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REVIEW

Microbial derived surface active compounds: properties and screening concept

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Abstract Biosurfactants are surface-active biomolecules that are produced by a variety of microorganisms. They have gained biotechnologist interest for high diversity and their efficient action in comparison to synthetic emulsifiers. So, we discussed a wide array of screening method based on direct and indirect surface and interfacial tension measurements. Also, this review describes biosurfactant physicochemical properties and natural role in the environment. Also, it presents their tolerance to extreme conditions of temperature, pH and ionic strength, low toxicity and biodegradability. Functional properties like emulsification, foaming, solubilizing and membrane permeabilizing activities were also discussed along with their related application.

Keywords Screening concept · Surface tension · Critical micellar concentration · Low toxicity and biodegradability · Functional properties · Higher efficiency

Introduction

Biosurfactants or microbial surfactants are chemical active compounds produced by micro-organisms with wide structural varieties. They are characterized by an amphiphilic structure with hydrophilic (peptide or amino-acids, polysaccharides…) and hydrophobic (fatty acid) moieties (Shoeb et al. 2013). They are generally synthesized by micro-organisms when growing on water immiscible substrates (Shoeb et al. 2013; Banat et al. 2010). Rhamnolipids of *Pseudomonas aeruginosa*, surfactin of *Bacillus subtilis*, emulsan of *Acinetobacter calcoaceticus* and sophorolipides of *Candida bombicola* are examples of the most known biosurfactants (Banat et al. 2010). They have the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and the interface, respectively with very low critical micelle concentration, none toxicity, highly biodegradability and tolerance to extreme conditions such as high temperature value, extreme pH, and high salinity (Kapadia Sanket and Yagnik 2013). Furthermore, biosurfactants offer numerous biological activities for increasing commercial importance. For this reasons, in the past few decades, they showed great economic interest, specifically, in agriculture field as a biocontrol agent and for their insecticide activity, in bioremediation for their role in hydrocarbon contaminant biodegradation and metal sequestering; in chemical industry, food processing, food additives, cosmetic, and pharmaceuticals field for their emulsifying, foaming, dispersant, and antiadhesive activities and in medicine for their antimicrobial, antitumor, antiviral, and anti-inflammatory activities (Banat et al. 2010).

Screening of biosurfactant producing strain

Generally, screening methods of biosurfactant producing strain are based on the physical effects of surfactants. Alternatively, the decreasing potency of surface and interfacial tension and ability of strains to interfere with hydrophobic interfaces can be explored. On the other hand, specific screening methods like the colorimetric CTAB agar assay are suitable only to a limited group of biosurfactants. To know, screening methods can give qualitative and/or quantitative results for biosurfactants production. For a first screening of isolates, qualitative methods are

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generally sufficient. Many studies reported the use of different screening method at the time and suggest their classification as primary, secondary and tertiary screening tools (Thavasi et al. 2011; Varjani et al. 2014). Here, we will discuss all the screening method developed for biosurfactant production as well as their properties along with some examples of their use. Table 1 resume the main screening methods developed to detect biosurfactants production, their advantages, disadvantages and applicability.

Screening method based on surface and interfacial tension

As defined by Satpute et al. (2010), surface tension is the force per unit length exerted by a liquid in contact with a solid or another liquid. It can also be considered as a measure of the free energy per unit area associated with a surface or an interface. Among the known liquids, water has highest surface tension value of 72 dyne/cm or mN/m which would be reduced upon the addition of surfactant. Interfacial tension is an intermolecular attractive force held within the molecules in a liquid (Satpute et al. 2010).

Biosurfactants or microbial derived surfactants are surface active molecules that have the ability to adsorb to surfaces or interfaces. On the basis of this famous properties, a wide array of screening concept for biosurfactant producing microbes are developed. They include the direct surface and interfacial tension measurement according to different method. Other screening methods have been developed that rely on the interfacial activity of the biosurfactants but that do not measure it directly such as the drop collapsing method, the oil spreading method… (Walter et al. 2010).

Methods involving direct measurement of the surface and interfacial tension Biosurfactant activities can be determined by measuring the changes in surface and interfacial tensions. In fact, it's the most reliable and common method to detect biosurfactant production and can be qualitative and quantitative. Surface tension at the air/water and oil/water interfaces can be easily measured with a tensiometer (Salihu et al. 2009). When a surfactant is added to air/water or oil/water systems at increasing concentrations, a reduction of surface tension is observed up to a critical level, above which amphiphilic molecules associate readily to form supramolecular structures like micelles, bilayers, and vesicle. This value is known as the critical micelle concentration (CMC). CMC is defined by the solubility of a surfactant within an aqueous phase and is commonly used to measure the efficiency of a surfactant. In fact, the values of surface tension, interfacial and CMC characterize each biosurfactant. Cooper and Goldenberg (1987), suggested biosurfactant production if a reduction of the surface tension of about 40 mN/m or less occurs. The direct measurement of surface tension can be done by different techniques:

Du-Nouy-Ring method

As suggested by Satpute et al. (2010), the Du-Nouy-Ring method is based on measuring the force required to detach a ring or loop of wire from an interface or surface. The detachment force is proportional to the interfacial tension. It can be measured with an automated tensiometer which is available from many manufacturers. The Du-Nouy-Ring assay is widely applied for screening of biosurfactant producing microbes (Plaza et al. 2006; Anyanwu et al. 2011; Pereira et al. 2013).

Stalagmometric method

According to Dilmohamud et al. (2005), the surface tension of a liquid can alternatively be measured with a Traube stalagmometer. The stalagmometric method is one of the most common methods for measuring surface tension. The principle is to measure the weight of the drops of the fluid falling from the capillary glass tube, and then calculate the surface tension of the fluid used. We know the weight of each drop of the liquid by counting the number of the drops falling out. From this we can determine the surface tension. It was adopted to detect biosurfactant production (Plaza et al. 2006; Abdel-Mawgoud et al. 2008). Nevertheless, Plaza et al. (2006) suggested that it is not recommendable due to the large variability they obtained in their results.

Pendant drop shape technique

The pendant drop shape technique is an optical powerful method for the measurement of interfacial tension properties from the shape of drops/bubbles (Hoorfar and Neumann 2006). It was firstly applied by Van der Vegt et al. (1991) for the screening of biosurfactant producing strain and can be used to monitor bacterial biosurfactant production. It's based on an idea suggesting that the shape of a liquid droplet depends greatly on the liquid surface tension. According to Tadros (2005), a drop of liquid is allowed to hang from the end of a capillary. It adopts an equilibrium profile that is a unique function of the tube radius, the interfacial tension, its density and the gravitational field. In fact, droplets of liquids with a low surface tension are more apt to deviate from a perfectly spherical shape than droplets of liquids with a high surface tension. It was applied by Chen et al. (2007), Safary et al. (2010) and Burch et al. (2011) for quantitative measurement of surface and interfacial tension.

