Sophorolipid Biosurfactants:

A New, Multifunctional Solution for Formulators Addressing Safety and Sustainability Concerns

By Dr. Lee Speight * and Charbel Cherfan *

Introduction

Consumers are becoming much more conscious of the ingredients in everyday products that they use on themselves and for their families. This has motivated cosmetic and personal care formulators to reevaluate product formulations to provide simpler, shorter ingredient lists. Brands such as Method, The Honest Company, and others have found success with minimalistic formulations by using bio-based ingredients. How are formulators able to maintain product performance with less ingredients?

One method is the strategic use of surfactants. Surfactants are powerful ingredients that allow formulators to do more with less, make water more wet, actives more soluble, and foam longer lasting and more luxurious. However, surfactants have become ingredients of concern to consumers, NGO's and regulators due to their environmental and health impacts – including palm deforestation, aquatic toxicity, skin irritations and exposure to 1,4-dioxane and other harmful trace chemicals.

Biosurfactants, or surfactants produced through the utilization of a microbial host organism, are a solution for formulations to address these safety and sustainability concerns. Surfactant molecules are amphipathic, containing both hydrophilic, water-loving and lipophilic, oil-loving distinct structural features. Biosurfactants have the same general performance parameters of petrochemical-derived surfactants and are robust emulsifiers, detergents and solubilizers. In addition, biosurfactants have been shown to have myriad additional functionalities unique to the microorganism that produces them. These multifunctional benefits can include antimicrobial, anti-inflammatory and wound-healing activities (Lydon, et al. 2017) (Ito, Araki and Hirata 2016) (Kim, et al. 2002).

Multifunctional Sophorolipids

One of the most promising biosurfactants for personal care and cosmetic formulations are sophorolipids. Sophorolipids are a type of glycolipid biosurfactant that was first identified in 1961 as a secondary metabolite of *Stramerella bombicola* (formerly *Candida bombicola)*, a type of yeast most commonly associated with honey bees (Gorin, Spencer and Tulloch 1961). Sophorolipids are amphipathic molecules composed of the disaccharide sophorose and a fatty acid hydrophobic residue **(Figure 1).** Sophorose itself is an interesting glucose derivative comprised of two glucose monomers linked by a usual β-1,2 bond. Like all surfactants, sophorolipids are composed of hydrophobic and hydrophilic domains, with the fatty acid residue and sophorose filling these roles respectively.

The novelty of sophorolipids compared to other biosurfactants, such as rhamnolipids, lies in their unique structure. Sophorolipid biosynthesis, discussed in detail below, begins with the terminal or subterminal oxidation of the alkane terminus of a fatty acid. This creates a fatty acid hydroxide that can then be etherified to the sophorose. Etherification allows for a more pH-resilient molecule than if the sophorose was esterified to the carboxylic acid portion of the fatty acid. Further, by connecting the fatty acid to the sophorose through the alkane terminus, the carboxylic acid is open for further functionalization. Sophorolipid-producing organisms have exploited this and create a large macrolactone by esterifying the already connected fatty acid to another hydroxyl of sophorose **(Figure 1, right).** Thus, there are two major classes of sophorolipids: linear sophorolipids with a free carboxylic acid and lactonic sophorolipids with the carboxylic acid masked as an ester. These classes can be further segregated into subclasses

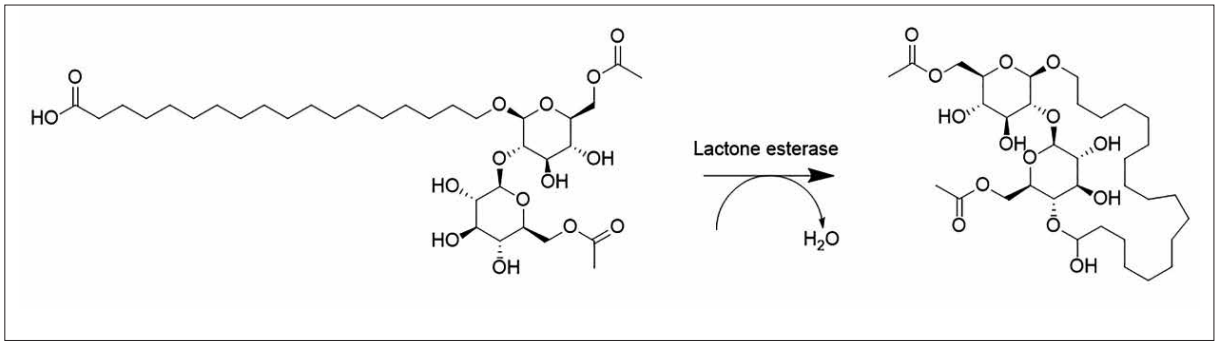


Figure 1: Structure of Sophorolipids. Linear stearic sophorolipid (left) and lactonic stearic sophorolipid (right)

* Locus Performance Ingredients

based on the extend of acetylation that occurs on sophorose hydroxyls and the carbon length and degree of unsaturation of the fatty acids used.

