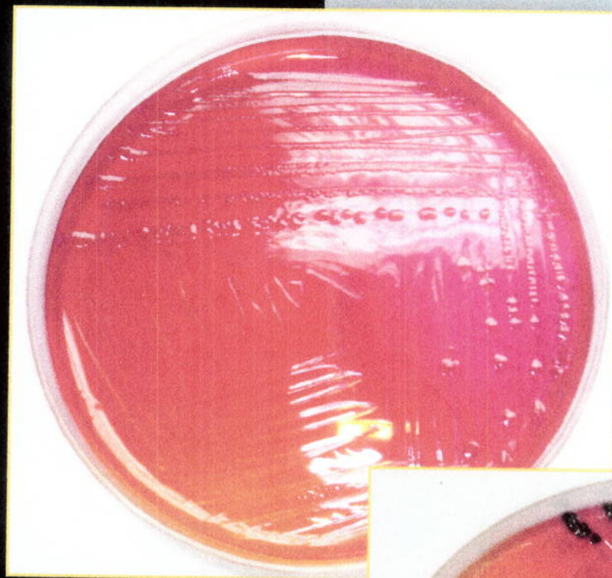
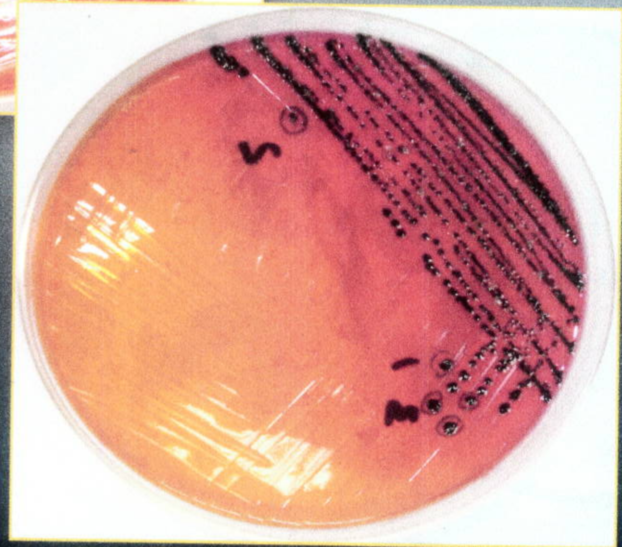
BIOSURFACTANTS

Research Trends and Applications





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6 Characterization, Production, and Applications of Lipopeptides

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INTRODUCTION

Surfactants are amphiphilic compounds that reduce the free energy of the system by replacing the bulk molecules of higher energy at an interface. They contain a hydrophobic portion with little affinity for the bulk medium and a hydrophilic group that is attracted to the bulk medium. Surfactants have been used industrially as adhesives; flocculating, wetting, and foaming agents; deemulsifiers; and penetrants (Mulligan and Gibbs, 1993). They are used for these applications based on their abilities to lower surface tensions and increase solubility, detergency power, wetting ability, and foaming capacity. Petroleum users have traditionally been the major users, as in enhanced oil removal applications by increasing the solubility of petroleum components (Falatko, 1991). They have also been used for mineral flotation and in the pharmaceutical industries. Typical desirable properties include solubility enhancement, surface tension reduction, the critical micelle concentrations (CMCs), wettability, and foaming capacity.

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Surfactants are classified as cationic, anionic, zwitterionic, and nonionic and are made synthetically from hydrocarbons, lignosulfonates, or triglycerides. Some common synthetic surfactants include linear alkyl benzenesulfonates, alcohol sulfates, alcohol ether sulfates, alcohol glyceryl ether sulfonates, α-olefin sulfonates, alcohol ethoxylates, and alkylphenol ethoxylates (Layman, 1985). Surfactants have many applications industrially with multiphasic systems. Sodium dodecyl sulfate (SDS, $C_{12}H_{25}$-SO_4^- Na^+) is a widely used anionic surfactant. The effectiveness of a surfactant is determined by surface tension lowering, which is a measure of the surface free energy per unit area or the work required to bring a molecule from the bulk phase to the surface (Rosen, 1978). These amphiphilic compounds (containing hydrophobic and hydrophilic portions) concentrate at solid–liquid, liquid–liquid, or vapor–liquid interfaces. An interfacial boundary exists between two immiscible phases. The hydrophobic portion concentrates at the surface while the hydrophilic is oriented toward the solution. A good surfactant can lower the surface tension of water from 72 to 35 mN/m and the interfacial tension (tension between nonpolar and polar liquids) for water against n-hexadecane from 40 to 1 mN/m. Efficient surfactants have a low CMC (i.e., less surfactant is necessary to decrease the surface tension) as the CMC is defined as the minimum concentration necessary to initiate micelle formation (Becher, 1965). In practice, the CMC is also the maximum concentration of surfactant monomers in water and is influenced by pH, temperature, and ionic strength.

An important factor in the choice of surfactant is the product cost (Mulligan and Gibbs, 1993). In general, surfactants are used to save energy and consequently energy costs (such as the energy required for pumping or mixing). Charge type, physicochemical behavior, solubility, and adsorption behavior are some important selection criteria for surfactants.

Some surfactants, known as biosurfactants, are biologically produced from yeast or bacteria (Lin, 1996). They can be potentially as effective with some distinct advantages over the highly used synthetic surfactants due to high specificity, biodegradability, and biocompatibility (Cooper, 1986).

Biosurfactants are grouped as glycolipids, lipopeptides, phospholipids, fatty acids, and neutral lipids (Bierman et al., 1987). Most of these compounds are either anionic or neutral, with only a few cationic ones. The hydrophobic parts of the molecule are based on long-chain fatty acids, hydroxy fatty acids, or α-alkyl-β-hydroxy fatty acids. The hydrophilic portion can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid, or alcohol. A wide variety of microorganisms can produce these compounds. The CMCs of the biosurfactants generally range from 1 to 200 mg/L and their molecular weights (MWs) from 500 to 1500 amu (Lang and Wagner, 1987).

LIPOPEPTIDE BIOSURFACTANTS

Lipopeptides are produced by a variety of microorganisms, including *Bacillus*, *Lactobacillus*, *Streptomyces*, *Pseudomonas*, and *Serratia* (Cameotra and Makkar, 2004; Georgiou et al., 1992). The lipopeptides are cyclic peptides with a fatty acyl chain. Various lipopeptides include surfactin (Roongsawang et al., 2003; Youssef et al., 2007), lichenysin A (Yakimov et al., 1995) or C (Jenny et al., 1991), B (Folmsbee et al., 2006), D (Zhao et al., 2010), bacillomycin (Roongsawang et al., 2003), fengycin

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(Vanittanakom and Loeffler, 1986), and iturin (Bonmatin et al., 2003). Surfactin is a cyclic heptapeptide, with antibacterial, antifungal, antiviral, and antitumor activities (Folmsbee et al., 2006; Zhao et al., 2010).

Lipopeptides have been tested in enhanced oil recovery and the transportation of crude oils (Hayes et al., 1986). They were demonstrated to be effective for antimicrobial activity and in the reduction of the interfacial tension of oil and water and the viscosity of the oil, the removal of water from the emulsions prior to processing, and the release of bitumen from oil sands. Although most biosurfactant-producing organisms are aerobic, a few anaerobic producers exist. *Bacillus licheniformis* JF-2 is an example, which would be well suited for in situ studies for enhanced oil recovery or soil decontamination (Javaheri et al., 1985).

Surfactin is the most studied lipopeptide and consists of a seven-amino acid sequence in a cyclical structure with a 13–16 carbon fatty acid (Kakinuma et al., 1969) and has two charged amino acids (glutamic and aspartic acids). In addition to surfactin, iturins and fengycins are also produced (Deleu et al., 1999). Their structures are shown in Figures 6.1 through 6.3. Iturins are cyclic peptides with seven amino acids and a ß-amino closure. Fengycin lipopeptides are ß-hydroxy fatty acids with an eight-member ring in an N-terminal decapeptide. At the C-terminal end, there is a tyrosine residue at position 3. This forms an eight-member lactone ring. Fengycin A and B vary at position 6. The A form has an Ala compared to the B form of a valine.

