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Biosurfactant: Bacterial Production, Properties, Classification and Applications

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Abstract

Biosurfactants (BS) are amphiphilic substances that are made by microbes like bacteria which due to their accumulation at the interface of immiscible liquids (water and oil) lower surface and interfacial tension. These compounds are environmentally benign substitutes for synthetic surfactants because of their biodegradability, low toxicity, and efficacy in harsh environmental settings. Exploring the taxonomy, characteristics, synthesis, and uses of bacteria that produce biosurfactants is the goal of this review. Based on their chemical makeup and molecular weight, they are classified including glycolipids, particulate surfactant, surfactin, iturin, polymeric surfactant, fatty acids, lichenysin and lipopeptides are among the classification. Pseudomonas, Bacillus, Alcanirorax, Rhodococcus, and Candida are some of the most well-known bacterial species that produce biosurfactants. They are used in detoxification of particular pollutant, prosper in challenging environmental circumstances, enhance the emulsification and solubilization of hydrophobic compounds, making them valuable in oil recovery and bioremediation. The diverse applications of biosurfactants, ranging from enhanced oil recovery and detergent formulation, medicine, food processing, pharmaceuticals and agriculture, underscore their industrial significance.

Keywords: Amphiphilic, Bioremediation, Biosurfactant, Emulsification and Surface tension.

INTRODUCTION

Biosurfactants are surface-active substances produced by microorganisms that have the capacity to reduce surface and interfacial tensions in solutions (Franzetti *et al.,* 2009). Biosurfactants are amphiphilic molecules that contain hydrophobic and hydrophilic parts that promote the presence of interfaces between fluids with different polarities. The hydrophilic part generally consists of one of the following structures: amino acids, anionic or cationic peptides, and carbohydrates. The hydrophobic tail is generally composed of peptides, proteins, or fatty acids that can be saturated or unsaturated (Farias *et al.,* 2021).

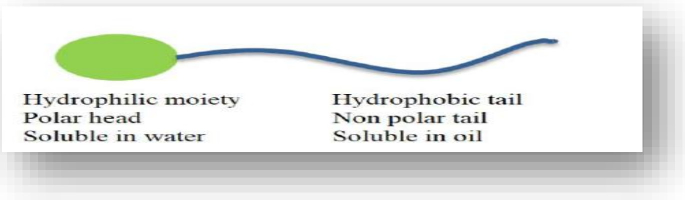

Figure 1: Structure of a Biosurfactant (Gürkök and Özdal 2021).

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Biosurfactant-producing bacteria have gained interest due to their non-toxic, biodegradable, and environmentally benign. They are classified as fatty acids, peptides, glycolipids, phospholipids, and lipopeptides corresponding to their chemical makeup or microbiological origin. Indeed, surfactants are microbial cells with strong cell surface hydrophobicity (Vijayakumar and Saravanan, 2015). In place of synthetic surfactants, these microbes produce surface-active chemicals that are safe for the environment. Because of their capacity to break down hydrophobic materials and operate in harsh environments, they are essential in the sectors of industry, medicine, and the environment.

Due to their stabilising, emulsifying (liquid-liquid mixture), foaming and cleaning qualities, antibacterial activities, and other qualities, they are widely used in the pharmaceutical, food, cosmetic, medical, and agricultural sectors (Nasiri and Biria, 2020).

Biosurfactants Producing Bacteria: According to numerous studies, the most frequently implicated genera in the synthesis of various forms of biosurfactants include *Pseudomonas, Bacillus, Rhodococcus,* and *Candida* (Varjani and Upasani, 2017). In accordance with Olivera *et al.* (2009), *Alcanirorax* is a gram-negative genus of Gammaproteobacteria (order Oceanospirillales), which are strictly aerobic marine obligate hydrocarbonoclasic bacteria (OHCB) that primarily use branching aliphatics and alkanes up to C32. When cultivated on hydrocarbons, the most well-known species in the genera, *Alcanivorax borkumensis*, generates a low molecular weight anionic glycolipid biosurfactant (Schneiker *et al.,* 2006).

