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Review

Production and potential biotechnological applications of microbial surfactants: An overview



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ABSTRACT

Microbial surfactants are amphipathic molecules that consist of hydrophilic and hydrophobic domains, which allow partition of two fluid phases of varying degree of polarity. They are classified into two main groups: bioemulsifier and biosurfactant, depending on their molecular weight. Microbial surfactants occur in various categories according to their chemical nature and producing organisms. These biomolecules are produced by diverse groups of microorganisms including fungi, bacteria, and yeasts. Their production is significantly influenced by substrate type, fermentation technology and microbial strains. Owing to inherent multifunctional properties and assorted synthetic aptitude of the microbes, microbial surfactants are mostly preferred than their chemical counterparts for various industrial and biomedical applications including bioremediation, oil recovery; as supplements in laundry formulations and as emulsion-stabilizers in food and cosmetic industries as well as therapeutic agents in medicine. The present review discusses on production of microbial surfactants as promising and alternative broad-functional biomolecules for various biotechnological applications.

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Abbreviations: SACs, Surface active compounds; CMC, Critical micelle concentration; CTAB, Cetyltrimethylammonium bromide; MIC, Minimum inhibitory concentration; MBC, Minimum bactericidal concentration; Akt, Threonine protein kinase; IC₅₀, Half-maximal inhibitory concentration; ST, Surface tension; mN/M, Millinewton per metre; E₂₄, Emulsification index; µg/mL, Microgram per milliliter; mg/mL, Milligram per milliliter; mg/L, Milligram per liter; g/L, Gram per litre; µL, Microlitre; mL, Millilitre; µm, Micrometre; nm, Nanometre; Da, Dalton; kDa, Kilodalton; v/v, volume per volume; °C, Degree Celsius; %, Percent; h, Hour; sec, Second.

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1. Introduction

Microbial surfactants (also known as surface-active compounds, SACs) are amphipathic and structurally diverse molecules that consist of hydrophilic and hydrophobic moieties, which form partition between liquid substances with different levels of polarity and hydrogen bonds (Santos et al., 2016). Usually, the hydrophobic moiety is a hydrocarbon chain consisting of long-chain fatty acids (saturated or unsaturated) whereas; the hydrophilic moiety may be ionic, non-ionic, amphoteric, amino acids or polysaccharides (Silva et al., 2014; Mao et al., 2015). They are divided into two groups namely, low molecular weight and high molecular weight SACs (Rosenberg and Ron, 1999). The low molecular weight SACs (biosurfactants) are recognized for superb surface activity by decreasing surface and interfacial tension between different phases and possess low critical micelle concentration (CMC) and stabilize emulsions (Batista et al., 2006; Uzoigwe et al., 2015). They consist of phospholipids, glycolipids, neutral lipids or fatty acids etc. with molecular weight of 500 to 1500 Da (Banat et al., 2010). On the contrary, bioemulsifiers are high molecular weight molecules that are more efficient in the formation and stabilization of oil–water or water–oil emulsions without reducing surface or interfacial tension (Gudiña et al., 2015b). They comprise of lipopolysaccharides, proteins, lipoproteins or glycoproteins, which contribute to their emulsification and emulsion stabilization (Rosenberg and Ron, 1999; Uzoigwe et al., 2015). These biomolecules are produced by plethora of microorganisms such as yeasts, bacteria, and filamentous fungi.

Due to their biological source, microbial surfactants are less toxic, biodegradable, environmentally compatible, highly selective and specific (in terms of their activities at extreme pH, temperatures and salinity) and can be produced from renewable and economical agro-industrial materials that are found in huge amounts. In addition, they possess akin or enhanced emulsifying activity in comparison to conventional chemical surfactants (Pereira et al., 2011; Banat et al., 2014).

Microbial surfactants possess distinct diverse functional properties including emulsification, wetting, foaming, corrosion-inhibition, dispersion, cleansing, surface activity etc., which make them a suitable candidate for various biotechnological applications such as bioremediation, microbially enhanced oil recovery; as additives in cleaning products and laundry formulations, and as emulsion-stabilizing agents in the food, cosmetic and pharmaceutical industries (Adetunji and Olaniran, 2018; Araújo et al., 2019; Mujumdar et al., 2019). This review, therefore, describes different

Table 1
Some surfactant-producing microorganisms.

