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# Biosurfactants and Bioemulsifiers Biomedical and Related Applications – Present Status and Future Potentials

Letizia Fracchia<sup>1</sup>, Massimo Cavallo<sup>1</sup>,

Maria Giovanna Martinotti<sup>1</sup> and Ibrahim M. Banat<sup>2</sup>

<sup>1</sup>*Department of Chemical, Food, Pharmaceutical and Pharmacological Sciences,*

*Drug and Food Biotechnology Center,*

*Università del Piemonte Orientale "Amedeo Avogadro", Novara,*

<sup>2</sup>*School of Biomedical Sciences, Faculty of Life and Health Sciences,*

*University of Ulster, Coleraine, N. Ireland,*

<sup>1</sup>*Italy*

<sup>2</sup>*UK*

## 1. Introduction

Many microorganisms are able to produce a wide range of amphipathic compounds, with both hydrophilic and hydrophobic moieties present within the same molecule which allow them to exhibit surface activities at interfaces and are generally called biosurfactants or bioemulsifiers. These surface-active compounds (SAC) are mainly classified according to their mode of action, molecular weight and general physico-chemical properties.

In literature, the terms 'biosurfactants' and 'bioemulsifiers' are often used interchangeably, however in general those that reduce surface and interfacial tension at gas-liquid-solid interfaces are called biosurfactants and those that mainly reduce the interfacial tension between immiscible liquids or at the solid-liquid interfaces leading to the formation of more stable emulsions are called bioemulsifiers or bioemulsans. The former group includes low-molecular-weight compounds, such as lipopeptides, glycolipids, proteins, while the latter includes high-molecular-weight polymers of polysaccharides, lipopolysaccharides proteins or lipoproteins (Smyth et al., 2010a, 2010c).

In heterogeneous systems, biosurfactants tend to aggregate at the phase boundaries or interfaces. They form a molecular interfacial film that affects the properties (surface energy and wettability) of the original surface. This molecular layer, in addition to lowering the surface tension in liquids, also lowers the interfacial tension between different liquid phases on the interfacial boundary existing between immiscible phases and therefore can have an impact on the interfacial rheological behaviour and mass transfer.

When at interfaces (solid- liquid, liquid-liquid or vapour-liquid), the hydrophobic moiety of the surface active molecules aggregates at the surface facing the hydrophobic phase (usually the oil phase) while the hydrophilic moiety is oriented towards the solution or hydrophilic phase (mainly water). Their diverse functional properties namely, emulsification, wetting,

foaming, cleansing, phase separation, surface activity and reduction in viscosity of heavy liquids such as crude oil, make them suitable for utilization for many industrial and domestic application purposes (Gautam & Tiagi, 2006; Franzetti et al., 2010a; Perfumo et al., 2010a; Satpute et al., 2010b).

During the past two decades biosurfactants have been under continuous investigation as a potential replacement for synthetic surfactants and are expected to have several industrial and environmental applications mainly related to detergency, emulsification, dispersion and solubilisation of hydrophobic compounds (Banat et al., 2000). In addition, biosurfactants present several advantages over surfactants of a chemical origin, particularly in relation to their biodegradability, environmental compatibility, low toxicity, high selectivity and specific activity at extreme temperatures, pH and salinity (Banat 1995a, 1995b). Due to all these properties, they have steadily gained increased significance in industrial and environmental applications such as bioremediation, soil washing, enhanced oil recovery and other general oil processing and related industries (Perfumo et al., 2010b). Furthermore, potential commercial applications in several other industries including paint, cosmetics, textile, detergent, agrochemical, food and pharmaceutical industries begin to emerge (Banat et al., 2000).

Numerous investigations in the field of biosurfactants/bioemulsifiers are leading to the discovery and description of many interesting chemical and biological properties and potential biomedical therapeutic and prophylactic applications. In this chapter we will focus on the most recent and appealing biomedical and therapeutic applications of biosurfactants and bioemulsifiers with special emphasis on the most recent results in the fields of biotechnology, nanotechnology and bioengineering.

## **2. Classification, properties and functional mechanisms of microbial surface-active compounds**

Microbial surface-active compounds are a range of structurally diverse molecules produced by different microorganisms and are mostly therefore classified by their structural features, the producing organism and their molecular mass. Their hydrophilic moiety is mainly comprised of an acid, peptide cations, or anions, mono-, di- or polysaccharides while their hydrophobic moiety can be an unsaturated or saturated hydrocarbon chains or fatty acids. The structural orientation on the surfaces and inter phases confers the range of properties, such as the ability to lower surface and interfacial tension of liquids and the formation of micelles and microemulsions between these different phases (Chen et al., 2010a, 2010b).

### **2.1 Low molecular weight compounds**

#### **2.1.1 Lipopeptides**

Microbial surface-active compounds can be roughly divided into low molecular weight molecules that efficiently reduce surface and interfacial tension (biosurfactants) (Fig. 1.), and high molecular weight polymers that stabilize emulsions but do not lower the surface tension as much (bioemulsans or bioemulsifiers) (Fig. 2.) (Neu, 1996; Rosenberg, 2006; Rosenberg & Ron, 1997; Smyth et al., 2010a, 2010c).

The most studied low-molecular-weight biosurfactant compounds are lipopeptides and glycolipids. Lipopeptides are mainly produced by members of the *Bacillus* species; they are composed of different families and each family is constituted of several variants, which can differ in their fatty acid chain and their peptide moiety (Dastgheib et al., 2008; Jacques, 2010; Thavasi et al., 2008, 2011).

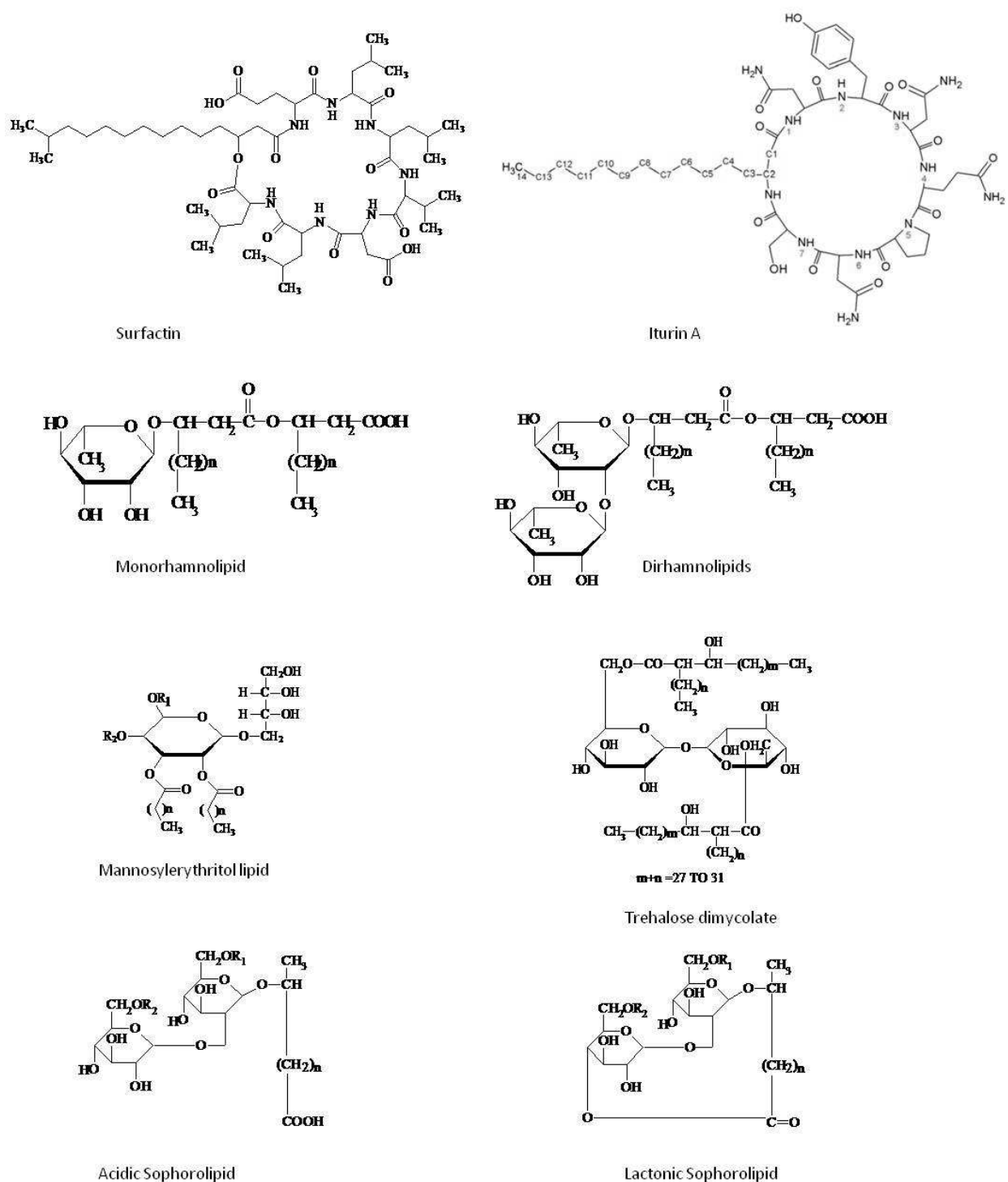


Fig. 1. Chemical structures of the main low molecular weight microbial surface active compounds reported; surfactin, iturin A, mono & di-rhamnolipids, mannosylerythritol lipids, dimycolates trehalose lipids, acidic and lactonic sophorolipids.

Surfactin, a cyclic lipopeptide produced by *Bacillus subtilis* is considered the most active biosurfactant discovered so far (Ron & Rosenberg, 2001). Surfactin was discovered by

Arima et al., (1968) from the culture broth of *Bacillus subtilis* and it was named thus due to its exceptional surfactant activity (Peypoux et al., 1999). Natural surfactins are a mixture of isoforms A, B, C and D which are classified according to the differences in their amino acid sequences and possess various physiological properties (Shaligram & Singhal, 2010). Surfactin is composed of a seven amino-acid ring structure coupled to a fatty-acid chain via a lactone linkage. Surfactin-A has L-leucine, surfactin-B has L-valine and surfactin-C has L-isoleucine at the amino acid position involved in the lactone ring formation with the C14–C15  $\beta$ -hydroxy fatty acid. The amino-acid residues may vary and the presence of these variants can be related to alterations in the culture conditions such as providing substrate containing some specific amino-acid residues in the culture media (Jacques, 2010).

Another surfactin related compound is lichenysin, a lipopeptide discovered in the supernatant of *Bacillus licheniformis* culture (Horowitz et al., 1990). Its chemical structure and physio-chemical properties are similar to surfactin (McInerney et al., 1990). In particular, lichenysin has Glutamine amino-acid in position 1 while surfactin has Glutamic acid. Other surfactin-like compounds are pumilacidin A, B, C, D, E, F and G, a complex of acylpeptide antibiotics isolated from *Bacillus pumilus* culture supernatants with interesting antiviral properties (Morikawa et al., 1992; Naruse et al., 1990). Among the lipopeptides belonging to the iturin family, iturin A is the most studied compound. It is a heptapeptide interlinked with a  $\beta$ -amino-acid fatty acid with carbon chain length from C14 to C17 (Peypoux, 1978, as cited in Jacques, 2010) produced by *Bacillus subtilis* strains reported to have antifungal activities (Besson et al., 1976).

Other members of the iturin family are iturin C, bacillomycin D, F, and Lc and mycosubtilin (Bonmatin et al., 2003). The family of fengycins includes fengycins A and B, lipodecapeptides which differ by their amino-acid residue in position 6 that can be Alanine or Valine and are known for their interesting fungitoxic and immunomodulating activities (Jacques, 2010). Other interesting lipopeptides are serrawettins, nonionic cyclodepsipeptide biosurfactants produced by *Serratia marcescens* (Matsuyama et al., 2010) and implicated with anti-tumor and anti-nematode activities.

### 2.1.2 Glycolipids

Are commonly mono or disaccharides compounds acylated with long chain fatty acids or hydroxyl fatty acids. Among them, rhamnolipids, mannosylerythritol lipids (MELs), sophorolipids and trehalolipids are the best-studied structural subclasses.

Rhamnolipids are glycosides, produced mainly by *Pseudomonas aeruginosa* and by the *Burkholderia* genus, that are composed of one (for mono-rhamnolipids) or two (for di-rhamnolipids) rhamnose sugar moieties linked to one or two  $\beta$ -hydroxyfatty acid chains (Perfumo et al., 2006; Raza, 2009). These molecules display high surface activities and many potential applications in the biomedical field due to their antibacterial, antifungal, antiviral, antiadhesive reported properties (Abalos et al., 2001; Cosson et al., 2002; Kim et al., 2000; Remichkova et al., 2008; Sotirova et al., 2008; Yoo et al., 2005). They have also been used in the preparation of nanoparticles (Palanisamy & Raichur, 2009; Xie et al., 2006) and microemulsions (Nguyen & Sabatini, 2009; Xie et al., 2007).

The mannosylerythritol (MELs) glycolipids are produced by the yeasts strains of the genus *Pseudozyma* sp. and *Ustilago* sp. from soybean oil or *n*-alkane (Arutchelvi & Doble, 2010). MELs are a mixture of partially acylated derivative of 4-*O*- $\beta$ -D-mannopyranosyl-D-erythritol,

containing C<sub>2:0</sub>, C<sub>12:0</sub>, C<sub>14:0</sub>, C<sub>14:1</sub>, C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>18:0</sub> and C<sub>18:1</sub> fatty acids as the hydrophobic groups (Bhattacharjee et al., 1970, as cited in Arutchelvi & Doble, 2010). Based on the degree of acetylation at C4 and C6 position, and their order of appearance on the thin layer chromatography, the MELs are classified into MEL-A, -B, -C and -D (Arutchelvi & Doble, 2010). MEL-A representing the diacetylated compound while MEL-B and MEL-C are monoacetylated at C6 and C4, respectively. The completely de-acetylated structure is known as MEL-D (Rau et al., 2005, as cited in Arutchelvi & Doble, 2010).

