



Review Article

Simple glycolipids of microbes: Chemistry, biological activity and metabolic engineering



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ABSTRACT

Glycosylated lipids (GLs) are added-value lipid derivatives of great potential. Besides their interesting surface activities that qualify many of them to act as excellent ecological detergents, they have diverse biological activities with promising biomedical and cosmeceutical applications. Glycolipids, especially those of microbial origin, have interesting antimicrobial, anticancer, antiparasitic as well as immunomodulatory activities. Nonetheless, GLs are hardly accessing the market because of their high cost of production. We believe that experience of metabolic engineering (ME) of microbial lipids for biofuel production can now be harnessed towards a successful synthesis of microbial GLs for biomedical and other applications. This review presents chemical groups of bacterial and fungal GLs, their biological activities, their general biosynthetic pathways and an insight on ME strategies for their production.

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1. Introduction

Lipid biotechnology research has focused to date on developing sustainable alternatives to depleting fossil fuels. One strategy was plant-derived fuel, biodiesel [1]. A main drawback of this approach

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is that oil and land allocated for biodiesel production compete with those allocated for human food consumption. Moreover, replacement of natural vegetations with plants used for biodiesel production generates long-term environmental concerns. Another strategy is to use lipids originating from microbes, called “single cell oil” (SCO) as substrates for biodiesel production. We believe that accumulating knowledge and developed biomolecular tools obtained from lipid engineering of oleogenic microbes can now be harnessed for the microbial production of lipid derivatives of added-value.

Glycosylation of organic molecules, including lipids, usually leads to derivatives of new and/or better physicochemical properties and biological activities [2,3] that reflect in higher market prices. Metabolic engineering of lipid derivatives has previously investigated polyunsaturated fatty acids [4] and fatty acid derivatives that are used as substrates for oleochemical industries, e.g. heterologous production of ricinoleic acids by *Y. lipolytica* [5]. Other added-value lipid derivatives of commercial interest include wax esters, polyhydroxyalkanoates (bioplastics), hydroxylated fatty acids, carotenoids, polyenic polymers [6] and glycolipids.

This review focuses on simple glycolipids (SGLs) as an important family of glycolipids (GLs) class. The importance of SGLs stems from the fact that this family of GLs comprises a wide range of bioactive molecules with potential biomedical, pharmaceutical and cosmetic applications [7,8]. Nonetheless, many simple GLs are limited commercially because of their still low yield and high cost of production, particularly of high purity simple GLs aimed for biopharmaceutical purposes.

We present the chemical groups of simple GLs, their microbial producers and their biological activities. Then, we describe the key biosynthetic enzymes and metabolic precursors involved in biosynthesis of simple GLs. Finally, we discuss metabolic engineering strategies for simple GLs production in native and heterologous hosts.

2. Definition and classification of simple glycolipids

The term glycolipids (GLs), in general, encompasses a wide diversity of structurally heterogeneous biological compounds that are produced by microbes, plants, animals and humans [9]. As their names suggest, they are composed of glycosyl and lipid moieties. The IUPAC uses the term GLs to broadly designate any compound containing one or more monosaccharide residues bound by glycosidic linkage to a hydrophobic moiety [10]. Our definition of GLs is even broader to include glycoside and non-glycoside GLs in which the sugar and lipid residues are linked together via glycosidic (e.g. O- or N-glycosidic linkages) and non-glycosidic linkages (e.g. ester or amide linkages), respectively (Fig. 1). The glycosyl residue can be mono-, di-, oligo or polysaccharides (e.g. glucose, cellobiose or glycan, respectively), alcohol sugars/polyols (like mannitol, erythritol or arabinol, etc.), amino sugars (like desosamine, etc) or sugar acids (like glucuronic acids). The lipid residue of GLs ranges from fatty acids, fatty alcohols, fatty amino alcohols, polyketides, sterols, hopanoids and carotenoids with different substitutions, chain lengths, saturation levels, branching and di-/oligo-/polymerizations.

Numerous classifications exist for GLs [10], the most convenient of which is their classification into simple and complex GLs [11–13] (Fig. 1). Simple GLs (SGLs), sometimes called saccharolipids [14], are two-component (glycosyl and lipid moieties) GLs in which the glycosyl and lipid moieties are directly linked to each other. Complex glycolipids (CGLs) are, however, structurally more heterogeneous, as they contain, in addition to the glycosyl and lipid moieties, other residues like glycerol (glycoglycerolipids), peptide (glycopeptidolipids), acylated-sphingosine (glycosphingolipids), or

other residues (Fig. 1). Polysaccharide-containing GLs, although containing no residues other than glycosyl and lipid moieties, are classified under complex glycolipids because of the complex nature of their polysaccharide residues; however, oligosaccharide-containing GLs are classified as simple GLs [13] (Fig. 1). Simple glycolipids addressed in this review are those of natural microbial origin, therefore, SGLs of synthetic or other biological origins are not mentioned.

3. Surfactant properties of simple glycolipids

Simple glycolipids (SGLs) are amphiphilic molecules as they comprise both the hydrophilic glycosyl and the lipophilic lipid residues. This amphiphilic nature confers surfactant activity to most GLs; those of which with pronounced surfactant activity are called biosurfactant. Compared to petroleum-derived (e.g. alkylbenzene sulfonates) or plant-based (e.g. alkyl polyglycosides) synthetic surfactants [15], microbially-produced SGL biosurfactants are mostly of higher surface activity, higher emulsifying power, lower critical micelle concentrations, higher biodegradability (compared to petroleum-derived surfactants), lower ecotoxicity [16] and lower protein denaturing potency [17–19]. The advanced properties of microbial SGLs are suggested to be attributed to a peculiar mosaic distribution of regions of polarity over the GL molecule, as well as to their branched or sometimes circular structures compared to synthetic surfactants [18]. Moreover, most SGLs are naturally produced as complex mixtures of congeners or homologues that vary in the number of glycosyl units and extent of their acylation, the number of conjugate lipid chains, their lengths, the extent of unsaturations and substitutions; these factors together contribute to their unique surfactant properties and behaviors [18].

Although the unique surface properties of some SGLs qualified some of them to be marketed as ecological surfactants [20,21], yet, their competitiveness in the detergent market is limited because of their higher prices compared to alkyl polyglycosides synthetic surfactants which are at least 50% less expensive. For example, the estimated cost of large-scale production of the SGLs: sophorolipid and rhamnolipid biosurfactants, are about US\$ 2.5–3/Kg [21,22] and US\$ 5–20/Kg [23], respectively, compared to US\$ 1–3/Kg for the synthetic alkyl polyglycoside surfactants [23].

Aside from their surface activities, nearly all natural SGLs have interesting biological activities, as described later, that let them occupy market niches not approachable by synthetic surfactants [24]. Noteworthy, the biological activities of SGLs are thought to stem from their surface activities [25].

4. Chemical groups and origins of microbial simple glycolipids

Microbially produced SGLs are classified in chemical groups based on their chemical structures so that every group comprises SGLs members sharing unique glycosyl and/or lipid moieties for SGLs produced by bacteria (Table 1) and fungi (Table 2). In this classification, some SGLs congeners are classified in separate groups when they originate from different microbial origins and vice versa. Under each SGL group, exhaustive list of its members, together with their chemical names, their microbial producers as well as their taxonomic phyla is mentioned (Tables 1 and 2). Furthermore, the confirmed chemical structures of representative or prototypic members of each SGL group are presented (Fig. 3).

Based on our survey of microorganisms producing SGLs, we found that 50% of all known microbial SGLs are produced by microbes belonging to the phylum *Actinobacteria* (Fig. 2). Second in rank to *Actinobacteria*, comes phylum *Proteobacteria* followed by

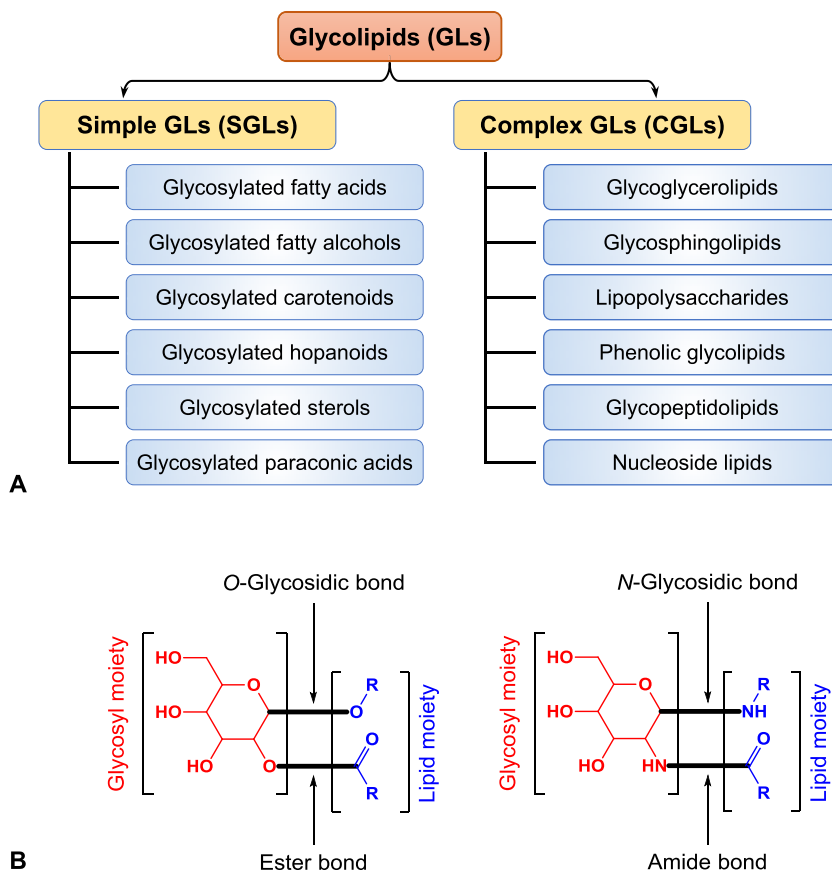


Fig. 1. Classification of glycolipids and main types of linkages between their glycosyl and lipid residues. (A) Simple glycolipids (SGLs) comprise glycolipids consisting of glycosyl and lipid residues only, whereas, complex glycolipids (CGLs) contain glycerol, ceramide, phenolic, peptide, nucleoside or polysaccharide residues in addition to the glycosyl and lipid residues. (B) Glycosyl and lipid residues are mainly linked via O-glycosidic and/or ester bonds, and less frequently via N-glycosidic and/or amide bonds.

the two major fungal phyla, *Ascomycota* and *Basidiomycota*, consecutively (Fig. 2).