Microorganism	Biosurfactant/ bioemulsifier	Reference
<i>Microbacterium</i> sp. MC3B-10	Microbactan	Camacho-Chab et al., 2013
<i>Pseudomonas aeruginosa</i> ATCC 9027	Rhamnolipids	Díaz De Rienzo et al., 2016
<i>Bacillus thailandensis</i> E264	Rhamnolipids	Díaz De Rienzo et al., 2016
<i>Pseudomonas fluorescens</i>	Viscosin	Banat et al., 2010
<i>Bacillus subtilis</i> SPB ₁	Surfactin/Iturin	Mnif et al., 2016
<i>Bacillus subtilis</i>	Subtilisin	Sutyak et al., 2008
<i>Bacillus subtilis</i> JB1	Subtilisin	Sung et al., 2010
<i>Acinetobacter calcoaceticus</i> PTCC 1316	Emulsan	Amani and Kariminezhad, 2016
<i>Acinetobacter radioresistens</i> KA53	Alasan	Toren et al., 2001
<i>Acinetobacter calcoaceticus</i> A2	Biodispersan	Rosenberg and Ron, 1989
<i>Leuconostoc mesenteroides</i>	Viscosin	Banat et al., 2010
<i>Serratia marcescens</i>	Serrawettin	Matsuyama et al., 2011
<i>Serratia rubidaea</i> SNAU02	Rhamnolipids	Nalini and Parthasarathi, 2018
<i>Gordonia</i> sp. BS29	Extracellular bioemulsan	Franzetti et al., 2009
<i>Pedobacter</i> sp. MCC-Z	Glycolipid-protein complex	Beltrani et al., 2015
<i>Streptomyces</i> sp. MAB36	Glycolipid	Manivasagan et al., 2014
<i>Rhodotorula babjevae</i> YS3	Sophorolipids	Sen et al., 2017
<i>Halomonas eurihalina</i>	Glycoprotein	Gutiérrez et al., 2007
<i>Debaryomyces polymorphus</i>	Carbohydrate-lipid complex	Nerurkar et al., 2009
<i>Paenibacillus</i> sp. #510	Oligosaccharide-lipid complex	Gudiña et al., 2015b
<i>Achromobacter</i> sp. HZ01	Lipopeptide	Deng et al., 2016
<i>Ochrobactrum pseudintermedium</i> C1	Glycoprotein	Bhattacharya et al., 2014
<i>Aspergillus ustus</i> MSF3	Glycolipoprotein	Kiran et al., 2009
<i>Penicillium chrosogenum</i> SNP5	Lipopeptide	Gautam et al., 2014
<i>Candida bombicola</i>	Sophorolipids	Felse et al., 2007
<i>Candida lipolytica</i> UCP 0988	Rufisan	Rufino et al., 2011
<i>Candida sphaerica</i> UCP 0995	Lunasan	Luna et al., 2011
<i>Yarrowia lipolytica</i> IMUFRJ 50,682	Yansan	Amaral et al., 2006
<i>Ustilago maydis</i>	Cellobiose lipids	Teichmann et al., 2007
<i>Pseudozyma fusiformata</i>	Mannosylerythritol lipids	Morita et al., 2007
<i>Trichosporon mycotoxinivorans</i> CLA2	Lipid-polysaccharide complex	Monteiro et al., 2012

Table 2
Major classes of microbial surfactants (Adetunji, 2017)

Class	Example
Glycolipid	Rhamnolipids Sphorolipids Trehalolipids
Lipopeptides and lipoproteins	Viscosin Serrawettin Polymyxin Gramicidin Subtilisin Surfactin Peptide-lipid
Fatty acids, neutral lipids and phospholipids	Fatty acids Neutral lipids Phospholipids
Polymeric surfactants	Emulsan Alasan Biodispersan Lipomanan Liposan Mannan lipid protein Carbohydrate-lipid-protein
Particulate surfactant	Protein PA Vesicles

techniques employed for screening of microorganisms for surfactant production. It further elucidates various nutritional and physicochemical parameters influencing production of these biomolecules for industrial and biomedical applications.

2. Surfactant-producing microorganisms

Over the years, there has been ceaseless drive to search for microorganisms with potential to produce surfactants with robust surface active or emulsifier properties (Silva et al., 2014). The amount of biosurfactant or bioemulsifier production is influenced by the type of microorganisms and their sources. These microorganisms are universally distributed and are found in water, soil, and harsh environments etc. (Ibacache-Quiroga et al., 2013; Yan et al., 2014). Table 1 illustrates a list of some surfactant-producing microorganisms.

3. Classification and chemical nature of microbial surfactants

Microbial surfactants are generally categorized based on their chemical components and microbial source (Banat et al., 2010; Vijayakumar and Saravanan, 2015). According to Rosenberg and Ron (1999), microbial surfactants are grouped into two: low molecular weight (glycolipids, lipopeptides, phospholipids) and high molecular weight surfactants (polymeric and particulate surfactants) (Table 2). Each class is discussed in details below:

3.1. Glycolipids

These are the frequently studied and common biosurfactants, comprising of carbohydrate moiety connected to aliphatic acids or hydroxyaliphatic acid via ether or ester group. The carbohydrate domain include rhamnose, mannose, glucose, galactose, galactose sulphate or glucuronic acid (Desai and Banat, 1997). The fatty acid component is akin to that of phospholipid of similar microorganisms. The most-studied glycolipids are rhamnolipids, mannosylerythriol lipids, trehalose lipids, cellobiolipids, and sphorolipids (Chrzanowski et al., 2012). However, rhamnolipids are noteworthy glycolipids produced by *Pseudomonas* sp. and consist of linkage of rhamnose and β -hydroxydecanoic acid molecules

(Jarvis and Johnson, 1949; Chong and Li, 2017). In this case, the hydroxyl group of one of the acids is linked to the reducing terminal of the rhamnose via a glycosidic bond whereas; the hydroxyl group of the other acid engages in esterification (Muthusamy et al., 2008).

