"Unlocking the potential of a glycolipid platform through chemical modification"

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ABSTRACT

Sophorolipids are an important class of glycolipid microbial biosurfactants because of their attractive surface-active and biological properties recently resulting in industrial production. However, their commercialization has been mainly limited to household detergent applications due to the minimal structural variation that can be produced via fermentation. The amphiphilic structure and the availability of the reactive sites on the sophorolipids make them suitable candidates for chemical modification which enables the generation of sophorolipid derivatives in a fast and systematic way. The latter is crucial to obtain a fundamental understanding of structure-activity relationship, in particular for high added-value applications. This review provides an overview of the recent developments in the chemical modifications of sophorolipids and novel types of related microbial glycolipids and their targeted applications.

Keywords: Chemical modification, green chemistry, microbial biosurfactants, sophorolipids, glycolipids, biosurfactants

INTRODUCTION

Sophorolipids (SLs) are a class of microbial glycolipid biosurfactants produced by certain yeast strains (e.g. *Starmerella bombicola*) using renewable carbon sources derived from first-generation biomass (e.g. glucose, fatty acids- alcohols) or even from waste- and side streams (e.g food waste, waste cooking oils, molasses etc.) [1], [2]. Currently SLs are amongst the three most representative microbial biosurfactants on the market owing to their relatively high production titers (>200 g L^{-1}), well-known surface-active properties as well as valuable biological activities (e.g. antimicrobial, antiviral, anticancer etc.) [3], [4], [5], [6]. Moreover, they are environmentally friendly alternatives to conventional surfactants due to their increased biodegradability and lower toxicity. Indeed, several international companies such as Evonik, Unilever, Henkel, Soliance, Saraya, MG Intobio, Holiferm, AmphiStar, Allied Carbon Solutions, SyntheZyme to name a few are investing in the development, and production of SLs [7].

Natural SLs typically consist of a mixture of lactonic sophorolipids (LSL) (1) and acidic sophorolipids (ASL) (2) with variation of the degree of acetylation at the 6' and 6'' positions of the sophorose head and the position of the glycosidic linkage of the sophorose moiety to the lipid tail (terminal (ω) and subterminal (ω-1)) (Figure 1). Usually, SLs occur as 20 major and up to 100 minor congeners. However, the low uniformity and the limited structure variability limits the large scale commercialization of SLs. Therefore, different modification strategies have been investigated to increase the structural variation based on classic SLs. An emerging technology lies in the (green) chemical modification of SLs which enables rapid generation of SL libraries in a systematic fashion [8]. Accordingly, chemical modification offers an effective way to establish the structure activity relationships to broaden the scope of the applications for the SL platform. More recently a novel microbial glycolipid platform was generated altering the biosynthetic pathway of SLs [9], [10], through genetic engineering of *Starmerella bombicola*, which offers a number of benefits compared to the use of classic SLs towards subsequent chemical derivatization (Figure 1).

