**Biosurfactants for sustainable soil management**

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**Abstract**

Biosurfactants, a diverse group of biologically-derived surface-active molecules, have garnered significant attention due to their unique properties and wide-ranging applications. Produced by microorganisms, biosurfactants exhibit exceptional surface tension reduction, emulsification, and foaming abilities, surpassing their synthetic counterparts in several aspects. This abstract provides an overview of biosurfactants, their production and their diverse applications.

**Keywords:** Biosurfactants, Microorganism

**Introduction**

Biosurfactants are amphiphilic chemicals obtained mostly from plants and microbes. Microbially manufactured bio-surfactants, on the other hand, have advantages over plant-based surfactants in terms of scale-up capability, quick production, and multifunctional characteristics. Plant-based biosurfactants offer outstanding emulsification qualities, but they are expensive to manufacture on a large scale. Furthermore, plant-based surfactants have challenges with solubility and hydrophobicity. The bioavailability and biodegradation kinetics of hydrophobic substances are dramatically influenced by biosurfactants. (Ahmad *et al*., 2016).

They normally increase the bioavailability of hydrophobic substances by decreasing their water repellence. Both stimulating and inhibiting effects of bio-surfactants have been observed based on the chemical properties of the bio-surfactants, the contaminants, and the physiology of the microorganisms. Biosurfactants perform a physiological role in enhancing the solubility and bioavailability of hydrophobic substances, as well as in stimulating microorganism swarming motility and in cellular signalling and differentiation processes. These biosurfactants play a role in biofilm development and can interact with a variety of microbial proteins. They can affect the enzyme conformation structure in this way, altering enzyme selectivity, activity, and functions, and thereby improving the breakdown of chemical pollutants. Biosurfactants may also help to ensure agricultural sustainability by serving as antimicrobial agents to combat illness. In this chapter, we focus on the usage and application of bio-surfactants for sustainable soil management, with the hope that bio-surfactants and bio emulsifiers will play a larger role in the future (Ahmad *et al*., 2018).

**Historical perspective and current status**

Bio-surfactants are surface active agents of biological origin that are beneficial due to their unique features and environmentally benign nature. Arima *et al.* (1968) first purified and described "surfactant," a biosurfactant generated by Bacillus subtilis. Since then, various researchers have studied bio-surfactants all over the world (Ahmad *et al.,* 2016). However, there are many aspects of their functionality that are still unknown. Despite the fact that several types of bio-surfactants have been isolated and characterized (Banat, 1995), there are five major categories of bio-surfactants: glycolipids, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric biosurfactants, and particulate biosurfactants. These bio-surfactants have applications in agriculture, pharmaceutical, food, cosmetics, and detergent industries. Bio-surfactants, particularly new generation surface active chemicals, can suit the needs of the modern market in natural products. Despite their strong demand in the organic market, commercial manufacturing is difficult due to their high cost and fermentation methods. According to one estimate, the cost of raw materials accounts for over 30 per cent of the total production cost in most biotechnological processes, including biosurfactant manufacturing. As a result, there is a growing interest in using low-cost renewable raw materials for such a process in order to make it economically viable. Process optimization is one method for making this technology economically viable. Biosurfactants are generally environmentally safe and long-lasting when used in agriculture. The size of the markets for surfactants and bio-surfactants, as well as the rate of market growth, often indicate the relative importance of these products (Ahmad *et al*., 2018).

**Sources of bio-surfactants**

Bio-surfactants are mainly produced by microorganisms and plants. However, microbially produced bio-surfactantsare receiving more attention due to ease in culturing, lower production cost, and greater functional properties.

1. **Plant-Based Bio-surfactants**

**Table 1: Plant based biosurfactants**

|  |  |  |
| --- | --- | --- |
| **Biosurfactant**  | **Source**  | **References**  |
| Lecithin  | Soybean oil seed, root mucilage of maize, lupin and wheat  | Read *et al.* (2003)  |
| Saponin  | Tea seed  | Wang *et al.* (2016) |
| Soybeans, broad beans, peanuts, kidney beans  | Xu *et al.* (2011)  |
| Chinese soapberry  | Zhou *et al.* (2013)  |
| Phospholipid  | Maize roots and lupin  | Read *et al.* (2003)  |
| Humic acid-like substance  | Soapnut plant  | Mukhopadhyay *et al.* (2013)  |

Saponins, lecithin, soy protein, and cyclodextrins are the most frequent plant-based biosurfactants (Table 1). Soybean has three naturally occurring biosurfactants: lecithin, soy protein, and soy saponin (Xu *et al.,* 2011). Lecithin is the most frequently utilized plant-based bio-surfactant and is mostly produced from soybean oil seed. Read *et al.* (2003) discovered lecithin in maize, lupin, and wheat root mucilage. This bio-surfactant has been shown to alter soil's physical and chemical properties. Vecino *et al. (*2014) investigated the surface tension activity of maize steep liquor derived from the corn milling industry and isolated the bio-surfactant present in this residue using organic solvents. Furthermore, employing chloroform as a possible organic solvent, the extraction of bio-surfactants from this liquor was optimized. Later, this lipopeptide surfactant was envisioned as a promising soil remediation alternative due to its high potential to promote the treatment of sewage sludge contaminated with polycyclic aromatic hydrocarbons.