MELs have recently gained attention due to their environmental compatibility, mild production conditions, structural diversity, self-assembling properties and versatile biochemical functions. In particular, interesting applications have been described in the biomedical field as antimicrobial, antitumor and immunomodulating molecules, in the biotechnological field for gene and drug delivery, and in cosmetic applications as skin moisturizers (Arutchelvi & Doble, 2010).

Sophorolipids are another extracellular glycolipids synthesized by some yeast species including *Candida bombicola*, *Candida apicola*, *Rhodotorula bogoriensis*, *Wickerhaminella domercqiae* and *Candida batistae* (Van Bogaert & Soetaert, 2010). They consist of two glucose units linked  $\beta$ -1,2. The 6- and 6'-hydroxyl groups are generally acetylated. The lipid portion is connected to the reducing end through a glycosidic linkage. The terminal carboxyl group of the fatty acid can be in the lactonic form or hydrolyzed to generate an anionic surfactant (Rosenberg & Ron, 1999). Sophorolipids have been reported suitable for a number of application in the biomedical field including use as antimicrobial, antiviral and anticancer. They also have been used in the synthesis of metal-bound nanoparticles in cosmetic and pharmacodermatological products (Van Bogaert & Soetaert, 2010).

Trehalose lipids are also a glycolipids containing trehalose as the sugar moiety which is a non-reducing disaccharide in which the two glucose units are linked in an  $\alpha$ , $\alpha$ -1,1-glycosidic linkage. It is the basic component of the cell wall glycolipids in *Mycobacteria* and *Corynebacteria* (Franzetti et al., 2010b). The most reported trehalose lipid is trehalose 6,6'-dimycolate, which is a  $\alpha$ -branched chain mycolic acid esterified to the C6 position of each glucose. Different trehalose containing glycolipids are known to be produced by several other microorganisms belonging to mycolates group, such as *Arthrobacter*, *Nocardia*, *Rhodococcus* and *Gordonia*. *Rhodococcus* genus in particular produced several types of trehalose lipids as reported by Lang & Philp (1998). These glycolipids vary in the number and overall chain length (C<sub>20</sub>-C<sub>90</sub>) of the esterified fatty acids. Beside their known industrial applications, trehalose lipids recently attracted attention to their functions in cell membrane interaction and their potential as antitumor therapeutic agents (Aranda et al., 2007, Harland et al., 2009, Imasato et al., 1990, Isoda et al., 1995, as cited in Shao, 2010; Ortiz et al., 2008, 2009; Zaragoza et al., 2009, 2010).

## 2.2 High molecular weight biosurfactants

These are generally grouped together as polymeric biosurfactants. They are produced by a number of different bacteria and are composed of lipoproteins, proteins, polysaccharides, lipopolysaccharides or complexes containing several of these structural types (Ron & Rosenberg, 2001; Rosenberg & Ron, 1997, 1999). The most commonly studied biopolymer is emulsan (Fig. 2.), a lipopolysaccharide isolated from *Acinetobacter calcoaceticus* RAG-1 ATCC 31012 with a molecular weight of around 1,000 kDa (Rosenberg et al., 1979).



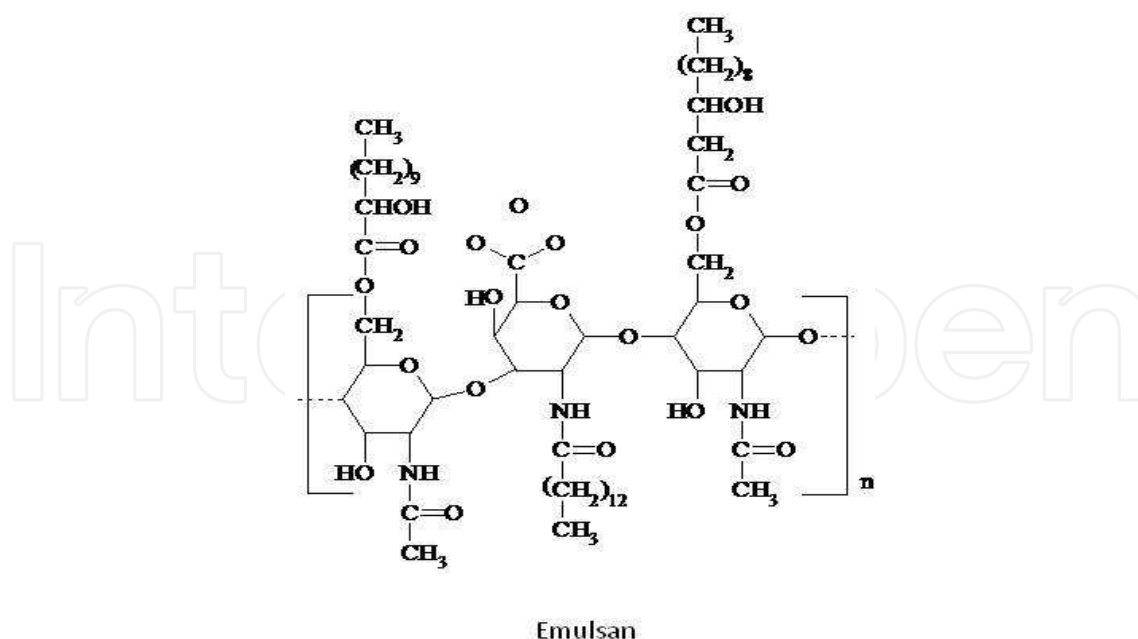


Fig. 2. Chemical structure of most known high molecular weight microbial surface active compound; emulsan.

RAG-1 emulsan is a complex of an anionic heteropolysaccharide and protein (Rosenberg & Kaplan, 1987, as cited in Rosenberg & Ron, 1999). Its surface activity is due to the presence of fatty acids, comprising 15% of the emulsan dry weight, which are attached to the polysaccharide backbone via O-ester and N-acyl linkages (Belsky et al., 1979, as cited in Rosenberg & Ron, 1999).

Another high molecular weight biosurfactant is alasan, a complex of an anionic polysaccharide and a protein with a molecular weight of around 1,000 kDa isolated from *Acinetobacter radioresistens* (Navon-Venezia et al., 1995, as cited in Smyth et al., 2010c). These high molecular weight biosurfactants generally possess effective emulsifying activity and are called bioemulsifiers. A large number of other polymeric compounds have been discovered but remain partially or totally uncharacterized (Smyth et al., 2010c). Little is known in general about these bioemulsifiers other than the producing organism and the overall chemical composition of the crude mixture. *Halomonas eurihalina* produces an extracellular sulfated heteropolysaccharide (Calvo et al., 1998, as cited in Rosenberg & Ron, 1999). *Pseudomonas tralucida* produced an extracellular acetylated polysaccharide that was effective in emulsifying several insecticides (Appaiah & Karanth 1991, as cited in Rosenberg & Ron, 1999).

Several bioemulsifiers are effective at high temperature, including the protein complex from *Methanobacterium thermoautotrophium* (De Acevedo et al., 1996, as cited in Rosenberg & Ron, 1999) and the protein-polysaccharide-lipid complex of *Bacillus stearothermophilus* ATCC 12980 (Gunjar et al., 1995, as cited in Rosenberg & Ron, 1999). Yeasts produce a number of emulsifiers, which are particularly interesting because of the food-grade status of several yeasts which allows use in food related industries. Liposan is an extracellular emulsifier produced by *Candida lipolytica* (Cirigliano & Carman, 1985, as cited in Rosenberg & Ron, 1999). It is composed of 83% carbohydrate and 17% protein. Mannanprotein emulsifiers are produced by *Saccharomyces cerevisiae* (Cameron et al., 1988, as cited in Rosenberg & Ron, 1999). Many of these bioemulsifiers have been used in the food, cosmetic, and petroleum industries (Rosenberg & Ron, 1999).

### 2.3 Properties and functions of biosurfactants

There is a growing interest in the study of the physicochemical and biological properties of biosurfactants because of their potential industrial applications (Cameotra & Makkar, 2004; Desai & Banat, 1997; Lang, 2002; Rodrigues et al., 2006a; Singh & Cameotra, 2004). The interesting biological activities displayed by these compounds constitute an added value to their potential uses (Lang et al., 1989; Lang & Wagner, 1993; Stanghellini & Miller, 1997, as cited in Sánchez et al., 2010). Due to these reasons, an intense research activity is currently directed toward identification of new biosurfactants and characterization of their chemical and biological properties (Biria et al., 2010; Morita et al., 2009a; Satpute et al., 2010a; Singh & Cameotra, 2004; Singh et al., 2007).

The most obvious property of biosurfactants compounds is their ability to effectively lower water surface tension, and a number of approaches that measure directly the surface activity of biosurfactants can be used as screening methods for their detection. Among them, the most frequently used as quick and simple techniques are the drop collapse (Bodour and Miller-Maier, 1998) and the oil spreading tests (Morikawa et al., 2000) (Fig. 3).

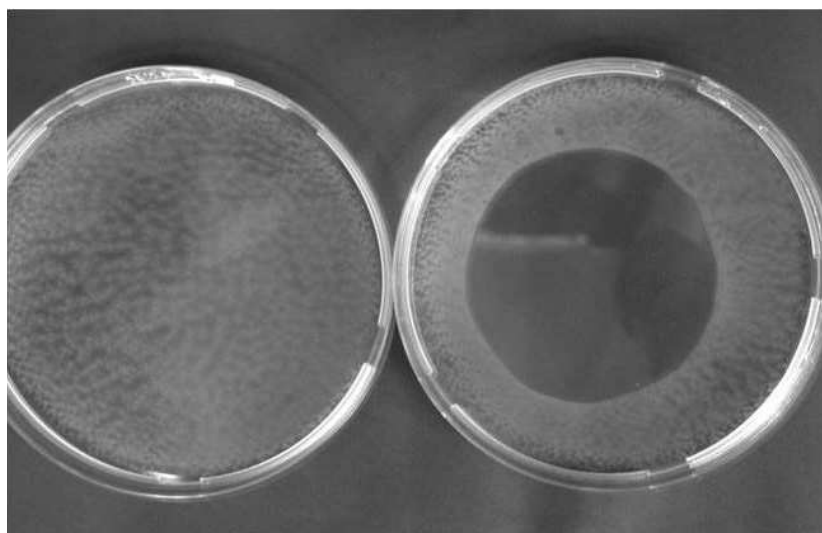


Fig. 3. Oil spreading test. This technique measures the diameter of clear zones caused when a drop of a biosurfactant-containing solution is placed on an oil-water surface (Morikawa et al., 2000). Photo courtesy by Fabrizio Rivardo.

An efficient biosurfactant can reduce the surface tension between pure water and air from 72mN/m to less than 30mN/m. Surfactin, as one of the most powerful biosurfactants, can reduce the surface tension of water from 72mN/m to 27mN/m (which is close to the minimum detectable value) at a concentration as low as 10  $\mu$ M (Seydlová & Svobodová, 2008). Rhamnolipids can similarly achieve such level of reduction (Hisatsuka et al., 1971, as cited in Muthusamy et al., 2008; Mohammad Abdel-Mawgoud et al., 2010). The sophorolipids from *T. bombicola* have been reported to reduce the surface tension to 33mN/m (Muthusamy et al., 2008) while MELs and trehalose lipids to less than 30mN/m (Arutchelvi & Doble, 2010; Shao et al., 2010).

As surfactant monomers are added into solution, the surface or interfacial tension will decrease until the biosurfactant reaches the critical micelle concentration (CMC). The CMC is defined as the minimum concentration necessary to initiate micelle formation (Becher, 1965). Above the CMC no further reduction in surface or interfacial tension is observed. At



the CMC, surfactant monomers begin to spontaneously associate into structured aggregates such as micelles, vesicles or continuous bilayers. These aggregates are produced as a result of numerous weak chemical interactions such as hydrophobic, van der Waals and hydrogen bonding (Maier, 2003; Raza et al., 2010). Since no chemical bonds are formed, these structures are fluid-like and are easily transformed from one state to another as conditions such as electrolyte concentration and temperature are changed (Lin, 1996).

The aggregate structure depends on the polarity of the solvent in which the surfactant is dissolved. In an aqueous solution, the polar head groups of a micelle will be oriented outward toward the aqueous phase, and the hydrophobic tails will associate in the core of the micelle within an oil-in-water micelle. In contrast, in oil, the polar head groups will associate in the center of the micelle while the hydrophobic tails will be oriented toward the outside within the water-in-oil micelle (Soberón-Chávez & Maier, 2010). Efficient surfactants have a low CMC, i.e. less surfactant is necessary to decrease the surface tension (Seydlová & Svobodová, 2008).

Biosurfactants are most effective and efficient at their CMC which can be 10–40 times lower than that of chemical surfactants, i.e. less surfactant is necessary to get a maximum decrease in surface tension (Desai & Banat, 1997). Another important property for industrial and biotechnological applications is that most biosurfactants surface activities are not affected by environmental conditions such as temperature and pH (Muthusamy et al., 2008) particularly those of glycolipids composition. It has been reported that lichenysin from *B. licheniformis* JF-2 was not affected by temperature (up to 50 °C), pH (4.5–9.0) and NaCl and Ca concentrations up to 50 and 25 g/l respectively. A lipopeptide from *B. subtilis* LB5a was also stable after autoclaving (121°C/20 min) and after 6 months at -18°C; the surface activity did not change from pH 5 to 11 and NaCl concentrations up to 20% (Muthusamy et al., 2008).