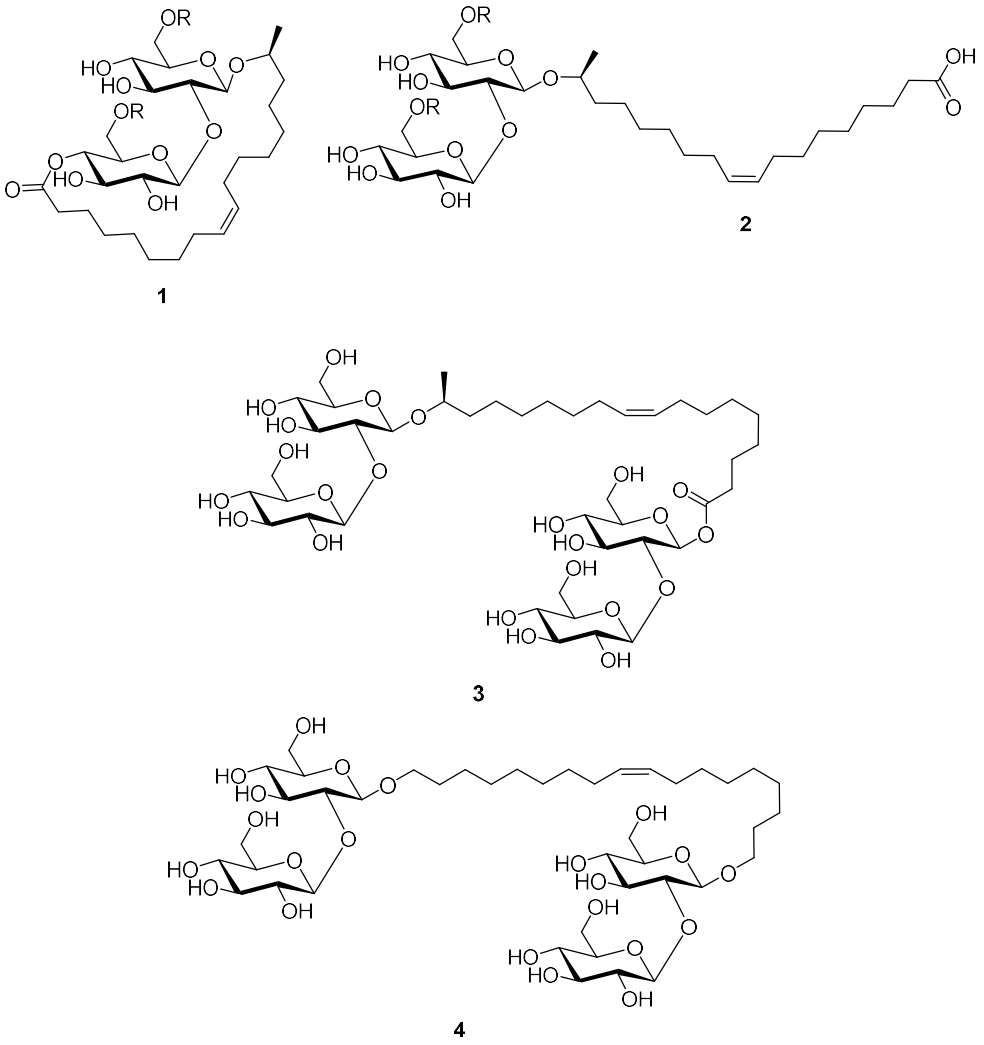

Figure 1. Chemical structures of natural lactonic (1) and acidic sophorolipids (2) produced by *Starmeralla bombicola.* The R groups represent either hydrogen or acetyl groups. Chemical structure of novel glycolipids produced by genetically engineered *Starmeralla bombicola* strains: nonacetylated bola sophorolipid (3) and nonacetylated bola sophoroside (4) [9], [10].

Chemical modifications towards new sophorolipid derivatives

Chemical modification of SLs can be performed by targeting the primary hydroxyl groups on the sophorose head (at the 6’ and 6’’ positions), the *cis* double bond on the lipid tail or the carboxyl functionality (COOH) at the end of the lipid tail (Figure 2).

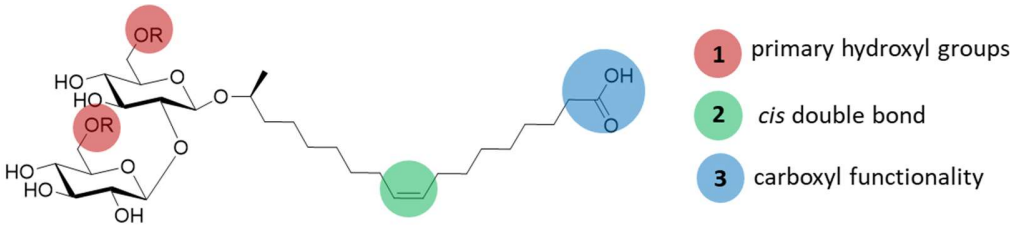

Figure 2. Sites that can be targeted for chemical modification shown on the acidic sophorolipid are highlighted (1: primary hydroxyl groups on the sophorose head group, 2: *cis* double bond in the lipid tail, 3: carboxyl functionality at the terminus of the lipid tail).

The susceptibility of these functional sites on SLs have been exploited throughout the years by researchers to increase the structural variation based on classic SLs, thereby expanding the scope of potential added-value applications [8]. More recently, novel glycolipids such as bola sophorosides and glucosides [11] have been developed and investigated as alternative platform molecules for green derivatization avoiding some key issues associated with classic SLs. The synthetic approaches targeting these sites for the chemical modification of SLs, produced by both wild-type and genetically modified yeast strains, are summarized in Figure 3 and briefly discussed in the following sections. Moreover, the performance of these SL derivatives in the respective application areas are summarized (Table 1).