3.2. Lipopeptides and lipoproteins

A well-studied lipopeptide is surfactin secreted from *Bacillus subtilis* ATCC 21332. Surfactin consists of amino acids connected to carboxyl and hydroxyl groups of a 14-carbon acid by lactone bond. It is regarded as the most effective biosurfactant with extraordinary surface activity at low concentrations (Kakimuna et al., 1969). A notable feature of surfactin is its potential to breakdown human red blood cells, leading to the formation of spheroplasts (Satpute et al., 2008).

3.3. Fatty acids, phospholipids and neutral lipids

Fatty acid and phospholipid biosurfactants are produced in huge amounts by microorganisms when cultivated in *n*-alkane-rich media. The proportion of hydrophilic and lipophilic moieties of the surfactants correlates with length of the hydrocarbon chain (Satpute et al., 2010). Phosphatidyl ethanolamine-containing vesicles secreted by *Acinetobacter* sp. form micro-emulsions of alkane-water while that produced by *Rhodococcus erythropolis* lowers the interfacial tension of mixture of hexadecane and water (Desai and Banat, 1997).

3.4. Polymeric biosurfactants

The most notable polymeric biosurfactants include lipomanan, alasan, liposan, emulsan etc. However, emulsan is regarded as an efficient bioemulsifier for the emulsification of hydrocarbon-water mixture at low concentrations (Zosim et al., 1982; Lang, 2002; Hatha et al., 2007). Emulsan and biodispersan produced by *Acinetobacter calcoaceticus* are the best-studied examples and consist of a heteropolysaccharide moiety covalently linked to fatty acids (Rosenberg et al., 1988). Liposan is an emulsifier produced by *Candida lipolytica* and comprises of carbohydrates (83%) and proteins (17%) (Cirigliano and Carman, 1984). Such glycoprotein complex is also produced by *Yarrowia lipolytica* (Shekhar et al., 2015). Others include mannan lipid protein (*Candida tropicalis*), protein PA (*Pseudomonas aeruginosa*) (Desai and Banat, 1997; Shekhar et al., 2015).

3.5. Particulate biosurfactants

The particulate biosurfactants such as extracellular membrane vesicles partition oil-water mixture and form micro-emulsion (at the interface), which facilitates uptake of alkane by microorganisms (Desai and Banat, 1997). A typical example includes vesicles produced by *Acinetobacter* sp., which consist of lipopolysaccharides, protein, and phospholipids (Kappeli and Finnerty, 1979; Vijayakumar and Saravanan, 2015).

4. Methods for detection of microbial surfactants

Advanced technologies for fast and dependable screening of microorganisms are essential for the discovery of novel surfactant-producing microbial strains (Maneerat, 2005; Satpute et al., 2010). Due to diverse functional and structural properties of the biomolecules, the use of single screening approach for the selection of surfactant-producing microorganisms has been very challenging in providing accurate and reliable results. Hence, the

Table 3
Advantages and disadvantages of microbial surfactant detection methods

Detection method	Advantage	Disadvantage
Oil spreading assay	Fast, simple, requires less sample volume and no specialized equipment	Biosurfactant detection is influenced by type and amount of oil used
Surface tension measurement	Precise, reliable and easy to use	Difficulties in concurrent measurement of different samples; prone to variations
Emulsification assay	Simple	Substrate specific; emulsion stability is influenced by physicochemical parameters
Drop collapse assay	Rapid, easy to carry out, requires no sophisticated equipment; less sample volume	Low sensitivity
CTAB agar plate method	Simple, permits different culture conditions	Specific for anionic biosurfactants; toxic and inhibit growth of some microbes
Blood haemolysis test	Easy to perform	Non-specific; unreliable; diffusion restriction of surfactants hinders clear zone formation

need for adoption of multiple screening tests concurrently for the selection of maximum number of biosurfactant or bioemulsifier producers among the population of isolated microorganisms. The different detection techniques often used for surfactant production are dependent on surface or emulsification activity etc. The advantages and disadvantages of these methods are highlighted in Table 3 and described below in details:

4.1. Oil-spreading assay

The oil spreading assay is carried out by the addition of crude oil (10 μ L) onto distilled water (40 mL) in a Petri dish, leading to the formation of thin oil layer. Thereafter, culture supernatants (10 μ L) are transferred on the oil–water surface (Morikawa et al., 2000). The displacement of oil and formation of clear zone show the presence of surfactant in the culture supernatant. The diameter of clear zone on the oil surface is tantamount to surfactant activity. The oil spreading method is rapid, sensitive and reliable technique to detect biosurfactant or bioemulsifier production by diverse microorganisms (Panjar et al., 2015; Kumar et al., 2015; Adetunji and Olaniran, 2019). In addition, it requires less sample volume and no specialized equipment, and can be employed when the activity and quantity of surfactant is low.

4.2. Surface tension measurement

Surface tension (ST) is a measure of free energy per unit area in relation to an interface or surface (Satpute et al., 2010). The direct measurement of ST of culture supernatants is carried out using tensiometer involving du Nuoy ring method, Wilhelmy plate method, maximum pull force method, or pendant drop method. The ST of deionized water is 72 mN/m; addition of surfactants causes a reduction in the ST (Satpute et al., 2010). Effectiveness of a biosurfactant depends on its ability to reduce the ST of water to below 40 mN/m (Abdel-Mawgoud et al., 2010). This is typical of a surfactin biosurfactant that reduced the ST of water to 27 mN/m (Cooper and Goldenberg, 1987; Banat, 1993). Similarly, a rhamnolipid biosurfactant secreted by *Pseudomonas aeruginosa* decreased the ST of water to about 30 mN/m (Dusane et al., 2010). This approach is precise and consistent for the screening of microorganisms for biosurfactant production (Bodour et al., 2003; Youssef et al., 2004; Salihu et al., 2009).