Table 1 Screening methods, advantages, disadvantages and applicability

Screening method	Advantages	Disadvantages	Applicability	References
Screening method based on surface and interfacial tension				
Methods involving direct measurement of surface and interfacial tension				
Du-Nouy-Ring method	Easy to make (can be measured with an automated tensiometer)	–	Widely applied for screening of biosurfactant producing microbes	Plaza et al. (2006), Anyanwu et al. (2011), Pereira et al. (2013)
Stalagmometric method	–	Large variability of the obtained results	Adopted to detect biosurfactant production	Plaza et al. (2006), Abdel-Mawgoud et al. (2008)
Pendant drop shape technique	Can be applied for quantitative measurement of surface and interfacial tension	–	Applied for the screening of biosurfactant producing strain and can be used to monitor bacterial biosurfactant production	Chen et al. (2007), Safary et al. (2010), Burch et al. (2011)
Wilhelmy plate method	Unlike a Du Noüy ring, no correction factors are required when calculating surface tensions when using the Wilhelmy plate	–	Used to measure equilibrium surface or interfacial tension at an air–liquid or liquid–liquid interface	Tuleva et al. (2002), Varadavenkatesan and Murty (2013)
Axisymmetric drop shape analysis by profile	Permit to assess bacterial biosurfactant production and to determine surface tension of biosurfactant solution	–	Permit to determine the contact angle and liquid surface tension from the profile of a droplet resting on a solid surface	van der Vegt et al. (1991), van Hoogmoed et al. (2000), Rodrigues et al. (2006)
Measurements based on surface/interfacial tension				
Agar plate overlaid with hydrocarbons	Easy to realize	Can't be applied for microbial strain that don't have the ability to degrade hydrocarbons	Applied for the screening of biosurfactant producing strain	Satpute et al. (2008), Cipinyté et al. (2011)
Drop collapse method	It's a sensitive and easy method to test for biosurfactant production Can also be made quantitative to determine biosurfactant concentration The stability of drops can correlates with surface and interfacial tension	–	Applied for the screening of biosurfactant producing strain	Thavasi et al. (2011), Plaza et al. (2006), Varadavenkatesan and Murty (2013), Johny (2013)
Assay of emulsification activity	Easy to realize Can be quantitative when measuring the generated turbidity or the emulsification index	Mainly qualitative Not specific for biosurfactants	Applied to detect biosurfactants production in the culture supernatant	Varjani et al. (2014), Chen et al. (2007), Johny (2013), Thavasi et al. (2011), Mnif et al. (2013)
Oil spreading test	Generally, the area of displacement by a surfactant-containing solution is directly proportional to the concentration of the biosurfactants tested and its diameter increased linearly with the surfactant quantity	–	Can be applied to detect biosurfactant production	Varadavenkatesan and Murty (2013), Johny (2013), Morikawa et al. (2000)
Microplate assay	Rapid and easy, permit to assay several samples at the same times, don't require special equipment, sensitive and allows an instantaneous detection of surface-active compounds.	Only qualitative	Used for the screening of biosurfactant producing strain	Chen et al. (2007), Johny (2013)

Table 1 continued

Screening method	Advantages	Disadvantages	Applicability	References
Penetration assay	It is simple and can be applied in high throughput screening	Only qualitative	Used for the screening of biosurfactant producing strain	Maczek et al. (2007)
Solubilization of crystalline anthracene	Simple and rapid screening method Can be quantitative	–	–	Willumsen and Karlson (1997)
Tilting glass slide test	Simple and easy to realize	Can be applied as a preliminary screening test to detect biosurfactant production	Followed by many authors to screen biosurfactant producing strain	Varadavenkatesan and Murty (2013), Tomar et al. (2013)
Screening method based on cell surface hydrophobicity				
Bacterial adherence to hydrocarbons	–	Only qualitative Indirect method to detect biosurfactant production	Assessed by many researchers evaluating biosurfactant production	Thavasi et al. (2011), Coimbra et al. (2009), Stoimenova et al. (2009)
Hydrophobic interaction chromatography (HIC)	Permits the simultaneous isolation and screening of biosurfactant producing bacteria	Indirect method	Applied for the simultaneous isolation and screening of biosurfactant producing bacteria	Pruthi and Cameotra (1997), Smyth et al. (1978)
Detection of biosurfactant production by thin layer chromatography	Can be applied to characterize the produced biosurfactant by using selective revelation reagents Direct colony-thin layer chromatographic technique can be applied for a rapid screening of biosurfactant strain	–	Can be used to detect biosurfactant production by separation of the supernatant on silica gel plate Generally, it's used to characterize the chemical nature of the produced biosurfactant	Anyanwu et al. (2011), Varadavenkatesan and Murty (2013), Matsuyama et al. (1991), Tuleva et al. (2002)
Replica plate assay: adhesion of bacteria to hydrophobic polystyrene	Simple assay and inexpensive way to identify an array of microbial strains for biosurfactant production	–	Permit a simultaneous isolation and identification of biosurfactant producing strain	Brzozowski et al. (2011)
Salt aggregation assay	Provides a simple means for identifying bacteria associated with the production of biosurfactants Gives a good estimation of the degree of cell surface hydrophobicity	–	Permits to evaluate cell surface hydrophobicity in relation to biosurfactant production	Andreu et al. (1995), Walencka et al. (2007)
Specific screening method				
Blue agar plate method	Simple and easy to realize	Permit only to detect glycolipids or other extracellular anionic biosurfactants Can be classified as a semi-quantitative assay	It was applied in many studies to detect rhamnolipid production	Tuleva et al. (2005), Varadavenkatesan and Murty (2013), Saravanan and Vijayakumar (2012)
Blood agar method	Simple and easy method to test for biosurfactant activity	Not specific as lytic enzymes can lead to clearing zone Can be limited by the poor diffusion of the target surfactants	Can be qualified as a preliminary method of screening of biosurfactant producing strain growing on hydrophilic substrates and must be complemented by emulsification activity and surface tension measurement	Thavasi et al. (2011), Varadavenkatesan and Murty (2013)

Wilhelmy plate method

A Wilhelmy plate is a thin plate that is used to measure equilibrium surface or interfacial tension at an air–liquid or liquid–liquid interface. In this method, the plate is oriented perpendicular to the interface, and the force exerted on it is measured (Tuleva et al. 2002; Varadavenkatesan and Murty 2013). The Wilhelmy plate consists of a thin plate usually on the order of a few square centimeters in area. The plate is often made from filter paper, glass or platinum which may be roughened to ensure complete wetting. In fact, the results of the experiment are irrelevant of the material used, as long as the material is wetted by the liquid (Butt et al. 2006). The plate is cleaned thoroughly and attached to a scale or balance via a thin metal wire. The force on the plate due to wetting is measured via a tensiometer or microbalance and used to calculate the surface tension (γ) using the Wilhelmy equation:

$$\gamma = \frac{F}{l\cos(\theta)}$$

where l is the wetted perimeter ($2w + 2d$; w is the plate width and d is the plate thickness) of the Wilhelmy plate and θ is the contact angle between the liquid phase and the plate. In practice the contact angle is rarely measured, instead either literature values are used, or complete wetting ($\theta = 0$) is assumed.

Unlike a Du Noüy ring, no correction factors are required when calculating surface tensions when using the Wilhelmy plate, assuming a zero contact angle.

Axisymmetric drop shape analysis by profile

As described by Noordmans and Busscher (1991), surface tension can be measured by the axisymmetric drop shape analysis by profile (ADSA-P). It is a technique developed in colloid and surface science to simultaneously determine the contact angle and liquid surface tension from the profile of a droplet resting on a solid surface (van der Vegt et al. 1991). Briefly, ADSA-P involves digitizing the circumference of a liquid droplet on a solid surface. The circumference of the droplet is fitted to the Laplace equation of capillarity, which yields the surface tension of the biosurfactant solution (Noordmans and Busscher 1991). It was adopted by many researchers to assess bacterial biosurfactant production and to determine surface tension of biosurfactant solution (van der Vegt et al. 1991; van Hoogmoed et al. 2000; Rodrigues et al. 2006).

Measurements based on surface/interfacial tension

Many other screening methods based on the surface activity of the biosurfactants but don't quantify it directly have been developed. We can state the screening by using agar plate overlaid with hydrocarbons, the quantification of the cell surface hydrophobicity, the oil spreading test, the assay of emulsification activity and the drop collapse method that are presented in the following.

Agar plate overlaid with hydrocarbons

As described by Satpute et al. (2008) and Cipinyté et al. (2011), newly isolated strains can be streaked on oil coated agar plates and incubated for 1 week at desired temperature. Colonies surrounded by an emulsified halo can be assumed as biosurfactant producers.

Drop collapse method

It was firstly developed by Jain et al. (1991). It's based on the ability of biosurfactant to destabilize liquid drop. If the liquid contain biosurfactant, drop disperses because a reduction of the interfacial tension between liquid and hydrophobic surface occurs. It is suggested and adopted by many researchers as a sensitive and easy method to test for biosurfactant production (Thavasi et al. 2011; Plaza et al. 2006; Varadavenkatesan and Murty 2013; Johny 2013). The method can also be made quantitative to determine biosurfactant concentration as drop stability depends on surfactant concentration (Mohammadipour et al. 2009). Also, the stability of drops can correlates with surface and interfacial tension.

