Appl Microbiol Biotechnol (2007) 76:23–34
DOI 10.1007/s00253-007-0988-7

MINI-REVIEW

Microbial production and application of sophorolipids

Inge N. A. Van Bogaert · Karen Saerens · Cassandra De Muynck · Dirk Develter · Wim Soetaert · Erick J. Vandamme

Received: 16 February 2007 / Revised: 4 April 2007 / Accepted: 6 April 2007 / Published online: 3 May 2007

Abstract Sophorolipids are surface-active compounds synthesized by a selected number of yeast species. They have been known for over 40 years, but because of growing environmental awareness, they recently regained attention as biosurfactants due to their biodegradability, low ecotoxicity, and production based on renewable resources. In this paper, an overview is given of the producing yeast strains and various aspects of fermentative sophorolipid production. Also, the biochemical pathways and regulatory mechanisms involved in sophorolipid biosynthesis are outlined. To conclude, a summary is given on possible applications of sophorolipids, either as native or modified molecules.

Keywords Sophorolipids · *Candida bombicola* · *Candida apicola* · Biosurfactant · Yeast

Introduction

In terms of production volume, surfactants belong to the most important classes of industrial chemicals with a current total world production of about 10 million ton per year. About half that volume is used in household and laundry detergents, the other half in a wide variety of industrial sectors, particularly the chemical, textile, food, and paper industry, cosmetics, personal, and health care, agriculture, etc.

The large majority of the currently used surfactants are petroleum-based and are produced by chemical means. These compounds are often toxic to the environment, and their use may lead to significant ecological problems, particularly in washing applications as these surfactants inevitably end up in the environment after use (Mann and Boddy 2000; Mann and Bidwell 2001). The ecotoxicity, bioaccumulation, and biodegradability of surfactants are therefore issues of increasing concern.

Glycolipid surfactants are composed of a carbohydrate head and a lipid tail. They are a class of nonionic surfactants that has significantly increased its market share during the last 10 years. They offer a vastly improved environmental compatibility as compared to traditional surfactants, combined with excellent functional properties. Whereas the first generation of glycolipids was produced from renewable resources through chemical means (e.g., alkylpolyglucosides, APGs), the second generation of glycolipids is obtained from renewable resources through biotechnological means; indeed, glycolipids produced by fermentation are now entering the market, particularly rhamnolipids and sophorolipids. These latter molecules consist of the dimeric sugar sophorose linked to a long chain hydroxy fatty acid. They posses good surface active properties and show excellent skin compatibility, a property that is very important for cosmetic and personal care applications. Furthermore, they can be used in various other sectors due to either their emulsifying, antimicrobial, or other beneficial properties. Sophorolipids are synthesized in high concentrations by nonpathogenic yeasts (in contrast to rhamnolipids, where the most productive strains are

I. N. A. Van Bogaert (✉) · K. Saerens · C. De Muynck · W. Soetaert · E. J. Vandamme
Laboratory of Industrial Microbiology and Biocatalysis,
Department of Biochemical and Microbial Technology,
Faculty of Bioscience Engineering, Ghent University,
Coupure Links 653,
9000 Ghent, Belgium
e-mail: Inge.VanBogaert@UGent.be

D. Develter
Ecover Belgium NV,
Industrieweg 3,
2390 Malle, Belgium

bacteria belonging to the species *Pseudomonas aeruginosa*). This fact makes sophorolipids particularly attractive for further commercial production and use.

Producing microorganisms

In the early 1960s of the past century, Gorin et al. (1961) were the first to describe an extracellular glycolipid synthesized by the yeast *Torulopsis magnoliae*. However, the authors reported in 1968 that the producing strain was incorrectly identified and was actually *Torulopsis apicola* (Hajsig), currently known as *Candida apicola*. The structure of the hydroxy fatty acid sophoroside mixture was elucidated as a partially acetylated 2-*O*-β-D-glucopyranosyl-D-glucopyranose unit attached β-glycosidically to 17-L-hydroxyoctadecanoic or 17-L-hydroxy-Δ9-octadecenoic acid (Tulloch et al. 1962; Tulloch and Spencer 1968).

In the same year, Tulloch et al. (1968a) also discovered a new sophorolipid from *Candida bogoriensis* (now known as *Rhodotorula bogoriensis*). The overall structure is similar to the sophorolipids of *Candida apicola*, but differs in its hydroxy fatty acid moiety: the sophorose unit is linked to 13-hydroxydocosanoic acid.

A third sophorolipid secreting yeast strain was identified by the same researchers as *Candida bombicola* (named initially *Torulopsis bombicola*); the glycolipids and production characteristics of this species are nearly identical to those of *Candida apicola* (Spencer et al. 1970). In 1998, Rosa and Lachance 1998 described the novel yeast species *Starmerella bombicola* and introduced it as the teleomorph of *Candida bombicola* based on the high 18S rDNA identity between both strains (more than 98%) and their ability to mate with each other to form ascospores.

Recently, Chen et al. (2006a) proved sophorolipid synthesis in a new strain of *Wickerhamiella domericqiae*. They observed more than six glycolipids and identified one of the three main products as 17-L-(-oxy)-octadecanoic acid 1,4″-lactone 6′,6″-diacetate, which is identical to the major component of the sophorolipids of *C. apicola* and *C. bombicola*.

Regarding the fact that the production of sophorolipids is not restricted to one single yeast species, but to a number of related microorganisms, it is not unlikely to presume that other species belonging or related to the *Wickerhamiella*, *Starmerella* and *Rhodotorula* clades are also capable to synthesize some sort of sophorolipid.

This review will mainly focus on the glycolipids synthesized by *C. bombicola* ATCC 22214. This is the strain preferred by most research groups active in the sophorolipid field; it can produce over 400 g/l sophorolipids and is used for commercial production and applications.

Structure and properties

As a surfactant molecule, sophorolipids are amphiphilic molecules interacting with the phase boundary in heterogeneous systems. They consist of a hydrophobic fatty acid tail of 16 or 18 carbon atoms and a hydrophilic carbohydrate head, sophorose. Sophorose is a glucose disaccharide with an unusual β-1,2 bond and can—in the case of sophorolipids—be acetylated on the 6′- and/or 6″-positions (Fig. 1). One terminal or subterminal hydroxylated fatty acid is β-glycosidically linked to the sophorose molecule. The carboxylic end of this fatty acid is either free (acidic or open form) or internally esterified at the 4″ or in some rare cases at the 6′- or 6″-position (lactonic form). The hydroxy fatty acid itself counts in general 16 or 18 carbon atoms and can have one ore more unsaturated bonds (Asmer et al. 1988; Davila et al. 1993). As such, the sophorolipids synthesized by *C. bombicola* are in fact a mixture of related molecules with differences in the fatty acid part (chain length, saturation, and position of hydroxylation) and the lactonization and acetylation pattern. Asmer et al. (1988) were the first to shed light on this structural variation. They separated the sophorolipid mixture by medium pressure liquid chromatography and thin layer chromatography, and mainly based on the lactonization and acetylation pattern, they put forward 14 components. However, differences in fatty acid length and hydroxylation pattern were not taken into account. Davila et al. (1993) separated the sophorolipid mixture by a gradient elution high-performance liquid chromatography (HPLC) method and used an evaporative light scattering for the detection of the individual sophorolipids. They spend special attention to the analysis of the fatty acid chain and identified over 20 components.