4.1. Bacterial simple glycolipids

Overall, bacterially produced simple glycolipids (SGLs) outnumbered fungally-produced ones (Fig. 2). A previous survey of about 16000 pooled natural bacterial metabolites revealed that about 20% of them are glycosylated, about 30% of these glycosylated metabolites are glycosylated lipids of which glycosylated macro-lactones/-lactams take a share of about 20% and other glycosylated lipids (including SGLs) take a share of 10% [26]. Nearly all glycosylated macro-lactones/-lactams are produced by members of the phylum *Actinobacteria*. We classified bacterially produced SGLs in 10 groups (Table 1).

4.2. Fungal simple glycolipids

Fungally produced simple glycolipids (SGLs) are less numerous than bacterially-produced ones (Fig. 2). Fungal SGLs are classified in 10 groups that are mainly produced by members of the phyla *Ascomycota* and *Basidiomycota* (Table 2).

5. Physiological roles of simple glycolipids

For most SGLs, the exact physiological roles to their native producers are not clearly known. Generally, SGLs are secondary metabolites that are not essential for cell viability. Nonetheless,

given their antimicrobial properties, SGLs are suggested to help producing organism dominate environmental niches by inhibiting the growth of other organisms [153]. In addition, SGLs are required to coordinate multicellular or group behaviors (biofilm formation and swarming) and enhance growth of producing organisms on hydrophobic carbon sources [154–156]. Some additional roles are assigned to specific SGLs like rhamnolipids, which are considered as virulence factors that modulate host immune response [29]. Similarly to their unglycosylated counterparts, glycosylated carotenoids are postulated to act as photoprotectants and antioxidants to protect organisms from injuries caused by free radicals and active oxygen species [106]. In thermophiles, glycocarotenoids are thought to stabilize and reinforce cell membranes [113]. Hopanoids are sterol analogues in bacteria. Similarly to sterol in eukaryotes, hopanoids and their glycosylated derivatives are thought to help stabilize and regulate membrane fluidity and permeability particularly during shifts in pH and other physicochemical conditions [117,157]. Sophorolipids are suggested to act as extracellular forms of carbon storage that can be recycled later under starvation conditions [154].

6. Bioactivities of simple glycolipids

Simple glycolipids (SGLs) have very interesting biological activities on other organisms ranging from viruses to human cells. Although the mechanism of these bioactivities is not definitively known, it is suggested that most of SGLs bioactivities arise from their surface activities. Collectively, many of them have antiviral,

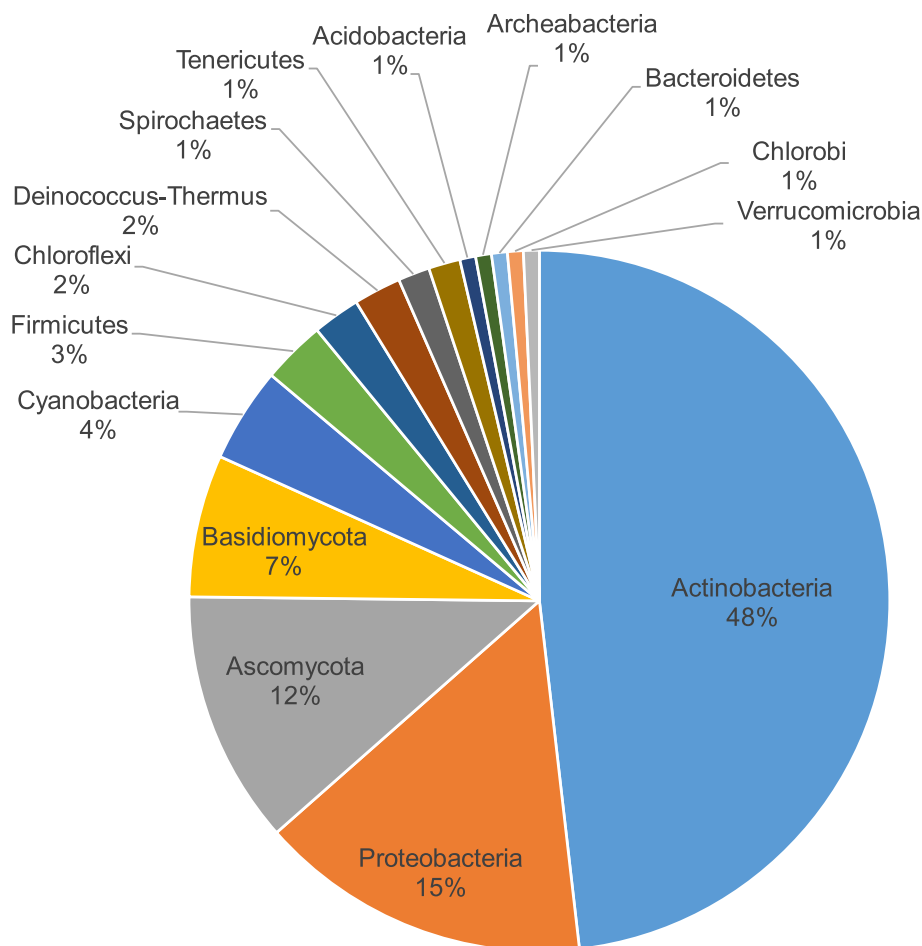


Fig. 2. Approximate distribution of microbial producers of simple glycolipids in different bacterial and fungal phyla. Incidences of microbial production of chemically unique SGLs in every microbial phylum were counted. Homologues or stereoisomers of the same chemically unique SGLs did not add into these calculations to avoid false overestimations. As an example, rhamnolipids (RLs) exist in two unique structures known so far, mono-rhamnolipids and di-rhamnolipids containing one and two rhamnose moieties, respectively, and are produced by proteobacterial species. Although these two RL congeners has several homologues varying in chain length of their lipid moiety, they were counted as two chemically unique SGLs in our calculations. Only the phylum of the microbial producer and not its genus and species identity that was taken into account; for example, although di-rhamnolipids are produced by different species of the genus *Pseudomonas* and *Burkholderia*, all di-rhamnolipids scored one hit in our calculations because all these di-rhamnolipids producers belong to the same phylum, *Proteobacteria*.

antimicrobial, anti-inflammatory and anticancer activities (Table 3). Many reviews are found in literature detailing the potential biomedical and cosmeceutical applications of biosurfactants in general, many of which are simple glycolipids [8,158–160].

7. Biosynthesis of simple glycolipids

With few exceptions, the exact biosynthetic steps of majority of simple glycolipids (SGLs) are not yet fully understood. Generally however, biosynthesis of SGLs implicates the supply and linking of glycosyl and lipid precursors. Pathways supplying glycolipid precursors are depicted later (Fig. 5) and are thought to play an important role in regulation of SGLs biosynthesis. Linking of glycosyl and lipid precursors is mostly via *O*-glycosidic or ester bonds (Fig. 1B) that are formed by glycosyltransferases (GT) (Fig. 4 B1, B2) [215] or acyltransferases (AT) [216] (Fig. 4 A1, A2), respectively. Glycosyltransferases catalyze the transfer of the sugar moiety from an activated glycosyl donor, usually sugar-nucleotide (Leloir GTs) or γ -phosphate (non-Leloir GTs), to a lipid acceptor (or a sugar acceptor for extending the sugar backbone of glycolipids), by making glycosidic bonds between the hydroxyl groups (nucleophile) of the acceptor and the anomeric carbon of the sugar donor (Fig. 4 B1, B2) [215]. Acyltransferases (AT) catalyze the transfer of

the lipid moiety from an activated acyl donor, mostly acyl-CoA or -ACP, to a glycosyl acceptor (or a lipid acceptor for extending the lipid backbone of the glycolipid) by making an ester bond between the hydroxyl group (nucleophile) of the acceptor and the acyl donor's carbonyl group [216] (Fig. 4 A1, A2).