Bacillus species in particular are some of the most well-known bacteria that produce biosurfactants. As an instance, *Bacillus methylotrophicus* USTBa was isolated from a petroleum reservoir and thrived on an aqueous medium with crude oil. Over 90% of the crude oil had been eliminated by *B. methylotrophicus* after 12 days of incubation. The bacteria developed a potent glycolipid-type biosurfactant, as evidenced by the culture medium's surface tension of 28 mN/m (Chandankere *et al.,* 2014).

Members of the genus *Pseudomonas* are frequently observed in coastal environments, while the majority of isolated species have been derived from terrestrial habitats (Bollinger *et al.,* 2020). On an array of hydrocarbon and non-hydrocarbon substrates, it thrives and yields rhamnolipids that can combine with kerosene and crude oil to form stable emulsions (Shahaliyan *et al.,* 2015).

In nature, *Acinetobacter* is widely distributed and is often found in marine habitats. Rhamnolipids with a Critical Micelle Concentration of 15 mg/L might additionally be synthesised by *A. calcoaceticus* (Hošková *et al.,* 2015).

Properties of Biosurfactants

- Biosurfactants are suitable for bioremediation and waste treatment because they can be broken down by bacteria and other microorganisms in soil or water (Fenibo *et al.,* 2019).
- Due to their production from raw materials and industrial waste, biosurfactants have a wide range of uses (Sharma *et al.,* 2015).
- They remain stable and functional under extreme pH, temperature, and salinity conditions
- The detoxification of particular pollutants is one of the specialised activities of biosurfactants, which are complex organic molecules (COMs) with their functional groups (Vijayakumar and Saravanan, 2015).

- They improve the solubilisation of hydrophobic substances by efficiently lowering surface and interfacial tension.
- Additionally, biosurfactants are easily included in food, pharmaceutical, and cosmetic goods due to their ability to reduce toxicity (Akbari *et al.,* 2019).

Classification of Biosurfactants

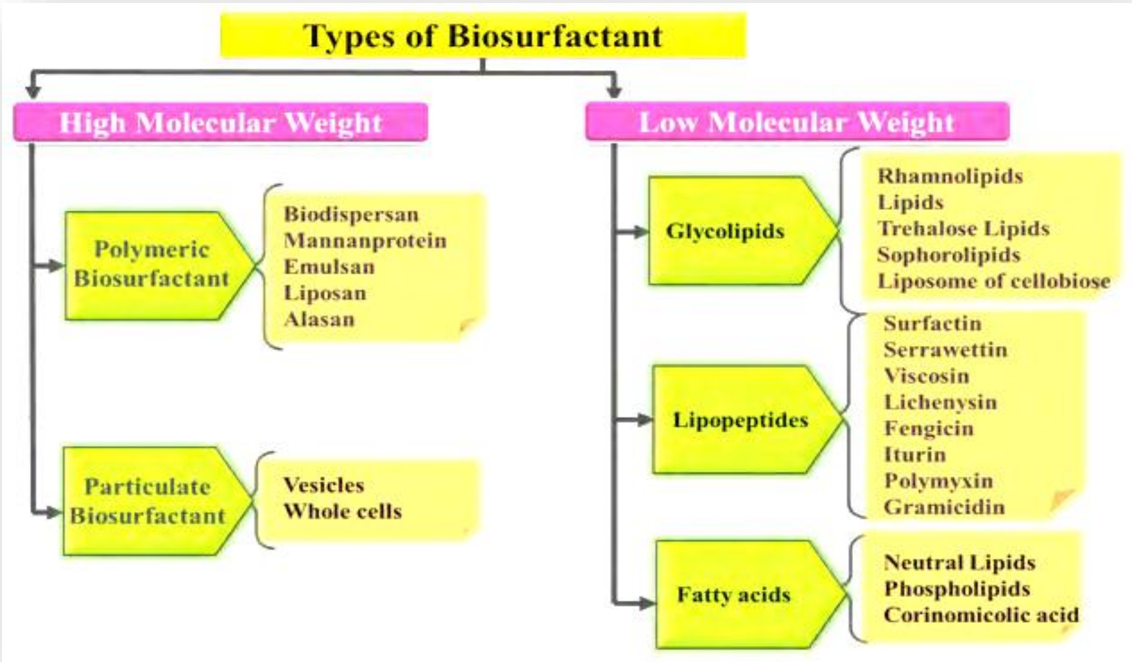

Fig 2: Types of Biosurfactant (Sarita *et al.,* 2023).

