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BIOSURFACTANTS

Production and Utilization—Processes, Technologies, and Economics

Edited by

Naim Kosaric

Fazilet Vardar Sukan

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Edited by
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Section I

Production

1 Types and Classification of Microbial Surfactants

Rudolf Hausmann and Christoph Syldatk

CONTENTS

Microorganisms produce manifold metabolites that do not seem to be necessary for their growth and survival. These metabolites are differentiated from primary metabolites and are usually designated as special metabolites, secondary metabolites, or natural products.

Many life processes require the presence of amphiphilic substances. Specifically, amphiphilics, for example, phospholipids, form the basis of all biological membranes. Although many cell components, such as fatty acids and phospholipids, generally, lower interfacial tension, additional specific compounds that lower interfacial tension are known from many microorganisms; they usually comprise unique structures. Such metabolites that lower interfacial tension are often secreted by microorganisms either into the culture medium or are integrated into the cell wall, thus permitting them to grow on or to take up hydrophobic substrates. They are often designated as "biosurfactants." These biosurfactants are among the few known microbial metabolites with bio-physically useful properties.

Owing to the lipid moiety, the extracellular compounds are assigned to the exolipids or "free" lipids. The majority of these exolipids is only formed under special, usually limiting growth conditions. A large number of type-specific, partially very unusual glycolipids, lipopolysaccharides, lipopeptides, and proteins are known. Despite the diversity in structures, all of these metabolites that lower surface tension are designated as biosurfactants.

The designation of biosurfactants for amphiphilic substances of microbial origin is to differentiate them from conventional synthetic surfactants. All in all, about 2000 different amphoteric structures of biological origin have been described. These substances were mainly interesting due to their antibiotics properties.

However, the term biosurfactant is sometimes synonymously used to refer to any natural surfactant or those obtained by chemical bonding of polar head groups and the hydrophobic tails, obtained from a natural source. Well-known examples of biosurfactants in a broader sense are soybean and egg yolk lecithins obtained from plant and animal sources, respectively, and alkyl polyglucosides (APGs) for chemically obtained surfactants from renewable sources.

All biosurfactants comprise at least one hydrophilic and one hydrophobic part due to their amphiphilic character. The molecular structure often also contains several hydrophobic and corresponding hydrophilic parts. The hydrophobic part usually comprises saturated or unsaturated fatty acids, hydroxyl fatty acids, or fat alcohols, with various other structures such as isoprenoids being possible as well. The chain length usually comprises between 8 and 18 carbon atoms. The hydrophilic part may be made up of either structurally relatively simple ester, hydroxyl, phosphate, or carboxyl groups, or of carbohydrates—such as mono, oligo, or polysaccharides—peptides or proteins. Many anionic and neutral biosurfactants are known. Cationic biosurfactants, in contrast, have been described extremely rarely, probably because they have a toxic effect, just like cationic surfactants in general.

Within the biosurfactants, the glycolipids form the greatest share, with the non-sugar component, the aglycone, being highly versatile. These structures are particularly interesting since many biosurfactants exhibit high efficiency at concurrently good biological degradability. They can also be produced from renewable resources.

Generally, biosurfactants are assigned the following properties beneficial for industrial use:

- Great structure diversity (about 2000 described biosurfactants)
- Beneficial surfactant properties
- Low eco-toxicity
- Antibiotic or bioactive effects
- Complete biological degradability
- Production from renewable resources

Although the biotechnological production of microbial surfactants has already been established so far, they have only been used in niche areas due to high production costs. A drastic reduction of production costs is, therefore, necessary to establish microbial surfactants as a general alternative to conventional surfactants also outside of the previous market niches.

There are numerous books, reviews, and original papers covering near-exhaustive aspects of natural surfactants and biosurfactants ranging from their application fields, microbial ecological, biotechnological, to chemical structure analysis.

A few of the selected books are those by Lang and Trowitsch-Kienast (2002), Sen (2010), Soberón-Chávez (2010), and general reviews are given by Satpute et al. (2010), Gutnick et al. (2011), and Merchant and Banat (2012). A thorough review of the chemical structures in the broadest sense covering natural surfactants is given in a series presented by Dembitsky (2004a,b, 2005a,b,c,d,e, 2006). This review focuses on low-molecular weight microbial surfactants with a well-defined structure prepared by fermentation covering the various types and classification of surfactants. The term biosurfactant is applied in its strictest sense referring exclusively to surfactants taken directly from microbial sources, without any organic synthesis.

In addition to the fact that they can be produced by renewable resources, biosurfactants are superior to their synthetic counterparts mainly by two essential characteristics: their structural diversity and the specific biological activity of many structures. It is evident that such additional properties exceeding pure reduction of surface tension make them particularly interesting for some applications.

Detailed consideration of the different biosurfactants regarding their actual application capacity is not possible for most biosurfactants, since the chemical structures and physical characterization of the surfactant properties alone are no indication of the performance properties in product formulations. For this, the corresponding biosurfactants must be available in quantities of about 0.1–1 kg. Thus, industrial product development of biosurfactants is limited to some few biosurfactants, including spiculisporic acid, sophorolipids, rhamnolipids, and mannosylerithritollipids.

Below, the best-known biosurfactants and, using some examples, the structural diversity and potential of microbial biosurfactants, in general, are illustrated based on selected structures.

1.1 LOW-MOLECULAR WEIGHT BIOSURFACTANTS

Usually, low-molecular weight biosurfactants are glycolipids or lipopeptides, but may also belong to the groups of simple fatty acids and free phospholipids. The best-examined glycolipids that reduce surface tension are acylated disaccharides with long-chain fatty acid or hydroxyl fatty acid residues. Lipopeptides comprise a peptide moiety that is synthesized by non-ribosomal peptidsynthases, linked to fatty acid or hydroxyl fatty acid residues.

1.2 FATTY ACIDS AND PHOSPHOLIPIDS

Some bacteria and fungi form free fatty acids or phospholipids when growing on *n*-alkanes (Desai and Banat 1997). Fatty acids can be produced by microbial oxidation of alkanes. A detailed overview of such fatty acids is provided by Rehm and Reiff (1981). The strongest reduction of surface and interface tensions is achieved by fatty acids with chain lengths of C12–C14. In addition to unbranched fatty acids, many more complex microbial fatty acids have been described that exhibit hydroxyl groups or other alkyl residues. Some of these complex fatty acids, such as corynomycol acids (Fujii et al. 1999), are strong surfactants.

Phospholipids are the main part of microbial membranes and are usually not present in an extracellular form. However, *Acinetobacter* sp. HO1-N secreted extracellular phospholipid vesicles were formed when growing on hexadecane. The strong surfactant effect of these vesicles was derived only indirectly via an optically clear micro-emulsion of hexadecane in water (Kappeli and Finnerty 1979, 1980). Kappeli and Finnerty (1979) also reported that some strains of *Aspergillus* produce phospholipids.

Rhodococcus erythropolis DSM 43215 also excreted phosphatidylethanolamines that lower surface tension and occur at growth on *n*-alkanes (Kretschmer et al. 1982). Phosphatidylethanolamine (Figure 1.1) is one of the most common phospholipids other than phosphatidylcholine and usually one of the main components of bacterial membranes.

1.3 GLYCOLIPIDS

Glycolipids comprising mono or oligosaccharides as well as lipid moieties form the most important group of low molecular weight biosurfactants. The saccharide part can comprise glucose, mannose, galactose, galactosesulfate, glucuronic acid, or rhamnose moieties. The lipid moiety comprises either saturated or unsaturated fatty acids, hydroxyl fatty acids or fat alcohols. The four biotechnologically important groups of microbial glycolipids are rhamnolipids, sophorolipids, trehaloselipids, and mannosylerytitollipids.

More than 250 glycolipids, including their chemical structures and biological activities, are described by Dembitsky (2004a,b).

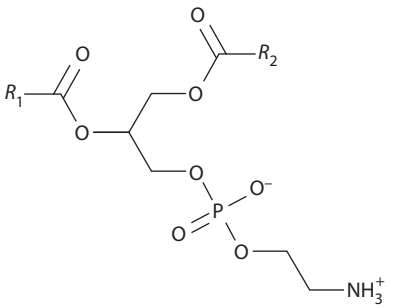

FIGURE 1.1 Phosphatidylethanolamine. R_1, R_2 = typically long, saturated or unsaturated, unbranched aliphatic chains.

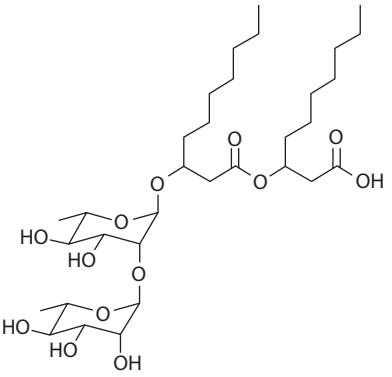

FIGURE 1.2 The characteristic di-rhamnolipid, α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-3-hydroxydecanoyl-3-hydroxydecanoate from *Pseudomonas aeruginosa*.

Rhamnolipids are mainly known from *Pseudomonas aeruginosa* and comprise one or two α-L-rhamnose units, linked via *O*-glycosidic linkage to one or two 3-hydroxyl fatty acid moieties. Natural rhamnolipids are present as mixtures of different congeners. The chain length of the 3-hydroxyl fatty acids varies between 8 and 16 carbon atoms, with 3-hydroxyl decanoic acid (Deziel et al. 2000) being predominant in *P. aeruginosa* and 3-hydroxyl tetradecanoic acid in *Burkholderia* species (Hörmann et al. 2010). The best-known rhamnolipid congener, being the α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-3-hydroxydecanoyl-3-hydroxydecanoate, is displayed in Figure 1.2. A rare rhamnolipid with three hydroxyl fatty acid parts has been described only for *Burkholderia plantarii* (Andrä et al. 2006). Abdel-Mawgoud et al. (2010) provide an overview of the diversity of known rhamnolipids.

Sophorolipids contain the disaccharide sophorose and may be present in two forms, the lactonic form and the open acid form (Nunez et al. 2001). There are many sophorolipid structures (Asmer et al. 1988) that have been mainly described for *Candida bombicola* (teleomorph *Starmerella bombicola*) and *C. apicola*. Predominantly, the hydrophobic part comprises a glycosidically bound 17-hydroxyoleic acid that is usually connected lactonically with the 4″ position of the sophorose, as well as acetyl residue in the 6′ and 6″ positions (Figure 1.3).

