

AN OVERVIEW OF RESEARCH ON BACTERIALLY GENERATED BIOSURFACTANTS

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Abstract

The productions of biosurfactants, which are surface-active biomolecules are produced by bacteria, fungi, and yeast, has become popular recently due to its many advantages over chemical surfactants. These advantages include lower toxicity, higher biodegradability, and better compatibility with the environment, higher foaming, high selectivity, and specific activity at extreme temperatures, pH levels, and salinities. Nowadays, practically all surfactants on the market are made chemically. Due in large part to their many functional qualities and the various ways that microorganisms can be synthesized, interest in biosurfactants has increased recently. Microbial biosurfactants have been discovered to have a wide range of uses in environmental protection, including soil and oil-contaminated industrial effluent biodegradation, oil spill control, and improved oil recovery. Microbial biosurfactants have potential use in food, cosmetics, oil, pharmaceutical/medicine, and biodegradation industries. Three key areas were covered in this review article: the many kinds of biosurfactants, the bacteria that produce the biosurfactants and the use of microbial biosurfactants.

KEY WORDS: Biosurfactant, microbes, biodegradation, contamination and bioemulsifier.

INTRODUCTION:

The ability of microorganisms to produce biomolecules such as extracellular organic acids or peroxides, enzymes, biosurfactant, and even quorum-sensing and quenching abilities dictates major ecological processes in the environment at different niches (Mishra et al., 2021, and Bose, S., et al., 2023). Microbial aggregation and assemblages known as biofilms are the outcome of the innate affinity of microorganisms for interfaces and their capacity (Davey, M.E. et al., 2000) to generate amphipathic surface-active agents (or biosurfactants) for dominating certain niches. According to Markande and Nerurkar (2019) and Neu (1996), these amphipathic biomolecules create a "conditioning film" on the surfaces that modify their initial characteristics and affect the interactions between microbe and interfaces (Markande, A.R., 2021).

According to Ron and Rosenberg (2001), biosurfactants are traditionally divided into two categories: low molecular weight biosurfactants and high molecular weight bio emulsifiers. However, over the past 20 years, the number of biosurfactants has increased dramatically, with an exponential rise in the last 10 years (Varjani and Upasani, 2017; Marcelino et al., 2020). Microbially derived biosurfactants and bioemulsifiers are suitable substitutes for their chemical equivalents (de Salek and Euston, 2019). There have been reports of microbial biosurfactants from freshwater and marine environments in addition to terrestrial ones (Kokare et al., 2007; Nerurkar et al., 2009; Surekha K Satpute et al., 2010; Kubicki et al., 2019; Varjani and Upasani, 2019).

Numerous microbes have been found to produce biosurfactants, but it has proven challenging to identify the genes, biochemical characteristics, or function of these compounds in microbial physiology (Mishra et al Numerous reviews (Rosenberg and Ron, 1997; Franzetti et al., 2009; Roy, 2017; Geetha et al., 2018; Satpute et al., 2010; Varjani and Upasani, 2021) have been published detailing the many facets of biosurfactant roles and possible applications.

