Bacillus licheniformis and Bacillus subtilis (Kuiper et al., 2022). It consists of a cyclic peptide with a β-hydroxy fatty acid chain, typically C16–C18. Lichenysin has potent antimicrobial properties, particularly against Gram-positive bacteria, and is being studied for potential medical and industrial applications, such as in the food and pharmaceutical industries (Kuiper et al., 2022).

Moreover, lichenysin's antimicrobial activity and ability to reduce surface tension have led to its consideration for use in biocontrol and environmental applications (Kuiper et al., 2022).

Phospholipids: Phospholipids are another class of important biosurfactants formed from glycerol, fatty acids, and phosphate-containing groups. These molecules, particularly phosphatidylcholine and phosphatidylethanolamine, play a crucial role in biological membranes and have shown surfactant properties (Gudipati et al., 2017). The surface activity of phospholipids is due to their amphiphilic nature, which allows them to form micelles, vesicles, and bilayers in aqueous solutions. Phospholipids derived from Lecithin (soybean or egg yolk) have applications in food, cosmetic, and pharmaceutical formulations due to their ability to stabilize emulsions and improve the bioavailability of drugs (Gudipati et al., 2017). In addition, phospholipids are known for their biodegradability and biocompatibility, making them environmentally friendly alternatives to synthetic surfactants (Liu et al., 2019).

Fatty Acids: Fatty acids such as lauric acid, myristic acid, and palmitic acid are also known to exhibit biosurfactant properties. These fatty acids can be produced by microorganisms through fermentation processes and are commonly used in various industrial applications such as cleaning agents, emulsifiers, and cosmetics (Santangelo et al., 2020). Microbial production of fatty acids through fermentation processes is of particular interest due to its sustainability compared to traditional chemical methods (Santangelo et al., 2020). The fatty acids produced in this manner have been shown to possess antimicrobial properties and are being studied for their potential in biofuel production as well as in bioremediation efforts (Liu et al., 2019). The amphiphilic nature of fatty acids allows them to form micelles in aqueous environments, which contributes to their ability to reduce surface tension and enhance the solubility of hydrophobic substances (Santangelo et al., 2020).

High Molecular Weight Polymeric Biosurfactants: Polymeric biosurfactants are high molecular weight molecules that often consist of repeating units of sugars and fatty acids. These biosurfactants typically have a backbone structure made up of three or four sugar molecules, to which fatty acids are attached (Rosenberg & Ron, 1997). Some well-known polymeric biosurfactants include emulsan, liposan, alasan, and other polysaccharide-protein complexes, which are recognized for their significant emulsifying properties and industrial applications (Puspita et al., 2015).

Emulsan: Emulsan is a complex acylated polysaccharide that is secreted extracellularly by gram-negative bacteria. It is an unbranched polysaccharide composed of three amino sugars: D-galactosamine, D-galactosaminouronic acid, and a dideoxydiaminohexose, in a 1:1:1 ratio. The side chains of emulsan are formed by bound O-acyl and N-acyl fatty acids, typically with carbon chain lengths ranging from 10 to 22 atoms, contributing 5-23% to the polymer's composition (Panilaitiset al., 2002). Acinetobacter calcoaceticus is known to produce emulsan with a molecular weight of around 1000 kDa, which has been extensively studied for its ability to act as an emulsifier in various industrial applications (Kim et al., 1997; Gorkovenkoet al., 1999). Even at very low concentrations (0.001-0.01%), emulsan demonstrates effective emulsifying properties for hydrocarbons in water (Zosim et al., 1982).

Alasan: Alasan is a high molecular weight, anionic polysaccharide produced by Acinetobacter radioresistens with a molecular weight of approximately 1,000 kDa (Smyth et al., 2010). This biosurfactant is known for its potent emulsifying activity, and because of its ability to effectively emulsify hydrocarbons, it is commonly referred to as a bioemulsifier. These high molecular weight biosurfactants are increasingly important in bioremediation and environmental applications due to their stability and effectiveness.

Liposan: Liposan is an extracellular emulsifying agent produced by the yeast Candida lipolytica. It consists of 83% carbohydrate and 17% protein and has gained attention for its potential use in food and beverage industries, especially in feed-grade applications (Cirigliano & Carman, 1985). Similarly, Saccharomyces cerevisiae produces a protein emulsifier called mannan, which exhibits excellent emulsifying activity on various oils, alkanes, and organic solvents (Cameron et al., 1988). These emulsifiers are crucial in many biotechnological processes where oil emulsification is required.

Hetero-polysaccharide Surfactants: Several extracellular acetylated polysaccharides and other heteropolysaccharide surfactants produced by bacteria such as Pseudomonas tralucida and Halomonaseurihalina have demonstrated effective emulsifying activity (Rosenberg & Ron, 1999). In addition, a protein complex produced by Methanobacteriumthermoautotrophium has shown significant bioemulsifying activity at high temperatures, further illustrating the diverse nature of polymeric biosurfactants. Bacillus species, such as Bacillus stearothermophilus ATCC 12980, also produce protein-polysaccharide-lipid complexes that function as bioemulsifiers, contributing to their utility in industrial applications (Smyth et al., 2010).

