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REVIEW

Mannosylerythritol lipids: a review

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Abstract Mannosylerythritol lipids (MELs) are surface active compounds that belong to the glycolipid class of biosurfactants (BSs). MELs are produced by *Pseudozyma* sp. as a major component while *Ustilago* sp. produces them as a minor component. Although MELs have been known for over five decades, they recently regained attention due to their environmental compatibility, mild production conditions, structural diversity, self-assembling properties and versatile biochemical functions. In this review, the MEL producing microorganisms, the production conditions, their applications, their diverse structures and self-assembling properties are discussed. The biosynthetic pathways and the regulatory mechanisms involved in the production of MEL are also explained here.

Keywords Glycolipid · *Pseudozyma* yeasts · Self-assembled structures · Mannosylerythritol lipids · Immunoglobulins

Introduction

Biosurfactants (BSs) or microbial surface active compounds are of recent interest due to their unique properties including structural diversity, higher biodegradability, lower toxicity, higher foaming ability, higher selectivity and specific activity at extreme conditions (pH, temperature and ionic strength) and mild production conditions when compared to the chemical surfactants. BSs are amphiphilic molecules with polar and non-polar domains [6, 13, 42, 53, 59]. There are five main classes of BSs, namely lipopeptides; glycolipids; fatty acids including neutral lipids and phospholipids; polymeric and particulate [6, 13, 53, 59]. Among these, the first two classes show wide range of applications in food, cosmetics, and pharmaceutical industries [3, 6, 57, 61, 62]. They are used in removing soil contaminants including heavy metals, oils and other toxic pollutants [13, 52, 53, 61]. Some of the BSs are potential antimicrobial agents and are suitable alternatives to synthetic antibiotics [3, 38, 57, 62].

Except for a few, the physiological roles of BSs in general are not yet completely characterized. BSs are essential for the motility (swarming and gliding) of the microorganisms. For example, surfactin production and flagellar biosynthesis are crucial for swarming motility in *B. subtilis* [13, 30]. Similarly, *Serratia marcescens* depends on serrawettin for surface locomotion and access to water repelling surfaces [13, 47]. 3-(3-Hydroxyalkanoyloxy) alkanoic acid (HAA), the intermediate in the rhamnolipid biosynthesis pathway, plays an essential role in swarming motility [7, 13]. BSs regulate the attachment and detachment of the microorganisms to or from solid substrates [13, 53, 58]. Rhamnolipids are essential to maintain the architecture of the biofilm [4, 13] and are considered as one of the virulence factors in *Pseudomonas* sp. [4, 13, 58]. Rhamnolipids, mannosylerythritol lipid and surfactin are a few examples of BSs showing antimicrobial and antibiotic properties. Thus they confer a competitive advantage to the organism during colonization and cell–cell competition [13, 58]. BSs enhance the accessibility of hydrocarbon substrates by forming small emulsion droplets and increase the surface area of the insoluble substrates [13, 53, 58]. BSs form

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complex (chelate) with heavy metals including Cd^{2+}, Pb^{2+}, Zn^{2+} and Hg^{2+} and hence reduce their toxicity [13, 52, 58]. They act as carbon and energy storage molecules and remain as a protective layer for the microorganisms against the environment with high ionic strength [41, 42, 58]. BSs may be the byproducts released in response to the environmental changes and may have a role in DNA transfer in natural environment, since they can enhance gene uptake via transfection [13, 23, 24, 58, 61].

Biosurfactant containing 4-*O*-*β*-D-mannopyranosyl-*meso*-erythritol as the hydrophilic group and a fatty acid and/or an acetyl group as the hydrophobic moiety is known as mannosylerythritol lipid (MEL) (Fig. 1) [43]. MEL is reported to be secreted by *Ustilago* sp. (as a minor component along with cellobiose lipid (CL)) [14], *Schizonella melanogramma* (shizonellin) [5], and *Pseudozyma* sp. (as a major component) [33]. Since the synthesis of MEL is not growth associated, it can also be produced by using resting (stationary phase) cells of yeast. MEL acts as an energy storage material in the yeast cells similar to triacylglycerols [42]. MELs are shown to reduce the surface tension of water to less than 30 mN m^{-1} [32, 37, 42, 45]. Even though BSs have a wide range of applications, in general, they are not yet commercially viable mainly due to their low yield leading to high production cost. However, MEL (165 g/L) and sophorolipid (above 400 g/L) are produced in high quantity [42] and hence may become economically attractive.

MEL: why?

The increasing interest in MELs could be attributed to their pharmaceutical applications [60, 73] and versatile biochemical functions including antitumor and differentiation inducing activities against human leukemia cells, rat peochromocytoma cells and mouse melanoma cells [25–27, 68, 71–73]. They can be used in the treatment of schizophrenia or diseases caused by dopamine metabolic dysfunction [67, 72] and microbial infections [37]. MELs are used in the purification of lectins (Immunoglobulin G) [9, 15, 16, 21, 39] and in the preparation of ice-slurry as an anti-agglomeration agent [41, 42]. Self-assembling properties [17–20, 39, 43, 70] of MELs are of recent interest because this behavior could be leveraged in gene transfection and drug delivery [23, 24, 42, 43, 65, 66]. The increasing number of novel MEL producers, their proven structural diversity, their self-assembling properties and myriad applications are discussed in this review.