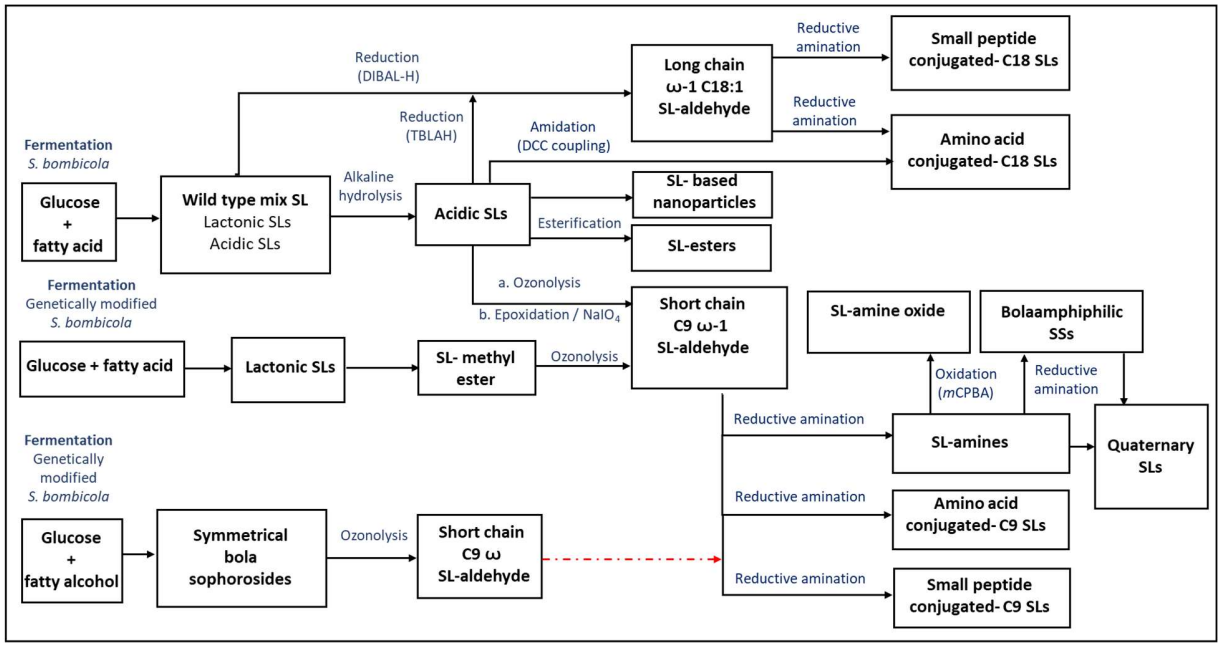

Figure 3. Block flow diagram for sophorolipid derivatives obtained via chemical modification of classic SLs and novel developed glycolipids.

Modifications of the sophorose head group

Head group modifications are typically performed by using enzymes (e.g. esterase and lipase) owing to their selective - in contrast to chemical modification - acylation capability of the primary hydroxyl groups on the sophorose head [12]. Therefore, the literature is scarce on the chemical modification of the sophorose head group. However, of note is the report by Zerkowski *et al.* describing the head group modification of stearic acid based crude SL with seven different amino acids through amide bond chemistry [13]. The obtained amino acid-SL conjugates displayed improved water solubility as well as good surfactant properties with minimum surface tensions below 40 mN m^{-1}. Although the non-selective acylation yielded the amino acid-SL derivatives as a mixture of isomers, this report is a fine example to showcase the potential of chemical modification to expand the structural variation of SLs.

Lipid tail modifications

Modification of the carboxy terminus of the lipid chain of SLs is usually performed via esterification or amidation. Through esterification, aliphatic esters of SLs, with varying chain lengths (C2 to C8), are typically obtained, whereas amino acids are coupled with ASLs via carbodiimide-mediated coupling reactions or via reductive amination with SL-aldehyde [14], [15]. Gross and co-workers investigated the interfacial tension reduction and emulsification performance of modified SL-esters compared to that of natural SLs in four different oil-water systems [16], [17], [18], [19]. The SL-esters were synthesized by the ring opening of natural LSL under alkaline conditions using ethanol, hexanol and 1-decanol as a reagent and solvent leading to the extension of the hydrophobic moiety of SL from 18 carbons to 20, 24, 28 carbons, respectively. Although there is not a clear trend with respect to the n-alkyl chain length of the ester moiety among the SL esters, an improvement in the reduction of the interfacial tension and emulsification properties with the obtained SL esters in all the tested oil-water systems compared to the 1:1 weight mixture of LSL and ASL was observed. The SL hexyl ester even surpassed the performance of commercial Triton in a crude oil-water system. Also, as depicted by the same research group in another study [20], tunable surfactant features (e.g., critical micelle concentration) can be achieved by simply varying the ester chain length.

Furthermore, the antimicrobial properties of a set of SL esters (ranging from SL-ethyl to -octyl ester) have been evaluated against *Escherichia coli* and a number of Gram-positive bacteria (i.e. *Bacillus. cereus, Bacillus subtilis, Staphylococcus aureus* and *Listeria innocua*) by determining the minimum inhibitory concentrations (MICs) [21]. The lowest MIC values were obtained with the SL-butyl ester against *B. cereus*, *B. subtilis and L. innocua*, which proved at least equally effective compared to the LSL or the benchmark antibiotic, streptomycin. While SL-esters with a shorter alkyl chain length ($n<4$ i.e. SL-methyl, -ethyl and -propyl ester) showed promising activities against all tested Gram positive bacteria, those with a longer alkyl chain length ($n>5$ i.e. SL-hexyl ester) scored poorly. The authors attribute this underperformance in antimicrobial activity with an increasing alkyl chain length ($n>5$) to the corresponding change in the hydrophilic-hydrophobic balance. The advantages of chemical modifications are further highlighted by the fact that modified SL ester derivatives ($n\geq4$) are three-fold more effective in killing *B. cereus*

compared to natural LSL. However, neither the modified SL-ester derivatives nor the natural LSL exhibited appreciable activity against *E. coli.* [22].

SL-ester derivatives have also drawn the attention of researchers as potential candidates in biomaterial applications (e.g., drug-loading, delivery, implantations etc.), in particular as biocompatible immunomodulatory agents. A recent example shows promising results obtained with the SL-ethyl ester and SL-butyl ester on the macrophage viability and the promotion of the phenotype transition in macrophage profile. The latter is an attribute deemed necessary for wound healing after biomaterial implantation [23].