Trehaloselipids are mainly known from *Mycobacterium* (Goren 1972), *Arthrobacter* (Suzuki et al. 1969), and *Rhodococcus* (Peng et al. 2007) species. They contain the disaccharide trehalose, which is acylated with long-chained, α-branched 3-hydroxyl fatty acids called mycol acids (Figure 1.4). These acyl groups are linked to the C6 and C6′ positions of trehalose in dimycolates, and at the C6 position in monomycolates. Additionally, other mycolic acid-containing glycolipids have been described (Lang and Philp 1998). The glycolipid 6,6′-dimycolyltrehalose is known as cord factor,

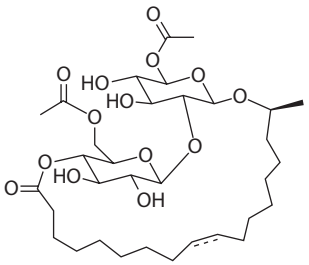

FIGURE 1.3 Characteristic lactonic sophorolipid from *Candida bombicola,* the 1,4″-lactone of 17-L-(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxyoctadecanoic acid 6′,6″-diacetate.

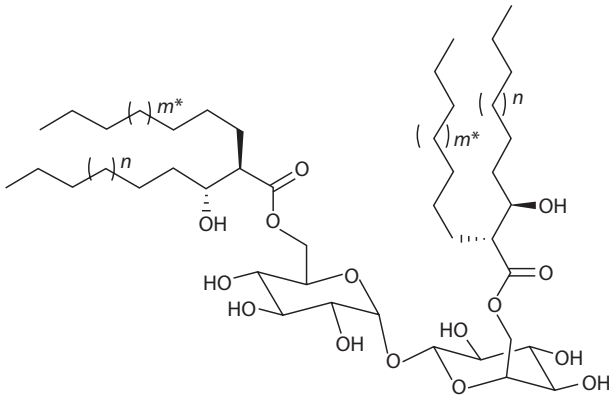

FIGURE 1.4 Trehalose-dicorynomycolate from *Rhodococcus erythropolis* $m + n = 18–22$. (*Partially unsaturated.)

and is an important virulence factor in mycobacteria infections (tuberculosis, leprosy) (Ryll et al. 2001; Kai et al. 2007). Asselineau and Asselineau (1978) provide an overview of mycobacterial trehaloselipids. The mycolic acids of the mycobacterial glycolipids are usually highly complex and comprise various functional groups, such as epoxy, ester, keto, methoxy, or cyclopropane groups (Barry et al. 1998).

Mannosylerythritollipids (MELs) comprise 4-*O*-β-D-mannopyranosyl-D-erythritol in their carbohydrate moiety, which may display diverse acylation patterns. The chain lengths of the acyl group vary considerably. MELs are mainly known from yeast species such as *Candida* (Kim et al. 1999) and *Pseudozyma* (formerly *Candida*) (Rau et al. 2005; Morita et al. 2008) and the closely related *Ustilago maydis* (Bolker et al. 2008). The typical MELs are illustrated in Figure 1.5, with the main components of the mixtures usually being MEL-A and MEL-B. Regarding the application potential, MELs are among the most promising glycolipids (Rau and Kitamoto 2008). One of the reasons for this is their suitability for pharmaceutical applications.

Glucoselipids are comparatively unusual glycolipids. Rubiwettin RG1 from *Serratia rubidaea* (Matsuyama et al. 1990) is a rhamnolipid-like exolipid comprising a glucose unit linked to two 3-hydroxyl fatty acids with chain lengths C14 and C10 as lipid main components. Another glucose lipid has been described by *Alcanivorax borkumensis* (Yakimov et al. 1998). The lipid moiety here comprises a 3-hydroxydecanacid tetramer (Schulz et al. 1991).

Cellobioselipids (ustilagin acids) are described as the second glycolipid group of *U. maydis* (Figure 1.6). The disaccharide cellobiose is glycosidically linked to the terminal hydroxyl group of a 15,16-dihydroxypalmitin acid (ustilagin acid A) or a 2,15,16-trihydroxypalmitin acid (ustilagin acid B). The cellobiose is substituted variably by acetyl or different acyl groups in the 6′ and 2″ positions (Bolker et al. 2008).

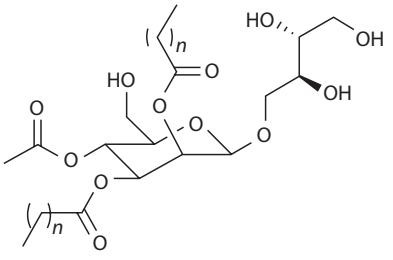

FIGURE 1.5 Typical mannosylerythritollipids (MELs) from *Pseudozyma* sp. $n = 6$, 8, 10, or 12.

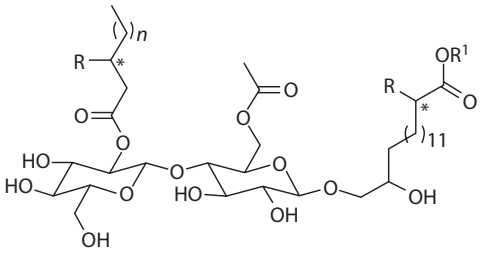

FIGURE 1.6 Cellobioselipide from *Ustilago maydis* R = OH or H; R^1 = H or CH_3; n = 1–14.

1.4 POLYKETIDEGLYCOSIDS

Although polyketides are typical secondary metabolites of microorganisms, comparatively few glycosylated polyketides have been described as biosurfactants. The polyketides with amphoteric properties include, among others, ionophoric and macrocyclical glycosides. The following lists two examples for microbially producible amphoteric polyketideglycosides.

The designation ionophore usually describes composite macrocyclical compounds that may form reversible chelates with ions and transport them as carriers through biological membranes otherwise impermeable to ions. The term is derived from "ion-carrying" (Greek "carrying" = phorós). Ionophorics are a functionally limited heterogeneous group of amphoteric molecules. In the narrower sense, they are not among the biosurfactants. Interesting glycosidic ionophores are the colopsinols A–E isolated from extracts of the marine dinoflagellate *Amphidinium* sp. (Y-5) (Kobayashi et al. 1999; Kubota et al. 1999, 2000; Kobayashi and Kubota 2007). The hydrophobic polyketide aglycone of colopsinol A (Figure 1.7) is formed by an aliphatic, linear C56 body that comprises two methyl, one methylide, two keto, five hydroxyl, two epoxy, and one sulfate-esterified tetrahydropyrane groups. The hydrophilic part is formed by the sugar component gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucose) and the sulfate ester.

An interesting bioactive polyketide from the macrolide group is elaiophylin (see Figure 1.8), a dimeric makrodiolidglycoside with side chains folded into a cyclic hemiketal and glycolized with 2-desoxy-l-fucose (Arcamone et al. 1959; Kaiser and Kellerschierlein 1981). Elaiophyline and similar derivatives are formed by various *Streptomycetes* (specifically *Streptomyces melanosporus*) and can be produced by fermentation, partially in large quantities (Haydock et al. 2004).

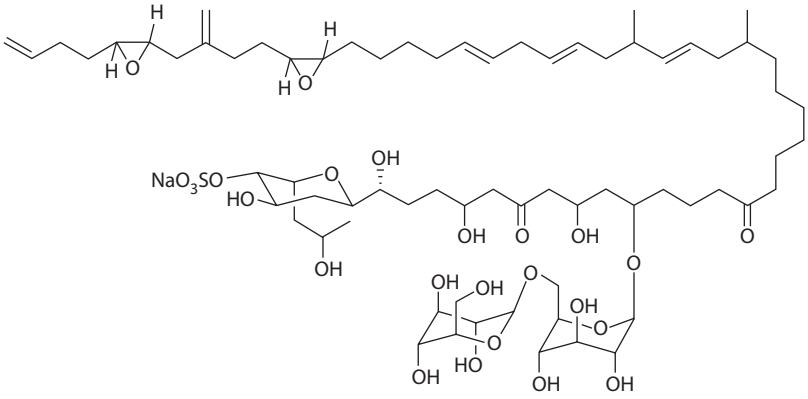

FIGURE 1.7 Colopsinol A from *Amphidinium* sp.

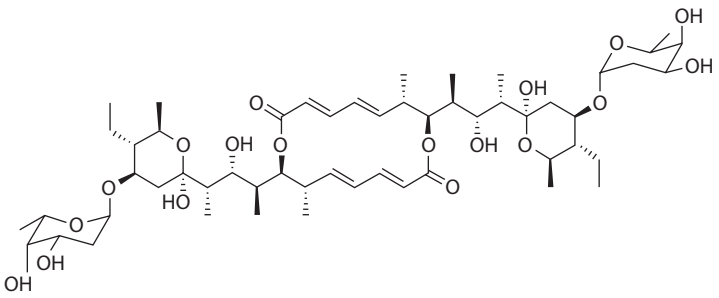

FIGURE 1.8 Elaiophylin from *Streptomyces melanosporus.*

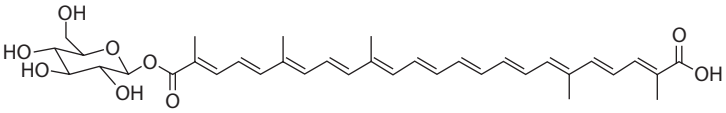

FIGURE 1.9 Carotenoid glycosiden from *Pseudomonas rhodos* and red-strain *Rhizobium lupine.*

1.5 ISOPRENOIDE AND CAROTENOID GLYCOLIPIDS

Microbial carotenoids are often formed by thermophilic bacteria. Therefore, it is assumed that the carotenoid formation is a protective mechanism to stabilize the cell membranes at high temperatures. Specifically in carotenoid glycosides (Figure 1.9), it is assumed that they are able to bridge the lipid double layer of microbial cell membranes to stabilize them at high temperatures (Yokoyama et al. 1995). As an example of this, the carotenoid glycosid b-D-glucosyl of the 4,4″-diapocarotene-6,6′-dioic acid (Figure 1.9) was isolated from the thermophilic microorganisms *Pseudomonas rhodos* and *Rhizobium lupini* (Liaaen-Jensen 1969; Kleinig et al. 1977; Kleinig and Broughton 1982a,b; Kleinig and Schmitt 1982).

1.6 LIPOPEPTIDES

Microbial lipopeptides are cyclic peptides that are acylated with a fatty acid. They are secreted into the growth medium by various microorganisms, including Gram-positive species, such as *Bacillus*, *Lactobacillus*, and *Streptomyces*, and Gram-negative species, such as *Pseudomonas* and *Serratia*. The natural lipopeptide that was first discovered was surfactin by *B. subtilis* (Arima et al. 1968). Many lipopeptides show not only a reduction of surface tension (Vater 1986) but also a strong antibiotic effect (Baltz et al. 2005). The best-known compounds of this class are certainly surfactin, polymyxin B, and the lipopeptidic antibiotic daptomycin by *Streptomyces roseosporus,* approved since 2003 (Eisenstein 2004; Baltz et al. 2005). Dexter et al. (2006) and Dexter and Middelberg (2007a,b) provide a current overview of the different groups of peptides and lipopeptides lowering surface tension. In addition to natural lipopeptides, synthetic lipopeptides have also been examined as antibiotics more frequently (Jerala 2007).