The natural structural diversity of sophorolipid biosurfactants and their ease of customization dramatically expands the color palette of options available to formulators. Linear sophorolipids are readily water soluble, form stable oil-in-water emulsions, and act as cleansers and detergents, while lactonic sophorolipids are less water soluble, form stable water-in-oil emulsions, and act as degreasers – removing oils and grime from delicate surfaces such as skin and faces. Lactonic sophorolipids are readily converted to the linear form with gentle hydrolysis and both forms can be blended together to achieve unique performance parameters.

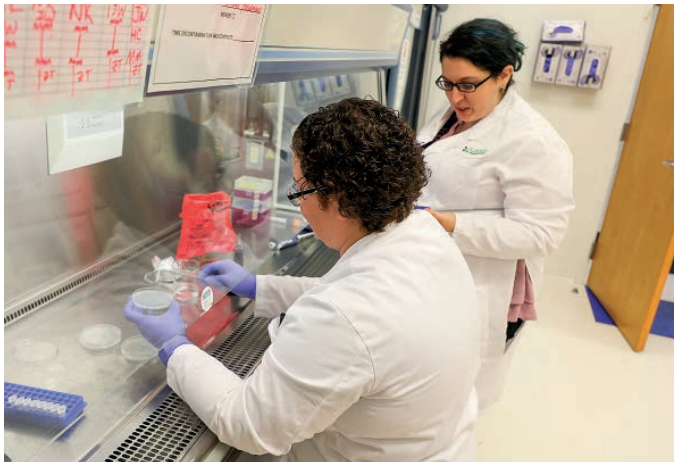
However, the commercial viability of biosurfactants has long been tenuous due to the complexity associated with managing the variability of products produced through fermentation. Chemicals produced from fermentation or other similar direct microbial production methods must be selectively separated from the producing organism and all other undesired metabolites. Typical fermentation products, such as ethanol, sugar and amino acids can easily be purified by distillation or recrystallization, the two principle methods of chemical purification at the commercial scale. Biosurfactants on the other hand, due to their large molecular weight and emulsion properties respectively, cannot use either of these methods of purification. Further, the typical fermentation yields of biosurfactants are low, making isolation more difficult.

Sophorolipid Production

The route to producing sophorolipids at commercially attractive prices and volumes involves a thorough understanding of the biosynthetic pathways involved in their fabrication. As the potential structural diversity of sophorolipids produced through natural fermentation methods is quite wide, careful analysis of the kinetics, gene promotors, enzyme inhibitors and agonists of each step in sophorolipid biosynthesis allows biochemists and engineers to optimize fermentation conditions for optimal productivity and structure selectivity. Herein is a short review of the contemporary research on each distinct step in sophorolipid biosynthesis **(Figure 2).**

Sophorolipid biosynthesis only occurs during the stationary phase of yeast growth. This occurs after nitrogen sources become limited and medium phosphate has been exhausted. At this point, the yeast requires a hydrophobic carbon source to begin sophorolipid production (de Oliveira, et al. 2014). The substrate scope for this requirement is broad and encompasses fatty alcohols, alkanes such as hexadecane, fatty acids, triglycerides, fatty acid esters, and fatty aldehydes. Triglycerides and fatty acid esters are hydrolyzed by excreted lipases before the free fatty acids are brought into the cell (de Oliveira, et al. 2014).

Import of hydrocarbon substrates into sophorolipid-producing yeast cells is a subject of curiosity to investigators. It was long assumed that a combination of passive diffusion and facilitated transport was responsible for hydrocarbon uptake by yeast, however, a review published in 2014 claimed that hydrocarbon import only occurs through carrier mediated processes (Kell 2018). A



subsequent study casts doubt on this theory and until a conclusive study is conducted, it is safe to assume a combination of methods are utilized by yeast cells to import hydrocarbons (Claus, Jezierska and Van Bogaert 2019).