When sophorolipids are solved in water, they lower the surface tension from 72.8 mN/m down to 40 to 30 mN/m, with a critical micelle concentration of 40 to 100 mg/l. The hydrophilic/lipophilic balance is 10 to 13, making sophorolipids useful as detergents or as stabilizers for oil-in-water emulsions. The different structural classes cause wide variation in physicochemical properties. Lactonized sophorolipids have different biological and physicochemical properties as compared to acidic forms. The biosurfactants' hydrophilic/lipophilic balance, foam formation capacity, and antimicrobial effects are all strongly influenced by the degree of lactone formation. In general, lactonic sophorolipids have better surface tension lowering and antimicrobial activity, whereas the acidic ones display better foam production and solubility. Also, the presence of acetyl groups can have a profound effect on the properties of biosurfactants. Indeed, acetyl groups lower the hydrophilicity of sophorolipids and enhance their antiviral and cytokine stimulating effects (Shah et al. 2005).

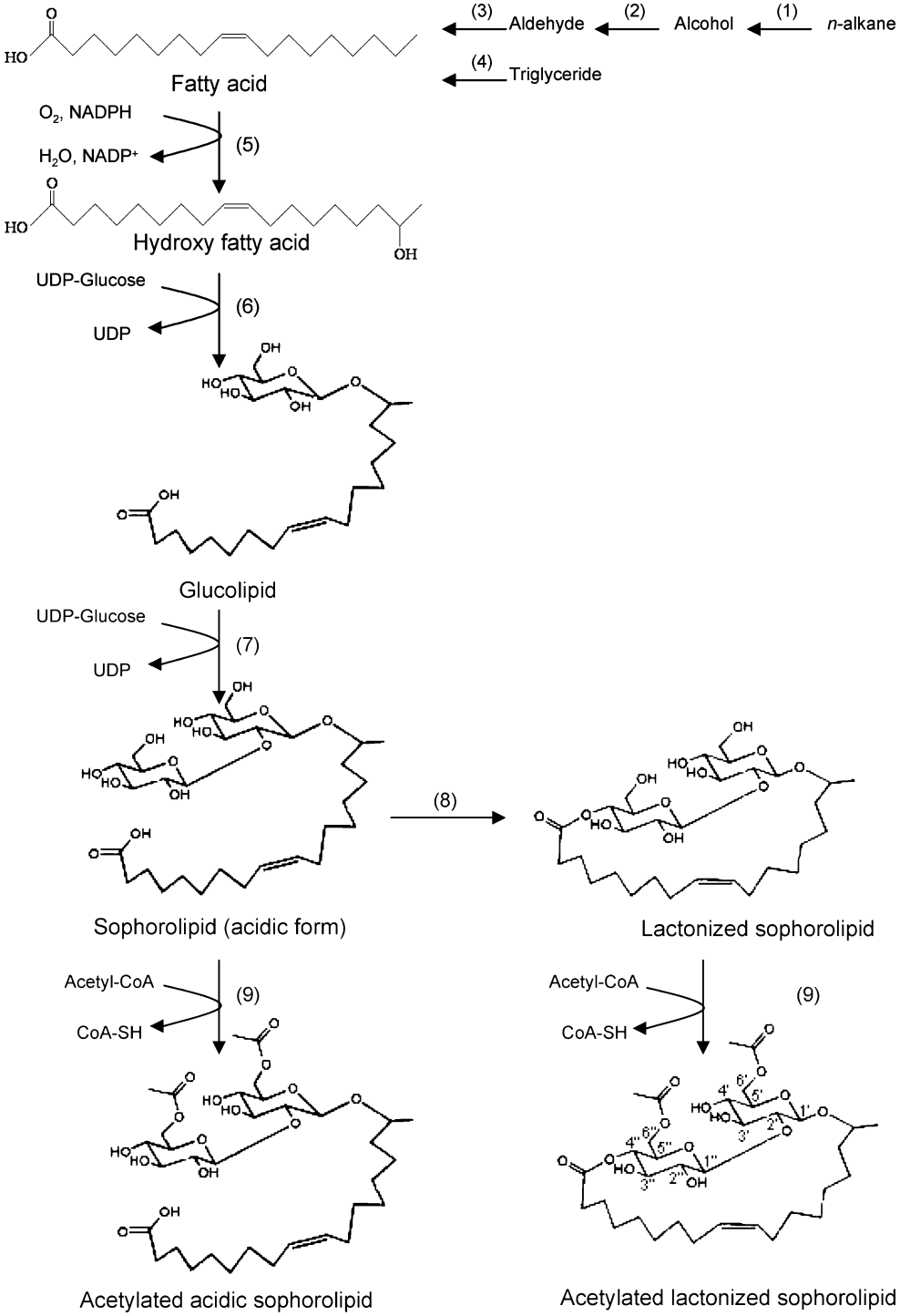

Fig. 1 Proposed sophorolipid biosynthetic pathway. *1*, cytochrome P450 monooxygenase; *2*, alcohol-dehydrogenase; *3*, aldehyde-dehydrogenase; *4*, lipase; *5*, cytochrome P450 monooxygenase; *6*, glucosyltransferase I; *7*, glucosyltransferase II; *8*, lactonesterase; *9*, acetyltransferase

Biosynthesis

Figure 1 gives a schematic overview of the biochemical pathways involved in sophorolipid synthesis. The building blocks for conventional sophorolipid synthesis are glucose and a fatty acid. Ideally, both can be provided in the production medium as such or, because free fatty acids can disturb the electron balance of the cells, sometimes fatty acid methyl or ethyl esters, or triglycerides are used. In this case, esterases will mediate gradual release of fatty acids. Since sophorolipid-producing yeast strains such as *C. bombicola* and *C. apicola* are capable of growing on alkanes, they posses the enzymes required for the terminal oxidation of alkanes, thereby generating fatty acids for further β-oxidation. Consequently, also alkanes or intermediates of the terminal oxidation pathway can act as feedstock. If no hydrophobic substrate is present in the medium, fatty acids will be formed *de novo* starting from

acetyl-CoA derived from glycolyze. On the other hand, and this especially when the glucose concentration is low, part of the fatty acids will be conducted toward the β-oxidation for cell maintenance instead of sophorolipid synthesis.

In a first step, the fatty acids are converted to a terminal (ω) or subterminal (ω-1) hydroxy fatty acid through the action of a membrane bound nicotinamide adenine dinucleotide phosphate (reduced form; NADPH) dependent monooxygenase enzyme, cytochrome P450 (Jones 1968). Lottermoser et al. (1996) identified two cytochrome P450 monooxygenase genes from *C. apicola* (European Molecular Biology Laboratory/GenBank accession numbers X76225 and X87640). Based on the amino acid similarity, they were classified into the CYP52 family, which comprises cytochrome P450 enzymes of yeasts capable of hydroxylating alkanes and/or fatty acids (Nelson 1998). However, the authors did not verify whether the corresponding gene products were involved in sophorolipid production or alkane assimilation, and if they were expressed at all. Yet, evolutionary history of cytochrome P450 genes is characterized by several events of gene duplication and conversion, resulting in a broad diversity among those genes also within the genome of a single organism, and it is not always clear what induces them or if they are expressed at all (Nebert and Gonzalez 1987; Nelson 1999). For *C. bombicola* ATCC 22214, we have identified five different cytochrome P450 monooxygenase genes belonging to the CYP52 family. One of them exposes very high similarity (91% AA identity) to the CYP52E2 gene of *C. apicola*, whereas the others probably belong to one or more new CYP52 subfamilies. The elucidation of their exact function is on its way.

In a second step, glucose is glycosidically coupled (position C1') to the hydroxyl group of the fatty acid through the action of a specific glycosyltransferase I. Experiments with ^{13}C-labeled glucose pointed out that the bulk of the added glucose first passed through glycolysis, in this way supplementing trioses for the gluconeogenesis of glucose for sophorolipid synthesis (Hommel et al. 1994). The transferase reaction requires nucleotide-activated glucose [uridine diphosphate (UDP)-glucose] as glucosyldonor (Breithaupt and Light 1982).