Assay of emulsification activity

It was described the first time by Panchal and Zajic (1978) as a qualitative test visualized by the formation of a creamy emulsion. In fact, an emulsion is defined as a heterogeneous system, composed of an immiscible liquid dispersed as microscopic droplet in another liquid continuous phase. Biosurfactants may stabilize (emulsifiers) or destabilize (deemulsifiers) the emulsion. To do, equal volumes (3 mL) of aqueous sample and n-hexadecane were added in the test tube and the mixture was thoroughly agitated in a vortex mixer for 2 min. Upon standing, a creamy emulsion was formed when an emulsifier was present. Therefore, an emulsification index (E24 %) was calculated by the following equation: E24 = (Height of an emulsion layer/Total height) × 100 (Varjani et al. 2014; Chen et al. 2007; Johny 2013). The most popularly used oil phase in emulsification test was kerosene and the ratio of the aqueous emulsifier and the oil phase was 2:3 (Rosenberg et al. 1979).

Also, the emulsification activity can be assayed by the ability of the surfactant to generate turbidity; due to suspended hydrocarbons in an aqueous assay system; that can

be quantified by a turbidimeter (Thavasi et al. 2011; Mnif et al. 2013).

Oil spreading test

Biosurfactant activity can be detected by the ability to provoke a clearing zone on an oil surface (Thavasi et al. 2011; Rodrigues et al. 2006). Generally, the area of displacement by a surfactant-containing solution is directly proportional to the concentration of the biosurfactants tested and its diameter increased linearly with the surfactant quantity. Many previous studies reported the use of the oil spreading test to detect biosurfactant production (Varadavenkatesan and Murty 2013; Johny 2013; Morikawa et al. 2000).

Microplate assay

Microplate assay is a qualitative assay for the presence of surfactants and was developed firstly by Vaux and Cottingham (2001). In fact, pure water in a hydrophobic well has a flat surface. The presence of surfactants in an aqueous solution causes some wetting at the edge of the well and the fluid surface becomes concave and takes the shape of a diverging lens. The assay is rapid and easy, permit to assay several samples at the same times and don't require special equipment. It's also sensitive and allows an instantaneous detection of surface-active compounds (Chen et al. 2007). Many researchers used the microplate assay to screen biosurfactant producing strain (Chen et al. 2007; Johny 2013).

Penetration assay

Penetration assay is a qualitative assay to detect biosurfactant production based on the contacting of two insoluble phases which leads to a color change. It was developed firstly by Maczek et al. (2007). For this assay, the cavities of a 96 well microplate are filled with 150 µL of a hydrophobic paste consisting of oil and silica gel. The paste is covered with 10 µL of oil. Then, the supernatant of the culture is colored by adding 10 µL of a red staining solution to 90 µL of the supernatant and is placed on the surface of the paste. If biosurfactant is present, the hydrophilic liquid will break through the oil film barrier into the paste and color will adsorb on the silica gel. So, the upper phase will change from clear red to cloudy white within 15 min. Biosurfactant free supernatant will turn cloudy but stay red. It is simple and can be applied in high throughput screening.

Solubilization of crystalline anthracene

Solubilization of crystalline anthracene, based on the solubilization of crystalline anthracene by the produced biosurfactant, is an indirect method for screening of biosurfactant producing bacteria. It's developed firstly by Willumsen and Karlson (1997). In fact, crystalline anthracene is added to the culture supernatant and incubated on a shaker at 25 °C for 24 h. The concentration of the dissolved hydrophobic anthracene is measured photometrically at 354 nm and correlates to the production of biosurfactant. This is a simple and rapid screening method.

Tilting glass slide test

It was elaborated for the first time by Persson and Molin (1987) as a preliminary screening test to detect biosurfactant production. In fact, a single colony is picked up from the Bushnell Haas agar plate and transferred on the surface of a sterile glass slide near one of the edges. It is then mixed with a droplet of 1 % saline water. The slide is gradually tilted to the other side and was examined for flow of a water droplet over its surface. Biosurfactant production is implied if water flows over the surface. It was followed by many authors to screen biosurfactant producing strain (Varadavenkatesan and Murty 2013; Tomar et al. 2013).

Screening method based on cell surface hydrophobicity

Several methods based on the change of cell surface hydrophobicity when producing biosurfactants can be used to screen biosurfactant producing strain. Generally, cell bound biosurfactant production is associated with high hydrocarbon uptake and therefore high surface hydrophobicity and microbes show low surface hydrophobicity when biosurfactant are released extracellularly (Franzetti et al. 2008). Thus, they are indirect methods for the screening of biosurfactant producing microbes. Nevertheless, several biosurfactant producing strain are selected on the basis of this methods (Pruthi and Cameotra 1997). A disadvantage is that the hydrophobicity of bacteria depends on physiological aspects like growth conditions or cellular age (Franzetti et al. 2008). Among the developed methods, we can describe the adhesion of bacteria to hydrocarbons test, chromatography based on hydrophobic interaction, adhesion of bacteria to hydrophobic polystyrene and aggregation test in the presence of increasing concentration of salt (Pruthi and Cameotra 1997).

Bacterial adherence to hydrocarbons Measurement of bacterial adhesion to hydrocarbons (BATH) assay was used to determine changes in cell surface hydrophobicity (Pruthi and Cameotra 1997). The cell surface hydrophobicity was measured for the first time by the bacterial adherence to hexadecane (BATH) (Rosenberg et al. 1980). The hydrophobicity was expressed as the percentage decrease in absorbance at 550 nm of the lower aqueous phase,

following the mixing procedure, compared with that of the cell suspension prior to mixing. It was assessed by many researchers evaluating biosurfactant production (Thavasi et al. 2011; Coimbra et al. 2009; Stoimenova et al. 2009).

Hydrophobic interaction chromatography (HIC) HIC is a chromatographic procedure based on hydrophobic interaction between the nonpolar groups on a hydrophobic chromatographic resin and the nonpolar regions of a particle. It was used by Rodrigues et al. (2006) as a technique for the purification and separation of biomolecules based on differences in their surface hydrophobicity.

As described by Pruthi and Cameotra (1997) the use of HIC is a reliable method that allows the simultaneous isolation and screening of microbes. It's also valid for comparative analysis of the hydrophobic properties of microorganisms. Thus, as proposed by Smyth et al. (1978), a bacterial suspension is drained into a gel bed of hydrophobized Sepharose. Hydrophobic microbes are retained by the gel and the degree of adsorption of the cells to the gel can be measured by the measurement of the turbidity or by bacteria counting in the elute (Smyth et al. 1978). For desorption of the adherent microbes, the ionic strength of the buffer is decreased.

Detection of biosurfactant production by thin layer chromatography Thin layer chromatography (TLC) techniques can be used to detect biosurfactant production by separation of the supernatant on silica gel plate. Also, it can be applied to characterize the produced biosurfactant by using selective revelation reagents. For example, lipopeptide appears as red spots in the presence of ninhydrin and glycolipid appears as yellow spots when anthrone reagent was used (Salihu et al. 2009). Generally, it's used to characterize the chemical nature of the produced biosurfactant (Anyanwu et al. 2011; Varadavenkatesan and Murty 2013). Nevertheless, direct colony-thin layer chromatographic technique can be applied for a rapid screening of biosurfactant strain as described by Matsuyama et al. (1991) as well as thin layer chromatography of supernatant to detect biosurfactant production as described by Tuleva et al. (2002).

Replica plate assay: adhesion of bacteria to hydrophobic polystyrene It consists of a simple assay for the identification and isolation of hydrophobic microbes and was developed firstly by Rosenberg (1981). It's based on the adherence of bacterial strains to hydrophobic polystyrene which correlates to cell surface hydrophobicity. As described by Pruthi and Cameotra (1997), this technique is an inexpensive way to identify an array of microbial strains for biosurfactant production simultaneously on readily available materials. Furthermore, the identification and isolation of potential strains might be combined in one step. It was assayed by Brzozowski et al. (2011) to evaluate the cell surface hydrophobicity of two *Lactobacillus* strains in relation to their biosurfactant production.

Salt aggregation assay It was firstly described by Lindahl et al. (1981) and provides a simple means for identifying bacteria associated with the production of biosurfactants. As showed by Pruthi and Cameotra (1997), this technique gives a good estimation of the degree of cell surface hydrophobicity. The cells are precipitated by increasing salt concentrations and the more hydrophobic the surface of the cells, the lower the salt concentration required to aggregate the cells. As positive control, all readings are compared to the reaction at the highest molarity. A bacterial suspension mixed with sodium phosphate without addition of salt is used as negative control. It was applied by Andreu et al. (1995) and Walencka et al. (2007) to evaluate cell surface hydrophobicity of vaginal *Lactobacillus* Species and *Saccharomyces cerevisiae* surface active producing strain respectively.