Moreover, unlike synthetic surfactants, microbial-produced compounds are easily degraded and are generally considered as low or non-toxic products and therefore, appropriate for pharmaceutical, cosmetic and food uses. Although little is known about the toxicity of microbial surfactants, some data in the literature suggest that they are less toxic than synthetic surfactants (Muthusamy et al., 2008). The synthetic anionic surfactant (Corexit) for example had an LC50 (lethal concentration to 50% of test species) against *Photobacterium phosphoreum* at approximately ten times lower concentrations than that for rhamnolipids, demonstrating the higher toxicity of the chemical-derived surfactant. It was also reported that biosurfactants needed higher effective concentration to decrease 50% of test population values (EC50) and were degraded faster than commercial dispersants. In another report, biosurfactants from *P. aeruginosa* were noted to have much less toxic and mutagenic activities when compared to synthetic surfactant Marlon A-350, which is widely used in the industry.

Understanding the functional mechanisms of biosurfactants and bioemulsifiers is of great help to discover interesting applications. Surfactin, one of the most powerful biosurfactants, is known to destabilize membranes disturbing their integrity and permeability (Bernheimer et al., 1970). This is due to changes in physical membrane structure or through disrupting protein conformations which alter important membrane functions such as transport and energy generation (Ortiz et al., 2008, 2009; Sánchez et al., 2009, 2010; Sotirova et al., 2008; Van Hamme et al., 2006; Zaragoza et al., 2009).

The molecular mechanisms of surfactin interactions with membrane structures have been described by Shaligram & Singhal (2010) and by Seydlová & Svobodová (2008). A key step for membrane destabilization and leakage is the dimerization of surfactin into the bilayer (Carrillo et al., 2003). The hypothetical mechanisms of surfactin interactions with

membranes exhibit a complex pattern of effects such as insertion into the lipid bilayers, chelating mono- and divalent cations, modification of membrane permeability by channel formation or membrane solubilization by a detergent-like mechanism. *In vitro*, the incorporation of surfactin into the membrane gives rise to dehydration of the phospholipid polar head groups and the perturbation of lipid packing which strongly compromise the bilayer stability, leading to the disturbance of the membrane barrier properties.

These structural fluctuations may well explain the primary mode of the antibiotic action and the other important biological effects of this lipopeptide (Carrillo et al., 2003). The extent of perturbation of the phospholipid bilayer correlates with the concentration of surfactin. At low concentrations surfactin penetrates readily into the cell membrane, where it is completely miscible with the phospholipids and forms mixed micelles. At moderate concentrations, the lipopeptide forms domains segregated within the phospholipid bilayer that may contribute to the formation of ion-conducting pores in the membrane leading to membrane disruption and permeabilization at high concentrations, showing a stronger activity than that of Triton (Heerklotz et al., 2007, as cited in Seydlová & Svobodová, 2008).

As biological amphiphilic molecules, biosurfactants naturally tend to self-assemble into hierarchically ordered structures using hydrogen bonding, hydrophobic and van der Waals interactions (Kitamoto et al., 2009). Glycolipids, and in particular MELs, are well known for their self-assembling properties, that are influenced by the stereochemistry of the saccharide head groups (Kitamoto et al., 2005). Some of glycolipid type surfactants, which possess relatively large hydrophilic head groups as compared to the hydrophobic parts, generally form micelles in a dilute aqueous solution. Other than spherical micelles, they also form oblate (disk-like) and prolate (rod-like) structures (Söderman, 2000, as cited in Kitamoto et al., 2009). As the surfactant concentration further increases, glycolipid/water systems start to form a range of liquid crystalline phases. In particular, glycolipid biosurfactants spontaneously self-assemble into a variety of molecular assemblies with well-defined and/or unique structures, such as sponge (L3), cubic (V2), hexagonal (H2), or lamellar ( $L_{\alpha}$ ) configurations (Imura et al., 2007, as cited in Kitamoto et al., 2009).

Among these molecular assemblies, vesicles are one of the most intensively studied ones. MELs in particular, due to their efficient molecular orientation property and effective balance between hydrophilic and hydrophobic groups, are able to form giant vesicles of diameter larger than 10  $\mu\text{m}$  (Kitamoto et al., 2002). In comparison, rhamnolipids show a pH-sensitive conversion of molecular assemblies due to the presence of a carboxyl group on the side chain (Kitamoto et al., 2002). This leads rhamnolipids to form micelles at pH more than 6.8, lipid particles at pH 6.6-6.2, lamella structures at pH 6.5-6.0, and finally vesicles in the size of 50-100 nm at pH 5.8-4.3. Glycolipid biosurfactant-based vesicles or bilayer membranes appear, thus, to be very promising for exploiting useful nanostructured materials and/or systems.

Another function of microbial surface-active molecules with interesting biotechnological potential is the ability to form stable emulsions (Fig. 4.). High molecular-mass biosurfactants are in general better emulsifiers than low-molecular-mass biosurfactants and are thus called bioemulsifiers. Bioemulsifiers, can form and stabilize oil in water or water in oil emulsions, but are not necessarily efficient detergents that are able to demonstrate remarkable surface tension reduction (Dastgheib et al., 2008). Emulsions can be produced with prolonged lifespan of months and years. Liposan, for example, does not reduce surface tension, but has been used successfully to emulsify edible oils (Cirigliano & Carman, 1985). Emulsan is an effective emulsifier at low concentrations (0.01-0.001%) representing emulsan-to-

hydrocarbon ratios of 1:100 to 1:1000, while exhibiting considerable substrate specificity (Ron & Rosenberg, 2001). Polymeric surfactants offer additional advantages because they coat droplets of oil, thereby forming stable emulsions. This property is especially useful for making oil/water emulsions for cosmetics and food industries (Muthusamy et al., 2008).

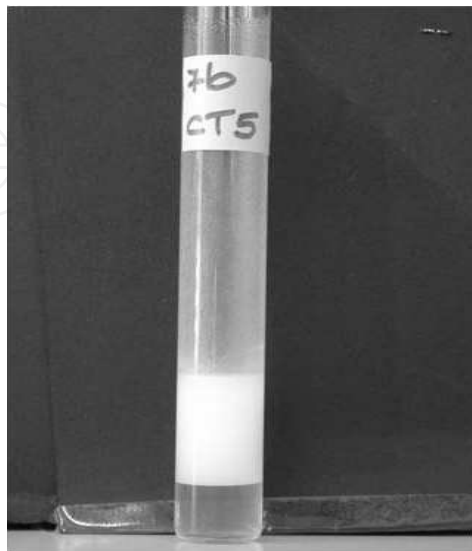


Fig. 4. Example of emulsion produced by the bioemulsifier extracted from the bacterial strain 7bCT5, isolated from a Cambodian soil. This emulsion is stable since 2009.

### 2.3.1 Natural roles

Although biosurfactants are produced by a large number of microorganisms and are clearly significant in many aspects of growth, it is difficult to generalize on their roles in microbial physiology. Due to their very diverse chemical structures and surface properties, different groups of biosurfactants may have different natural roles in the growth of the producing microorganisms and probably provide advantages in a particular ecological niche. Ron & Rosenberg, (2001) and Van Hamme et al., (2006) recently reviewed the physiological roles of microbial surface-active compounds. Some biosurfactants are essential for the motility of the microorganisms (gliding and swarming). For example, serrawettin plays a fundamental role for surface locomotion and access to water repelling surfaces for *Serratia marcescens* whereas surfactin, together with flagellar biosynthesis, are crucial for swarming motility in *B. subtilis* (Arutchelvi et al., 2008; Van Hamme et al., 2006). Bioemulsifiers also play an important role in regulating the attachment-detachment of microorganisms to and from surfaces (Van Hamme et al., 2006).

In addition, bioemulsifiers are involved in cell-to-cell interactions such as bacterial pathogenesis, quorum sensing and biofilm formation, maintenance and maturation. Rhamnolipids, for example, are essential to maintain the architecture of the biofilms and are considered as one of the virulence factors in *Pseudomonas* sp. (Arutchelvi et al., 2008; Ron & Rosenberg, 2001; Van Hamme et al., 2006). Rhamnolipids, mannosylerythritol lipid and surfactin show antimicrobial and antibiotic properties thus conferring a competitive advantage to the organism during colonization and cell-cell competition. In addition cellular differentiation, substrate accession and resistance to toxic compounds are all roles attributed to microbial surface-active compounds. Their most widespread role however is believed to be the interaction between microbes and insoluble substrates such as

hydrocarbons. Some biosurfactants/bioemulsifiers enhance the growth of bacteria on hydrophobic water-insoluble substrates by increasing their bioavailability, presumably by increasing their surface area, desorbing them from surfaces and increasing their apparent solubility (Neu, 1996; Ron & Rosenberg, 2001; Van Hamme et al., 2006).

### 3. Biomedical applications of microbial surface-active compounds

The use and potential commercial applications of biosurfactants in the medical field have increased during the past decade. Their antibacterial, antifungal and antiviral activities make them relevant molecules for applications in combating many diseases and as therapeutic agents. Furthermore, biosurfactants are generally considered safer than synthetic pharmaceuticals, due to their biological origin. Their pertinence in these fields is related to their biological properties such as the ability to disrupt membranes leading to cell lysis and metabolite leakage through increased membrane permeability and hence antimicrobial activity. Moreover, similarly to organic-conditioning films, their ability to partition at the interfaces can affect the adhesion properties of cells/microorganisms. Biomedical applications of biosurfactants have been thoroughly described (Banat et al., 2010; Cameotra & Makkar, 2004; Rodrigues et al., 2006a; Rodrigues & Teixeira, 2010; Seydlová & Svobodová, 2008; Singh & Cameotra, 2004).

#### 3.1 Antimicrobial activity of biosurfactants

The search for new antimicrobial drugs remains a major concern nowadays because of the newly emerged pathogenic microorganisms and traditional others which have become virtually unresponsive to existing antibiotics. In fact, no novel or effective chemical antibiotics have been discovered during the last few decades (Hancock & Chappelle, 1999). Microbial metabolites have been recognized as a major source of compounds endowed with ingenious structures and potent biological activities (Donadio et al., 2002). Among these, some biosurfactants have been reported to be suitable alternatives to synthetic medicines and antimicrobial agents and may therefore be used as effective and safe therapeutic agents (Banat et al., 2000; Cameotra & Makkar, 2004; Singh & Cameotra, 2004).

##### 3.1.1 Antibacterial activity

Lipopeptides have the most potent antimicrobial activity and have been a subject of several studies on the discovery of new antibiotics. The antibiotic activity is due to the ability of molecules of lipopeptide biosurfactants to self-associate and form a pore-bearing channel or micellular aggregate inside a lipid membrane (Carrillo et al., 2003; Deleu et al., 2008). Surfactin, in particular, has been associated with several physical and biological actions, such as antimicrobial, antiviral, anti-mycoplasma and haemolytic activities. It can penetrate into the membrane through hydrophobic interactions, thus influencing the ordering of the hydrocarbon chains and thus varying the membrane thickness (Bonmatin et al., 2003). Such membrane disruptions are a nonspecific mode of action and are advantageous for action on different cell membranes of both Gram-positive and Gram-negative bacteria (Lu et al., 2007). It has been suggested that such action by surfactin type peptides on membrane integrity rather than other vital cellular processes may perhaps constitute the next generation of antibiotics (Rodrigues & Teixeira, 2010). Similar bioactive fractions from the marine *Bacillus circulans* biosurfactant had antimicrobial action against various Gram-positive and Gram-negative pathogenic and semi-pathogenic bacteria including *Micrococcus flavus*, *Bacillus*



*pumilis*, *Mycobacterium smegmatis*, *Escherichia coli*, *Serratia marcescens*, *Proteus vulgaris*, *Citrobacter freundii*, *Proteus mirabilis*, *Alcaligenes faecalis*, *Acetobacter calcoaceticus*, *Bordetella bronchiseptica*, *Klebsiella aerogenes* and *Enterobacter cloacae* (Das et al., 2008). The chemical identity of this bioactive biosurfactant fraction showed overlapping patterns with that of surfactin lipopeptides and lichenysin. Mild antimicrobial action was also observed against methicillin-resistant *Staphylococcus aureus* (MRSA) and other MDR pathogenic strains. The biosurfactant was also found to be nonhaemolytic in nature thus indicating possible use as a drug in antimicrobial chemotherapy.

Very recently Huang et al., (2011) evaluated antimicrobial activity of surfactin and polylysine against *Salmonella enteritidis* in milk using a response surface methodology and showed *S. enteritidis* to be very sensitive to both molecules with minimum inhibitory concentrations of 6.25 and 31.25 µg/mL, respectively. The optimization of antimicrobial activity indicated that *S. enteritidis* could be reduced by 6 orders of magnitude at a temperature of 4.45°C, action time of 6.9 h, and concentration of 10.03 µg/mL (surfactin/polylysine weight ratio, 1:1).

In addition to surfactin, *Bacillus subtilis* strains produce a broad spectrum of bioactive peptides with great potential for biomedical applications, such as fengycin (Vanittanakom et al., 1986) and the iturin compounds: iturins (Besson et al., 1978; Peypoux et al., 1978), mycosubtilins (Peypoux et al., 1986), and bacillomycins (Peypoux et al., 1984), all of which are amphiphilic surface and membrane-active compounds with potent antimicrobial activities. Huang et al., (2007) reported that a lipopeptide antimicrobial substance produced by *B. subtilis* fmbj strain, which is mainly composed of surfactin and fengycin, was able to inactivate endospores of *B. cereus* through damaging the surface structure of the spores as seen by Transmission Electron Microscopy.