The nomenclature of the lipopeptides has been rather chaotic; hence, similar lipopeptides of one group are often called by different names, while other groups are summarized under one name. An example of this is the group of surfactins. In contrast to proteins, lipopeptides are not formed ribosomally by translation of an mRNA, but by special, non-ribosomal peptidsynthases, in which one module each leads to the addition of an amino acid, ring closure, and acylation (Peypoux et al. 1999).

Surfactin from *Bacillus subtilis* is a cyclic lipopeptide (Figure 1.10) comprising seven amino acids and different 3-hydroxyl fatty acids. The main component is the 3-hydroxyl-13-methyl-myristin

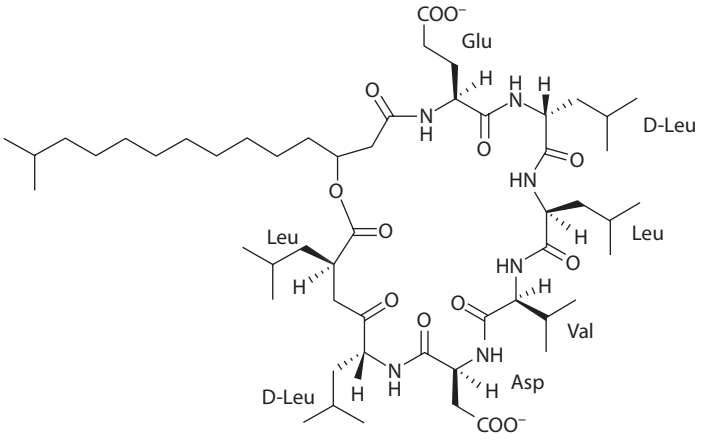

FIGURE 1.10 Representative surfactin from *B. subtilis.*

acid. Surfactin is a very good surfactant that also has antibacterial properties (Arima et al. 1968; Kakinuma et al. 1968; Peypoux et al. 1999; Ongena and Jacques 2008). It is synthesized by a linear, non-ribosomal peptide synthase, the surfactin synthase. When dissolved, it shows a characteristic saddle-like conformation that is essential for the wide bioactive range of surfactin (Hue et al. 2001). In addition to surfactin, *B. subtilis* produces two other lipopeptides as well, specifically Iturin and Fengycin.

Polymyxines are a group of cationic, branched, cyclic dekapeptides. The polymyxines A–E have been insulated from different strains of *Bacillus polymyxa* since 1947 (Stansly 1949). Polymyxine B is a decapeptide with eight amino acids forming a ring and linked to a branched fatty acid. The lipopeptid gained a certain importance as an antibiotic (Zavascki et al. 2007; Kwa et al. 2008; Landman et al. 2008).

Viscosin from *Pseudomonas fluorescens, P. libanensis*, and *P. viscosa* is a cyclic lipopeptide that reduces surface tension. The structure contains hydrophobic amino acids, linked to a fatty acid (Neu and Poralla 1990; Neu et al. 1990; Saini et al. 2008).

Serrawettin is a group of cyclodepsipeptides produced by *Serratia marcescens.* Serrawettin W1 or Serratamolide (Figure 1.11) is a rotationally symmetric cyclodepsipeptide, produced non-ribosomally by an aminolipid synthetase (Li et al. 2005). It comprises two serine and mainly two 3-hydroxydecan acid parts (Wasserman et al. 1962; Matsuyama et al. 1985). Serrawettin W2 is a cyclic lipopeptide composed of five different amino acids and 3-hydroxydecanoic acid and is required for swarming motility in *S. marcescens* (Matsuyama et al. 1992; Lindum et al. 1998). Serrawettin W2 shows antimicrobial properties and is sensed and avoided by nematodes (Pradel et al. 2007). Serratamolide and serrawettin W2 have been used in studies of therapeutic activity and proapoptotic effects in cancer research (Escobar-Diaz et al. 2005; Soto-Cerrato et al. 2005).

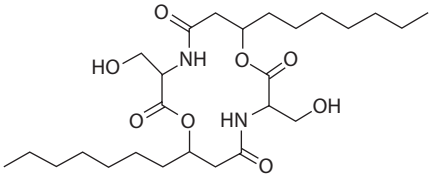

FIGURE 1.11 Serratamolide from *Serratia marcescens.*

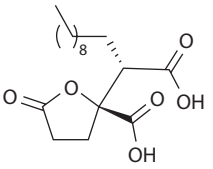

FIGURE 1.12 Spiculisporic acid from *Penicillium spiculisporum*.

1.7 SPICULISPORIC ACID

Spiculisporic acid (Figure 1.12) (4,5-dicarboxy-γ-oentadecanolacton) is formed as a secondary metabolite of *Penicillium spiculisporum* and accumulates in the culture fluid, from which needle-shaped crystals can be acquired by acid precipitation and subsequent recrystallization. The maximum titer reported by Tabuchi et al. (1977) is up to 110 g/L after a cultivation time of 10 days.

The commercial potential of spiculisporic acid has been examined comprehensively. It is not only interesting as a biosurfactant due to its availability, but also for its unique structure and environmental compatibility (Tabuchi et al. 1977; Ishigami et al. 2000).

1.8 HIGH-MOLECULAR WEIGHT BIOSURFACTANTS

High-molecular weight polymeric biosurfactants are produced by many bacteria of different species. They are polysaccharides, proteins, lipopolysaccharides, lipoproteins, or complex mixtures of these referred to as lipoheteropolysaccharides. The best-known high-molecular weight biosurfactants are emulsans that are formed by various prokaryotes, including *Archaea*, Gram-positive, and Gram-negative bacteria. The emulsan of *Acinetobacter* types is best known, however (Sar and Rosenberg 1983; Rosenberg and Ron 1997). Two of these *Acinetobacter* emulsans, RAG-1 (Figure 1.13) and BD4 emulsan, have been examined in more detail.

Emulsan is a highly effective emulsifier even in low concentrations of 0.01–0.001%. The emulsifying effect is relatively specific: pure aliphatics, aromatics, or cyclical hydrocarbons are not emulsified, while many mixtures of aliphatic and aromatic compounds are effectively emulsified (Rosenberg and Ron 1999).

The RAG-1 emulsan of *Acinetobacter* sp. ATCC 31012 (RAG-1) is not a defined polysaccharide, but a complex mix of high-molecular exopolysaccharides and lipopolysaccharides. The exopolysaccharide is probably a anionic polysaccharide with a molecular weight of 200–250 kDa (Dams-Kozlowska et al. 2008). The lipopolysaccharide of RAG-1 emulsan probably has a polysaccharide part of D-galactosamine, D-galactosaminuronic acid, and di-amino-6-deoxy-D-glucose at a ratio of 1:1:1.

The amino groups are either acetylated or amidically linked to a 3-hydroxyl butyric acid. The lipid moiety comprises singly unsaturated fatty acids with a chain length of 10–18 C-atoms, linked to the saccharid moiety either via *O*-acyl- or *N*-acyl links. The lipid share makes up the emulsan by up to 23% (w/w) (Zhang et al. 1999).

The *Acinetobacter calcoaceticus* BD4 emulsan, in contrast, is a complex protein–polysaccharide mixture. The polysaccharide moiety of the BD4 emulsan comprises repeating heptasaccharides that are built from L-rhamnose, D-glucose, D-glucuronic acid, and D-mannose at a ratio of 4:1:1:1. It is interesting that neither the extracellular protein nor the polysaccharide reduce surface tension in their pure forms (Kaplan et al. 1985, 1987). A similarly complex biosurfactant that also comprises polysaccharide and protein moieties is alasane from *Acinetobacter radioresistens* KA53 (Navon-Venezia et al. 1995, 1998). The polysaccharide moiety is rather unusual due to covalently bound alanine. The emulsifying effect of alasane is essentially due to one of the alasane proteins (45 kDa), which has a stronger emulsifying effect than the alasane as such (Toren et al. 2002).

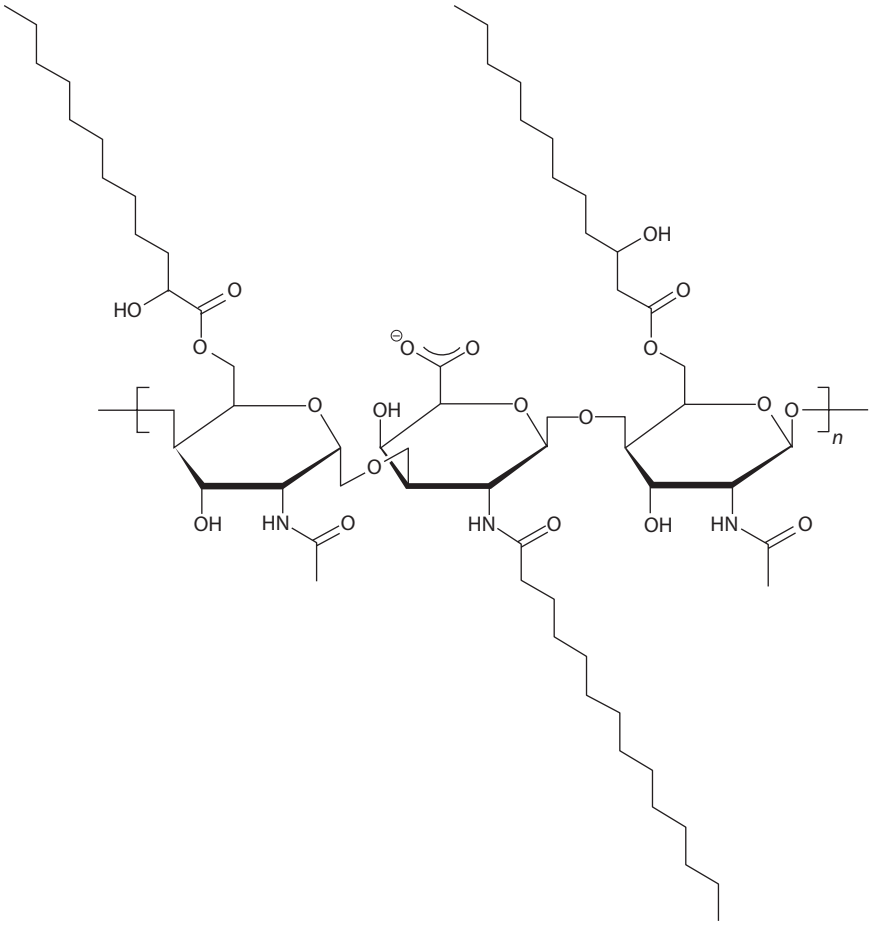

FIGURE 1.13 RAG-1 emulsan from *Acinetobacter* sp. ATCC 31012.

Gutierrez et al. (2008) describe another high-molecular glycoprotein with surfactant effect from *Pseudoalteromonas* sp. TG12, called PE12 by them. PE12 has a molecular weight in excess of 2000 kDa. PE12 is noticeable because it comprises xylose at an unusually high ratio (28%).