In a subsequent step, a second glucose is glycosidically coupled to the C2' position of the first glucose moiety by glycosyltranferase II. Both glycosyltransferases involved in sophorolipid synthesis of *R. bogoriensis* were partially purified. The two enzyme activities could however not be separated and highly purified samples exhibit a single major band of 52 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Esders and Light 1972; Breithaupt and Light 1982). Therefore, it remains open for discussion whether the consecutive glucose transfers are carried out by two different (but copurified) enzymes or by one and the same (multi)enzyme. It is supposed that sophorolipid synthesis in *C. bombicola* involves analogous enzymes.

The sophorolipids obtained after the action of glucosyltransferase II are as such detected in the sophorolipid mixture as the acidic, nonacetylated molecules. The majority of the sophorolipids are however further modified by both internal esterification (lactonization) and by acetylation of the carbohydrate head. Lactonic sophorolipids are formed by an esterifaction reaction of the carboxyl group of the hydroxy fatty acid with a hydroxyl group of sophorose (Fig. 1). The vast majority of lactones are esterified at the 4″-position, whereas a small percentage is esterified at either the 6′- or 6″-position (Asmer et al. 1988). Since several commercial lipases are able to introduce such a 6′- or 6″-ester linkage, it is suggested that those bonds are formed by cellular lipases, whereas esterification at the 4″-position is believed to be catalyzed by a specific lactone esterase. Neither of both esterases has been identified in *C. bombicola* or in other sophorolipid producing species.

The acetylation at the 6′- and/or 6″-position is carried out by an acetyl-coenzyme A (CoA) dependent acetyl transferase. The transferase from *R. bogoriensis* has been partially purified (Esders and Light 1972; Bucholtz and Light 1976), but the corresponding enzyme has not yet been identified in *C. bombicola*.

The fermentation process

Culture conditions

Together with mannosylerythritol lipids, sophorolipids are the only surfactants produced in large quantities by yeasts. *C. bombicola* ATCC 22214 is mainly used as producing strain, whereas some research groups in Germany prefer (red) working with *C. apicola* IMET 42747 (Hommel et al. 1987). This latter strain is however closely related to *C. bombicola*, and mechanisms and characteristics for sophorolipid production can be considered more or less the same for both yeast species.

The optimal growth temperature of *C. bombicola* ATCC 2214 is 28.8°C (information from the National Collection of Yeast Cultures, UK). Gobbert et al. (1984) pointed out that the optimal temperature for sophorolipid formation is 21°C. The authors themselves however advised to use higher temperatures for better handling (e.g., sample taking and oil addition). Most fermentations are run at 25 or 30°C. The amount of obtained sophorolipids 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 to the fermentation at 30°C (Casas and Garcia-Ochoa 1999).

During the exponentional growth phase, pH drops tremendously and must further be maintained at the value of 3.5 by the addition of NaOH for optimal sophorolipid production (Gobbert et al. 1984). This low pH and the antimicrobial effect of sophorolipids protect the fermentation broth against contamination, even when fed-batch processes of more than 200 h are run. Sophorolipid synthesis starts when the yeast cells enter stationary phase and takes place under conditions of nitrogen limitation, making the synthesis not growth-associated (Davila et al. 1992). Albrecht and colleagues linked the synthesis also to phosphate depletion and suggested the following mechanism. Phosphate- or nitrogen-limiting conditions cause a decline in the specific activities of NAD^+- and $NADP^+$-dependent isocitrate dehydrogenase, which leads to accumulation of isocitrate and subsequently citrate in the mitochondria. Both are transported into the cytosol and citrate is cleaved by adenosine triphosphate (ATP): citrate synthase to give rise to acetyl-CoA, the precursor for fatty acid synthesis (Albrecht et al. 1996).

Oxygen supply is very important throughout the whole fermentation process; the yeast cells are very sensitive to oxygen limitation during their exponentional growth, and good aeration conditions are important for sophorolipid production. Guilmanov et al. (2002) investigated the effect of aeration by means of shake-flask experiments. The optimal aeration for high sophorolipid yield, expressed in terms of oxygen transfer rate, lays between 50 and 80 mM O_2/l h^{-1}.

Hydrophilic and lipophilic carbon sources and their influence on sophorolipid formation

In addition, when the yeast cells are supplied with only one type of carbon source, such as glucose (Hommel et al. 1994) or *n*-alkanes (Jones and Howe 1968), sophorolipid formation is observed. The production is however considerably higher when two types of carbon sources, a hydrophilic (glycidic) and a hydrophobic (lipidic) one, are provided. In most cases, glucose is used as the hydrophilic carbon source. Sucrose can also act as substrate, but the obtained sophorolipid level is lower (Klekner et al. 1991). In an attempt to reduce substrate costs, cheese whey was proposed as hydrophilic carbon source. Zhou and Kosaric (1993, 1995) first investigated the fermentation with galactose and lactose, the main sugar components of whey. *C. bombicola* was not able to grow when only lactose was present, but when also olive, canola, or safflower oil was supplemented, growth and sophorolipid formation were observed. Daniel et al. (1998a) then investigated the production of sophorolipids in a medium with deproteinized whey concentrate and rapeseed oil. They obtained high levels of sophorolipid production (280 g/l), but strangely enough, the lactose was not consumed, nor could β-galactosidase activity in the supernatant or crude cell extract be detected. Still, the same researchers managed to fully use the deproteinized whey as a substrate for sophorolipid production by a two-stage cultivation process. They first cultivated the oleaginous yeast *Cryptococus curvatus* on the whey. The cells, which accumulated a high single cell oil level, were harvested and disrupted and then served as lipidic substrate for the *C. bombicola* cells (Daniel et al. 1998b). Furthermore, also low-cost soy molasses can act as glucose substitute, but again, lower yields were observed (Solaiman et al. 2004). As *C. bombicola* was originally isolated from honey, some researchers have tested it as a substrate. It was however only supplemented at the end of the fermentation process when the initially added glucose was consumed (Pekin et al. 2005).

A lot of substrates can act as hydrophobic carbon source: oils, fatty acids, and their corresponding esters, alkanes, etc. The level of sophorolipid formation during fermentations based on alkanes as hydrophobic feed-stock largely depends on the chain length of the used substrate. Hexadecane and octadecane give the best production yields. They appear to be directly converted into hydroxy fatty acids and incorporated into the sophorolipid molecules, in this way strongly influencing the fatty acid composition of the sophorolipid mixture. Davila et al. (1994), for example, detected over 70% of hydroxylated hexadecanoic and octadecanoic acid for fermentations on hexadecane and octadecane, respectively. Shorter alkanes are only to a minor extent incorporated, whereas the vast majority is either elongated to C16 or C18 fatty acids, or metabolized via β-oxidation. More or less the same is true for eicosane (*n*-C20) or longer alkanes. A few percentages of eicosane could be detected in the sophorolipid fatty acid moiety, whereas none were observed for the longer alkanes. The alkanes are metabolized by β-oxidation, either completely or partially to give rise to shorter fatty acids which can be incorporated into the sophorolipid molecules. The yields are however higher when compared to the shorter alkanes and even comparable to those obtained for *n*-C16 and *n*-C18 (Tulloch et al. 1962; Jones and Howe 1968).

The same trend is observed for fatty acids or fatty acid methyl esters. The best results are obtained for oleic acid (C18:1). It turns out that the fatty acid chain length determines the rate and position of hydroxylation and consequently governs the incorporation into the sophorolipid molecule. Pentadecanoic acid is too short for hydroxylation, but palmitic acid (22.55 Å) is not. It is predominantly hydroxylated at the terminal position. The longer the chain, the more the terminal/subterminal oxidation ratio declines; for stearic acid (25.05 Å) for example, no terminal oxidation is observed while this does occur for oleic (24.22 Å) and

heptadecanoic acid (23.80 Å). Linolenic acid (C18:3), however, does not follow this rule, no sophorolipids with this fatty acid tail were ever observed. It is suggested that the 15–16 double bound is too close to the enzymatic reaction site (Tulloch et al. 1962; Davila et al. 1994). In both sophorolipid mixtures on alkanes and fatty acids with direct incorporation, desaturation is observed (Brett et al. 1971; Davila et al. 1994).

