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Biosurfactants in Pharmaceutical Industry (A Mini-Review)

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Abstract: Biosurfactants can be served as green alternatives in a variety of applications including bioremediation, pharmaceuticals, agricultural disease control and cosmetics. Biosurfactant mixtures produced by microbes and they are genus- and sometimes species-specific. Because of their short fatty acid tails and polar head groups, biosurfactants are highly sticky and both hydrophilic and hydrophobic. In pharmaceutics, biosurfactants can be used for gene delivery and recovery of intracellular products as well as they can be served as antimicrobial substances and emulsifying agents.

Key words: Biosurfactant, gene delivery, pharmaceutics, bioemulsifier, antimicrobial activity

INTRODUCTION

A surfactant is an amphiphilic agent with both lipophilic and hydrophilic structural moieties in its molecule. Surfactants are widely used for industrial, agricultural, food, cosmetic and pharmaceutical applications. Most of these compounds are chemically synthesized and potentially cause environmentally and toxically problems (Schramm *et al*., 2003; Makkar and Rockne, 2003). However, it is only in the past few decades that surface active molecules of microbial origin, referred to as biosurfactants, have gained considerable interest (Desai and Banat, 1997; Healy *et al*., 1996). Biosurfactants are surface-active metabolites produced by microorganisms when grown on water miscible or oily substrates: they either remain adherent to microbial cell surfaces or are secreted in the culture broth (Abouseouda *et al*., 2008; Correa-Bicca *et al*., 1999; Cunha *et al*., 2004; Das and Mukherjee, 2007). They possess the characteristic property of reducing the surface and interfacial tensions using the same mechanisms as chemical surfactants (Singh *et al*., 2007). Microbial surfactants constitute a diverse group of surface-active molecules and are known to occur in a variety of chemical structures, such as glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, phospholipids and polymeric and particulate structures (Desai and Banat, 1997). The features that make them commercially promising alternatives to chemically synthesized surfactants are their lower toxicity, higher biodegradability and hence, greater environmental compatibility, better foaming properties (useful in mineral processing) and stable activity at extremes of pH, salinity and temperature (Amiriyan *et al*., 2004; Batista *et al*., 2006; Bento *et al*., 2005; Bhattacharyya *et al*., 2003; Bodour *et al*., 2003; Cameotra and Makkar, 2004; Chen *et al*., 2007; Christofi and Ivshina, 2002; Cohen and Exerowa, 2007). Unlike chemical surfactants, which are mostly derived from petroleum feedstock, these molecules can be produced by microbial fermentation processes (Fusconi *et al*., 2010; Silva *et al*., 2009) using cheaper agrobased substrates and waste materials (Fox and Bala, 2000; Maneerat, 2005; Nitschke *et al*., 2004; Panilaitis *et al*., 2007; Rashedi *et al*., 2005; Rivardo *et al*., 2009; Rivera *et al*., 2007; Rodrigues *et al*., 2006a). Although, most biosurfactants are considered to be secondary metabolites, some may play essential roles for the survival of biosurfactant-producing microorganisms through facilitating nutrient transport or microbe-host interactions or by acting as biocide agents (Dehghan-Noudeh *et al*., 2005; Déziel *et al*., 1996; Fernandes *et al*., 2007;

Rodrigues *et al.*, 2006a). Biosurfactant roles include increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding (Kavamura and Esposito, 2010; Hoffiman *et al.*, 2010), bacterial pathogenesis and biofilm formation (Fiechter, 1992; Gautam and Tiagi, 2006; Healy *et al.*, 1996; Simoes *et al.*, 2010).

In various industrial processes, they are potentially useful surface-active agents for emulsion (Dhakephalkar *et al.*, 2010; Amaral *et al.*, 2009; Huang *et al.*, 2010). Polymerization, wetting, foaming, phase dispersion, emulsification and de-emulsification (Kosaric, 1992; Martinez-Checa *et al.*, 2007). Biosurfactants have also been found to possess several properties of therapeutic and biomedical importance (Gudiana *et al.*, 2010). They have antibacterial, antifungal (Joshi *et al.*, 2008) and antiviral properties; they inhibit fibrin clot formation and they have anti-adhesive action against several pathogenic microorganisms (Mulligan, 2005; Rodrigues *et al.*, 2004, 2006a; Singh and Cameotra, 2004).