SL amides are another targeted class of SL derivatives. The rationale behind synthesizing amino-acid conjugated SLs is typically to improve the antimicrobial activity of SLs. SL amides are typically synthesized via a carbodiimide-mediated coupling reaction of SL acid with amino acids or amino acid alkyl esters. An earlier example on amino acid conjugated SLs documents their antibacterial, anti-HIV and spermicidal activities [15]. All tested derivatives displayed antibacterial and antiviral activities, with the leucine conjugated-SL ethyl ester being the most effective derivative. However, none of the derivatives were significantly spermicidal, suggesting that they are selective in the lysis of membranes. However, the point should also be made that in vitro observations can differ quite drastically from real in vivo effects. Overall, esterified SL-amino acid derivatives showed better antiviral activities compared to their non-esterified analogues showing that increased hydrophobicity plays a key role. However, it has been noted by the authors that previously synthesized monoacetylated SL-ethyl ester (MAEE-SL) outperformed the leucine conjugated-SL ethyl ester against all the tested organisms except *Moraxella sp*, suggesting that the degree of acetylation of the sophorose head group may also be a contributor to the lytic action. Similarly, Tang et.al. synthesized arginine and leucine conjugated-SLs and tested their efficiencies in parallel with the ASL, as food preservative agents against eight (including *E. coli* and *Aspergillus flawus*) different pathogens that can cause health problems such as diarrhea, vomiting and food poisoning etc. [24]. By conjugating ASL with arginine and leucine, an increased anti-microbial activity was targeted through the introduction of cationic charge and increased hydrophobicity [25]. Amongst the tested derivatives, arginine conjugated-SL showed effective and broad anti-microbial activities toward all tested pathogens with MIC values equal or lower

than 250 mg/L, which makes them promising alternatives to chemical preservatives. The increased activity could be explained by the increased adhesion of SLs to the negatively charged bacteria due to the positive charge of the resulting conjugate. On the other hand, no anti-microbial activities were observed with the hydrophobic leucine conjugated-SLs (compared with the arginine conjugated) against the tested pathogens indicating the need to optimize a cationic charge-hydrophobicity balance. The anti-microbial activity of ASL and its derivatives against *E. coli* and the *mildew A. flawus* is limited. The lack of activity against *E. coli* is probably attributed to the stronger cell membrane of gram-negative bacteria.

ASLs have also been employed as reducing and stabilizing agents in the synthesis of metal nanoparticles. Owing to their biocompatibility, selective interactions with proteins and more importantly water dispersibility, SLs are increasingly investigated for the synthesis of such metal nanoparticles in biomedical applications. For example, Singh *et al.* synthesized SL based silver nanoparticles (AgNPs-SLs), which were more potent against Gram-positive bacteria (*B. subtilis and S. aureus*) and especially Gram-negative (*Pseudomonas aeruginosa*) bacteria compared to natural SLs [26]. SLs capping provides a better interaction with the outer layer of Gram-negative bacteria cells (lipopolysaccharides/phospholipids) while silver ions allow a better penetration inside the bacterial cell once the AgNPs-SLs are close to the outer layer. Recently, Shikha *et al*. reported similar outcomes with SL based gold nanoparticles (AuNPs-SLs) where the AuNPs-SLs showed high efficacy against both Gram-positive (*S. aureus*) and Gram-negative (*Vibrio cholerae*) bacteria, but particularly against Gram-negative bacteria [27]**.** A similar activity was observed with the SL capped zinc oxide nanoparticles against the Gram-negative bacterium *Salmonella enterica* and fungus *Candida albicans* [28]. As such, SL based metal nanoparticles overcome the aforementioned resistance of gram-negative bacteria towards amino acid conjugated SL derivatives, SL-esters or natural SLs [29], [30].

Modifications targeting the double bond

The susceptibility of the double bond present in the lipid tail of SLs has been exploited targeting the formation of new SL derivatives. The production of short chain SLs has been reported by Develter and Fleurackers [31]. While the alkaline hydrolysis of SL-esters yielded C5 to C12 SL-derivatives, the ozonolysis of ASL yielded C9 ASL, the latter proving to be a superior wetting agent

than the alkylpolyglucoside Simulsol AS48. Peng *et al.* performed a ring opening cross metathesis of LSLs followed by ethanolysis to obtain SL-derivatives with varying alkyl chain lengths (e.g. C10 to C14) [32]. The latter were shown as effective surfactants for lowering the surface tension at the air – water interface making them suitable candidates for domestic and industrial cleaning applications [33]. Following these earlier works, Delbeke *et al*. introduced the oxidative cleavage of the double bond on SLs via ozonolysis as a synthetic approach. In this synthesis strategy, the production of SL-aldehyde is targeted to enable further chemical derivatizations. Briefly, the SL-aldehyde was produced by ozonolysis of peracetylated SL-methyl ester followed by a reductive work-up [34], [35]. Indeed, this approach has been proliferative in generating a plethora of SL derivatives including SL amines, SL amine oxides, bolamphiphilic SLs and quaternary ammonium SLs [34], [36], [37], [38], [39]. Among all the derivatives, quaternary ammonium SLs with long alkyl chains on the nitrogen atom (i.e. dodecyl, pentadecyl and octadecyl) proved to be the most effective derivatives in terms of antimicrobial activity and transfection efficacy. The utility of the sophorose head group in antimicrobial activity was proven by comparing the activity of the SL derivatives to their corresponding deglycosylated analogues. More interestingly, the antimicrobial activities of above mentioned quaternary ammonium SLs derivatives were higher by a factor of 100 compared to LSL- and ASLs showing the improved antimicrobial properties obtained by chemical derivatization. The lack of antimicrobial activity of the SL amine oxide derivatives against the tested microorganisms suggests the necessity of the quaternary ammonium group. These studies proved that the SL aldehyde can be considered as a platform molecule to introduce different functionalities, thereby increasing the structural variation of the SL platform. This strategy was further improved by the same research group towards a safer, greener and a scalable process by replacing the LSL with so called symmetrical bola sophorosides (sym. bola SS) [11]. The latter was produced by a genetically modified yeast strain where two sophorose moieties are symmetrically linked to a hydrophobic linker with terminal glycosidic bonds [10]. Due to the increased hydrophilic character of the symmetrical bola SSs, water was used as solvent in which the ozonolysis was performed. Besides being a green solvent, using water helps to reduce the total number of process steps by eliminating the necessity of the subsequent work-up after ozonolysis and also helps to reduce the safety risks by trapping the