Particulate Biosurfactants: Particulate biosurfactants, including extracellular vesicles and whole microbial cells, play an important role in the emulsification and uptake of hydrocarbons by microorganisms. Extracellular vesicles, composed of proteins, phospholipids, and lipopolysaccharides, help microbes partition hydrocarbons, forming microemulsions that facilitate alkane uptake (Neu, 1996; Desai & Banat, 1997). For example, Acinetobacter species, when grown on hexadecane, produce vesicles with a buoyant density of 1.158 g/cm3, which are involved in hydrocarbon uptake (Kappeli& Finnerty, 1979; Perfumoet al., 2010). Similarly, Pseudomonas marginal also forms vesicles that act as biosurfactants. Additionally, many species of Cyanobacteria, as well as non-hydrocarbon degraders and some pathogens, have been observed to exhibit strong affinities for hydrocarbon-water and air-water interfaces. In these cases, the microbial cells themselves may serve as natural surfactants (Karanth et al., 1999).

PROPERTIES OF BIOSURFACTANTS:

The commercial adoption of biosurfactants has seen consistent growth, owing to the numerous benefits they provide over synthetic alternatives. These advantages include:High surface and interface activity, Emulsion formation and breaking properties, Long-term stability of emulsions, often lasting months or even years, High biodiversity and ease of production, Low

toxicity, Tolerance to various environmental factors, such as temperature, pH, and ionic strength and Biodegradability.

Biosurfactants are recognized for their exceptional surface and interface activity, significantly reducing water's surface tension. For instance, they can lower the critical micelle concentration (CMC) by 10–40 times compared to chemical surfactants, allowing for lower quantities to achieve desired surface tension reductions (Desai & Banat, 1997). An effective biosurfactant can reduce water's surface tension from 72 to 35 mN/m, and the interfacial tension between water and hexadecane can be reduced from 40 to 1 mN/m (Mulligan et al., 1999; Wang et al., 2008). Among biosurfactants, rhamnolipids and surfactin can lower water's surface tension to 25–26 mN/m, and sophorolipids to 33 mN/m. Additionally, these biosurfactants reduce interfacial tension to between 1–5 mN/m (Cooper et al., 1981; Cooper & Cavalero, 2003).

For high molecular weight biosurfactants, liposan stands out due to its ability to either stabilize or destabilize emulsions, even though it doesn't significantly impact surface tension. Liposan has been used successfully to emulsify edible oils (Cirigliano & Carman, 1985; Joice et al., 2014; Santos et al., 2016). Emulsions produced by polysaccharide biosurfactants demonstrate exceptional stability, lasting for months or even years. This stability is due to these biosurfactants' ability to effectively coat oil droplets (Velikonja &Kosaric, 1993; Sousa et al., 2012). This property makes them particularly valuable in industries like food and cosmetics, where long-lasting emulsions are essential.

Biosurfactants, produced by microorganisms, are chemically diverse, ranging from low to high molecular weight compounds, and their chemical composition determines their specific applications. In contrast to synthetic surfactants, biosurfactants are eco-friendly and biodegradable, which makes them ideal for environmental applications like petroleum spill cleanup and bioremediation (Mulligan, 2005; Mohan et al., 2006). They are generally considered low-toxicity or non-toxic, even for applications in pharmaceuticals, cosmetics, and the food industry. Research comparing the toxicity of synthetic surfactants with biosurfactants shows that biosurfactants degrade more quickly, making them safer for commercial use. Notably, one synthetic surfactant, sucrose-stearate, degrades faster than some biosurfactants, possibly due to its structural similarity to glycolipids (Poremba et al., 1991; Flaszet al., 1998). Additionally, Marlon A-350, a widely used synthetic surfactant, has shown significantly higher toxicity and mutagenicity than emulsions produced by Pseudomonas aeruginosa.

Biosurfactants also exhibit resilience under various environmental conditions such as temperature, pH, and salinity. For example, lichenysin, a glycolipid produced by Bacillus licheniformis JF-2, can withstand temperatures up to 50°C, while LB5a, a lipopeptide from Bacillus subtilis, remains stable even after exposure to autoclave temperatures of 121°C for 20 minutes and at –18°C for up to 6 months. LB5a's surface activity remains unchanged across a pH range of 5 to 11 and in NaCl concentrations as high as 20%. Similarly, lichenysin is stable at pH values ranging from 4.5 to 9.0 and in salt concentrations up to 50% (Cooper et al., 1981).

Fig. 2.8: Applications of Biosurfactants

APPLICATIONS OF BIOSURFACTANTS:

Biosurfactants have gained significant attention for their wide range of applications in various industries, especially those requiring enhanced emulsification properties, including biomedicine, petroleum, food, and cosmetics.

Medicine:

Biosurfactants have numerous applications in the medical field, with several studies extensively reviewing their potential (Mukherjee et al., 2006; Fathabadet al., 2011; Shoeb, 2015; Joshi et al., 2015).