**Microbially Produced Bio-surfactants**

Microorganisms such as yeasts, bacteria, and some filamentous fungus can produce bio-surfactants with a variety of molecular structures and surface activity. Through the oxidation process, microorganisms gain energy for development from various carbon sources. The combination of carbon sources with insoluble substrates promotes intracellular diffusion and the synthesis of several compounds. There has recently been a surge in interest in isolating microorganisms that produce tension active compounds with good surfactant properties, such as low critical micelle concentration, low toxicity, and high emulsifying activity. The literature identifies bacteria of the genera Pseudomonas and Bacillus as excellent makers of biosurfactants; however, numerous additional genera have also been identified for this function. For example, extracted the biosurfactant bound to the cells of *Lactobacillus pentosus* grown on hemi cellulosic hydrolysates from Grape Marc.

**Table 2: Bio-surfactant producing bacteria**

|  |  |  |
| --- | --- | --- |
| **Sl. No.**  | **Microorganism**  | **Biosurfactant**  |
| 1.  | *Pseudomonas sp*  | Ornithine lipids  |
| 2.  | *Pseudomonas fluorescens*  | Viscosin  |
| 3.  | *Pseudomonas aeruginosa*  | Rhamnolipids  |

**Table 3: Bio-surfactant producing fungi**

|  |  |  |
| --- | --- | --- |
| **Sl. No.**  | **Microorganism**  | **Biosurfactant**  |
| 1.  | *Candida antarctica*  | Mannoserthritol lipid  |
| 2.  | *Candida bombicola*  | Sophorous lipids  |
| 3.  | *Penicillium chrysogenum*  | Polyketide derivative  |
| 4.  | *Yarrowia lipolytica*  | Carbohydrate complex  |

**Table 4: Bio-surfactant producing yeast**

|  |  |  |
| --- | --- | --- |
| **SL. No.**  | **Microorganism**  | **Biosurfactant**  |
| 1  | *Debaryomyces polymorphus*  | Carbohydrate complex  |
| 2.  | *Saccharomyces cerevisiae*  | Mannanoprotein  |
| 3.  | *Pseudozyma aphidis*  | Mannoserthritol lipids  |

**Classification of Bio-surfactants**

**Based on molecular weight**

1. **Low molecular weight biosurfactants:** Examples-Glycolipids, phospholipids and lipopeptides

Low-molecular-mass biosurfactants are efficient in lowering surface and interfacial tensions, whereas high-molecular-mass biosurfactants are more effective at stabilizing oil-in-water emulsions. Low molecular weight biosurfactants have their molar mass ranging between 0.5 and 1.5 kDa, while bio-emulsifiers can reach up to 500 kDa

1. **High molecular weight biosurfactants:** Example-Polysaccharides, proteins, lipopolysaccharides, lipoproteins

**Based on charge/ nature of their polar groups**

1. **Anionic biosurfactant**: Sulfopon sodium coco sulfates
2. **Cationic biosurfactant**: Protelan AGL sodium Nα-cocoyl glutamate
3. **Amphoteric biosurfactant**: Lecithins
4. **Nonionic biosurfactant**: Mono- and diglyceride mixtures

**Biosurfactant production**

Bushnell Haas broth is used as the production medium, and it is infected with a 24-48h old bacterial culture prepared in Nutrient broth medium or a 144-168h old fungal culture prepared in potato dextrose and broth medium, all of which are kept at room temperature and shaken. Allow the infected culture to grow for 7-10 days under optimal conditions. To get clear sterile supernatant, centrifuge the culture broth at 10000 rpm for 15 minutes to remove the cells.

**Biosurfactant recovery**

1. **Cold acetone precipitation method**

Three liters of cold acetone were added to the crude biosurfactant solution and left to stand for 10 hours at 4°C. The precipitate was recovered by centrifugation at 10,000 rpm for 20 minutes, and the resulting pellet was used as a partially purified biosurfactant, which was then evaporated to dryness to eliminate residual acetone before being dissolved in sterile water.