Lichenysin, pumilacidin and polymyxin B (Grangemard et al., 2001; Landman et al., 2008; Naruse et al., 1990; Yakimov et al., 1995) are other antimicrobial lipopeptides produced by *Bacillus licheniformis*, *Bacillus pumilus* and *Bacillus polymyxa*, respectively. Polymyxin B, in particular, due to its high affinity for the lipid moieties of lipopolysaccharide, has shown antibacterial activities against a wide variety of Gram-negative pathogens. Being a cationic agent, it binds to the anionic bacterial outer membrane, leading to a detergent effect that disrupts membrane integrity. Important nosocomial pathogens such as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp. are usually susceptible to polymyxins and considerable activity has been reported against *Salmonella* spp., *Shigella* spp., *Pasteurella* spp., and *Haemophilus* spp. (Landman et al., 2008).

Another promising example of an antimicrobial lipopeptide that is under commercial development is daptomycin (Cubicin®). It has been approved for the treatment of skin infections by the FDA in 2003 (Giuliani et al., 2007, as cited in Seydlová & Svobodová, 2008). Daptomycin produced by *Streptomyces roseosporus* has been shown to be highly active against multiresistant bacteria such as MRSA (Tally & De Bruin, 2000, as cited in Seydlová & Svobodová, 2008). Another lipopeptide with antimicrobial activity and other interesting biological properties is viscosin, a cyclic lipopeptide from *Pseudomonas* (Saini et al., 2008).

Glycolipids, both rhamnolipids (Abalos et al., 2001; Benincasa et al., 2004) and sophorolipids (Kim et al., 2002; Van Bogaert et al., 2007) also have shown interesting antimicrobial activities (Fig. 5). Benincasa et al., (2004) reported that a mixture of six rhamnolipides homologues performed very well against *Bacillus subtilis* with a MIC of 8 µg/mL. Mannosylerythritol lipids (MEL-A and MEL-B) produced by *Candida antarctica* strains have also been reported to exhibit antimicrobial action against Gram-positive bacteria (Kitamoto et al., 1993).



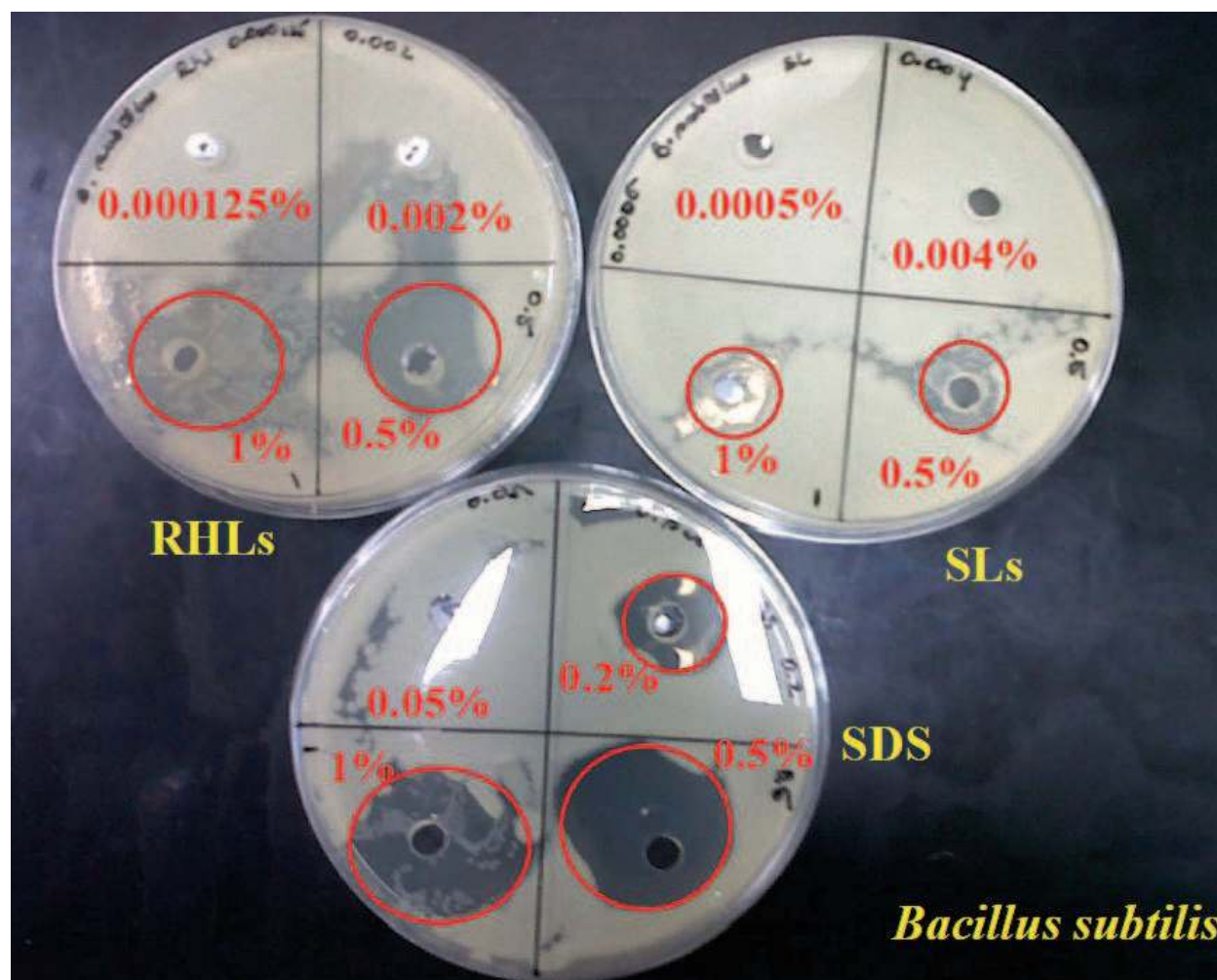


Fig. 5. Measuring antimicrobial activity for rhamnolipids, sophorolipids and SDS at various concentrations above and below the CMC for these surface active molecules against *Bacillus subtilis*, red circles showing clearing/inhibition zones.

Very recently, Nitschke et al., (2010) reported rhamnolipids produced by *P. aeruginosa* LBI with antimicrobial activity against several bacteria and fungi, including *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Mucor miehei* and *Neurospora crassa*. Another glycolipid, flocculosin, a cellobiose lipid produced by the yeast-like fungus *Pseudozyma flocculosa*, was particularly effective against *Staphylococcus* species, including MRSA. Its antibacterial activity was not influenced by the presence of common resistance mechanisms against methicillin and vancomycin and it was able to eliminate *C. albicans* cells in a very short period of time (Mimee et al., 2009).

Trehalose lipids produced by *Tsukamurella* sp. strain DSM 44370 together with trisaccharide and tetrasaccharide lipids also showed some activity against Gram-positive bacteria, with the exception of the pathogenic strain *Staphylococcus aureus*, whereas Gram-negatives were either slightly or not inhibited at all (Vollbrecht et al., 1999, as cited in Franzetti et al., 2010b). Studies carried out to elucidate the molecular interactions between this biosurfactant and the lipidic component of the membrane showed that trehalose lipid increased the fluidity of phosphatidylethanolamine and phosphatidylserine membranes and formed domains in the fluid state and did not modify the macroscopic bilayer organization (Ortiz et al., 2008, 2009).

### 3.1.2 Antiviral activity

Antiviral effects have also been reported for surfactin and its analogues (Naruse et al., 1990). More effective inactivation of enveloped viruses, such as retroviruses and herpes viruses, were noted compared to non-enveloped viruses, suggesting that inhibitory action links may be due to physico-chemical interactions with the virus envelope (Vollenbroich et al., 1997a). Antiviral activity of some lipopeptides therefore may take place as a result of the viral lipid envelope and capsid disintegration due to ion channels formation, with consequent loss of the viral proteins involved in virus adsorption and/or penetration (Jung et al., 2000; Seydlová & Svobodová, 2008).

*In vitro* experiments showed that both surfactin and fengycin produced by *B. subtilis* fmbj were able to inactivate cell-free virus stocks of porcine parvovirus, pseudorabies virus, newcastle disease virus and bursal disease virus and could effectively inhibit infections and replication of these viruses (Huang et al., 2006).

Sophorolipids are also claimed to have activity against human immunodeficiency virus (Shah et al., 2005) and trehalose lipids (namely trehalose dimycolate, TDM) conferred higher resistance to intranasal infection by influenza virus in mice though inducing proliferation of T-lymphocytes bearing gamma/delta T-cell receptors, associated with the maintenance of acquired resistance to the infection (Hoq et al., 1997, as cited in Franzetti et al., 2010b).

Rhamnolipid alginate complex also showed significant antiviral activity against herpes simplex virus types 1 and 2. In particular, they significantly inhibited the herpesvirus cytopathic effect in the Madin-Darby bovine kidney cell line (Remichkova et al., 2008). The suppressive effect of the compounds on herpes simplex virus replication was dose-dependent and occurred at concentrations lower than the critical micelle concentration.

### 3.1.3 Anti mycoplasma activity

Some investigations have shown interesting anti-mycoplasma effects for surfactins. Mycoplasma contamination in cell culture is a frequently occurring serious limitation to biomedical research, particularly when it affects the irreplaceable cell lines which ultimately ends up destroyed. Earlier studies showed that surfactin treatment of mammalian cells that had been contaminated with mycoplasmas permitted specific inactivation of mycoplasmas without significantly damaging effects on cell metabolism in the culture (Vollenbroich et al., 1997b). In a more recent study, surfactin was used to eliminate mycoplasma from an extensively infected irreplaceable hybridoma cell line (Kumar et al., 2007). There were apparent indications of limited elimination, suggesting the possible use of surfactin in achieving total decontamination. However, it was observed that surfactin was toxic to the infected hybridoma cells plated at various cell densities and exposure times, therefore it was suggested that preliminary tests should be carried out to determine the cytotoxicity of surfactin before use in decontamination.

Another study confirmed surfactin potential to eliminate mycoplasma cells independently of the target cell, which is a significant advantage over the mode of action of conventional antibiotics (Fassi et al., 2007). This study also reported that surfactin exhibited a synergistic effect in combination with enrofloxacin, and resulted in mycoplasma-killing activity of about two orders of magnitude greater than when the molecules were used separately.

### 3.1.4 Antifungal activity

The antifungal activities of biosurfactants have long been known, although their action against human pathogenic fungi has been rarely described (Abalos et al., 2001; Chung et al.,

2000; Tanaka et al., 1997). The previously mentioned cellobiose lipid flocculosin isolated from *Pseudozyma flocculosa*, was shown to display *in vitro* antifungal activity against several pathogenic yeasts, associated with human mycoses, including *Candida lusitanae*, *Cryptococcus neoformans*, *Trichosporon asahii* and *Candida albicans* (Mimee et al., 2005). This product positively inhibited all pathogenic strains tested under acidic conditions and showed synergistic activity with amphotericin B. Moreover, no significant cytotoxicity was detected when tested against human cell lines. In nature, flocculosin is part of *P. flocculosa* biocontrol arsenal against other fungi. Recent reports however have suggested that flocculosin is also used by *P. flocculosa* as a nutrient source when experiencing food limitation and the molecule is rapidly deacylated under alkaline conditions losing its antimicrobial activity which may explain conflicting results concerning the antimicrobial activity of this class of glycolipids (Mimee et al., 2009).

Other antifungal activity of biosurfactants against phytopathogenic fungi has also been described. It has been recently demonstrated that glycolipids, such as cellobiose lipids (Kulakovskaya et al., 2009, 2010) and rhamnolipids (Debode et al., 2007, Banat et al., 2010) and cyclic lipopeptides (Tran et al., 2007, 2008, as cited in Banat et al., 2010), including surfactin, iturin and fengycin (Kim et al., 2010; Arguelles-Arias et al., 2009, Chen et al., 2009, Grover et al., 2010, Mohammadipour et al., 2009, Snook et al., 2009) can all have varying degrees of antimicrobial activities.

### 3.2 Antiadhesion activity of biosurfactants

Microbial biofilms formation on medical and technical equipment is an important and mostly hazardous occurrence, especially as the bacteria within such biofilms usually become highly resistant to antibiotics and adverse environmental challenges. Several approaches have been adopted in order to limit pathogen colonization. Strict hygienic practices by healthcare personnel such as hand washing and regular disinfection of equipment and environment become of grave importance. However, it should be noted that routine disinfection is becoming controversial as frequent application becomes less effective (Dettenkofer et al., 2004, 2007, Kramer et al., 2006, as cited in Falagas & Makris, 2009).

#### 3.2.1 Biofilms on medical devices

Device-related infections are often identified as having a biofilm aetiology and biofilm formation can occasionally be facilitated by the host inflammatory response molecules which can make adhesion to the surface of the device easier (Hall-Stoodley et al., 2004). Almost all kind of surfaces are suitable to be colonized by biofilms (Donlan & Costerton, 2002). Biomedical devices are not the exception, biofilms are often found on the surface of urinary catheters (Stickler, 2008), central venous catheters (Petrelli et al., 2006), heart valves (Litzler et al., 2007), voice prostheses (Buijssen et al., 2007), contact lenses (Imamura et al., 2008), hip prostheses (Dempsey et al., 2007) and intrauterine devices (Chassot et al., 2008).

Current biofilm preventive strategies are essentially aimed at coating medical surfaces with antimicrobial agents, a process not always successful (Basak et al., 2009; von Eiff et al., 2005). Bacteria in biofilms become highly resistant to antibiotics, and so they evade host defenses withstanding antimicrobial chemotherapy (Morikawa, 2006). Since nosocomial infections remain an important problem even for hospitals with strict infection control programmes, infection control measures remain highly sought after (Falagas & Makris, 2009). Development of successful technologies based on the biofilm formation and growth control is expected to be a major breakthrough in the clinical practice and preventive medicine.