With hydrocarbons inside the cell and sufficient glucose present, the hydrocarbons are activated through oxidation by cytochrome P450 in a NADPH dependent process. If exogenous hydrocarbons are not available, cells can produce them through fatty acid synthesis. Cytochrome P450 is membrane bound and regulated to only be expressed during the stationary phase of the yeast life cycle. The enzyme activates the hydrocarbon by oxidation of the α or β carbon of a linear hydrocarbon to produce a hydroxyl. This hydroxyl is used as the nucleophile in the next step of sophorolipid production (de Oliveira, et al. 2014) (Baccile, et al. 2017).

A common misconception when considering the structure of sophorolipids is that the glucose moieties come directly from glucose fed to the yeast. This is incorrect however, as the glucose in the media is utilized in glycolysis, and UDP-glucose – the substrate for the glycosylation of the hydroxylated hydrocarbon – is produced from gluconeogenesis (de Oliveira, et al. 2014). There are two distinct glycosylation enzymes involved in the assembly of the sophorose group. Glucosyltransferase I (UgtA1) activates UDP-glucose and facilitates the nucleophilic attack by the hydroxylated hydrocarbon. This forms an ether linkage at the C1 position of glucose and effectively produces a glycolipid. Glucosyltransferase II (UgtA2) also activates UDP-glucose, but here the nucleophile is the hydroxyl on C2 of glucose from the newly formed glycolipid (de Oliveira, et al. 2014) (Baccile, et al. 2017). This step forms the sophorose group in its entirety by the formation of the β1-2 bond, as well as the first molecule that can be called a sophorolipid.

At this point, the yeast cell has created a linear nonacetylated sophorolipid. Acetylation occurs at the C6 and C6' hydroxyls of the glucose moieties by the well-known enzyme acetyltransferase using acetyl-CoA. The extent of acetylation is difficult to estimate or control due to the various methods of producing acetyl-CoA (most readily available from glycolysis and fatty acid metabolism) as well as the activity of extracellular lipases (de Oliveira, et al. 2014). Extracellular lipases are of particular concern as the next step in sophorolipid biosynthesis following acetylation is cellular export.