Concerning the fate of SGLs, one report showed that the floculosin GL can be degraded by its producing yeast, *Pseudozyma flocculosa*, which feeds on it under nutrient limitations [153]. Glycolipids could theoretically be hydrolyzed by one or more of the following enzymes. First, glycoside hydrolases (GH) that hydrolyze the sugar-sugar or sugar-lipid glycosidic bonds (Fig. 4 B1, B2) [153,217]. Second, carbohydrate esterases (CE) hydrolyze the sugar-lipid ester bonds (Fig. 4 A2). Lipid esterases (LE), also known as lipases, hydrolyze lipid-lipid ester bonds (Fig. 4 A1) in glycolipids with multimeric hydroxyalkanoate lipid moieties e.g. rhamnolipids (Fig. 3). This hypothesis is corroborated by reports showing the hydrolysis of polymeric hydroxyalkanoates, which share the same lipid moieties as rhamnolipids, by the action of microbial lipases/esterases [218–220]. Nonetheless, the metabolic fate of SGLs is one of the subjects that require thorough investigations.

Among the poorly studied aspects in SGLs metabolism also are the transport SGLs across microbial membranes. Some SGLs require active transport for their exportation out of the cell, like cellobiose

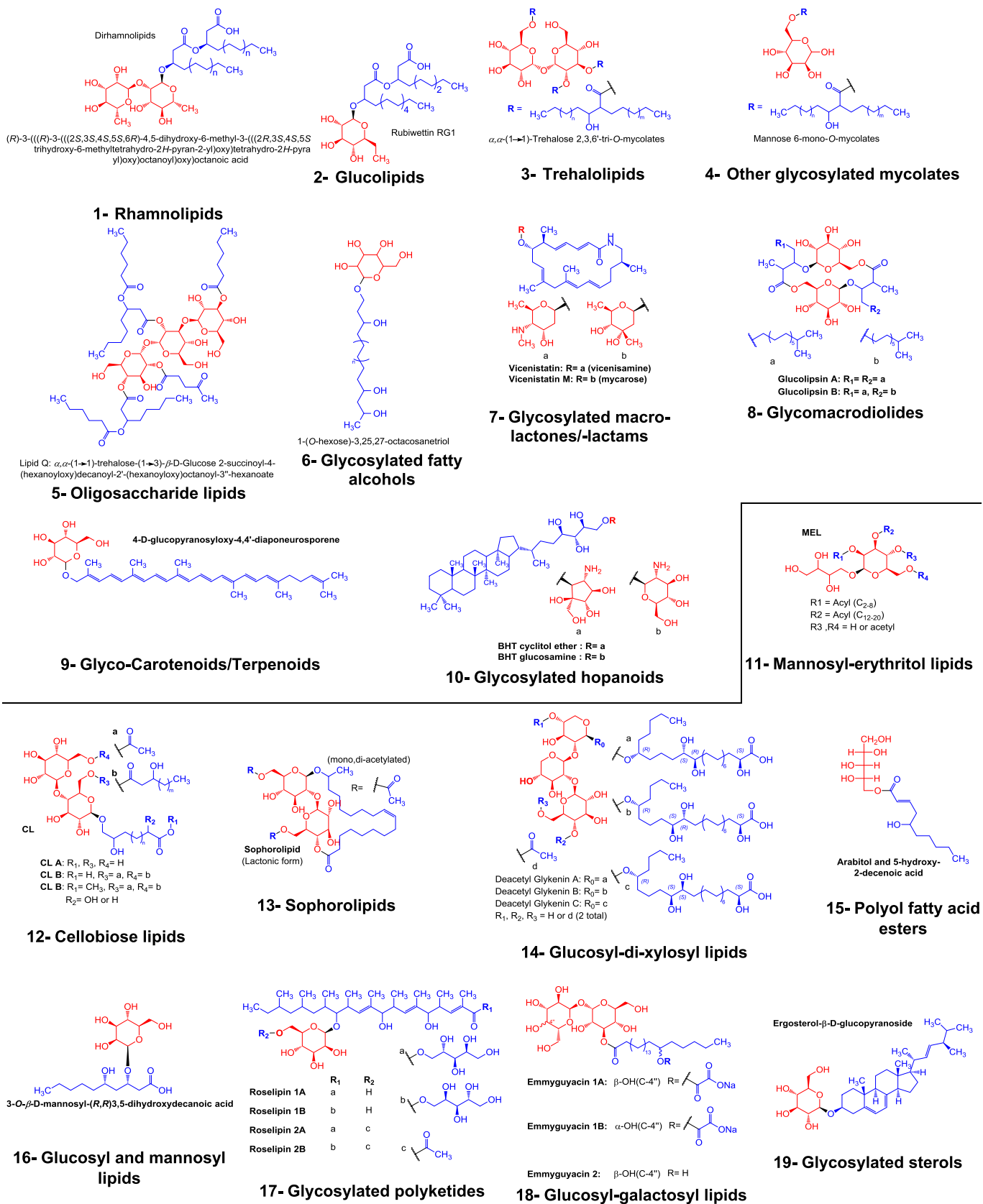


Fig. 3. Structures of prototypic members of bacterial and fungal simple glycolipid (SGL) groups. The glycosyl and lipid residues are colored in red and blue, respectively. Bacterial and fungal SGLs are represented in the upper and lower halves (separated by a line) of the figure, respectively. The representative structure of fungally produced glycosylated paraconic acids (20th group of SGLs) is not given as their structures have been debated [27,28].

Table 1
Chemical groups and members of bacterial simple glycolipids as well as names and phyla of native producers.