Antimicrobial and Anti-adhesive Activity:

Biosurfactants affect the permeability of microbial cell membranes, often acting as detergents (Zhao et al., 2013; Duarte, 2014; Fracchia et al., 2012; Corvis et al., 2006). Several biosurfactants, such as lipopeptides, have shown strong antibacterial, antifungal, and antiviral properties (Fathabadet al., 2011). For example, Surfactin, Lichenysin, Pumilacidin, Iturin, and Fengycins are commonly known lipopeptides with antimicrobial effects. Bacillus circulans, a marine species, produces a biosurfactant with potent antimicrobial properties against both

Gram-positive and Gram-negative pathogens, including multidrug-resistant strains (Das et al., 2008; Fracchia et al., 2012).

Biosurfactants also inhibit pathogen adhesion, which has significant implications for therapeutic and probiotic applications. For instance, the pre-coating of vinyl urethral catheters with surfactin significantly reduces biofilm formation against Salmonella species, Escherichia coli, and Proteus mirabilis (Rodrigues et al., 2006). Additionally, Krishnasamy et al. (2008) demonstrated that biosurfactants effectively inhibited the adhesion of Candida albicans to silicone rubber. Other studies have also shown biosurfactants to reduce the adhesion of uropathogenic Enterococcus faecalis (Krishnasamy et al., 2008).

Biosurfactants have been explored for their anti-HIV properties, with studies indicating their ability to inhibit the growth of HIV in leukocytes (Desai & Banat, 1997; Krishnaswamy et al., 2008). Sophorolipids from Candida bombicola and their derivatives, such as sophorolipid diacetate ethyl ester, have demonstrated potent spermicidal and virucidal activities, making them suitable candidates for use in vaginal microbicides (Krishnaswamy et al., 2008).

Anti-cancer Activity:

Certain microbial extracellular glycolipids (MELs) have been shown to induce cell differentiation rather than proliferation in human pro-myelocytic leukemia cells. MELs enhanced acetylcholine esterase activity in PC 12 cells, disrupting the cell cycle at the G1 phase and leading to partial cellular differentiation. This suggests the potential use of MELs as novel anti-cancer agents (Krishnaswamy et al., 2008).

Gene Delivery and Other Applications:

Biosurfactants play a vital role in the efficient and safe delivery of nucleotides into mammalian cells, which is crucial for clinical gene therapy applications (Fathabad, 2011). They are also used to stimulate stem fibroblast metabolism and treat respiratory failure in premature infants caused by deficiencies in pulmonary protein complexes. The cloning and fermentation of biosurfactant molecules in bacteria are expanding their medical applications (Krishnaswamy et al., 2008). Additionally, bacterial lipopeptides, combined with antigens, serve as non-toxic, non-pyrogenic immuno-adjuvants, as demonstrated with Iturin A and herbicolin A (Fathabad, 2011).

Food Industry:

Biosurfactants play an essential role in the food industry, particularly in the formation and stabilization of emulsions. They help control the aggregation of fat globules, improve food texture, stabilize aerated systems, and extend the shelf-life of starch-containing products (Kachholz&Schlingmann, 1987). In bakery and ice-cream production, biosurfactants help control consistency, slow down staling, and aid in solubilizing flavor oils. Rhamnolipid surfactants, for example, improve the stability, texture, and volume of dough, enhancing the quality of bakery products, buttercream, and frozen desserts (Van Haesendonck&Vanzeveren, 2004; Castro et al., 2015).

Cosmetic Industry:

Biosurfactants find diverse applications in cosmetics due to their properties such as emulsification, foaming, water-binding, spreading, and wetting, which influence the viscosity and consistency of products. They are increasingly replacing chemically synthesized surfactants and are used in products like bath items, acne pads, anti-dandruff shampoos, toothpaste, and insect repellents (Fathabadet al., 2011). Additionally, biosurfactants act as solubilizers and cleansers with antimicrobial activity, making them valuable in various cosmetic formulations.

Petroleum Industry:

Biosurfactants have versatile applications in the petroleum industry, particularly in cleaning up oil spills and other petroleum contaminations. They also facilitate the bioremediation of hydrocarbons and improve the recovery of petrochemical products. Studies have shown that rhamnolipid-producing Pseudomonas aeruginosa strains enhance the degradation of hydrocarbons (Itoh & Suzuki, 1972; Das et al., 2010; Viramontes-Ramos, 2010; Pacwa et al., 2011). Biosurfactants increase microbial access to insoluble substrates such as hydrocarbons by enhancing their solubility and bioavailability, thus aiding biodegradation (Zhang & Miller, 1992; Hunt et al., 1994). This mechanism, where biosurfactants increase the solubility of organic compounds above their Critical Micelle Concentration (CMC), helps microorganisms assimilate hydrocarbons more efficiently (Chang et al., 2008). The use of biosurfactants is crucial in bioremediation efforts to clean polluted environments (Calvo et al., 2009).

Biosurfactants like rhamnolipids and surfactin, produced by Pseudomonas aeruginosa and Bacillus subtilis respectively, have been shown to increase the solubility of petroleum mixtures and stimulate indigenous microorganisms for enhanced biodegradation of diesel-contaminated soil (Whang et al., 2008). Additionally, Gordonia species, which produce emulsans, have been found to degrade crude oil effectively when grown on polycyclic aromatic hydrocarbons (PAHs) (Franzetti et al., 2008).

