Biosurfactants: A General Overview

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Abstract This Microbiology Monographs volume covers the current and most recent advances in the field of microbial surfactants. There is increasing interest in microbial biosurfactants for several reasons. First, biosurfactants are considered environmentally "friendly" since they are relatively nontoxic and biodegradable. Second, biosurfactants have unique structures that are just starting to be appreciated for their potential application to many different facets of the industry, ranging from biotechnology to environmental cleanup. The aim of this introductory chapter is to give a general overview of biosurfactants, their properties, their relationship to the synthetic surfactant industry, and their distribution in the environment.

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1 Introduction

The demand for new specialty surfactants in the agriculture, cosmetic, food, pharmaceutical, and environmental industries is steadily increasing. Since these surfactants must be both effective and environmentally compatible, it is natural to turn to the microbial world to try to meet this demand (Banat et al. 2000; Mukherjee et al. 2006). Each chapter in this volume focuses on one class of biosurfactant produced by different microorganism and is written by one or more experts who work with these fascinating molecules. These reviews include the physicochemical properties of biosurfactants, their role in the physiology of the microbe that produces it, the biosynthetic pathway for their production, including the genetic regulation, and potential biotechnological applications.

2 Physicochemical Properties, Formation of Micelles, and Other Aggregates

The term surfactant encompasses a wide variety of compounds, both synthetic and biological, all of which have tensioactive properties. These molecules are amphiphilic in nature, having both hydrophilic and hydrophobic domains that allow them to exist preferentially at the interface between polar and nonpolar media (Table 1, Fig. 1). Thus, surfactants tend to accumulate at interfaces (air–water and oil–water) as well as at air–solid and liquid–solid surfaces. Accumulation of surfactants at interfaces or surfaces results in the reduction of repulsive forces between dissimilar phases and allows the two phases to mix and interact more easily. Specifically, surfactants can reduce surface (liquid–air) and interfacial (liquid–liquid) tension. In fact, the effectiveness of a surfactant is determined by its ability to lower the surface tension, which is a measure of the surface free energy per unit area required to bring a molecule from the bulk phase to the surface. The physicochemical characteristics that define a surfactant are its abilities to enhance the apparent water solubility of hydrophobic compounds, to form water–hydrocarbon emulsions, and to reduce surface tension (Desai and Banat 1997).

Typically, effective surfactants lower the surface tension between water and air from 72 to 35 mN/m and the interfacial tension between water and *n*-hexadecane from 40 to 1 mN/m. As surfactant monomers are added into the solution, the surface or interfacial tension will decrease until the surfactant concentration reaches what is known as the critical micelle concentration (cmc). 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, and lamellae (continuous bilayers). These aggregates form as a result of numerous weak chemical interactions between the polar head groups and the nonpolar tail groups including hydrophobic, Van der Waals, and hydrogen bonding. The cmc for any surfactant is dependent on the surfactant structure as

Table 1 Examples of the more common biosurfactants and their origin

Head group	Biosurfactant	Microorganism
Fatty acids, neutral lipids, and phospholipids	Fatty acid	*Corynebacterium lepus* (Cooper et al. 1981)
	Neutral lipid	*N. erythropolis* (Kretschmer et al. 1982)
	Phospholipid	*Thiobacillus thiooxidans* (Knickerbocker et al. 2000)
Lipopeptides	Surfactin	*Bacillus subtilis*, *Bacillus pumilus A* (Seydlova and Svobodova 2008)
	Viscosin	*Pseudomonas fluorescens*, *P. libanensis* (Laycock et al. 1991)
	Serrawettin	*Serratia marcescens* (Matsuyama et al. 1992)
Glycolipids	Mannosylerythritol lipids	Genus *Pseudozyma* (*yeast*), *Candida antartica*, *Ustilago maydis* (Kitamoto et al. 2002)
	Sophorolipids	*C. batistae*, *T. bombicola*, *C. lypolytica*, *C. bombicola*, *T.apicola*, *T.petrophilum*, *C. bogoriensis* (Van Bogaert et al. 2007)
	Rhamnolipids	*Pseudomonas* sp., *P. aeruginosa* (Reiling et al. 1986)
	Trehaloselipids	*Rhodococcus* sp., *Arthrobacter* sp., *R. erythropolis*, *N. erythropolis* (Lang and Philp 1998)
	Cellobiolipids	*Ustilago zeae*, *Ustilago maydis* (Hewald et al. 2005)
Polymeric	Emulsan	*Acinetobacter calcoaceticus* (Rosenberg and Ron 1999)
	Biodispersan	*A. calcoaceticus* (Rosenberg and Ron 1997)
	Mannan–lipid–protein	*C. tropicalis* (Rosenberg and Ron 1999)
	Alasan	*A. radioresistens* (Navonvenezia et al. 1995)
Siderophore	Flavolipids	*Flavobacterium* (Bodour et al. 2004)