4.3. Emulsification activity

Among the functional properties of biosurfactant/bioemulsifier is emulsification, which involves phase separation of two immiscible liquids (Satpute et al., 2010; Inès and Dhouha, 2015). It is measured by calculating emulsification index (E_{24}), as described by Cooper and Goldenberg (1987). In this method, equal volume of

hydrocarbon-based compounds and culture supernatants (2:2) is mixed together for 120 sec before cooling at room temperature for 24 h. The E_{24} is measured by dividing the height of the emulsion layer by the total height of liquid. Bioemulsifiers including emulsan and liposan produced by strains of *Acinetobacter calcoaceticus* and *Candida lipolytica*, respectively have been reported to exhibit higher emulsification activity (Satpute et al., 2010; Uzoigwe et al., 2015). These bioemulsifiers have high molecular weight of approximately 1000 kDa with tensile strength and resistance to shearing, a major factor contributing to their high emulsifying activities (Desai and Banat, 1997). Emulsan formed stable emulsion at low concentration, thus considered as the most potent emulsion stabilizer (Ron and Rosenberg, 2001). In addition, biosurfactants which are lipopeptide (e.g. surfactin) and glycolipid (e.g. rhamnolipids) in nature have also been reported to stabilize emulsions (Kim et al., 1997; Benincasa et al., 2004). Several authors have employed emulsification index as a technique for the detection of biosurfactant or bioemulsifier production from various microorganisms (Ben et al., 2015; Ndlovu et al., 2016; Adetunji and Olaniran, 2019).

4.4. Drop collapse method

The drop collapse assay is used for the screening of biosurfactant and/or bioemulsifier-producing microorganisms by measuring surface tension of the culture supernatants (Jain et al., 1991). It is based on destabilization of oil–water surface by the surfactants (Walter et al., 2010). The surface activity of the organisms is evident by the spread of the culture supernatants over a hydrophobic layer because of interfacial tension that exists between the oil–water surface and the supernatant (Hsieh et al., 2004; Walter et al., 2010). In this assay, an aliquot of culture supernatant is added onto an oil-coated surface for 60 sec. The spread or collapse of the aliquot sample suggests the presence of surfactants in the culture supernatant (Jain et al., 1991; Bodour and Miller-Maier, 1998; Walter et al., 2010). This is due to reduction in interfacial tension between the oil–water surface. Conversely, the absence of biosurfactants in the culture sample causes the aliquot to remain stable possibly due to its repellent by the hydrophobic surface. The stability of the culture supernatant is influenced by the surfactant concentration (Bodour and Miller-Maier, 1998; Bodour et al., 2003). A low concentration of surfactants in the culture supernatant permits insensitivity and inaccuracy of the drop collapse assay. This owes to the fact that, a high amount of surfactant is needed for a noticeable collapse of the culture supernatant on oil surface (Satpute et al., 2008; Walter et al., 2010). This technique is simple, quick, and requires no sophisticated equipment and large sample volume. It has been used for the screening of numerous microorganisms for biosurfactant or bioemulsifier production (Ibrahim et al., 2013; Panjar et al., 2015).

4.5. Cetyltrimethylammonium bromide agar plate method

The cetyltrimethylammonium bromide (CTAB) agar plate method is employed to detect glycolipid or other anionic surfactant-producing microorganisms (Siegmond and Wagner, 1991). In this technique, the microorganisms are grown in a medium consisting of CTAB and methylene blue. The production of anionic surfactant by the microbial strains is indicated by the formation of dark blue halos around the colonies. This technique is simple, specific for anionic biosurfactants and can be applied directly on agar plates or liquid broth using different substrates or temperatures. However, CTAB is noxious and inhibits the growth of some microorganisms (Soltanighias et al., 2019).

4.6. Blood haemolysis test

This is a qualitative screening test for the detection of biosurfactant-producing microorganisms. It was developed by Mulligan et al. (1984) to determine the ability of biosurfactant to lyse red blood cells. Bacterial cultures are inoculated on blood agar plate and then incubated at desired temperature for 48 h. The lysis of the blood cells is visualized by the formation of colorless halos around the colonies, indicative of the presence of biosurfactant-producing organisms. However, this method has been considered non-specific and unreliable (Youssef et al., 2004; Satpute et al., 2008).

5. Microbial surfactant production

Microbial surfactants are produced extracellularly in aqueous media or intracellularly by microorganisms in the presence of water soluble and/or water insoluble substrates *via de novo* pathway and/or assembly from other substrates with consequent variations in structure or production domain within the organisms (Gautam and Tyagi, 2006; Satpute et al., 2010). The production of these biomolecules is dependent on appropriate selection of microbial strains, substrate type, and fermentation technology (Marchant and Banat, 2012; Marchant et al., 2014).