In order to be effective, a surface-active agent must have a combination of hydrophobic and hydrophilic elements, which makes the molecule amphipathic (most biosurfactants). This allows the agent to have an effective active area on both types of surfaces hydrophobic and hydrophilic (Neu, 1996). The natural qualities and corresponding various purification methods which vary depending on the biomolecules involved were examined by Fenibo et al., 2019. Specific moieties were detected in more than 160 biomolecules in various combinations, out of the 108 biosurfactants that were reported and examined in this work. Roughly one in four biosurfactants that have been described thus far contain either lipid (23.8%) or carbohydrates as mono- or oligosaccharides (24.8%) as their main component. The minor components of the biosurfactants documented up to this point are proteins (15.8%), polysaccharides (13.4%), and amino acids, often known as peptides (9.9%). 3.5% of biosurfactants contained unidentified components that made up the surface-active agents, while 8.9% of biosurfactants contained additional biomolecules like fatty acids or alkyl derivatives. According to Markande et al. (2013), very few biosurfactants have ever been described as proteinaceous. The biosurfactants of bacteria (22.6%), actinobacteria (27.3%), algae and cyanobacteria (20%), fungi (31%), and yeasts (33.3%) are composed of carbohydrates and oligosaccharides. Additionally, it was observed that the polymeric carbohydrates, also known as polysaccharides, were found in bacteria (14.3%), actinobacteria (9.1%), algae and cyanobacteria (20%), fungus (10.3%), and yeasts (11.1%). Previous authors' lists of biosurfactants generated by fungi have agreed with the findings of this review (Araújo et al., 2021 and Bhardwaj et al., 2013). No reports of carbohydrates or polymeric carbohydrates (polysaccharides) from archaeal surface-active agents have been made. Biosurfactant contents of bacteria (12%), actinobacteria (18.2%), algae and cyanobacteria (10%), and fungi (3.4%) were found to consist of amino acids and peptides; these constituents were not found in archaea or yeasts (Baldeweg et al. 2019) used NMR investigations to investigate a family of amphiphilic hex peptides and Malpinins and cyclo-pentapeptides (Malpibaldins) from the fungus *Mortierella alpine*. Numerous reports of lipopeptides from a variety of microorganisms mostly *Bacillus* species have been made by (Geissler et al., 2019). However, a number of amphipathic molecules (Mycolic and Corynemycolic including oligosaccharide lipids, non-trehalose glycolipids, macrocyclic, and trehalose-lipid esters) were reported by Kügler et al. (2015).

Classification of biosurfactant based on this chemical nature

The main criteria used to categorize biosurfactants are their origins and chemical structures. Usually, an amino acid, peptide, mono-, di-, or polysaccharide makes up the hydrophilic top. Usually, (Mulligan et al., 2014). While a linear, branched, saturated, unsaturated, or hydroxylated fatty acid makes up the hydrophobic end. biosurfactants are categorized according to their chemical makeup and the microorganisms that produce them. (Desai and Banat, 1997; Muthusamy et al., 2008; Christofi and Ivshina, 2002).

Glycolipids

These carbohydrates combine long chain aliphatic acid or hydroxylaliphatic acid to form the most widely used and readily accessible biosurfactant (Christofi and Ivshina, 2002). Either ether or ester groups are used to make the link. Among the glycolipids, rhamnolipids, trihalolipids, and sorbolipids are the most well-known biosurfactants (Mallik et al., 2022). The best researched glycolipids are those produced by *Pseudomonas* sp. (Jarvis et al., 1949), *Pseudozyma antarctica* (Kitamoto et al. 1990), and *Rhodococcus* sp., *Nocardia* sp., *Arthrobacter* sp., and *Mycobacterium* sp. (Lang and Wagner, 1987). *Ustilago maydis* produces cellobiolipids (Teichmann et al. 2007b), while *Candida* sp. produces sophorolipids (Cooper and Paddock, 1984).

Lipopeptides and lipoprotein

Numerous cyclic lipopeptides are generated, such as lipopeptide and decapeptide antibiotics. These have a polypeptide chain connected to a lipid. *Bacillus* sp. produces surfactin, a (Sreelakshmi, K.P., et al., 2024). A lipopeptide comprising seven amino acids linked to a carbon acid with carboxyl and hydroxyl groups. (Kroneislová, et al., 2024). The capacity of surfactin to lyse mammalian erythrocytes and generate spheroplasts is a crucial feature (Bernheimer and Avigad, 1970).

Generally speaking, the molecules in this family of biosurfactants are made up of cyclic peptides connected to fatty acids. (Liu, K., et al., 2020). These antibiotic-like compounds are known to be produced by a number of bacteria including *Bacillus subtilis*, which also generates subtilisin and surfactin. *Bacillus subtilis* and *subtilis* and *B. licheniformis* produce lichenysin and peptide-lipid (Yakimov et al., 1997; Begley et al., 2009), *Pseudomonas fluorescens* synthesizes viscosin (Banat et al. 2010), *Serratia marcescens* synthesizes serranawettin (Lai et al. 2009).