Furthermore, oils (especially those of vegetable origin) are widely used as lipidic carbon source. The most common vegetable oils are comprised of saturated or unsaturated fatty acids with chain lengths of 16 or 18 carbon atoms, making them an ideal substrate for direct incorporation and the consequent high sophorolipid production and yield. Examples of tested oils are canola, corn, safflower, sunflower, olive, rapeseed, grape seed, palm, coconut, fish, and soybean oil (Cooper and Paddock 1984; Lee and Kim 1993; Davila et al. 1994; Zhou and Kosaric 1995; Rau et al. 1996; Kim et al. 1997; Casas and Garcia-Ochoa 1999; Cavalero and Cooper 2003; Pekin et al. 2005). As vegetable oils are renewable resources, sophorolipid synthesis with those carbon sources contributes to the environmental friendly character of this surfactant. Furthermore, waste streams such as biodiesel by-product streams, soybean dark oil and waste frying oil can be used (Ashby et al. 2005; Kim et al. 2005; Fleurackers 2006). It is difficult to compare the effects of various oil sources because different media and culture conditions were used. Casas and Garcia-Ochoa (1999), however, compared olive, grape seed, sunflower, corn, and coconut oil and concluded that sunflower oil gave the best results. Coconut oil is less suitable, as it in fact contains high amounts of lauric and myristic acid. Davila et al. (1994) tested palm, sunflower, fish, and rapeseed oil. The latter one turned out to be most suitable for sophorolipid production due to its high oleic acid content. These researchers could further increase the production and yield from 255 g/l and 0.53 g/g to 340 g/l and 0.65 g/g when using methyl or ethyl esters of the rapeseed oil. Nonincorporated substrates, either alkanes, fatty acids, or esters, are mainly oxidized to CO_2.

Brakemeier et al. (1995, 1998a) developed a method to circumvent the length-dependent and restricted incorporation. They used secondary alcohols (C12 to C16) as the lipophilic carbon source. The majority of the substrate was incorporated into the glycolipids without any further modifications, although small percentages of (ω-1)-alkandiols and hydroxy fatty acids were observed. The resulting compounds display better surface active properties as compared to native sophorolipids. Another remarkable event is the possible coupling of sophorose units at both sites of the alkandiols, leading to a glycolipid with two sophorose units separated by a hydrocarbonic spacer; these molecules are however only slightly soluble in water. The greatest disadvantage of this method is the high cost of secondary alcohols. Primary alcohols are much cheaper substrates, but are most likely converted to fatty acids and metabolized in the β-oxidation route. The metabolization can nevertheless be avoided in favor of the incorporation in sophorolipids by increasing the level of glucose (150 g/l) and yeast extract (4 g/l). Tests with 2-, 3-, or 4-dodecanones showed that those ketones were reduced into their corresponding alcohols by *C. bombicola* and subsequently incorporated into sophorolipid molecules (Brakemeier et al. 1998b).

Effect of other medium components on production

The level of sophorolipid production largely depends on the medium composition and the addition of both the glycidic and lipidic carbon source. Tulloch et al. (1968b) obtained 40 g/l using a simple medium containing glucose, yeast extract, urea, and *n*-octadecane. Certain researchers kept using this medium and could increase the sophorolipid formation by better control of the fermentation process and/or stepwise addition of the hydrophobic carbon source (Asmer et al. 1988; Rau et al. 1996; Casas and Garcia-Ochoa 1999; Guilmanov et al. 2002; Cavalero and Cooper 2003; Solaiman et al. 2004). Pekin et al. (2005), for instance, managed to produce up to 400 g/l sophorolipids. Others use media comprising potassium phosphate, citrate, and ammonium instead of urea and minerals such as iron, magnesium, and calcium and achieve similar results (Daniel et al. 1998b; Davila et al. 1994). Typically, a carbon conversion yield between 60 and 70% is obtained.

Yeast extract is essential for both cell growth and sophorolipid formation. Substitution with ureum or peptone negatively influenced the biomass and glycolipids yield. The most favorable concentration remains however unclear. Cooper and Paddock (1984) set the optimum at 5 g/l, whereas Zhou et al. (1992) obtained the highest sophorolipid concentration using only 2 to 3 g/l yeast extract. Casas and Garcia-Ochoa (1999) further decreased the yeast extract content to 1 g/l. They pointed out that higher concentrations were favorable for the development of biomass but decreased the glycolipids production due to depletion of the carbon sources by cell growth. It stays however tricky to draw conclusions because all three research groups set this optimum using different media.

Factors influencing the sophorolipid composition

As discussed in the above section, the fatty acid moiety of the sophorolipid molecules is to a large extent influenced by the supplemented hydrophobic carbon source. The glycidic part as such always is sophorose, whatever the employed culture conditions or hydrophilic carbon source.

However, the sophorose unit can be mono-or diacetylated and esterified to the fatty acid carboxylic site. What exactly determines the degree of lactonization remains unclear, but the lactonic/acidic balance is to a certain point influenced by fermentation conditions. Especially when the alkanes hexadecane, heptadecane, or octodecane are used, 85% or more of the sophorolipids are diacetylated lactones. This high structural homology involves another interesting phenomenon: the sophorolipids tend to form white crystals instead of the traditional brown and viscous oily mixture. This feature makes them relatively easy to isolate (Davila et al. 1994; Cavalero and Cooper 2003). Such high degrees of lactonization are more difficult to achieve with oils or fatty acids, but can be obtained in fed-batch fermentation with limited feeding of the lipidic carbon source (Davila et al. 1994; Rau et al. 1996). According to Davila et al. (1994), sophorolipids produced from oils always exhibited a higher level of diacetylated lactones than the ones produced from the corresponding esters. In addition, the fatty acid composition of the lipidic carbon source also influences the sophorolipid composition. When substrates rich in polyunsaturated fatty acids are used (e.g., sunflower or linseed oil), increased levels of acidic sophorolipids were observed. Loss of flexibility of the sophorolipidic fatty acid tail due to the two double bounds can possibly hamper internal esterification.

The partition in structural classes also tends to depend on the level of yeast extract in the medium. A concentration of 1 g/l mainly triggers the lactonic form, whereas 20 g/l lead to the synthesis of acidic sophorolipids. Furthermore, the same investigators noticed a higher concentration of acidic sophorolipids in shake-flask compared to fermentor experiments, suggesting that also the oxygen supply is a determining factor (Casas and Garcia-Ochoa 1999). Finally, the lactonic/acidic balance seems to change during the course of fermentation. In the beginning, acidic forms are predominant, whereas later on, conversion to the lactonic and acetylated forms is observed (Davila et al. 1992). It is therefore suggested that in energetically less favorable conditions, more acidic structures are found (Davila et al. 1997).

Downstream processing

On laboratory scale, sophorolipids can be extracted from the culture broth with organic solvents such as ethyl acetate. Residual lipidic carbon source can however be coextracted and cause difficulties during further applications. For this reason, additional extraction with hexane is most frequently used, but other solvents such as pentane (Cavalero and Cooper 2003) or t-butyl methyl ether (Rau et al. 2001) can also be applied.