Specific screening method

Blue agar plate method It is an excellent technique developed by Siegmund and Wagner (1991) that has been used generally for detection of glycolipids or other extracellular anionic biosurfactants. Bacterial isolates were streaked on mineral salts agar medium supplemented with a carbon source and cetyltrimethylammonium bromide (CTAB: 0.0005 %)–methylene blue (MB: 0.0002 %). So, as anionic biosurfactant forms insoluble ion pair with the cationic CTAB-MB, the formation of dark blue halo around the culture is considered as positive for biosurfactant production. It can be classified as a semi-quantitative assay. It was applied in many studies to detect rhamnolipid production (Tuleva et al. 2005; Varadavenkatesan and Murty 2013; Saravanan and Vijayakumar 2012).

Blood agar method As mentioned by Walter et al. (2010), the lysis of erythrocytes cells has been recommended as a simple and easy method to test for biosurfactant activity. The hemolytic activity can be due to the formation of mixed micelles that interact with the lipid bilayers of the erythrocyte membrane causing therefore an osmotic lysis. A linear increase of the diameter of lysis on blood agar can be observed as function of the biosurfactant concentration. In fact, as suggested by Mulligan et al. (1984), it's qualified as not specific, as lytic enzymes can lead to clearing zone and it can be limited by the poor diffusion of the target surfactants. Thus, it can give a lot of false negative and false positive results. For these, it can be qualified as a preliminary method of screening of biosurfactant producing strain growing on

hydrophilic substrates and must be complemented by emulsification activity and surface tension measurement as processed by many authors (Thavasi et al. 2011; Varadavenkatesan and Murty 2013).

Biosurfactants properties and main application

Physicochemical properties of biosurfactants: surface tension reduction and critical micelle concentration (CMC)

The word surfactant is an abbreviation for surface active agent. A surfactant is characterized by its tendency to adsorb at surfaces and interfaces. Examples of interfaces involving a liquid phase include suspension (solid–liquid), emulsion (liquid–liquid) and foam (liquid–vapour). Another general and fundamental property of biosurfactants is that monomers in solutions tend to form aggregates, called micelles (Pacwa-Plociniczak et al. 2011). The concentration at which micelles start to form is called critical micelle concentration (CMC). Micelle formation, or micellization, can be viewed as an alternative mechanism to adsorption at the interfaces for removing hydrophobic groups from contact with the water, thereby reducing the free energy of the system (Pacwa-Plociniczak et al. 2011). CMC values can be considered as an intrinsic characteristic of each biosurfactant measuring its efficiency and purity. The most recognized properties of biologically produced surfactant their ability to decrease the surface tension (ST) of water and the interfacial tension (IFT) between two non-miscible liquids. The most efficient biosurfactant decrease the surface tension of water from 72 mN/m to a value less or equal to 30 mN/m. For each biosurfactant, a minimal decrease of the surface tension can be reached at the critical micelle concentration. As presented by Whang et al. (2008), two biosurfactants with rhamnolipid and lipopeptide nature (produced by correspond *P. aeruginosa* J4 and *B. subtilis* ATCC 21332) have critical micelle concentration (CMC) values of about 50 and 45 mg/L respectively and were able to reduce surface tension to less than 30 dynes/cm from 72 dynes/cm. Table 2 reviewed microbial surfactants and their producing strain, critical micelle concentration and surface tension reduction.

Effect of certain physic-chemical characteristics of biosurfactants on CMC values and surface tension decrease

Biosurfactants can be distinguished as to whether they are efficient or effective surface-active agents. Efficiency is measured by the concentration of surfactant required to produce some significant reduction in the surface tension of water called critical micelle concentration, while effectiveness is measured by the minimum value to which it can lower the surface tension. The critical micelle concentration usually increases with increase in the length of the hydrophobic part of the surfactant and decreases with increased unsaturation or branching. However, effectiveness increases with these changes and depends upon the cohesiveness of the hydrophobic groups in the surfactant.

Effect of certain physic-chemical characteristics on the effectiveness of biosurfactant

For lowering surface and interfacial tensions, the most active saturated fatty acids are in the range C12–C14. In addition to straight-chain fatty acids, microorganisms produce complex fatty acids containing hydroxyl groups and alkyl branches that are recognized by their higher effectiveness. As suggested by Zajic et al. (1983), lower cohesiveness permitted to attain lower surface tension value. Usually branched chain hydro-carbons are more effective surfactants than long-chained hydrocarbons as they have lower cohesiveness. In fact, Yakimov et al. (1996) studied the structure–function relationship of surface-active lipopeptides by analysis of the activities of structurally characterized compounds and proved that fractions of lichenysin A with branched beta-hydroxy acids in the lipid tail demonstrated lower surface-tension activity than the fractions of lichenysin A having straight beta-hydroxy acids. Other studies depicted the modulation of surface tension reduction with the total fatty acid content of the produced biosurfactant under different nutrients conditions (Amézcua-Vega et al. 2007).

Moreover, environmental parameters like pH, temperature and ionic strength don't have pronounced effect on the effectiveness of certain biosurfactant. However, other studies suggested that ionic strength can modulate the surface activity of certain microbial derived surfactants and became more efficient. In a study conducted by Singer et al. (1993), the interfacial tension of a glycolipid biosurfactant decreased dramatically when the glycolipid was blended with 0.5 % pentanol and 1.7 % (w/v) sodium chloride. NaCl, $MgCl_2$ and $CaCl_2$ were consistently found to activate the surface activity of the *B. licheniformis* BAS50 derived lipopeptide (Yakimov et al. 1995). Huszcza and Burczyk (2003) demonstrated that salt addition can increase surface activity of a *B. coagulans* derived biosurfactant (the interfacial tension decreased from 4.5 mN/m to 0.94 mN/m by the addition of 5 wt % of NaCl). Qiao and Shao (2010) reported the increase of oil spreading capacity of a proline lipid biosurfactant in presence of 3–12 % NaCl; 50 mmol/L (4.76 %) $MgCl_2$ and 0–3 mmol/L (0.332 %) $CaCl_2$.

Table 2 Critical micelle concentration and surface tension reduction of microbial derived surfactant

Producing strain	Biosurfactant	CMC value	Surface tension	References
Bacterial derived biosurfactants				
Flavobacterium sp. strain MTN11	Flavolipids	0.3 g/L	26.0 mN/m	Bodour et al. (2004)
P. aeruginosa J4	Rhamnolipid	50 mg/L	30 mN/m	Whang et al. (2008)
Pseudomonas aeruginosa SP4	Rhamnolipid	200 mg/L	29 mN/m	Pornsunthorntawee et al. (2008)
Rhodococcus erythropolis 51T7	Trehalose lipids	0.037 g/L	–	Marqués et al. (2009)
Rhodococcus sp. SD-74	Succinoyl trehalose lipid	5.6×10^{-6} M	19 mN/m	Tokumoto et al. (2009)
Rhodococcus sp. strain TA6	Lipids and glycolipids	–	30 mN/m	Shavandi et al. (2011)
Oleomonas sagaranensis AT18	Glycolipid	8 mg/L	25 mN/m	Saimmai et al. (2012b)
Inquilinus limosus KB3	Lipopeptide	9 mg/L	25 mN/m	Saimmai et al. (2013)
Rhodococcus sp., strain PML026	Trehalolipid	250 mg/L	29 mN/m	White et al. (2013)
Halobacteriaceae archaeon AS65	Lipopeptide	10 mg/L	25.5 mN/m	Chooklin et al. (2014)
Streptomyces sp. SS 20	Glycolipid	0.3 g/L	34.2 mN/m	Hayder et al. (2014)
B. methylotrophicus USTBa	Glycolipid	0.035 g/L	28 mN/m	Chandankere et al. (2014)
Ochrobactrum anthropi 2/3	Glycolipid	8.0 mg/L	25 mN/m	Nopart et al. (2014)
Yeast derived biosurfactants				
Tsukamurella spec.	Oligossaccharide lipids	–	30 mN/m	Vollbrecht et al. (1999)
Starmerella bombicola MTCC 1910	Sophorolipids	–	36.2 mN/m	Vedaraman and Venkatesh (2010)
Pseudozyma churashimaensis sp. nov.	Mannosylerythritol lipids	1.7×10^{-6}M	29.2 mN/m	Morita et al. (2011)
Pseudozyma parantarctica JCM 11752	Mannosylribitol lipid	1.6×10^{-6} M	23.7 mN/m	Morita et al. (2012)
Pseudozyma parantarctica JCM 11752	Mannosylarabitol lipid	1.5×10^{-6} M	24.2 mN/m	Morita et al. (2012)
Candida sphaerica UCP0995	Glycolipid	0.25 g/L	25 mN/m	Luna et al. (2012)
Candida lipolytica UCP 0988	Lipopeptide	0.3 g/L	25 mN/m	Rufino et al. (2014)
Actinomycete derived biosurfactants				
Streptomyces amritsarensis sp. nov.	Lipopeptide	0.2 %	37 mN/m	Sharma et al. (2014)
Streptomyces species B3	Glycolipid	110 mg/L	29 mN/m	Khopade et al. (2012)
Fungal derived biosurfactants				
Pichia caribbica	Xylolipid	1 mg/L	35.9 mN/m	Joshi-Navare et al. (2014)