Common name: Chemical names (C _x : chain length of fatty acid chains)	Producer	Phylum
Bacteria		
1- Rhamnolipids		
Monorhamnolipids: α -L-rhamnopyranosyl-R,R-3-(3'-hydroxyalkanoyloxy)alkanoate (C ₈₋₁₆)	Spp. of <i>Pseudomonas</i> and <i>Burkholderia</i> [29]	Proteobacteria
Dirhamnolipids: α -L-rhamnopyranosyl-(1-2)- α -L-rhamnopyranosyl-R,R-3-(3'-hydroxyalkanoyloxy)alkanoate (C ₈₋₁₆)	Spp. of <i>Pseudomonas</i> and <i>Burkholderia</i> [29]	Proteobacteria
2- Glucolipids		
Rubiwettin RG1: β -D-glucopyranosyl 3-(3'-hydroxytetradecanoyloxy)decanoate	<i>Serratia rubidaea</i> [30].	Proteobacteria
3- Trehalolipids		
α,α -(1-1)-Trehalose 6-mono-O-mycolates	<i>Rhodococcus erythropolis</i> [31]	Actinobacteria
α,α -(1-1)-Trehalose 2,3-di-O-mycolates	<i>Tsakumurella</i> sp. [32]	Actinobacteria
Cord factor: α,α -(1-1)-Trehalose 6,6'-di-O-mycolates	Spp. of <i>Mycobacterium</i> , <i>Rhodococcus</i> , <i>Arthrobacter</i> , <i>Nocardia</i> and <i>Gordonia</i> [31,33]	Actinobacteria
α,α -(1-1)-Trehalose 2,3,6'-tri-O-mycolates	<i>Rhodococcus aurantiacus</i> [34]	Actinobacteria
STL-1, α,α -(1-1)-Trehalose 2,2'-di-O-succinoyl-3,4-di-O-alkanoates	<i>Rhodococcus erythropolis</i> [35]	Actinobacteria
STL-2, α,α -(1-1)-Trehalose 2,3,4-mono-O-succinoyl-di-O-alkanoates	<i>Rhodococcus erythropolis</i> [35]	Actinobacteria
STL-3, α,α -(1-1)-Trehalose 2,3,4,2'-mono-O-succinoyl-tri-O-alkanoates	Spp. of <i>Rhodococcus</i> [36,37], <i>Arthrobacter</i> [38]	Actinobacteria
4- Other glycosylated (non-trehalose containing) mycolates		
Sucrose 6-mono-O-mycolates	Spp. of <i>Arthrobacter</i> , <i>Corynebacterium</i> , <i>Nocardia</i> , <i>Brevibacterium</i> [39,40]	Actinobacteria
Fructose 6-mono-O-mycolates	Spp. of <i>Arthrobacter</i> , <i>Corynebacterium</i> , <i>Nocardia</i> , <i>Brevibacterium</i> [39,40]	Actinobacteria
Fructose 1,6-di-O-mycolates	Spp. of <i>Arthrobacter</i> , <i>Corynebacterium</i> , <i>Nocardia</i> , <i>Brevibacterium</i> [39,40]	Actinobacteria
Glucose-6- β -hydroxy- α -hexadecenoyl-eicosenoate	<i>Brevibacterium thiogenitalis</i> [41]	Actinobacteria
Mannose 6-mono-O-mycolates	<i>Arthrobacter</i> sp. [42]	Actinobacteria
Maltose 6-mono-O-mycolates	<i>Arthrobacter</i> sp. [42]	Actinobacteria
Maltose 6,6'-di-O-mycolates	<i>Arthrobacter</i> sp. [42]	Actinobacteria
Maltotriose 6,6',6''-tri-O-mycolates	<i>Arthrobacter</i> sp. [42]	Actinobacteria
Cellobiose 6-mono-O-mycolates	<i>Arthrobacter</i> sp. [42]	Actinobacteria
5- Trehalose-containing Oligosaccharide lipids		
Lipid Q: β -D-glucose-(1-3)- α,α -(1-1)-trehalose hexanoyl-succinoyl-3-(hexanoyloxy)octanoate-3-(hexanoyloxy)decanoate	<i>Rhodococcus</i> sp. [43,44]	Actinobacteria
GL2: β -D-glucose-(1''-2')- α,α -(1-1)-trehalose 4,6,2'',3'' tetra-O-alkanoates (C ₈₋₁₀)	<i>Tsakumurella</i> sp. [32]	Actinobacteria
GL3: β -D-glucose-(1''-2')- α,α -(1-1)-trehalose-(6'-1''')- β -D-galactose-4,6,2'',3''-tetra-O-alkanoates (C ₈₋₁₀)	<i>Tsakumurella</i> sp. [32]	Actinobacteria
β -D-glucose-(1-3)- α,α -(1-1)-trehalose-(6-1)- β -D-glucose-(6-1)- β -D-glucose mono-O-succinoyl-hepta-O-alkanoate (C ₂₋₈)	<i>Nocardia corynebacteroides</i> [45–47]	Actinobacteria
4,6-(1-Carboxyethylidene)-3-O-Me- β -D-glucose-(1-3)-4,6-(1-carboxyethylidene)- β -D-glucose-(1-4)- β -D-glucose-(1-6)- α,α -(1''-1')-trehalose-4''-O-alkanoyl-6'-O-alkenoate	<i>Mycobacterium smegmatis</i> [48,49]	Actinobacteria
6- Glycosylated fatty alcohols		
Alkane 1,2-diol glycoside; Hexose 1-(O-hexose)alk-2-yl alkanoate (Diol = C ₁₉₋₂₀ , alkanoate = C ₁₄₋₁₆)	<i>Roseiflexus castenholzii</i> [50]	Chloroflexi
1-(O-hexose)-3,25-hexacosanediol and its homologue: 1-(O-hexose)-3,27-octacosanediol	Spp. of cyanobacteria e.g. <i>Anabaena</i> , <i>Nodularia</i> , <i>Calothrix</i> , <i>Synechococcus</i> [51]	Cyanobacteria
1-(O-hexose)-3-keto-25-hexacosanol and its homologue: 1-(O-hexose)-3-keto-27-octacosanol		Cyanobacteria
1-(O-hexose)-3,25,27-octacosanetriol		Cyanobacteria
1-(O-hexose)-3-keto-25,27-octacosanediol OR its isomer: 1-(O-hexose)-27-keto-3,25-octacosanediol		Cyanobacteria
7- Glycosylated macro-lactones/-lactams		
Barsilolinide A/B/C: 2-deoxy- α -L-fucopyranoside of C ₃₂ -membered macrolactone	<i>Nocardia brasiliensis</i> [52,53]	Actinobacteria
Fluvirucins: amino sugar glycosides of C ₁₄ -membered macrolactam	Spp. of <i>Actinomadura</i> , <i>Streptomyces</i> , <i>Microtetraspora</i> and <i>Saccharotrix mutabilis</i>	Actinobacteria
Vicenistatin: amino sugar (vicenisamine) glycoside of C ₂₀ -membered macrolactam	<i>Streptomyces</i> sp. [54,55]	Actinobacteria
Vicenistatin M: D-mycarose glycoside of C ₂₀ -membered macrolactam	<i>Streptomyces erythreus</i> and <i>Nocardia</i> spp. and other <i>Streptomyces</i> spp. [56]	Actinobacteria
Erythromycins A, B, D, C, E, F and Erythromycin esters (C ₁₄ -membered macrolactam glycosides)	<i>Streptomyces antibioticus</i> [57]	Actinobacteria
Oleandomycin (C ₁₄ -membered macrolactam glycosides)	<i>Streptomyces felleus</i> and <i>S. narbonensis</i> [56]	Actinobacteria
Pikromycin, Narbomycin, 5-O-mycaminosyl-narbonolide (C ₁₄ -membered macrolactam glycosides)		Actinobacteria
10,11-Dihydropikromycin, Kayamicin (C ₁₄ -membered macrolactam glycosides)	<i>Streptomyces narbonensis</i> [56]	Actinobacteria
Spinosyns (Tetracyclolide) containing forosamine (amino sugar) and tri-O-methyl rhamnose	<i>Saccharopolyspora spinosa</i> [56,58]	Actinobacteria
Lepicidin A	<i>Saccharopolyspora spinosa</i> [56]	Actinobacteria
Leucomycins, Josamycin, Platenomycins, Medicamycin, Espinomycins	<i>Streptomyces kitasatoensis</i> [56]	Actinobacteria
Carbomycin B, platenomycins W1/W2, Niddamycin, Midecamycin A3/A4	<i>Streptomyces platenis</i> [56]	Actinobacteria
Acumycin (cirramycin B), Cirramycin F and derivatives	<i>Streptomyces griseoflavus</i> , <i>S. fradiae</i> , <i>S. flocculus</i> [56]	Actinobacteria
Chalcomycin, Neutramycin	<i>Streptomyces bikiniensis</i> , <i>S. rimosus</i> , <i>S. hirsutus</i> [56]	Actinobacteria
Aldgamycin F, E and Swalpamycin	<i>Streptomyces lavendulae</i> , <i>S. avidinii</i> , <i>S. amandii</i> (for swalpamycin) [56]	Actinobacteria

Table 1 (continued)