6. Parameters influencing microbial surfactant production

Bioprocess parameters including carbon and nitrogen sources, temperature, pH, metal ions, agitation speed etc. influenced the growth of microorganisms for surfactants' production. Interaction of these parameters with each other affects the kinetics of microbial surfactant production (Yaraguppi et al., 2020). The various nutritional and physicochemical parameters that influence the yield of microbial surfactants are discussed in details below:

6.1. Carbon sources

The common carbon sources that are incorporated in fermentation media for microbial surfactant production include carbohydrates, fats & oils, and hydrocarbon groups (Nurfarahin et al., 2018). These carbon sources are classified as water-soluble (e.g. glucose, sucrose, glycerol) and water insoluble (such as crude oils, vegetable oils), and affect the composition of biosurfactants (Prabhu and Phale, 2003; Cunha et al., 2004). However, among the carbon sources, glucose is mostly utilized by microorganisms due to its easier metabolism *via* glycolytic pathway for the generation of desired metabolites (Nurfarahin et al., 2018). Biosurfactant secretion by *Pseudomonas aeruginosa* MTCC 7815 when cultivated in growth media containing glucose, glycerol, fructose and starch was assessed. Maximum E_{24} (76.77%) and lowest ST (34.53 mN/

m) was recorded in a medium containing glucose as a carbon source (Tomar and Srinikethan, 2016).

On the other hands, biosurfactant production in the presence of water-insoluble substrates eases mass transfer of the substrates across the cell surface for microbial growth (Campos et al., 2013; Nurfarahin et al., 2018). These carbon sources are consumed by microorganisms as a building block for the synthesis of hydrophilic and hydrophobic moieties of the biosurfactant (Weber et al., 1992; Desai and Banat, 1997). Govindammal and Parthasarathi (2013) investigated the influence of glucose, petroleum-based substrates, waste fried vegetable oil, and coconut oil cake on biosurfactant production by *Pseudomonas fluorescens* MFS03 isolated from mangrove forest soil. Vegetable oil and coconut oil were reported as promising substrates for biosurfactant production. Similarly, Sim et al. (1997) studied the effect of vegetable oils (canola and soybean oils) and glucose on rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* UW-1. Results recorded showed a 10–12-fold increase in the yield of rhamnolipid when vegetable oil instead of glucose was utilized as the sole carbon source. As a result, various authors have suggested the use of vegetable oil as inexpensive and renewable substrates for biosurfactant production (Desai and Banat, 1997; Banat et al., 2000, 2014; Satpute et al., 2017). Vegetable oil represents one of the first substrates reported for high yields of biosurfactants (Robert et al., 1989). Several co-workers have reported the use of olive oil as the best carbon source for biosurfactant production (Kiran et al., 2010; Noudeh et al., 2010; Adetunji and Olaniran, 2019).

6.2. Nitrogen sources

Nitrogen sources form another most essential nutrient for microbial growth and production of biosurfactants (Santos et al., 2016). Various nitrogen sources (organic and inorganic) are employed for biosurfactant production (Abdel-Mawgoud et al., 2010). The influence of different nitrogen sources namely, NaNO_3 , $(\text{NH}_4)_2\text{SO}_4$, and $\text{CH}_4\text{N}_2\text{O}$ on biosurfactant production by *Pseudomonas aeruginosa* when cultivated in mineral salt medium was investigated (Santa Anna et al., 2002). Sodium nitrate was more effective when compared to other nitrogen sources, resulting in higher rhamnolipid production of 3.16 g/L. Enhanced sophorose lipids secretion by *Torulopsis bombicola* and *Candida bombicola* have been obtained in the presence of yeast extract and urea as nitrogen sources (Deshpande and Daniels, 1995). Ammonium nitrate and yeast extract have also been reported as the best nitrogen sources for optimum production of mannosylerythritol lipids by *Candida* sp. (Sarubbo et al., 2007; Rufino et al., 2008). Furthermore, addition of amino acids (such as aspartic acid, asparagine, glycine and glutamic acid) in growth media enhances microbial biosurfactant production (Duvnjak et al., 1983). This is notable of two- & four-folds improvements in the yields of lichenysin A by *Bacillus licheniformis* BAS50 following addition of L-glutamic acid and L-asparagine, respectively in the growth media (Yakimov et al., 1996). Surprisingly, depletion of nitrogen sources by microorganisms during stationary growth phase improves biosurfactant production (Patel and Desai, 1997). This causes optimum biosurfactant production with great effects on the composition of the biomolecules.