Effect of certain physic-chemical characteristics on CMC values

The CMC depends on several physical–chemical factors like surfactant structure, composition, temperature, ionic strength, and the presence and types of organic additives in the solutions. As suggested by Satpute et al. (2010), CMC values of nonionic micelles depend on lipophilic and hydrophilic groups, whereas for ionic micelles, length of lipophilic group and charge are crucial. Generally, the CMC decreases and the surface-active agent became more efficient as the hydrophobicity of the detergent increases. Other properties that directly affect the CMC are the characteristics of the hydrophobic and hydrophilic groups and solution additives such as electrolytes. To conclude, the ratio and composition of the homologues, the presence of unsaturated bonds, the branching and length of the alkylic chain, or the size of the hydrophilic head group of the surfactant can all affect the CMC values (Haba et al. 2003).

Effects of the hydrophilic group on CMC values

Variations in the hydrophilic head group affect the detergent CMC. Generally, the high hydrophilic character of the molecules in the mixture directly affects micellization, resulting in higher CMC values and therefore less efficient surface active agents. Also, the ionic charge of the hydrophilic group affects the micellization behavior. Detergents containing ionic head groups have a higher CMC than those containing nonionic head groups (Rosen 2004). This is due to electronic repulsion between the head groups of neighboring detergent monomers within the micelles. Detergents containing zwitterionic head groups tend to have smaller CMCs than those containing ionic head groups.

Effects of the hydrophobic group on CMC values

As described by Rosen (2004), the physical characteristics of the hydrophobic group can also have varying effects on the CMC of a particular detergent. In general, the CMC decreases as the number of carbon atoms in the alkyl chain increases up to approximately 16–18 carbons (for straight chain alkyls) (Rosen 2004). Above this point, detergents become lipid like and do not form discrete micelles. In general, carbon atoms on branched hydrophobic chains have about half the effect on the CMC as carbon atoms on straight chains. A carbon–carbon double bond increases the CMC compared to the corresponding saturated compound; compounds with *cis* double bonds have a higher CMC than compounds with *trans* double bonds. For microbial derived surface active compounds, assuming that the unsaturated rhamnolipid molecules are involved in micellization, the presence of unsaturations affects the conformation of the molecules in the micelles and therefore alters the CMC values. So, the CMC values shown by the unsaturated compounds are greater than those of the corresponding saturated. This could be attributed to the steric factor in micelle formation (Yakimov et al. 1996; Mata-Sandoval et al. 1999).

When waste frying oils were used as a carbon source, a mixture of eleven rhamnolipid homologues containing 18.95 % of unsaturated hydrophobic fatty acid were obtained and surface tension measurements yielded a CMC value of 108.8 mg/L larger than that of the mixture in which all the hydrophobic chains were saturated with a minimum of surface tension of 32.8 mN/m and an interfacial tension of about 1 mN/m against kerosene (Haba et al. 2003). A similar behavior is reported for a rhamnolipid mixture derived from *P. aeruginosa* AT10 with a 27 % of unsaturated components with a CMC value of 150 mg/L (Abalos et al. 2001). Moreover, Benincasa et al. (2010) reported the production rhamnolipid mixture contained 31 % of unsaturated fatty acids by *P. aeruginosa* LBI grown on soapstock having a CMC value of 120 mg/L and a surface tension of 24 mN/m and an interfacial tension of 1.31 mN/m. When rhamnolipid homologues with saturated fatty acid were produced as major components by *P. aeruginosa* UG2 on corn oil, CMC values were smaller of about 37 and 38 mg/L (Mata-Sandoval et al. 1999).

Other studies suggested that the presence of longer fatty acid chains increased the hydrophobicity of the molecules and decrease the critical micelle concentration (CMC). As follow, a mixture of rhamnolipid surfactants, obtained from corn oil rich in $Rh_2C_{10}C_{12}$ and $Rh_2C_{10}C_{12:1}$ species, had a CMC of 37 mg/L and a surface tension of 36 mN/m (Mata-Sandoval et al. 1999). However, species with a high content of $Rh_2C_{10}C_{10}$, had a CMC of 53 mg/L and a surface tension of 31 mN/m (Mata- Sandoval et al. 1999).

Effects of electrolytes and environmental factors on CMC values

Electrolytes tend to reduce the CMC of detergent solutions. Addition of electrolytes decreases the repulsion between similarly charged ionic head groups within a micelle and therefore, the detergent monomers can pack tightly and the CMC is reduced. Addition of salts to solutions containing nonionic detergents also reduces CMC values. The lower the CMC of a surfactant, the more efficient it is, and the more favorable the economics of employing it in a commercial process become. The CMCs of many biosurfactants were considerably lower in an electrolyte solution than in distilled water (Kretschmer et al. 1982). As suggested by Özdemir et al. (2004) the CMC values of rhamnolpids biosurfactants and the minimum surface tension at the CMC depends greatly on the pH of the solution. Marqués et al. (2009) proved the fluctuation of the *Rhodococcus erythropolis* 51T7 glycolipid CMC/critical aggregation concentration (CAC) as function of the pH value; 0.05 g/L at pH 7.4 and 0.034 g/L at pH 4. In a study conducted by Champion et al. (1995); rhamnolipid morphology using cryo-transmission electron microscopy was demonstrated to be a function of pH. As pH increased, it changed from lamellar, to vesicular, to miceller mophology. Also, cadmium seemed to stabilize rhamnolipid vesicle structures as shown by an increase in vesicle number and a decrease in vesicle diameter; in contrast, octadecane favored the micellar structure as shown by the complete absence of vesicles (Champion et al. 1995).

Hydrophilic and lipophilic balance (HLB)

It's an intrinsic property of biosurfactants represented by an arbitrary scale of 0–20, wherein the most hydrophilic materials have highest number. The HLB scale denotes the ability of surfactant to form emulsions of water-in-oil or oil-in-water by comparing with surfactants of known HLB values and properties. Generally, bio-emulsifiers are classified according to their hydrophile–lipophile balance (HLB); those having a low HLB are strong lipophiles and used as water-in-oil emulsifiers, whereas those having a high HLB are strong hydrophiles and used as oil-in-water emulsifiers. According to Christofi and Ivshina (2002), emulsifiers with low HLB values less than 3 are characterized as surface films, those having an HLB between 3 and 6 are lipophilic and favor stabilization of water-in-oil emulsification and between 7 and 9 are recognized as wetting agents. Whereas emulsifiers with HLB values between 8 and 15 favor oil in-water emulsification, for those having HLB between 13 and 15 they are detergents and those having HLB values between 15 and 18 can be recognized as solubilizing agents. Regarding literature

reviews and studies, the HLB of trehalose lipids produced by *Rhodococcus erythropolis* 51T7 was of about 11 (Marqués et al. 2009); of di-, tri-, and tetrasaccharide lipids biosurfactants produced by *Tsukamurella* spec. were between 8 and 10 (Vollbrecht et al. 1999).