1.9 PROTEINS

Proteins are a very interesting class of little-explored and biotechnologically hardly used biosurfactants. Naturally occurring foams often contain protein degradation products or special foaming proteins. A particularly interesting example of this is the ranaspumines of tropical frogs that use foam nests to protect their eggs. These proteins comprise different structures with foam-stabilizing properties. Another strongly foaming protein is latherin, mainly known from horse sweat (Cooper and Kennedy 2010).

A better-known class is that of hydrophobines, small proteins secreted by fungi (Wessels 1997, 2000; Wosten and Wessels 1997; Linder 2009).

1.10 CONCLUSIONS

Microorganisms are able to form a wide range of metabolites that reduce surface tension. They are either secreted into the culture medium or integrated into the cell wall, usually, permitting

growth on or in the reception of hydrophobic substrates. These "biosurfactants" usually show a very low critical micelle concentration as compared with chemically produced surfactants, are biodegradable, and often have interesting bio-active properties. Therefore, they are also very interesting for industrial applications in food, cosmetics, and pharmaceutics.

Many of the above biosurfactants appear to be naturally optimized for the corresponding microorganisms, but they are not perfect for industrial applications in the present form, and therefore only used in niche areas so far. Therefore, the search for microbial producers of new structures, for example, in unusual biotopes, is still very interesting. In the meantime, novel metagenom-based screening methods have come into use here as well.

In the still low number of established microbial production procedures, it turns out that modern molecular–biological methods may not only permit higher product concentrations, but also clearly improve molecule structures regarding specific applications. Both the above yeast-produced sophorolipids that have long been known in literature and the MELs have become important platform composites for many different industrial applications by modifying their basic structures in the meantime. This is expected for bacterial rhamnolipids in future as well.

Another still existing limitation in biosurfactants is the much higher production costs as compared with chemically produced surfactants, preventing widespread use of these interesting compounds. The economic efficiency of the production processes strongly depends on suitable and effective methods of isolation and cleaning of the products formed in all cases.

All in all, biosurfactants produced based on renewable resources are currently about to leave their niche position and become an industrial reality.

REFERENCES

Abdel-Mawgoud, A. M., F. Lépine et al. 2010. Rhamnolipids: Diversity of structures, microbial origins, and roles. *Applied Microbiology and Biotechnology*, **86**: 1323–1336.

Andrä, J., J. Rademann et al. 2006. Endotoxin-like properties of a rhamnolipid exotoxin from *Burkholderia* (*Pseudomonas*) *plantarii*: Immune cell stimulation and biophysical characterization. *Biological Chemistry* **387**(3): 301–310.

Arcamone, F. M., C. Bertazzoli et al. 1959. Melanosporin and elaiophylin, new antibiotics from *Streptomyces melanosporus* (sive *Melanosporofaciens*) n. sp. *Giorn Microbiol* **7**(3): 207–216.

Arima, K., A. Kakinuma et al. 1968. Surfactin a crystalline peptidelipid surfactant produced by *Bacillus subtilis*—Isolation characterization and its inhibition of fibrin clot formation. *Biochemical and Biophysical Research Communications* **31**(3): 488–494.

Asmer, H. J., S. Lang et al. 1988. Microbial-production, structure elucidation and bioconversion of sophorose lipids. *Journal of the American Oil Chemists Society* **65**(9): 1460–1466.

Asselineau, C. and J. Asselineau 1978. Specific lipids in mycobacteria. *Annales de Microbiologie* **A129**(1): 49–69.

Baltz, R. H., V. Miao et al. 2005. Natural products to drugs: Daptomycin and related lipopeptide antibiotics. *Natural Product Reports* **22**(6): 717–741.

Barry, C. E., R. E. Lee et al. 1998. Mycolic acids: Structure, biosynthesis and physiological functions. *Progress in Lipid Research* **37**(2–3): 143–179.

Bolker, M., C. W. Basse et al. 2008. *Ustilago maydis* secondary metabolism—From genomics to biochemistry. *Fungal Genetics and Biology* **45**: S88–S93.

Cooper, A. and M. W. Kennedy 2010. Biofoams and natural protein surfactants. *Biophysical Chemistry* **151**(3): 96–104.

Dams-Kozlowska, H., M. P. Mercaldi et al. 2008. Modifications and applications of the *Acinetobacter venetianus* RAG-1 exopolysaccharide, the emulsan complex and its components. *Applied Microbiology and Biotechnology* **81**(2): 201–210.

Dembitsky, V. M. 2004a. Astonishing diversity of natural surfactants: 1. Glycosides of fatty acids and alcohols. *Lipids* **39**(10): 933–953.

Dembitsky, V. M. 2004b. Chemistry and biodiversity of the biologically active natural glycosides. *Chemistry & Biodiversity* **1**(5): 673–781.

Dembitsky, V. M. 2005a. Astonishing diversity of natural surfactants: 2. Polyether glycosidic ionophores and macrocyclic glycosides. *Lipids* **40**(3): 219–248.

Dembitsky, V. M. 2005b. Astonishing diversity of natural surfactants: 3. Carotenoid glycosides and isoprenoid glycolipids. *Lipids* **40**(6): 535–557.
Dembitsky, V. M. 2005c. Astonishing diversity of natural surfactants: 4. Fatty acid amide glycosides, their analogs and derivatives. *Lipids* **40**(7): 641–660.
Dembitsky, V. M. 2005d. Astonishing diversity of natural surfactants: 5. Biologically active glycosides of aromatic metabolites. *Lipids* **40**(9): 869–900.
Dembitsky, V. M. 2005e. Astonishing diversity of natural surfactants: 6. Biologically active marine and terrestrial alkaloid glycosides. *Lipids* **40**(11): 1081–1105.
Dembitsky, V. M. 2006. Astonishing diversity of natural surfactants: 7. Biologically active hemi- and monoterpenoid glycosides. *Lipids* **41**(1): 1–27.
Desai, J. D. and I. M. Banat 1997. Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews* **61**(1): 47–64.
Dexter, A. F., A. S. Malcolm et al. 2006. Reversible active switching of the mechanical properties of a peptide film at a fluid–fluid interface. *Nature Materials* **5**(6): 502–506.
Dexter, A. F. and A. P. J. Middelberg 2007a. Peptides as functional surfactants. *1st International Conference on Multiscale Structures and Dynamics of Complex Systems*, Beijing, People's Republic of China.
Dexter, A. F. and A. P. J. Middelberg 2007b. Switchable peptide surfactants with designed metal binding capacity. *Journal of Physical Chemistry C* **111**(28): 10484–10492.
Deziel, E., F. Lepine et al. 2000. Mass spectrometry monitoring of rhamnolipids from a growing culture of *Pseudomonas aeruginosa* strain 57RP. *Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids* **1485**(2–3): 145–152.
Eisenstein, B. I. 2004. Lipopeptides, focusing on daptomycin, for the treatment of Gram-positive infections. *Expert Opinion on Investigational Drugs* **13**(9): 1159–1169.
Escobar-Diaz, E., E. Lopez-Martin et al. 2005. AT514, a cyclic depsipeptide from *Serratia marcescens*, induces apoptosis of B-chronic lymphocytic leukemia cells: Interference with the Akt/NF-Î°B survival pathway. *Leukemia* **19**(4): 572–579.
Fujii, T., R. Yuasa et al. 1999. Biodetergent—IV. Monolayers of corynomycolic acids at the air–water interface. *Colloid and Polymer Science* **277**(4): 334–339.
Goren, M. B. 1972. Mycobacterial lipids—Selected topics. *Bacteriological Reviews* **36**(1): 33.
Gutierrez, T., T. Shimmield et al. 2008. Emulsifying and metal ion binding activity of a glycoprotein exopolymer produced by *Pseudoalteromonas* sp. strain TG12. *Applied and Environmental Microbiology* **74**(15): 4867–4876.
Gutnick, D. L., H. Bach et al. 2011. 3.59—*Biosurfactants. Comprehensive Biotechnology* (2nd edition). Burlington, Academic Press: pp. 699–715.
Haydock, S. F., T. Mironenko et al. 2004. The putative elaiophylin biosynthetic gene cluster in *Streptomyces* sp DSM4137 is adjacent to genes encoding adenosylcobalamin-dependent methylmalonyl CoA mutase and to genes for synthesis of cobalamin. *Journal of Biotechnology* **113**(1–3): 55–68.
Hörmann, B., M. M. Muller et al. 2010. Rhamnolipid production by *Burkholderia plantarii* DSM 9509(T). *European Journal of Lipid Science and Technology* **112**(6): 674–680.
Hue, N., L. Serani et al. 2001. Structural investigation of cyclic peptidolipids from *Bacillus subtilis* by high-energy tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* **15**(3): 203–209.
Ishigami, Y., Y. J. Zhang et al. 2000. Spiculisporic acid. Functional development of biosurfactant. *Chimica Oggi-Chemistry Today* **18**(7–8): 32–34.
Jerala, R. 2007. Synthetic lipopeptides: A novel class of anti-infectives. *Expert Opinion on Investigational Drugs* **16**(8): 1159–1169.
Kai, M., Y. Fujita et al. 2007. Identification of trehalose dimycolate (cord factor) in *Mycobacterium leprae*. *Febs Letters* **581**(18): 3345–3350.
Kaiser, H. and W. Kellerschierlein 1981. Structure elucidation of elaiophylin—Spectroscopy and chemical degradation. *Helvetica Chimica Acta* **64**(2): 407–424.
Kakinuma, A., G. Tamura et al. 1968. Wetting of fibrin plate and apparent promotion of fibrinolysis by surfactin a new bacterial peptidlipid surfactant. *Experientia* **24**(11): 1120–1121.
Kaplan, N., E. Rosenberg et al. 1985. Structural studies of the capsular polysaccharide of *Acinetobacter calcoaceticus* Bd4. *European Journal of Biochemistry* **152**(2): 453–458.
Kaplan, N., Z. Zosim et al. 1987. Reconstitution of emulsifying activity of *Acinetobacter calcoaceticus* Bd4 emulsan by using pure polysaccharide and protein. *Applied and Environmental Microbiology* **53**(2): 440–446.
Kappeli, O. and W. R. Finnerty 1979. Partition of alkane by an extracellular vesicle derived from hexadecane-grown *Acinetobacter*. *Journal of Bacteriology* **140**(2): 707–712.