Common name: Chemical names (C _x : chain length of fatty acid chains)	Producer	Phylum
Spiramicins	<i>Streptomyces ambofaciens</i> [56,59]	Actinobacteria
Tylosins	<i>Streptomyces fradiae</i> , <i>S. hygroscopicus</i> [56,60]	Actinobacteria
Concanamycins	<i>Streptomyces diastatochromogenes</i> [56]	Actinobacteria
Tetrins and related compounds, Maduralide	<i>Streptomyces</i> sp. [56]	Actinobacteria
Pimaricin	<i>Streptomyces natalensis</i> [56]	Actinobacteria
Colubricidin A	<i>Streptomyces</i> sp. [56,61]	Actinobacteria
Nystatin	<i>Streptomyces noursei</i> [62]	Actinobacteria
Amphotericin B	<i>Streptomyces nodosus</i> [63]	Actinobacteria
Oasomycins, Desertomycins	<i>Streptomyces cinnamoneus</i> (previously <i>Streptoverticillium baldacci</i>)	Actinobacteria
Rapamycin	<i>Streptomyces hygroscopicus</i> [64]	Actinobacteria
Avermectins	<i>Streptomyces avermectilis</i> [65]	Actinobacteria
PM100117 and PM100118	<i>Streptomyces caniferus</i>	Actinobacteria
8- Glycomacrolidolides (glycosylated macrocyclic dilactones)		
Glucolipin A, B: dilactone of two glucosides of 3-hydroxy fatty acids C ₁₉ /C ₁₉	<i>Streptomyces purpurogeniscleroticus</i> , <i>Nocardia vaccinii</i> [66]	Actinobacteria
Fattiviracin A1: dilactone of two glucosides of 3-,17-, ω-1-trihydroxy fatty acids C ₂₄ /C ₃₃	<i>Kibdelosporangium albatum</i> [67]	Actinobacteria
Cycloviracin B1 and B2: dilactones glucosides of 3-,19-, ω-1-trihydroxy fatty acids (C _{22–28}) and of 3-,17-, ω-1-trihydroxy fatty acids (C _{22–24})	<i>Streptomyces microflavus</i> [67]	Actinobacteria
Elaiophylins, Efomycin G	<i>Streptomyces</i> spp. [68]	Actinobacteria
Halichoblelides A, B, C	<i>Streptomyces</i> spp. [69–71]	Actinobacteria
Bispolides A1, A2, A3, B1, B2a, B2b and B3	<i>Microbispora species</i> [72]	Actinobacteria
Macroviracins A-D: related to fattiviracin and cycloviracins	<i>Streptomyces</i> sp. [73]	Actinobacteria
9- Glyco-carotenoids/-terpenoids:		
9.1-Acyclic glyco-carotenoids		
Rhodopsin glucoside	<i>Halorhodospira abdelmalekii</i> , <i>H. halochloris</i> [74]	Proteobacteria
Dihydroxylycopene mono-/di-glucosides and their acyl (C _{12:0} or C _{14:1}) derivatives	<i>Halorhodospira abdelmalekii</i> , <i>H. halochloris</i> [74]	Proteobacteria
D-Glucosyl 4,4'-diapocarotene-6,6'-dioic acid	<i>Pseudomonas rhodos</i> [75], <i>Rhizobium lupini</i> [76,77]	Proteobacteria
1'-glucosyloxy-3',4'-didehydro-1',2'-dihydro-ψ,ψ-carotene monoester	<i>Chondromyces apiculatus</i> [78], <i>Myxococcus fulvus</i> [79]	Proteobacteria
Staphyloxanthin: α-D-glucopyranosyl 1-O-(4,4'-diaponeurosporen-4-olate) 6-O-(12-methyltetradecanoate)	<i>Staphylococcus</i> spp. [80]	Firmicutes
4-D-glucopyranosyloxy-4,4'-diaponeurosporene	<i>Streptococcus faecium</i> [81]	Firmicutes
Hydroxy-diaponeurosporene glucoside esters	<i>Heliorestis</i> sp. [82]	Firmicutes
Rhodopin β-D-glucoside, Rhodopinal β-D-glucoside	<i>Rhodopseudomonas acidophila</i> , <i>Rhodospirillum tenue</i> and <i>Rhodocyclus purpureus</i> [83]	Proteobacteria
Oscillaxanthin: 1,1'-dihydroxy-2,2'-di-β-L-rhamnosyl- 1,2,1',2'-tetrahydro-3,4,3',4'-tetrahydrolycopene	<i>Oscillatoria rubescens</i> [84]	Cyanobacteria
Bacterioruberin mono- and di-glycosides	<i>Unidentified Halophilic bacterium</i> [85]	Proteobacteria
Diapolyopenedioic acid xylosyl esters A, B, and C	<i>Rubritalea squalenifaciens</i> [86]	Verrucomicrobia
Methyl 5-glucosyl-5,6-dihydro-apo-4,4'-lycopenoate	<i>Planococcus maritimus</i> [87]	Firmicutes
Vancoresmycin	<i>Amycolatopsis</i> [88]	Actinobacteria
9.2-Monocyclic glyco-carotenoids		
Salinixanthin	<i>Salinibacter ruber</i> [89], <i>Rhodothermus marinus</i> [90]	Bacteroidetes
Phleixanthophyll, 4-ketophleixanthophyll	<i>Mycobacterium phlei</i> [91]	Actinobacteria
Phleixanthophyll palmitate: (2'-S)-1'-[(6-O-palmityl-β-D-glucopyranosyl)oxy]-3',4'-didehydro-1',2'-dihydro-β,ψ-caroten-2'-ol	<i>Nocardia</i> sp. [92]	Actinobacteria
1'-[(6-O-acyl-β-D-glucopyranosyl)oxy]-1',2'-dihydro-β,ψ-caroten-4-one	<i>Rhodococcus rhodochrous</i> [93,94]	Actinobacteria
Myxobactone	<i>Myxococcus fulvus</i> [79,95]	Proteobacteria
Myxobactin	<i>Myxococcus fulvus</i> [96]	Proteobacteria
Keto-myxocoxanthin glucoside ester (Myxobactone ester)	<i>Roseiflexus castenholzii</i> [97]	Chloroflexi
OH-γ-carotene glucoside laurate: 1'-[(6-O-lauryl-β-D-glucopyranosyl)oxy]-1',2'-dihydro-β,ψ-carotene	<i>Chlorobium tepidum</i> [98]	Chlorobi
OH-chlorobactene glucoside laurate; 1'-[(6-O-lauryl-β-D-glucopyranosyl)oxy]-1',2'-dihydro-φ,ψ-carotene	<i>Chlorobium tepidum</i> [98]	Chlorobi
OH-γ-carotene glucoside ester derivative	<i>Chloroflexus aurantiacus</i> [99]	Chloroflexi
1'-β-glucopyranosyl-3,4,3',4'-tetrahydro-1',2'-dihydro-β,ψ-caroten-2-one	<i>Meiothermus ruber</i> [100]	Deinococcus-Thermus
Myxoxanthophyll like glyco-carotenoid: (3R,2'S)-myxol-2'-(2,4-di-O-methyl-α-L-fucoside)	<i>Synechocystis</i> sp. [101]	Cyanobacteria
Sioxanthin; (2'S)-1'-β-D-glucopyranosyloxy-3',4'-didehydro-1',2'-dihydro-φ,ψ-caroten-2'-ol	<i>Salinispora</i> sp. [102]	Actinobacteria
9.3-Bicyclic glyco-carotenoids		
Corynexanthin monoglycoside	<i>Corynebacterium</i> sp. [103]	Actinobacteria
Corynexanthin diglycoside	<i>Arthrobacter</i> sp [104]	Actinobacteria
Sarcixanthin monoglycosides	<i>Curtobacterium flaccumfaciens</i> [105], <i>Micrococcus luteus</i> [106], <i>M. yunnanensis</i> [107].	Actinobacteria
Sarcixanthin diglucosides	<i>Micrococcus luteus</i> [106], <i>M. yunnanensis</i> [107].	Actinobacteria
Zeaxanthin mono- and di-glucosides	<i>Erwinia herbicola</i> , <i>Rhodobacter sphaeroides</i> [108]	Proteobacteria
Zeaxanthin mono- and di-rhamnosides (mainly Z-isomers), Zeaxanthin diglucoside	<i>Sulfolobus shibatae</i> [109]	Archaeobacteria
Zeaxanthin mono- and di-rhamnosides	<i>Corynebacterium autotrophicum</i> (<i>Xanthobacter autotrophicus</i>) [110]	Proteobacteria
Aastaxanthin dirhamnoside	<i>Sphingomonas astaxanthinifaciens</i> [111]	Proteobacteria
Myxocoxanthin rhamnoside	<i>Sorangium compositum</i> [112]	Proteobacteria

(continued on next page)

Table 1 (continued)

Common name: Chemical names (C _x : chain length of fatty acid chains)	Producer	Phylum
Thermozeaxanthin-13, -15, and -17 (Zeaxanthin mono- β -D-glucoside-branched fatty acid esters)	<i>Thermus thermophilus</i> [113]	Deinococcus-Thermus
Thermobiszeaxanthin-13-13, -13-15, and -15-15 (Zeaxanthin di- β -D-glucoside-branched fatty acid esters)	<i>Thermus thermophilus</i> [113]	Deinococcus-Thermus
Adonixanthin and astaxanthin glucosides	<i>Agrobacterium aurantiacum</i> [114,115]	Proteobacteria
Decaprenoxanthin mono- and diglucoside (2R,6S,2'R,6'S)-(2,2'-bis(4-hydroxy-3-methyl-2-butenyl) _{ε,ε} -carotene) di- β -D-glucoside	<i>Corynebacterium glutamicum</i> [116]	Actinobacteria
10- Glycosylated hopanoids/sterols		
Bacteriohopanetetrol cyclitol ether	<i>Chloracidobacterium thermophilum</i> [117,118]	Acidobacteria
BHT cyclitol	<i>Burkholderia cenocepacia</i> [119], <i>Zymomonas mobilis</i> [120]	Proteobacteria
BHT glucosamine	<i>Burkholderia cenocepacia</i> [119], <i>Zymomonas mobilis</i> [120,121]	Proteobacteria
O- α -D-Glucuronopyranosyl BHT	<i>Rhodospirillum rubrum</i> [122]	Proteobacteria
Cholesteryl- α -D-glucopyranoside	<i>Helicobacter pylori</i> [123]	Proteobacteria
Cholesteryl-6-O-tetradecanoyl- α -D-glucopyranoside, Cholesteryl-6-O-dodecanoyl- α -D-glucopyranoside	<i>Helicobacter pylori</i> [123], <i>H. felis</i> , <i>H. muridarum</i> , <i>H. mustelae</i> , <i>H. fennelliae</i> , and <i>H. cinaedi</i> [124]	Proteobacteria
Cholesteryl-6-O-acyl- β -D-galactopyranoside	<i>Borrelia burgdorferi</i> , <i>B. garinii</i> and <i>B. afzelii</i> [125]	Spirochaetes
Cholesteryl-6-O-acyl- β -D-glucopyranoside	<i>Borrelia hermsii</i> [125]	Spirochaetes
Cholesteryl- β -D-glucopyranoside and its 3,4,6-triacyl derivatives	<i>Mycoplasma gallinarum</i> [126]	Tenericutes
Cholesteryl- α,α' -di-D-glucoside; α -D-glucopyranosyl-(1 \rightarrow 3)-(O-acyl)- α -D-glucopyranosyl-(1 \rightarrow 3)-cholesterol	<i>Acholeplasma axanthum</i> [127]	Tenericutes

lipids, mannosylerythritol lipids [221] and sophorolipids [222], whereas, many other SGLs are thought to passively diffuse out of the cell.

To give a general overview, we present the general biosynthetic map of SGLs showing the diversity of the immediate glycosyl and lipid precursors of SGLs and the pathways furnishing them (Fig. 5).

8. Metabolic engineering of simple glycolipids

Metabolic engineering can be employed to satisfy demand for simple glycolipids (SGLs) by offering solutions to the main challenges facing their production and commercialization. The most important challenge is the high cost of production of SGLs at high purities to qualify for medical or cosmeceutical applications (usually >90–95% purities are required) [21,22]. This high cost stems from a multiplicity of factors including the inherent low yield/productivity of microbial SGLs, costly raw nutritive materials, expensive biosafety containment measures when using pathogenic SGLs producers, expensive/laborious foam control and expensive downstream processing and purification. Furthermore, SGLs are in many cases naturally produced as mixture of homologues/congeners that are difficult to separate; this makes the study and attribution of a specific activity to a specific SGL homologue/congener unattainable. Lastly, there is accumulating evidence that SGLs biosynthesis is tightly regulated in native producers e.g. rhamnolipids production in *Pseudomonas aeruginosa* [226] and sophorolipids production in *Starmerella bombicola* [222]. These tight genetic and metabolic regulations possibly explain the limited improvement in SGL yields using simple optimization media components and process conditions in native SGL producers. One should not be misled, however, by the extraordinarily high GL yields reported in literature that are obtained through media optimization, particularly for rhamnolipids [231]. Such reports are questionable due to different quantification methods used that vary in their specificity and/or sensitivity. Standardized protocols for SGLs quantification were made recently available [232,233] and are expected to profoundly minimize discrepancies in quantification values in glycolipid research.