Temperature, pH and ionic strength tolerance

Biosurfactants are reported stable at various temperature, pH and salinity (Salihu et al. 2009; Augustin and Tene Hippolyte 2012). A lipopeptide derived from *B. subtilis* LB5a is highly stable at 121 °C for 20 min and even after 6 months was found to be stable at −18 °C; the surface activity did not change from pH 5 to pH 11 and NaCl concentrations up to 20 % (Nitschke and Pastore 2006). A *Leucobacter komagatae* derived lipopeptide showed a strong stability of its emulsification property to heat treatment up to 100 °C (or its autoclaving at 121 °C) and salt addition (up to 16 % NaCl did not cause a significant effect on E24) (Saimmai et al. 2012a). Similarly, an *Oleomonas sagaranensis* and a *Candida sphaerica* derived glycolipids and an *Inquilinus limosus*; *Halobacteriaceae archaeon* and *B. subtilis* derived lipopeptides showed a strong thermal and pH stability with respect to surface tension reduction and emulsification activity with a promising activity at high level of salt concentration (Chooklin et al. 2014; Luna et al. 2012; Pathak and Keharia 2014; Saimmai et al. 2012b, 2013). As demonstrated by Khopade et al. (2012) and Nopart et al. (2014), *Streptomyces* and *Ochrobacterum anthropi* derived surfactant were effective at very low concentrations over a wide range of temperatures, pH and salt concentrations. Similarly, a *Rhodococcus* derived glycolipid and *P. fluorescens* derived rhamnolipid were stable during exposure to high salinity (10 % NaCl), elevated temperatures (120 °C for 15 min) and within a wide pH range (4.0–10.0) (Shavandi et al. 2011; Abouseoud et al. 2007). A trehalolipid produced by *Rhodococcus* sp. produced emulsions that were stable to a wide range of conditions; pH 2–10, temperatures of 20–100 °C and NaCl concentrations of 5–25 % w/v (White et al. 2013). The activity and stability of the glycolipid bioemulsifier produced by *Streptomyces* sp. SS 20 was effective over a wide range of temperature (30–100 °C), pH (3–7) and salt concentration of 3 % (w/v) with a greater emulsion stability in the presence of liquid mono-aromatic compounds enabling its use in the petroleum hydrocarbons industry, e.g. in enhanced oil recovery, and in bioremediation process (Hayder et al. 2014). With the oil spreading test, a proline lipid derived from the hydrocarbon-degrading bacterium *Alcanivorax dieselolei* B-5 showed reasonable stability towards pH, temperature and salinity (Qiao and Shao 2010).

Also, the antifungal activity of certain lipopeptides exhibited higher stability towards extreme temperature, a wide range of pH values and salinities (Joshi et al. 2008; Zhao et al. 2010). Moreover, previous study demonstrated that the insecticide activity of SPB1 lipopeptide biosurfactant towards *Ephestia kuehniella* could withstand environmental stresses such as extreme pH and temperature and sunlight/UV radiation (Ghribi et al. 2012).

These interesting properties offer the opportunities for the biosurfactants to be investigated in extreme environment for microbial enhanced oil recovery and in situ biodegradation of oil sludge (Saimmai et al. 2012a, b, 2013; Nopart et al. 2014; Joshi et al. 2008; Qazi et al. 2013). Also, they enable their use in industrial processes for food and pharmaceutics frequently involving exposure to extreme conditions of temperature, pressure, pH and ionic strength.

Low toxicity

Owing to its natural origins, biosurfactants are recognized as low or non-toxic. Munstermann et al. (1992) showed the reduced or equal toxicity of microbial derived surface active compounds (Trehalose dicorynomycolate and Trehalose tetraester from *R. erythropolis* and Rhamnolipids from *P. aeruginosa*) towards different synthetic surfactants. A biosurfactant from *P. aeruginosa* was considered non-toxic and non-mutagenic in comparison to a synthetic one "Marlon A-350" widely used in industry (Flasz et al. 1998). In a study conducted by Ivshina et al. (1998), a series of microbial derived surfactants (glycolipid of *R. ruber* IEGM 231; trehalose dicorynomycolate of *R. erythropolis*, trehalose tetra ester of *R. erythropolis* and rhamnolipids of *P. aeruginosa*) inhibited luminescence of 50 % of *Vibrio fisheri* in comparable or higher concentration than synthetic surfactants. Edwards et al. (2003) reported a comparison of acute and chronic toxicity of three synthetic surfactants and three microbiological derived surfactants (rhamnolipid, emulsan, biological cleanser PES-51) towards *Mysidopsis bahia* and *Mendidia beryllina.* They proved that PES-61 (synthetic surfactant) and Emulsan were the least toxic whereas Triton X-100 (synthetic) was the most toxic. As suggested by Dehghan-Noudeh et al. (2005), *B. subtilis* derived lipopeptide pose haemolytic activity to human erythrocyte lower than cationic surfactants (CTAB, TTAB, BC) and anionic SDS. As presented by Das and Mukherjee (2005), *P. aeruginosa* derived biosurfactant do not pose detrimental effect to heart, lung, liver and kidney and interfere in blood coagulation in normal clotting time. Hirata et al. (2009) proved a lower cytotoxicity of sophorolipid on human keratinocytes the same as surfactin. In a study conducted on keratinocytes and fibroblast, a *Rhodococcus* sp. 51T7

derived trehalose tetraester was demonstrated less irritating to skin the commercial than the commercial surfactant SDS and could be used in galenic or cosmetic applications (Marqués et al. 2009).

Also, as suggested by Kuyukina et al. (2007), the glycolipid complex synthesized by *Rhodococcus ruber* actinobacteria is not toxic. It caused no stimulation or inhibition of the experimental animal behavioral activity and no deaths and body weight loss were observed over 14-day (Kuyukina et al. 2007). Also, it exhibits no appreciable effect on proliferative activity of peripheral blood leukocytes (Kuyukina et al. 2007). Additionally, in a study conducted by Gein et al. (2011) glycolipid biosurfactant from *Rhodococcus ruber* displayed no cytotoxicity against human lymphocytes and therefore could be proposed as a potential immunomodulating and antitumor agent.

A biosurfactant produced by *Candida sphaerica* demonstrated no toxicity against seeds of *Brassica oleracea*, *Chicoria intybus* and *Solanum gilo* or the micro crustacean *Artemia salina* employed as a bioindicator (de Souza Sobrinho et al. 2013). Moreover, in another study conducted by Luna et al. (2013) and Rufino et al. (2014) a *Candida sphaerica* UCP 0995 glycolipid and *Candida lipolytica* UCP 0988 lipopeptide and derived biosurfactant showed no toxicity against different vegetable seeds: *Brassica oleracea*, *Solanum gilo* and *Lactuca sativa* L. and the micro-crustacean *Artemia salina*. Moreover, a glycolipid biosurfactant derived from *B. methylotrophicus* USTBa does not pose any inhibitory effect on seed germination and root elongation of vegetable seeds namely, *Triticum aestivum*, *Raphanus sativus*, *Vigna radiate*, and *Brassica napus* (Chandankere et al. 2014). Similarly, in a study conducted by Camacho-Chab et al. (2013), the bioemulsifier produced by by *Microbacterium* sp. MC3B-10 was not toxic to *Artemia salina* nauplii.

In acute toxicity study done on outbred male albino mice, Kuyukina et al. (2007) showed that the glycolipid produced by *R. ruber* IEGM 231 had no effects on central nervous system and did not exhibit any deaths, weight losses and changes in behavior. Similarly, Hwang et al. (2009) showed that *B. subtilis* surfactin C was not toxic to adult of Sparague-Dawley rats. Nevertheless, highest doses significantly decreased body weight with normal food and water consumption and histhopathological tests (liver, lungs, heart, spleen, adrenals, kidneys, thyroid glands, testes, ovaries) showed significant increase in liver weigh with 1 and 2 (g/kg) doses (zonal necrosis of hepathic vein) (Hwang et al. 2009). *In vivo* toxicity of a lipopeptide biosurfactant produced by *B. subtilis* SPB1 was conducted on male mice by determining the *LD50* values and investigating the effect of daily intra-peritoneal injection of determined doses of SPB1 lipopeptide biosurfactant on hematological and serum biochemical properties of mice (Sahnoun et al. 2014). We observed that SPB1 biosurfactant having an *LD50* value of 475 mg/kg had no significant adverse effect on hematological parameters and serum biochemical data for a daily intake of doses lower than 47.5 mg/kg of body weight (Sahnoun et al. 2014). In another study, acute toxicity tests involving two species of marine larvae, *Mysidopsis bahia* (shrimp) and *Menidia beryllina* (fish), demonstrated low toxicity of the biosurfactant JE1058BS produced by *Gordonia* sp. (Saeki et al. 2009). Thus, reduced toxicity makes biosurfactants more suitable for industrial and environmental applications such as bioremediation.