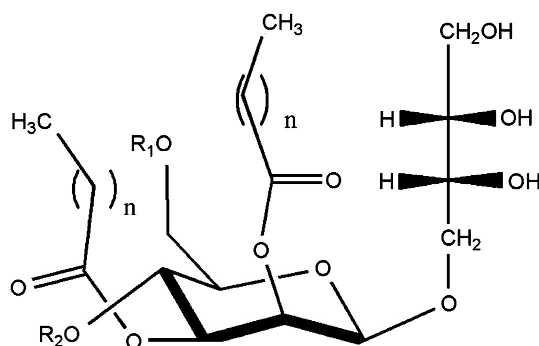

Fig. 1 Structure of MEL [43] (MEL-A: $R_1 = R_2 = Ac$; MEL-B: $R_1 = Ac$, $R_2 = H$; MEL-C: $R_1 = H$, $R_2 = Ac$: $n = 6–10$)

Structural diversity of MEL and its microbial sources

MELs are secreted by several microorganisms and they are first noted as oily compounds in the cultured suspension of *Ustilago* maydis PRL-627 by Haskins et al. [14]. This microorganism was shown to produce MEL along with CL when glucose was used as the carbon source [2, 8, 14]. Bhatttacharjee et al. [1] characterized MEL as a glycolipid, especially as a mixture of partially acylated derivative of 4-*O*-*β*-D-mannopyranosyl-D-erythritol, containing C2:0, C12:0, C14:0, C14:1, C16:0, C16:1, C18:0 and C18:1 fatty acids as the hydrophobic groups. *Schizonella melanogramma* was the second microorganism identified as a MEL producer (schizonellin), but not in association with CL [5]. Table 1 summarizes the structural variants of the MELs and the corresponding microbial sources. These variants arise due to the following reasons:

- Number and position of the acetyl group on mannose or erythritol or both.
- Number of acylation in mannose.
- Fatty acid chain length and their saturation.

The acyl residues in MEL ranged from C_7 to C_{14} and their proportions varied depending upon the carbon source used. For example, *Candida* sp. B-7 (currently known as *Pseudozyma antarctica*) produced 4-*O*-(2′, 6′-di-*O*-acyl-*β*-D-mannopyranosyl)-D-erythritol with high yield (25–36 g/L), when it was grown on *n*-alkane or triglycerides at 30 °C for 7 days but not with carbohydrate as the carbon source [29]. Based on the degree of acetylation at C4 and C6 position, and their order of appearance on the thin layer chromatography (TLC), the MELs (from *P. aphidis*) are classified as MEL-A, -B, -C and -D. MEL-A representing the diacetylated compound while MEL-B and MEL-C are monoacetylated at C6 and C4, respectively. The completely deacetylated structure is known as MEL-D [54]. Mixtures of these four MELs but predominantly MEL-A and MEL-B were isolated from *Candida antarctica* strain T34 [33]. Excellent surface active and antimicrobial properties of MELs isolated from T-34 were reported by Kitamoto et al. [37].

Kurtzmanomyces sp. I-11 produces a novel MEL composed of a sugar moiety, 6-*O*-*β*-D-mannopyranosyl-

Table 1 Structural variants of MEL and their corresponding microbial sources

Microorganism	Sugar	Fatty acid profile	Type of MEL (major component)	References
Ustilago nuda PRL-627	4-*O*-β-D-mannopyranosyl-D-erythritol	$C_{2:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$	NA	[1]
Candida (*Pseudozyma*) sp. B7	4-*O*-β-D-mannopyranosyl-D-erythritol	C_7–C_{14}	MEL-B7	[29]
Pseudozyma antarctica T-34	4-*O*-β-D-mannopyranosyl-erythritol	$C_{8:0}$ (27.26%), $C_{10:0}$ (21.28%), $C_{10:1}$ (27.22%)	MEL-A and MEL-B (it produce all four types)	[34, 40]
Ustilago maydis DSM 4500 and ATCC 1482	4-*O*-β-D-mannopyranosyl-D-erythritol	C14:1 (43%), C6:0 (20%) and $C_{16:1}$ (12%)	MEL-A	[63]
Schizonella melanogramma	4-*O*-β-D-mannopyranosyl-D-erythritol	$C_{14:0}$, $C_{16:1}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$	Schizonellin A and schizonellin B (similar to MEL-A and -B)	[5]
Kurtzmanomyces sp. I-11	6-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	$C_{8:0}$ (36.4%), $C_{12:0}$ (11.9%), $C_{14:2}$ (25.9%)	MEL-I-11	[28]
Candida sp. SY16	6-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	$C_{6:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{14:1}$	MEL-A	[31]
Pseudozyma aphidis DSM 70725	4-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	$C_{10:0}$, $C_{10:1}$, and $C_{8:0}$	MEL-A	[54]
Pseudozyma rugulosa NBRC 10877	4-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	$C_{8:0}$ (28.09%), $C_{10:0}$ (21.68%), $C_{10:1}$ (22.94%)	MEL-A	[48]
Pseudozyma hubeiensis KM-59	4-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	C_6, C_{12} and C_{16}	MEL-C (65%)	[45, 46]
Pseudozyma shaxiensis	4-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	$C_{16:0}$, $C_{16:1}$, $C_{16:2}$, and $C_{14:1}$	MEL-C	[10]
P. tsukubaensis JCM 10324T	1-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	C_8, C_{12}, C_{14}	MEL-B with new diastereomer of sugar	[12, 49]
P. fusiformata	4-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	C_6 and C_8	MEL-A	[49]
Pseudozyma graminicola CBS 10092	4-*O*-β-D-mannopyranosyl-(1→ 4)-D-*meso*-erythritol	$C_{6:0}$, $C_{8:0}$, $C_{12:0}$, $C_{12:1}$, $C_{14:0}$, and $C_{14:1}$	MEL-C (85%)	[50]
P. antarctica and *P. parantarctica*	4-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	$C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$	Monoacylated MEL	[11]
P. antarctica and *P. rugulosa*	4-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	$C_{18:1}$, $C_{18:0}$, $C_{10:0}$, $C_{10:1}$, $C_{16:0}$, and $C_{8:0}$	Triacylated MEL	[44]
P. siamensis CBS 9960	4-*O*-β-D-mannopyranosyl-(1 → 4)-D-*meso*-erythritol	$C_{14:2}$, $C_{16:0}$, $C_{16:1}$, $C_{16:2}$ (C_2 or C_4) at the C-2′ position and (C_{16}) at the C-3′ position of the mannose moiety	MEL-C mixture of monoacetylation and diacetylation	[57]