Kappeli, O. and W. R. Finnerty 1980. Characteristics of hexadecane partition by the growth-medium of *Acinetobacter* sp. *Biotechnology and Bioengineering* **22**(3): 495–503.
Kim, H. S., B. D. Yoon et al. 1999. Characterization of a biosurfactant, mannosylerythritol lipid produced from *Candida* sp SY16. *Applied Microbiology and Biotechnology* **52**(5): 713–721.
Kleinig, H. and W. J. Broughton 1982a. Carotenoids of rhizobia. 5. Carotenoid-pigments in a red strain of rhizobium from *Lotononis bainesii* Baker. *Archives of Microbiology* **133**(2): 164–164.
Kleinig, H. and W. J. Broughton 1982b. Carotenoid pigments in a red strain of Rhizobium, from *Lotononis bainesii* Baker. *Arch. Microbiol.* **133**: 164–174.
Kleinig, H., W. Heumann et al. 1977. Carotenoids of *Rhizobia*. 1. New carotenoids from *Rhizobium lupini*. *Helvetica Chimica Acta* **60**(1): 254–258.
Kleinig, H. and R. Schmitt 1982. On the biosynthesis of C30 carotenoic acid glucosyl esters in *Pseudomonas rhodos*—Analysis of Car-mutants. *Zeitschrift Fur Naturforschung C-A Journal of Biosciences* **37**(9): 758–760.
Kobayashi, J., T. Kubota et al. 1999. Colopsinol A, a novel polyhydroxyl metabolite from marine dinoflagellate *Amphidinium* sp. *Journal of Organic Chemistry* **64**(5): 1478–1482.
Kobayashi, J. I. and T. Kubota 2007. Bioactive macrolides and polyketides from marine dinoflagellates of the genus *Amphidinium*. *Journal of Natural Products* **70**(3): 451–460.
Kretschmer, A., H. Bock et al. 1982. Chemical and physical characterization of interfacial-active lipids from *Rhodococcus erythropolis* grown on normal-alkanes. *Applied and Environmental Microbiology* **44**(4): 864–870.
Kubota, T., M. Tsuda et al. 1999. Colopsinols B and C, new long chain polyhydroxy compounds from cultured marine dinoflagellate Amphidinium sp. *Journal of the Chemical Society-Perkin Transactions* **1**(23): 3483–3487.
Kubota, T., M. Tsuda et al. 2000. Colopsinols D and E, new polyhydroxyl linear carbon chain compounds from marine dinoflagellate *Amphidinium* sp. *Chemical & Pharmaceutical Bulletin* **48**(10): 1447–1451.
Kwa, A. L., V. H. Tam et al. 2008. Polymyxins: A review of the current status including recent developments. *Annals Academy of Medicine Singapore* **37**(10): 870–883.
Landman, D., C. Georgescu et al. 2008. Polymyxins revisited. *Clinical Microbiology Reviews* **21**(3): 449–465.
Lang, S. and J. C. Philp 1998. Surface-active lipids in rhodococci. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* **74**(1–3): 59–70.
Lang, S. and W. Trowitsch-Kienast 2002. *Biotenside*. Stuttgar, Leipzig, Wiesbaden, B.G. Teubner.
Li, H., T. Tanikawa et al. 2005. *Serratia marcescens* gene required for surfactant serrawettin W1 production encodes putative aminolipid synthetase belonging to nonribosomal peptide synthetase family. *Microbiology and Immunology* **49**(4): 303.
Liaaen-Jensen, S. 1969. Selected examples of structure determination of natural carotenoids. *Pure and Applied Chemistry* **20**: 421–448.
Linder, M. B. 2009. Hydrophobins: Proteins that self assemble at interfaces. *Current Opinion in Colloid & Interface Science* **14**(5): 356–363.
Lindum, P. W., U. Anthoni et al. 1998. N-Acyl-L-homoserine lactone autoinducers control production of an extracellular lipopeptide biosurfactant required for swarming motility of *Serratia liquefaciens* MG1. *Journal of Bacteriology* **180**(23): 6384–6388.
Matsuyama, T., M. Fujita et al. 1985. Wetting agent produced by *Serratia marcescens*. *FEMS Microbiology Letters* **28**(1): 125–129.
Matsuyama, T., K. Kaneda et al. 1990. Surface-active novel glycolipid and linked 3-hydroxy fatty-acids produced by *Serratia rubidaea*. *Journal of Bacteriology* **172**(6): 3015–3022.
Matsuyama, T., K. Kaneda et al. 1992. A novel extracellular cyclic lipopeptide which promotes flagellum-dependent and -independent spreading growth of *Serratia marcescens*. *Journal of Bacteriology* **174**(6): 1769–1776.
Merchant, R. and I. M. Banat 2012. Microbial biosurfactants: Challenges and opportunities for future exploitation. *Trends in Biotechnology* **30**(11): 558–565.
Morita, T., M. Konishi et al. 2008. Production of glycolipid biosurfactants, mannosylerythritol lipids, by *Pseudozyma siamensis* CBS 9960 and their interfacial properties. *Journal of Bioscience and Bioengineering* **105**(5): 493–502.
Navon-Venezia, S., E. Banin et al. 1998. The bioemulsifier alasan: Role of protein in maintaining structure and activity. *Applied Microbiology and Biotechnology* **49**(4): 382–384.
Navon-Venezia, S., Z. Zosim et al. 1995. Alasan, a new bioemulsifier from *Acinetobacter radioresistens*. *Applied and Environmental Microbiology* **61**(9): 3240–3244.

Neu, T. R., T. Hartner et al. 1990. Surface-active properties of viscosin—A peptidolipid antibiotic. *Applied Microbiology and Biotechnology* **32**(5): 518–520.

Neu, T. R. and K. Poralla 1990. Emulsifying agents from bacteria isolated during screening for cells with hydrophobic surfaces. *Applied Microbiology and Biotechnology* **32**(5): 521–525.

Nunez, A., R. Ashby et al. 2001. Analysis and characterization of sophorolipids by liquid chromatography with atmospheric pressure chemical ionization. *Chromatographia* **53**(11–12): 673–677.

Ongena, M. and P. Jacques 2008. *Bacillus* lipopeptides: Versatile weapons for plant disease biocontrol. *Trends in Microbiology* **16**(3): 115–125.

Peng, F., Z. Liu et al. 2007. An oil-degrading bacterium: *Rhodococcus erythropolis* strain 3C-9 and its biosurfactants. *Journal of Applied Microbiology* **102**(6): 1603–1611.

Peypoux, F., J. M. Bonmatin et al. 1999. Recent trends in the biochemistry of surfactin. *Applied Microbiology and Biotechnology* **51**(5): 553–563.

Pradel, E., Y. Zhang et al. 2007. Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences* **104**(7): 2295–2300.

Rau, U. and D. Kitamoto. 2009. Mannosylerythritol lipids: Production and downstream processing. In: D. Hayes et al. (eds.), *Biobased Surfactants and Detergents: Synthesis, Properties, and Applications*. Vol. 51–77. AOCS Press, Urbana, USA.

Rau, U., L. A. Nguyen et al. 2005. Fed-batch bioreactor production of mannosylerythritol lipids secreted by *Pseudozyma aphidis*. *Applied Microbiology and Biotechnology* **68**(5): 607–613.

Rehm, H. J. and I. Reiff 1981. *Mechanisms and Occurrence of Microbial Oxidation of Long-chain Alkanes*. Berlin/Heidelberg, Springer.

Rosenberg, E. and E. Z. Ron 1997. Bioemulsans: Microbial polymeric emulsifiers. *Current Opinion in Biotechnology* **8**(3): 313–316.

Rosenberg, E. and E. Z. Ron 1999. High- and low-molecular-mass microbial surfactants. *Applied Microbiology and Biotechnology* **52**(2): 154–162.

Ryll, R., Y. Kumazawa et al. 2001. Immunological properties of trehalose dimycolate (cord factor) and other mycolic acid-containing glycolipids—A review. *Microbiology and Immunology* **45**(12): 801–811.

Saini, H. S., B. E. Barragan-Huerta et al. 2008. Efficient purification of the biosurfactant viscosin from *Pseudomonas libanensis* strain M9–3 and its physicochemical and biological properties. *Journal of Natural Products* **71**(6): 1011–1015.

Sar, N. and E. Rosenberg 1983. Emulsifier production by *Acinetobacter calcoaceticus* strains. *Current Microbiology* **9**(6): 309–313.

Satpute, S. K., A. G. Banpurkar et al. 2010. Methods for investigating biosurfactants and bioemulsifiers: A review. *Critical Reviews in Biotechnology* **30**(2): 127–144.

Schulz, D., A. Passeri et al. 1991. Marine biosurfactants.1. Screening for biosurfactants among crude-oil degrading marine microorganisms from the North-Sea. *Zeitschrift für Naturforschung C-A Journal of Biosciences* **46**(3–4): 197–203.

Sen, R. 2010. *Biosurfactants*. New York, Springer.

Soberón-Chávez, G. 2010. *Biosurfactants: From Genes to Applications*. Springer.

Soto-Cerrato, V., B. Montaner et al. 2005. Cell cycle arrest and proapoptotic effects of the anticancer cyclodepsipeptide serratamolide (AT514) are independent of p53 status in breast cancer cells. *Biochemical Pharmacology* **71**(1–2): 32–41.

Stansly, P. G. 1949. The polymyxins—A review and assessment. *American Journal of Medicine* **7**(6): 807–818.

Suzuki, T., K. Tanaka et al. 1969. Trehalose lipid and alpha-branched-beta-hydroxy fatty acid formed by bacteria grown on N-alkanes. *Agricultural and Biological Chemistry* **33**(11): 1619.

Tabuchi, T., I. Nakamura et al. 1977. Factors affecting production of open-ring acid of spiculisporic acid by *Penicillium spiculisporum*. *Journal of Fermentation Technology* **55**(1): 43–49.

Toren, A., E. Orr et al. 2002. The active component of the bioemulsifier alasan from *Acinetobacter radioresistens* KA53 is an OmpA-like protein. *Journal of Bacteriology* **184**(1): 165–170.

Vater, J. 1986. Lipopetides, an attractive class of microbial surfactants. In: Kilian, H.-G. and Weiss, A. (eds.), *Progress in Colloid and Polymer Science*. Berlin/Heidelberg, Verlag, Springer: **72**: 12–18.

Wasserman, H. H., J. J. Keggi et al. 1962. Structure of serratamolide. *Journal of the American Chemical Society* **84**(15): 2978–2982.

Wessels, J. G. H. 1997. Hydrophobins: Proteins that change the nature of the fungal surface. *Advances in Microbial Physiology, Vol. 38*. R. K. Poole. **38:** 1–45.

Wessels, J. G. H. 2000. Hydrophobins, unique fungal proteins. *Mycologist* **14**(4): 153–159.

Wosten, H. A. B. and J. G. H. Wessels 1997. Hydrophobins, from molecular structure to multiple functions in fungal development. *Mycoscience* **38**(3): 363–374.

Yakimov, M. M., P. N. Golyshin et al. 1998. *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *International Journal of Systematic Bacteriology* **48**: 339–348.

Yokoyama, A., G. Sandmann et al. 1995. Thermozeaxanthins, new carotenoid-glycoside-esters from thermophilic eubacterium *Thermus thermophilus*. *Tetrahedron Letters* **36**(27): 4901–4904.

Zavascki, A. P., L. Z. Goldani et al. 2007. Polymyxin B for the treatment of multidrug-resistant pathogens: A critical review. *Journal of Antimicrobial Chemotherapy* **60**(6): 1206–1215.