Although their cost-effectiveness is still unclear, chemical synthesis of GL could overcome many of the problems of SGLs production. Nonetheless, chemical synthesis of SGLs is confronted also by many other limitations and concerns. First, the difficult

stereoselective synthesis of glycolipids which are mostly chiral molecules. An attempt to chemically synthesize mono-rhamnolipid, that is naturally produced as α -L-rhamnopyranosyl-R- β -hydroxydecanoyl-R- β -hydroxydecanoate, resulted in the inevitable co-production of three other diastereomers with different configurations of the β -hydroxyl groups (R,R; R,S; S,S; S,R) and different surface activities [234–236]. Second, certain ecological/health issues are associated with synthetic approaches that most probably involve the use of non-sustainable petrochemical substrates and generate toxic waste products [237]. Thirdly, the biodegradability and toxicity issues of co-produced new-to-nature SGLs diastereomers require attention and investigation.

Genetic engineering and synthetic biology could offer promising ecological solutions to current challenges facing SGLs production, particularly after recent advances in metabolic engineering and tools for cloning and heterologous expression of large biosynthetic pathways. The following sections discuss some of the metabolic engineering strategies for SGLs production.

8.1. Engineering heterotrophic carbon source utilization

Raw nutritive materials accounts for approximately more than 85% of the total estimated production/operation costs of SGLs [21]. A wide range of low-cost renewable raw materials were suggested for SGLs production [238,239], yet, the capacity of GL producers to utilize these raw materials should be investigated or genetically engineered in the selected production host. A successful example of the latter is the engineering of *P. aeruginosa* strain to utilize whey waste for RLS production via heterologous expression of *E. coli lac* genes [240]. Likewise, bacterial and fungal GL producers could be engineered to utilize cheap waste lignocellulosic wastes [241–243]. Although enhancing the utilization of waste oils by expression of lipid esterases seems a good strategy given the low cost and high GL yields [244,245] associated with these oily carbon sources, these carbon sources are, however, cumbersome during recovery of glycolipids as they necessitate extra steps for their removal adding to the net cost of glycolipids recovery [246].

8.2. Heterologous expression of GL biosynthetic pathway

Containment of biosafety level 2 organisms contribute remarkably in the operational costs of simple glycolipids (SGLs)

Table 2

Chemical groups and members of fungal simple glycolipids as well as names and phyla of their native producers.

Common name: Chemical names (C _x : chain length of fatty acid chains)	Producer	Phylum
Fungi		
1- Mannosyl-erythritol lipids (MEL, Ustilipids) and MEL congeners		
MEL	<i>Ustilago maydis</i> , <i>Pseudozyma</i> (<i>Candida</i>) <i>antarctica</i> [128,129], <i>Kurtzmanomyces</i> [130]	Basidiomycota
MEL	<i>Geotrichum candidum</i> [131]	Ascomycota
Mannosylmannitol lipids (MML), mannosylribitol lipids (MRL) and mannosylarabitol lipids (MAL)	<i>Pseudozyma parantarctica</i> [132]	Basidiomycota
2- Cellobiose lipids (CL, Ustilagic acids)		
Cellobiose (β -D-Glc-(1 \rightarrow 4)- β -D-Glc) 2''-O-hexanoic acid 1-O-16- ω , ω -1-dihydroxyhexadecanoate or 1-O-16- ω , ω -1, α -trihydroxy hexadecanoate	<i>Ustilago maydis</i> [128]	Basidiomycota
Cellobiose 6'-O-acetyl-2''-O- β -hydroxyalkanoyl-1-O-16- ω , ω -1-dihydroxyhexadecanoate or 1-O-16- ω , ω -1, α -trihydroxy hexadecanoate methyl ester	<i>Ustilago maydis</i> [128], <i>Pseudozyma fusiformata</i> [133]	Basidiomycota
Cellobiose 16-O- ω , ω -1-dihydroxyhexadecanoate or 1-O-16- ω , ω -1, α -trihydroxy hexadecanoate	<i>Symptodiomyopsis paphiopedili</i> [134]	Basidiomycota
Microcin: cellobiose 2'',3'',4'',6'',6'-penta-O-acetyl-1-O-16- ω , α -dihydroxyhexadecanoate	<i>Cryptococcus humicola</i> [135]	Basidiomycota
Flocculosin: 2-(2',4'-diacetoxy-5'-carboxy-pentanoyl) octadecyl cellobioside	<i>Anthracoystis</i> (<i>Pseudozyma</i>) <i>focculosa</i> [136]	Basidiomycota
3- Sophorolipids		
Sophorose (β -D-Glc-(1 \rightarrow 2)- β -D-Glc) -1-O-16- ω , ω -1-dihydroxyalkanoate or 1-O-16- ω , ω -1, α -trihydroxy alkananoate (C ₁₆ -C ₁₈ :0-2)	<i>Starmerella</i> (<i>Candida</i>) <i>bombicola</i> , <i>Candida apicola</i> and other spp. [137]	Ascomycota
Sophorose (β -D-Glc-(1 \rightarrow 2)- β -D-Glc) -1-O-16- ω , ω -1-dihydroxyalkanoate or 1-O-16- ω , ω -1, α -trihydroxy alkananoate (C ₁₆ -C ₁₈ :0-2)	<i>Cryptococcus curvatus</i> [137]	Basidiomycota
Sophorose 6'-mono-O-acetyl or 6',6'-di-O-acetyl -1-O-16- ω , ω -1-dihydroxyalkanoate or 1-O-16- ω , ω -1, α -trihydroxy alkananoate (C ₁₆ -C ₂₀ :1)	<i>Starmerella</i> (<i>Candida</i>) <i>bombicola</i> , <i>Candida apicola</i> and other spp. [137], <i>Wickerhamiella domercqiae</i> [138,139]	Ascomycota
Sophorose lipid lactonic/ring form, lactonization of free carboxyl group with C-4'' or C-6'' (intramolecular ester bonds)	<i>Starmerella</i> (<i>Candida</i>) <i>bombicola</i> , <i>Candida apicola</i> and other spp. [137]	Ascomycota
Dimeric and trimeric sophorolipids (intermolecular ester bonds between carboxyl of one molecule to C-4'' of another molecule)	<i>Candida</i> spp. [140]	Ascomycota
4- Glucosyl-di-xylosyl lipids (Glykenins)		
Glykenins A, B, C: O- β -D-glucose-(1 \rightarrow 2)-O- β -D-xylose-(1 \rightarrow 2)-O- β -D-xylose tetrahydroxyhexacosanoic acids, mono-di or tri-acetylated	Basidiomycetous sp. [141]	Basidiomycota
5- Polyol fatty acid esters (Liamocins and their congeners)		
Liamocins	<i>Aureobasidium pullulans</i>	Ascomycota
Mannitol and pentitol esters of 3-D-hydroxypalmitic and 3-D-hydroxystearic acids	<i>Rhodotorula glutinis</i> and <i>Rhodotorula graminis</i>	Basidiomycota
6- Glucosyl and mannosyl lipids		
Monoglucosyloxyoctadecenoic acid	<i>Aspergillus niger</i> [142]	Ascomycota
Halymecin B: mannosylated tetramer of 3,5-dihydroxydecanoic acid	<i>Fusarium</i> sp. [143]	Ascomycota
Halymecins F: acetylated halymecin B, halymecin G: mannosylated trimer of 3,5-dihydroxydecanoic acid	<i>Simplicillium lamellicola</i> [144]	Ascomycota
(3R,5R)-3-O- β -D-mannosyl-3,5-dihydroxydecanoic acid	<i>Simplicillium lamellicola</i> [144]	Ascomycota
7- Glycosylated polyketides		
Roselipin 1, 2: 2,4,6,8,10,12,14,16,18-nonamethyl-5,9,13-trihydroxy-2E,6E,10E-icosenoic acid mannosylated (+-acetylated) at C-13, D-arabitol ester [145,146]	<i>Gliocladium</i> [145,146]	Ascomycota
TMC-151 A ~ F: 2,4,6,8,10,12,14,16,18-nonamethyl-5,9,13-trihydroxy-2E,6E,10E-icosenoic acid mannosylated (+-acetylated) at C-13, D-mannitol ester [145,146]	<i>Gliocladium catenulatum</i> [147]	Ascomycota
TMC-154: isomeric form of roselipin 1 and TMC-171 A ~ C: as roselipin 3 but esterified to mannitol	<i>Gliocladium</i> [148]	Ascomycota
Roselipins 3A to 3E: 14,15-dehydro derivatives of roselipin 1A/B	<i>Clonostachys candelabrum</i> [149]	Ascomycota
Cladionol A: 15-mannosyl-2,4,6,8,10,12,14,16,18,20-decamethyl-3,7,11,15-tetrahydroxy-4E,8E,12E-docosenoic acid arabitol ester	<i>Gliocladium</i> [150]	Ascomycota
8- Glucosyl-galactosyl lipids		
Emmyguyacin 1A: α -D-glucopyranosyl- α -D-galactopyranose 3'-O-hydroxydocosanoate with 17-((carboxycarbonyl)oxy) group of oxalate ester at OH of C-17	<i>Fungal species</i> [151]	NA
Emmyguyacin 1B: Trehalose 3'-O-docosanoate with 17-((carboxycarbonyl)oxy) group of oxalate ester at OH of C-17	<i>Fungal species</i> [151]	NA
Emmyguyacin 2: as emmyguyacin 1A without the oxalate ester	<i>Fungal species</i> [151]	NA
9- Glycosylated sterols		
Ergosterol- β -D-glucopyranoside	<i>Pichia pastoris</i> , <i>Sordaria macrospora</i> , <i>Rhynchosporium secalis</i> [152]	Ascomycota
10- Glycosylated paraconic acids		
Gobienines A/B/C (non-confirmed structure [28])	<i>Acarospora gobiensis</i> (Lichen) [27]	Ascomycota

production. Moreover, working with pathogenic or opportunistic pathogens presents a health risk to manufacturing personnel as well as to public and environment. Heterologous expression of GL biosynthetic genes in hosts that are Generally Recognized As Safe (GRAS) is, therefore, a promising solution as it would require a less

costly biosafety level 1 manufacturing facility.