Biosurfactants also contribute to microbial enhanced oil recovery (MEOR), a process where specific microorganisms are utilized to enhance oil production from marginal reservoirs. The versatile metabolic processes of these microorganisms are exploited for increased oil yield (Cassia et al., 2014; Pacwa Płociniczaket al., 2011). Microorganisms like Bacillus subtilis, Pseudomonas aeruginosa, and Torulopsisbombicola are utilized for oil recovery and spill cleanup (Das & Mukherjee et al., 2006; Banat et al., 2014; Al-Wasifyet al., 2014).

Nanotechnological Applications:

The use of nanoparticles has significantly advanced various fields, driven by their large surface area to volume ratio and well-defined properties. Among them, silver nanoparticles (Ag-NP) are particularly well-known for their high electrical and thermal conductivity and enhanced stability (Chang & Yen, 1995; Shiraishi & Toshima, 2000; Krutyakovet al., 2008). Biosurfactants are valuable in nanotechnology as stabilizing agents for nanoparticle preparation, including Ag-NPs and nanozirconia (Kitamotoet al., 2005; Reddy et al., 2009). For example, rhamnolipids have been shown to stabilize Ag-NPs in water-in-oil microemulsions (Xie et al., 2005; Płazaet al., 2006; Banat, 2014), while sophorolipids serve as capping and reducing agents for silver nanoparticle formation (Kasture et al., 2008). Furthermore, glycolipid biosurfactants from Brevibacterium casei MSA19 have been used to

stabilize silver nanoparticles, contributing to the growing applications of biosurfactants in nanotechnology (Kiran et al., 2010; Farias et al., 2014).

Natural surfactins have also been shown to stabilize superparamagnetic iron oxide nanoparticles and cadmium sulfide nanoparticles, preventing aggregation and enhancing their application potential in various fields (Liao et al., 2010; Singh et al., 2011; Kiran et al., 2010, 2014; Farias et al., 2014; Ali et al., 2013).

SCREENING OF BIOSURFACTANTS AND THEIR PRODUCTION:

Biosurfactant-producing microorganisms primarily belong to genera such as Bacillus, Pseudomonas, Rhodococcus, and various yeast species. These microorganisms are also significant in the bioremediation of hydrocarbons and petroleum-based spills (Bodouret al., 2003; Singh et al., 2011; Patowaryet al., 2016). While some organisms show specific activities, recent metagenomic research suggests that in natural settings, the efficient bioconversion processes are often carried out by microbial consortia working in synergy rather than individual organisms (Kumar et al., 2011). Besides the well-known terrestrial environments, marine ecosystems, mangroves, and their sediments have become important sources for isolating biosurfactants and bioemulsifiers (Maneeratet al., 2006; Anandaraj and Thivakaran, 2010; Burgos et al., 2011; Saimmaiet al., 2012). Unique biosurfactants produced by extremophiles and halophilic bacteria, such as Acinetobacter species and Kocuria marina, are gaining attention due to their enhanced stability and potential for a variety of applications. Despite numerous reports on microorganisms producing biosurfactants, the most recent developments are summarized in Table 2.1.

Table 2.1: Organisms Producing Biosurfactants, Their Types, and Potential Applications

Organism	**Type of Biosurfactant**	**Potential Applications**	**Reference**
Acinetobacterium spp.	Lipopolysaccharide-protein	Used in drug formulations	Dehghan-Noudehet al., 2007
Azotobacter chroococcum	Lipopeptide	Environmental applications	Thavasiet al., 2008
Bacillus subtilis BS5	Lipopeptide	Bioremediation of hydrocarbon-contaminated sites	Abdel-Mawgoudet al., 2008
Bacillus subtilis HOB2	Lipopeptide	Enhanced oil recovery, bioremediation of soil and marine environments, food industries	Haddad et al., 2008

Bacillus subtilis ZW-3	Lipopeptide	Pharmaceutics, environmental protection, cosmetics, oil recovery	Wang et al., 2008
Bacillus velezensis H3	Lipopeptide	Industrial strain for lipopeptide production	Liu et al., 2010
Brevibacteriumaureum MSA13	Lipopeptide	Environmental molecules for bioremediation	Kiran et al., 2010
Burkholderiaplantari DSM9509	Rhamnolipid	Detergents and pharmaceutical industry	Hormann, 2010
Calyptogenasoyoae	Mannosyl-erythritol lipid	Bioremediation processes in marine environments	Konishi et al., 2010
Candida bombicola	Sophorolipids	Environmental applications	Daverey and Pakshirajan, 2010 a, b
Halomonas	Glycolipid	Antimicrobial activity, anticancer activity	Donio et al., 2013
Micrococcus luteus BN56	Trehalose tetra ester	Bioremediation of oil-contaminated environments	Tuleva et al., 2009
Nocardiopsis alba MSA10	Lipopeptide	Bioremediation	Gandhimathiet al., 2008
Nocardiopsislucentensis MSA04	Glycolipid	Bioremediation in marine environments	Kiran et al., 2010
Pseudomonas aeruginosa BS20	Rhamnolipid	Bioremediation of hydrocarbon-contaminated sites	Abdel-Mawgoudet al., 2009
Pseudomonas aeruginosa UFPEDA 614	Rhamnolipid	Bioremediation	Neto et al., 2008