NA not available

(1 → 4)-*O*-*meso*-erythritol and a mixture of fatty acids [C8:0 (36.4%), C12:0 (11.9%) and C14:2 (25.9)] [28] as the lipophylic group. *Candida antarctica* KCTC 7804 which was isolated from an oil contaminated site, produced a MEL containing 6-*O*-acetyl-2, 3-di-*O*-alkanoyl-*β*-D-mannopyranosyl-(1 → 4)-*O*-*meso*-erythritol. The lipophilic group of this MEL is a mixture of hexanoic, dodecanoic, tetradecanoic and tetradecenoic fatty acids. The acetyl group is linked at the C-6 position of the D-mannose [31]. *Pseudozyma rugulosa* NBRC 10877 is reported to produce from soybean oil a mixture of MEL-A (68%), MEL-B (12%) and MEL-C (20%) consisting of C_8 and C_{10} fatty acids as the hydrophobic moiety [48]. *P. fusiformata, P. parantarctica* and *P. tsukubaensis* are identified as MEL producers and of these three strains; *P. parantarctica* produces the highest quantity of MEL (30 g/L). Interestingly, *P. parantarctica* and *P. fusiformata* produce mainly MEL-A, whereas *P. tsukubaensis* produces MEL-B. The strains of *Pseudozyma* are classified into four groups such as, the first group produces largely MEL; the second produces both MEL and other biosurfactants; the third produces mainly MEL-B; and the fourth produces non-MEL BS [49]. Hence the production of MEL can be used as an important taxonomic index to identify *Pseudozyma* yeasts [10, 49–51].

MEL-C is produced by *Pseudozyma hubeiensis* and the major fatty acids in it are C_6, C_{12}, and C_{16} which are different from those of the other MELs reported so far [45]. *P. shanxiensis* produces a hydrophilic MEL consisting of a mixture of 4-*O*-[(2′,4′-di-*O*-acetyl-3′-*O*-alka(e)noyl)-*β*-D-mannopyranosyl]-D-erythritol (Fig. 2) and 4-*O*-[(4′-*O*-acetyl-3′-*O*-alka(e)noyl-2′-*O*-butanoyl)-*β*-D-mannopyranosyl]-D-erythritol (Fig. 3) and this has much shorter chain (C_2 or C_4) at the C-2′ position of the mannose moiety compared to other classes of MELs which comprise of a medium chain length acid (C_8 to C_{14}) at the C-2′ position [10, 51]. *Pseudozyma siamensis* CBS 9960 is found to accumulate higher amounts of glycolipid than its closely related species, *P. shanxiensis*, which is a known MEL-C producer. The former produces a mixture of different types of MEL-C, namely (>84% of the total) 4-*O*-[(2′,4′-di-*O*-acetyl-3′-*O*-alka(e)noyl)-*β*-D-mannopyranosyl]-D-erythritol and 4-*O*-[(4′-*O*-acetyl-3′-*O*-alka(e)noyl-2′-*O*-butanoyl)-*β*-D-mannopyranosyl]-D-erythritol. This MEL-C possess a short-chain length acid (C_2 or C_4) at the C-2′ position and a long-chain length acid (C_{16}) at the C-3′ position of the mannose moiety, and thus, the hydrophobic part is considerably different from that of conventional MELs, which have two medium-chain length acids (C_{10}) at the C-2′ and C-3′ positions [51]. *P. antarctica* and *P. rugulosa* with soybean oil as the carbon source produce the most hydrophobic triacylated MEL namely 1-*O*-alka(e)noyl-4-*O*-[(4′,6′-di-*O*-acetyl-2′,3′-di-*O*-alka(e)noyl)-*β*-D-mannopyranosyl]-D-erythritol (Fig. 4) [9]. *P. antarctica* T-34

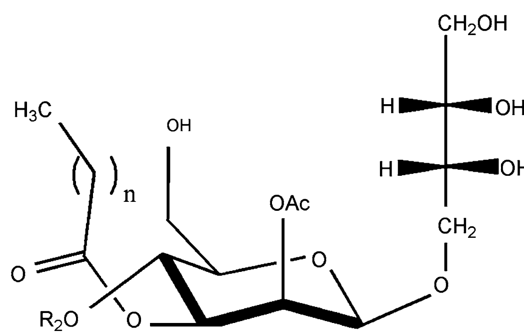

Fig. 2 Structure of 4-*O*-[(2′,4′-di-*O*-acetyl-3′-*O*-alka(e)noyl)-*β*-D-mannopyranosyl]-D-erythritol; R_2 = Ac; n = 12–16