Heterologous expression of rhamnolipids (RLs) in non-pathogenic hosts has received much attention because of the large commercial potential of RLs and because the main and best RLs producer is the opportunistic pathogenic bacterium

Table 3
Biological activities of different chemical groups of microbial simple glycolipids.

Chemical group of simple glycolipids (SGLs)	A	B	C	D	E	F	G	H	I	J	K	L
	Antibacterial	Antifungal	Antiviral	Antiparasitic	Anticancer	↑ Cell differentiation ^a	Immunomodulatory	Antioxidant	Antiadherent (Biofilm, wounds)	Neuronal activity	Sperm immobilizing activity	↓ Diacylglycerol acyl transferase 2 ^a
Bacterial SGLs												
1 Rhamnolipids	A1	B1	C1	D1			G1		I1			
2 Glycolipids (Rubiwettin)												
3 Trehalolipids			C3		E3	F3	G3					
4 Other glycosylated mycolates												
5 Oligosaccharide lipids					E5							
6 Glycosylated fatty alcohols												
7 Glycosylated macro-lactones/-lactams	A7	B7	C7	D7	E7		G7					
8 Glycomacrodilolides	A8	B8	C8	D8	E8		G8					
9 Glyco-carotenoids/-terpenoids							G9	H9				
10 Glycosylated hopanoids												
Fungal SGLs												
11 Mannosyl-erythritol lipids	A11				E11	F11		H11		J11		
12 Cellobiose lipids	A12	B12										
13 Sophorolipids			C13			F13	G13		I13		K13	
14 Glucosyl-di-xylosyl lipids (Glykenins)	A14											
15 Polyol fatty acid esters	A15				E15							
16 Glucosyl and mannosyl lipids	A16				E16							
17 Glycosylated polyketides			C17	D17								L17
18 Glucosyl-galactosyl lipids			C18									
19 Glycosylated sterols												
20 Glycosylated paraconic acids												
References:												
A1: [23]			B8: [161,162]			D7: [58,61,65]			F3: [36,163,164]			H9: [165]
A7: [56]			B12: [135,166–168]			D8: [169,170]			F11: [171–175]			H11: [129]
A8: [72,176,177]			C1: [178]			D17: [149]			F13: [171]			I1: [179–181]
A11: [182]			C3: [183]			E3: [47,184]			G1: [185]			I13: [186,187]
A12: [188–190]			C7: [191]			E5: [32,47]			G3: [192,193]			J11: [131]
A14: [194]			C8: [56,73]			E7: [195]			G7: [53,64,196]			K13: [197]
A15: [198–200]			C13: [197]			E8: [70,71,176]			G8: [201]			L17: [202]
A16: [144]			C17: [203]			E11: [204,205]			G9: [206]			
B1: [23,158,207–212].			C18: [151]			E15: [198]			G13: [213,214]			

^a The signs ↑ and ↓ denotes for stimulation and inhibition, respectively.

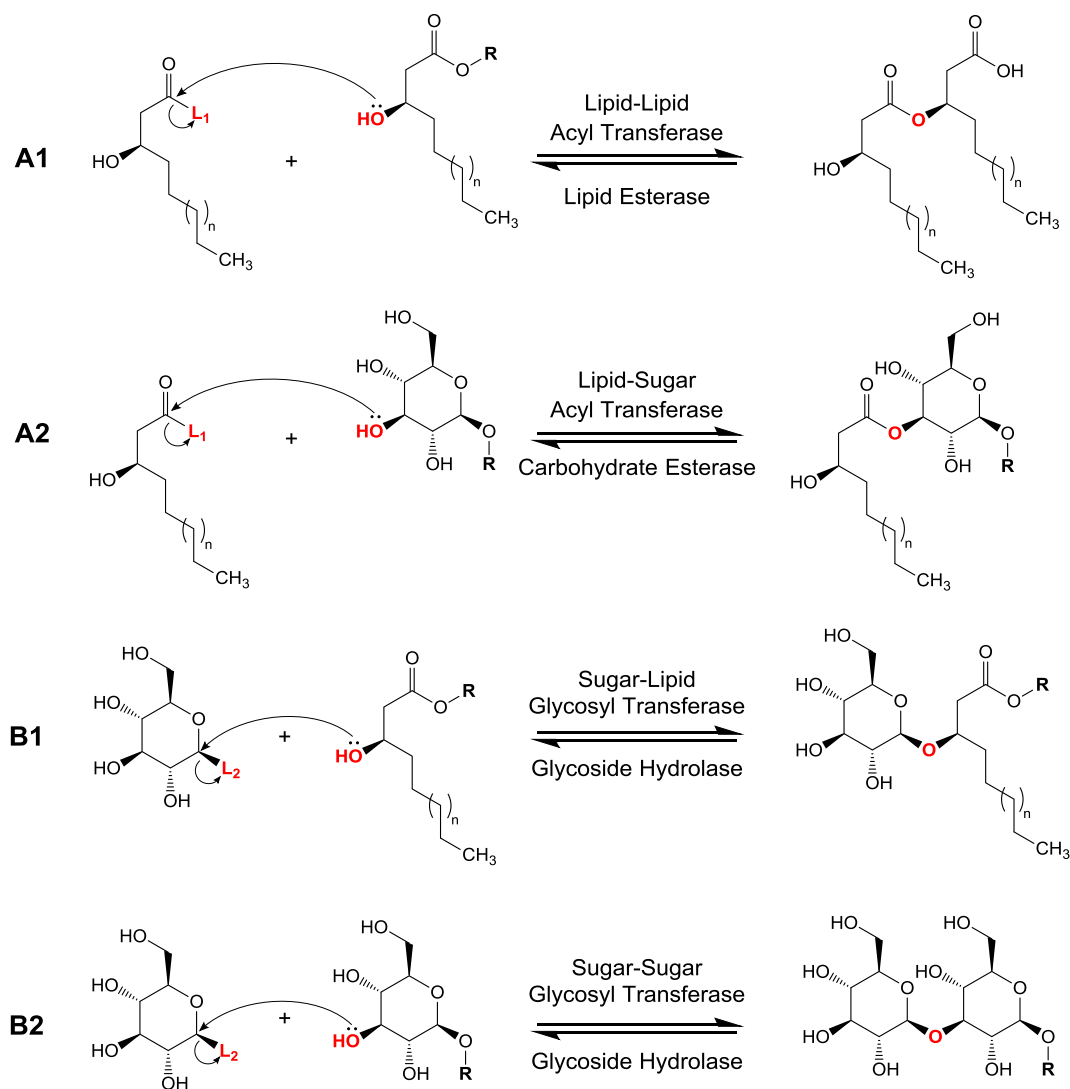


Fig. 4. Key enzymes of glycolipid biosynthesis and hydrolysis. Last steps of glycolipid biosynthesis involves linking of sugar and lipid moieties via either or both Acyl Transferases (AT) (**A1** and **A2**, forward reactions) and Glycosyl Transferases (GT) (**B1** and **B2**, forward reactions) which catalyze the ester and glycosidic bonds formation, respectively. Glycolipids are catabolized or broken down by Lipid Esterase (LE), Carbohydrate Esterases (CE) and Glycoside Hydrolases (GH) that hydrolyze the bond between alkyl-alkanoate ester, acyl-sugar ester and glycosidic bonds, respectively (reverse reactions). **L1**: Coenzyme A (CoA-S-) or Acyl Carrier Protein (ACP-S-) activating groups on acyl donors; **L2**: Nucleotides or phosphates activating groups on glycosyl donors. **R**: any substitution that could be glycosyl, lipid, or glycolipid units. Notes: β -glucose and *R*-3-hydroxyalkanoate are used as examples of any sugar and hydroxyl fatty acid of any chain length (*n*), respectively. Hydrolysis reactions do not generate activated products.