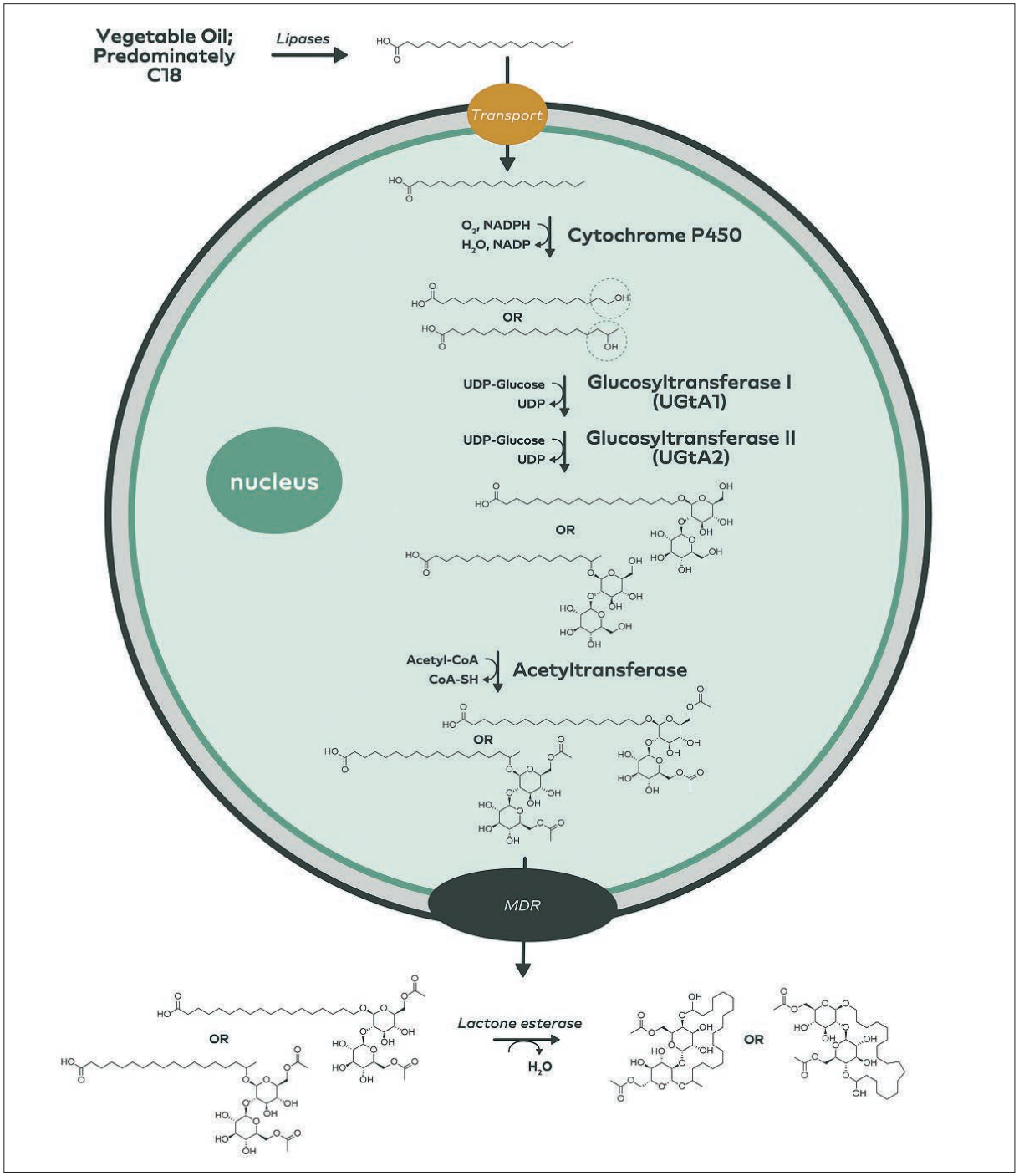


Figure 2: Biosynthesis of Sophorolipids. Terminal and subterminal oxidation of stearic acid is shown to illustrate sophorolipid structural diversity.

Like hydrocarbon import, there are conflicting literature reports on the method of sophorolipid export from yeast cells. Hypotheses including vesicles, passive and active transport have been discussed, but it seems a consensus is forming around the theory that an ATP dependent transporter is required to remove sophorolipids from the cell. Van Bogaert and coworkers identified a gene for a putative multidrug resistance protein (MDR) in the sophorolipid gene cluster for *S. bombicola* (Van Bogaert, et al. 2013). This protein has 49% structural match with a known MDR from *Aspergillus* species that has been shown to facilitate the export of toxins and antibiotics, making it a good candidate for exporting complex molecules such as sophorolipids. The team tested the hypothesis that the MDR they identified could be a SL-specific transporter by creating a series of knock-out strains. Each of the MDR-knock-out strains failed to produce more than 10% of the sophorolipid yield of wild type strains, suggesting that the MDR identified is responsible, in whole or part, for effective sophorolipid cellular export (Lodens, et al. 2020).

After the sophorolipids have been exported from the cell, an excreted enzyme, lactone esterase, catalyzes the intramolecular esterification of the free fatty acid to the C4' hydroxyl of the sophorose group. This is a dehydration reaction, producing one molar equivalent of water for each lactonized sophorolipid. Control of the extend of sophorolipid lactonization can be achieved by longer fermentation runs to give maximum time for the lactonization enzyme to produce sophorolipids with a high lactonic to linear ratio (de Oliveira, et al. 2014). Baccile and coworkers detailed the process of selectively producing acetylated linear so-

phorolipids through the use of a lactone esterase knock-out strain created by Ciesielska and team (Baccile, et al. 2017) (Ciesielska, et al. 2014).

Sophorolipid biosynthesis concludes with the formation of lactonic sophorolipids. During actual production, the sophorolipids are produced during the yeast's stationary phase, which typically occurs within 24 to 48 hours of the beginning of fermentation (de Oliveira, et al. 2014). Sophorolipid production can then continue, debatably indefinitely, as long as sufficient glucose and hydrocarbon substrate are provided. Several continuous and semi-continuous sophorolipid fermentation protocols have been described in the literature, yet despite this, sophorolipids commercialization has been limited to date.

The work of Baccile's group is an important development as one of the factors that has limited sophorolipid commercialization is the large structural diversity of sophorolipids. As it is possible to produce both lactonic and linear sophorolipids, and each one has distinct performance parameters in formulations, producers must decide which, or both, sophorolipid form to produce. One solution has been to produce a standardized 50:50 blend of lactonic to linear sophorolipids. These sophorolipids typically are produced by harvesting the crude predominately lactonic sophorolipid, removing excess feedstock oil and/or fatty acids through extraction and hydrolyzing a portion of the lactonic sophorolipids to the linear form with hydroxide salts. However, the hydrolysis process is nonselective and cleaves the acetyl groups from the sophorose moiety. This can result in an acidic, vinegar odor in the sophorolipid product that must be removed by vacuum or steam stripping.