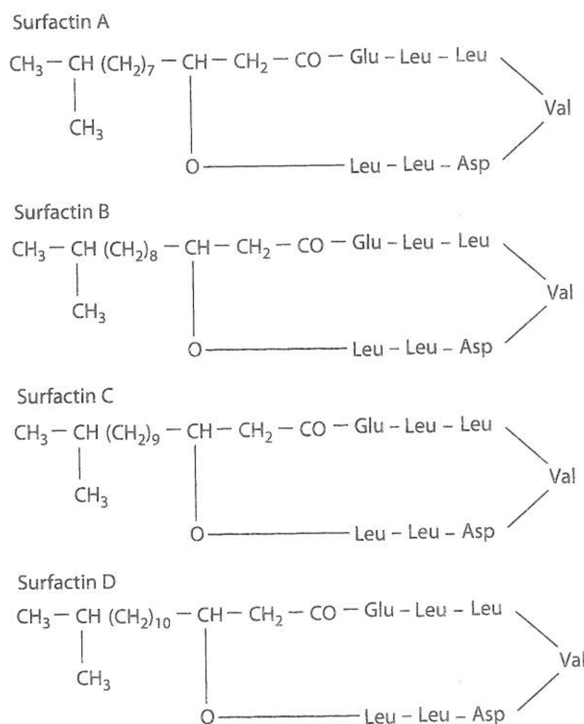

FIGURE 6.1 Structures of various forms of surfactin. (Adapted from Janek, T. et al., *Bioresour. Technol.*, 101, 6118, 2010.)

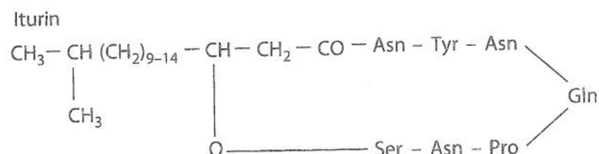

FIGURE 6.2 Structure of iturin.

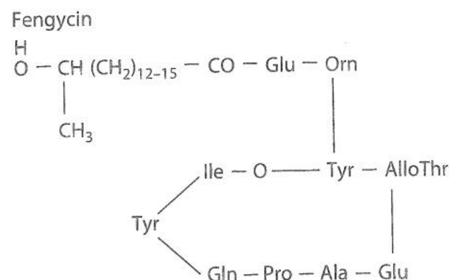

FIGURE 6.3 Structure fengycin.

The fatty acid normally varies from 14 to 18 carbons in length (Arima et al., 1968; Matsuyama et al., 1992; Roongsawang et al., 2003). Fengycin has three charged amino acids (two glutamic acids and an ornithine). The CMC is 6.25 mg/L.

Other lipopeptides have also been studied. They include lichenysin from *B. licheniformis* (Grangemard et al., 1999; Horowitz et al., 1990), arthrofactin from *Arthrobacter* sp. (now known as *Pseudomonas* sp. MIS38) (Morikawa et al., 1993; Roongsawang et al., 2003), pumilacidin from *P. pumilus* (Naruse et al., 1990), and serrawettin from *Serratia marcescens* (Matsuyama et al., 1992). Others include massetolide A (Sen and Swaminathan, 1997), putisolvins I and II (Kuiper et al., 2004), and pumilacidin (Naruse et al., 1990). As can be seen, there is no generally accepted nomenclature based on the structure. *Pseudomonas* lipopeptides include viscosin, amphisin, tolassin, and syringomycin (Raaijmakers et al., 2006). Viscosin has nine amino acids with a 3-hydroxy fatty acid, whereas amphisin has 11 amino acids linked to a similar fatty acid (Soresen et al., 2001). A comparison of the CMC of some lipopeptides is shown in Table 6.1.

TABLE 6.1
CMC Values of Various Isolated Lipopeptides

Lipopeptide	Microbial Source	CMC (mg/L)	Reference
Fengycin	*B. circulans* DMS-2	10–13	Sivapathasekaran et al. (2009, 2010)
Surfactin	*B. subtilis*	17	Deleu et al. (1999)
Surfactin and fengycin	*B. subtilis*	10 and 11	Lin et al. (1998)
Biosurfactant	*B. licheniformis*	0.6	Barros et al. (2008)
Lipopeptide	*Rhodococcus* sp.	23.7	Peng et al. (2008)

Synthesis of the lipopeptides is performed based on a series of enzymes for each step of amino acid addition, ring closure, and acylation (Peypoux et al., 1999). This makes genetic manipulation difficult for enhanced production. Most studies are concentrated on growth optimization and isolation of overproducers (Peypoux et al., 1999) with the exception of a few studies (Gu et al., 2007; Nakayama et al., 1997; Ohno et al., 1995; Peypoux et al., 1999).

Most of the focus has been on higher-priced applications due to the low yields and high cost of the media. Higher-volume low applications including environmental remediation, enhanced oil recovery, laundry soaps, and polymerization of emulsions, need the development of low-cost substrates such as agroindustrial wastes (Makkar and Cameotra, 1999; Mukherjee et al., 2006) and isolation techniques, bioreactor design, and higher yields.

Surfactin

B. subtilis produces surfactin, one of the first lipopeptides found in 1968 (Figure 6.1). It has seven amino acids bonded to the carboxyl and hydroxyl groups of a 14-carbon acid (Kakinuma et al., 1969) and has blood clotting properties. Surfactin concentrations as low as 0.005% reduce the surface tension to 27 mN/m, making it a powerful biosurfactant. The CMC can be as low as 10 mg/L (Dae et al., 2006). The interfacial tension for hydrocarbon–water interfaces can be less than 1 mN/m.

The primary structure of surfactin was determined many years ago by Kakinuma et al. (1969). It is a heptapeptide with a β-hydroxy fatty acid within a lactone ring structure. The seven amino acids are bonded to the carboxyl and hydroxyl groups of a 14-carbon acid. More recently, the three-dimensional structure was determined by ^{1}H NMR techniques (Bonmatin et al., 1995). Surfactin folds into a β-sheet structure, which resembles a horse saddle both in aqueous solutions and at the air/water interface (Ishigami et al., 1995). The solubility and surface-active properties of the surfactin are dependent on the orientation of the residues. The fatty acyl chain with the hydrophobic residues formed one face, while the two carboxylic acid side chains form a claw structure enabling the chelation of heavy metals (Bonmatin et al., 1994; Gallet et al., 1999; Magetdana and Ptak, 1992). This property has been evaluated in soil remediation studies (Mulligan et al., 1999).

Mixtures of surfactin produced by *B. subtilis* have been characterized by mass spectrometry (Hue et al., 2001). A combination of liquid-secondary ion mass spectrometry (LSI-MS) and high-energy tandem mass spectrometry (MS/MS) showed that the amino acid composition or length of the acyl chain can vary from 12 to 16 carbons. Leucine and isoleucine can also be differentiated. Data obtained from protonated and cationized fragments were also useful for structural characterization. They are known as A, B, C, and D forms (Figure 6.1).

Surfactin Production

Most biosurfactants are produced from hydrocarbon substrates (Syldatk and Wagner, 1987). Production can be growth associated. In this case, they can either use the emulsification of the substrate (extracellular) or facilitate the passage of the substrate through the membrane (cell membrane associated). Biosurfactants, however, are also produced from carbohydrates, which are very soluble. Gram-positive and gram-negative

bacteria can produce cyclic lipopeptides. The different structures can lead to different properties. The biosurfactants have been postulated to enhance the growth on hydrocarbons and, in this case, may influence the ecology of the host sponge.

Solid-state fermentation using okara, a soybean curd residue, has been performed by Ohno et al. (1995). Other substrates studied have included starch (Sandrin et al., 1990), cassava waste (Santos et al., 2000), molasses (Makkar and Cameotra, 1997b), soybean (Kim et al., 2009), and potato wastes (Fox and Bala, 2000). Surfactin yields from an autoclaved purified starch were 0.154 g/g. Low solid potato effluents exhibited a 66% lower surfactin yield than the purified starch (Thompson et al., 2001). It has been postulated that higher yields result from nutritional limitations.

Surfactin yields during production are low (0.02 g/g glucose) (de Roubin et al., 1989). Addition of iron and manganese can enhance concentrations to 0.7 g/L (Rosenberg, 1986). Further work on iron addition performed by Wei and Chu (1998) determined that addition of 1.7 mM of iron can lead to the production of up to 3.5 g/L of surfactin and enhanced biomass production. Alkaline addition is required to overcome the decrease in pH to below 5 due to acid formation. Further studies by Wei and Chu (2002) showed the effect of manganese on nitrogen utilization and subsequently surfactin production. A 0.1 mM magnesium sulfate concentration increased almost ninefold the surfactin level to 2.6 g/L. Wei et al. (2007) subsequently used the Taguchi method to optimize surfactin production with regard to the presence of Mg, K, Mn, and Fe. They found that K and Mg were critical. Kinsinger et al. (2003, 2005) further determined that concentrations of the four ions could be optimized and allowed the production of 3.34 g/L of surfactin.