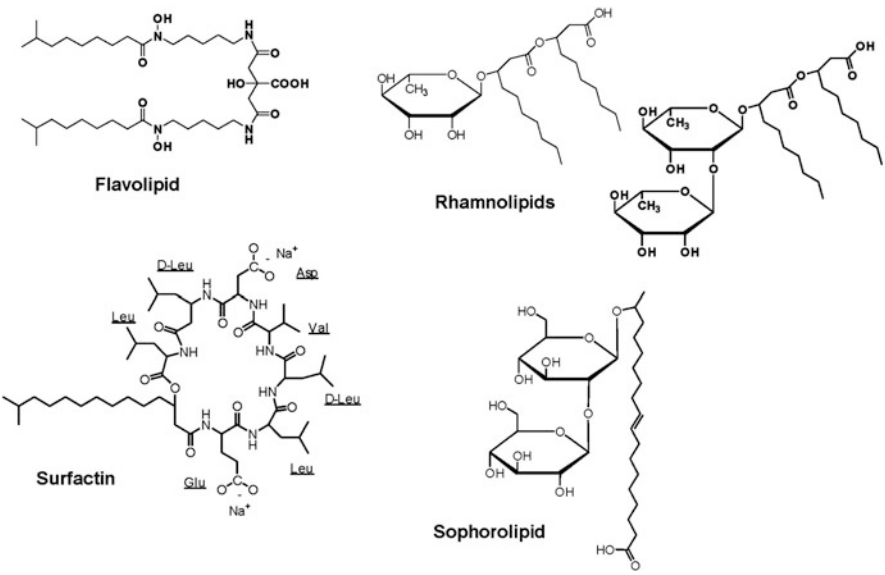

Fig. 1 Representative structures of biosurfactants

well as the pH, ionic strength, and temperature of the solution. Further, the aggregate structure is dictated by the polarity of the solvent in which the surfactant is dissolved. For example, 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 (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 (water in oil micelle).

Biosurfactants are produced by plants and animals as well as microorganisms. A large body of research has been carried out on surfactants produced by bacteria, yeast, and fungi, and the aim of this book is to provide a review of the various microbial surfactants that have been identified. Microbial surfactants have an amazingly wide range of chemical structures (Fig. 1) and each appears to play different roles in the life cycle of the producing microorganism (Ron and Rosenberg 2001). Biosurfactants display important biological activities, including antibiotic, antifungal, insecticidal, antiviral, immunomodulator, and antitumoral activities. These activities are the basis for growing interest in a number of specialty applications such as biological control of pests in agriculture (Stanghellini and Miller 1997), cancer treatment (Saini et al. 2008), and wound healing (Piljac et al. 2008; Stipcevic et al. 2006). The biosurfactants that have been studied in most detail include the rhamnolipids produced by *Pseudomonas aeruginosa* (Soberon-Chavez et al. 2005) and different *Burkholderia* species (Dubeau et al. 2009), and surfactin, a lipopeptide that is synthesized by *Bacillus subtilis* (Mulligan 2005). These are examples of effective biosurfactants that can each reduce the surface tension between pure water and air, from 73 mN/m to less than 30 mN/m.