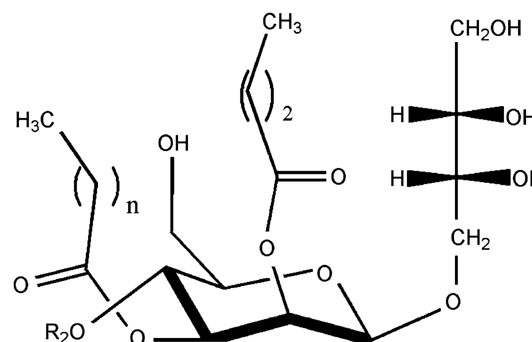

Fig. 3 Structure of 4-*O*-[(4′-*O*-acetyl-3′-*O*-alka(e)noyl-2′-*O*-butanoyl)-*β*-D-mannopyranosyl]-D-erythritol; R_2 = Ac; n = 12–16

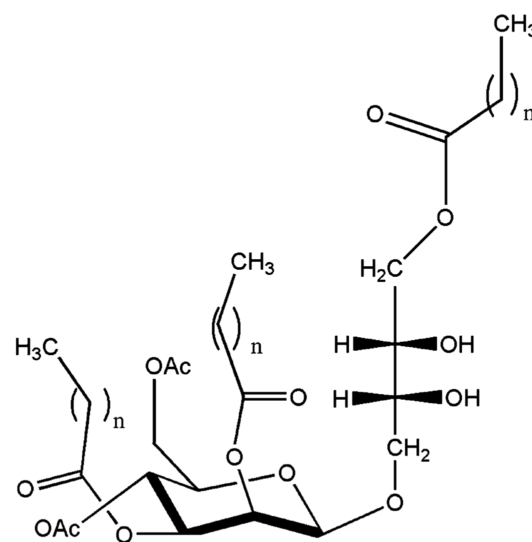

Fig. 4 Structure of triacylated MEL; n = 6–10; m = 6–16

produces a hydrophilic monoacylated MEL, 4-*O*-(3′-*O*-alka(e)noyl-*β*-D-mannopyranosyl)-D-erythritol (Fig. 5), with glucose as the carbon source. This MEL reduces the surface tension of water to 33.8 mN/m at a critical micelle concentration (CMC) of 3.6×10^{-4} M, and it has a hydrophilic–lipophilic balance of 12.15. This CMC is 100-fold

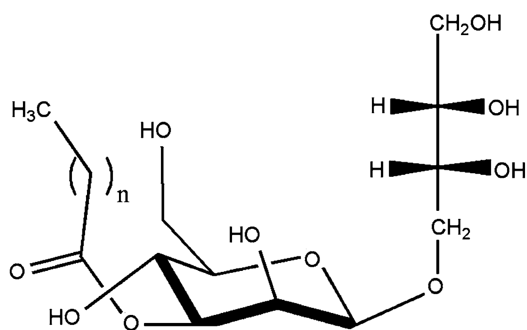

Fig. 5 Structure of monoacylated MEL; $n = 4$–14

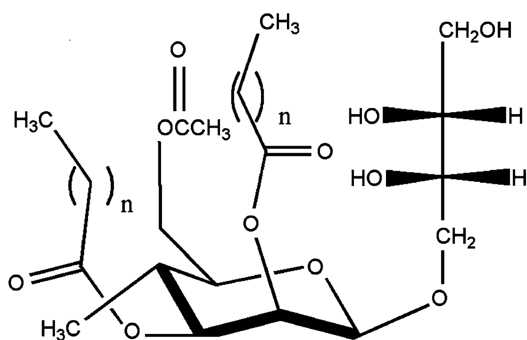

Fig. 6 Diastereomer of MEL-B from *P. tsukubaensis*; $n = 6$–12

higher than that of the other MELs reported [11]. A strain of *P. hubeiensis* KM-59 produces a hydrophilic MEL-C namely (4-*O*-[4′-*O*-acetyl-2′,3′-di-*O*-alka(e)noyl-*β*-D-mannopyranosyl]-D-erythritol) [46]. *Pseudozyma graminicola* CBS 10092 also produces MEL-C. It has C_6, C_8 and C_{14} acids and is considerably different from the other MEL-C producing *Pseudozyma* strains such as *P. antarctica* and *P. shanxiensis* [50].

Pseudozyma tsukubaensis also produces an unusually different carbohydrate structure namely, 1-*O*-*β*-(2′,3′-di-*O*-alka(e)noyl-6′-*O*-acetyl-D-mannopyranosyl)-D-erythritol (Fig. 6). The configuration of this erythritol moiety in this MEL is opposite to that of the known MEL-B or to other MELs reported [12]. Table 2 summarizes the surface activities of various homologs of MEL.

Microbial production condition

The production of MEL in shake flask is reported while very few attempts have been made to produce it in a fermentor. Seed cultures are prepared by inoculating cells grown on slants into test tubes containing a growth medium [4% glucose, 0.3% $NaNO_3$, 0.03% $MgSO_4$, 0.03% KH_2PO_4, 0.1% yeast extract (pH 6.0)] at 25 °C on a reciprocal shaker (150 rpm) for 2 days [10, 33, 34, 56]. Exactly 1 mL of the seed culture is transferred to a 300 mL Erlenmeyer flask containing 30 mL of the basal medium [4% olive oil, 0.3% $NaNO_3$, 0.03% $MgSO_4$, 0.03% KH_2PO_4, 0.1% yeast extract (pH 6.0)], which is then incubated on a rotary shaker (220 rpm) at 28 °C for 7 days.