1. **Acid precipitation method**

Biosurfactant can also be precipitated by lowering the pH of the cell-free broth culture to 2.0 with 6 N HCl and leaving it at 4°C overnight. Centrifugation (8000 rpm for 15 minutes at 20°C) is used to collect the precipitated pellets, which are then dissolved in sterile distilled water. Following that, the pH is corrected to 8.0 using 1 N NaOH for future usage.

1. **Ammonium sulphate precipitation**

Precipitation with ammonium sulphate is used to precipitate high-molecular-weight biosurfactants such as emulsan and bio-dispersion (protein-rich substances). A varied concentration of ammonium sulphate is employed depending on the type of biosurfactant. In the instance of ammonium sulphate precipitation, the rhamnolipid is precipitated by salting out the product, which is then refined via dialysis and lyophilized.

**Screening of Isolates for Biosurfactant Production**

1. **Drop collapse test**

The drop collapse test is a simple and quick way to assess a microbe's ability to produce biosurfactants. This test requires no additional equipment and only a tiny amount of microbiological sample. Drops of oil are deposited on the slide, and then 10l of the microbiological sample is added by piercing the drop with a micropipette without disturbing the dome-shaped oil. The drop collapse test is judged positive if the drop collapsed within 1 minute.

1. **Blood agar haemolysis**

This is used to screen microorganisms for their potential to create biosurfactants. For testing haemolytic activity, a blood agar plate containing 5% sheep blood is employed. Positive strains cause blood cell lysis and form a colourless, transparent ring around the colonies. As a result, bacteria that exhibit positive blood haemolysis are classified as biosurfactant makers. The underlying principle strategy of the subsequent screening was that biosurfactants promote erythrocyte lysis. The assay also predicts the surface activity of microorganisms that produce biosurfactants. The blood agar method is recommended as a preliminary screening method that should be supplemented with other techniques based on surface activity measures.

1. **Oil displacement activity**

This is a quick and simple procedure that requires only a small amount of sample. It is used when the activity and quantity of biosurfactant are low due to its great accuracy. The organism that produces biosurfactants displaces the oil (increases its diameter) and spreads it in the water. The increase in diameter of oil caused by surfactant activity is measured. If there is a biosurfactant in the supernatant, the oil is displaced and a clearing zone forms. Surfactant activity is proportional to the diameter of this clearing zone on the oil surface. This is also known as the oil spreading technique.

**CTAB agar plate method**

Siegmund and Wagner (1991) invented the CTAB (Cethyl trimethyl ammonium bromide) agar plate method. It's a semi-quantitative test for detecting extracellular glycolipids or other anionic surfactants. The CTAB agar assay is a convenient screening approach for anionic biosurfactants. The downside of this technique is that CTAB is toxic since it inhibits the growth of certain microorganisms. The cationic surfactant is found in cethyl trimethyl ammonium bromide. Microbes growing on the plate secrete anionic surfactants, which combine with cethyl trimethyl ammonium bromide and methylene blue (basic dye) to generate a dark blue, insoluble ion. As a result, productive colonies are encircled by dark blue halos.

**Applications of bio-surfactants**

* Bioremediation of contaminated agricultural soil.
* Plant growth promotion by elimination of phytopathogens (Ayele *et al.,* 2021).
* Application in pesticide industries.

**Advantages of bio-surfactants**

* Biodegradability, Low Toxicity
* Biocompatibility
* They can be produced from cheap raw materials that are easily available in large quantities
* It exhibits emulsification capacity
* Specificity
* Tolerance to temperature, pH and ionic strength
* They are ecologically accepted due to their property of maintaining sustainability (Shadia, 2017)
* Low critical micelle concentration

**Disadvantages of bio-surfactants**

* Expensive large-scale production
* Difficulty in obtaining pure substances
* Strong foam formation

**Conclusion**

In conclusion, biosurfactants represent a remarkable class of natural molecules with immense potential and versatility. These biologically-derived surface-active compounds offer a wide array of benefits over their synthetic counterparts, making them attractive for various industrial applications.

One of the most significant advantages of biosurfactants is their eco-friendly nature and low toxicity, which aligns with the growing global focus on sustainability and environmental protection. Their production by microorganisms, such as bacteria and fungi, offers a sustainable alternative to chemical surfactants, reducing the environmental burden associated with conventional surfactant manufacturing. In agriculture, biosurfactants can enhance nutrient availability and soil remediation, promoting sustainable and efficient farming practices. Their potential to support microbial interactions and biofilm formation enhances biodegradation processes, contributing to environmental cleanup and pollution mitigation.

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