To eliminate biofilm formation novel compounds capable of specifically targeting biofilm growth while causing no adverse toxicity to the environment of application are needed. Several reports have suggested that, in addition to their direct action against pathogens, biosurfactants are able to interfere with biofilm formation, modulating microbial interaction with interfaces (Federle & Bassler 2003; Merk et al., 2005; Neu, 1996; Rasmussen & Givskov, 2006; Rodrigues et al., 2006b, 2006c; Rodrigues et al., 2007).

Surfactin for example has shown to be an important part of a list of biofilm controlling agents. Surfactin is able to inhibit biofilm formation of *Salmonella typhimurium*, *S. enterica*, *E. coli* and *Proteus mirabilis* in polyvinyl chloride wells, as well as vinyl urethral catheters (Mireles et al., 2001). Many *Salmonella* species are important opportunistic pathogens of the urinary tract system. Recently, two lipopeptide biosurfactants, produced by *B. subtilis* V9T14 and *B. licheniformis* V19T21, showed the ability to selectively inhibit biofilm formation by pathogenic strains on polystyrene (Rivardo et al., 2009). In particular, *S. aureus* ATCC 29213 and *E. coli* CFT073 biofilm formation were decreased by 97% and 90%, respectively. V9T14 biosurfactant was active on the Gram-negative strain yet ineffective against the Gram-positive while the opposite was observed for V19T21 biosurfactant. These effects were observed either by coating the polystyrene surface with these compounds or by adding the biosurfactant to the inoculum. The chemical characterization of V9T14 lipopeptide biosurfactant carried out by LC/ESI-MS/MS revealed that it was composed of 77% of surfactin and of 23% of fengycin (Pecci et al., 2010).

The activity of AgNO<sub>3</sub> combined with the lipopeptide biosurfactant V9T14 has also been studied against a preformed *E. coli* biofilm on the Calgary Biofilm device (Rivardo et al., 2010). Results indicated that the activity of silver can be synergistically enhanced by the presence of V9T14, allowing a reduction in the quantity of silver used to achieve greater antimicrobial impact. The concentrations of silver in the silver-biosurfactant solutions were 129 to 258 fold less than the concentrations needed when silver was used alone. In another study, the V9T14 biosurfactant in association with antibiotics led to a synergistic increase in the efficacy of antibiotics in *E. coli* CFT073 biofilm inhibition and, in some combinations, to total eradication of the uropathogenic strain biofilm (Rivardo et al., 2011).

In another recent work, marine bacterial culture supernatants of *Bacillus pumilus* and *B. indicus* significantly inhibited the initial attachment process and biofilm formation and dispersal of mature biofilms of *Vibrio* spp. strains (Nithya & Pandian, 2010). The bacterial supernatants also reduced the surface hydrophobicity of *Vibrio* spp. which is one of the important requirements for biofilm development. Valle et al., (2006) observed that distinct serotypes of group II capsular polysaccharides, produced by the uropathogenic *E. coli* (UPEC strain CFT073) behaved like surface-active polymers that displayed anti-adhesion properties. The treatment of abiotic surfaces with group II capsular polysaccharides drastically reduced both initial adhesion and biofilm development of important nosocomial pathogens.

More recently, the effect of different temperatures on the anti-adhesive activity of surfactin and rhamnolipid biosurfactants was tested on polystyrene surfaces, regarding the attachment of *Staphylococcus aureus*, *Listeria monocytogenes*, and *Micrococcus luteus* (Zeraik & Nitschke, 2010). Surfactin inhibited bacterial adhesion at all tested conditions, and its activity increased with the decrease in temperature, giving a 63–66% adhesion reduction in the bacterial strains at 4°C. Rhamnolipids promoted a slight decrease in the attachment of *S. aureus* but were not as effective. The ability of rhamnolipid biosurfactant to inhibit adhesion of microorganisms to silicone rubber was also investigated in a parallel-plate flow chamber (Rodrigues et al., 2006c). The results showed an effective reduction in the initial deposition

rates and in the number of bacterial cells adhering after 4h, for several microorganisms. Moreover, perfusing the flow chamber with biosurfactant containing solution followed by the passage of a liquid–air interface produced high detachment (96%) of adhered cells for several microorganisms. These capabilities have a lot of implications regarding biofilm formation and microbial contamination and establishments on such biomedical devices made of such compounds.

Antibiofilm activity was also reported for a glycolipid biosurfactant isolated from another marine bacterium *Brevibacterium casei* MSA19 against pathogenic biofilms *in vitro* (Kiran et al., 2010a). The purified glycolipid disrupted the biofilm formation under dynamic conditions and the biofilm-forming capacity of both mixed culture and individual human and fish pathogenic strains was significantly inhibited at 30 mg/mL glycolipid. Raya et al., (2010) analyzed the effects of rhamnolipids and shear on initial attachment of *Pseudomonas aeruginosa* PAO1 in glass flow chambers. The presence of rhamnolipids significantly reduced the initial attachment of PAO1, even at the low concentration of 13 mg/L. Prewashing the cells with a 100 mg/L rhamnolipid solution, however, did not affect the attachment significantly. The initial cell attachment increased with increasing shear at the very low shear range (up to 3.5–5.0 mN/m<sup>2</sup>), however the attachment could be minimized with further increase of the shear.

The biosurfactant Lunasan produced by the yeast *Candida sphaerica* UCP0995 completely inhibited the adhesion of *Streptococcus agalactiae*, *Streptococcus sanguis* 12, *Streptococcus mutans*, *Streptococcus mutans* NS, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida albicans* on plastic tissue culture plates at a concentration of 10 mg/ml and ≈92% inhibition of adhesion occurred for *Pseudomonas aeruginosa* (Luna et al., 2011). Lunasan, tested at the same concentration, also showed antimicrobial activity against the strains *Streptococcus oralis* (68%), *Candida albicans* (57%), and *Staphylococcus epidermidis* (57.6%). The same research group also described antiadhesive and antimicrobial activities of Rufisan, a biosurfactant produced by the yeast *Candida lipolytica* UCP 0988 (Rufino et al., 2011). Crude biosurfactant showed anti-adhesive activity at ≥0.75 mg/L against most of the microorganisms tested (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus mutans* NS) and the anti-adhesive property was proportional to the concentration of the biosurfactant while antimicrobial activities were also observed at higher biosurfactant concentrations.

In conclusion, the anti-adhesive activity of biosurfactants against several pathogens indicates their potential utility as coating agents for medical insertional materials that may lead to a reduction in a large number of hospital infections without the need for use of synthetic drugs and chemicals.

### 3.2.2 Biofilms on food processing surfaces

In addition to the treatment of biomaterials used for medical devices, biosurfactants have also been used in the pre-treatment of material surfaces found in food-processing environments. Pathogenic bacteria implicated in food-borne illness outbreaks are able to form biofilms on food contact surfaces that are more resistant to sanitation than free-living cells (Kalmokoff et al., 2001; Kim et al., 2006; Stepanovic et al., 2004). The pre-conditioning of surfaces using microbial surface-active compounds may be an interesting strategy for preventing the adhesion of food-borne pathogens to solid surfaces. Meylheuc et al., (2006b) demonstrated that the preconditioning of stainless steel surfaces with an anionic biosurfactant produced by *Pseudomonas fluorescens* reduced the number of *L. monocytogenes* LO28-adhering cells and thus favoured the bactericidal activities of the disinfectants sodium hypochlorite (NaOCl) and peracetic acid/hydrogen peroxide (PAH).



Similarly, biosurfactants obtained from *Lactobacillus helveticus* and *P. fluorescens* were able to inhibit the adhesion of four *Listeria* strains to stainless steel (Meylheuc et al., 2006a). Whichever strain of *L. monocytogenes* used in combination with biosurfactants, the anti-adhesive biological coating developed both reduced the total adhering flora and the viable and culturable adherent bacteria on stainless steel surfaces. More recently, another group investigated the effect of rhamnolipid and surfactin biosurfactants on the adhesion of the food pathogens *E. sakazakii*, *L. monocytogenes* and *S. enteritidis* to polypropylene and stainless steel surfaces (Nitschke et al., 2009). Preconditioning with surfactin, rather than rhamnolipid, caused a reduction in the number of adhering cells particularly of *L. monocytogenes* and to some extent *E. sakazakii* on stainless steel. Surfactin showed a significant decrease in the adhesion on polypropylene of all strains. The adsorption of surfactin on polystyrene also reduced the adhesion of *S. enteritidis*- and *L. monocytogenes*-growing cells. In addition, surfactin was able to delay bacterial adhesion within short contact periods using non-growing cells or longer contact periods using growing cells.

Other antimicrobial and antiadhesive properties of a biosurfactant produced by *Lactobacillus paracasei* ssp. *paracasei* A20 isolated from a Portuguese dairy plant were also described (Gudiña et al., 2010). The biosurfactant had antimicrobial activity against a broad range of microorganisms including the pathogenic *C. albicans*, *E. coli*, *S. aureus*, *S. epidermidis* and *Streptococcus agalactiae* while exhibiting a considerable antiadhesive activity against a wide range of microorganisms.

The activity demonstrated by biosurfactants suggests that they could be considered as new tools in developing strategies to prevent or delay microbiological colonization of industrial plant surfaces used in foodstuffs preparation.

### 3.3 Probiotics biosurfactants activity

Probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". They have been reported to have positive effects on the maintenance of human health (Gupta & Garg 2009). Interest in probiotics has gained great significance due to the increasing antimicrobial resistance of bacteria worldwide. Evidence suggests that probiotic organisms may have a role in lowering the incidence or the duration of antibiotic-related diarrhea, contributing to the prevention or treatment of vaginal candidiasis, bacterial vaginosis and recurrent lower urinary tract infections. Furthermore, they encourage improved immunological defense responses and can decrease the activity of numerous toxic antimetabolites (Falagas et al., 2006a, 2006b, 2007).

Probiotics mechanisms of action vary, however, some are known to produce various antimicrobial agents such as organic acids, hydrogen peroxide, carbon peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins (Merk et al., 2005). In addition, probiotics have long been known also for the capacity to interfere with the adhesion and formation of biofilms of pathogens to epithelial cells of urogenital and intestinal tracts (Reid et al., 1998, 2001). The mechanisms of this interference include the release of surface active molecules (Gudiña et al., 2010; Rodrigues et al., 2006d). Hong et al., (2005) reported the production of antimicrobial lipopeptides by *Bacillus* probiotics products as the main mechanisms by which they inhibit the growth of pathogenic microorganisms in the gastrointestinal tract. Similarly, competition with other microorganisms for adherence to epithelial cells as well as biosurfactants production are well known mechanisms used by *Lactobacillus* probiotics to interfere with vaginal pathogens (Barrons & Tassone, 2008; Cribby et al., 2008; Falagas et al., 2007).

Several investigators have pointed to evidence that probiotic type microorganisms and their biosurfactants may antagonize the growth of nosocomial pathogens on inanimate surfaces (Rodrigues et al., 2004a, 2004b, 2006b, 2006c; Walencka et al., 2008). Falagas & Makris, (2009) reviewed studies involving *in vitro* experiments on the potential role of probiotics microorganisms and their products in the inhibition of bacterial or fungal colonisation of artificial surfaces, such as vinyl urethral catheters and silicon rubber voice prostheses (Busscher et al., 1997, 1998; Velraeds et al., 1996, 1997, 2000; Rodrigues et al., 2004a, 2004b, 2006b, Van der Mei et al., 2000). The majority of the investigators examined the preconditioning of the materials surfaces with probiotic biosurfactants, while others added probiotic biosurfactant producing strains to examine adhesion or biofilm development. It was generally demonstrated that both probiotics microorganisms alone (mainly *Streptococcus thermophilus* and *Lactobacillus* spp. strains) or their biosurfactants were able to antagonize growth and development of potentially pathogenic microorganisms including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp., *Enterococcus faecalis*, *Candida albicans*, *Candida tropicalis* (Busscher et al., 1997; Van Hoogmoed et al., 2000).

Rodrigues et al., (2004a) demonstrated that the biosurfactant obtained from the probiotic bacterium *Lactococcus lactis* 53 was able to inhibit the adhesion of bacterial pathogens to silicone rubber with an adsorbed biosurfactant layer. Adhesion of yeasts was also decreased in the presence of biosurfactant, but to a lesser extent. In another work, using an artificial throat model, the same authors showed that biosurfactants obtained from probiotic strains greatly reduced microbial numbers on voice prostheses and induced a decrease in the airflow resistance of voice prostheses after biofilm formation, which may prolong the lifetime of indwelling silicone rubber voice prostheses (Rodrigues et al., 2004b).

In a more recent work, it was demonstrated that the preconditioning of silicon rubber with a biosurfactant produced by the strain *Streptococcus thermophilus* A reduced adhering bacterial pathogens by up to 97% and adhering *Candida* spp. by up to 70% (Rodrigues et al., 2006b). Velraeds et al., (1996) also reported on the inhibition of adhesion of pathogenic enteric bacteria by a biosurfactant produced by a *Lactobacillus* strain and later showed that the biosurfactant caused an important dose-related inhibition of the initial deposition rate of *E. coli* and other bacteria adherent on both hydrophobic and hydrophilic substrata (Velraeds et al., 1997).

Another interesting application area that is gaining increased interest relates to probiotics use in preventing oral infections (Çaglar et al., 2005; Hatakka et al., 2007; Köll et al., 2008; Meurman, 2005; Meurman & Stamatova, 2007). Van Hoogmoed et al., (2004) demonstrated that *Streptococcus mitis* biosurfactant inhibited adhesion of *Streptococcus sobrinus* HG 1025 and *Streptococcus mutans* ATCC 25175 to bare enamel, while *S. mitis* biosurfactant was able to inhibit the adhesion of *S. sobrinus* HG 1025 to salivary pellicles. The authors later reported that these reductions may be attributed to increased electrostatic repulsion between the bacteria and the biosurfactant-coated pellicles (Van Hoogmoed et al., 2006).