Isolation of glycolipid is carried out by solvent extraction. Equal volumes of ethyl acetate and culture medium are mixed together and are allowed to separate in a separating funnel. Solvent layer is collected and evaporated. This fraction is analyzed using TLC on silica plates (Silica gel 60F; Wako) with a solvent system consisting of chloroform/methanol/7 N ammonia solution (65:15:2, by vol). The compounds on the plates are located by charring them at 110 °C for 5 min after spraying a mixture of anthrone and sulfuric acid [10, 34, 56]. The concentrated glycolipids are dissolved in chloroform and then purified by silica gel (Wako-gel C-200) column chromatography using a gradient elution technique with chloroform–acetone (10:0–0:10, vol/vol) solvent mixtures [10, 56]. Table 3 summarizes the production conditions and the corresponding yield in the preparation of MEL by *Pseudozyma* sp.

Rau et al. [56] reported the production of MEL in a bioreactor and the various downstream processing strategies for its recovery and purification. Solvent extraction, adsorption on different types of Amberlite XAD followed by solvent extraction and heating the culture suspension to 110 °C for 10 min were the three downstream methods reported. The last method gave the highest yield of 93 wt% with a purity of 87 wt%, whereas the solvent extraction method gave only 79 wt% yield with 100 wt% purity [56].

Table 2 Structural variants of MEL and their surface activities

Type of MEL	Critical micelle concentration (M)	Surface tension (mN/m)	Interfacial tension (mN/m)	References
MEL-A	2.7×10^{-6}	28.4	2.1	[37]
MEL-B	4.5×10^{-6}	28.2	2.4	[37]
MEL-C (from *P. hubeiensis* KM-59)	6.0×10^{-6}	25.1	–	[46]
MEL-C (from *P. graminicola* CBS 10092)	4.0×10^{-6}	24.2	–	[50]
MEL-C (from *P. siamensis* CBS 9960)	4.5×10^{-6}	30.7	–	[57]
Monoacylated MEL	3.6×10^{-4}	33.8	–	[11]

Table 3 Effect of the culture conditions and carbon source on the production and composition of MEL by *Pseudozyma* sp.

Type of MEL	Yield (g/L)	Conditions	References
MEL-B7	30	*n*-Alkane or vegetable oils; batch culture	[29]
Mixture of MEL-A, -B and -C	47	Soybean oil and resting cells; batch culture	[35]
Mixture of MEL-A, -B, -C and D	40	Vegetable oils, yeast extract and batch culture	[34]
	30	Sunflower oil; batch culture	[34]
Mixture of MEL-A, -B and -C	140	Octadecane; batch culture	[40, 42]
Mixture of MEL-A, -B, and -C	165	Soybean oil and fed batch reactor	[55]
Mixture of MEL homologs	95	Glucose:soybean oil (1:1) and intermittent addition of soybean—fed batch culture	[32]
MEL-B	25	Soybean oil batch culture	[12, 45]
MEL-C	15	Soybean oil batch culture	[45]
MEL-C	76.3	Soybean oil and fed batch reactor	[46]

Biosynthetic pathway of MEL

Kitamoto et al. [37, 43] attempted to relate the substrate and the corresponding product structures. When fatty alcohols or acids of chain length of C_n were used, the products formed were with the chain length of C_{n-2}, C_{n-4}, C_{n-6}, etc. [36, 42]. The authors concluded that the products were the β-oxidation intermediates of the substrates supplied.

2-Bromooctanoic acid [38, 42] (strong inhibitor of the fatty acid β-oxidation) inhibited the production of MEL and the degree of inhibition increased with the increase in the chain length of the substrate supplied. The cerulenin (strong inhibitor to de novo fatty acid synthesis) did not affect the fatty acid composition of MEL. In general, once the fatty acid entered the β-oxidation pathway, it was completely oxidized to an acetyl CoA and the intermediates did not leak out from this cycle [42, 64]. Two types of β-oxidation cycles are present in mammals, namely peroxisomal partial β-oxidation and mitochondrial complete β-oxidation. The former is responsible for the shortening of long and very long chain fatty acids which are then converted into acetyl CoA in the latter cycle [42, 69]. The biosynthetic "chain shortening" pathway of MEL is different from the three generally known pathways in microorganisms, namely, de novo synthesis, chain elongation and intact incorporation [42].

Self-assembling properties of MEL

Self-assembly (SA) can be defined as the spontaneous and reversible organization of molecular units into ordered structures by non-covalent interactions without the application of external force or stimulus. Any amphiphilic molecule possesses this ability. For example, ionic and nonionic surfactants at high concentrations can self-assemble into three-dimensional ordered lyotropic liquid crystals including sponge, cubic, lamella, and hexagonal phases. Natural sugar-based surfactants exhibit the above-mentioned behavior and in addition they posses several advantages over the synthetic ones including high biodegradability, eco-friendly, molecular recognition and less-toxicity. They can self-assemble into specific lyotropic liquid crystalline phases which are stabilized by hydrogen bonds between the sugar moieties. Chirality of the sugar also affects their lyotropic and thermotropic phase behaviors [20, 39]. MEL exhibits the above-mentioned lyotropic liquid crystalline phases. The four classes of MEL with variation in their hydrophilicity show different self-assembling properties including liposomes, self-assembled monolayer, lamella phase, sponge phase, liquid lyotropic crystals and bicontinuous cubic phase [17–21, 39, 43, 70].