Neutral lipids and phospholipids as they thrive on n-alkanes, a variety of bacteria and yeast produce significant amounts of phospholipid surfactants and fatty acids (Cirigliano and Carman, 1985). The length of the hydrocarbon chain in each structure directly relates to the hydrophilic and lipophilic balance (HLB). Large amounts of phospholipids fatty acids, or neutral lipids are secreted by a number of yeasts, bacteria, and fungi (Srivastava et al., 2022) that may thrive on hydrophobic substrates like alkanes in order to facilitate the uptake of the carbon source. *Acinetobacter sp.*, *Pseudomonas sp.*, *Micrococcus sp.*, *Micrococcus sp.*, *Candida sp.*, *Penicillium sp.*, *Candida lepus*, *Nocardia erythropolis*, *Thiobacillus thiooxidans*, and *Aspergillus sp.* are a few examples (Kappeli and Finnerty, 1979). Phosphatidylethanolamine-rich vesicles that form optically transparent microemulsions of alkanes in water are generated in *Acinetobacter sp.* (Kappeli and Finnerty, 1979). (Pandhi, N. and Shrinivasan, S., 2020). produced by *Rhodococcus erythropolis* grown on n-alkane reduces the interfacial tension between hexadecane and water to less than 1 mN/m and a critical micelle concentration (CMC) of 30 mg/l (White, D. A., et al., 2013, Kretschmer et al. 1982). Bile salts are synthesized by *Myroidesmyroides sp.*, (Desai and Banat, 1997).

Biosurfactants made of polymers like Alasan, liposan, lipomanan, emulsan, and a few more polysaccharide-protein complexes are the most researched polymeric biosurfactants. (Luft, Luciana et al., 2022). An extracellular powerful polyanionic amphipathic heteropolysaccharide bioemulsifier is produced by *Acinetobacter calcoaceticus* RAG-1 (Rosenberg et al. 1979). According to Zosim et al. (1982), emulsan may effectively emulsify hydrocarbons in water, even at concentrations as low as 0.001 to 0.01%. Produced by the yeast *Yarrowia lipolytica*, produces liposan is an extracellular watersoluble emulsifier consisting of 83% carbohydrates and 17% protein, a carbohydrate-protein complex (Cirigliano and Carman 1984) cite two other examples: protein PA produced by *Pseudomonas aeruginosa* and mannan lipid protein made by *Candida tropicalis*.

Polymeric biosurfactants are made up of several parts. The most researched ones, emulsan and biodispersan, are produced by *Acinetobacter calcoaceticus* and have a heteropolysaccharide backbone to which fatty acids are covalently attached (Rosenberg et al. 1988). Differential biosurfactants the process by which extracellular membrane vesicles separate hydrocarbons from a microemulsion which is crucial for the uptake of alkanes by microbial cells. *Acinetobacter sp.* (Hommel, Rolf K. et al., 1991). vesicles have a buoyant density of 1.158 cubic g/cm, a diameter of 20–50 nm, and a composition of lipopolysaccharide, phospholipids, and protein (KappeliTomar, Sonica, and Finnerty, 1979).

Ionic charge classification of biosurfactants (Clint, 1992 and Bir Pal Singh ,2014). The kind of polar group is typically used to identify chemically produced surfactants. Group hydrophilic Usually usually called the "head group," the hydrophilic group of a surfactant is either strongly polar or charged. A nian (Ghosh et al.,2020) These consist of sulphonate and sulphates, as well as conventional soaps and the first synthetic detergents. Caustic (Saleem et al., 2024). These are typically chemicals that are alkyl pyridinium, imidazolinium, or quaternary ammonium. Amphoteric zwitterionic surfactants are utilized as sulphobetaines or betaines.(Sarkar et al 2024). These substances are found in infant shampoos and toilets because they are less irritating to the eyes and cause less irritation on skin than anionic chemicals. Distinctive surfactants Usually, ethoxylates predominate in these. (Ahmed, Fahim Uddinet al., 2009).