Recently, biosurfactants received much attention in nanobiotechnology criteria (Koopmans and Aggeli, 2010; Palanisamy, 2008; Rodriguez *et al.*, 2010; Cevc and Vierl, 2010; Solanki and Murthy, 2010; Reddy *et al.*, 2009; Palanisamy and Riachur, 2009).

Surface-active compounds produced by microorganisms are of two main types, those that reduce surface tension at the air-water interface (biosurfactants) and those that reduce the interfacial tension between immiscible liquids, or at the solid-liquid interface (bioemulsifiers). Biosurfactants usually exhibit emulsifying capacity but bioemulsifiers do not necessarily reduce surface tension (Kakugawa *et al.*, 2002; Konishi *et al.*, 2007; Langer *et al.*, 2006; Freitas *et al.*, 2009).

Here, we discuss the role and applications of biosurfactants focusing on medicinal pharmaceutical perspectives.

BIOSURFACTANT CLASSIFICATION

Unlike chemically synthesized surfactants, which are classified according to the nature of their polar grouping, biosurfactants are categorized mainly by their chemical composition and their microbial origin. According to the studies of Desai and Banat (1997) and Gautam and Tiagi (2006), biosurfactants, based on the structure of their hydrophilic part, are mainly classified into 5 categories:

- Glycolipids
- Lipopeptides
- Fatty acids
- Polymer type
- Particulate biosurfactants (Desai and Banat, 1997; Gautam and Tiagi, 2006).

Glycolipids

Most known biosurfactants are glycolipids. They are carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids. Among the glycolipids, the best known are rhamnolipids, trehalolipids and sophorolipids (Morita *et al.*, 2006; Sullivan, 1998; Thanomsub *et al.*, 2007).

Lipopeptides and Lipoproteins

A large number of cyclic lipopetides including decapeptide antibiotics (gramicidins) and lipopeptide antibiotics (polymyxins) possess remarkable surface-active properties.

Fatty Acids, Phospholipids and Neutral Lipids

Several bacteria and yeasts produce large quantities of fatty acid and phospholipid surfactants during growth on n-alkanes.

Polymeric Biosurfactants

The best-studied polymeric biosurfactants are emulsan, liposan, mannoprotein and other polysaccharide-protein complexes.

Particulate Biosurfactants

Extracellular membrane vesicles partition hydrocarbons to form a microemulsion which plays an important role in alkane uptake by microbial cells (Monteiro *et al*., 2007; Mukherjee *et al*., 2006; Ortiz *et al*., 2006).

Thus the majority of biosurfactants include low-molecular-weight glycolipids (GLs), lipopeptides (LPS), flavolipids (FLs), phospholipids and high-molecular-weight polymers such as lipoproteins, lipopolysaccharide-protein complexes and polysaccharide-protein-fatty acid complexes. Biosurfactants have a great deal of structural diversity. The common lipophilic moiety of a biosurfactant molecule is the hydrocarbon chain of a fatty acid, whereas the hydrophilic part is formed by ester or alcohol groups of neutral lipids, by the carboxylate group of fatty acids or amino acids (or peptides), organic acid in the case of flavolipids, or, in the case of glycolipids, by the carbohydrate (Rodrigues *et al*., 2006b; Ruiz-Garc *et al*., 2005; Santa Annal *et al*., 2002; Singh *et al*., 2007).

Potential Applications of Biosurfactants in Industries

Surfactants offer extraordinary benefits to many industries. They are involved in infinite number of different industrial processes and physicochemical phenomenon; increasing mobility, increasing solubility, lubrication, removing soil or scouring (Pei *et al*., 2009; Lai *et al*., 2009), wetting, rewetting, softening, retarding dyeing rate, fixing dyes, making emulsions, stabilizing dispersions, coagulating suspended solids, making foams (Hirata *et al*., 2009), preventing foam formation and defoaming (Kosaric, 1992; Zang and Miller, 1992; Zouboulis *et al*., 2003). The most significant application of biosurfactants was studied in bioremediation for example in removing heavy metals from soils (Asci *et al*., 2010; Wang and Mulligan, 2009a, b; Gusiatin *et al*., 2009; Mullingan, 2009; Frazetti *et al*., 2009; Nayak *et al*., 2009).