Formation of thermodynamically stable vesicle by MEL

Mannosylerythritol lipids form thermodynamically stable vesicle ($L_{\alpha 1}$) from the sponge phase (L_3), when they are mixed with L-α-dilauroylphosphatidyl choline(DLPC) [18]. Since they are negatively curved lipids, mixing DLPC (phospholipids) with this sponge phase, induces the fusion of both the lipids and favors their distribution in the inner monolayer of the vesicle. This causes the asymmetric distribution of the two lipids leading to the formation of thermodynamically stable vesicle (Fig. 7). Smallest sized vesicles (633.3 nm) are obtained at a DLPC mole fraction of 0.3 and it is stable at 25 °C for more than 3 months. Such vesicles formed from the natural glycolipids are most preferable for drug delivery and gene transfer due to their biocompatibility when compared to their synthetic counterparts [18].

Multilamellar vesicles and large unilamellar vesicles

Imura et al. [19] studied the self-assembling properties of MEL-A and -B by using fluorescence-probe spectroscopy, dynamic light scattering (DLS) spectroscopy, freeze–fracture

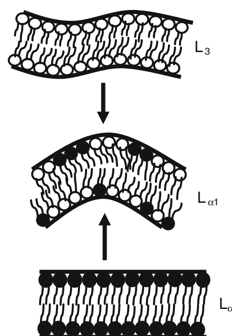

Fig. 7 Mechanism of formation of thermodynamically stable vesicles by MEL-A/DLPC mixture [16]. L_3 MEL sponge phase; $L_{\alpha 1}$ thermodynamically stable vesicle; L_α Large multilamellar vesicle (○ - MEL-A negatively curved lipid; ● - DLPC zero curved lipid)

transmission electron microscopy (FF-TEM) and synchrotron small/wide-angle X-ray scattering (SAXS/WAXS) spectroscopy. They observed that the MELs self-assemble into large unilamellar vesicles (LUV) just above their critical-aggregation concentration (CAC), which was found to be 4.0×10^{-6} M and 6.0×10^{-6} M for MEL-A and MEL-B, respectively. Above a CAC (II) value of 2.0×10^{-5} M, MEL-A is found to drastically change into sponge like structure (L_3) which is composed of a network of randomly connected bilayers with a water-channel diameter of 100 nm and resembles a multicomponent synthetic surfactant system. This channel dimension is relatively large when compared to those obtained with synthetic surfactants. MEL-B, which has a hydroxyl group at the C-4′ position instead of an acetyl group on mannose gives only one CAC. The self-assembled structure of MEL-B seems to gradually move from LUV to multilamellar vesicles (MLV) with lattice constant of 4.4 nm [19].

Lyotropic-liquid-crystalline phases of MEL

At high concentrations, the formation of an inverted hexagonal phase (H_2) for MEL-A and a lamella phase (L_α) for MEL-B was clearly observed by using polarized optical microscopy. These results indicate that the difference in the spontaneous curvature between MEL-A and MEL-B molecules is due to an extra acetyl group present in MEL-A. This difference decides the direction of their self-assembly. The microorganisms are able to engineer distinct self-assembled structures by varying the substitutions on the polar head group [19].

Imura et al. showed on the phase diagram that MEL-A is able to self-assemble into a variety of distinctive lyotropic liquid crystals including L_3, bicontinuous cubic (V_2), and lamellar (L_α) phases. The lattice constants are estimated to be 11.39 and 3.58 nm for V_2 and L_α, respectively. Differential scanning calorimetry (DSC) measurement revealed that the phase transition enthalpies from these Lyotropic liquid crystals (LCs) to the fluid isotropic (FI) phase are in the range of 0.22–0.44 kJ/mol. L_3 region of MEL-A is spread considerably over a wide temperature range (20–65 °C) when compared to that of other surfactants [20]. This is probably due to its unique structure which is molecularly engineered by the microorganisms.

MEL-B from *P. tsukubaensis* (yield 20 g/L) has 1-*O*-*β*-(2′,3′-di-*O*-alka(e)noyl-6′-*O*-acetyl-D-mannopyranosyl)-D-erythritol (Fig. 6) [12], which is a diastereomer of the conventional MEL-B. This new diastereomer self-assembles into a lamellar (L_α) phase over a remarkably wide range of concentrations and temperatures whereas MEL-A (diacetyl) forms L_3, V_2, and L_α phases. It is observed that the difference in the number of acetyl groups and the configuration of erythritol moiety play an important role in the aqueous-phase behavior of MEL/water mixture. The interplanar distance (*d*) of L_α phase of MEL-B is estimated to be 4.7 nm at low concentrations (≤60 wt%) and this d-spacing decreases with increase in concentration (~3.1 nm at >60%). This phase is found to be stable up to 95 °C and below a concentration of 85% (wt). The melting temperature of the liquid crystalline phase dramatically decreases with increase in MEL-B concentration (above 85 wt%). The MEL-B is able to form vesicle of dimension 1–5 μm [70]. MEL-C from *P. Siamensis* CBS 9960 forms liquid crystal phases such as hexagonal (H) and L_α phases at a wide range of concentrations [51].

Coacervate formation of natural glycolipids

Coacervate is a spherical aggregation of lipid molecules making up a colloidal inclusion, which is held together by hydrophobic forces. Coacervate formation by MEL-A and vesicle formation by MEL-B have been observed and as mentioned before this difference arises due to the presence of 4′-*O*-acetyl group in MEL-B [17].