Zhang, J. W., S. H. Lee et al. 1999. Surface properties of emulsan-analogs. *Journal of Chemical Technology and Biotechnology* **74**(8): 759–765.

2 Sophorolipids
Microbial Synthesis and Application

I.N.A. Van Bogaert, K. Ciesielska, B. Devreese, and W. Soetaert

CONTENTS

2.1 INTRODUCTION

Sophorolipids are glycolipidic biosurfactants extracellularly produced by several yeast species. Owing to the nonpathogenic character of the production host and the high yields, there is quite some commercial interest in these molecules. Indeed, to date sophorolipids are available in the market and find applications in real-life products. Further research is currently being conducted in order to broaden their application range, either by evaluating their behavior in new applications or by modifying the sophorolipid chemical structure.

2.2 STRUCTURE AND PROPERTIES

Sophorolipids are glycolipid biosurfactants consisting of a sophorose sugar head and a hydrophobic fatty acid tail. Sophorose is a glucose disaccharide with a β-1,2 bond and can be acetylated at the

6′ and/or 6″ positions. To the sophorose, a single terminal or subterminal hydroxylated fatty acid (C16 or C18) is β-glycosidically linked. The carboxylic end of this fatty acid can be free (acidic/open form) or internally esterified at the 4″ position of the sophorose head (lactone form) (Figure 2.1). Rarely, the esterification occurs at the 6′- or 6″ position. The hydroxyl fatty acid can contain one or more unsaturated bonds (Asmer et al. 1988). The main producers are yeast species belonging to the *Starmerella* clade. They synthesize sophorolipids as a mixture of molecules that differ in the fatty acid part (chain length, saturation, and position of hydroxylation), in the acetylation pattern as well in lactonization (Davila et al. 1993).

The exact structure of the sophorolipid isoform has immense influence on the physicochemical properties. Lactonic sophorolipids have enhanced biological properties (e.g., inhibiting effects), and have a better capacity to lower the surface tension, whereas the acidic sophorolipids are more soluble and are better foam formers (Van Bogaert et al. 2011). Despite the fact that di- or monoacetylated sophorolipids are less soluble, they have better antibacterial (Lang et al. 1989), antiviral, and cytokine stimulating effects (Shah et al. 2005). Concerning the surfactant properties however, one must bear in mind that there is a natural synergy among the different compounds occurring in the natural blend (Hirata et al. 2009a).

Sophorolipids lower the surface tension from 72.8 mN/m down to 40–30 mN/m, even in the presence of salts (Hirata et al. 2009b) and in a wide temperature range (Nguyen et al. 2010). The reported critical micelle concentration (CMC) values range from 11 to 250 mg/L, depending on the applied measuring methods and conditions (Develter and Lauryssen 2010). Even with this variation, these values are about two orders of magnitude lower compared with chemical-derived surfactants, adding to their environmental friendly profile and in certain applications circumventing the problems related to a higher price, as less product is needed.

The minimal dynamic surface tension is 32.1 mN/m, but is only achieved in semi-static conditions (at least 40 s) and high concentrations. Nevertheless, one can observe a fast drop in surface tension even at short bubble lifetimes of 30 ms, rendering sophorolipids potentially suitable for dynamic applications such as spray-on coating and cleaning, this is in contrast to other glycolipids (Develter and Lauryssen 2010). Sophorolipids are low-foaming surfactants even at high concentrations (Hirata et al. 2009b). Moreover, they are good wetting agents illustrated by their ability to decrease the contact angle of water on polyvinyl chloride (PVC) from 110° to 80° at a minimal concentration of 36 mg/L (Develter and Lauryssen 2010). Both features render sophorolipids ideal components for hard surface cleaning products and dishwashing rinse aids (EP1445302).

As can be expected from biosurfactants, sophorolipids were demonstrated to be readily biodegradable as determined by the standard manometric respirometry and stable metabolite studies OECD 301C and 301F (Hirata et al. 2009b; Renkin 2003). Aquatic toxicity is 10-fold less compared with conventional surfactants and *Daphnia* reproduction is not affected at all (Renkin 2003).

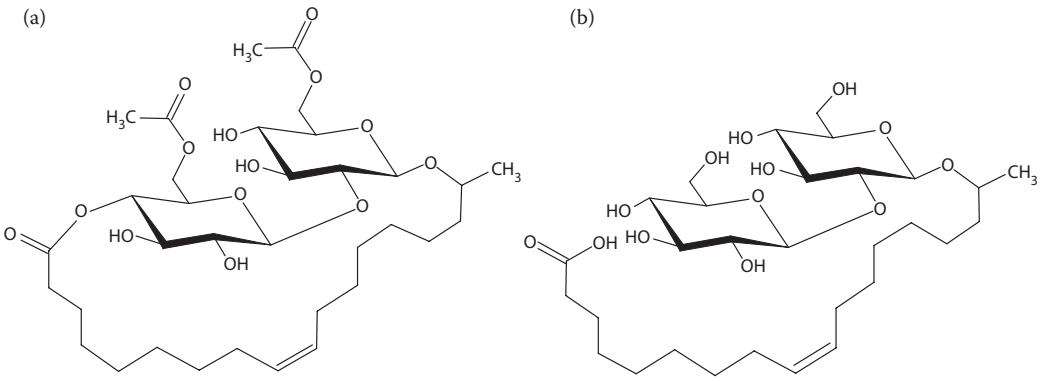

FIGURE 2.1 (a) Lactonic diacetylated sophorolipid. (b) Acidic nonacetylated sophorolipid.

Furthermore, tests with acidic sophorolipids and the natural blend pointed out that they are not irritating to the skin, do not trigger allergic reactions, and have an oral safety level that is greater than or equal to 5 mL/kg weight (US 5756471). Cytotoxicity was evaluated by the dimethylthiazol-diphenyltetrazoliumbromide method with human epidermal keratinocytes and was proven to be low (Hirata et al. 2009b).

While sophorolipids are demonstrated to be noncytoxic and do not influence aquatic systems, they inhibit growth of some fungi and Gram-positive bacteria, such as *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus, Streptococcus faecium, Propionibacterium acnes*, and *Corynebacterium xerosis*. These latter ones are the causal agents of acne and dandruff, respectively, rendering them attractive ingredients for cosmetic, hygienic, and pharmaco-dermatological products as described below (Hommel et al. 1987; Kim et al. 2002; Lang et al. 1989; Mager et al. 1987). The mode of action most likely involves interactions with the cellular membrane, as demonstrated for *B. subtillis* where increased leakage of intracellular enzymes was detected upon treatment (Kim et al. 2002). Further biological activities are discussed in the application part.

2.3 SUPRAMOLECULAR ASSEMBLY

Self-assembly is a process in which single components organize themselves into a structure or pattern as a result of specific local interactions, without external forces. Self-assembly of sophorolipids is entropically driven and takes place above the CMC. Beyond this point, surfactants organize themselves in water such that their polar head groups become oriented toward the water, whereas the hydrophobic tails cluster together. This orientation leads to the formation of various superstructures such as micelles, vesicles, or multilayers.

Acidic sophorolipids, especially, received a lot of attention because of their unique structural features including an asymmetrical polar head size (disaccharide vs. COOH) and a kinked hydrophobic core (*cis*-9-octadecenoic chain; Zhou et al. 2004). Different techniques have been employed to investigate the various structures of the self-assembly including light microscopy, small- and wide-angle x-ray scattering, Fourier transform infrared spectroscopy, and dynamic laser light scattering. The degree of ionization of the -COOH group greatly influences the sophorolipid self-assembly (Baccile et al. 2012). When the degree of ionization increases, negative charges at the micellar surfaces are introduced, which initiate changes in shape, aggregation state, and surface properties. Micelles are formed at low ($pH < 5$) and medium ($5 < pH < 8$) degrees of ionization. At high ionization ($pH > 8$), large net-like aggregates are observed.

In the same study of Baccile et al., the morphology of the assemblies was evaluated as a function of sophorolipid concentration. For sophorolipid concentrations <1 wt%, spherical micelles having an average radius of 3.0 nm existed. At a sophorolipid concentrations ≥1 wt%, micelles were no longer spherical and started to elongate from $c > 0.5$ wt%, and at $c = 5$ wt%, a cylindrical micelle shape was observed.

There are some contradictory reports on the formation of giant twisted and helical ribbons. Zhou et al. (2004) report on the formation of giant ribbons depending on the pH and time for the C18:1-*cis* sophorolipids, while Baccile et al. (2012) did not observe these structures and Dhasaiyan et al. (2013) reported giant ribbons exclusively for C18:1-*trans* sophorolipids.

2.4 SOPHOROLIPID-PRODUCING ORGANISMS

Candida apicola was the first species described to have produced sophorolipids (Gorin et al. 1961; formerly called *Torulopsis magnolia*). However, for industrial purposes, the most applied producer is *Starmerella* (syn. *Candida*) *bombicola*, a nonpathogenic yeast isolated from the honey of *Bombus* sp. (the bumble-bee), by Spencer in 1970 (Spencer et al. 1970). The name *Starmerella bombicola* was proposed by taxonomists as they discovered the new clade *Starmerella* to which *Candida bombicola* was classified based on high 18S rDNA identity (Rosa and Lachance 1998). Strains from the

Starmerella clade are fermentative and utilize a few carbon sources, such as glucose, galactose, raffinose, and sucrose. They are osmotolerant, which indicates a specialization toward a microenvironment with a high osmotic pressure such as nectar. In 2010, Kurtzman discovered new members of the *Starmerella* genus producing sophorolipids: *C. stellata, C. riodocensis*, and *Candida* sp. *NRRL Y-27208* (Kurtzman et al. 2010). All these species produce predominantly acidic sophorolipids in contrast to *S. bombicola* and *C. apicola,* which produce mostly the lactone form. Interestingly, in *Candida* sp. *NRRL Y-27208* a novel form of dimeric and trimeric sophorose containing sophorolipids was identified by MALDI-TOFMS (Figure 2.2). Surprisingly, some of these compounds were also detected in very minor amounts in the *S. bombicola* sophorolipid mixture (Price et al. 2012). Furthermore, Konishi et al. (2008) discovered *C. batistae,* which produces mainly diacetylated acidic sophorolipids containing mostly terminally hydroxylated octadecanoic acid as the lipid tail. Imura et al. (2010) described *C. floricola* TM 1502 as a new sophorolipid producer, secreting mainly diacetylated acidic sophorolipids. Generally, in the last 3 years, the interest in the *Starmerella* clade of organisms increased. Other new strains were isolated from flowers and fruits, such as *Starmerella* sp. nov. (Sipiczki 2013), *Starmerella jinningensis* sp. nov. (Li et al. 2013), and *Candida kuoi* sp. (Kurtzman). Only the last one was shown to produce acidic sophorolipids similar to *C. batistae, C. riodocensis,* and *C. stellata*.