explosive ozonides formed during ozonolysis. Moreover, the high heat capacity of water enables performing the ozonolysis at room temperature. C9 ASL or AGL and C9 SL or GL aldehyde were obtained in one step via ozonolysis starting from bola sophorosides/glucosides respectively so that they can be employed in the synthesis of new SL derivatives as discussed above.

As a testament to the industrial interest in SL derivatization, the production of the SL aldehyde handle has been also the subject of a recent patent application [40]. While C9 SL-aldehyde was obtained either via ozonolysis or via a four-step reaction sequence (i.e. epoxide ring formation, ring opening to a vicinal diol and oxidative cleavage with $NaIO_4$), the C18:1 SL aldehyde was produced by reduction of natural SLs with ate complexes (i.e. TBLAH and DIBAL-H). Subsequently, both SL-aldehydes were subjected to reductive amination with amino acids and small chain peptides yielding a new set of amino acid conjugated SL derivatives. The latter were tested as active ingredients in the formulations of disinfecting consumer products.

The alkene moiety on the lactonic SL was recently exploited via epoxidation to attain poly(ethylene glycol) attached non-ionic biosurfactants [41]. Briefly, following the epoxidation, the epoxide ring was opened with five different poly(ethylene glycol) with varying chain lengths including both diols (PEG) and mono methoxy-terminated (MePEG) yielding non-ionic derivatives of SLs. The calculated HLB values based on the Griffin method revealed that these SL-derivatives could be used as wetting agents, oil-water emulsifiers, detergents and solubilizing agents. The tunability is achieved by being able to attach varying PEGs to the natural SL.

	SL- derivatives	Potential applications	Notes
Head Group modification	Head group modification with amino acids	Charged SL-derivatives	Increased water solubility and improved surface-tension properties [13].
Lipid tail modification	SL-esters: SL-EE, SL-HE and SL-DE	Emulsifying agents	Improved emulsification properties compared to mixture of classic SLs in four different oil-water systems (i.e. lemon oil-water, aqueous Arabian oil-water, almond oil-water and crude oil-water[16], [17], [18], [19].
	SL-esters: SL-ME, SL-EE, SL-PrE, SL-BE, SL-PE, SL-HE, SL-OE	Antibacterial agents Biocompatible immunomodulatory agents	Moderate selectivity against tested Gram-positive human pathogens. SL-BE showed the best selectivity [21]. SL-EE and SL-BE suppressed M1 polarization and also promoted M2 polarization in lipopolysaccharide-stimulated macrophages [23].
	Amino acid conjugated-SLs	Antimicrobial and anti-viral agents	All tested derivatives displayed antibacterial and antiviral activities against the tested pathogens, with the leucine conjugated-SL ethyl ester being the most effective derivative [15].

	Amino acid conjugated-SLs	Food preservative agents	Arginine conjugated-SL showed effective and broad anti-microbial activities toward all tested pathogens [24].
Modifications targeting the double bond	Amino acid conjugated-SLs	Antimicrobial and anti-viral agents	The derivatives performed well in the formulations of consumer products as disinfecting agents [40].
	SL-based nanoparticles	Antibacterial agents	The derivatives showed high potency against tested Gram-positive bacteria and especially Gram-negative bacteria compared to classic SLs [26], [27], [28].
	Short chain SLs (C5-C12), (C10-C14)	Wetting agents	Suitable candidates for domestic and industrial cleaning applications [31], [32].
	Quaternary ammonium SLs	Antimicrobial and Gene transfection agents	Derivatives with long alkyl chains on the nitrogen atom (i.e. dodecyl, pentadecyl and octadecyl) proved to be the most effective derivatives [34], [36], [39].

Table 1. An overview of the SL-derivatives from selected papers and their applications (tested or targeted) discussed in this review. Abbreviations: SL: Sophorolipid, SL-ME: SL-methyl ester, SL-EE: SL-Ethyl ester, SL-PrE: SL-Propyl ester, SL-BE: SL-Butyl ester, SL-PE: SL-Pentyl ester, SL-HE: SL-Hexyl ester, SL-OE: SL-Octyl ester, SL-DE: SL-Decyl ester.

Future perspectives

The literature reports of both academic and industrial researchers demonstrate that the chemical modification of sophorolipids is a promising approach to increase the valorization potential of the SL platform and biosurfactants in general. However, further research and development is required to facilitate the commercial adoption of these SL derivatives.