Pseudomonas alcaligenes	Rhamnolipid	Environmental applications	Oliveira et al., 2009
Pseudomonas fluorescens BD5	Lipopeptide	Bioremediation and biomedicine	Janek et al., 2010
Pseudomonas koreensis	Lipopeptide	Biocontrol agent	Hultberg et al., 2010
Pseudomonas libanensis M9-3	Lipopeptide	Environmental and biomedical applications	Saini et al., 2008
Pseudoxanthomonas sp. PNK-04	Rhamnolipid	Environmental applications	Nayak et al., 2009
Pseudozymasiamensis CBS 9960	Mannosyl-erythritol lipid	Promising yeast biosurfactant	Morita et al., 2008
Pseudozymagraminicola CBS 10092	Mannosyl-erythritol lipid	Washing detergents	Morita et al., 2008
Pseudozymahubeiensis	Glycolipid	Bioremediation of marine oil pollution	Fukuoka et al., 2008
Pseudozyma antarctica	Mannosyl-mannitol lipid	Emulsifiers and/or washing detergents	Morita et al., 2009
Rhodococcuserythropolis 3C-9	Glucolipid, trehalose lipid	Oil spill cleanup operations	Peng et al., 2007
Rhodococcus sp. TW53	Lipopeptide	Bioremediation of marine oil pollution	Peng et al., 2008
Rhodococcuswratislaviensis BN38	Glycolipid	Bioremediation applications	Tuleva et al., 2008

To isolate microorganisms with desirable biosurfactant production capabilities, a two-fold strategy is commonly employed. In addition to the dilution plating method, enrichment culturing has proven especially effective in identifying novel biosurfactant producers (Schulz et al., 1991; Mercade et al., 1996; Huy, 1999; Rahman et al., 2002; Van Hamme et al., 2003; Bento et al., 2005). Hydrocarbon-based sources such as C14 and C15 n-alkanes, Poly Aromatic Hydrocarbons (PAHs), sludge oil, and petroleum-polluted soils are found to be ideal carbon sources that stimulate biosurfactant production. Other factors, including nutrient availability and physical conditions, also significantly influence biosurfactant yields. Several primary screening methods, including the agar plate method (with or without enrichment), the blue agar

plate method, and tests for hemolytic activity, are routinely employed to identify biosurfactant producers (Morikawa et al., 1992; Banat, 1993; Carrillo et al., 1996).

Agar Plate Overlaid with Hydrocarbons:

When biosurfactant-producing organisms are cultured on oil-coated agar plates (either mineral salt or complete media) and incubated for a week, they form a visible halo around the colony due to emulsification of the oil. This simple and effective technique is commonly used for primary screening to detect biosurfactant producers (Morikawa et al., 1992; Shoebet al., 2015).

Blue Agar Plate Method:

The Cetyl Trimethyl Ammonium Bromide (CTAB) agar plate method is specifically designed to screen for glycolipids and bioemulsifiers. Anionic biosurfactants react with CTAB and methylene blue to form insoluble complexes, resulting in a blue halo around the colony, indicating biosurfactant production. This method is a reliable way to detect anionic biosurfactants (Siegmund and Wagner, 1991; Mohammad Abdel Mawgoudet al., 2010; Tahzibiet al., 2004; Gunther et al., 2005; Tuleva et al., 2005; Satpute et al., 2010). A semi-quantitative assay can also be used to detect extracellular glycolipids or anionic surfactants.

Hemolytic Activity:

The blood agar method is a simple qualitative test for identifying biosurfactant-producing organisms, as recommended by Mulligan et al. (1984). It involves culturing microorganisms on solid media such as Luria agar (LA) or nutrient agar (NA) supplemented with 5% fresh whole blood (Banat, 1993; Carrillo et al., 1996). After incubation, the presence of hemolysis indicates the production of surface-active compounds. While this test is useful for preliminary screening, it is less reliable as a sole indicator of biosurfactant activity, especially for biosurfactants with poor diffusion properties (Youssef et al., 2004; Satpute et al., 2010a; Joice et al., 2014). Hemolytic activity may fail to detect biosurfactants that cannot diffuse efficiently through the agar, preventing the lysis of blood cells (Satpute et al., 2008; Thavasiet al., 2011).

TESTS FOR THE PRODUCTION OF BIOSURFACTANTS

The confirmation of biosurfactant production can be achieved through various methods, including the drop collapse method, oil spreading technique, and emulsification activity. Additional techniques such as direct surface/interfacial tension measurements, cell surface hydrophobicity tests (e.g., BATH assay, Du-Nouy-Ring method), and colony thin-layer chromatography are also employed to identify biosurfactant production (Rosenberg et al., 1980; Matsuyama et al., 1987; Bodour and Miller-Maier, 1998; Morikawa et al., 2000; Ellaiah et al., 2002; Satpute et al., 2010; Mukherjee et al., 2023).