6.3. Physicochemical parameters

Physicochemical parameters such as pH, temperature, metal ions, oxygen requirements and agitation speed play a crucial role in influencing the growth and/or metabolic activity of microorganisms for biosurfactant production. This is remarkable of maximum rhamnolipid production recorded by *Pseudomonas* sp. at optimum pH range of 6.0–6.5 with a drastic decline at pH greater than 7.0

(Guerra-Santos et al., 1984). In addition, optimum incubation temperature for maximum biosurfactant secretion differs, depending on the physiology of the organisms. As a consequence, microorganisms are cultivated under varying temperatures for high yields of biosurfactant. Metal ions such as Mg^{2+} , K^+ , Mn^{2+} and Fe^{2+} act as a key cofactor for the enzyme-catalyzed biosynthetic pathways of biosurfactant production (Wei et al., 2007; Chen et al., 2015). However, excessive availability of these metal ions may be toxic to microorganisms. Enhanced surfactin production by *Bacillus subtilis* in the presence of Mn^{2+} , Fe^{2+} , and Mg^{2+} has been reported (Gudiña et al., 2015a). Moreover, oxygen supply and agitation speed contribute significantly to the kinetics of biosurfactant production by *Acinetobacter calcoaceticus* RAG-1 (Wang and Wang, 1990) and *Bacillus subtilis* (Sheppard and Cooper, 1990). Furthermore, incubation period plays a crucial role in microbial surfactant production since the biomolecules are produced at different time intervals. For instance, maximum bioemulsifier production was reported by *Acinetobacter* sp. Ab9-ES at 168 h when cultivated in growth media supplemented with olive oil whereas, optimum biosurfactant yield was recorded by *Aeribacillus pallidus* YM-1 at 10 h when grown in glucose-containing media (Zheng et al., 2012; Adetunji and Olaniran, 2019).

7. Potential applications of microbial surfactants

In recent years, there have been incessant demands for biosurfactants owing to their multifunctional properties and production by various microorganisms. In addition, of utmost importance is the environmental compatibility of these biomolecules due to their biodegradability and less toxicity when compared to synthetic surfactants. These outstanding features of biosurfactants permit their application as alternatives to chemical surfactants in a wide range of biotechnological applications including petroleum, agriculture, food processing, cosmetics, detergents, leather, textile, paper and pharmaceutical industries (Rodrigues et al., 2006; Banat et al., 2010; Gudiña et al., 2016). Furthermore, biosurfactants are used for the recovery of oil residue from storage tank, clean-up of oil spills and bioremediation of contaminated soil and water (Sobrinho et al., 2013; Silva et al., 2014). A summary of the potential applications of biosurfactants in various industries is presented in Table 4. The major biotechnological applications are discussed in details below:

7.1. Bioremediation

Microbial surfactants enhance the dispersal and solubility of contaminants in aqueous phase and further increase the bioavailability of the hydrophobic substrates to microbes with subsequent elimination of such pollutants through biodegradation, thus eliminating the need for additional process and results in lesser operational costs (Damasceno et al., 2012; Olkowska et al., 2012). Numerous studies have demonstrated the potential applications of biosurfactants in environmental decontamination (Hu et al., 2013; Chaprão et al., 2015). Maier and Soberon-Chavez (2000) reported that rhamnolipid promotes the degradation of hydrocarbon-based pollutants. The use of rhamnolipid and surfactin for the degradation of diesel-contaminated soil and water has also been reported (Whang et al., 2008). Gusmão et al. (2010) assessed the potential of crude biosurfactants produced by *Candida glabrata* UCP 1002 in the remediation of soil–water–hydrophobic pollutant system. Removal efficiency of 92.6% oil was recorded. In another study, a novel biosurfactant, denoted lunasan produced by *Candida sphaerica* UCP 0995 was found to remove 95% of motor oil from contaminated site (Luna et al., 2011).

Table 4
Some potential biotechnological applications of microbial surfactants

Industry	Application	Role	Reference
Detergent	Laundry detergents	Additive for improved performance and stain removal	Vijayakumar and Saravanan, 2015.
Medicine	Pharmaceuticals and therapeutics	Antibacterial agents; antiadhesive agents; antifungal agents; antiviral agents; antimycoplasma agents; antitumoral agents; anti-inflammatory agents; anticoagulant agents; anticancer agents	Banat et al., 2010, 2014; Mnif and Ghribi, 2015; Gudiña et al., 2016
Agriculture	Biocontrol; Biofertilizers	Emulsifying agents; dispersing agents; improvement of soil quality; plant pathogen elimination; enhances bioavailability of nutrients for beneficial plant-associated microbes; root colonizers	Sachdev and Cameotra, 2013; Mnif and Ghribi, 2016
Cosmetics	Skin care products	Emulsifying agents; foaming agents; antimicrobial agents; cleansers	Vijayakumar and Saravanan, 2015
Nanotechnology	Nanoparticle production	Stabilization; adsorption; dispersion; emulsification	Biswas and Raichur, 2008; Reddy et al., 2009; Farias et al., 2014. Banat et al., 2010; Pacwa-Plociniczak et al., 2011; Silva et al., 2014
Bioremediation	Removal of pollutants from contaminated soil and water; clean-up of oil spills; microbial-enhanced oil recovery	Dispersion; emulsification; desorption; solubilization; anti-corrosive agents; foaming agents; surface tension reduction	Banat et al., 2010; Pacwa-Plociniczak et al., 2011; Silva et al., 2014
Food	Emulsification; de-emulsification; functional ingredient	Stabilization of emulsion; phase dispersion; lowering of surface and interfacial tension; Improvement of texture, shelf life and consistency; Emulsifiers; control of fat globule agglomeration, food preservation	Campos et al., 2013; Mnif and Ghribi, 2016