Also other microorganisms, not belonging to the *Starmerella* clade, were reported to produce sophorolipids. *Wickerhamiella domercqiae*, a strain isolated from oil waste, was shown to secrete

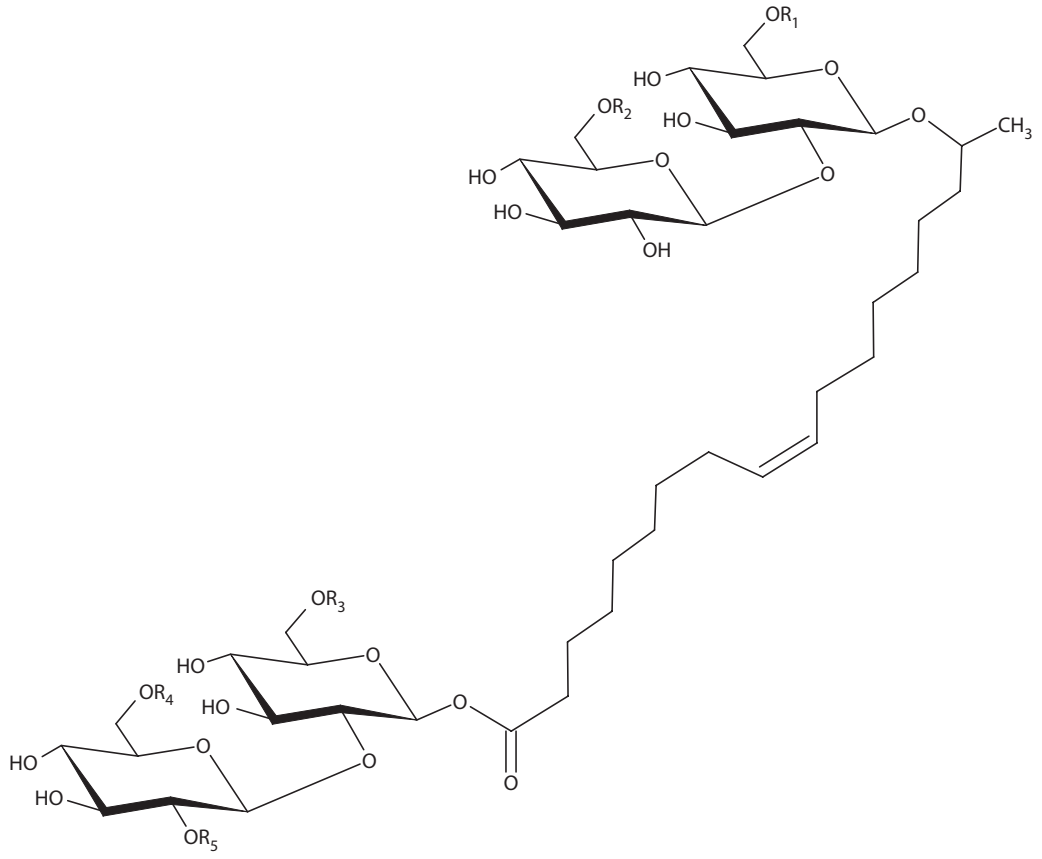

FIGURE 2.2 The structure of dimeric and trimeric sophorolipids. R_1, R_2, R_3, R_4 = –H or $–COCH_3$ independently of each other. For *Starmerella bombicola* R_5 = –H, for *Candida* sp. *NRRL Y-27208* R_5 = –H, a fatty acid, a fatty acid with a sophorose or a fatty acid with a sophorose and another fatty acid attached to it.

molecules that are almost identical to the major components of sophorolipids produced by *S. bombicola* and *C. apicola* (Chen et al. 2006a). Moreover, *W. domercqiae* is also able to produce lactonic diacetylated sophorolipids, with 17-hydroxyoctadecanoic acid as a lipid, in a high yield. Sophorolipid production was also reported in the thermotolerant yeast *Pichia anomala*. However, the yield was very low and the structural properties of the products have not been described (Thaniyavarn et al. 2008). Finally, Tulloch et al. found a new form of sophorolipids produced by *Candida* (now *Rhodotorula) bogoriensis*. Its structure differs from the sophorolipids of *S. bombicola* in the hydroxy fatty acid moiety, which is 13-hydroxyl dodecosanoic acid (C22; Tulloch et al. 1968).

2.5 SOPHOROLIPID BIOSYNTHESIS

Generally, sophorolipid yields increase extremely when both hydrophilic and hydrophobic carbon sources are present in the medium (e.g., glucose and vegetable oil, respectively). This can be explained by the fact that when only a hydrophilic substrate is present, sophorolipid synthesis requires *de novo* fatty acid synthesis at the cost of additional energy, and the efficiency of the process drops. However, when a hydrophobic substrate is present, it is directly incorporated into the sophorolipids (Hommel et al. 1994b). Different hydrophobic carbon sources have already been tested: from alkanes, fatty acids, alcohols to esters. Interestingly, only those with a chain length similar to *de novo* produced sophorolipids (C16–C18) seem to be easily incorporated and result in high fermentation yields. Among the most favored are rapeseed oil/esters that contain mainly C18:1 and C18:2 fatty acids (Davila et al. 1994). In contrast, a rather poor integration of oils originating from coconut and meadow foam is observed, probably because they contain either medium or very long chain fatty acids (Van Bogaert et al. 2010).

This preference for C16 and C18 fatty acids can be explained by the specificity of the cytochrome P450 monooxygenase, which is proposed to be the first enzyme in the sophorolipid pathway and which hydroxylates mainly C16:0, C18:1, and C18:0 fatty acids. Therefore, in case of a hydrophobic source with a different carbon chain length, the fungus needs to either shorten or lengthen the fatty acid chains, or, most likely, completely degrade them. In this case, *de novo* synthesis of the sophorolipid hydrophobic moiety is required.

Biologically derived sophorolipids can be modified by chemo-enzymatic processes as described for some of the applications. Yet, all existing methods are expensive and time consuming. The availability of genetically modified strains able to produce new-to-nature molecules with different properties would bypass the need for these additional steps and purifications. However, in order to create such strains, the knowledge about sophorolipid biosynthesis needs to be improved.

Sophorolipid biosynthetic enzymes are organized in a gene cluster, a feature quite typical for fungal secondary metabolites (Van Bogaert et al. 2013; Figure 2.3b). The initial step involves the terminal or subterminal hydroxylation of a fatty acid by an endoplasmic reticulum (ER)-associated cytochrome P450 enzyme (Figure 2.3a). Its expression during sophorolipid production was confirmed at the transcription level by real-time reversed transcription PCR (Van Bogaert et al. 2010). Next, a glucose molecule is bound to the hydroxylated fatty acid-forming glucolipids. Then, a second glucose is attached creating an acidic sophorolipid. As described by Saerens et al. (2011a,c), those two reactions are carried out by two glucosyltransferases, UGTA1 and UGTB1, which use UDP-glucose as donor. The obtained acidic nonacetylated sophorolipids can be secreted as such or, alternatively, they first undergo an acetylation on the sophorose 6′ and/or 6″ hydroxyl groups by an acetyl-CoA-dependent acetyltransferase before secretion (Saerens et al. 2011b). Secretion in the extracellular environment is mediated by active transport: an ATP-dependent multidrug resistance protein is responsible for majority of the translocation (Van Bogaert et al. 2013). Outside the cell, the molecules then can undergo lactonization catalyzed by a specific lactone esterase (Ciesielska 2013; Van Bogaert et al. 2011). The gene is not localized in the sophorolipid biosynthetic cluster and most likely evolved independently, which is supported by the fact that some organisms do not produce lactonic sophorolipids at all and that its expression profile in *S. bombicola* differs from the profile of the cluster genes.

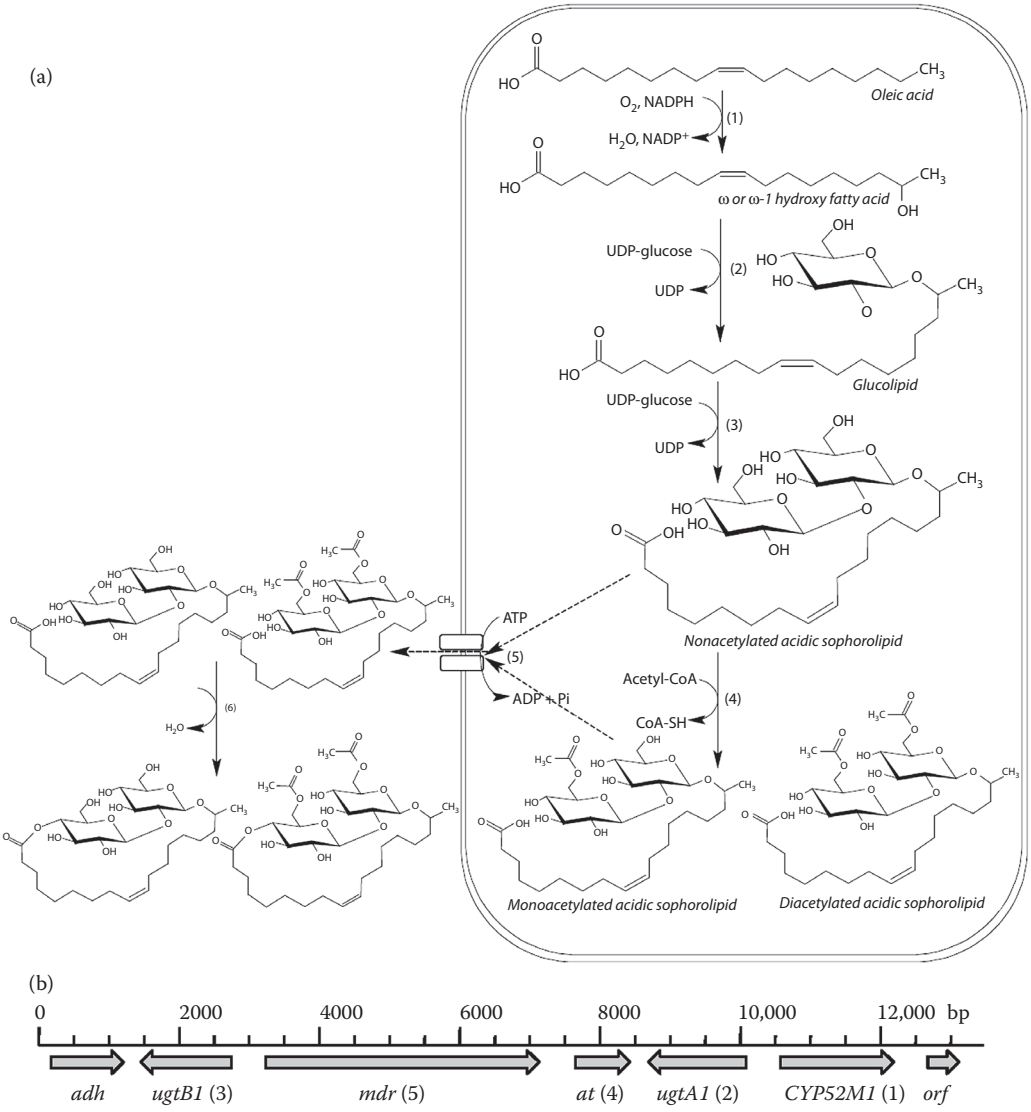

FIGURE 2.3 (a) Theoretical sophorolipids biosynthesis pathway. (b) Sophorolipid gene cluster. (1) Cytochrome P450 monooxygenase CYP52M1, (2) UDP-glucosyltransferase *ugtA1*, (3) UDP-glucosyltransferase *ugtB1*, (4) acetyltransferase *at*, (5) sophorolipid transporter *mdr*, *adh*: putative alcohol dehydrogenase, *orf*: open-reading frame with unknown function.