- Firstly, research focusing on the selective production of specific SLs or sophorosides by fermentation need to be pursued. Highly selective production of targeted glycolipids would then increase the overall process yields and efficiencies. The research on chemical modification of SLs is mostly conducted at laboratory-scale (i.e. obtaining products at g-scale). Consequently, the scope of application testing becomes limited with the available quantities. Without the tested and proven use of SL derivatives in high-value applications, the research on the scale-up of chemical modification is not justified thereby creating a vicious cycle. However, the scale-up research needs to be performed to enable the testing of SL derivatives in a broad range of applications. As the length- and time-scale parameters (e.g. vessel volume, heat transfer area, feed tube dimensions, heating/cooling time, mixing time and vessel charge/discharge etc.) will be different at the larger scale, the behavior of the chemical reactions as well as the physical operations will be also altered. Therefore it is essential to investigate physicochemistry of these chemical reactions at scale as well.

- With respect to application areas, the coupling of SLs with (Active Pharmaceutical Ingredients) APIs to obtain hybrid materials deserves more attention in order to develop medical applications.

- Last but not least, life-cycle analysis studies and techno-economic analysis with respective hot spot analyses of the integrated fermentation and chemical modification process would help to determine where further optimization should focus.

Conclusion

The chemical modification of SLs has been an emerging area of research. The reactive sites on SLs have been exploited by researchers to increase the structural variation of the sophorolipid

platform, with varying degrees of success. The rapid and systematic generation and evaluation of SL libraries allows to establish structure-function relationships. With respect to head-group modification, improved water solubility and surfactant properties were obtained through the non-selective acylation of the sophorose head, despite the end-product being an isomer. On the other hand, increased antimicrobial activities were achieved with the lipid-tail modifications. For example, SL-esters with shorter *n*-alkyl chains (n<4) and amino acid conjugated-SLs (i.e. leucine and arginine conjugated) displayed higher potency, in particular towards Gram-positive bacteria. This observation suggests that an optimum balance of cationic charge-hydrophobicity is required for an effective biological activity. Furthermore, SL based metal nanoparticles were more potent against *E. coli* compared to the other SL derivatives, (SL-esters and amino acid conjugated-SLs) and the natural SLs. Additionally, oxidative cleavage of the olefinic moiety of SLs produces shorter SL derivatives, but more importantly generates a useful handle, SL-aldehyde, for further chemical derivatization. The yields of this reaction were increased by using bola sophorosides instead of natural sophorolipids. The SL aldehyde allows the introduction of a nitrogen atom to the SL structure via reductive amination (e.g. SL amines, amino acid conjugated). Subsequent quaternization of SL-amines provides a fixed positive charge on the SLs that makes them attractive as antimicrobial- and gene transfection agents. Overall, the sophorolipid platform is expanding at a rapid pace thanks to the opportunities provided by the chemical modification and further biotechnological achievements.

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REFERENCES

[1] X. Ma, L. Meng, H. Zhang, L. Zhou, J. Yue, H. Zhu, R. Yao, Sophorolipid biosynthesis and production from diverse hydrophilic and hydrophobic carbon substrates, Appl. Microbiol. Biotechnol. 104 (2020) 77–100. https://doi.org/10.1007/s00253-019-10247-w.

[2] P. Jiménez-Peñalver, A. Rodríguez, A. Daverey, X. Font, T. Gea, Use of wastes for

sophorolipids production as a transition to circular economy: state of the art and perspectives, Rev. Environ. Sci. Biotechnol. 18 (2019) 413–435. https://doi.org/10.1007/s11157-019-09502-3.

[3] W.Y. Cho, J.F. Ng, W.H. Yap, Sophorolipids — Bio-Based Antimicrobial Formulating Agents, Molecules. 27 (2022) 1–24.

[4] S. Claus, I.N.A. Van Bogaert, Sophorolipid production by yeasts: a critical review of the literature and suggestions for future research, Appl. Microbiol. Biotechnol. 101 (2017) 7811–7821. https://doi.org/10.1007/s00253-017-8519-7.

[5] T. Rebecca Miceli, T. David Corr, M. Margarida Barroso, N. Dogra, A. Richard Gross, Sophorolipids: Anti-cancer activities and mechanisms, Bioorganic Med. Chem. 65 (2022) 116787. https://doi.org/10.1016/j.bmc.2022.116787.

[6] S. Dierickx, M. Castelein, J. Remmery, V. De Clercq, S. Lodens, N. Baccile, S.L. De Maeseneire, S.L.K.W. Roelants, W.K. Soetaert, From bumblebee to bioeconomy: Recent developments and perspectives for sophorolipid biosynthesis, Biotechnol. Adv. 54 (2022) 107788. https://doi.org/10.1016/j.biotechadv.2021.107788.

[7] Market Synopsis, Sophorolipids Mark. By Strain, By Appl. (Soaps Deterg. Anti-Microbial Agents, Pers. Care Prod. By End-Use (Pharmaceutical Ind. FMCG Ind. By Reg. Forecast to 2030. (2022) 20–23. https://www.emergenresearch.com/industry-report/sophorolipids-market (accessed January 2, 2023).

[8] E.I.P. Delbeke, M. Movsisyan, K.M. Van Geem, C. V. Stevens, Chemical and enzymatic modification of sophorolipids, Green Chem. 18 (2015) 76–104. https://doi.org/10.1039/c5gc02187a.