New biosurfactant molecules produced by probiotic bacteria are reported from dairy products and environment. Recent work by Walencka et al., (2008) demonstrated that surfactants obtained from three *Lactobacillus acidophilus* strains inhibited *S. epidermidis* and *S. aureus* biofilm integrity and formation. Moreover, surfactant addition to preformed mature biofilms accelerated their dispersal and altered the characteristics of the biofilm morphology. A novel xylolipid biosurfactant from *Lactococcus lactis*, a probiotic strain isolated from a traditional Indian fermented dairy product, showed a good antibacterial activity against clinical pathogens of *E. coli* and MRSA strains (Saravanakumari & Mani, 2010). Xylolipid was non-pathogenic and safe for oral consumption and dermal applications,

suggesting that it could be safely used as a therapeutic agent or as a preservative in food or cosmetic products.

In another recent work, a biosurfactant producing strain, *Lactobacillus* sp. CV8LAC, isolated from fresh cabbage, showed interesting antiadhesive activity against two *C. albicans* pathogenic biofilm-producing strains (CA-2894 and DSMZ 11225) (Fracchia et al., 2010). The CV8LAC biosurfactant significantly inhibited the adhesion of fungal pathogens to polystyrene microtiter plates in pre-coating and co-incubation experiments. In pre-coating assays, biofilm formation of strain CA-2894 was reduced by 82% at concentration of 312.5 µg/mL while that of strain DSMZ 11225 was reduced by 81% at 625 µg/mL. In co-incubation assays, biofilm formation of the two strains was inhibited by 70% at 160.5 µg/well and by 81% at 19.95 µg/well, respectively. It was interesting to note that no inhibition of both *C. albicans* planktonic cells was observed, thus indicating that the biosurfactant displayed specific anti-biofilm formation but not antimicrobial activity.

Considering their importance for human health and their recognized safety, environmental probiotic organisms may, thus, represent a safe and effective intervention for infection control purposes. Probiotics themselves or their products (biosurfactants), could be applied to patient care equipment, such as tubes or catheters, with the aim of decreasing the colonisation of these sites by nosocomial pathogens and potentially impede a central step in the pathogenesis of nosocomial infections (Falagas & Makris, 2009).

### 3.4 Other promising biological activities

Biosurfactants have been shown to have many other roles in biomedical application. Some of the most powerful molecules (eg. surfactin, mannosylerythritol lipids (MELs), trehalose lipids) are known to have anti-inflammatory, anti-tumour, immunosuppressive and immunomodulating functions, in addition to other properties such as self-assembling, human cells stimulation and differentiation, interaction with stratum corneum lipids, cell-to-cell signaling, hemolytic activity.

#### 3.4.1 Anti-tumor activity

Recently, it has been demonstrated that these interesting microbial products can control a variety of mammalian cell functions. They are considered to participate in various intercellular molecular recognitions such as signal transduction, cell differentiation, cell immune response, etc. (Osada, 1998). Cao et al., (2010) demonstrated that surfactin induces apoptosis in human breast cancer MCF-7 cells through a ROS/JNK-mediated mitochondrial/caspase pathway. In a more recent work, they investigated the reactive oxygen species (ROS) and Ca<sup>2+</sup> impact on mitochondria permeability transition pore (MPTP) activity, and MCF-7 cell apoptosis induced by surfactin (Cao et al., 2011). The results showed that surfactin initially induced the ROS formation, leading to the MPTP opening accompanied with the collapse of mitochondrial membrane potential which lead to an increase in the cytoplasmic Ca<sup>2+</sup> concentration. In addition, cytochrome c was released from mitochondria to cytoplasm through the MPTP which activated caspase-9, eventually inducing apoptosis.

In another study, viscosin, an effective surface-active cyclic lipopeptide recovered from *Pseudomonas libanensis* M9-3, inhibited the migration of the metastatic prostate cancer cell line, PC-3M, without visible toxicity effects (Saini et al., 2008). More recently, lipopeptides (namely isoforms of surfactins and fengycins) derived from a marine *Bacillus circulans* DMS-

2 showed interesting cytotoxic activity against cancer cell lines (Sivapathasekaran et al., 2010). The purified lipopeptides at a concentration of 300  $\mu\text{g}/\text{mL}$  showed more than 90% inhibition of proliferation on both colon cancer cell lines HCT 15 and HT 29 after 24 h treatment and the antiproliferative activity of lipopeptides was observed in a dose dependent manner.

Significant effects against both tumor cell lines as compared to non-tumor cell line were also observed, thus indicating the selective inhibitory activity of these molecules. Serratamolide AT514, cyclic depsipeptide from *Serratia marcescens*, belonging to the group of serrawettins, has also been reported to be a potent inducer of apoptosis of several cell lines derived from various human tumors and B-chronic lymphocytic leukemia cells, primarily involving the mitochondria-mediated apoptotic pathway and interference with Akt/NF- $\kappa\text{B}$  survival signals (Escobar-Díaz et al., 2005, as cited in Matsuyama et al., 2010). Biological studies of AT514 using human B-lymphocytes are now in progress for clinical applications of AT514 in the field of medical oncology.

Interesting anti-tumor activities has also been reported for glycolipids. Mannosylerythritol lipids (MELs) are among the most promising biosurfactants known due to their versatile interfacial and biochemical actions. Interesting studies, thoroughly reviewed by Kitamoto et al. (2002) and by Arutchelvi & Doble, (2010), have shown that MEL-A and MEL-B display excellent growth inhibition and differentiation-inducing activities against human leukemia cells including myelogenous leukemia cell K562, promyelocytic leukemia cell HL60, and the human basophilic leukemia cell line KU812, as well as growth inhibition activity of mouse melanoma B 16 cells. Recently Chen et al., (2006) also demonstrated that a sophorolipid produced from the yeast *Wickerhamiella domercqiae* induced apoptosis in H7402 human liver cancer cells by blocking cell cycle at G1 phase and partly at S phase, activating caspase-3, and increasing  $\text{Ca}^{2+}$  concentration in cytoplasm.

### 3.4.2 Anti-inflammatory activity

Byeon et al., (2008) observed that surfactin was able to downregulate LPS-induced nitric oxide production in RAW264.7 cells and primary macrophages by inhibiting NF- $\kappa\text{B}$  activation, suggesting a good potential as a bacterium-derived anti-inflammatory agent. Selvam et al., (2009) studied the effect of *B. subtilis* PB6, a natural probiotic, on plasma cytokine levels in inflammatory bowel disease and colon mucosal inflammation. The strain was found to secrete surfactins which are known to inhibit phospholipase A2, involved in the pathophysiology of inflammatory bowel disease. In animal experiments carried out in rat models for trinitrobenzene sulfonic acid-induced colitis, oral administration of PB6 as a probiotic suppressed colitis as measured by mortality rate and changes in colon morphology and weight gain. Plasma levels of pro-inflammatory cytokines were also significantly lowered and the anti-inflammatory cytokine significantly increased after the oral administration of PB6, supporting the concept that PB6 inhibits PLA2 by secreting surfactins.

In another work, surfactin isomers derived from the mangrove bacterium *Bacillus* sp. (No. 061341) showed interesting anti-inflammatory activities (Tang et al., 2010). In particular, this class of cyclic lipopeptide showed strong inhibitory properties on the overproduction of nitric oxide and the release of IL-6 in LPS-induced murine macrophage cell RAW264.7. Moreover, structure-activity relationship studies revealed that the existence of the free carboxyl group in the structure of surfactin isomer was crucial as to the anti-inflammatory activities. An interesting recent study explored the mechanisms responsible for surfactin-induced anti-inflammatory actions in the context of periodontitis caused by *Porphyromonas*



*gingivalis*, the major pathogen of periodontal disease (Park et al., 2010). The Authors observed that surfactin significantly reduces pro-inflammatory cytokines, including tumor necrosis factor- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6, and IL-12, through suppression of nuclear factor- $\kappa$ B activity in *P. gingivalis* LPS-stimulated THP-1 human macrophage cells, in a Heme oxygenase-1 (HO-1)-dependent fashion. Furthermore, surfactin treatment effectively induces HO-1 expression, a major defense in response to oxidative stress.

These observations support the potential of surfactin as a candidate in strategies to prevent caries, periodontitis, or other inflammatory diseases.

### 3.4.3 Immuno-modulatory action

Park & Kim, (2009) studied the role of surfactin in the inhibition of the immunostimulatory function of macrophages through blocking the NK- $\kappa$ B, MAPK and Akt pathway. This provided a new insight into the immunopharmacological role of surfactin in autoimmune disease and transplantation. Their work indicated that surfactin has potent immunosuppressive capabilities which suggested important therapeutic implications for transplantation and autoimmune diseases, including allergy, arthritis and diabetes.

A biosurfactant glycolipid complex from *Rhodococcus ruber* was also shown to activate the production of IL-1 $\beta$  and TNF- $\alpha$  cytokines without modifying the production of IL-6, thus suggesting good prospects for further studies of immunomodulating and antitumor activities (Kuyukina et al., 2007).

### 3.4.4 Other biomedical related properties

Han et al., (2008) observed that high surfactin micelle concentration affected the aggregation of amyloid  $\beta$ -peptide (A $\beta$  (1-40)) into fibrils, a key pathological process associated with Alzheimer's disease. Fengycin, another lipopeptide biosurfactant is also able to cause membrane perturbations (Deleu et al., 2008). Recent results by Eeman et al., (2009) emphasized the ability of fengycin to interact with the lipid constituents of the stratum corneum extracellular matrix and with cholesterol. Another interesting property of surfactin and its synthetic analogues is the ability to alter the nanoscale organisation of supported bilayers and to induce nanoripple structures with intriguing perspectives for biomedical and biotechnological applications (Bouffieux et al., 2007; Brasseur et al., 2007; Francius et al., 2008).

Morita et al., (2010) investigated the cell activating property of MELs using cultured fibroblast and papilla cells, and a three dimensional cultured human skin model. The diacetylated MEL (MEL-A) produced from soybean oil significantly increased the viability of the fibroblast and of the papilla cells over 150% compared with that of control cells, suggesting potential use as new hair growth agent stimulating the papilla cells. Using a three-dimensional cultured human skin model, Morita et al., (2009b) observed that the viability of the SDS damaged cells was markedly improved by the addition of MEL-A in a dose-dependent manner. This demonstrated that MEL-A also had a ceramide-like moisturising activity toward the skin cells. Similarly, (Kitagawa et al., 2007, as cited in Worakitkanchanakul et al., 2008) reported that MEL-B shows excellent moisturizing properties, equivalent to those of natural ceramides, toward human skin.

Trehalose lipids also display various interesting biological activities mainly due to their great tendency to partition into phospholipid membranes (Ortiz et al., 2008, 2009). In particular, the trehalose lipid was suggested to incorporate into the membrane bilayers and produce structural perturbations, which might affect the function of both phosphatidylethanolamine and phosphatidylserine membranes. Zaragoza et al., (2010) observed that a succinoyl trehalose



lipid produced by *Rhodococcus* sp. caused the swelling of human erythrocytes followed by hemolysis at concentrations well below its critical micellar concentration. They concluded that trehalose lipid caused the hemolysis of human erythrocytes by a colloid-osmotic mechanism, most likely by formation of enhanced permeability domains, or “pores” enriched with biosurfactant, within the erythrocyte membrane.

Permealization of biological and artificial membranes was also reported to be induced by *Pseudomonas aeruginosa* dirhamnolipid (Sánchez et al., 2010). In particular, it caused the hemolysis of human erythrocytes through a lytic mechanism, as shown by the similar rates of K<sup>+</sup> and hemoglobin leakage, and by the absence of effect of osmotic protectants. Scanning electron microscopy showed that the addition of the biosurfactant changed the usual disc shape of erythrocytes into that of spherocochinocytes.

#### 4. Biotechnological and nanotechnological applications of surface-active compounds

Biosurfactants, have been increasingly attracting attention in the field of nanotechnology (Kitamoto et al., 2005, 2009). During the last decade, unique properties of biosurfactants, like versatile self-assembling and biochemical properties, which are not usually observed in conventional chemical surfactants, have been reported (Kitamoto et al., 2005, 2009). In recent years, the development of new functional structures and/or systems using self-assembly of amphiphilic molecules has evolved into a dynamic and rapidly growing area of nanotechnology (Ariga et al., 2007, Shimizu et al., 2005, as cited in Kitamoto et al., 2009) due to their ability to self-assemble into hierarchically ordered structures using hydrogen bonding, hydrophobic and van der Waals interactions as mentioned earlier.

Mannosylerythritol lipids (MELs) show the most interesting self-assembling properties and numerous related potential applications (Kitamoto et al., 2009). Konishi et al., (2007), Imura et al., (2007, 2008), and Ito et al., (2007), for example, developed and studied the kinetics of interactions in carbohydrate ligand systems composed of self-assembled monolayers of mannosylerythritol lipid-A (MEL-A) serving as a high-affinity, easy to handle and low-cost ligand system for immunoglobulin G and M and lectins..