Self-assembled monolayer structures of MEL

MEL-A has been used as a model to understand the role of molecular interaction between glycolipids and proteins in biological recognition events including cell adhesion, signal transduction and immune function. MEL exhibits highly ordered self-assemblies and affinity towards glycoproteins. Self-assembled monolayers (SAMs) of MEL on the alkanethiol SAMs using MEL-A bilayer membrane structures are called supported "Hybrid Bilayer Membranes" (HBMs) (Fig. 8). The interaction between the HBMs and two different immunoglobulins namely HIgG and HIgM was studied by using surface plasmon resonance (SPR) and atomic force microscopy (AFM). The affinity constants (K_a) between SAM and HIgG and HIgM are 9.4×10^6 M^{-1} and 5.4×10^6 M^{-1}, respectively. The binding affinity between the self-assembled monolayer of MEL-A and HIgG is 25 times higher than that observed with *Staphylococus aureus* protein A which is very commonly used for the purification of HIgG. Also it is six times higher

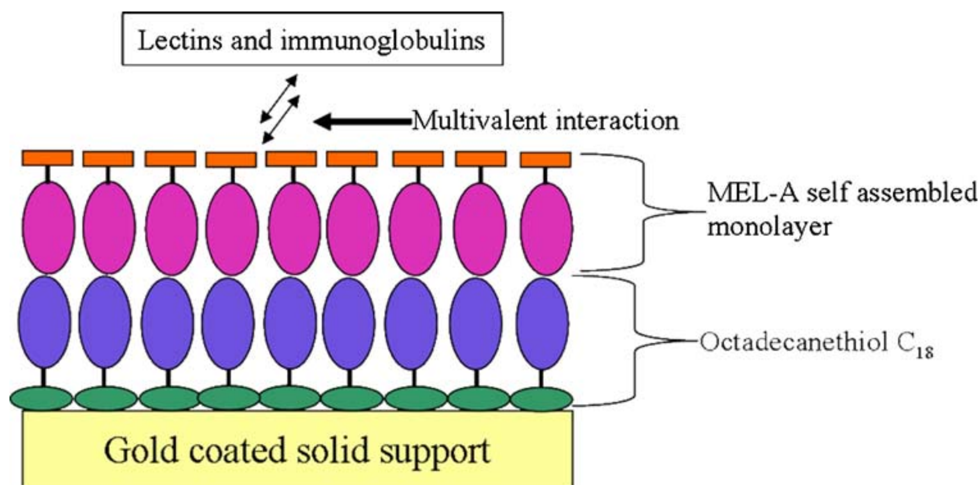

Fig. 8 Schematic representation of a hybrid bilayer membrane and molecular interaction [19]

than that observed with MEL-A and poly (2-hydroxyethyl methacrylate) (PHEMA) [21]. MEL-A SAMs on alkanethiolates are well aligned along the surface when compared to those on polymer surfaces prepared by the solvent evaporation method. Although the individual carbohydrate and protein interaction is relatively weak ($K_a \sim 10^3$ to 10^4 M^{-1}), the high binding affinity observed here can be attributed to the multivalent effect of the self-assembled monolayer of MEL [19, 21, 43]. Table 4 summarizes the different classes of MELs and their corresponding self-assembling properties.

Applications

MELs are considered as molecules of multifunctionality due to their exceptional surface activity; high yield (above 100 g/L); biocompatibility; self-assembling properties; antimicrobial activities; and biochemical functions [13, 59].

Antimicrobial activity of MEL

Both MEL-A and MEL-B show strong activity against gram positive bacteria, weak activity against gram negative bacteria and no activity against fungi. The minimum inhibitory concentrations against gram-positive bacteria are significantly lower than those of sucrose and sorbitan monoesters of fatty acids. Other MELs accumulated in the cell of *Schizonella melanogramma* also exhibit antibacterial and antifungal activities [5]. Matsumura et al. reported that mannopyranosyl groups are the most effective amongst the *n*-alkyl glycosides tested against microorganisms. It may not be difficult to enhance the antimicrobial activity of the present MELs further by the chemical modification of the sugar moiety [37].

MEL induces cell differentiation and apoptosis

MEL-A and -B have excellent growth inhibition and differentiation-inducing activities against human leukemia cells including myelogenous leukemia cell K562 [26], promyelocytic leukemia cell HL60 [25, 26] and the human basophilic leukemia cell line KU812 [25, 42]. Both inhibit the growth of HL60 cells at concentrations between 5 and 10 μM and also induce changes to their morphology. They (at 10 mg/L) also induce granulocytic differentiation [27, 42] and inhibit the activity of phospholipid- and Ca^{2+}-dependent protein kinase C in HL 60 cells. MEL-A inhibits the serine/threonine phosphorylation of a 30 kDa protein in HL-60 cells and the

Table 4 Homologs of MEL and their self-assembling properties

Type of MEL	Size of the vesicles (μm)	Critical aggregation concentration (M)	Self-assembling structures	Referenes
MEL-A	1–20	4.0×10^{-6}	Spherical droplet, Large unilamellar vesicles (LUV) sponge (L_3), bicontinuous cubic (V_2) and lamella	[17–20, 39]
Conventional MEL-B	1–20	6.0×10^{-6}	Giant vesicles, multi lamellar vesicles (MLV)	[17, 19, 39]
MEL-B (new diastereomer *P. tsukubaensis*)	1–5	NA	Lamellar (L_α) phase over a wide concentration and temperature ranges, vesicle	[67]
MEL-C	NA	NA	Myelines and lamella (L_α)	[46]
MEL-C from *P. siamensis* CBS 9960	NA	NA	Hexagonal (H) and lamella (L_α)	[57]