Yields of 0.14 g/g sugar have been obtained using peat as a substrate after hydrolysis with 0.5% sulfuric acid for 1 h at 120°C (Sheppard and Mulligan, 1987). Citric acid addition to glucose media could also enhance production (de Roubin et al., 1989). In attempts to influence the metabolic pathway, glutamic acid, leucine, aspartic acid, and valine were added to the media but did not enhance production. Nitrogen, however, was a significant factor in surfactin production. Doubling ammonium nitrate concentrations from 0.4% to 0.8% increased yields by a factor of 1.6, while organic nitrogen addition did not have any benefit.

Other investigators (Davis et al., 1999) found that surfactin yields were highest in nitrate-limited oxygen-depleted conditions, followed by ammonium-limited (0.075 g surfactin per g biomass), oxygen-depleted conditions (0.012 g/g biomass), and carbon-limited, oxygen-depleted conditions (0.0069 g/g biomass).

A strain of *B. subtilis* was able to produce biosurfactant at 45°C at high NaCl concentrations (4%) and a wide pH range (4.5–10.5) (Makkar and Cameotra, 1997a,b). It was able to remove 62% of the oil in a sand pack saturated with kerosene and thus could be used for in situ oil removal and cleaning sludge from sludge tanks.

Makkar and Cameotra (2002) studied another strain of *B. subtilis* MTCC2423. They found it preferred sodium or potassium nitrate (3 g/L) or urea (1 g/L). Magnesium concentrations of 2.43 mM and calcium concentrations of 0.36 mM were optimal for biosurfactant yield. Unlike for previous studies for *B. subtilis* by de Roubin et al. (1989), aspartic acid, asparagine, glutamic acid, valine, and lysine increased biosurfactant production by 60%. While glycine and leucine addition had no affect, alanine and arginine decreased production. Production was good even at high concentrations of NaCl (up to 4%) and pH values from 4.5 to 10.5.

Solid carriers have also been evaluated for surfactin yield enhancement (Yeh et al., 2005). Activated carbon and expanded clay were added at concentrations of 133 g/L. Surfactin at concentrations of 2150 and 3300 mg/L were obtained for each carrier, respectively. Activated carbon was more appropriate for the fermentation process and seemed to increase cell growth and thus yield. A summary of the yields of surfactin can be seen in Table 6.2.

Das et al. (2009) determined that antimicrobial activity was obtained from a glucose substrate, instead of sucrose, starch, and glycerol. Emulsifying lipopeptide biosurfactants from *Azotobacter chroococcum* can be produced from oil (crude, waste motor lubricant) and peanut oil cake (Thavasi et al., 2009).

Production of another lipopeptide, brevifactin, was characterized and optimized by the marine strain *Brevibacterium aureum* MSA13 (Kiran et al., 2010).

TABLE 6.2
Production of Surfactin

B. subtilis Strain	Substrate	Surfactin Yield or Concentration	Reference
ATCC 21332	Synthetic or semisynthetic peat hydrolysate	100–250 mg/L	Arima et al. (1968); Cooper et al. (1981)
RB14	Aqueous two phase	160 mg/L 350 mg/L	Sheppard and Mulligan (1987)
	Semisynthetic	250 mg/L	Drouin and Cooper (1992)
	Solid-state okara	200–250 mg/kg wet mass	Ohno et al. (1992) Ohno et al. (1992)
MI113 (pC12)	Semisynthetic	350 mg/L	
MI113 (pC12)d	Solid-state okara	2000 mg/kg wet mass	Ohno et al. (1992)
ATCC 55033	Semisynthetic		Ohno et al. (1992)
		3500–4300 mg/L	Carrera et al. (1992)
Mutant strain of ATCC 21332	Synthetic	2000–4000 mg/L 550 mg/L	Carrera et al. (1993a,b) Mulligan et al. (1989)
C9 (KCTC 8701P) ATCC 21332	Glucose with modified salts and oxygen limitation	7.0 g/L	Kim et al. (1997)
ATCC 21332	Glucose and mineral salts with iron	3.5 g/L	Wei and Chu (1998)
ATCC 21332	Glucose with oxygen and nitrogen depletion	0.44 g/L	Davis et al. (1999)
MTCC 2423	Purified starch	0.154 g/g	Fox and Bala (2000)
SD 901 ATCC 21332	Sucrose with mineral salts	1.23 g/L 8,000–50,000 mg/L	Makkar and Cameotra (2002)
Isolate	Bean extract		Yoneda et al. (2006)
	Solid carriers	2150–3300 mg/L	Yeh et al. (2005)
	Sucrose with foam collection	0.25 g/g	Amani et al. (2010)

Source: Adapted from Shaligram, N.S. and Singhal, R.S, *Food Technol. Biotechnol.*, 48: 119–134, 2010.

Various agro and industrial solid waste substrates including molasses, olive oil, and acrylamide were evaluated. The biosurfactant was stable over the pH range of 5–9, and up to 5% NaCl and a temperature of 121°C. The surface tension was 28.6 mN/m. The lipopeptide was characterized as an octadecanoic acid methyl ester with four amino acids pro–leu–gly–gly. This lipopeptide, thus, could have potential for microbial enhanced oil recovery and oil spill remediation.

LIPOPEPTIDE PRODUCTION REACTOR DESIGN AND OPTIMIZATION

Free and immobilized cells of *B. subtilis* ATCC 21332 were grown to produce surfactin and fengycin (Chtioui et al., 2010). Although the production of both biosurfactants was enhanced by two to four times, fengycin was particularly improved. N-heptane was used for extracting the biosurfactant. A continuous extraction with a liquid membrane called petraction was used, but the stripping was too slow. Further optimization is needed. Petraction was also employed by Dimitrov et al. (60) for surfactin. At pH 5.65, 97% recovery was achieved compared to 83% at pH 6.05 in 4 h. However, approximately 90% was removed in 30 min.

Further studies were performed using a rotating disk bioreactor (Chtioui et al., 2012). Cells were immobilized on the rotating disks. Foaming did not occur as the aeration was bubbleless. Fengycin production was favored (838 mg/L) compared to surfactin (212 mg/L). Increasing the number of disks improved the production of both products. Surfactin production was more correlated with improved oxygenation, while the fengycin production was related to more biofilm formation.

A two-phase reactor with polyethylene glycol and dextran (D-40) was evaluated for surfactin production by *B. subtilis* ATCC 21332 (Drouin and Cooper, 1992) in a cyclone reactor. Cells accumulated in the dextran phase and surfactin in the other phase. This enabled the separation of surfactin from the cells to decrease cell inhibition.

An airlift reactor in batch mode was employed to enhance aeration with a potato process effluent as the substrate (Noah et al., 2002). A 0.5 vvm air flow rate enabled surfactin removal. Conditions of a large inoculum, pH control, and the use of a pressurized reactor optimized the growth of *B. subtilis* over indigenous bacteria. Noah et al. (2005) subsequently used a chemostat and low solid potato effluents. At 0.5 vvm, a surfactin concentration increased to 1.1 g/L was obtained at high agitation rates (400 rpm).

Martinov et al. (2008) studied aeration in a stirred tank reactor with foaming. Different agitators were tested due to the decrease in aeration in the presence of surfactin. A low shear impeller Narcissus maintained stable k_La values while reducing foaming. Studies by Yeh et al. (2006) indicated however that agitation rates above 350 rpm and aeration above 2 vvm lead to higher foaming levels that caused low surfactin production and low of cells. A k_La of 0.012/s was optimal.

Sen and Swaminathan (1997, 2004) studied surfactin production by *B. subtilis 3256*. Maximal production (1.1 g/L) was at 37.4°C, pH 6.75, agitation of 140 rpm, and aeration of 0.75 vvm. Primary inoculum age (55–57 h) of 5%–6% by

volume and secondary inoculation of (4–6 h) 9.5% by volume were also important for optimizing surfactin production.

Gancel et al. (2009) investigated lipopeptide production during cell immobilization on iron-enriched polypropylene particles. Immobilization improved biosurfactant production by up to 4.3 times. The amount of fengycin to surfactin varied depending on the iron content of the pellets. Highest surfactin (390 mg/L) and fengycin (680 mg/L) production was at 0.35% iron.