Biodegradability

Generally, owing their natural origin, microbial-derived surface active compounds are easily degraded unlike synthetic surfactants. Kim et al. (2002) reported an efficient biodegradation of Mannosylerythritol lipid biosurfactant (MEL) produced by *Candida antarctica* towards LAS and SDS. These encourage the use of microbial derived compounds as alternative of synthetic surfactants in bioremediation. Mohan et al. (2006) indicated that rhamnolipid are easy biodegradable under aerobic and anaerobic conditions whereas Triton X-100 is non-biodegradable under anaerobic conditions and partially biodegradable under aerobic conditions. Similarly, Hirata et al. (2009) showed that sophorolipid, surfactin and arthrofactin was easy biodegradable in comparison to synthetic surfactants which showed no biodegradability after 8 days. Pei et al. (2009), Bafghi and Fazaelipoor (2012) and Chrzanowski et al. (2012) discussed an efficient and good biodegradability of the rhamnolipid biosurfactants. Likewise, Lima et al. (2011) demonstrated the biodegradability of five biological surfactants (produced by two *Bacillus* sp., *Flavobacterium* sp., *Dietzia maris* and *Arthrobacter oxydans*) that is much higher than of synthetic SDS.

Efficiency towards chemical surfactants

Regarding literature reviews and studies, the efficiency of a microbial derived surfactant towards synthetic emulsifiers was described. At 0.05 mg/mL, surfactin showed a higher foaming capacity in comparison to Sodium dodecyl sulfate and Bovine serum albumin that form unstable foams during the foaming process and cannot produce the required foam volume (Razafindralambo et al. 1996). In some laboratory experiments, it was found that *P. aeruginosa* derived rhamnolipid biosurfactant and *R. ruber* derived biosurfactant are more efficient in residual oils or hydrocarbons removal than the synthetic surfactant, Tween 60 as suggested by Scheibenbogen et al. (1994) and Kuyukina et al. (2005) respectively. Otherwise, a monorhamnolipid was

demonstrated more effective in enhancing removal of residual hydrocarbon from soil than sodium dodecyl sulfate (SDS) and polyoxyethylene (20) sorbitan monooleate (Bai et al. 1997). When using monorhamnolipid, 22 % of residual hydrocarbon was removed in contract to 0 and 6.1 % when using Sodium dodecyl sulfate (SDS) and polyoxyethylene (20) sorbitan monooleate respectively (Bai et al. 1997). A monoacylglycerols glycolipid produced by *Candida ishiwadae* exhibited higher surfactant activities tested by the drop collapse test than several artificial surfactants such as sodium dodecyl sulphate, Triton and Tween (Thanomsub et al. 2004). In a similar way, *Candida antarctica* derived biosurfactants were demonstrated more effective in improving the biodegradation of crude oil than chemical surfactants (Hua et al. 2004). Tuleva et al. (2008) and Thavasi et al. (2011) suggested the effectiveness emulsification activity of a *R. wratislaviensis* derived trehalose tetraester and *P. aeruginosa* derived lipopeptide towards Triton X-100 and Triton X-100 and Tween 20 and Tween 80 respectively. Chandran (2010) reported the effectiveness of surface activity evaluated by means of the drop collapse test and oil displacement tests towards chemical surfactants. Pereira et al. (2013) showed that a *B. subtilis* lipopeptide surfactin have better interfacial-activity and oil recovery efficiency than common chemical surfactants, thus being more attractive to be applied in Microbial Enhanced Oil Recovery. A *B. methylotrophicus* USTBa derived glycolipid was demonstrated more effective than SDS in hydrocarbon emulsification (Chandankere et al. 2014). Similarly, others studies indicated the superior performance of the biosurfactant over synthetic surfactants SDS in terms of mobilization of oil pollutants from the contaminated soil (Andreu et al. 1995; Siegmund and Wagner 1991; Janek et al. 2010). Nevertheless, *B. subtilis* derived lipopeptide were shown slightly better than Tween 20, Tween 40, Tween 60 and Triton X 100 except for the SDS (Pathak and Keharia 2014). Also, Morita et al. (2010) demonstrated the effectiveness of the mannosylerythriol lipids as moisturizing agent to repair the damaged hair towards ceramide and lauryl glucoside. Regarding the oil displacement test, the minimum active dose of a proline lipid was largely below those of chemical surfactants suggesting a best efficiency (Qiao and Shao 2010).

Vaz et al. (2012) suggested the efficiency of the biosurfactant recovered from *B. subtilis* EG1 towards commercial chemical surfactants showing a similar thermal stability and equal or superior capacity to form emulsions with *n*-hexadecane enabling its potential use in several industries. Moreover, it exhibited an interesting anti-adhesive activity against *Staphylococcus aureus* and *Escherichia coli* when no particular trend or special effect could be assigned to the use of commercial chemical surfactants as anti-adhesives (Vaz et al. 2012).

Generally, biosurfactants are characterized by smaller critical micelle concentration (CMC) than the synthetic surfactants making them better and more efficient. For example, CMC values of rhamnolipid biosurfactant and Rokanol NL6 were of about 0.07 and 0.12 g/L (Medrzycka et al. 2009). Therefore, rhamnolipid was more efficient in washing out oil from the ground with a maximal oil removal for biosurfactant solution about 22 %, while for synthetic surfactant 14 % (Medrzycka et al. 2009). Also, it was characterized by a little smaller solubilisation efficiency towards the synthetic surfactant Rokanol NL6 (Pastewski et al. 2008). At a like manner, a crude biosurfactant produced by *P. aeruginosa* SP4 showed better surface activity than two synthetic surfacatant SDS and Pluronic F-68 with a superior properties in both heat and pH stabilities (Pornsunthorntawee et al. 2008). In fact, it reduced the surface tension of pure water to 29.0 mN/m with a CMC value of approximately 200 mg/L in contrast to 42.8 and 28.6 mN/m, corresponding to the CMC values of approximately 350 and 1280 mg/L, for Pluronic F-68 and SDS respectively (Pornsunthorntawee et al. 2008). Although, recent studies showed that *P. aeruginosa* derived rhamnolipid exhibited better surface activity and frothability when compared with conventional flotation frothers, methyl isobutyl carbinol, pine oil, Dowfroth-250 and Aerofroth-65 (Khoshdast et al. 2012).

The efficiency of microbial derived surface active compounds towards chemical synthetized surfactants and their robust characteristics are very beneficial for applications under extreme conditions of temperature and pH, such as in oil recovery and in the bioremediation of a polluted environment. Moreover, they have great potential for biomedical applications due to its robust heat tolerance even after being submitted to autoclave sterilization.

Functional properties and related application

In addition to the surface activity potential, biosurfactants possessed others functional properties like emulsification, wetting capacities and foaming. Also, they can act as enhancers of hydrocarbons solubility and mobility. Owing an anionic character, biosurfactants can complex metals. Also, biosurfactant are well known by their membrane permeabilization properties as they can induce pore formation in lipid bilayer membrane. This permits their use as antimicrobial, hemolytic and insecticide agents.

Emulsification and foaming properties

Emulsification corresponds to a dispersion of one liquid into another (as microscopic droplets) leading to the mixing of two immiscible liquids. This property is especially useful for making oil/water emulsions for environment,

cosmetics and food. The crude rhamnolipid biosurfactant formed stable water-in-oil microemulsions with crude oil and various types of vegetable oils, but not with short-chain hydrocarbons (Pornsunthorntawee et al. 2008). Aromatic and aliphatic hydrocarbons and several plant oils were good substrates for the bioemulsifiers produced by *B. subtilis* K1, *Inquilinus limosus* and *Halobacteriaceae archaeon* as suggested by Pathak and Keharia (2014), Saimmai et al. (2013) and Chooklin et al. (2014) respectively. In contrast, Saimmai et al. (2012a, b) reported the effectiveness of a lipopeptide bioemulsifier to emulsify hydrocarbons towards vegetable oils (soybean oil, palm oil and olive oil). These properties enabled biosurfactant use in many domains like emulsifiers for environment, food industry, cosmetics and pharmaceutics.