The sophorolipids produced from these methods have strong performance in formulations that require a surfactant with hydrophilic-lipophilic balance (HLB) parameters between 10 – 13. The HLB scale, which goes from 0 – 20, describes the balance of hydrophilic to lipophilic structural regions in a surfactant molecule. Surfactants with values between 10 – 13 are water dispersible, oil-in-water emulsifiers and detergents. These surfactants have some foaming ability, but higher HLB values result in higher foam.

Sophorolipid Advancements

Locus Performance Ingredients (Locus PI) is a new U.S.-based sophorolipid producer that has chosen a different strategy in which the company embraces the structural diversity of sophorolipids. Locus PI's parent company, Locus Fermentation Solutions (Locus FS), has developed a modular fermentation platform that allows for much higher sophorolipid productivity than traditional fermentation methods at a fraction of the typical capital cost. With this advancement in fermentation technology, Locus PI is able to produce a line of high purity sophorolipids, branded as Ferma™ S Pure, at commercially viable volumes and prices. This advantage is due to Locus PI's focus on providing formulation flexibility to customers through its modular fermentation platform and its ability to produce both low HLB, predominantly lactonic sophorolipids and high HLB, predominantly linear sophorolipids.

Instead of standardizing the sophorolipid product to compensate for sophorolipid product structural diversity, scientists at Locus FS and Locus PI undertook a thorough analysis of each enzymatic step in the biosynthesis of sophorolipids. This project yielded key proprietary process improvements to induce the optimal conditions for each enzymatic step to limit structural diversity naturally. The result is a high purity, predominately lactonic form sophorolipid, commercialized as Ferma™ SL Pure.

Ferma™ SL Pure is a unique sophorolipid for cosmetic and personal care formulators. It is strongly lipophilic with 65 % of the sophorolipid present in the lactonic form and has a high degree of sophorse acetylation. With a total sophorolipid content of 60%, Ferma™ SL Pure is a highly concentrated biosurfactant and has optimal activity in applications that require a surfactant HLB between 2 – 7. The typical critical micelle concentration (CMC) of Ferma™ SL Pure is 62 mg/L, indicating activity at extremely low concentrations. Optimal applications for Ferma™ SL include defoaming, water-in-oil emulsions and enhancing wetting properties, in formulations such as stick deodorants, essential oil-based aromatherapy products and perfumes.

Predominately lactonic form Ferma™ SL Pure is one of the sophorolipid biosurfactant offerings of Locus PI. For formulators requiring surfactants with an HLB value at the other end of the scale, Locus PI produces Ferma™ SH Pure, a sophorolipid product containing a predominately linear form. Like its lipophilic analog, Ferma™ SH Pure is highly concentrated (60% active) but finds best use in applications that require a surfactant HLB higher than 15. As the structure is more disordered than Ferma™ SL Pure, Ferma™ SH Pure's CMC is higher, with typical values around 70 mg/L. Formulations such as shampoos, body and face washes and water-based essential oil products are ideal applications for Ferma™ SH Pure. The Ferma™ S Pure line of products provides personal care and cosmetic formulators with sophorolipid-based biosurfactants at commercially viable volumes and cost-in-use competitive pricing. This offering differs when compared to the typical commercialization strategy for sophorolipids, where producers mitigate the variability in fermentation structural diversity

by creating a single, standardized product. The disadvantage of this strategy is that formulators are unable to capture the unique multifunctionality of each distinct form of the sophorolipid biosurfactant. These standardized sophorolipids result in products best suited to mid-HLB scale applications. To empower formulators to capture the full value of sophorolipids, Locus PI has taken a different path. In addition to offering biosurfactants at both ends of the HLB scale, Ferma™ S products have been designed and tested to be perfectly compatible with one another. This allows formulators the freedom to create their own unique blends of Ferma™ S to maximize the value of their products.

Formulating personal care and cosmetic products to keep up with clean beauty trends does not have to be difficult or costly. Sophorolipids are a cost-effective and environmentally friendly solution to remove or reduce skin irritating sulfates, remove 1,4-dioxane containing ethoxylates and eliminate palm-derived ingredients from consumer products. Thanks to the recent technology advancements such as those developed by Locus PI, high activity biosurfactants like Ferma™ S are enabling the simplification of ingredient lists with ingredients that are 100% GMO-free and available for customization to personal care and cosmetic formulators today.

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