Guez et al. (2008) evaluated the influence of oxygen transfer rate on the production of the lipopeptide mycolysin by *B. subtilis* ATCC6633. A respiratory activity monitoring system used for the study showed that oxygen metabolism has an effect on the homologue production and that the regulatory system is complex. Chenikher et al. (2010) examined the ability to control the specific growth rate for the production of surfactin and mycosubtilin. Most feeding strategies do not take into account the loss of the biomass with the foam. This must be taken into account to enable the maintenance of the specific growth rate and subsequently production. The growth rate of 0.05/h was maintained.

An integrated foam collector was integrated for biosurfactant production to study parameters for scale-up (Amani et al., 2010). The best conditions were 300 rpm and 1.5 vvm for a surfactant yield on sucrose of 0.25 g/g. K_La of 0.01/s was achievable in shake flasks and bioreactors, and this could potentially be used for scale-up.

MEASUREMENT AND CHARACTERIZATION TECHNIQUES

Enhanced surfactin production can be determined by blood agar plate screening due to hemolysis by surfactin (Mulligan et al., 1984). To verify that the isolates are biosurfactant producers, then the cultures must be grown and the surfactin levels determined. The most common technique for determining surfactant concentration is surface tension measurement and CMC determination. HPLC is also frequently used. An assay based on hemolysis was used for the analysis of surfactin in the fermentation broth. It was determined that the method could be used as a quick low-technology method of surfactin analysis.

Huang et al. (2009) compared blood plate hemolysis, surface tension, oil spreading, and demulsification. Surface tension measurement followed by demulsification tests allowed isolation of a demulsification strain *Alcaligenes* sp. S-Xj-1, which produced a lipopeptide that was able to break O/W and W/O emulsions.

Knoblich et al. (1995) studied surfactin micelles by ice embedding and transmission electron cryomicroscopy. The micelles found were ellipsoidal with dimensions of 19, and 11 nm in width and length,respectively or spherical with a 5–9 nm in diameter, at pH 7. However, at pH 9.5, the micelles were more cylindrical with width and length dimensions of 10–14, and 40–160, or spherical with diameters of 10–20 nm. Addition of 100 mM NaCl and 20 mM $CaCl_2$ at pH 9.5 formed small spheres instead of the cylindrical micelles.

Hue et al. (2001) examined the use of a combination of LSI-MS and MS/MS for the characterization of the mixtures of surfactin produced by *B. subtilis.* Amino acid composition was determined, and the length of the acyl chain was shown to vary from 12 to 15 carbons. Leucine and isoleucine could be differentiated.

Biosurfactant proteins produced by *Lactobacillus fermentum* RC-14 have also been identified by a ProteinChip-interfaced mass spectrometer (Reid et al., 2002). Five tryptic peptide sequences by collision-induced dissociation tandem mass spectrometry were identified following on-chip digestion of collagen-binding proteins. This may lead to the determination of the factors that are responsible for antistaphylococcal activity.

^{1}H-NMR was used by Bonmatin et al. (1994) to show that surfactin can have two conformations depending on the pH. The saddle-like structure is bidentate with the two charged amino acids as sites for cation binding.

SANS studies were performed to study the characteristics of surfactin (Shen et al., 2009). At pH 7.5, the aggregation number was only 20, and the diameter of the micelles was 50 Å with a hydrophobic core of 22 Å radius. It is postulated that the leucines are in the hydrophobic core, which is consistent with its foaming characteristics. Further work (Shen et al., 2010) showed the solubilization of diphenylcarbamyl chloride.

Pecci et al. (2010) characterized the biosurfactants produced by *B. licheniformis* V9T14 strain. This strain exhibited antimicrobial activity that inhibited biofilm formation of human pathogens. LC-ESI–MS/MS analyses were used, and fengycin and surfactin homologues were determined. Fractionation was further performed by silica gel chromatography. C13, C14, and C15 surfactin homologues were found plus C17 fengycins A and B. Other C14–C16 fengycin homologues were also confirmed. Most of the surfactin (61.3%) was in the C15 form with an MW of 1035. The two most common forms of fengycin A and B, respectively, were the C17 of MW 1477 (25.1%) and 1505 (55.1%). The LC-ESI–MS/MS proved useful for the characterization of the lipopeptides.

An oil emulsification test was used to screen for biosurfactants and bioemulsifiers for strains from a sea mud (Liu et al., 2010). A *B. velezensis* H3 strain was isolated and could produce biosurfactants on starch and ammonium sulfate. C14 and C15 surfactins were discovered, which could lower the surface tension to 25.7 and 27.0 mN/m, respectively, from pH 4 to 10. CMCs were in the order of 10^{-5} mol/L. Antimicrobial properties were shown. The yield however was only 0.49 g/L. Highest yields of up to 50 g/L have been previously found by a strain on maltose and soybean flour (Yoneda et al., 2006).

GENETICS OF LIPOPEPTIDE PRODUCTION

Ultraviolet radiation mutation between argC4 and hisA1 on the genetic map led to a strain that produced 3.5 times surfactin (Mulligan et al., 1989). Another technique included random mutagenesis by N-methyl-N′ nitro-N-nitrosoguanidine of *B. licheniformis,* where an increase in surfactin production of 12-fold was obtained (Lin et al., 1998). Tsuge et al. (2001) not only found that the *yerP* gene is involved in surfactin resistance in the strain but also evaluated if this gene was involved in surfactin production. Although the *sfp* gene was inserted into the strain, production was low. Therefore, it did not appear that the *yerP* gene was linked to surfactin production.

Washio et al. (2010) analyzed the genetics of arthrofactin production by *Pseudomonas* sp. MIS38. Arthrofactin are cyclic lipopeptides that function as

antibiotics, immunosuppressants, antitumor agents, siderophores, and surfactants. Schwartzer et al. (2003) postulated it to be superior as a biosurfactant to surfactin and is necessary for the swarming and biofilm formation by the bacteria. Mutants from gene insertion were isolated that did not produce the biosurfactant, gaining some info on the synthesis.

Ion beam implantation has also been utilized for generating high surfactin–producing mutant of *B. subtilis* (Liu et al., 2006). N^+ is implanted by this method. Gong et al. (2009) indicated that a mutant using this technique on the concentration of a crude surfactin of 12.2 g/L could be produced from 6.5 g/L of biomass. It is not known what the effect of the implantation has on the metabolism of the microorganisms.

Chelardi et al. (2012) also studied the motility of *B. subtilis*. They confirmed that *swrA* gene is needed for swarming, and surfactin increases surface wettability to allow swarming on low humidity surfaces.

Various oil reservoirs of salinities from 2.1% to 15.9% were examined to determine the presence of biosurfactant-producing strains (Simpson et al., 2011). The presence of surfactin (*srfA3*) and lichenysin (*licA3*) genes to evaluate the potential for biosurfactant production potential was confirmed. Subsequently, nutrient addition was performed to stimulate production. This confirmed the ability to biostimulate biosurfactant production in an oil reservoir for oil recovery.

EXTRACTION OF LIPOPEPTIDES

Crude extraction of lipopeptides is summarized in Table 6.3 but is usually by chemical extraction. For example, surfactin can be extracted by acid precipitation (pH 2) followed by solvent extraction by methanol (Vater et al., 2002). For *Pseudomonas* lipopeptides, multiple extraction by the ethyl acetate can be used (Kuiper et al., 2004).

Purification of lipopeptides is important for subsequent industrial application. Thin layer chromatography, HPLC, gel permeation chromatography, ion exchange chromatography, and ultrafiltration have been used. HPLC by reverse phase chromatography in particular is often used with the detection by UV absorbance or mass spectrometry to provide some information on the molecular mass of the components. Ultrafiltration with 30 kDa (UF-1) and 10 kDa (UF-II) cutoff membranes was employed in a single step (Sivapathasekaran et al., 2011). The recovery was higher with the 10 kDa membrane (89%) compared to 73% with the 30 kDa. Purity was also higher (83% compared to 78%). The product was a mixture of surfactin and fengycin.