Drop Collapse Method

The drop collapse method is a qualitative assay used to detect the presence of biosurfactants (Jain et al., 1991; Walter et al., 2010). This method relies on the destabilization of liquid droplets by biosurfactants. In this test, droplets of a cell suspension or culture supernatant are placed on an oil-coated solid surface. If the liquid lacks surfactants, the polar water molecules are repelled from the hydrophobic surface, and the droplets remain stable. Conversely, if surfactants are present, the droplets spread or collapse due to reduced interfacial tension. The stability of the droplets is influenced by surfactant concentration and correlates with surface and interfacial tension (Jain et al., 1991; Das et al., 2010). This method can serve both

qualitative and quantitative purposes, with micrometers used to measure drop size across varying volumes or concentrations of culture filtrate (Bodour and Miller-Maier, 1998; Joice et al., 2014). Notably, this assay can be adapted for automated screening using microplates and has been widely utilized for screening microorganisms that produce biosurfactants (Bodouret al., 2003; Youssef et al., 2004; Batista et al., 2006; Plaza et al., 2006; Maczek et al., 2007).

Oil Spread Method

The oil spread assay is a modification of the drop collapse method and can be employed as both a qualitative and quantitative method for screening biosurfactant producers. In this assay, the area of oil displacement is directly proportional to the concentration of biosurfactant present in the solution (Morikawa et al., 2000). The oil spreading assay is recognized for its simplicity and efficiency, making it suitable for detecting biosurfactant production even at low concentrations (Youssef et al., 2004; Plaza et al., 2006; Burchet al., 2011; Walter et al., 2010).

Emulsification Assay

The emulsification assay, originally developed by Cooper and Goldenberg (1987), serves as an indirect method for screening biosurfactant production. Patil and Chopade (2001) later modified this method into a quantitative emulsification assay, categorizing results into emulsification units. This assay allows for the comparison of biosurfactant-producing cultures based on their emulsification index (Ellaiah et al., 2002). Additionally, it can facilitate the simultaneous detection of biosurfactant production and hydrocarbon degradation activity on agar plates by overlaying with hydrocarbons (Kokareet al., 2007). While emulsification activity is a useful criterion for screening potential biosurfactant producers, it does not always correlate with surface activity (Bonilla et al., 2005; Plaza et al., 2006). Notably, a significant number of isolates that were positive for the degradation of kerosene, hexadecane, benzene, toluene, and crude oil also produced biosurfactants. Measuring the emulsification of hydrocarbons in culture broth has proven beneficial for selecting carbon and energy sources for biosurfactant production (Bento et al., 2005; Plaza et al., 2006; Chen et al., 2007; Thavasiet al., 2011).

Surface/Interfacial Activity

Various methods have been employed to measure surface activity, serving as supplementary techniques to confirm the presence of biosurfactant-producing microbes.

Direct Surface/Interfacial Tension Measurements:

Direct measurement of interfacial or surface activity in culture supernatants is a straightforward screening method suitable for preliminary assessments of biosurfactant-producing microbes (Lin, 1996; Ramkrishna Sen et al., 2010; Walter et al., 2010). However, this method has limitations, as the range of measurement is restricted. Surface tension decreases with increasing surfactant concentration until the critical micelle concentration (CMC) is reached. Beyond this point, further increases in biosurfactant concentration do not result in a detectable decrease in surface tension. Consequently, two cultures may exhibit the same surface tension despite significant differences in concentration. Serial dilution can address this issue, as dilution typically results in a steep increase in surface tension (Makkar

and Cameotra, 1997; Morikawa et al., 2000; Batista et al., 2006). Additionally, measurements can be influenced by pH and ionic strength.

Cell Surface Hydrophobicity Assay:
The cell hydrophobicity assay measures changes in surface hydrophobicity related to bacterial adherence to hydrocarbons (Rosenberg et al., 1980). The release of extracellular biosurfactants mediates hydrocarbon uptake, altering surface hydrophobicity, which can be quantified (Bouchez-Naitaliet al., 1999). This method has been effectively used to screen for efficient biosurfactant producers, as there is a direct correlation between cell surface hydrophobicity and biosurfactant production (Pan et al., 2006; Maneeratet al., 2006). Cultures producing cell-bound biosurfactants associated with hydrocarbons exhibit a high surface hydrophobicity index, making this assay a valuable screening method (Franzetti et al., 2008).

Du-Nouy-Ring Method:
The Du-Nouy-Ring method assesses the ability of a culture filtrate to reduce the surface tension of a liquid medium to 40 mN/m or less, indicating potential biosurfactant production (Cooper, 1986; Walter et al., 2010). Willumsen and Karlson (1997) noted that a good biosurfactant producer can reduce the surface tension of the growth medium by ≥20 mN/m compared to distilled water. While this method requires specialized equipment, its accuracy and ease of use are advantageous. However, it has limitations, such as the inability to read multiple samples simultaneously and the requirement for larger sample volumes, which cannot be diluted (Bodour and Miller-Maier, 1998). The Du-Nouy-Ring method measures the force needed to detach a wire ring from an interface, with the detachment force being proportional to interfacial tension. This measurement is typically conducted using an automated tensiometer, and the platinum ring is flamed before use to eliminate contamination. Alternatively, a platinum plate, known as a Wilhelmy plate, can be employed in a similar manner (Tadros, 2005; Tuleva et al., 2005; Wei et al., 2005; Chen et al., 2015).