A proteomic comparison between sophorolipid inducing and noninducing conditions in *S. bombicola* demonstrated a coordinated synthesis of the enzymes of the sophorolipid biosynthetic cluster. In the stationary phase, their levels were two orders of magnitude higher than in exponentially grown cells. The same clear upregulation was detected in RNA-sequencing experiments (Ciesielska et al. 2013).

2.6 REGULATION OF SOPHOROLIPID BIOSYNTHESIS

Sophorolipids are mainly secreted during the stationary phase and their presence is not obligatory for cell viability. Hence, they can be considered as secondary metabolites. The molecules are

produced at certain limiting conditions in excess of a carbon source and, therefore, it is suggested that they act as an external carbon sink.

In other fungal organisms producing glycolipids, for example, MELS and cellobiose lipids, the expression of the clustered genes is controlled by a transcription factor that is activated in response to a changing environment. For example, in *Ustilago maydis,* the production of biosurfactants occurs after activation upon nitrogen limitation of a transcription factor present in the cluster (Teichmann et al. 2010). In the *S. bombicola* sophorolipid cluster, no transcription factor was detected (Van Bogaert et al. 2013). However, for *C. apicola* it is suggested that the ammonium ion concentration influences for sophorolipid production (Hommel et al. 1994a). Also Davila et al. (1992) described that sophorolipid production in *S. bombicola* is connected with nitrogen limitation. Later, Albrecht et al. (1996) followed the nitrogen and phosphate concentration during *S. bombicola* growth and concluded that sophorolipid production occurred at total phosphate exhaustion and nitrogen limitation. It was also proposed that under these conditions NAD(+)- and NADP(+)-dependent isocitrate dehydrogenase (NAD/P-ICDH) has a declined specific activity. This, together with a normal isocitrate synthase activity, causes the release of excessive citrate into the cytosol where ATP-dependent citrate lyase converts it into oxaloacetate and acetyl-CoA, the building block of fatty acids. Albrecht et al. followed the activity of NAD and NADP-ICDH during growth in a bioreactor and also tested the cofactor's influence on the activity of those enzymes. It was suggested that the decline in specific activity of NAD/P-ICDH is not regulated at the enzyme activity but at the enzyme synthesis level.

2.7 THE SOPHOROLIPID PRODUCTION PROCESS

2.7.1 Fermentation Parameters

As mentioned above, sophorolipid synthesis in not associated with actively growing cells, but with stationary phase. Hence, production only starts after a nonproductive growth phase of 1 or 2 days, but can be maintained at good production rates for over 1 week when the carbon sources are kept available by, for example, a fed-batch fermentation. Indeed, as explained in Section 2.5, production is optimal when both glucose (hydrophilic carbon source) and vegetable oil (hydrophobic C-source) are supplied. A correct feeding rate of both turns out to be important for productivity and influences the sophorolipid composition (balance lactonic vs. acidic form) as well (Davila et al. 1997). Besides the carbon sources, the medium should contain an (organic) nitrogen source, such as yeast extract or corn steep liquor, and favorable, but not essential, buffering agents or small amounts of minerals.

In general, most fermentations are carried out at 25°C or 30°C. The amount of obtained sophorolipid is nearly identical for both temperatures, whereas for fermentations at 25°C, biomass growth is lower, and the glucose consumption rate is higher as compared with the fermentation at 30°C (Casas and Garcia-Ochoa 1999).

After initiation of the fermentation process, pH is allowed to drop spontaneously till 3.5 and should be maintained at this point for optimal sophorolipid production (Gobbert et al. 1984). Throughout the whole fermentation process, the culture broth should be supplied with sufficient oxygen; the yeast cells are very sensitive to oxygen limitation during their exponential growth and cannot grow anaerobically. Furthermore, good aeration conditions are important for sophorolipid production as the cytochrome P450 monooxygenase uses molecular oxygen.

When looking at general product yield, values of over 400 g/L can be achieved (Daniel et al. 1998; Pekin et al. 2005). In the referred cases, the volumetric productivities were 0.92 and 0.7 g/L/h, due to the prolonged incubation times. However, higher volumetric values of, for instance, 1.9 and 2.1 g/L/h can be obtained with a respective yield of 365 and 317 g/L sophorolipids (Davila et al. 1997; Kim et al. 2009). Recently, Gao et al. could further increase productivity till 3.7 g/L/h, thanks to fermentations run at very high cell density (Gao et al. 2012). An overview of the most remarkable productivities is given in Table 2.1.

TABLE 2.1
Overview of Remarkable Sophorolipid Production Methods with Respect to Yields

Characteristics	Production (g/L)	C-Yield (g/g)	Time (h)	Volumetric Productivity (g/L/h)	References
10% glucose 10% animal fat	120	0.58	68	2.4	Deshpande and Daniels (1995)
10% glucose 10.5% safflower oil	137		192	0.7	Zhou and Kosaric (1993)
10% glucose 10.5% rapeseed oil	160		216	0.7	Zhou and Kosaric (1995)
30% rapeseed oil 10% deproteinized whey concentrate lactose not consumed	280		280	1.0	Daniel et al. (1998)
Single cell-oil from *Cryptococcus curvatus* grown on deproteinized whey concentrate 40% rapeseed oil added after single cell oil consumption	422		145 +410	0.8–1.0	Daniel et al. (1998b)
Turkish corn oil Glucose Honey added when glucose depleted	>400	>0.6	436	>0.9	Pekin et al. (2005)
Fed batch of glucose and octadecane	175	0.33	165	1.1	Davila et al. (1994)
Fed batch of glucose and rapeseed FAEE	340	0.65	165	2.1	
Fed batch of glucose and rapeseed oil	255	0.53	165	1.5	
11% glucose 10% soybean oil fed batch	120	0.6	110	1.1	Lee and Kim (1993)
10% glucose 10% sunflower oil resting cells	120	0.6	200	0.6	Casas and Garcia-Ochoa (1999)
Glucose Oleic acid Fed batch Crystals if limited oil feeding	180		200	0.9	Rau et al. (1996)
Glucose Rapeseed oil Fed batch	300	0.68	125	2.4	Rau et al. (2001)
4% oleic acid and 10% glucose at start additional fed batch focus on aeration (50–80 mM O_2/L/h)	350		>week	1–1.5	Guilmanov et al. (2002)
Glucose: 30–40 g/L Rapeseed oil	365		192	1.9	Kim et al. (2009)
Glucose Tallow fatty acid residue Fed batch	120	0.41	240	0.5	Felse et al. (2007)
Glucose Rapeseed oil High dry cell weight	200		54	3.7	Gao et al. (2012)

FAEE = fatty acid ethyl ester.

2.7.2 Substrates

Glucose is the hydrophilic substrate of choice. Other mono- or disaccharides can also act as substrate: fructose, mannose, saccharose, maltose, raffinose, sucrose, and lactose, yet at the expense of productivity (Gobbert et al. 1984; Klekner et al. 1991; Zhou and Kosaric 1993). In this respect, cheaper substrates or waste streams were evaluated as well. Again, glycerol can be used, but has a negative effect on the overall yield (Ashby et al. 2006). The same is true for sugarcane or soy molasses, which contain a number of sugars that are nonfermentable for *S. bombicola* (e.g., stachyose). However, molasses is a source of protein and nitrogen and can (partially) replace the conventional nitrogen source (Solaiman et al. 2007; Takahashi et al. 2011).

Several types of molecules can act as hydrophobic carbon source: oils, fats, fatty acids, their corresponding esters, alkanes, or waste streams containing one of them. In general, fatty acid methyl- or ethyl esters derived from vegetable oils result in improved yields compared to their corresponding oils and both perform better than alkanes (Table 2.1; Davila et al. 1994). However, the presence of residual hydrophobic carbon source in the final sophorolipids is a bigger issue for fatty acid esters due to their corrosive and irritating properties.

Free fatty acids of a specific length can be used as well, although there are some constraints such as a melting temperature above room temperature for the saturated fatty acids and effects on the cell's electron balance. As can be expected from the enzyme preferences and the native sophorolipid fatty acid tail lengths, oleic acid performs bests (Asmer et al. 1988). Consequently, vegetable oils rich in oleic acid, such as rapeseed oil, promote sophorolipid production (US 5900366). In this respect, also the high oleic sunflower oil would be a good choice. As this oil came to be widely available only recently due to expiring of a patent, no reports on fermentations with this substrate are consultable yet, but the fatty acid profile with over 80% oleic acid (vs. 55–75 for rapeseed oil) looks promising.

Analogue to fatty acids, the effectiveness of alkanes depends on their chain length. Hexadecane, heptadecane, and octadecane achieve the best production yields and appear to be directly converted into hydroxy fatty acids and incorporated into the sophorolipid molecules, this way strongly influencing the fatty acid composition of the sophorolipid mixture (Cavalero and Cooper 2003; Davila et al. 1994).

Nonincorporated hydrophobic substrates are mainly oxidized to CO_2 by beta-oxidation and for the best-performing substrates a carbon conversion yield between 60% and 70% is obtained (see also Table 2.1).

2.7.3 Product Recovery

On laboratory scale, recuperation of sophorolipids from the culture broth is generally done by organic solvent extraction with, for example, ethyl acetate. Residual hydrophobic carbon source is co-extracted and can be removed by additional treatment with hexane. For some applications, sophorolipids are extracted with pentanol (Baccile et al. 2013) or *t*-butyl methyl ether (Rau et al. 2001).

For larger scale and industrial applications, physical separation methods are preferred. Sophorolipids are heavier than water, allowing to centrifuge them down or to just decant them from the fermentation medium after heating (salting out). Further elimination of water and impurities may be required. In general, the fed-batch fermentation needs to be stopped when too high concentrations are reached resulting in high viscosity with stirring and aeration problems. In this respect, separation strategies integrated with the production process could prolong the production phase. Ultrasound separation was evaluated and cells could be retained at high efficiencies and viability, but the sophorolipid purification yield can still be improved (Palme et al. 2010). Foam fractionation is another option often suggested for biosurfactants, but this process has not been established as yet for sophorolipids. Cyclic fermentation with intermediate sedimentation and harvesting steps can be applied as well (US5879913).