As sophorolipids are heavier than water, it is often possible to centrifuge them down or to just decant the sophorolipids from the fermentation medium, after heating if necessary. This method is very convenient when working with large volumes and high yields. Further elimination of water and impurities can be achieved by addition of polyhydric alcohols and subsequent distillation (Inoue et al. 1980). Ultrafiltration is also often used in the recovery of biosurfactants, but this technique has not been optimized yet for sophorolipids (Mulligan and Gibbs 1990).

As discussed in the previous section, certain fermentation conditions can give rise to high concentrations of lactonic sophorolipids resulting in a crystalline product, which is obviously much easier to separate and purify. Hu and Ju (2001) developed a method for the separation of lactonic sophorolipids from the crude, viscous mixture based on crystallization in phthalate and phosphate buffers. If one is interested in a specific sophorolipid structure, e.g., for pharmaceutical applications, chromatographic purification with silica gel or preparative reversed phase columns is necessary (Lin 1996).

Physiological role of sophorolipids

Synthesis of biosurfactants is often associated with the assimilation of hydrophobic substrates (Ito et al. 1980); this theory is however not commonly accepted for sophorolipid formation, especially because the molecules are also formed when no hydrophobic substrate is present and in amounts largely exceeding the concentration required for emulsification. As sophorolipid synthesis is associated with nitrogen starvation, it is suggested that formation of glycolipids is some sort of overflow metabolism, by means of extracellular storage material. This hypothesis is supported by the findings of Hommel et al. (1994), regarding the biosynthesis of the sophorose moiety, which resembles the trehalose synthesis of *Saccharomyces cerevisiae* under anaerobic conditions. It was also demonstrated that sophorolipids can be used as sole carbon source (Garcia-Ochoa and Casas 1997). As *C. bombicola* and *C. apicola* by nature occur in environments with high osmotic strength, sophorolipid production may be a way of dealing with the high sugar concentrations by converting, storing, and making them less available for other organisms. Furthermore, sophorolipids display antimicrobial activity against certain yeasts such as *Candida* and *Pichia* species (Ito et al. 1980) and Gram-positive bacteria. The mono- and diacetylated lactones have the strongest inhibitory effect (Lang et al. 1989). We believe that the physiological role of sophorolipid synthesis is extracellular carbon source storage, combined with deal-

ing with a high-sugar niche and defending this against other competing microorganisms.

Applications of native sophorolipids

The most recognized feature of sophorolipids is their ability to act as a surfactant. Surfactants are widely used in the food, pharmaceutical, cosmetic, and cleaning industries. Throughout the years, surfactants have been produced from petrochemical raw materials. During the last decades, the environmental awareness has become a more important issue in the study, development, and application of surfactants. In this respect, biosurfactants such as sophorolipids offer the advantages of biodegradability, low ecotoxicity and the production on renewable-resource substrates.

The Japanese company Saraya (http://www.saraya.com) has commercialized sophoron, a dish washer containing sophorolipids as cleaning agent (Futura et al. 2002). Sophorolipids can also be applied in laundry detergents (Hall et al. 1996). As sophorolipids are nonionic molecules, they preserve their surface lowering properties despite high salt concentrations. In addition, they are active across a wide temperature range. One feature one must keep however in mind is their instability at pH higher than 7.0 to 7.5: beyond this point, irreversible hydrolysis of the acetyl groups and ester bonds is observed. The emulsifying properties of sophorolipids can also be exploited in the petroleum industry; they are useful in secondary oil recovery, in removing hydrocarbons from drill material, and in the regeneration of hydrocarbons from dregs and muds (Baviere et al. 1994; Marchal et al. 1999; Pesce 2002). Sophorolipids can also be applied for decontaminating porous media such as soils and groundwater tables polluted by hydrocarbons (Ducreux et al. 1997) and in the removal of heavy metals from sediments (Mulligan et al. 2001). Furthermore, the emulsifying property of sophorolipids can be used in the food industry to improve the quality of wheat flour products (Akari and Akari 1987) and in the cold storage transportation in air conditioning systems for the prevention of ice particle formation (Masaru et al. 2001).

Moreover, sophorolipids find application in various cosmetic formulations. The French company Soliance (http://www.groupesoliance.com) produces sophorolipid-based cosmetics for body and skin. In addition to its role as emulsifier, the glycolipid acts as a bacteriocidal agent in the treatment of acne, dandruff, and body odors (Mager et al. 1987). Furthermore, they are claimed to trigger several beneficial events regarding the protection of hair and skin, making them attractive components in cosmetic, hygienic, and pharmaco-dermatological products. They stimulate the dermal fibroblast metabolism and collagen neosynthesis, inhibit free radical and elastase activity, posses macrophage-activating and fibrinolytic properties, and act as desquamating (i.e., eliminating the surface portion of the protective layer of the epidermis as part of the wound healing process) and depigmenting agents (Hillion et al. 1998; Borzeix 1999; Maingault 1999). Sophorolipids are also believed to stimulate the leptin synthesis through adipocytes, in this way reducing the subcutaneous fat overload (Pellecier and André 2004).

As mentioned above, sophorolipids posses antimicrobial properties. For this reason, they can be applied in germicidal mixtures suitable for cleaning fruits and vegetables (Pierce and Heilman 1998). The antimicrobial action is not merely restricted toward bacteria, sophorolipids also act as antifungal agents against plant pathogenic fungi such as *Phytophthora* sp. and *Pythium* sp. (Yoo et al. 2005), inhibit algal bloom (Gi 2004), and are even claimed to have antihuman immunodeficiency virus and sperm-immobilizing activities (Shah et al. 2005). Other medical beneficial effects of sophorolipids are their ability to trigger cell differentiation instead of cell proliferation and the inhibition of protein kinase C activity of the human promyelocytic leukemia cell line HL60. The anticancer action is not caused by a simple detergent-like effect but is attributed to a specific interaction with the plasma membrane (Isoda et al. 1997). The above-described experiment was conducted with a crude sophorolipid mixture. Chen et al. (2006a), however, conducted anticancer tests with the purified diacetylated lacton. This component exhibits cytotoxic effects on several human cancer cell lines. The cytotoxic effect on the human liver cancer cells H7402 was further investigated and turned out to be attributed to the molecule's ability to induce apoptosis (Chen et al. 2006b). Furthermore, sophorolipids tend to decrease mortality caused by septic shock in a rat model. It stays however a matter of debate whether the effect is caused by direct modulation of immune and inflammatory responses or by the antibacterial properties of the sophorolipid molecules (Bluth et al. 2006; Napolitano 2006).

Finally, sophorolipids are a source of difficult-to-synthesize ω and ω-1 hydroxy fatty acids. Those fatty acids can be used in polymerization reactions or can be lactonized into macrocyclic esters, which find application in the perfume and fragrance industry (Inoue and Miyamoto 1980). Zerkowski and Solaiman (2006) produced fatty amines starting from sophorolipid derived 17-hydroxy oleic acid. The compounds could be of interest in the preparation of highly functionalized polymers and surfactants.

Chemical or enzymatic modified sophorolipids and their applications

The simplest modification of sophorolipids, which at the same time reduces their structural variability, is conversion into the deacetylated acidic form by alkaline hydrolysis in an aqueous environment. If one wants to obtain sophorolipids merely lacking acetyl groups, enzymes such as acetylesterase (E.C. 3.1.1.6) or cutinase from *Fusarium solani* must be used. Acetylesterase removes both acetyl groups, whereas cutinase specifically hydrolyses the 6′ position (Asmer et al. 1988; De Koster et al. 1995).