Direct Colony-Thin Layer Chromatographic Technique
In the direct colony-thin layer chromatographic technique, bacterial mass is placed directly on a pre-developed thin-layer chromatography (TLC) plate (using a chloroform: methanol mixture in a 2:1 ratio) to characterize biosurfactant producers. After drying the bacterial mass, the plate is developed using a solvent mixture of chloroform, methanol, and 5M ammonia (85:25:4 v/v). The resulting chromatogram reveals the characteristic lipid compositions of the organism (Matsuyama et al., 1987). This method is rapid, easy to perform, and does not require specialized equipment.

The biosynthetic pathways for the synthesis of the major hydrophobic and hydrophilic moieties of biosurfactants have been studied and documented (Hommel and Ratledge, 1993; Syldatk and Wagner, 1987). These studies proposed various pathways for the synthesis of distinct biosurfactants, which are subsequently linked. The synthesis of hydrophilic and hydrophobic moieties occurs via independent pathways that are substrate-dependent. The production of biosurfactants by several bacteria and yeast has been shown to be inducible in the presence of hydrocarbons, alkanes, or glycerides. For instance, the induction of

sophorolipid synthesis was observed with the addition of long-chain fatty acids to the growth medium (Tulloch et al., 1961; Rapp et al., 1979; Chakrabarty, 1985). Conversely, the presence of specific chemicals can repress biosurfactant production. For example, the addition of D-glucose, acetate, and tricarboxylic acids drastically reduced the synthesis of rhamnolipids by Bacillus subtilis and liposan by Candida lipolytica (Duvnjak et al., 1982; Gobbertet al., 1984; Cirigliano and Carman, 1985). Limiting the concentrations of magnesium, calcium, potassium, sodium salts, and trace elements significantly influences the yields of biosurfactants produced by Bacillus subtilis DSM 2659 (Guerra-Santos et al., 1984; Banat et al., 2012). Additionally, the synthesis of certain biosurfactants correlates with nitrogen exhaustion in the media or the onset of the stationary phase (Santos et al., 2016; Guerra-Santos et al., 1984; Ramana and Karanth, 1989). Iron limitation has been shown to reduce biosurfactant production in Pseudomonas fluorescens, while the addition of iron and manganese salts stimulates biosurfactant production in Bacillus subtilis and Pseudomonas putida (Cooper et al., 1981; Persson et al., 1988; Ochsner et al., 1994; Sharma et al., 2014).

Large-scale economic production of biosurfactants has primarily been limited to specific strains of Pseudomonas, Bacillus, and Candida species (Mukherjee et al., 2006). To enhance biosurfactant production efficiency, agro-industrial by-products have been explored as substrates, utilizing both yeasts and bacteria (Makkar and Cameotra, 2002). For example, anionic glycolipids have been produced using groundnut oil refinery residues and corn steep liquor substrates by Candida species, while Pseudomonas aeruginosa has been utilized for production using cashew apple juice (Raza et al., 2007). The use of motor oil, soap, and frying oil as substrates has also proven successful, although downstream processing can incur higher costs. Additionally, rapeseed oil has been employed for the production of extracellular biosurfactants by Rhodococcus species (BS32) (Ruggeri et al., 2009). Glycerol, a by-product of biodiesel production, has been widely used as a substrate due to its availability in substantial quantities (Zhang et al., 2016). The implementation of oxygen control devices and statistical tools has led to the optimization of cultural conditions, resulting in significant increases in biosurfactant yields (Kronembergeret al., 2008). For instance, the production of biosurfactants by Rhodococcus species MTCC 2574 was effectively enhanced using response surface methodology (RSM), where the yield of biosurfactant increased from 3.2 to 10.9 g/L when n-hexadecane was used as the substrate (Mutaliket al., 2008). Similarly, the production of cell-bound glycolipids by Gordonia species BS29 increased five-fold through the application of RSM (Franzetti et al., 2009). These optimization methods have also been successfully applied to enhance biosurfactant production in Bacillus circulans MTCC 8281 (Sivapathasekaranet al., 2010). Furthermore, a 3.5-fold increase in biosurfactant yield was achieved by developing a genetic algorithm using artificial neural networks (Pal et al., 2009). Experimental and modeling data relating biosurfactant production and biomass formation in Pseudomonas aeruginosa as a model organism were fitted to the Leudeking-Piret model using nonlinear regression and the least-squares method. This model provided a satisfactory representation of biomass growth, with an average percentage error of 5.74% in biosurfactant production and 10.172% in microorganism concentration (Haba et al., 2000).