Table 1 below lists the latest discoveries in the biotechnological and nanotechnological fields applicable to biosurfactants, and in particular the latest successful results of mannosylerythritol lipids (MELs) application in the enhancement of the gene transfection efficiency of cationic liposomes as well as some interesting applications of glycolipids and other biosurfactants in drug delivery and gene therapy. Biosurfactants use as a “green” alternative for high-performance nanomaterials production and, in particular, for the synthesis and stabilization of metal-bound nanoparticles will also be described.

| Biosurfactant type          | Activity/application  | Study  |
|-----------------------------|---|--|
| Mannosylerythritol lipids-A | Ligand system for immunoglobulin G and M and lectins                          | Konishi et al., (2007);<br>Imura et al., (2007, 2008),<br>Ito et al., (2007)   |
|                             | DNA capsulation and membrane fusion with anionic liposomes                    | Ueno et al., (2007a)   |
|                             | <i>In vitro</i> promotion of gene transfection mediated by cationic liposomes | Inoh et al., (2001, 2004, 2010); Igarashi et al., (2006); Ueno et al., (2007b) |

| Biosurfactant type             | Activity/application  | Study                             |
|--------------------------------|---|-----------------------------------|
|                                | <i>In vivo</i> promotion of liposome-mediated gene transfection   | Inoh et al., (2009)               |
|                                | Herpes simplex virus thymidine kinase gene therapy  | Maitani et al., (2006)            |
|                                | Water-in-oil microemulsions   | Worakitkanchanakul et al., (2008) |
|                                | Increase membrane fluidity of monolayers composed of L- $\alpha$ -dipalmitoylphosphatidylcholine (DPPC) | Kitamoto et al., (2009)           |
| Mannosylerythritol lipids-B    | Self-assembling and vesicle-forming activity  | Worakitkanchanakul et al., (2008) |
| Rhamnolipids and sophorolipids | Deuterated rhamnolipids and sophorolipids   | Smyth et al., (2010b)             |
|                                | Cadmium sulfide nanoparticles   | Singh et al., (2011)              |
|                                | Biocompatible microemulsions of lecithin/rhamnolipid/sophorolipid biosurfactants                        | Nguyen et al., (2010)             |
| Rhamnolipids                   | Silver nanoparticles with antibiotic microbial activity   | Kumar et al., (2010)              |
|                                | Nickel oxide nanoparticles by microemulsion technique   | Palanisamy & Raichur, (2009)      |
|                                | Silver nanoparticles  | Xie et al., (2006)                |
|                                | ZnS nanoparticles   | Narayanan et al., (2010)          |
|                                | Microemulsions  | Xie et al., (2005, 2007)          |
|                                | Alcohol-free microemulsions   | Nguyen & Sabatini, (2009)         |
| Sophorolipids                  | Cobalt nanoparticles  | Kasture et al., (2007)            |
|                                | Silver nanoparticles  | Kasture et al., (2008)            |
|                                | Sophorolipid-coated silver and gold nanoparticles with antibacterial activity                           | Singh et al., (2009, 2010)        |
|                                | Biocompatible microemulsions of lecithin/rhamnolipid/sophorolipid biosurfactants                        | Nguyen et al., (2010)             |
| Glycolipid biosurfactant       | Silver nanoparticles  | Kiran et al., (2010b)             |
| Fengycin and surfactin         | Enhancers for the skin accumulation of aciclovir  | Nicoli et al., (2010)             |
| Surfactin                      | Surfactin-mediated synthesis of gold nanoparticles  | Reddy et al., (2009)              |
|                                | Cadmium sulfide nanoparticles   | Singh et al., (2011)              |

Table 1. Examples of recent biosurfactant applications in the biotechnological and nanotechnological fields.

#### 4.1 Liposomes and gene transfection

Gene transfection into the cells is a fundamental technology not only for molecular and cellular biology processes but also a clinical gene therapy (Ueno et al., 2007b). Although several methods for gene transfection have been investigated (Felgner et al., 1989, Fujiwara, 2000, Gao & Huang, 1991, Hatakeyama et al., 2007, Nishiyama et al., 2005, Ueno et al., 2007b), more efficient and safe systems are still needed (Ueno et al., 2007b). Among the various methods, lipofection using cationic liposomes is considered to be a promising method for introducing foreign gene to the targeted cells due to their high transfection efficiency, low toxicity and immunogenicity, ease of preparation and targeted application (Farhood et al., 1992, Felgner et al., 1989, Kogure et al., 2007, Lasic, 1998, Nakanishi, 2003, Inoh et al., 2010). The physicochemical properties of cationic liposomes, such as lipid packing density, shape, and zeta-potential, have a significant effect on gene transfection efficiency (Lin et al., 2003, Takeuchi et al., 1996, Wittenberg et al., 2008, Xu et al., 1999, as cited by Inoh et al., 2010).

Inoh et al., (2001) reported that MEL-A promoted DNA transfection efficiency mediated by cationic liposomes. Confocal laser scanning microscopic analysis showed the distribution of lipids and oligonucleotide DNA in MEL-A-containing liposome-DNA complex in the plasma membrane and the nucleus of target cells at 1 h after the addition of complex (Inoh et al., 2004). This suggests that MEL-A induces the membrane fusion between the target cells and the cationic liposomes, accelerating the efficiency of gene transfection significantly. Similarly, Igarashi et al., (2006) reported that MEL-A significantly increased the cellular association and the efficiency of gene transfection mediated by cationic liposomes in human cervix carcinoma Hela cells. Analysis of flow cytometric profiles clearly indicated that the amount of DNA associated with the cells was rapidly increased and sustained by addition of MEL-A to the liposome. Confocal microscopic observation also indicated that the MEL-lipoplex distributed widely in the cytoplasm and DNA presence was intensely detected in cytoplasm around the nucleus.

The above results suggested that MEL-A increased gene expression by enhancing the association of the lipoplexes with the cells in serum and, thus, MEL-liposome may prove a significant nonviral vector for gene transfection and gene therapy.

In an attempt to explain how MEL-A-containing liposomes could accelerate gene transfection, Ueno et al., (2007a) examined MEL-containing liposomes properties such as their activity for DNA capsulation and membrane fusion abilities of cationic liposomes with artificial anionic liposomes. They observed that MEL-A-containing liposomes exhibited high activity in DNA incapsulation and membrane fusion with anionic liposomes, which are important properties for gene transfection. On the other hand, MEL-B- and MEL-C-containing liposomes only increased either the incapsulation or the membrane fusion. Ueno et al., (2007b) further examined the mechanism of the transfection mediated by cationic liposomes with NBD-conjugated MEL-A and reported that MEL-A distributed on the intracellular membranes through the plasma membranes of target cells, while the cationic liposomes with MEL-A fused to the plasma membranes within 20–35 min. Thereafter, they noted that the oligonucleotide released from the vesicles was immediately transferred to the nucleus. They therefore suggested that MEL-A was capable of promoting the transfection efficiency of target cells by inducing membrane fusion between liposomes and the plasma membrane of these cells.

Recently Kitamoto et al., (2009) demonstrated that monolayers composed of L- $\alpha$ -dipalmitoylphosphatidylcholine (DPPC) containing MEL-A had greater membrane fluidity

than those containing only DPPC. It was also reported that unsaturated fatty acids in MEL-A significantly influenced surface pressure and packing density in the monolayer and thus the physicochemical properties of MEL-A and MEL-A/lipids (Imura et al., 2008). Transfection efficiency of nano vectors with MEL-A was investigated *in vivo* on tumor cells in the mouse abdominal cavity (Inoh et al., 2009). When a complex of the nano vectors with MEL-A and plasmid DNA was injected intraperitoneally into C57BL/6J mice bearing B16/BL6 tumors, the biosurfactant significantly increased liposome-mediated gene transfection to the mouse tumor cells. The transfection efficiency of the plasmids into the solid tumors by the cationic liposomes of cholesteryl-3beta-carboxyamidoethylene-N-hydroxyethylamine (OH-Chol) with MEL-A increased by approximately 100-fold compared to that by the commercially available DC-Chol cationic liposomes without MEL-A. This suggests that nonviral vectors with MEL-A are very useful for gene transfection *in vivo*. The mechanisms of gene delivery by nano vectors with MEL-A and the numerous biological activities of these biosurfactants have been described by Nakanishi et al., (2009) and Kitamoto et al., (2009).

Inoh et al., (2010) further investigated the effects of unsaturated fatty acid ratio within the MEL-A compound on the physicochemical properties and gene delivery into cells of cationic liposomes using MEL-A with three different unsaturated fatty acid (USF) component ratios. Gene transfer efficiency of cationic liposomes containing MEL-A (containing 21.5% USF) was much higher than that of those containing MEL-A (containing 9.1%USF) and MEL-A (containing 46.3%USF). In particular, MEL-A (21.5% USF)-containing cationic liposomes induced highly efficient membrane fusion after addition of anionic liposomes and led to subsequent DNA release.

Imaging analysis revealed that MEL-A (21.5% USF)-containing liposomes fused with the plasma membrane and delivered DNA into the nucleus of NIH-3T3 cells, MEL-A (46.3% USF)-containing liposomes fused with the plasma membrane did not deliver DNA into the nucleus, and MEL-A (9.1% USF)-containing liposomes neither fused with the plasma membrane nor delivered DNA into the nucleus. These results suggest that the MEL-A unsaturated fatty acid ratio significantly affects transfection efficiency due to changes in membrane fusion activity and the efficiency of DNA release from the liposomes. Mannosylerythritol lipid-B (MEL-B) with a different configuration of the erythritol moiety was found to self-assemble into a lamellar phase over remarkably wide concentration and temperature ranges; furthermore it showed great potential as a vesicle-forming lipid, suggesting its potential application in drug and gene delivery as well as in transdermal delivery systems (Worakitkanchanakul et al., 2008). In another work, a liposome vector containing betasitosterol beta-D-glucoside biosurfactant-complexed DNA was successfully used for herpes simplex virus thymidine kinase gene therapy (Maitani et al., 2006).

#### 4.2 Biosurfactants potential in drug delivery

Properties such as detergency, emulsification, foaming and dispersion make biosurfactants interesting molecules with potential application in the field of drug delivery (Faivre & Rosilio, 2010). MEL-A for example has much higher emulsifying activity with soybean oil and tetradecane than polysorbate 80 (Kitamoto et al., 2009) and is able to form stable water-in-oil microemulsions without addition of co-surfactant or salt (Worakitkanchanakul et al., 2008).

Rhamnolipids and sophorolipids have also been mixed with lecithins to prepare biocompatible microemulsions in which the phase behavior was unaffected by changes in



temperature and electrolyte concentration, making them desirable for cosmetic and drug delivery applications (Nguyen et al., 2010). In 1988, rhamnolipid liposomes were patented as drug delivery systems, useful as microcapsules for drugs, proteins, nucleic acids, dyes and other compounds, as biomimetic models for biological membranes and as sensors for detecting pH variations. These novel liposomes were described as safe and biologically decomposable, with suitable affinity for biological organisms, stable and with long service and shelf life.

The potential of lipopeptides, fengycin and surfactin to act as enhancers for the transdermal penetration and skin accumulation of aciclovir was also recently investigated (Nicoli et al., 2010) to elucidate any possible synergistic effect between surfactin and fengycin associated with anodal iontophoresis. It was demonstrated that these lipopeptides did not enhance aciclovir transport across the skin (not even when associated with iontophoresis) although they increased aciclovir concentration in the epidermis by a factor of 2 (Nicoli et al., 2010).

Microemulsion produced using biosurfactant are thermodynamically stable and their isotropic systems that form spontaneously-consisting of microdomains of oil or water stabilized by an interfacial film - in addition to their long-term stability, easy preparation and high solubilization capacity are considered to be very promising liquid vehicles for future drug delivery systems (Date et al., 2008, as cited in Faivre & Rosilio, 2010).

### 4.3 Nanoparticles

Another interesting application for natural surfactant is the the synthesis of metal-bound nanoparticles as an alternative environmentally friendly technology (Sharma et al., 2009). Nanomaterials synthesis and use has been an active research area due to interesting properties of the nanomaterials as compared to bulk material use (Palanisamy & Raichur, 2009). Metal nanoparticles have been explored in various fields such as catalysis, mechano- and electrical applications and biomedical uses (Van Bogaert & Soetaert, 2010). The reduction in size gives rise to size dependent effects such as high surface to volume ratio, lower melting point, changes in electronic structure and changes in lattice structure and interatomic distances which in turn affect the processing parameters (Liveri, 2006, as cited in Palanisamy & Raichur, 2009).

The use of gold nanoparticles, in particular, is currently undergoing a dramatic expansion in the field of drug and gene delivery, targeted therapy and imaging technologies (Boisselier & Astruc, 2009; Pissuwan et al., 2009, 2011). Potential therapeutic applications of gold compound and gold nanoparticles also include anti-HIV activity, anti-angiogenesis, anti-malarial activity, anti-arthritis activity and biohydrogen production (Kalishwaralal et al., 2010). Silver nanoparticles are also been reported to possess anti-fungal activity, anti-inflammatory effect, anti-viral, anti-angiogenesis and anti-platelet activity (Kalishwaralal et al., 2010).

Reddy et al., (2009) successfully synthesized surfactin-mediated gold nanoparticles and investigated the effects of proton concentrations and temperature on the morphology of the obtained nanoparticles. It was demonstrated that the nanoparticles synthesized at pH 7 and 9 remained stable for 2 months, while aggregates were observed at pH 5 within 24 h. Moreover, the nanoparticles formed at pH 7 were uniform in shape and size and were polydispersed and anisotropic at pH 5 and 9. The nanoparticles synthesized produced at room temperature were monodispersed and were more uniform when compared to those formed at 4°C. More recently they also carried out a biological synthesis of gold and silver

nanoparticles using the bacteria *Bacillus subtilis*. Gold nanoparticles were synthesized both intra- and extracellularly, while silver nanoparticles were exclusively formed extracellularly (Reddy et al., 2010). According to the Authors the nanoparticles were stabilized by the surface-active molecules i.e., surfactin or other biomolecules released into the solution by *B. subtilis*.