NA not available

tyrosine phosphorylation of 55-, 65-, 95-, 135-kDa proteins in K562 cells [42]. Therefore, MEL appears to directly affect intracellular signal transduction through phosphate cascade systems. Both induce significantly the neurite outgrowth of rat pheochromocytoma PC-12 cells and partial cellular differentiation [42, 68]. MEL-A has been recently demonstrated to inhibit the growth of mouse melanoma B 16 cells in a dose-dependent manner. At 10 μM concentration it causes the condensation of chromatin, fragmentation of DNA, and arrest of sub-G1, which indicates that the cells are undergoing apoptosis. MEL is also found to induce tyrosinase activity and enhances the production of melanin [71]. This trigger of mouse melanoma B 16 cells may be through a signaling pathway that involves protein kinase C_α (PKC_α) [73].

Purification of glycoprotein

MEL-A, -B and -C exhibit high binding affinity to human immunoglobulins [15, 16, 39, 43]. They also show binding affinity towards Ig M and other glycoproteins. Self-assembled monolayer of MEL-A show six times higher affinity than the immobilized MEL-A in PHEMA [21]. All these results show their potential application as a ligand for the purification of lectins.

Vehicles for gene delivery

MELs can be used as a vehicle for gene and drug delivery due to their ability to form thermodynamically stable vesicles with the ability to fuse with the membrane [23, 24, 43, 65, 66]. Among various biosurfactants, MEL-A provides the highest sufficiency [24]. It remarkably accelerates the adhesion of positively charged liposome–DNA complex to the cell membrane and incorporates this complex into the cell [23, 43].

Inhibit ice agglomeration

Ice slurry systems are finding wide applications as environmental friendly cold thermal storage units, especially as air conditioners [22, 42]. In these units, there is a possibility for the ice particles to agglomerate or grow together and block the pipeline, causing superfluous power loads thereby leading to loss of efficiency. MEL at low concentration (10 mg/L) gets adsorbed on the ice surface and suppresses the agglomeration of ice particles. Their effective performance has also been tested successfully in a large scale model (300 L) [22, 41, 42].

Future perspectives

The past 60 years of research on MEL has proved that it is the most promising microbial extracellular glycolipid ever known because of its high yield, excellent surface activity, diverse biochemical functions, biocompatibility and its wide range of applications. However, the commercial viability of MEL depends upon its production cost which has to be significantly lowered with the help of recent developments in bioprocess technology together with the advanced separation techniques. Hence the following research topics need to be explored:

- Reduce the production cost by using cheaper raw material, optimize the production conditions together with downstream processes.
- Engineer the overproducing mutant and recombinant strains.
- Develop structure-activity relationship to tailor the compounds with the required features. This can be done by making derivatives (by chemical or enzymatic synthesis and modifications) and analyzing their corresponding performance.
- Study on the various factors that determine the self-assembling properties to enhance their scope of applications in the biomedical field.
- Identify the enzymes involved in their biosynthesis and regulate or augment the production of only one type of MEL with higher yield.
- Determine the role of cell wall components (of *Candida* sp. *Torulopsis* sp. and *Pseudozyma* sp.) and understand how they tolerate high levels of MEL. This may provide new approaches to increase the yield as well as this knowledge could be extrapolated for other classes of BSs.
- Since MEL can interact with glycoproteins (lectins), use them as model systems to study the mechanism of different biochemical functions.
- Application of MEL as a vehicle for drug and gene delivery.
- Establish mechanisms of antimicrobial and antitumor activities of MEL.
- Similarity studies on the enzymes involved in the synthesis of different classes of glycolipid (rhamnolipids, sophorolipids, cellobiose lipids, MEL, etc.) may give insight into the active site specificity of enzymes for the synthesis of particular kind of glycolipid.
- Understand the role of MEL production in microbial physiology.

Conclusion

Mannosylerythritol lipid, 2,3-di-*O*-alka(e)noyl-*β*-D-mannopyranosyl-(1 → 4)-O-*meso*-erythritol, partially acetylated at C4 and/or C6 position, is produced by *Ustilago* sp., *Schizonella* sp., and *Pseudozyma* sp. The type of MEL produced varies with the strains and the species of

Pseudozyma. Hence its production can be used as an important taxonomic index to identify *Pseudozyma* yeast. This is considered as a most promising biosurfactant because of its high yield, excellent surface activity, diverse biochemical functions and biocompatibility. MELs exhibit interesting self-assembling properties which enhance their efficiency in gene transfection. Recent research on the ability of MEL-A to form water-in-oil microemulsion without the use of a cosurfactant seems to add an impetus to the development of the microemulsion technology which is limited due to the fact that they require cosurfactants, salts or alcohols for stabilization. MEL induces differentiation of several carcinoma cell lines. They exhibit antimicrobial and antitumor activities. The high binding affinity of the monolayer of MEL-A to the immunoglobulins helps in the purification of lectins. However their large scale production and recovery processes have to be optimized in order to compete economically with the chemical surfactants. Even though enormous information is available on the growing number of microorganisms that produce MEL with unique physicochemical properties, very less knowledge is available on the enzymes involved in each step of its synthesis. Its regulation, structure–function relationship, mechanism of its biochemical functions like differentiation on carcinoma cell lines are poorly understood and needs major research thrust.

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