The utilization of microbial surfactants serves as a promising strategy for the biotreatment of oily wastewater (Rahbari-Sisakht et al., 2017). Zhang et al. (2009) applied rhamnolipids for the treatment of oily wastewater in an aerated active sludge system. There was an increase (17.7%–63%) in the removal efficiency of oil at rhamnolipid concentration of 11.2 mg/L and 20 °C. However, at 25 °C, the removal efficiency of oil was above 80% (with rhamno-

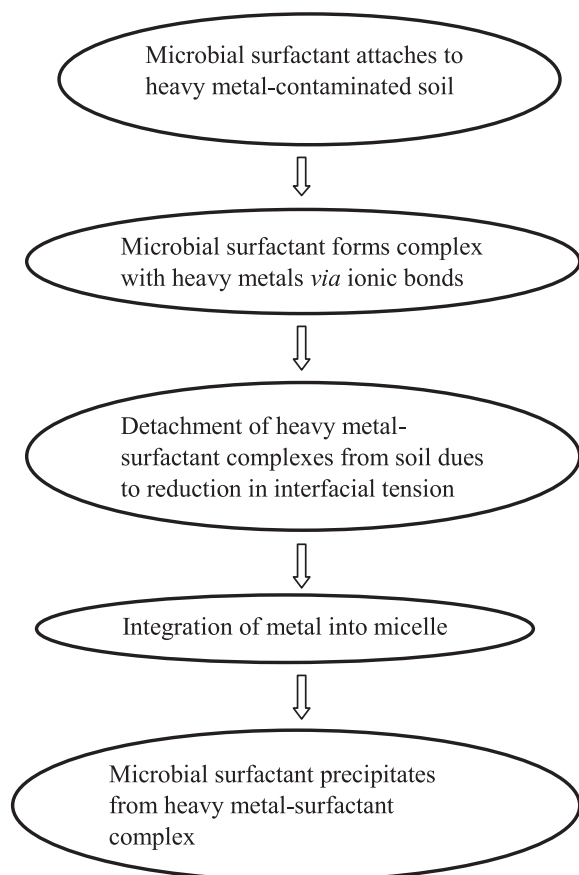


Fig. 1. Schematic diagram showing mechanism of action of microbial surfactant in the removal of heavy metals from contaminated site.

lipids) when compared to 22.3% (without rhamnolipids). Significant improvement of 24% (without rhamnolipids) to 92% (with rhamnolipids) in the removal of oil was recorded at rhamnolipid concentration of 22.5 mg/L for 24 h at 20 °C. The enhanced remediation of oils was possibly due to better solubility and lower interfacial tension of the rhamnolipids. In another study, Yan et al. (2012) used a rhamnolipid secreted by *Pseudomonas aeruginosa* F-2 for the reclamation of oil from a contaminated sludge. Removal efficiency (91.5%) of oil was reported.

The rapid raise in the quantity of heavy metals and radionuclides in polluted sites in many developed countries impacts serious environmental and health hazards via food chain or direct contact with contaminated soil and water (Chakraborty and Das, 2014; Mao et al., 2015). The heavy metals bind to the soil surface as ions or precipitates of metallic compounds. They are recovered from the contaminated sites via surfactant-mediated complexation and ion exchange (Swarnkar et al., 2012; Santos et al., 2016) (Fig. 1). Dahrzma and Mulligan (2007) assessed the removal efficacy of heavy metals from sediments collected from the Lachine Canal in Canada using rhamnolipid biosurfactant. Rhamnolipid concentration of 0.5% (v/v) led to removal efficiencies of Cu (37%), Ni (27%) and Zn (13%). Biosurfactants produced by *Pseudomonas* sp. and *Alcaligenes* sp. have been employed for the floatation and removal of calcite and scheelite. Recovery efficiencies of 95% CaWO₄ and 30% CaCO₃ were recorded in comparison to conventional surfactant, found to be incapable of separating the minerals (Nitschke and Pastore, 2002). Similarly, Juwarkar et al. (2007) investigated the potential of rhamnolipid for the removal of cadmium and lead from contaminated soil samples. The rhamnolipid biosurfactant removed both free and weakly bound forms of cad-

mium and lead from the tested soil, a trend not noticeable in the aqueous control systems without biosurfactant.

7.2. Food industry

Microbial surfactants are employed in food industries for lowering of surface and interfacial tension as well as formation and stabilization of emulsions. Emulsification is significant for consistency and texture formation in food as well as dispersion of immiscible substances and solubilization of aromas (Campos et al., 2014). Usually, the emulsifiers stabilize emulsion by controlling cluster of fat globules; stabilization of aerated systems coupled with improving longevity and altering rheological characteristics of food products (Campos et al., 2013). Enhancement in the stability of dough; volume, texture and conservation of bakery products following addition of rhamnolipid biosurfactants has been reported (Van Haesendonck and Vanzeveren, 2004). Remarkably, bioemulsifiers produced by *Candida utilis* and *Saccharomyces cerevisiae* are used in processed salad dressings and for stabilization of emulsions in food products, respectively (Torabizadeh et al., 1996; Campos et al., 2015).