In addition of all these benefits, biosurfactants have a large number of bioactivities: inhibit bacterial growth (Flagas and Makris, 2009; Sabate *et al*., 2009), toxic effects, immune stimulant, tumor growth inhibition, antibiotic, cell lysis (haemolysis) (Dehghan-Noudeh *et al*., 2005), plant pathogenicity (Joshi *et al*., 2008), effects on migration of human neutrophils, respiratory action (anti- asthma activity), food digestion (Nitschke and Costa, 2007), inhibition of cell wall synthesis, fungicidal properties (Joshi *et al*., 2008) or enzyme stimulation, bio regulatory effects, etc. Hence they play part in many processes in nature (Rodrigues *et al*., 2006b).

These molecules have an unlimited number of uses that involves every industry and every aspect of life: oil industry, pharmaceuticals, testing quality of condoms, hygiene an cosmetics, cement, beer and beverages, textiles, paint, detergents and cleaning (Rai and Mukherjee, 2010) and food processing (Arauz *et al*., 2009). However, the applications depend on applied properties and the mechanism of action.

Potential Applications of Biosurfactants in Pharmaceutics

Kakugawa *et al*. (2002), as well as Mukherjee *et al*. (2006) demonstrated that the biosurfactants could have a wide range of applications in pharmaceutical fields.

Gene Delivery

The establishment of an efficient and safe method for introducing exogenous nucleotides into mammalian cells is critical for basic sciences and clinical applications such as gene therapy. Among

various methods for gene transfection, (Zhang *et al*., 2010; Fujita *et al*., 2009; Liu *et al*., 2010) lipofection using cationic liposomes is considered to be a promising way to deliver foreign gene to the target cells without side-effects. Although, several kinds of cationic liposomes for lipofection have been developed, further studies are still required to develop a non-viral vector which has comparable efficiency to viral vectors (Inoh *et al*., 2001, 2004; Kitamato *et al*., 2002; Maitani *et al*., 2007; Okayama *et al*., 1997).

Kitamato *et al*. (2002) demonstrated that in comparison with commercially available cationic liposomes, liposomes based on biosurfactants show increasing efficiency of gene transfection. Ueno *et al*. (2007) have been developing some new techniques and methodologies for the liposome-based gene transfection. They introduced biosurfactants in this field. They examined MEL-A-containing liposomes for gene transfection.

Immunological Adjuvants

Bacterial lipopeptides constitute potent non-toxic and non-pyrogenic immunological adjuvants when mixed with conventional antigens. A marked enhancement of the humoral immune response was obtained with the low molecular mass antigens iturin AL, herbicolin A and microcystin (MLR) coupled to poly-L-lysine (MLR-PLL) in rabbits and in chickens (Rodrigues *et al*., 2006b).

Inhibition the Adhesion of Pathogenic Organisms to Solid Surfaces

Biosurfactants have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or to infection sites (Das *et al*., 2009); thus, prior adhesion of biosurfactants to solid surfaces might constitute a new and effective means of combating colonization by pathogenic microorganisms (Rivardo *et al*., 2009). Pre-coating vinyl urethral catheters by running the surfactin solution through them before inoculation with media resulted in a decrease in the amount of biofilm formed by *Salmonella typhimurium*, *Salmonella enterica*, *E. coli* and *Proteus mirabilis* (Rodrigues *et al*., 2004). Furthermore, Rodrigues *et al*. (2004) demonstrated that biosurfactants greatly reduced microbial numbers on prostheses and also induced a decrease in the airflow resistance that occurs on voice prostheses after biofilm formation.

Recovery of Intracellular Products

Surfactants have also been used to permeabilise or lyse cells after fermentation as part of the protocol for recovery of intracellular products. Reverse micelle solutions were used for selective permeabilization of *Escherichia coli* to facilitate extraction of penicillin acylase. This process can be achieved by using biosurfactants (Singh *et al*., 2007).

It is well known that highest efficiencies in terms of overall release of intracellular proteins from microbial cells are achieved through aggressive mechanical cell disintegration methods. However, in addition to releasing intracellular proteins, these methods solubilize most of the protein components associated with cell walls, organelles and membranes. More selective permeabilization, achieved by using reagents which render the cell envelope more porous are beneficial for selective release of target proteins where the objective is to obtain an extracted product with a high specific activity or where further protein purification is required. Biosurfactants can be the reagents of choice for membrane permeabilization (Desai and Banat, 1997).