Classification Based on Molecular Weight

Low-Molecular-Weight Biosurfactants: The biosurfactants with the lowest molecular weight typically consist of glycolipids or lipopeptides. The most researched glycolipids are rhamnolipids, trehalolipids, and sophorolipids that are disaccharides that have been acylated with hydroxyl or long-chain fatty acids (Saharan *et al.,* 2011).

High-Molecular-Weight Biosurfactants: Ron and Rosenberg state that a number of bacterial species belonging to different genera produce exocellular polymeric surfactants, which are composed of proteins, polysaccharides, lipopolysaccharides, or complex blends of these biopolymers. They are also referred to as bioemulsans, which are more effective at stabilising water-and-oil emulsions. According to Desai and Banat, typical structure of a biosurfactant consists of a hydrophilic moiety made up of amino acids or peptides, anions or cations, mono-, di-, or polysaccharides, and a hydrophobic moiety composed of derivatives of unsaturated, saturated, or fatty acids (Eduardo *et al.,* 2011).

Classification Based on Chemical Composition

Glycolipids: The majority of biosurfactants are glycolipids which are composed of mono-, di-, tri-, and tetra-saccharides in combination with one or more aliphatic or hydroxyaliphatic acid chains.

I. **Rhamnolipids:** Glycolipids containing one or two rhamnose molecules linked to one or two hydroxydecanoic acid molecules are known as rhamamnolipids. *Pseudomonas aeruginosa* is the frequently investigated source of them (Lourith and

Kanlayavattanakul, 2009). As per Soberón-Chávez *et al.* (2021), they have the ability to significantly lower the surface tension of water and have great promise for bioremediation of habitats contaminated with heavy metals and/or oil. Additionally, the amount of carbons and the presence of unsaturation in the fatty acid chain can change (Abeer *et al*., 2018).

II. **Sophorolipids**: They are generally produced by yeast species such as *Torulopsis sp.* and *Candida sp.* and are used in stabilizing oil/water emulsions, in cosmetics with their wetting-moisturizing properties, and in medicine with their antimicrobial, anti-inflammatory and immune system regulatory effects (Gaur *et al*., 2019). They are composed of a dimeric carbohydrate called sophorose and a long-chain hydroxyl fatty acid connected by a glycosidic link.

III. **Trehaloselipids:** The disaccharide trehalose, which is connected to mycolic corrosive at C-6 and C-6, is linked to the majority of types of *Mycobacterium, Corynebacterium,* and *Nocardia. Trichomonas Kproteases* derived from *Arthrobacter sp*. additionally, according to Nuneza *et al.* (2003), *Rhodococcus erythropolis* decreased the surface pressure and interfacial strain in the culture stock.

Lipopeptides and Lipoproteins: Lipopeptide biosurfactant is mostly produced by strains of *Bacillus* and *Pseudomonas* (Coutte *et al.,* 2017). Lipopeptides produced from *Pseudomonas*, such as putisolvin and viscosin, are composed of a brief oligopeptide connected to a fatty acid tail exhibiting a high level of surface and biological activity (Götze and Stallforth, (2020)). This lipopeptide, which is characteristic of *B. subtilis*, has been extensively researched due to its superior surface activity, antibacterial properties, and emulsification abilities (Nelson *et al.,* 2020).