The foaming characteristic of surfactin can be used to remove it during fermentation (Figure 6.4). At a concentration of 0.05 mg/L, it is comparable to SDS and bovine serum albumin (Razafindralambo et al., 1996). Low agitation speeds in the fermentor were beneficial for the removal of high concentrations of surfactin in the collected foam (Davis et al., 2001). Between 10 and 30 h, stirrer speeds of 146 and 166 rpm led to surfactin concentrations of 1.67 and 1.22 g/L, respectively, and agitation rates of 269 rpm produced concentrations of only 75 mg/L. Overall recovery of the produced surfactin in the foam was over 90%. Makkar and Cameotra (2001) also used foam fractionation to recover surfactin

TABLE 6.3
Recovery of Surfactin by Foam Collection

Producing Organism	Description	Foam Generation	Recovery%[a]	Enrichment Factor[b]	Reference
B. subtilis BBK 006	Recovery during batch operation	Not controlled	92.3	55.0	Chen et al. (2006a)
B. subtilis BBK 006	Recovery during continuous operation	Not controlled	28.7	50.1	Chen et al. (2006b)
B. subtilis ATCC 21331	Separate foam fractionation of cell-free broth	Controlled	97.1	2.9	Davis et al. (2001)
	Separate foam fractionation of cell broth	Controlled	97.3	1.7	
	Recovery during batch operation under oxygen depletion and low agitation (146 rpm)	Not controlled	90.0	34.0	

Source: Modified from Winterburn, J.B. and Martin, P.J, *Biotechnol. Letters*, 34(2): 187–195.

[a] Recovery = $(C_f V_f / C_i V_i)$, where C_f, C_i are the foam and initial surfactin concentrations and V_i, V_f are the initial liquid and foam volumes.

[b] Enrichment = C_f/C_i.

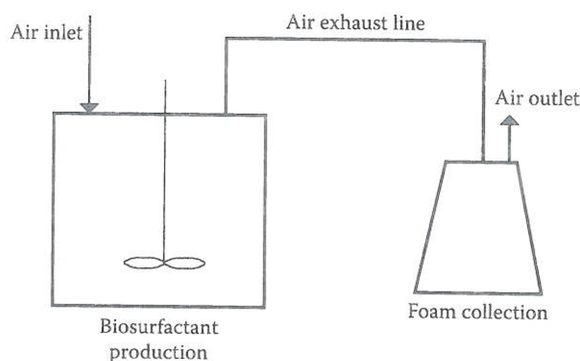

FIGURE 6.4 Recovery of surfactin by foam collection.

by *B. subtilis* MTCC 2423. Using sucrose as the substrate, yields of 4.5 g/L were obtained at 45°C. Other studies are shown in Table 6.4.

Liu et al. (2007) evaluated different conditions for adsorption for surfactin on activated carbon. pH values from 6.5 to 8.5 and 30°C were optimal. Adsorption onto activated carbon was studied to incorporate surfactin removal with production (Montastruc et al., 2008). Adsorption capacities were about 30 mg of surfactin

TABLE 6.4
Extraction Processes for Lipopeptides

Process	Biosurfactants Recovered by Each Method
Adsorption	Lipopeptides
Foam fractionation	Surfactin
Precipitation by acid	Surfactin
Ultrafiltration	Surfactin

Source: Adapted from Desai, J.D. and Banat, I.M., *Microbiol. Mol. Biol. Rev.*, 61, 47, 1997.

per gram of activated carbon and slightly lower (20 mg/g) from culture media. Ninety percent of the surfactin could then be removed by pure methanol.

Two resins (AG1-X4 and XAD-7) for the adsorption of surfactin were evaluated by Chen et al. (2008). Sorption capacities were 1.76 and 0.41 g/g, respectively. The large micelles decreased sorption in the resins.

An automated collection method was used to isolate fengycin produced by *B. subtilis* (Glazyrina et al., 2008). A flounder was used to remove the surfactant concentrated at the surface. The fraction removed was nine times higher in concentration than the bulk solution. No solvents or foam fractionation was required.

Foaming has been used by Davis et al. (2001) as a recovery process with a stirrer speed of 146 rpm lead to an enrichment of 34% and 90% recovery surfactin from the cell broth. Although the enrichment was low (1.7), the recovery was 97%. Higher speeds (204 and 269 rpm) caused high levels of foaming. With a chemostat at a dilution of 0.2/h, a high factor of enrichment was shown (Chen et al., 2007).

Chen et al. (2008a) used hexane to extract surfactin from the fermentation broth of *B. subtilis* ATCC 21332 with a microporous polyvinylidene fluoride hollow fiber module (0.2 μm pore size). The micelles did not easily pass through the pores and were sorbed onto the membrane material. Desorption by ethanol from the membranes improved the surfactin purity to 78%. Further work by Chen et al. (2008b) was performed by acid precipitation and redissolution with NaOH. Ethyl acetate was shown to be a better extractant than hexane. However, the addition of ammonium cations of Aliquat 336 could bind to surfactin and enhance the extraction to 92% for a 3 g/L concentration. Recoveries of 90% and 88% could be achieved with NaCl or ammonium sulfate addition to ethanol/water.

Membrane Lipopeptide Recovery

Ultrafiltration membranes can be used to retain micelles of surfactin and other lipopeptides as they are larger than monomers (Mulligan and Gibbs, 1990) as shown in Figure 6.5. Sen and Swaminathan (2005) demonstrated the purification of surfactin with a polymeric membrane. Optimal flux (260 L/m^2-h) and a 166-fold concentration factor were obtained at a pH 8.5. Chen et al. (2007) used a two-step ultrafiltration process. Ultrafiltration membranes of 100 kDa were used to recover the micelles

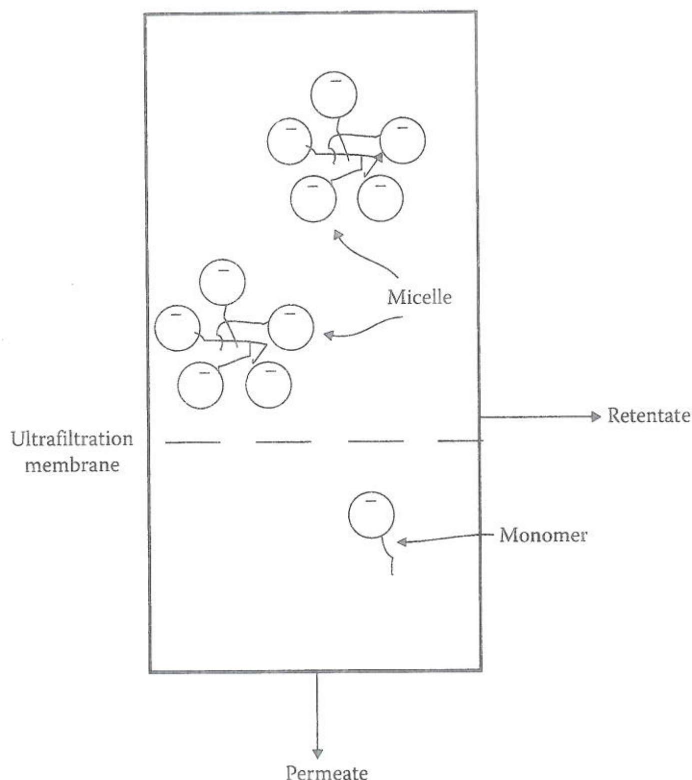

FIGURE 6.5 Removal of metals from contaminated soil by surfactin.

while 1 kDa MW cutoff nanomembranes were used for concentrating the monomers. Recoveries of 97% were high, but purities were low (55%). Purities were increased by using a two-step ultrafiltration process with 100 kDa MW cutoff membranes. The surfactin recovered in the permeate of one membrane was then passed through the other membrane to remove salts. Recoveries were 72% with an 83% purity. Chen et al. (2008a) also evaluated two different membranes of the same MW cutoff (100 kDa). Although the cellulose ester membrane gave a higher recovery (97% compared to 88%) than the polyethersulfone membrane, it was not recommended due to the flux decrease from concentration polarization, gel formation, and amino acid sorption at the membrane. Further studies by Chen et al. (2008c) indicated that a combination of salting out and membrane filtration could increase yields and purity.

STRAIN ISOLATION

Gandhimathi et al. (2009) isolated a lipopeptide from a marine sponge-associated actinomycete, *Nocardia alba* MSA10. The lipopeptide exhibited the properties of lipase production, and demulsification, hemolytic, antibiotic, and surface activities. The solvents, ethyl acetate diethyl ether and dichloromethane, were used for

TABLE 6.5
Lipopeptide-Producing Marine Organisms

Producing Microorganism	Chemical Composition	Properties	Reference
Bacillus licheniformis BAS50	Lichenysin of MW 1006–1034	Surface tension reduction to 28 mN/m, CMC of 12 mg/L Antibacterial activity	Yakimov et al. (1995)
Bacillus circulans	Novel biosurfactant	Antimicrobial activity	Mukherjee et al. (2009)
Azotobacter chroococcum	Lipid: protein (31.3:68.7)	Emulsify ability for waste motor oil, crude oil, diesel, kerosene, naphthalene, anthracene, xylene	Thavasi et al. (2009)

Source: Adapted from Satpute et al., 2010.

extraction. The substrate glucose and peptone were used for production, which was optimal at pH 7, 30°C, and 1% salinity. It was stable between 4 and 9 and up to 80°C. Other marine lipopeptide-producing isolates are shown in Table 6.5.