A number of semi-polar substances, including amine oxides, sulphoxides, and phospholine oxides, are also members of this class of surfactants. (Mu, Linyu, 2020) Blending these kinds typically include anionic and non-ionic groups, like alkyl ethoxysulfates. Shampoos and dishwashing detergents include this surfactant, which is gentle on skin and cannot be avoided when skin contact occurs. Group hydrophobic these surfactant types often referred to as "tail" mostly consist of simple hydrocarbon groups (Braathen, L.R., et al., 2017).

Numerous microbes that are employed in industrial settings to remove waste products have been isolated from sources of contaminated soil, effluents, and discharge point waste water. (Naidoo, et al., 2014). As a result, these can develop on substrates that other microorganisms that don't produce biosurfactants which may find toxic. Numerous microbial biosurfactants are accessible (Jimoh, et al., 2019). The characteristics of the carbon substrate, as well as the concentrations of nitrogen, phosphorus, magnesium, iron, and manganese ions in the medium (Guerra-Santos et al., 1986; Haferburg et al., 1986; Abu-Ruwaida et al., 1991) and culture conditions (pH, temperature, agitation, dilution rate, etc.) all have a major impact on their type, quantity, and quality (Georgiou et al., 1992). Priority must be given to the selection of microorganisms for the use of microbial enhanced oil recovery, as these organisms depend on a variety of environmental factors, including temperature, pressure, pH, and salinity (Khire et al., 1994).

Bacteria

One important factor in the synthesis of biosurfactants is bacteria. The other species that are involved in the production of biosurfactants after the dominant genus, *Pseudomonas* (Aparna, A., et al., 2012). According to Coelho et al., 2003, marine *Pseudomonas sp.* strain GU 104 degrades quinoline to produce biosurfactants report on *Pseudomonas* strains that produce polymeric biosurfactants is available. (Husain et al. 1997), revealed that *Pseudomonas nautica*, which was isolated from the Mediterranean Sea coastal region produced extracellular biosurfactants with emulsifying properties (Ghaleb, Rania N., and Hemlata Bhosale 2024). Different types of emulsifiers are produced by microorganisms based on different types of hydrocarbons and carbon sources. (Desai et al., 1988) work amply demonstrates the hydrocarbon degradation to produce a trehalose lipid-o-dialkyl monoglycerides-protein emulsifier by *Aspergillus fluorescens* (Karnwal, Arun, 2021).

The main bioproducts of *Bacillus* species are lipopeptides, subtilisin, lichenysin, surfactin, and lipid protein complexes derived from various marine environment thermotolerant and halotolerant *Bacillus licheniformis* JF2 was isolated. (Sankhyan et al., 2024) which was helpful for in situ microbial enhanced oil recovery. Only two strains of *Bacillus subtilis* were reported to have this ability for the synthesis of biosurfactants (McInerney, J. J., et al., 2003) screened more than 22 microorganisms for the ability to synthesise biosurfactants under thermophilic conditions. *Acinetobacter sp.* is a gram-negative bacterium that is commonly found in marine environments and is widely distributed in nature (Cameotra et al., 2003). Owing to their adaptable characteristics, the above bacteria has garnered significant interest from numerous investigators (Choudhary et al 2024). Numerous other sources have also been thoroughly examined, aside from the maritime environment (Cornell, S. E., et al 2003).

According to MacCormack and Fraile (1997), *Acinetobacter sp.* is essential for the bioremediation processes which plays a significant role in the degradation of hydrocarbons (Juni, 1978). *Acinetobacter calcoaceticus* RAG-1 was discovered to produce a surfactant namely (Nerurkar et al.2009) after being isolated from the Mediterranean Sea (Reisfeld et al., 1972). This surfactant is used in the market under the brand name "Emulsan". *Rhodococcus sp.* produces glycolipid surface-active molecules. *Rhodococcus erythropolis* strain 3C-9 an oil-degrading bacterium was isolated by Peng et al. (2007) from the seaside soil of the Island of Xiamen, which is situated on the west bank of the Taiwan Strait.