The worldwide surfactant industry was valued at $20 billion in 2006 with the United States/Canada, Western Europe, and China accounting for approximately 70% of the market (Janshekar et al. 2007). Surfactants are used in a wide variety of industries that produce household and industrial cleaners, personal care products, and in various types of manufacturing including food processing and the production of plastics, paints and coatings, textiles, pulp and paper, and agricultural products. These compounds are also used in the specialty chemical market as components of cosmetic products, pharmaceuticals, emulsifiers, wetting agents, and in the synthesis of fine chemicals. Presently, the vast majority of surfactants used are synthetic; however, in light of their unique chemical characteristics, biosurfactants have been recognized for their potential utility in many applications in a number of recent reviews, e.g., (Bonmatin et al. 2003; Desai and Banat 1997; Kosaric 2001; Kralova and Sjoblom 2009; Lang and Wullbrandt 1999; Maier and Soberon-Chavez 2000; Nitschke and Costa 2007; Ritter 2004; Rodrigues et al. 2006a; Ron and Rosenberg 2001; Rosenberg and Ron 1999; Singh et al. 2007).

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. Thus far, the only commercially available biosurfactants are rhamnolipids and surfactin. Rhamnolipids are produced by a small number of companies such as Jeneil Biosurfactant Company (JBR products) and Rhamnolipid, Inc. (http://www.rhamnolipidholdings.com). However, even these companies do

not always have readily available product for sale, underscoring the tenuous nature of the commercial availability of biosurfactants to date. One of the reasons that there are commercial sources of rhamnolipids is that they are the only biosurfactant thus far that has been approved by US Environmental Protection Agency for use in food products, cosmetics, and pharmaceuticals (Nitschke and Costa 2007).

Despite the current limitations to commercial production of biosurfactants, there is great interest in these materials since they are considered to be "green" alternatives to synthetic surfactants (Ritter 2004). Biosurfactants are considered relatively nontoxic and biodegradable, but perhaps more importantly, the chemical structure of biosurfactants is unique and exhibits great structural diversity including glycolipids, lipopeptides, fatty acids and neutral lipids, siderophore–lipids, and polymeric surfactants (Table 1). Biosurfactants are intriguing, in that they are produced as complex mixtures of up to 40 congeners where the hydrophilic head groups are fairly conserved and the hydrophobic tail groups have considerable variation (Bodour et al. 2004; Monteiro et al. 2007). Component congeners within these complex mixtures can have remarkably different properties, and behavioral differences between biosurfactant classes can be equally divergent. In contrast to common synthetic surfactants that typically possess alkyl chains of ten or more carbon units, many biosurfactants possess surprisingly short alkyl chains. Such structures enhance the aqueous solubility of these biosurfactants but render Van der Waals attractive interactions relatively weak. Despite this structural attribute, biosurfactants do aggregate in solution, supplemented by intermolecular forces such as hydrogen bonding. Moreover, not only do these biosurfactants aggregate in solution, but they also exhibit powerful surfactant activity at both liquid and solid surfaces. Thus, it seems that nature has uniquely and intentionally positioned these biosurfactants to possess characteristics that lie at the boundary between conventional hydrophilic and hydrophobic organic molecules. The structures of these biosurfactants are quite elegant and, in many cases, defy conventional chemical intuition that would predict little surface activity. Despite their aqueous solubility, they can have remarkably low cmcs when compared to structurally similar synthetic surfactants. For example, nonionic (i.e., low pH), multicomponent monorhamnolipid mixtures, in which the heptyl chain congener is most prevalent, have cmc values that range from <1 μM to ~10 μM depending on solution ionic strength (Lebron-Paler 2008). This cmc value increases as the pH increases and the rhamnolipids become deprotonated, reaching values on the order of ~100 μM at pH 8. In contrast, the nonionic alkyl glucosides and glucamides of comparable alkyl chain length that are structurally similar to the rhamnolipids at pH 4 have cmc values on the order of 10^{-3} M or higher (Nickel et al. 1992; Soderman and Johansson 2000; Zhang and Marchant 1996), at least one order of magnitude and in many cases several orders of magnitude greater than those of the rhamnolipids. As a result of their remarkable properties, there is tremendous interest in these molecules for uses as diverse as bioremediation of organics (e.g., Chen et al. 2005; Garcia-Junco et al. 2003; Mulligan 2009; Olivera et al. 2000; Schippers et al. 2000; Shin et al. 2005; Urum and Pekdemir 2004; Uysal and Turkman 2005) and metals (e.g., Dahrazma and Mulligan 2007; Mulligan et al. 2001; Neilson et al. 2003; Ron and Rosenberg