Sriram et al. (2011a) isolated a lipopeptide from a metal-tolerant strain of *B. cereus* NK1. The strain was tolerant to ferrous sulfate, zinc, and lead. Biofilm inhibition by pathogens and antimicrobial activities against fungi, and gram-positive and -negative bacteria were indicated. The strain was resistant to various antibiotics including ampicillin, bacitracin, erythromycin, and rifampicin but was less resistant to others. The lipopeptide had a CMC of 45 mg/L with a surface tension of 36 mN/m. The metal resistance of the strain could enable it to be used for remediation purposes.

Another strain, *Escherichia fergusonii* KLU01 was isolated by Sriram et al. (2011b) from an oil-contaminated soil. It was able to produce a biosurfactant of CMC of 36 mg/L, with emulsification properties, and excellent stability over a range of pH (4–10), temperature (20°C–100°C), and various salts. The strain was also tolerant against manganese, lead, iron, nickel, copper, and zinc.

Jing et al. (2011) also isolated a strain of *B. subtilis* JA-1 from an oil reservoir. The strain was able to grow at temperatures of 60°C. Surface tension could be reduced to 28.3 mN/m with a CMC of 48 mg/L. The biosurfactant was stable up to pH 12, 121°C, and salt concentrations of up to 14%. The strain thus could be potentially useful for enhanced oil recovery.

Ismail et al. (2012) isolated a crude oil–emulsifying *Bacillus* sp. I-15 from oil contamination. The surface tension was reduced to 42 mN/m and the CMC was 200 mg/L. It could potentially be beneficial for natural attenuation of oil contamination.

Ghojavand et al. (2011) studied a strain of *B. mojavensis* that produced a biosurfactant. The strain could tolerate high salinities (up to 10% NaCl) and temperatures up to 55°C and could grow under anaerobic conditions. The biosurfactant could reduce the surface tension to 27 mN/m. Emulsification stability, however,

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was poor. Previous work by Ghojavand et al. (2008) isolated thermotolerant, halotolerant, and facultative biosurfactant-producing *B. subtilis* strains as shown by the 16S ribosomal deoxyribonucleic acid gene. These strains could potentially be used for enhanced oil recovery. Another strain *B. mojavensis* XH1 was studied by Li et al. (2012). A biodemulsifier was produced and isolated by ethanol extraction and then sephadex and silicon gel column chromatography. A response surface methodology was used to optimize the media for production. Biodemulsifier yield increased to 2.07 g/L.

A strain of *B. mojavensis* (PTCC 1696) (Ghojavand et al., 2012) was isolated from an oil field. The biosurfactant produced by the strain could reduce the surface tension to 26.7 mN/m. Biosurfactant was added for water flooding to enhance oil recovery from a low-permeability carbonate reservoir. Although the concentration of the surfactant was low (0.1 g/L), it showed potential for the oil removal. Costs of the purified surfactin for biomedical research are in the range of $10 per mg compared to $2–$4 per kg for emulsion formulations.

A licheniformin biosurfactant was produced by *B. licheniformis* MS3. The lipopeptide contained the amino acids, Gly, Ala, Val, Asp, Ser, Gly, Tyr, and a lactone ring with a fatty acid moiety at the N-terminal amino acid residue (structure). The MW was determined as 1438 Da. The surface tension could be lowered to 38 mN/m by the isolated biosurfactant at a concentration of 15 mg/L. Isolation was performed using an electroflotation column. It was stable over a range of temperatures (45°C–85°C) and from pH 3 to 11.

Janek et al. (2010) isolated lipopeptides produced by *Pseudomonas fluorescens* BD 5 from the arctic. The biosurfactants were named pseudofactin I and II. They were cyclic with good emulsification abilities for plant oils and hydrocarbons, comparable to that of surfactin (Abdel-Mawgoud et al., 2008). Pseudofactin II reduced the surface tension to 31.5 mN/m with a CMC of 72 mg/L. Approximately 10 mg/L was recovered. The yield of pseudofactin I was 1/12th that of the other form. They could potentially be used for various medical and biotechnological applications (Figure 6.6).

A strain of *B. mycoides* was isolated from an oil field (Najafi et al., 2010). The isolate produced a lipopeptide derivative that could lower the surface tension to 34 mN/m. To optimize production, a response surface methodology was employed. Optimal production of surfactin of 3.3 g/L were obtained at 16.6 g/L glucose substrate concentration, 39°C, pH 7.4, and total salt concentration of 55.4 g/L.

A response surface methodology was also employed by Wei et al. (2010) for fengycin production. The *B. subtilis* F29-3 strain had been isolated from a potato farm. The fengycin was isolated by acid precipitation at pH 2 followed by ultrafiltration and nanofiltration for purification. The media design that was optimal included mannitol, soybean meal, sodium nitrate, and magnesium sulfate and increased production by almost threefold (3.5 g/L).

Another strain *Brevibacillus brevis* (Wang et al., 2010) was isolated from an oil field. It consisted of Asp, Glu, Val, and Leu in a ratio of 1:1:1:4 like surfactin. The surface tension was 26.8 mN/m and the CMC was 9×10^{-6} M. The MWs of the various fractions varied from 1008 to 1035, depending on the C13–C15 hydrocarbon portion. This was the first time this species was shown to produce surfactin.

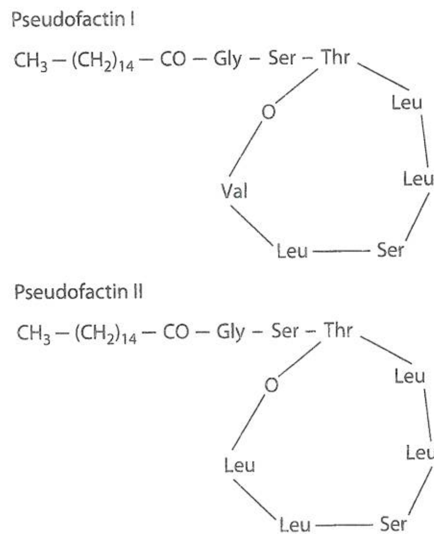

FIGURE 6.6 Structure of pseudofactin. (Adapted from Janek, T. et al., *Biores. Technol.*, 101, 6118, 2010.)

A *B. subtilis* strain was isolated from a refinery soil by Fonseca et al. (2007). A factorial design and response surface analysis indicated that the optimal C/N ratio was 3 and agitation rate was 250 rpm. The minimum surface tension was 31 mN/m. The lipopeptide on preliminary characterization was shown to be different from surfactin.

Low-cost substrates that have been used include cassava wastewater, sludge palm oil, vegetable oil refinery waste, and molasses. Raw glycerol from biodiesel production was investigated by de Faria et al. (2011). The strain *B. subtilis* LSFM-05 was grown on this substrate and produced C14/Leu7 surfactin with a CMC of 70 μM. The surfactant was collected in the foam and was produced at a concentration of 1.37 g/L. The surface tension was 29.5 mN/m. An esterified glutamic acid present in the surfactin differed from the commercial Sigma-Aldrich.

Pemmaraju et al. (2012) isolated biosurfactant-producing strains from an oily sludge using a combination of surface tension reduction, hemolytic activity, emulsification activity, drop-collapse assay, and cell surface hydrophobicity studies. Up to 6.9 g/L of surfactant (a mixture of surfactins, iturins, and fengycins) was produced by *B. subtilis* DSVP23 and is growth-associated. C12–30 hydrocarbons (saturates and aromatics) could be degraded within 5 days, indicating the potential for bioremediation by this strain.

Lipopeptides were isolated from *Paenibacillus* sp. (HRAC30) (Canova et al., 2010). The lipopeptides were characterized as a surfactin and was C15 lipopeptide of MW 1036. It was extracted using ethyl acetate and then elution through a Sephadex column. The compound was shown to be an active phytopathogen suppressor and could be used to control *Rhizoctonia solani*, a pathogen for commercial crops. The minimum inhibitory concentration was 14 μg/mL, which is almost as effective as iturin.