The majority of the other modifications are carried out at the carboxylic end of the fatty acid. The first reported one is the synthesis of sophorolipid alkyl esters in 1971 to enhance the characteristics of prepared food products such as bakery and oily emulsions. The authors found that the beneficial effects of the molecules increased with the chain length of the ester (Allingham 1971). Zhang et al. (2004) observed the same trend. They synthesised and compared the properties of sophorolipid methyl, ethyl, propyl, and butyl esters and found that the critical micelle concentration decreases to about one half per additional carbon group to the ester moiety. Ashby et al. (2006) obtained sophorolipid methyl esters by simply applying fatty acid methyl esters of soy oil as the hydrophilic carbon source. However, the sophorolipid methyl esters comprise only 48% of the total mixture, and no esterification is observed when ethyl- or propyl-soyate are used. The authors suggest that a lipase with only a partial activity against methyl groups may be responsible for this phenomenon. There are a number of patents on the use of sophorolipid esters in cosmetics (Abe et al. 1981).

The sugar moiety hydroxyl groups of the sophorolipid ester can be substituted with hydroxyalkyl groups, giving rise to hydroxypropyl-etherified glycolipids ester. These esters have been used in pencil-shaped lip rouge, lip cream, and eye shadow, in powdered compressed cosmetic material as well as in aqueous solutions (Kawano et al. 1981a, b).

Sophorolipid esters can be reacetylated at the 6′- and 6″-positions by Lipase Novozyme 435 from *Pseudozyma* (*Candida*) *antarctica*. When sophorolipid methyl esters are reacted with the acylating agent at a concentration less than equimolar, lactonic sophorolipids are formed. The fatty acid carboxyl group is however linked to the sophorose unit by the 6″- instead of the 4″-hydroxyl found in the native form. The lactonic sophorolipid can further be acetylated by Novozym 435 to lead to the formation of a 6′-monoacyl derivate (Bisht et al. 1999). Singh et al. (2003) on the other hand developed a method for the direct regioselective acetylation of sophorolipid ethyl esters. With Novozym 435 or Lipase PS-C, they could mediate monoacetylation at the 6′- or 6″-position, respectively.

Furthermore, the ester itself can be subjected to further alterations. Carr and Bisht (2003) started from sophorolipid methyl esters with all free hydroxyl groups of the sugar moiety blocked by peracylation. Novozym 435 turned out to be the only enzyme that could transesterify the peracetylated sophorolipid methyl esters with 1-butanol or 2-methylpropanol. Nuñez et al. (2003) applied more or less the same strategy to obtain a galactopyranose-sophorolipid. After reacetylation of a sophorolipid methyl ester, they transesterified it with 1,2-3,4-di-*O*-isopropylidene-D-galactopyranose and again Novozym 435 as a catalyst. After acidic removal of the sugar hydroxyl group protection, a galactopyranose C6-linked to the carboxylic end of a nonacetylated sophorolipid molecule was obtained. Other researchers synthesized amide derivates with Novozym 435 and stated that those derivates may have potential as tuneable immunoregulators. The introduction of methacryl or tyrosine groups on the other hand allows the molecules to be functional in polymerization processes (Singh et al. 2003). Sophorolipids can, however, also be subjected to direct enzymatic polymerization. Hu and Ju (2003) optimized the reaction conditions for lipase-mediated conversion of diacetylated lactonic sophorolipids to monoacetylated lactonic sophorolipids, which were in the same reaction polymerized to oligomers and polymers, probably through ring-opening polymerization. Four different lipases were tested and polymerization took place in all cases, demonstrating the high promiscuity of lipase enzymes.

A recent developed family of derivatives are the amino acid sophorolipid conjugates. The amino acids are coupled to the carboxylic end of acidic sophorolipids by using (di) carbodiimide. In this way, the nonionic sophorolipid can be converted to a cationic, zwitterionic, or anionic surfactant with increased water solubility and polar head groups that allow further chemical derivatization (Zerkowski et al. 2006). Azim et al. (2006) evaluated the antibacterial, antiHIV, and spermicidal activity of their conjugates. All molecules exhibited the desired action, but leucine-sophorolipid was the most effective one.

A lot of examples are given on modification of the fatty acid carboxylic end and the sophorose acetylation pattern, but as far as we know, only one report on alteration of the sugar moiety itself is available. Rau and coworkers (1999) subjected deacetylated acidic sophorolipids to various glycosidases. Hesperidinase turned out to be the most active in the specific release of one glucose molecule. The surface active properties of this glucolipid are comparable to those of the acid sophorolipid, but its solubility in water is smaller.

Perspectives

Sophorolipids belong to the most promising biosurfactants. In contrast to rhamnolipids, synthesized by *Pseudomonas aeruginosa*, the producing strains are nonpathogenic. Very high production yields can be achieved (over 400 g/l), and this is based on renewable resources or even waste streams. The current production price amounts to 2 to 5 €/kg, depending on substrate cost and production scale. As sophorolipids find applications in the cleaning, environmental, and food industry as well as in the personal care, cosmetic, and pharmaceutical sectors, it is clear that their economical competitiveness depends on their final utilization. If sophorolipids are for example used in the cleaning industry, they have to vie with other environmentally friendly surfactants, such as the alkyl-polyglucosides (APGs), which have a market price of 2 €/kg. However, in the cosmetic or pharmaceutical sectors, higher price dimensions are standard, and therefore, sophorolipids can easily compete.

A lot of research has been performed on the optimization of the fermentation process, but as far as we know, hardly any work has been published on the genetics of the producing yeast strain. However, genetic engineering of these yeast species could open up perspectives for higher yields and modification of the glycolipid mixture produced. In this context, we are developing an efficient transformation and selection system for *C. bombicola* (Van Bogaert et al. 2007) and are isolating several genes involved in sophorolipid synthesis.

References

Abe Y, Inoue S, Ishida A (1981) Cosmetic composition of skin and hair treatment. US patent 427340

Akari S, Akari Y (1987) Method of modifying quality of wheat flour product. Japanese patent 61205449

Albrecht A, Rau U, Wagner F (1996) Initial steps of sophoroselipid biosynthesis by *Candida bombicola* ATCC 22214 grown on glucose. Appl Microbiol Biotechnol 46:67–73

Allingham RP (1971) Sophoroside esters in prepared food products. US patent 3622344

Ashby RD, Nunez A, Solaiman DKY, Foglia TA (2005) Sophorolipid biosynthesis from a biodiesel co-product stream. J Am Oil Chem Soc 82:625–630

Ashby R, Solaiman DKY, Foglia TA (2006) The use of fatty acids to enhance free acid sophorolipid synthesis. Biotechnol Lett 28:253–260

Asmer HJ, Lang S, Wagner F, Wray V (1988) Microbial-production, structure elucidation and bioconversion of sophorose lipids. J Am Oil Chem Soc 65:1460–1466

Azim A, Shah V, Doncel GF, Peterson N, Gao W, Gross R (2006) Amino acid conjugated sophorolipids: a new family of biological active functionalized glycolipids. Bioconjug Chem 17:1523–1529

Baviere M, Degouy D, Lecourtier J (1994) Process for washing solid particles comprising a sophoroside solution. US patent 5326407

Bisht KS, Gross RA, Kaplan DL (1999) Enzyme-mediated regioselective acylations of sophorolipids. J Org Chem 64:780–789

Bluth MH, Kandil E, Mueller CM, Shah V, Lin YY, Zhang H, Dresner L, Lempert L, Nowakowski M, Gross R, Schulze R, Zenilman ME (2006) Sophorolipids block lethal effects of septic shock in rats in a cecal ligation and puncture model of experimental sepsis. Crit Care Med 34:188–195

Borzeix CF (1999) Use of sophorolipids comprising diacetyl lactones as agent for stimulating skin fibroblast metabolism. World patent 99/62479

Brakemeier A, Lang S, Wullbrandt D, Merschel L, Benninghoven A, Buschmann N, Wagner F (1995) Novel sophorose lipids from microbial conversion of 2-alkanols. Biotechnol Lett 17:1183–1188