2002; Sandrin et al. 2000; Tan et al. 1994; Torrens et al. 1998; Wang and Mulligan 2009; Wen et al. 2009), cosmetic additives (Hayase and Ishihata 2006; Pianelli et al. 2002; Yoneda et al. 2001), pharmaceutical preparations (Piljac et al. 2008; Singh and Cameotra 2004; Stipcevic et al. 2005, 2006), and coatings resistant to bacteria (Meylheuc et al. 2006; Rodrigues et al. 2004; Rodrigues et al. 2006b; Vollenbroich et al. 1997).

3 Biosurfactant Production in the Environment

Biosurfactant production by bacteria has been studied mainly from the perspective of biotechnological potential. Thus, biosurfactant-producing organisms have been isolated from a wide diversity of environments including soil, sea water, marine sediments, oil fields (Yakimov et al. 1998) and even extreme environments (Cameotra and Makkar 1998). Not only are biosurfactant producers widely distributed but also many different microbes produce biosurfactants. Bacterial genera described to produce surfactants include: *Pseudomonas*, *Rhodococcus*, *Mycobacterium*, *Nocardia*, *Flavobacterium*, *Corynebacterium*, *Clostridium*, *Acinetobacter*, *Thiobacillus*, *Bacillus*, *Serratia*, *Arthrobacter*, and *Alcanivorax* (Bodour et al. 2003). Some of these genera have multiple species that produce different kinds of surfactants. For example, *P. aeruginosa* produces rhamnolipids, and several *Pseudomonas* species, including *P. fluorescens*, produce cyclic lipopeptides (CLP) (Raaijmakers et al. 2006), which are similar to surfactin and other CLP produced by *Bacillus*.

There are still relatively few studies that have addressed the frequency and distribution of biosurfactant producers in the environment. These studies suggest that only a small fraction of the community is capable of biosurfactant production unless a selective pressure exists. For example, one report determined the distribution of culturable bacteria that were able to produce biosurfactants from undisturbed and contaminated sites (Bodour et al. 2003). Twenty sites were sampled resulting in 1,305 isolates of which 45, or 3.4% of the total, were found to be biosurfactant producers. Of the 45 biosurfactant producers, there were 16 unique isolates, which included *B. subtilis*, *P. aeruginosa*, *Pseudomonas* sp., and a *Flavobacterium* sp. Biosurfactant producers were obtained from a majority, but not all, of the soils tested. Of the three soils tested that were co-contaminated with both organics and metals, 8.4% of the 203 isolates obtained produced surfactants, suggesting that some environments may have greater selective pressure for biosurfactants production than others. The biosurfactant produced by the *Flavobacterium* isolate was subsequently identified and represents an entirely new class of biosurfactants, the flavolipids (Bodour et al. 2004).

In a more recent study, (Toribio et al. unpublished) screened for biosurfactant production in a group of 700 bacterial isolates, mainly *Pseudomonas*, taken over a 4 year period (2003–2005 and 2007) from the extremely oligotrophic water column at Cuatro Ciénegas Basin in the Mexican state of Coahuila (Souza et al. 2006). Only six of the isolates obtained produced a biosurfactant including one *P. aeruginosa*,

one *Pseudomonas moseelii*, three *Pseudomonas koreensis*, and one *Serratia marcescens* (Toribio et al. unpublished). Although these results are not completely comparable with the results discussed previously (Bodour et al. 2003), because the screening for bacteria was done in oligotrophic water samples and was biased for *Pseudomonas*, the screening was not restricted to this genus.