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Another species, *Paenibacillus alvei* (Najafi et al., 2011), was isolated from an oil field. Biosurfactant production was optimized with central composite rotatable design response surface methodology. The biosurfactant reduces the surface tension to 35 mN/m. A glucose concentration of 13 g/L, temperature of 35°C, a 51 g/L total salt concentration, and pH 6.9 were the optimal conditions.

A strain of *B. amyloliquefaciens* was isolated from crude oil (Sang-Cheol et al., 2010). Lipopeptides of molecular mass of 1086.9 and 1491.2 *m/z* were determined. The higher MW corresponds to fengycin B. However, as the structure differed from fengycin A and B forms, it was designated as fengycin S. Due to its properties of emulsification, it could be used for oil spills.

Rufino et al. (2012) produced a lipopeptide from a yeast *Candida lipolytica*. A waste soybean oil residue was used as the substrate. The surface tension was 25 mN/m, and the CMC of the crude form was 0.03% and consisted of 50% protein, 20% lipid, and 8% carbohydrate.

Pseudomonas aeruginosa strains MTCC7815 and MTCC7812 were studied for solubilization and metabolism of fluorene, pyrene, and phenanthrene (Bordolai and Konwar, 2009). Pyrene and fluorene were solubilized by these two lipopeptide-producing strains, which enhanced growth. The surface tension was reduced to 35 mN/M by these strains, and the CMC were 100 and 110 mg/L for MTCC7815 and MTCC 7812, respectively. Previous studies (Bordolai and Konwar, 2008) showed that these biosurfactants were very stable (pH 2.5–1) and up to 100°C. Crude oil–saturated sand pack studies indicated that 50%–60% of the oil could be recovered from room temperature to 90°C, indicating potential for enhanced oil recovery. Glucose and glycerol were the best carbon sources.

Saimmai et al. (2013) isolated strains from mangrove sediments that produce biosurfactants. Many pollutants such as hydrocarbons are found in the sediments, and thus biosurfactant production could enhance the uptake of these pollutants by the bacteria. They identified a strain of *S. ruminantium* CT2 that produced the lipopeptide for the first time. It grew the best on molasses and could reduce the surface tension to 25.5 mN/m with a CMC of 8 mg/L. Maximum production was 5 g/L. Ethyl acetate could be used to extract the biosurfactant from the broth. The biosurfactant was characterized as a lipopeptide similar to surfactin. It showed good pH and temperature stability and could enhance motor oil removal from contaminated sand, ability to solubilize polycyclic aromatic hydrocarbons, and antimicrobial activity.

PROPERTIES AND APPLICATIONS OF LIPOPEPTIDES

Surfactin addition improved the mechanical dewatering of peat by greater than 50% at very low concentrations (0.0013 g/g wet peat) by altering the flow characteristics of the trapped water within the peat particles (Cooper et al., 1986). Surfactin has also shown the ability to inhibit blood coagulation and protein denaturation, to accelerate fibrinolysis, and to have antimyoplasmic properties (Vollenbroich et al., 1997). Mycoplamata leads to respiratory inflammation, urogenital tract diseases, and cofactors in the AIDS (Vollenbroich et al., 1997) pathogenesis. Antibiotic therapy is not effective against mycoplasmata, but surfactin can cause leakage of the

plasma membrane and finally disintegration. One disadvantage is the competition with proteins. Endoflaxacin coaddition allowed a synergistic effect (Seydlová and Svobodová, 2008). Cao et al. (2009) showed that when the lipopeptide from *B. natto* TK-1 was tested against MCF-17 human breast cancer cells, it indicated antitumor behavior. The inhibition was as the G2/M phase of growth.

Other potential medical benefits for surfactin have been identified. For example, surfactin can reduce the inflammatory activity of the lipopolysaccharides against eukaryotic cells (Seydlová and Svobodová, 2008). Surfactin C was better than surfactin A, B, or D for anti-inflammatory activity, antiviral activity against herpes simplex virus (HSV-1 and 2), semliki forest virus, simian immunodeficiency virus, vesicular stomatitis virus and feline calicivirus.

Das et al. (2008) isolated at a biologically active fraction from a marine *B. circulans* by methanol extraction and HPLC fractionation. One of the fractions showed surface tension lowering to 28 mN/m and antimicrobial activity against gram-positive and gram-negative pathogenic and semipathogenic bacteria. Unlike surfactin, however, it was not hemolytic.

The adhesion of bacteria and biofilm formation are the first steps to bacterial infection.

Therefore, inhibition of this can reduce the growth of pathogenic bacteria. Lipopeptides can reduce adhesion and biofilm formation, and thus this was studied (Das et al., 2009). Higher concentrations of purified surfactant (10 mg/mL) could reduce adhesion by over 80% of several strains and over 70% for biofilm inhibition. This indicates the potential in biomedical application as bacteria in biofilms are resistant to antibiotics. The presence of *Salmonella typhimurium, Salmonella enterica, Escherichia coli*, and *Proteus mirabilis* could be reduced in PVCs and vinyl urethral catheters by running surfactin through the catheter before use (Seydlová and Svobodová, 2008).

Rivardo et al. (2010) investigated the effect of lipopeptide addition with silver on *E. coli* biofilm inhibition. The biosurfactant V9T19 was obtained from *B. licheniformis*. Silver is a well-known disinfectant. Adding the lipopeptide was able to reduce the amount of silver required by 129- to 258-fold, demonstrating its synergistic effect.

Singh and Cameotra (2004) describe the biomedical properties of surfactin and iturin A produced by *B. subtilis*. Tanaka et al. (1997) has also described the antiviral properties of surfactin. The mechanism has been postulated to be related to disruption of the virus lipid membrane.

Lima et al. (2011a) investigated the biodegradability of various surfactants. Lipopeptides were obtained from various strains *Bacillus* sp. LBBMA 111A, *B. subtilis* LBBMA 155, and *Arthrobacter oxydans* LBBMA 201. SDS, a synthetic surfactant, was also compared. Although biosurfactants are supposed to be more biodegradable, there have been few studies on this. Pure and mixed cultures were studied for the biodegradation tests, and carbon dioxide emissions were monitored. The biodegradation of the biosurfactants was significantly more than the synthetic surfactant, indicating their potential for environmental applications. Subsequent work by Lima et al. (2011b) indicated that these biosurfactants could remove phenanthrene and cadmium for contaminate soil in combination with an inorganic ligand iodide.

Reddy et al. (2009) evaluated surfactin as a stabilizing agent for the synthesis of silver nanoparticles. The stability of the nanoparticles was determined and found to be stable for a period of 2 months in the presence of surfactin. pH and temperature conditions affected particle size. Surfactin as a stabilizing agent is renewable, less toxic, and biodegradable, and thus an environmentally friendly additive.

In addition, the presence of two negative charges, one on the aspartate and the other on the glutamate residue of surfactin, enables the binding of various metals such as calcium, barium, lithium, magnesium, manganese, and rubidium (Thimon et al., 1992). Eliseev et al. (1991) also showed that a *Bacillus* species could release oil at low concentrations of 0.04 mg/L from oil-saturated columns.

A strain of *B. subtilis* isolated from contaminated sediments (Olivera et al., 2000) could produce surfactin. A crude form was then added to ship bilge waste to enhance biodegradation. Although aliphatic and aromatic compounds in a nonsterile environment were degraded more quickly in the presence of the biosurfactant, N-C17 pristane and N-C18 phytane degradation were not.

Using a technique called micellar enhanced ultrafiltration, Mulligan et al. (1999) studied the removal of various concentrations of metals from water by various concentrations of surfactin by a 50,000 Da MW cutoff ultrafiltration membrane. Cadmium and zinc rejection ratios were superior (close to 100%) at pH values of 8.3 and 11, while copper rejection ratios were the highest at pH 6.7 (about 85%). The addition of 0.4% oil as a cocontaminant slightly decreased the retention of the metals by the membrane. The ultrafiltration membranes also indicated that metals became associated with the surfactin micelles as the metals remained in the retentate and did not pass through into the permeate as illustrated in Figure 6.5. The ratio of metals to the surfactin was determined to be 1.2:1, which was only slightly different from the theoretical value of 1 mol metal: 1 mol surfactin due to the two charges on the surfactin molecule.

Batch soil washing experiments were performed to evaluate the feasibility of using surfactin from *B. subtilis* for the removal of heavy metals from a contaminated soil and sediments (Mulligan et al., 1999). Compared to minimal amounts for the control, 0.25% surfactin with 1% NaOH removed 25% of the copper and 6% of the zinc from the soil and 15% of the copper and 6% of the zinc from the sediments. A series of five washings of the soil with 0.25% surfactin with 1% NaOH removed 70% of the copper and 22% of the zinc. Ultrafiltration, octanol–water partitioning, and zeta potential measurement determined that surfactin was able to remove the metals by sorption and complexation at the soil interface, then desorption of the metal through interfacial tension lowering and fluid forces into solution and finally micellar complexation (Figure 6.5).