Surfactin produced by *Bacillus amyloliquefaciens* KSU-109 was also used for the synthesis of cadmium sulfide nanoparticles which remained stable up to six months without compromising their functionality (Singh et al., 2011). This kind of nanoparticles works as semiconductors with unique optical properties and tunable photo-luminescence allowing potential applications in solar energy conversion, nonlinear optical, photoelectrochemical cells and heterogeneous photocatalysis (Singh et al., 2011). In addition, surfactin produced by strain KSU-109 was easily extracted and used without further purification for nanoparticles stabilization under ambient conditions (Singh et al., 2011). Such simple, inexpensive and environmental friendly procedure of obtaining surfactin offers a further advantage of use in nanobiotechnology for the large-scale production of highly stable metal nanoparticles.

Both rhamnolipids and sophorolipids have also been successfully used for the synthesis and stabilization of metal-bound nanoparticles. Purified rhamnolipids from *P. aeruginosa* strain BS-161R were used to synthesize silver nanoparticles which exhibited good antibiotic activity against both Gram-positive and Gram-negative pathogens and *Candida albicans*, suggesting their broad spectrum antimicrobial activity (Kumar et al., 2010). In another work, a glycolipid biosurfactant produced from sponge-associated marine bacteria *Brevibacterium casei* MSA19, using agro-industrial and industrial waste as substrate, were used as a "green" stabilizer for the synthesis of stable and uniform silver nanoparticles (Kiran et al., 2010b). The biosurfactant acted as stabilization agent and prevented the formation of aggregates.

Palanisamy & Raichur, (2009) also described a simple and eco-friendly method for synthesizing spherical nickel oxide nanoparticles by microemulsion technique using rhamnolipids as alternative surfactant. The synthesized nanoparticles were found to be fully crystalline and spherical in shape with uniform distribution and increasing the pH of the solution decreased the size of the nanoparticles. Xie et al., (2006) were also able to synthesize silver nanoparticles in rhamnolipid reverse micelles while in another study rhamnolipids were used as capping agents for the synthesis of ZnS nanoparticles in aqueous medium (Narayanan et al., 2010).

Sophorolipids were also tested for use in nanoparticles synthesis and reported to be good reducing and capping agents for cobalt and silver particles (Kasture et al., 2007, 2008, as cited in Van Bogaert & Soetaert, 2010). Singh et al., (2009) demonstrated the antibacterial activity of sophorolipid-coated silver and gold nanoparticles against both Gram-positive and -negative bacteria. They also verified that sophorolipid-coated gold nanoparticles were more cyto and geno-compatible with respect to silver nanoparticles (Singh et al., 2010). They also plan to investigate these nanoparticles suitability for medical and diagnostic applications.

Recently, methodologies for the biological synthesis of metal nanoparticles using microbes have also been described (Narayanan & Sakthivel, 2010; Kalishwaralal et al., 2010; Reddy et al., 2010). In addition Smyth et al., (2010b) reported on the production of selectively deuterated rhamnolipids and sophorolipids using deuterated substrates. The production of such deuterated biosurfactants, in particular, or other bioactive microbial products in general, in which distinct pattern of labeling could be achieved resulting in varying molecular

weight products and or stereochemistry unrecognised by existing degradative enzymes is very important. Such molecules would have great future implications with regards to efficacy and/or persistence or the development of resistance for some bioactives particularly in biomedical related applications.

#### 4.4 Microemulsions

Microemulsions are thermodynamically stable, isotropic dispersions of oil, water and surfactant (Rosen, 1989, as cited in Nguyen et al., 2010). Microemulsion systems produce high solubilization capacity and ultralow interfacial tensions of oil and water, making them desirable in practical applications such as enhanced oil recovery, drug delivery, food and cosmetic applications (Bourrel & Schechter, 1988, Kogan & Garti, 2006, Komesvarakul et al., 2006, Lawrence & Rees, 2000, Vandamme, 2002, Yuan et al., 2008, as cited in Nguyen et al., 2010). Xie et al., 2005 demonstrated that rhamnolipids could be successfully used to form microemulsions using medium chain alcohols as cosurfactant. Subsequently, the same Authors observed that the phase behavior and microstructure of these microemulsions were rational to the conformational changes of rhamnolipid molecules at the interface of oil/water (Xie et al., 2007). Microemulsion technique using oil-water-surfactant mixture has also emerged as a promising method for nanoparticle synthesis and can be used to synthesize different types of particles (Eastoe et al., 2006, as cited in Palanisamy & Raichur, 2009). Palanisamy & Raichur, (2009), for example, successfully used rhamnolipids as the surfactant to synthesize spherical nickel oxide nanoparticles by microemulsion technique. In another work, Nguyen & Sabatini, (2009) were able to formulate alcohol-free microemulsions using rhamnolipid biosurfactant and rhamnolipid mixtures.

Lecithin-based microemulsions have proven to be desirable in biocompatible formulations due to their tendency to mimic the phospholipid nature of cell membranes (Nguyen et al., 2010). In a recent report Nguyen et al., (2010) formulated and evaluated microemulsions of lecithin/rhamnolipid/sophorolipid biosurfactants with a range of oils. Sophorolipid played an important role as the hydrophobic component in these formulations and the phase behavior of these biocompatible microemulsions did not change significantly with changing temperature and electrolyte concentration, making them desirable for cosmetic and drug delivery applications.

#### 4.5 A survey over biotechnological commercial applications and patents of biosurfactants and bioemulsifiers

Due to their broad-range of functional properties and the diverse synthetic capabilities of microbes, biological surfactants and emulsifiers have been recently used in various industries like detergents and soaps, petroleum, textile, agriculture, cosmetic, medicine and food (Banat et al., 2000, 2010). Due to their environmental acceptability, biodegradability and lower toxicity, they are generally accepted as good candidates to substitute synthetic surfactants. Commercial applications of biosurfactants and bioemulsifiers in the biotechnological field are mainly related to the oil industry, enhanced oil recovery and bioremediation technologies (Desai & Banat, 1997). However, interesting marketable products and patents have been issued in the last few years in the health care and cosmetic industries, reviewed by Shete et al., (2006) and Banat et al., (2010).

Sugar-based biosurfactants, sophorolipids in particular, are very attractive in these fields, because of their good detergency, emulsifying, foaming and dispersing properties (Faivre & Rosilio, 2010). Sophorolipids are better solubilizers than emulsifiers, but their derivatives

containing propylene glycol have excellent hygroscopic properties and are applied as moisturizer or softener in cosmetic products (Faivre & Rosilio, 2010). For example, a product containing 1 mol of sophorolipid and 12 mol of propylene glycol has excellent skin compatibility and is used commercially as a skin moisturizer (Yamane, 1987, as cited in Desai & Banat 1997). Sophorolipid is commercially used by Kao Co. Ltd. as a humectant for cosmetic makeup brands such as Sofina. This company has developed a fermentation process for sophorolipid production, and after a two-step esterification process, the product finds application in lipstick and as moisturizer for skin and hair products (Inoue et al., 1979 a, 1979b, as cited in Desai & Banat, 1997). Moreover, sophorolipids are also believed to stimulate the leptin synthesis through adipocytes, in this way reducing the subcutaneous fat overload (Pellecier & André, 2004, as cited in Van Bogaert & Soetaert, 2010).

The French company Soliance (<http://www.groupe-soliance.com>) produces sophorolipid-based cosmetics for the body and skin and the Korean MG Intobio Co. commercializes Sopholine cosmetics (Van Bogaert & Soetaert, 2010). They are also found in cleaning soap mixtures (Ecover™ products). Despite the high number of scientific publications and patents, industrial surfactin applications still remain quite limited (Jacques, 2010). Sold by SIGMA and SHOWA DENKO for analytical or laboratory purposes, the compound is also available in several Japanese cosmetic products.

During the last decades, many patents have been issued worldwide in relation with applications of biosurfactants and bioemulsifiers in the health care field (Shete et al., 2006). Bioemulsifiers produced by *Acinetobacter calcoaceticus*, for examples, have been used in shampoos and soaps against acne and eczema and in personal care products. The skin cleansing cream and lotion containing these bioemulsifiers have, among other properties, the ability to interfere with microbial adhesion on skin or hair (Hayes et al., 1989, 1990, 1991, 1992, as cited in Shete et al., 2006). Viscosin and analogues have been patented as antibacterial, antiviral, antitrypanosomal therapeutic compounds that inhibit the growth of *Mycobacterium tuberculosis*, Herpes simplex virus 2 and/or *Trypanosoma cruzi* (Burke et al., 1999, as cited in Shete et al., 2006). *Lactobacillus* biosurfactants have also been patented as inhibitors of adherence and colonization of bacterial pathogens on medical devices (Reid et al., 2000).

Another interesting patented area is related to antimicrobial biosurfactant peptides produced by probiotic strains able to selectively bind to collagen and inhibit infections around wounds at the site of implants and biofilms associated with infections in mammals (Howard et al., 2002, as cited in Shete et al., 2006). Sophorolipids, in particular have been the object of many patents as moisturizing agents and for the amelioration of skin physiology, skin restructuring and repair (Shete et al., 2006). Sophorolipids are also used for the treatment of skin, as an activator of macrophages, and as agent in fibrinolytic healing, desquamating and depigmenting process (Maingault, 1999 as cited in Shete et al., 2006). A germicidal composition containing fruit acids, a surfactant and a sophorolipid biosurfactant, able to kill in 30 seconds 100% of *E. coli*, *Salmonella* and *Shigella*, has been patented for cleaning fruits, vegetable, skin and hair (Pierce & Heilman, 2001).

Rhamnolipids in comparison have been patented in a process to make some liposomes and emulsions (Ishigami & Suzuki 1997; Ramiisse et al., 2000) both important in the cosmetic industry. More recently an activator and anti-aging agent containing MEL as active ingredient has been patented (Suzuki et al., 2010). Another recent invention is directed to polymeric acylated biosurfactants that can self-assemble or auto-aggregate into polymeric micellar structures useful in topically-applied dermatologic products (Owen & Fan, 2010).



Another patent has been deposited about a biosurfactant composition produced by a new *B. licheniformis* strain, with anti-adhesion activity against biofilm producer microbial pathogens (Martinotti et al., 2009).

## 5. Conclusions and perspectives

As evidenced by the growing number of publications on the topic of biosurfactants, there is an increasing interest in the study of these molecules and their potential applications. The demand for new specialty surfactants in the agriculture, cosmetic, food, pharmaceutical, and environmental industries is steadily increasing and biosurfactants, as effective and environmentally compatible compounds, perfectly meet this demand (Banat et al., 2000, 2010; Mukherjee et al., 2006).

The most important limitation for the commercial use of biosurfactants is the complexity and high cost of production, which has limited the development of their use on a large scale (Soberón-Chávez & Maier, 2010). However, the proven antimicrobial, anti-adhesive, immune-modulating properties of biosurfactants and the recent successful applications in gene therapy, immunotherapy and medical insertion safety suggest that it is worth persisting in this field. Moreover, in pharmaceutical and biomedical sectors, the high cost of production could be compensated for by the small amounts of product required. In fact, it has been elucidated that biosurfactants used as pharmaceutical agents are needed only in very low concentrations (Cameotra & Makkar, 2004). Prerequisites for making biosurfactant production more profitable and economically feasible include optimized growth/production conditions and novel and efficient multi-step downstream processing methods as well as the use of recombinant varieties of microorganisms or selected hyperproducing mutants, which can grow on a wide range of cheap renewable substrates (Muthusamy et al., 2008).

Recent advances in the area of biomedical application are probably going to take the lead due to higher potential economic returns. Moreover, due to their self-assembly properties, new and fascinating applications in nanotechnology are predicted for biosurfactants (Kitamoto et al., 2009; Palanisamy, 2008; Reddy et al., 2009). In-depth studies of their natural roles in microbial competitive interactions, cell-to-cell communication, pathogenesis, motility and biofilm formation and maintenance could suggest improved and interesting future applications.

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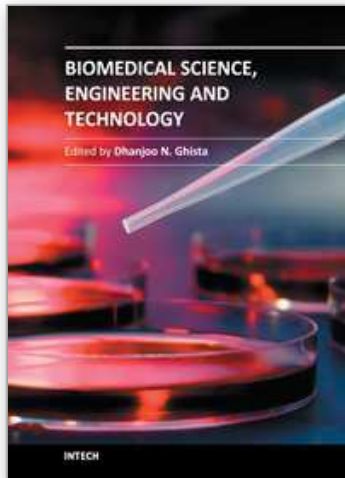
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This innovative book integrates the disciplines of biomedical science, biomedical engineering, biotechnology, physiological engineering, and hospital management technology. Herein, Biomedical science covers topics on disease pathways, models and treatment mechanisms, and the roles of red palm oil and phytochemical plants in reducing HIV and diabetes complications by enhancing antioxidant activity. Biomedical engineering covers topics of biomaterials (biodegradable polymers and magnetic nanomaterials), coronary stents, contact lenses, modelling of flows through tubes of varying cross-section, heart rate variability analysis of diabetic neuropathy, and EEG analysis in brain function assessment. Biotechnology covers the topics of hydrophobic interaction chromatography, protein scaffolds engineering, liposomes for construction of vaccines, induced pluripotent stem cells to fix genetic diseases by regenerative approaches, polymeric drug conjugates for improving the efficacy of anticancer drugs, and genetic modification of animals for agricultural use. Physiological engineering deals with mathematical modelling of physiological (cardiac, lung ventilation, glucose regulation) systems and formulation of indices for medical assessment (such as cardiac contractility, lung disease status, and diabetes risk). Finally, Hospital management science and technology involves the application of both biomedical engineering and industrial engineering for cost-effective operation of a hospital.

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