Thus, in the recovery of purified intracellular proteins use of selected biosurfactants to permeabilise cells with selective protein release represents a promising purification option. In selecting biosurfactants for these applications the primary consideration is the efficiency and selectivity of the surfactant in permeabilizing cells with the selective release of the desired product. It is also important to insure the chosen biosurfactant has no negative impact on the stability or activity of the product since biosurfactants may bind to proteins and other bioactive molecules (Desai and Banat, 1997; Singh *et al*., 2007).

Antimicrobial Activity

The diverse structures of biosurfactants confer them to display versatile performance (Ajesh and Sreejith, 2009; Zhao *et al*., 2010). By its structure, biosurfactant is supposed to exert its toxicity on the cell membrane permeability as a detergent like effect. One useful property of many biosurfactants is their antimicrobial activity (Rahman and Ano, 2009). Several biosurfactants have strong antibacterial, antifungal and antiviral activity. Other medically relevant uses of biosurfactants include their role as anti-adhesive agents to pathogens, making them useful for treating many diseases and as therapeutic and probiotic agents. The MEL, a glycolipid biosurfactant, exerted the growth inhibition and differentiation-inducing activities against human leukemia cell lines by directly affect intracellular signal transduction through phosphate cascade system (Tabatabaee *et al*., 2005; Tahzibi *et al*., 2004; Techaoei *et al*., 2007; Thaniyavarn *et al*., 2006).

Das *et al*. (2009) have reported a biosurfactant produced by marine *B. circulans* that had a potent antimicrobial activity against Gram-positive and Gram-negative pathogenic and semi-pathogenic microbial strains including MDR strains.

Fernandes *et al*. (2007) investigated the antimicrobial activity of biosurfactants from *Bacillus subtillis* R14 against 29 bacterial strains. Their results demonstrated that lipopeptides have a broad spectrum of action, including antimicrobial activity against microorganisms with multidrug-resistant profiles (Fernandes *et al*., 2007).

Rodrigues *et al*. (2006c) mentioned in their review about biosurfactants that MELs produced by *Candida antartica*, rhamnolipids produced by *P. aeruginosa* and lipopeptides produced by *B. subtilis*31 and *B. licheniformis* have been shown to have antimicrobial activities (Rodrigues *et al*., 2006d).

Biosurfactants for Cosmetics

Many biosurfactant properties such as emulsification and de-emulsification, foaming, water binding capacity, spreading and wetting properties effect on viscosity and on product consistency, can efficiently be utilized by the above industry.

Surfactants as emulsifiers, foaming agents, solubilizers, wetting agents, cleansers, antimicrobial agents, mediators of enzyme action in various dosage forms like creams, lotions, liquids, pastes, powders, sticks, gels, films, sprays could be used and may be replaced by biosurfactants (Tugrul and Cansunar, 2005; Tuleva *et al*., 2002; Ueno *et al*., 2007; Urum and Pekdemir, 2004; Villeneuve, 2007; Youssef *et al*., 2007).

Cosmetic products using surfactants including; insect repellents, antacids, bath products, acne pads, antidandruff products, contact lens solution, hair colours and care products, deodorants, nail care, body massage accessories, lipsticks, lip makers, eye shades, mascaras, soap, tooth pastes and polishes, denture cleansers, adhesives, antiperspirants, lubricated condoms, baby products, foot care, mousses, antiseptics, shampoos, conditioners, shampoos, conditioners, shave and depilatory products, moisturizers, health and beauty products (Schramm *et al*., 2003). All of these applications could be replaced by using biosurfactants.

CONCLUSION

Chemically synthesized surface-active compounds are widely used in the pharmaceutical, cosmetic, petroleum and food industries. However, with the advantages of biodegradability and production on renewable-resource substrates, biosurfactants may eventually replace their chemically synthesized counterparts. So far, the use of biosurfactants has been limited to a few specialized applications because biosurfactants have been economically uncompetitive. There is a need to gain a greater understanding of the physiology, genetics and biochemistry of biosurfactant-producing strains and to improve process technology to reduce production costs (Youssef *et al*., 2004, 2007; Zang and Miller, 1992; Zouboulis *et al*., 2003).

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