Surfactin: The conventional structure of surfactin is a β-hydroxy fatty acid chain with 13 to 15 carbons linked to a peptide ring with seven L and D amino acids (deOliveira *et al.,* 2021).
Iturin: Iturin was first isolated from a soil sample collected in Ituri, Zaire, which is currently part of the Democratic Republic of the Congo (Raaijmakers *et al.,* 2010). Iturin is a lipopeptide belonging to the second family that is produced by certain *Bacillus subtilis* strains as well as other closely related *Bacillus* species, including *B. amyloliquefaciens*.

Lichenysin: Numerous biosurfactants generated by the bacteria *Bacillus licheniformis* have good pH, temperature, and salt stability and function efficiently together. Their physicochemical properties and structure are similar to those of surfactin. (Roy, 2017).

Neutral Lipids, Phospholipids and Fatty Acids: *Acinetobacter* sp. and other yeasts and bacteria produce phosphatidylethanolamine-rich vesicles that dissolve alkanes in water to form micro-emulsions that are optically transparent. Although fatty acids are widely used in the food industry, phospholipids have been shown to be useful for gene carrier systems due to their membrane-like structure. According to Santos *et al.* (2018), phospholipid biosurfactants comprise substances such lysolecithin and lecithin.

Polymeric Surfactants: It is reported that the genus *Acinetobacter* produces complex polymeric surfactants with a high molecular weight that are referred to as bioemulsifiers. Oil/water type emulsions and bioremediation procedures use biodispersan and emulsan (Shekhar *et al.,* 2015). Polymeric biosurfactants, which are high weight molecular biopolymers, can be made up of several types of biopolymers such as proteins, lipopolysaccharides, polysaccharides, and lipoproteins.

Particulate Biosurfactants: In order for microbial cells to absorb alkanes, hydrocarbons must be divided into a micro emulsion by extracellular membrane vesicles called particulate biosurfactants. According to Santos *et al.* (2018), *Acinetobacter* sp. vesicles are composed of protein, phospholipids, and lipopolysaccharide and they have a buoyant density of 1.158 cubic g/cm and a diameter of 20–50 nm.

Applications of Biosurfactant Producing Bacteria

Application in Cosmetic Industry: These surfactants are employed in insect repellents, antacids, bath products, acne pads, anti-dandruff products, contact lens solutions, baby products, mascara, lipsticks, toothpaste, dentine cleansers, and a host of other applications (Gharaei-Fathabad 2011). They are also used as emulsifiers, foaming agents, solubilizers, wetting agents, cleansers, antimicrobial agents, and mediators of enzyme action. Surfactants are typically used in cosmetics to use *Pseudomonas fluorescens* lipase (use percentage = 90%) to convert tallow to glycerol (1.5:2) (Siebenhaller *et al.,* 2017).

Application in Food Processing Industry*:* Currently, ethoxylated monoglycerides (EMGs) made from synthesised oligopeptides and fatty acid esters (FAEs) such as sorbitan or ethylene glycol, glycerol, lecithin, and its derivatives are employed as emulsifiers in the global food industry (Tan *et al.,* 2018). The study demonstrated the exceptional efficacy of rhamnolipids against *Liesterai monocytogenes*, a gram-positive bacteria that is one of the most aggressive food-borne pathogens and has been linked to an extraordinary number of food-borne deaths. Nonetheless, the investigation demonstrated that while the impact of rhamnolipids was bacteriostatic, the incorporation of nisin significantly enhanced the biosurfactant's efficacy (Magalhães and Nitschke, 2013).

Application of Biosurfactant in Microbial Enhanced Oil Recovery: In conventional oil recovery, microbial surfactants are commonly employed. Studies have demonstrated that some microbes can accelerate the breakdown of hydrocarbons, making them useful for oil spill management (Gomes *et al.,* 2018). To increase yield recovery, emulsion breakdown that occurs at various stages of petroleum extraction and processes can be taken advantage of by employing the demulsifying properties of certain biosurfactants. According to Almatawah (2017), microbial surfactants that lower surface tension can accumulate distinct oil from the bottom of tanks.