Brakemeier A, Wullbrandt D, Lang S (1998a) *Candida bombicola*: production of novel alkyl glycosides based on glucose/2-dodecanol. Appl Microbiol Biotechnol 50:161–166

Brakemeier A, Wullbrandt D, Lang S (1998b) Microbial alkyl-sophorosides based on 1-dodecanol or 2-, 3-or 4-dodecanones. Biotechnol Lett 20:215–218

Breithaupt TB, Light RJ (1982) Affinity–chromatography and further characterization of the glucosyltransferases involved in hydroxydocosanoic acid sophoroside production in *Candida bogoriensis*. J Biol Chem 257:9622–9628

Brett D, Howling D, Moris LJ, James AT (1971) Specificity of the fatty acid desaturases. The conversion of saturated to monoenoic acids. Arch Biochem Biophys 143:535–547

Bucholtz ML, Light RJ (1976) Acetylation of 13-sophorosyloxydocosanoic acid by an acetyltransferase purified from *Candida bogoriensis*. J Biol Chem 251:424–430

Carr JA, Bisht KS (2003) Enzyme-catalyzed regioselective transesterification of peracylated sophorolipids. Tetrahedron 59:7713–7724

Casas JA, Garcia-Ochoa F (1999) Sophorolipid production by *Candida bombicola*: medium composition and culture methods. J Biosci Bioeng 88:488–494

Cavalero DA, Cooper DG (2003) The effect of medium composition on the structure and physical state of sophorolipids produced by *Candida bombicola* ATCC 22214. J Biotechnol 103:31–41

Chen J, Song X, Zhang H, Qu YB, Miao JY (2006a) Production, structure elucidation and anticancer properties of sophorolipid from *Wickerhamiella domercqiae*. Enzyme Microb Technol 39:501–506

Chen J, Song X, Zhang H, Qu YB, Miao JY (2006b) Sophorolipid produced from the new yeast strain *Wickerhamiella domercqiae* induces apoptosis in H7402 human liver cancer cells. Appl Microbiol Biotechnol 72:52–59

Cooper DG, Paddock DA (1984) Production of a biosurfactant from *Torulopsis bombicola*. Appl Environ Microbiol 47:173–176

Daniel HJ, Otto RT, Reuss M, Syldatk C (1998a) Sophorolipid production with high yields on whey concentrate and rapeseed oil without consumption of lactose. Biotechnol Lett 20:805–807

Daniel HJ, Reuss M, Syldatk C (1998b) Production of sophorolipids in high concentration from deproteinized whey and rapeseed oil in a two stage fed batch process using *Candida bombicola* ATCC 22214 and *Cryptococcus curvatus* ATCC 20509. Biotechnol Lett 20:1153–1156

Davila AM, Marchal R, Vandecasteele JP (1992) Kinetics and balance of a fermentation free from product inhibition-sophorose lipid production by *Candida bombicola*. Appl Microbiol Biotechnol 38:6–11

Davila AM, Marchal R, Monin N, Vandecasteele JP (1993) Identification and determination of individual sophorolipids in fermentation products by gradient elution high-performance liquid chromatography with evaporative light-scattering detection. J Chromatogr 648:139–149

Davila AM, Marchal R, Vandecasteele JP (1994) Sophorose lipid production from lipidic precursors-Predictive evaluation of industrial substrates. J Ind Microbiol 13:249–257

Davila AM, Marchal R, Vandecasteele JP (1997) Sophorose lipid fermentation with differentiated substrate supply for growth and production phases. Appl Microbiol Biotechnol 47:496–501

De Koster C, Heerma W, Pepermans HAM, Groenewegen A, Peters H, Haverkamp J (1995) Tandem mass spectrometry and nuclear magnetic resonance spectroscopy studies of *Candida bombicola* sophorolipids and product formed on hydrolysis by cutinase. Anal Biochem 230:135–148

Ducreux J, Ballerini D, Baviere M, Bocard C, Monin N (1997) Composition containing a surface active compound and glycolipids and decontamination process for a porous medium polluted by hydrocarbons. US patent 5654192

Esders TW, Light RJ (1972) Glucosyl- and acetyltransferases involved in the biosynthesis of glycolipids from *Candida bogoriensis*. J Biol Chem 247:1375–1386

Fleurackers SJJ (2006) On the use of waste frying oil in the synthesis of sophorolipids. Eur J Lipid Sci Technol 108:5–12

Futura T, Igarashi K, Hirata Y (2002) Low-foaming detergent compositions. World patent 03/002700

Garcia-Ochoa F, Casas JA (1997) Process for the production of sophorose by *Candida bombicola*. Spanish patent 2103688

Gi KE (2004) Red tide inhibiting agent comprising yellow earth and biodegradable sophorolipid. Korean patent 20040083614

Gobbert U, Lang S, Wagner F (1984) Sophorose lipid formation by resting cells of *Torulopsis bombicola*. Biotechnol Lett 6:225–230

Gorin PAJ, Spencer JFT, Tulloch AP (1961) Hydroxy fatty acid glycosides of sophorose from *Torulopsis magnoliae*. Can J Chem 39:846–855

Guilmanov V, Ballistreri A, Impallomeni G, Gross RA (2002) Oxygen transfer rate and sophorose lipid production by *Candida bombicola*. Biotechnol Bioeng 77:489–494

Hall PJ, Haverkamp J, Van Kralingen CG, Schmidt M (1996) Laundry detergent composition containing synergistic combination of sophorose lipid and nonionic surfactant. US patent 5520839

Hillion G, Marchal R, Stoltz C, Borzeix CF (1998) Use of a sophorolipid to provide free radical formation inhibiting activity or elastase inhibiting activity. US patent 5756471

Hommel R, Stuwer O, Stuber W, Haferburg D, Kleber HP (1987) Production of water-soluble surface-active exolipids by *Torulopsis apicola*. Appl Microbiol Biotechnol 26:199–205

Hommel RK, Weber L, Weiss A, Himmelreich U, Rilke O, Kleber HP (1994) Production of sophorose lipid by *Candida (Torulopsis) apicola* grown on glucose. J Biotechnol 33:147–155

Hu YM, Ju LK (2001) Purification of lactonic sophorolipids by crystallization. J Biotechnol 87:263–272

Hu YM, Ju LK (2003) Lipase-mediated deacetylation and oligomerization of lactonic sophorolipids. Biotechnol Prog 19:303–311

Inoue S, Miyamoto N (1980) Process for producing a hydroxyfatty acid ester. US patent 4201844

Inoue S, Kiruma Y, Kinta M (1980) Dehydrating purification process for a fermentation product. US patent 4197166

Isoda H, Kitamoto D, Shinmoto H, Matsumura M, Nakahara T (1997) Microbial extracellular glycolipid induction of differentiation and inhibition of the protein kinase C activity of human promyelocytic leukemia cell line HL60. Biosci Biotechnol Biochem 61:609–614

Ito S, Kinta M, Inoue S (1980) Growth of yeasts on normal-alkanes-Inhibition by a lactonic sophorolipid produced by *Torulopsis bombicola*. Agric Biol Chem 44:2221–2223

Jones DF (1968) Microbiological oxidation of long-chain aliphatic compounds. Part V. Mechanism of hydroxylation. J Chem Soc (C):2827–2833

Jones DF, Howe R (1968) Microbiological oxidation of long-chain aliphatic compounds. Part I. Alkanes and Alk-1-enes. J Chem Soc (C) 2801–2808

Kawano J, Suzuki T, Inoue S, Hayashi S (1981a) Powdered compressed cosmetic material. US patent 4305931

Kawano J, Suzuki T, Inoue S, Hayashi S (1981b) Stick-shaped cosmetic material. US patent 4305929