7.3. Medicine

Several microorganisms are resistant to a variety of antimicrobial agents due to misuse of the chemotherapeutic agents. Thus, persistent and chronic infectious diseases emerge, constituting a serious public health concern (Coates et al., 2011). In addition, in the last decades, the discovery of new antibiotics has reduced significantly, due to challenges in identification of novel and hyperactive compounds with subsequent high costs needed for their development. Therefore, there is an urgent need for the discovery of antimicrobial drugs characterized with wide therapeutic properties.

Biosurfactants are employed in medicine owing to their bactericidal, virucidal and fungicidal activities, amongst others, which are measured in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Sophorolipids from *Candida bombicola* inhibited bacterial growth with a MIC of approximately 30 and 1 mg/mL in a contact time of 2 and 4 h, respectively for *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027 as well as 6 and 1 mg/mL in a contact time of 4 h for *Staphylococcus aureus* ATCC 6358 and *Bacillus subtilis* ATCC 6633, respectively (Joshi-Navare and Prabhune, 2013). Biosurfactant produced by *Pediococcus dextrinicus* SHU1593 elucidated antibacterial activity against *Enterobacter aerogenes* and *Escherichia coli* at a concentration of 25 mg/mL (Ghasemi et al., 2019). These biomolecules exhibit similar or better antimicrobial activity when compared to synthetic antibiotics possibly due to their self-association and pore formation in the cell membrane structure (Deleu et al., 2008). For instance, biosurfactant (glycolipid) from *Streptomyces* sp. MAB36 exhibited akin antifungal activity against *Aspergillus niger* and *Candida albicans* in comparison to synthetic nystatin (Manivasagan et al., 2014). In another study, conventional antibiotic, chloramphenicol was found to be less efficacious against *Escherichia coli* and *Staphylococcus epidermidis* when compared to biosurfactant produced by *Nocardiopsis dassonvillei* MAD08 (Selvin et al., 2009).

Furthermore, some biosurfactant compounds possessed a substantial antiadhesive and antibiofilm activity. Antibiofilm activity of glycolipid biosurfactant produced by *Brevibacterium casei* MSA19 was studied (Kiran et al., 2010). The biosurfactant removed pre-formed biofilms of all the tested pathogenic microbial strains when applied at a concentration of 30 µg/mL. Similarly, biosurfactant lunasan produced by *Candida sphaerica* was employed as an antiadhesive agent at a concentration of 10 mg/mL for the prevention of biofilm formation by *Pseudomonas aeruginosa* and *Streptococ-*

cus sp. Approximately 80–92% adherence inhibition was recorded (Luna et al., 2011) whereas, biosurfactant rufisan exhibited antiadherence activity against most of the tested microorganisms including *Streptococcus sanguis*12, *Streptococcus agalactiae*, *Streptococcus oralis* J22, *Streptococcus mutans*, *Streptococcus mutans* HG, and *Streptococcus mutans* NS at a concentration beyond the CMC of 0.3% (Rufino et al., 2011).

Biosurfactants have been highlighted as potential anticancer agents by interfering with cancer development processes. These metabolites are involved in several intracellular molecular recognition steps consisting of signal transduction, cell differentiation and cell immune response etc. (Rodrigues et al., 2006). For instance, surfactin biosurfactants are commonly used as potential anti-cancer agent against many cancer cell lines (Dey et al., 2015). Iturin A produced by *Bacillus megaterium* has been found to significantly impair proliferation and inhibit the Akt signaling network, resulting in apoptosis induction in breast cancer cells (Dey et al., 2015). In addition, lipopeptide somocystinamide A secreted from *Lyngbya majuscula* demonstrated noteworthy cytotoxicity against lung, breast and prostate cancer cells with IC₅₀ values between 1.3 μM and 970 nM, based on the cancer model (Wrasidlo et al., 2008).

7.4. Cosmetic industry

Biosurfactants are used for different purposes in cosmetic industries due to their multifunctional properties including foaming, water-binding capacity, emulsification, wetting, demulsification, and spreading, which allow these compounds to be utilized efficiently in cosmetic products such as solubilizers, anti-dandruff shampoo, soap, creams, cleansers, hair conditioners and other dermatological products (Youssef et al., 2007; Shekhar et al., 2015). In comparison to chemically synthesized surfactants, which render allergic and irritation effects to skin, microbial surfactants are better skin compatible (Williams, 2009; Ferreira, et al., 2017). Lipopeptide biosurfactants with moisturizing and anti-wrinkle properties demonstrated less toxicity to human cells. Thus, they are employed as supplements in skin care products (Mandal et al., 2013). In addition, monoglyceride produced from *Pseudomonas fluorescens* is a commonly used surfactant in the cosmetic industry (McNeill and Yamane, 1991).

8. Conclusions and recommendations

The global market for microbial surfactants has increased significantly in the last decades. This owed to the outstanding functional properties of these biomolecules, forming a foundation for exploration in bioremediation, medicine, cosmetics, and food industries, amongst others. The production of microbial surfactants is influenced by apt choice of microbial strains, substrate type, and fermentation technology. However, commercial production of microbial surfactants formed a major constraint from economic viewpoint. Optimization of fermentation parameters using cheaper and renewable substrates coupled with robust downstream processing methods could pave the way for profitable and cost-effective surfactant production. In addition, the use of recombinant and mutant hyper-producing microorganisms could provide high yields surfactant production that would be beneficial for industrial applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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