CONCLUSION

Surfactin has very interesting surfactant properties. Potential medical applications are related to antiinflammatory, antiviral, antibiotic, and antiadhesive activities. However, the economics are not competitive due to poor yields and the requirement for expensive and complex substrates. Portillo-Rivera et al. (2009) have postulated that biosurfactant costs can be as low as $0.50 per liter from molasses sugarcane. Low-cost purification methods are also needed as downstream costs can account for 60% of

the cost (Mukherjee et al., 2006). Purity of the product is also a major consideration. Ninety-eight percent pure surfactin is sold for $10 per mg but can be reduced to $2–$4 per kg for tank cleaning or oil recovery applications (Bognolo, 1999). Although more information is available concerning the biosynthesis of surfactin, there is still a lack of information regarding the secretion, metabolic route, primary cell metabolism, and physicochemical properties of the biosurfactant. Research is thus required to accelerate the knowledge in this area and possibly will enhance the applications of the surfactant. New forms of surfactin and other lipopeptides could also become available.

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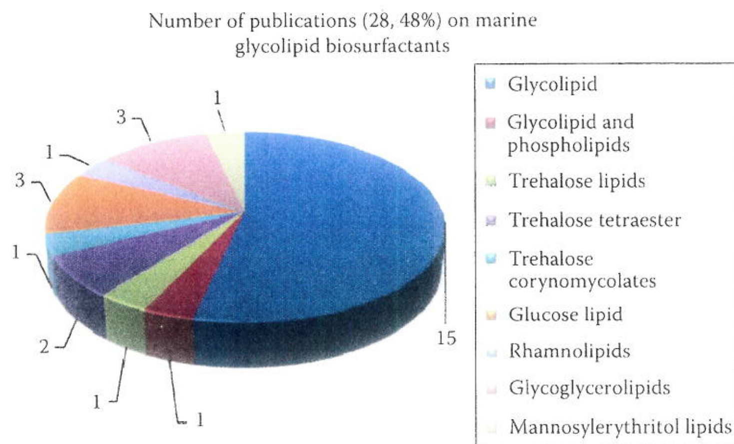

FIGURE 5.1 Breakdown compilation of publication on marine glycolipid biosurfactants.

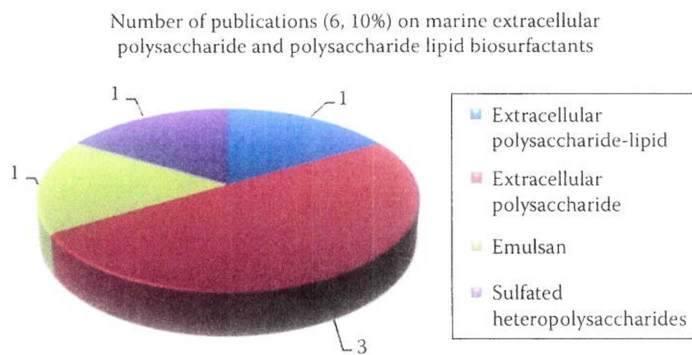

FIGURE 5.2 Breakdown compilation of publication on marine extracellular polysaccharide and polysaccharide lipid biosurfactants.

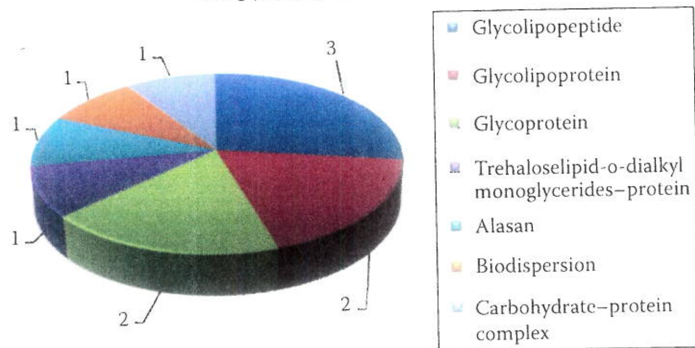

FIGURE 5.3 Breakdown compilation of publication on marine glycolipopeptide, glycolipoprotein, and glycoprotein biosurfactants.

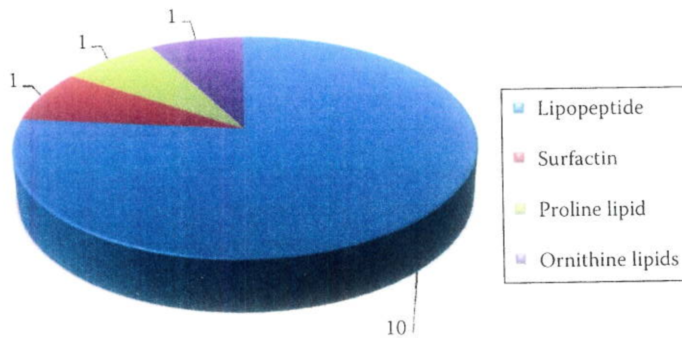

FIGURE 5.4 Breakdown compilation of publication on marine lipopeptide and lipoprotein biosurfactants.

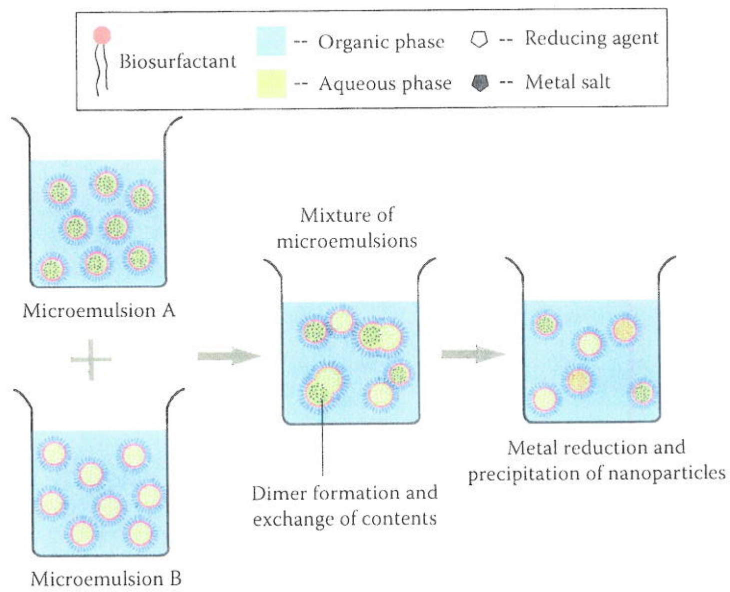

FIGURE 9.1 Mechanism for microemulsion-based nanoparticles synthesis. (Adapted from Capek, I., *Adv. Colloid Interface Sci.*, 110, 49, 2004.)

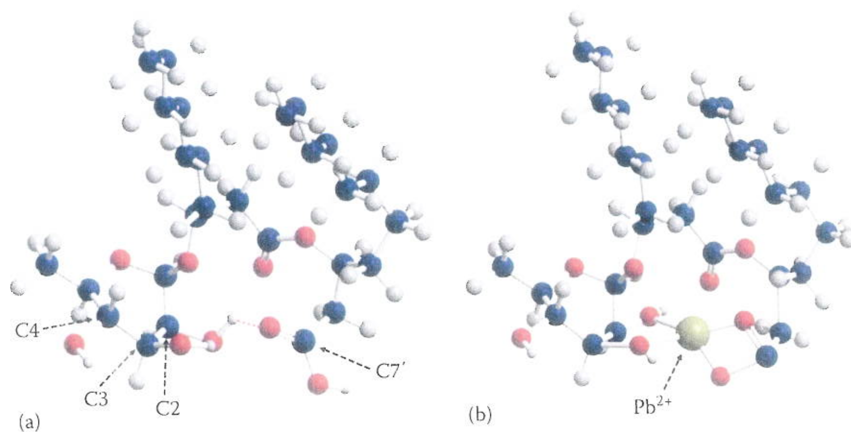

FIGURE 11.3 (a) An energy-minimized molecular mechanics model of monorhamnolipid (C10, C10) showing the oxygen-rich cavity that may serve as a cation binding pocket. (b) A model showing how a Pb^{2+} ion might interact with the binding pocket of monorhamnolipid (C10, C10).