Pseudomonas aeruginosa [247]. One example is the successful expression of rhamnolipids biosynthetic genes of *P. aeruginosa* in non-pathogenic bacteria, namely *P. putida* [246] and *P. fluorescens* [248] as well as in *E. coli* [249,250], the best of which was recombinant *P. putida* [246], though, all recombinant strains produced RLs at much lower yields than the native producer. Interestingly, the non-pathogenic strain *P. chlororaphis* is naturally producing mono-rhamnolipids and not di-rhamnolipids as it lacks the gene coding for the second rhamnosyltransferase, *rhIC* [251]. Heterologous expression of *rhIC* from *P. aeruginosa* in *P. chlororaphis* resulted in production of di-RL at concentration more than twice that of mono-RL [251]. *Burkholderia kururiensis* is another nonpathogenic heterologous host that was successfully engineered for RLs production [252]. Further engineering strategies were reviewed for overproduction of RL [253].

Several glycosylated carotenoids (C₄₀ and C₅₀), e.g. glucosides of decaprenoxanthin and sarcinaxanthin as well as zeaxanthin, were successfully engineered in *Corynebacterium glutamicum* by

overexpression of genes coding for lycopene cyclization, hydroxylation and glycosylation [254].

Interestingly, 17 genes were heterologously expressed in an *E. coli* strain that is already producing 6-deoxyerythronolide B precursor to produce the glycosylated macrolide, erythromycin C [255]. The cloned genes encoded the deoxysugar, desosamine, biosynthetic enzymes and the enzymes converting 6-deoxyerythronolide B to erythromycin C [255,256].

Selection criteria for candidate hosts for heterologous glycolipid production should include, in addition to being non-pathogenic, to be natively tolerant to high concentrations of the target SGLs if high productivities are sought [246]. This is particularly important for SGLs which mostly demonstrate antimicrobial activities.

Moreover, the candidate host should, preferably, abundantly produce the precursors required for SGLs biosynthesis. A good approach would be starting with analysis of the intracellular concentration of lipid and glycosyl precursors. One example is the evaluation of the *R*-specific enoyl-CoA hydratase-2 (ECH-2) activity

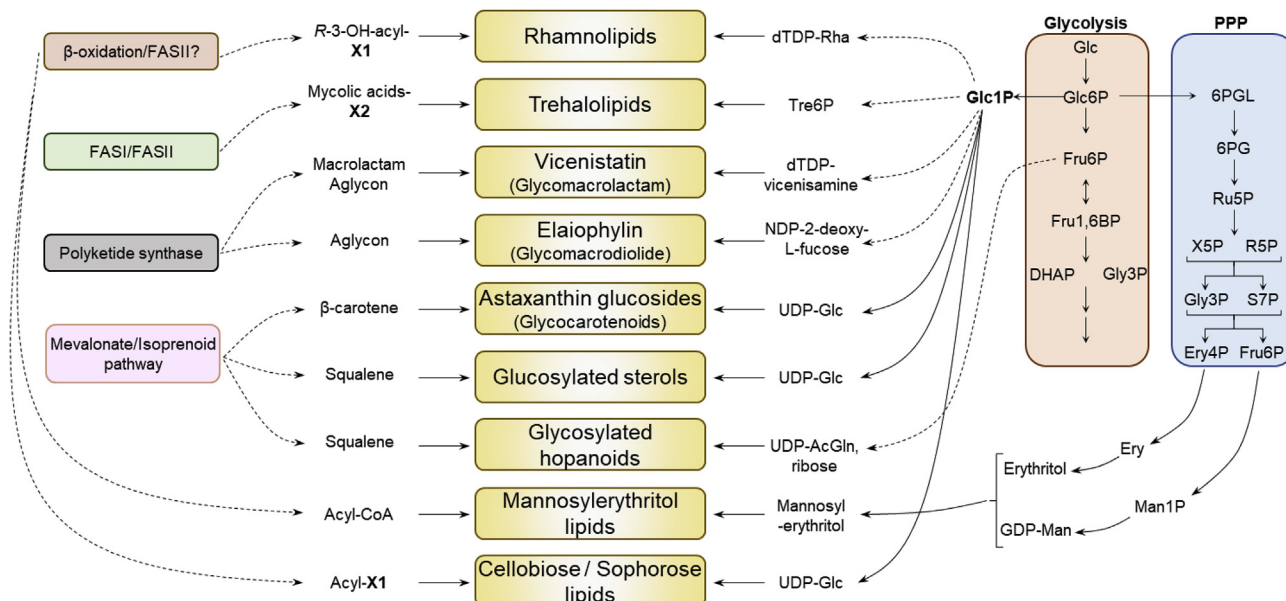


Fig. 5. Sugar and lipid precursors of prominent members of simple glycolipid groups and their furnishing pathways. Biosynthesis of simple glycolipids that harbor glucoside units, like glucosides of astaxanthin [115] and zeaxanthin [223] as well as glucosylated sterols [224], cellobiose and sophorose lipids [225], require UDP-glucose as glycosyl donor. Peculiar glycosyl donors that are activated in other ways than UDP are required in case of rhamnolipids [226], trehalolipids [227], vicenistatin [228] and elaiophyllin [68]. All glycosyl donors are derived from glucose -1-phosphate except that of glycosylated hopanoids whose glycosyl donors is derived from β -D-fructofuranose-6-phosphate and ribose [119]. Mannosylerythritol lipids are expected to derive the glycosyl unit, mannosylerythritol, from erythritol and GDP-mannose which originate from D-erythrose-4-phosphate and β -D-fructofuranose-6-phosphate intermediates of the pentose phosphate pathway as described in the yeast *Yarrowia lipolytica* [229,230]. The lipid moiety originates mostly from fatty acid synthesis and/or β -oxidation except glycosylated macrolides, carotenoids and sterols/hopanoids whose lipid moiety is furnished from the polyketide for the former and from mevalonate/isoprenoid pathway for the latter two groups. **6PGL**: 6-phosphogluconolactone; **6PG**: 6-phosphogluconic acid; **Ru5P**: D-Ribulose-5-phosphate; **R5P**: D-Ribose-5-phosphate; **X5P**: D-Xylulose-5-phosphate; **S7P**: D-Sedoheptulose-7-phosphate; **Gly3P**: D-Glyceraldehyde-3-phosphate; **DHAP**: Dihydroxyacetone phosphate; **Ery4P**: D-Erythrose-4-phosphate; **Fru6P**: β -D-Fructofuranose-6-phosphate; **Fru1,6BP**: β -D-Fructofuranose-1,6-bisphosphate; **dTDP-Rha**: dTDP-rhamnose; **Tre6P**: Trehalose-6-phosphate; **Glc**: D-Glucopyranose; **Glc1P**: Glucose-1-phosphate; **Glc6P**: Glucose-6-phosphate; **UDP-glc**: Uridine diphosphate glucose; **UDP-AcGln**: Uridine diphosphate N-acetylglucosamine; **Man1P**: Mannose-1-phosphate; **GDP-Man**: GDP-mannose; **R-3-OH-acyl-X1**: R-3-hydroxy acyl-X1 (X1 = -CoA/-ACP); **X2** = Mycolyl Carrier Protein. **PPP**: Pentose Phosphate Pathway. Dashed lines means that multiple biosynthetic steps are involved.

in crude cell lysate of target host organism as this predicts the potential of this host to synthesize R-3-hydroxyalkanoate precursors [257] that form the lipid part of many R-3-hydroxyfatty acid-containing glycolipids, e.g. rhamnolipids and rubiwettins. The ECH-2 activity was recently reported to be significantly implicated in rhamnolipids biosynthesis [258].

Oleaginous yeasts, like *Yarrowia lipolytica* and *Rhodospiridium toruloides*, are potential candidates in view of their already high lipid flux [259,260]; therefore, they are supposed to have abundant lipid precursors for GLs biosynthesis.

8.3. Blocking competing pathways

Blocking competitive pathways is an important strategy that is expected to enhance GLs biosynthesis. One example is blocking polyhydroxyalkanoates (PHA) synthesis in *P. aeruginosa* that changed the distribution of produced rhamnolipids (RLs) congeners by doubling the amount of produced mono-rhamnolipids relative to di-rhamnolipids [258]. Also, PHA mutant of *P. putida* was used to enhance heterologous production of RLs [246].

8.4. Tailoring the GL pool composition

Most simple glycolipids (SGLs) are naturally produced in mixtures of congeners and homologues like rhamnolipids [29] and sophorolipids [222]. For studying the functions and properties of each GL species in these natural mixtures, engineered production of purified GL should be sought. This can be achieved by selectively knocking out the genes coding for biosynthesis of specific congeners or forms. A recent example is the production of sophorolipids

pool enriched to 88% in the acidic non-lactonized free form by using a mutant strain of *Starmerella bombicola* [261] that is defective in lactone esterase [262].

9. Summary and perspectives

More than 5 decades of glycolipids research has led to the discovery of a huge number of simple microbial glycolipids, around 140 of which are cited in this review. In spite of the many publications demonstrating their great biomedical potential, the majority of discovered simple glycolipids are still unable to translate into commercial products because of their high cost of production mainly stemming from low biological yields. Metabolic engineering has the potential to overcome this cost problem particularly after the revolutionary developments in genetic engineering and synthetic biology techniques that were witnessed in the last 5 years.

This review is an attempt to structure the literature available on simple glycolipids aiming at providing metabolic engineers with an outlook on glycolipids and their biosynthesis. It highlights some of aspects and details that are still missing in the biosynthesis, transport and catabolism of glycolipids that need to be pursued and applied profitably in engineering cost-effective microbial glycolipid producers.

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