Kim SY, Oh DK, Lee KH, Kim JH (1997) Effect of soybean oil and glucose on sophorose lipid fermentation by *Torulopsis bombicola* in continuous culture. Appl Microbiol Biotechnol 48:23–26

Kim HS, Kim YB, Lee BS, Kim EK (2005) Sophorolipid production by *Candida bombicola* ATCC 22214 from a corn-oil processing byproduct. J Microbiol Biotechnol 15:55–58

Klekner V, Kosaric N, Zhou QH (1991) Sophorose lipids produced from sucrose. Biotechnol Lett 13:345–348

Lang S, Katsiwela E, Wagner F (1989) Antimicrobial effects of biosurfactants. Fett Wiss Technol Fat Sci Technol 91:363–366

Lee KH, Kim JH (1993) Distribution of substrates carbon in sophorose lipid production by *Torulopsis bombicola*. Biotechnol Lett 15:263–266

Lin SC (1996) Biosurfactants: recent advances. J Chem Technol Biotechnol 66:109–120

Lottermoser K, Schunck WH, Asperger O (1996) Cytochromes P450 of the sophorose lipid-producing yeast *Candida apicola*: Heterogeneity and polymerase chain reaction-mediated cloning of two genes. Yeast 12:565–575

Mager H, Röthlisberger R, Wzgner F (1987) Use of sophorolse-lipid lactone for the treatment of dandruffs and body odeur. European patent 0209783

Maingault M (1999) Utilization of sophorolipids as therapeutically active substances or cosmetic products, in particular for the treatment of the skin. US patent 5981497

Mann RM, Bidwell JR (2001) The acute toxicity of agricultural surfactants to the tadpoles of four Australian and, two exotic frogs. Environ Pollut 114:195–205

Mann RM, Boddy MR (2000) Biodegradation of a nonylphenol ethoxylate by the autochthonous microflora in lake water with observations on the influence of light. Chemosphere 41:1361–1369

Marchal R, Lemal J, Sulzer C, Davila AM (1999) Production of sophorolipid acetate acids from oils or esters. US patent 5900366

Masaru K, Takashi N, Yoji A, Kazuo N, Tatsu N, Sumiko T, Jotaro N (2001) Composition for high-density cold storage transportation. Japanese patent 2001131538

Mulligan CN, Gibbs BF (1990) Recovery of biosurfactants by ultrafiltration. J Chem Technol Biotechnol 47:23–29

Mulligan CN, Yong RN, Gibbs BF (2001) Heavy metal removal from sediments by biosurfactants. J Hazard Mater 85:111–125

Napolitano LM (2006) Sophorolipids in sepsis: Antiinflammatory or antibacterial? Crit Care Med 34:258–259

Nebert DW, Gonzalez FJ (1987) P450 genes: structure, evolution, and regulation. Annu Rev Biochem 56:945–993

Nelson DR (1998) Cytochrome P450 nomenclature. Methods Mol Biol 107:15–24

Nelson DR (1999) Cytochrome P450 and the individuality of species. Arch Biochem Biophys 369:1–10

Nunez A, Foglia TA, Ashby R (2003) Enzymatic synthesis of a galactopyranose sophorolipid fatty acid-ester. Biotechnol Lett 25:1291–1297

Pekin G, Vardar-Sukan F, Kosaric N (2005) Production of sophorolipids from *Candida bombicola* ATCC 22214 using Turkish corn oil and honey. Eng Life Sci 5:357–362

Pellecier F, André P (2004) Cosmetic use of sophorolipids as subcutaneous adipose cushion regulation agents and slimming application. World patent 2004/108063

Pesce L (2002) A biotechnological method for the regeneration of hydrocarbons from dregs and muds, on the base of biosurfactants. World patent 02/062495

Pierce D, Heilman TJ (1998) Germicidal composition. World patent 98/16192

Rau U, Manzke C, Wagner F (1996) Influence of substrate supply on the production of sophorose lipids by *Canidida bombicola* ATCC 22214. Biotechnol Lett 18:149–154

Rau U, Heckmann R, Wray V, Lang S (1999) Enzymatic conversion of a sophorolipid into a glucose lipid. Biotechnol Lett 21:973–977

Rau U, Hammen S, Heckmann R, Wray V, Lang S (2001) Sophorolipids: a source for novel compounds. Ind Crops Prod 13:85–92

Rosa C, Lachance M (1998) The yeast genus *Starmerella* gen. nov. and *Starmerella bombicola* sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow. Int J Syst Bacteriol 48:1413–1417

Shah V, Doncel GF, Seyoum T, Eaton KM, Zalenskaya I, Hagver R, Azim A, Gross R (2005) Sophorolipids, microbial glycolipids with anti-human immunodeficiency virus and sperm–immobilizing activities. Antimicrob Agents Chemother 49:4093–4100

Singh SK, Felse AP, Nunez A, Foglia TA, Gross RA (2003) Regioselective enzyme-catalyzed synthesis of sophorolipid esters, amides, and multifunctional monomers. J Org Chem 68:5466–5477

Solaiman DKY, Ashby RD, Nunez A, Foglia TA (2004) Production of sophorolipids by *Candida bombicola* grown on soy molasses as substrate. Biotechnol Lett 26:1241–1245

Spencer JFT, Gorin PAJ, Tulloch AP (1970) *Torulopsis bombicola* sp. n. Antonie Van Leeuwenhoek 36:129–133

Tulloch AP, Spencer JFT (1968) Fermentation of long-chain compounds *by Torulopsis apicola*. IV. Products from esters and hydrocarbons with 14 and 15 carbon atoms and from methyl palmitoleate. Can J Chem 46:1523–1528

Tulloch AP, Spencer JFT, Gorin PAJ (1962) The fermentation of long-chain compounds by *Torulopsis magnoliae*. Can J Chem 40:1326–1338

Tulloch AP, Spencer JFT, Deinema MH (1968a) A new hydroxy fatty acid sophoroside from *Candida bogoriensis*. Can J Chem 46:345–348

Tulloch AP, Hill A, Spencer JFT (1968b) Structure and reactions of lactonic and acidic sophorosides of 17-hydroxyoctadecanoic acid. Can J Chem 46:3337–3351

Van Bogaert INA, De Maeseneire SL, De Schamphelaire W, Develter D, Soetaert W, Vandamme EJ (2007) Cloning, characterisation and functionality of the orotidine-5′-phosphate decarboxylase gene (*URA3*) of the glycolipid producing yeast *Candida bombicola*. Yeast 24:201–208

Yoo DS, Lee BS, Kim EK (2005) Characteristics of microbial biosurfactant as an antifungal agent against plant pathogenic fungus. J Microbiol Biotechnol 15:1164–1169

Zerkowski JA, Solaiman DKY (2006) Synthesis of polyfunctional fatty amines from sophorolipid-derived 17-hydroxy oleic acid. J Am Oil Chem Soc 83:621–628

Zerkowski JA, Solaiman DKY, Ashby R, Foglia TA (2006) Head group-modified sophorolipids: Synthesis of new cationic, zwitterionic, and anionid surfactants. J Surfactants Deterg 9:57–62

Zhang L, Somasundaran P, Singh SK, Felse AP, Gross R (2004) Synthesis and interfacial properties of sophorolipid derivatives. Colloid Surf A Physicochem Eng Asp 240:75–82

Zhou QH, Kosaric N (1993) Effect of lactose and olive oil on intracellular and extracellular lipids of *Torulopsis bombicola*. Biotechnol Lett 15:477–482

Zhou QH, Kosaric N (1995) Utilization of canola oil and lactose to produce biosurfactant with *Candida bombicola*. J Am Oil Chem Soc 72:67–71

Zhou QH, Kleckner N, Kosaric N (1992) Production of sophorose lipids by *Torulopsis bombicola* from safflower oil and glucose. J Am Oil Chem Soc 69:89–91