Two distinct biosurfactant types are produced by *Rhodococcus erythropolis* (Shulga V.I., and P. Sigmund 1990) and *Rhodococcus sp.* (Singer and Finnerty, 1990;). The primary use of *Halomonas sp.* is in the synthesis of EPS with emulsifying capabilities. In Terra Nova Bay Station, Ross Sea, Antarctica, a bacterial strain known as *Halomonas* ANT-3b was isolated from the sea ice-seawater interface (Rizzo et al 2021). This strain is responsible for producing emulsifying glycolipids. *Halomonas sp.* also produces glycoprotein-based bioemulsifiers (protein and uronic acids), which have been chemically and physically characterised by (Hussein et al., 2021). *Myroides* is a rod-shaped, pigmented, gram-negative, aerobic, non-motile bacterium that is typically found in marine environments (Saticioglu et al., 2023). Most of the research was done on *Myroides* strain sp. SM1, a bioemulsifier-producing strain that was isolated from the seawater that had been contaminated with oil in Songkhla Lake, Thailand (Maneerat et al., 2007). When compared to other surfactants, *Myroides sp.* SM1 has a higher surface activity for oil displacement as it grows well, emulsifies weathered crude oil, and produces an

extracellular bioemulsifier (Shekhar et al., 2015). A complex of L-ornithine lipids, including L-ornithine and a different couple of iso-3-hydroxy fatty acid and iso-fatty acid wide temperature range (Fardami et al., 2022). However, at high salt concentrations and extreme pH values, its emulsification activities quickly decline (Maneerat and Phetrong, 2007).

Due to their ability to stick to weathered crude oil, cell associated surface active molecules from *Myroides sp.* exhibit strong emulsification activity (Maneera and Dikit, 2007). Authors have documented the production of surfactants by a variety of fungal species from various origins. When compared to other fungal species, *Candida sp.* is the most widely distributed species that has been documented from various sources for surfactant production (Desai, Jitendra D., and Ibrahim M. Banat, 2007). *Candida bombicola* has been reported by Casas and Garcia-Ochoa (1999) to produce sophorolipids. One of the well-known fungi for producing lipid, carbohydrate, and protein-based bioemulsifiers is *Yarrowia lipolytica*. During the growth phase, this bioemulsifier based on polysaccharides can make the cells more hydrophobic. It was demonstrated by (Zinjarde and Pant 2002) that when a cell enters stationary phase, extracellular bioemulsifier production does occur. Another kind of bioemulsifier is called "yansan," (de Jesús et al 2014). which is made from the Brazilian wild strain of *Yarrowia lipolytica*, IMUFRJ 50682, in a fermentation medium with glucose added as a supplement (Groenewald, et al., 2014). Ceramide It has also been reported that a unique yeast isolate can produce a biosurfactant/bioemulsifier with a good emulsification index (80%) when combined with crude oil and kerosene (Panjar et al., 2015).

Additionally, it has been demonstrated to extract 76% of crude oil from pollutants (Oloke and Glick, 2005). When compared to other yeast species, *Pseudozyma sp.* is primarily known for producing biosurfactants. *Torulopsis petrophilum* was isolated by Cooper and Paddock (1983) in order to produce sophorolipids. Together with *Ustilago maydis* and *Schizonella melanogramma*, *Kurtzmanomyces sp.* I-11 is a novel MEL producer. Kakugawa et al. (2002) isolated *Kurtzmanomyces sp.* I-11 for the production of Mannosylerythritol lipids (MEL).

Antimicrobial activity

Well-researched biosurfactants including Fengycin, Iturin, Rhamnolipids and Surfactin, have been shown to have antimicrobial properties (Kourmentza et al., 2017) While numerous biosurfactants have been documented for their antifungal, antibacterial and antihelminth properties, their potential has been enhanced by recent research on these properties in light of the pervasive reports of antibiotic resistance (Thipe, Velaphi C., et al 2023). Recent analysis of biosurfactant activities against common antibiotic-resistant bacterial and fungal pathogens was conducted by (Bhawsar et al., 2011). Despite the possibility that biosurfactant activity has some wound healing properties (Sana et al., 2018), there have been reports of pharmaceutical applications for biosurfactants, including as adjuvants for vaccines (Makkar and Cameotra, 2002). Furthermore, it has been reported that the biosurfactants also work against phytopathogenic fungi (Sharma et al., 2017).

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