EXTRACTION AND CHARACTERIZATION OF BIOSURFACTANTS

The total cost of producing any biotechnological product, including biosurfactants, is significantly influenced by downstream processing steps (Mukherjee et al., 2006). Common techniques for extraction include precipitation, solvent extraction, and chromatographic purification. The extraction of low molecular weight biosurfactants employs various solvent systems based on their hydrophobicity and hydrophilic-lipophilic balance (HLB) values. For instance, rhamnolipids can be extracted with ethyl acetate after acid precipitation, while n-hexane is used for the extraction of sophorolipids. Other biosurfactants are typically extracted using a chloroform-methanol mixture. Lipopeptides are acidified to precipitate and then extracted using methanol (Smyth et al., 2010a; Balan, 2017). High molecular weight biosurfactants are usually extracted from culture broth using ammonium sulfate precipitation, followed by purification through dialysis. Alternative techniques for high molecular weight biosurfactants include TCA/acetone precipitation, acid ethanol, and chloroform/methanol precipitation (Smyth et al., 2010b). The cost of solvents in these extraction techniques contributes to the overall production cost, prompting interest in exploring methods to make the process economically viable for commercial-scale biosurfactant production.

Recent advancements focus on developing methods that minimize the bulk use of organic solvents, thereby reducing costs and hazards. In the past decade, optimized high-performance liquid chromatography (HPLC) has been employed for the purification of lipopeptides (Smyth et al., 2010a, b; Sivapathasekaranet al., 2010). The separation and purification of fenugycin were achieved by pressing and harvesting the liquid surface layer after fermentation (Glazyrinaet al., 2008). Liquid membrane extraction has gained traction for the recovery of surfactin, achieving an efficiency of 97% (Dimitrov et al., 2008; Chen and Juang, 2015; Liu, 2010). Although recombinant strains of Bacillus and Acinetobacter species are known to produce biosurfactants, Pseudomonas recombinant species have been extensively utilized due to their adaptability for commercial production. Random mutagenesis using gamma rays or N-Methyl-N'-nitrosoguanidine has been shown to increase rhamnolipid production by 2-3 fold. However, the complexity of the transcriptional regulatory network in Pseudomonas aeruginosa complicates the modulation of rhamnolipid production (Mukherjee et al., 2006). The rhlABRI operon of Pseudomonas aeruginosa has been genetically engineered and cloned into E. coli or non-pathogenic Pseudomonas species to enhance production (Cabrera-Valladares et al., 2006; Cha et al., 2008). Transposon-mediated chromosomal integration of the rhamnosyl transferase 1 complex into E. coli and Pseudomonas aeruginosa has resulted in gene stability (Wang et al., 2007). While the regulatory mechanisms of several biosurfactants remain incompletely understood, further studies could yield valuable insights to enhance yields (Hsueh et al., 2007).

Given the diverse groups of biosurfactants produced by microorganisms, characterizing these compounds is crucial. High-performance liquid chromatography (HPLC) is a key analytical method for separating samples based on hydrophobicity. The purified compounds obtained can be further characterized using various techniques

including mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and infrared (IR) spectroscopy. These techniques provide detailed information about the molecular structure, functional groups, and overall composition of the biosurfactants.

Mass Spectrometry (MS): Mass spectrometry is a powerful analytical technique used to determine the molecular weight and structure of biosurfactants. It can provide insights into the molecular composition and fragmentation patterns of the compounds, allowing for the identification of specific biosurfactant types (Smyth et al., 2010a). Coupling MS with chromatographic techniques, such as HPLC, enhances the resolution and specificity of the analysis, enabling the detection of complex mixtures of biosurfactants (Liu et al., 2010).

Nuclear Magnetic Resonance (NMR) Spectroscopy: NMR spectroscopy is another essential tool for characterizing biosurfactants. It provides detailed information about the molecular structure, including the arrangement of atoms and the presence of functional groups. NMR can be used to confirm the identity of biosurfactants and to study their interactions with other molecules (Smyth et al., 2010b). This technique is particularly useful for elucidating the structure of lipopeptides and glycolipids, which are common types of biosurfactants.

Infrared (IR) Spectroscopy: Infrared spectroscopy is employed to identify functional groups within biosurfactants based on their characteristic absorption bands. This technique is useful for determining the presence of specific chemical bonds, such as hydroxyl, carbonyl, and ester groups, which are critical for understanding the properties and behavior of biosurfactants (Balan, 2017). IR spectroscopy can be used in conjunction with other analytical methods to provide a comprehensive characterization of biosurfactants.

Other Characterization Techniques: In addition to the aforementioned techniques, other methods such as gas chromatography (GC), thin-layer chromatography (TLC), and elemental analysis can also be employed to characterize biosurfactants. GC is particularly useful for analyzing volatile compounds, while TLC can be used for preliminary separation and identification of biosurfactants (Matsuyama et al., 1987). Elemental analysis provides information on the elemental composition of biosurfactants, which can be important for understanding their chemical behavior and potential applications.

CONCLUSION

The production and application of biosurfactants represent a promising area of research and development. With the increasing demand for sustainable and eco-friendly alternatives to synthetic surfactants, biosurfactants offer a viable solution. Continued research into the optimization of production processes, characterization techniques, and applications will further enhance the commercial viability of biosurfactants, paving the way for their widespread use in various industries. Future studies should focus on understanding the regulatory mechanisms governing biosurfactant production and exploring novel microbial strains and substrates to maximize yields and reduce production costs.

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