[9] L. Van Renterghem, S.L.K.W. Roelants, N. Baccile, K. Uyttersprot, M.C. Taelman, B. Everaert, S. Mincke, S. Ledegen, S. Debrouwer, K. Scholtens, C. Stevens, W. Soetaert, From lab to market: An integrated bioprocess design approach for new-to-nature biosurfactants produced by Starmerella bombicola, Biotechnol. Bioeng. 115 (2018) 1195–1206. https://doi.org/10.1002/bit.26539.

[10] Roelants, Sophie L. K. W., Van Renterghem, Lisa, Soetaert, Wim, J. Remmery, Improved bola SS production WO2020104582A1, 2020.

[11] M. Pala, J. Everaert, A. Ollivier, R. Raeymaekers, K. Quataert, S.L.K.W. Roelants, W. Soetaert, C. V Stevens, Ozonolysis of Symmetrical Bola Sophorosides Yields Key Precursors for the Development of Functional Sophorolipid Derivatives, (2022). https://doi.org/10.1021/acssuschemeng.2c03004.

[12] A. Sembayeva, B. Berhane, J.A. Carr, Lipase-mediated regioselective modifications of macrolactonic sophorolipids, Tetrahedron. 73 (2017) 1873–1880. https://doi.org/10.1016/j.tet.2017.02.043.

[13] J.A. Zerkowski, D.K.Y. Solaiman, R.D. Ashby, T.A. Foglia, Head group-modified sophorolipids: Synthesis of new cationic, zwitterionic, and anionic surfactants, J. Surfactants Deterg. 9 (2006) 57–62. https://doi.org/10.1007/s11743-006-0375-x.

[14] J.A. Carr, K.S. Bisht, Enzyme-catalyzed regioselective transesterification of peracylated sophorolipids, Tetrahedron. 59 (2003) 7713–7724. https://doi.org/10.1016/S0040-4020(03)01213-4.

[15] A. Azim, V. Shah, G.F. Doncel, N. Peterson, W. Gao, R. Gross, Amino acid conjugated sophorolipids: A new family of biologically active functionalized glycolipids, Bioconjug.

Chem. 17 (2006) 1523–1529. https://doi.org/10.1021/bc060094n.

[16] A. Koh, R. Gross, A versatile family of sophorolipid esters: Engineering surfactant structure for stabilization of lemon oil-water interfaces, Colloids Surfaces A Physicochem. Eng. Asp. 507 (2016) 152–163. https://doi.org/10.1016/j.colsurfa.2016.07.089.

[17] A. Koh, A. Wong, A. Quinteros, C. Desplat, A. Richard Gross, Influence of Sophorolipid Structure on Interfacial Properties of Aqueous-Arabian Light, J Americ Oil Chem Soc. (2016) 107–119.

[18] A. Koh, R.J. Linhardt, R. Gross, Effect of Sophorolipid n-Alkyl Ester Chain Length on Its Interfacial Properties at the Almond Oil-Water Interface, Langmuir. 32 (2016) 5562–5572. https://doi.org/10.1021/acs.langmuir.6b01008.

[19] A. Koh, R. Gross, Molecular editing of sophorolipids by esterification of lipid moieties: Effects on interfacial properties at paraffin and synthetic crude oil-water interfaces, Colloids Surfaces A Physicochem. Eng. Asp. 507 (2016) 170–181. https://doi.org/10.1016/j.colsurfa.2016.07.084.

[20] A. Koh, K. Todd, E. Sherbourne, R.A. Gross, Fundamental Characterization of the Micellar Self-Assembly of Sophorolipid Esters, Langmuir. 33 (2017) 5760–5768. https://doi.org/10.1021/acs.langmuir.7b00480.

[21] F. Totsingan, F. Liu, R.A. Gross, Structure–activity relationship assessment of sophorolipid ester derivatives against model bacteria strains, Molecules. 26 (2021) 1–9. https://doi.org/10.3390/molecules26103021.

[22] S. Gruenheid, H. Le Moual, Resistance to antimicrobial peptides in Gram-negative bacteria, FEMS Microbiol. Lett. 330 (2012) 81–89. https://doi.org/10.1111/j.1574-

6968.2012.02528.x.

[23] P. Diaz-Rodriguez, H. Chen, J.D. Erndt-Marino, F. Liu, F. Totsingan, R.A. Gross, M.S. Hahn, Impact of Select Sophorolipid Derivatives on Macrophage Polarization and Viability, ACS Appl. Bio Mater. 2 (2019) 601–612. https://doi.org/10.1021/acsabm.8b00799.

[24] Y. Tang, M. Jin, T. Cui, Y. Hu, X. Long, Efficient Preparation of Sophorolipids and Functionalization with Amino Acids to Furnish Potent Preservatives, J. Agric. Food Chem. 69 (2021) 9608–9615. https://doi.org/10.1021/acs.jafc.1c03439.

[25] C. Zhou, Y. Wang, Structure–activity relationship of cationic surfactants as antimicrobial agents, Curr. Opin. Colloid Interface Sci. 45 (2020) 28–43. https://doi.org/10.1016/j.cocis.2019.11.009.

[26] S. Singh, P. Patel, S. Jaiswal, A.A. Prabhune, C. V. Ramana, B.L.V. Prasad, A direct method for the preparation of glycolipid-metal nanoparticle conjugates: Sophorolipids as reducing and capping agents for the synthesis of water re-dispersible silver nanoparticles and their antibacterial activity, New J. Chem. 33 (2009) 646–652. https://doi.org/10.1039/b811829a.