Application in Pharmaceutical Industry*:* More recently, it was reported that two marine actinobacterial strains—*Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8 isolated from marine sediment in the Gulf of Suez, Egypt, produced exceptionally stable bacterial starch that is useful in the production of pharmaceuticals and antifouling (Hamed *et al.,* 2021). Biosurfactants are helpful in the fight against illnesses that are resistant to drugs because of their antimicrobial and anti-biofilm properties.

Application of Biosurfactant in Petroleum*:* The International Energy Agency reports that exotic crude oils, such as heavy and extra-heavy oils, are progressively supplanting medium and light oils in petroleum output. In nations including the USA, Canada, China, Mexico, and Venezuela, heavy and extra-heavy crude oils account for at least half of the recoverable oil resources (Cerón-Camacho 2013). They help disperse and break down oil spills in both land and marine habitats and also enhance oil extraction and breaks down petroleum waste.

Application in Commercial Laundry*:* According to Mukherjee (2007), biosurfactants, such as Cyclic Lipopeptide (CLP), retain their surface-active properties even when heated to high temperatures and are stable across a broad pH range of 7.0–12.0. Their outstanding compatibility and stability with conventional laundry detergents, together with their good emulsion formation capabilities with vegetable oils, made them a desirable addition to laundry detergent formulations (Das and Mukherjee 2007).

Application in Agriculture: Microbe-associated molecular patterns (MAMPs) are signalling pathways that are known to influence plant immunity. According to Makkar and Rockne (2003), surfactants are also thought to facilitate the adsorption of microbes to polluted soil particles, reducing the length of the diffusion channel between the site of absorption and the microorganisms' site of bio uptake. Because of their insecticidal activity against the fruit fly *Drosophila melanogaster*, they show promise for usage as biopesticides (Mulligan, 2005).

Application in Oil Industry: When biosurfactants from three distinct bacteria were tested for their bioremediation performances, Xia *et al.* (2011) found that even when utilised at severe levels of salinity, temperature, pH, and metal ions, they all showed good promise in oil recovery. *Pseudomonas aeruginosa, Bacillus subtilis,* and *Rhodococcus erythropolis* were the three bacteria that were employed; *P. aeruginosa* had the greatest emulsification index of 80%. Since only around 30% of oil contamination can be removed by traditional primary and secondary approaches, these extremely effective bioremediation agents are being employed to clean up soil contaminated by heavy metals and hydrocarbons. Most potential biosurfactants are produced by *Bacillus licheniformis* JF-2, which is isolated from the water accumulation that is injected into the oil field.

Application in Medicine*:* It has been discovered that biosurfactants prevent pathogenic organisms from adhering to solid surfaces or infection sites. Rodrigues *et al.* (2006) showed that pre-coating vinyl urethral catheters with a surfactin solution before inoculating them with media reduced the amount of biofilm that *Proteus mirabilis, Salmonella enterica, E. coli* and *Salmonella typhimurium* formed. Strong antibacterial, antifungal, and antiviral activities have been found in various biosurfactants, according to Gharaei-Fathabad (2011). These surfactants act as anti-adhesive agents to pathogens, which makes them effective in treating a wide range of diseases in addition to their usage as therapeutic and probiotic agents. For use in basic sciences and clinical applications, he asserted that the development of a reliable and secure technique for introducing exogenous nucleotides into mammalian cells is essential.

Furthermore, biosurfactants help make heavy metals more bioavailable and easier to remove from contaminated areas, some biosurfactants exhibit anti-tumor properties. Research on bacteria that produce biosurfactants is progressing quickly, with an emphasis on scale-up, cost-effective manufacturing, and the investigation of novel industrial and medicinal uses.

CONCLUSION

Bacteria that produce biosurfactants are an environmentally benign substitute for synthetic surfactants in a variety of sectors such as cosmetics, agriculture and petroleum recovery due to their effectiveness in bioremediation, low toxicity, stability under extreme environmental conditions. When it comes to solving industrial and environmental problems, their special qualities and adaptability make them indispensable. Achieving cost-effective large-scale production and realising their full potential require on-going research and technological innovation.

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