Based on the results of these two studies, the fraction of culturable isolates that produce biosurfactants is small. However, it is possible and even likely that other biosurfactant-producing populations were present in the soil and water samples tested but were not enriched by the screening conditions used. Despite the limitations of the screening methods used in these studies, a diverse group of biosurfactant-producing organisms was obtained, including one novel biosurfactant producer (*Flavobacterium* sp.), suggesting that a more exhaustive screening has the potential to yield other new biosurfactant producers.

The question remains: what is the role of biosurfactant production in the ecology of environmental bacteria? Although many roles have been proposed based on pure culture studies, researchers are just beginning to explore how biosurfactants function in situ. Since biosurfactant production requires valuable resources and energy from the producing isolate, it is likely that their production provides an advantage in the competition for resources or in protection under harsh environmental conditions. For example, (Toribio et al. unpublished) propose that biosurfactant production by *P. koreensis* isolates from the Cuatro Ciénegas Basin might play an ecological role in survival in this extremely oligotrophic environment, enabling the bacteria that produce these tensioactive compounds to restrain the growth of competitors when growing as a part of biofilms or bacterial mats. As a second example, a study of 57 polyaromatic hydrocarbon-degrading isolates obtained from hydrocarbon-contaminated soil sites showed that 67% of the isolates produced surfactants, suggesting that biosurfactants production is an important characteristic in this subset of microorganisms (Willumsen and Karlson 1997). Interestingly, the production of biosurfactants by these isolates did not necessarily correlate with their ability to degrade hydrocarbons. As a final example, a recent study examined heterotrophic bacteria that were cultured from a mine tailings site. The mine site is characterized by low pH (2.5–4) and high metal content (up to 4 g/kg of arsenic and lead respectively). Total cultural counts on R2A agar (at neutral pH) were approximately 600 CFU/g tailings, a very low count, which indicates the high level of stress in this environment. Five unique isolates were obtained from this study and all of them produce biosurfactants, suggesting that this may be an important survival trait for microbes in this site (Solis-Dominguez and Maier unpublished).

4 Conclusions

The chapters presented in this book provide in-depth reviews of the best-studied major groups of biosurfactants discovered thus far including the rhamnolipids, surfactin and related lipopeptides, the serrawettins, trehalose lipids, mannosyl

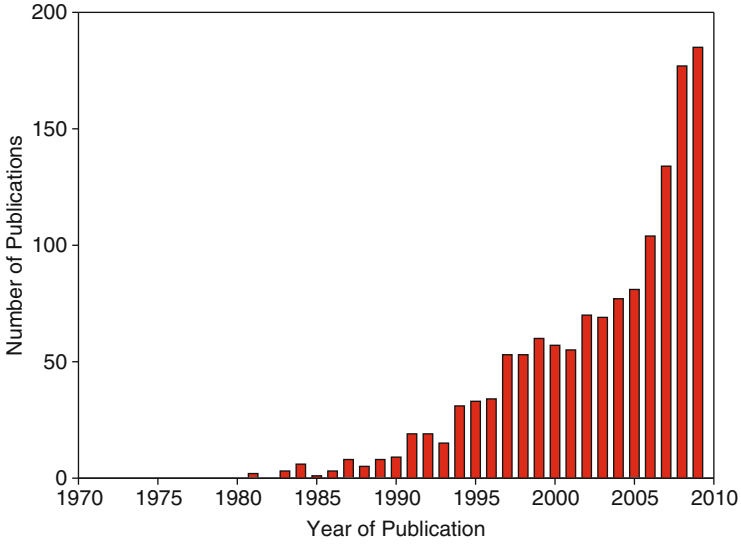

Fig. 2 The number of publications obtained from a year by year search for the term "biosurfactant" on the Thomson Reuters ISI Web of Knowledge search platform (2009)

erythriol lipids, and sophorolipids. There is a rapidly increasing body of research on these molecules as well as other newly discovered biosurfactants as evidenced by the rapidly growing number of publications on the topic of biosurfactants (Fig. 2). We expect in the next 5–10 years, as yields increase and production costs decrease, new biosurfactants continue to be discovered, and the chemistry and potential applications of these molecules are better understood, that biosurfactants will begin to compete favorably with synthetic surfactants in the surfactant industry, particularly in specialty surfactant markets.

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