Moreover, biosurfactants are characterized by interesting foaming activities (Abouseoud et al. 2007; Hirata et al. 2009; Morita et al. 2010) permitting their use as cleaning agents and detergents as well as in oil industry, laundry detergent formulation and cosmetic.

Solubilization and mobilization properties; role to enhance hydrocarbon biodegradation

Owing to their surface activity, biosurfactants can act as enhancers of hydrocarbon solubility and mobility from contaminated soil increasing therefore their solubility and availability for hydrocarbon degrading bacteria. Abouseoud et al. (2010) reported the efficiency of a rhamnolipid biosurfactant in increasing the solubility of naphthalene, permitting its potential use in bioremediation of polycyclic aromatic hydrocarbons (PAH) contamination in extreme environments. Previous studies reported the potential application of biosurfactant in stimulating indigenous microorganisms for enhanced bioremediation of hydrocarbon-contaminated soil and water (Anyanwu et al. 2011; Saravanan and Vijayakumar 2012; Whang et al. 2008; Zou et al. 2014; Saimmai et al. 2012a, 2013; Nopart et al. 2014). In this aim, Whang et al. (2008) demonstrated the usefulness of rhamnolipid and surfactin type biosurfactant in increasing diesel dissolution permitting therefore their use for enhanced biodegradation of diesel-contaminated water and soil. In fact, with the addition of 40 mg/L surfactin, diesel biodegradation efficiency increases from 40 to 94 %; for rhamnolipid, 80 mg/L permitted en enhancement of diesel biodegradation from 40 to 100 % (Whang et al. 2008). Moreover, lipopeptide from *Serratia marcescens* NSK-1, *Bacillus siamensis* and *Acinetobacter baylyi* ZJ2 were reported highly efficient in enhancing crude oil degradation as described by Anyanwu et al. (2011), Saravanan and Vijayakumar (2012) and Zou et al. (2014) respectively. Saimmai et al. (2012a, 2013) and Nopart et al. (2014) discussed the effectiveness of a glycolipid and lipopeptide type bioemulsifiers in enhancing PAHs solubility and lubricating oil removal from contaminated sand and its biodegradation.

Metal sequestering property; role in bioremediation

Also, the anionic charge of certain microbial surfactants offers them the opportunity to complex certain heavy metals extremely toxic to the environment. Therefore, several studies reported the use of biosurfactants in bioremediation of metal-contaminated soils. Nopart et al. (2014) and Chooklin et al. (2014) studied the effectiveness of a glycolipid and lipopeptide surface active compounds in cadmium and lead removal from aqueous solution with a better efficiency at concentration below the CMC.

Membrane permeabilization properties; biosurfactants as antimicrobial, hemolytic and insecticide agents for biomedicine and agriculture

Furthermore, biosurfactant are well known by their membrane permeabilization properties as they can induce pore and ion channels formation in lipid bilayer membrane. Therefore, they have the ability to destabilize membranes disturbing their integrity and permeability and can act as antimicrobials, hemolytic agent and insecticide compounds.

In this aim, biosurfactants are well recognized by their antimicrobial activity against a wide array of bacteria (Saimmai et al. 2012a, b, 2013; Nopart et al. 2014) and fungi (Tomar et al. 2013; Joshi et al. 2008; Zhao et al. 2010). For example, *Candida bombicola* derived sophorolipids inhibited bacterial growth of both gram negative and gram positive bacteria with MIC of about 30 and 1 μg/mL at a contact time of 2 and 4 h respectively for *E. coli* (ATCC 8739); *P. aeruginosa* (ATCC 9027) and 6 and 1 μg/mL for *S. aureus* (ATCC 6358), *B. subtilis* (ATCC 6633) respectively at a contact time of 4 h (Joshi-Navare and Prabhune 2013). Glycolipid biosurfactant from marine *Brevibacterium casei*; a potent antibacterial agent disrupted the biofilm formation against mixed pathogenic biofilm bacteria (Kiran et al. 2010). The mycelia growth of *Aspergillus flavus* and *Colletotrichum gloeosporioides* was considerably reduced with increasing concentration of surfactin, and 36, 54, 84, and 100 % inhibitions of mycelia growth were, respectively, observed at 20, 40, 80, and 160 mg/L after 7 days of incubation (Mohammadipour et al. 2009). These properties enable the use of biosurfactant in biomedicine to combat pathogenic bacteria invasion and as biocontrol agent to combat pest crop and phytopathogenic fungi invasion. In this aim, Mimee et al. (2009) reported the antibacterial activity of flocculosin, a

cellobiose lipid produced by the yeast-like fungus *Pseudozyma flocculosa*, against clinical bacterial isolates and the pathogenic yeast *Candida albicans*. An *Ustilago maydis* derived cellobiose lipids had in vivo phytopathogenic fungi inhibition as infection of tomato leaves by the plant pathogenic fungus *Botrytis cinerea* was prevented by co-inoculation the biosurfactant producing fungi sporidia (Teichmann et al. 2007). Also, Arutchelvi and Doble (2010) reported the possible use of a rhamnolipid biosurfactant as a biocontrol agent against phytopathogens (*Fusarium proliferatum* and *Aspergillus niger*) and exploited for biomedical applications.

Others studies reported the ability of microbial surface active compounds to disorganize larvae cell integrity causing their death offering therefore a wide array of application in agriculture field to protect crop from pest invasion. Rhamnolipid biosurfactant were also reported as effective insecticidal agents towards various larvae; the Green Peach Aphid *Myzus persicae* (Kim et al. 2011) and *Rhyzopertha dominica* larvae (Ahmed et al. 2012). Recently, a lipopeptide biosurfactant derived from *B. subtilis* SPB1 was demonstrated as an efficient biological control agent against the Egyptian cotton leaf worm (*Spodoptera littoralis*), the olive moth *Prays oleae* and the third instar larvae *Ephestia kuehniella* (Lepidoptera: Pyralidae) (Ghribi et al. 2012).

The ability of erythrocytes lysis permits the use of biosurfactants as potent inhibitors of fibrin clot formation. Vallet-Gely et al. (2010) reported the hemolytic activity of entolysin, a new cyclic lipopeptide produced by *P. entomophila.* Also, syringopeptin syringomycin from *P. syringae* pv. *syringae* were reported to exhibit a membrane-permeabilizing activities in human red blood cells and in bilayer lipid membranes (Agner et al. 2000).

Moreover, microbial surfactants-amphiphilic compounds can interact with interfaces and inhibit the adhesion of microorganisms to different surfaces and are therefore as anti-adhesive agents for food and bio pharmaceutics industry. Pseudofactin produced by *P. fluorescens* BD5 lowered the adhesion of bacterial strains of five species and and two *Candida albicans* strains; pathogenic microorganisms which are potential biofilm formers on catheters, implants and internal prostheses; to three types of surfaces (glass, polystyrene and silicone) (Janek et al. 2012). *Candida sphaerica* derived Lunasan inhibited the adhesion between 80 and 92 % of *P. aeruginosa*, *Streptococcus agalactiae* and *Streptococcus sanguis* at a concentration of 10 mg/mL (Luna et al. 2011).

Conclusion

Biosurfactants are generally produced by a wide variety of microbes, bacteria, fungi and yeast. They are secreted extracellulary or attached to parts of cells, predominantly during growth on water immiscible substrates. Many literature reviews reported the physiological roles of microbial surface-active compounds. Owing to their great physico-chemical properties and surface activity, low toxicity and biodegradability, great tolerance to extreme conditions of pH, temperature and salinity and efficiency to synthetic emulsifiers; biosurfactants offers great potential of large scale application in many fields. They are interesting candidates in environment for bioremediation as enhancer of hydrocarbon solubility, mobility and biodegradation and as metal sequestering compounds. In biomedicine and agriculture, they are efficient antimicrobials agents to against pathogenic fungi and bacteria. Also, the offers great challenges for industrial application as emulsifying, foaming, moisturizing and stabilizing agents. For this, several screening methods based on surface and interfacial tension reduction, cell surface hydrophobicity and other specific method were developed permitting the exploration of a wide array of microbial producing strain.

Conflict of interest The authors report no declaration of interest.

Ethical standard All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent Informed consent was obtained from all individual participants included in the study.

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