[27] S. Shikha, S.R. Chaudhuri, M.S. Bhattacharyya, Facile One Pot Greener Synthesis of Sophorolipid Capped Gold Nanoparticles and its Antimicrobial Activity having Special Efficacy Against Gram Negative Vibrio cholerae, Sci. Rep. 10 (2020) 1–13. https://doi.org/10.1038/s41598-019-57399-3.

[28] G. Basak, D. Das, N. Das, Dual role of acidic diacetate sophorolipid as biostabilizer for ZnO nanoparticle synthesis and biofunctionalizing agent against Salmonella enterica and Candida albicans, J. Microbiol. Biotechnol. 24 (2014) 87–96.

https://doi.org/10.4014/jmb.1307.07081.

[29] V. Shah, D. Badia, P. Ratsep, Sophorolipids having enhanced antibacterial activity [1], Antimicrob. Agents Chemother. 51 (2007) 397–400. https://doi.org/10.1128/AAC.01118-06.

[30] K. Kim, D. Yoo, Y. Kim, B. Lee, D. Shin, E.K. Kim, Characteristics of sophorolipid as an antimicrobial agent, J. Microbiol. Biotechnol. 12 (2002) 235–241.

[31] D. Develter, S. Fleurackers, A method for the production of short chained glycolipids, 2008. http://www.google.com/patents/EP1953237A1?cl=en.

[32] Y. Peng, F. Totsingan, M.A.R. Meier, M. Steinmann, F. Wurm, A. Koh, R.A. Gross, Sophorolipids: Expanding structural diversity by ring-opening cross-metathesis, Eur. J. Lipid Sci. Technol. 117 (2015) 217–228. https://doi.org/10.1002/ejlt.201400466.

[33] C. Ahn, V.K. Morya, E.K. Kim, Tuning surface-active properties of bio-surfactant sophorolipids by varying fatty-acid chain lengths, Korean J. Chem. Eng. 33 (2016) 2127–2133. https://doi.org/10.1007/s11814-016-0082-x.

[34] E.I.P. Delbeke, B.I. Roman, G.B. Marin, K.M. Van Geem, C. V. Stevens, A new class of antimicrobial biosurfactants: Quaternary ammonium sophorolipids, Green Chem. 17 (2015) 3373–3377. https://doi.org/10.1039/c5gc00120j.

[35] E.I.P. Delbeke, J. Everaert, E. Uitterhaegen, S. Verweire, A. Verlee, T. Talou, W. Soetaert, I.N.A. Van Bogaert, C. V. Stevens, Petroselinic acid purification and its use for the fermentation of new sophorolipids, AMB Express. 6 (2016). https://doi.org/10.1186/s13568-016-0199-7.

[36] E.I.P. Delbeke, O. Lozach, T. Le Gall, M. Berchel, T. Montier, P.A. Jaffrès, K.M. Van Geem,

C. V. Stevens, Evaluation of the transfection efficacies of quaternary ammonium salts prepared from sophorolipids, Org. Biomol. Chem. 14 (2016) 3744–3751. https://doi.org/10.1039/c6ob00241b.

[37] E.I.P. Delbeke, S.L.K.W. Roelants, N. Matthijs, B. Everaert, W. Soetaert, T. Coenye, K.M. Van Geem, C. V. Stevens, Sophorolipid Amine Oxide Production by a Combination of Fermentation Scale-up and Chemical Modification, Ind. Eng. Chem. Res. 55 (2016) 7273–7281. https://doi.org/10.1021/acs.iecr.6b00629.

[38] E.I.P. Delbeke, J. Everaert, O. Lozach, T. Le Gall, M. Berchel, T. Montier, P.A. Jaffrès, P. Rigole, T. Coenye, M. Brennich, N. Baccile, S.L.K.W. Roelants, W. Soetaert, I.N.A. Van Bogaert, K.M. Van Geem, C. V. Stevens, Synthesis and Biological Evaluation of Bolaamphiphilic Sophorolipids, ACS Sustain. Chem. Eng. 6 (2018) 8992–9005. https://doi.org/10.1021/acssuschemeng.8b01354.

[39] E.I.P. Delbeke, J. Everaert, O. Lozach, T. Le Gall, M. Berchel, T. Montier, P.A. Jaffrès, P. Rigole, T. Coenye, M. Brennich, N. Baccile, S.L.K.W. Roelants, W. Soetaert, I.N.A. Van Bogaert, K.M. Van Geem, C. V. Stevens, Lipid-Based Quaternary Ammonium Sophorolipid Amphiphiles with Antimicrobial and Transfection Activities, ChemSusChem. 12 (2019) 3642–3653. https://doi.org/10.1002/cssc.201900721.

[40] L. Speight, D. Hagaman, A. Morris, N. Callow, T. Dixon, C. Cherfan, Enhanced Sophorolipid Derivatives, (2022).

[41] J.K. Ogunjobi, C.R. McElroy, J.H. Clark, D. Thornthwaite, O.E. Omoruyi, T.J. Farmer, A class of surfactants: Via PEG modification of the oleate moiety of lactonic sophorolipids: Synthesis, characterisation and application, Green Chem. 23 (2021) 9906–9915.

https://doi.org/10.1039/d1gc02247d.