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Minireview

Characteristics of mannosylerythritol lipids and their environmental potential

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ABSTRACT

Mannosylerythritol lipids (MELs) are promising biosurfactants containing two glycosyl derivatives and various fatty acids, which are mainly secreted by *Pseudozyma* as well as *Ustilago*. In this review, the latest research is demonstrated on production conditions, structural diversity, self-assembling properties and versatile biochemical functions of MELs. The genetic study and synthetic pathways, which mainly influence the type and yield of MELs production. Due to the excellent surface activity, biocompatibility and restorative function, MELs can be used in enviornmental industry, which has not been widely noted. In this paper, the current status of research on enviornmental potential of MELs has been discussed including petroleum degradation, bioconversion of chemical wastes and enhanced bioremediation of amphiphilic wastes.

1. Introduction

Mannosylerythritol lipids (MELs) are one of the most promising biosurfactants,[1] which were first described in 1956 by Boothroyd.[2] MELs are amphiphilic molecules with 4-O-β-D-mannopyranosyl-erythritol or 1-O-β-D-mannopyranosyl-erythritol as a hydrophilic headgroup and fatty acyl groups as the hydrophobic unit.[3–5] MELs usually have one or two acetyl groups at C-4′ and/or C-6′ of the mannose moiety. MEL-A is di-acetylated, whereas MEL-B and MEL-C are mono-acetylated at C-4′ and C-6′, respectively.[6,7]

MEL-A, MEL-B and MEL-C can be separated from microbial metabolites, which are produced by microorganism. However, a new type of MEL homologs having no acetyl groups, namely MEL-D, commonly can only be derived by enzymatic synthesis from MEL-B by *Pseudozyma tsukubaensis* or *Ustilago scitaminea*.[6,8] MELs are produced by fungal strains such as *Ustilago* sp[9,10] or *Pseudozyma* sp.[9,11] In addition, MELs are produced by *Pseudozyma* sp as relatively high quantities while they are produced as relatively low quantities by *Ustilago* sp.[12] The most common MEL producing species of the genus *Pseudozyma* are *Pseudozyma antarctica*, *Pseudozyma aphidis*, *Pseudozyma rugulosa*, and *Pseudozyma parantarctica*, which mainly produce diacetylated derivatives of MEL-A with small amounts of MEL-B and MEL-C.[13–15] Almost all vegetable oils (except palm oil and coconut oil) have been found to serve as a good carbon source for the production of MELs by various *Pseudozyma* sp. Soybean oil, olive oil, and safflower oil are the best carbon sources for bioproduction. *P. rugulosa* and *P. parantarctica* produced the most amount of MEL with soybean oil when compared to other vegetable oils tested (safflower oil, soybean oil, palm oil, corn oil, olive oil, rapeseed oil, and coconut oil).[14,16] On the other hand, the type of nitrogen source also considerably affected MEL formation. Sodium nitrate (0.3%, w/w) was clearly the best nitrogen source while ammonium nitrate and ammonium sulfate were not suitable for *Pseudozyma* sp to produce MELs.[17]

Because of their versatile biochemical actions as well as excellent interfacial properties as bio-based surfactants, MELs have been applied in many fields.[18] Their pharmaceutical potential applications are extensive,[19–21] such as differentiation-inducing activities against human leukemia cells,[22] rat peochromocytoma cells,[23] and mouse melanoma cells,[19,24,25] and inhibiting the secretion inflammatory mediators from mast cells.[21] They also can be used in the treatment of schizophrenia or diseases caused by dopamine metabolic dysfunction[19,26] and microbial infections.[18] Due to their high binding affinity, MELs are used in the purification of lectins

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and immunoglobulins.[27–31] In the preparation of ice-slurry, MELs became antiagglomeration agents.[32,33] In addition, MEL-A, acetylated at C-4′ and C-6′ dramatically increases the efficiency of gene transfection mediated by cationic liposomes.[34–38]

MELs' high biodegradability, mild production conditions and variety of functions would broaden their application in new technology areas,[3] especially in environmental protection. Previous studies focus on the excellent interfacial properties and high biodegradability of MELs to apply them in the biodegradation of petroleum compounds.[39] However, their self-assembling properties, repairing cells ability and being separated as bioconversion products from crude by fungi, which can be used in environmental protection are rarely reported. In this review, the latest progresses of research and advancement in MELs are summarized, and their environmental potential is also discussed.

2. Structural analysis of MELs

The structure of MELs contain two parts, i.e. sugar moiety and fatty acid profile. Variety of MELs arise due to three reasons as the number and the position of acetyls group on mannose, number of acyl groups in mannose and erythritol, and fatty acid chain length with their saturability.[12,17]

2.1. Sugar moiety analysis

In most cases, MELs contain 4-O-β-D-mannopyranosyl-erythritol as their sugar moiety or a hydrophilic unit. According to the degree of acetylation at C-4′ and C-6′ position in mannopyranosyl, MELs are classified as MEL-A, MEL-B, MEL-C and MEL-D (Figs. 1 and 2).[7] MEL-A represents the diacetylated compound whereas MEL-B and MEL-C are monacetylated at C-6′ and C-4′, respectively. The completely deacetylated structure is attributed to MEL-D.[40] However, a novel type of MEL was found by Morita et al., named as mono-acylated and tri-acetylated MEL, in which C-2′, C-4′, and C-6′ of mannopyranosyl are linked with OAc.[41] It is structurally and interfacially different from conventional MELs. When C-2′, C-4′, and C-6′ link with OH, it is another type of MEL called mono-acylated MEL (Fig. 3), which is one of microbial products by fungus from a glucose-rich medium.[42] While in the area of erythritol, Fukuoka et al.[43] discovered a diastereomer type of MEL-B. Its sugar moiety was identified to be 1-O-β-D-mannopyranosyl-erythritol, stereochemically different from the 4-O-β-D-mannopyranosyl-erythritol of conventional MELs. Moreover, in 2009, mannosyl-mannitol lipid, which possesses mannitol (C6 sugar alcohol) as the hydrophilic part instead of erythritol was reported by Morita et al.[11]

2.2. Fatty acid profile analysis

Bhattacharjee et al.[44] characterized MELs, which contain $C_{2:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$ and $C_{18:1}$ fatty acids as the hydrophobic groups. In 1983, Kawashima et al.[45] enriched a mutant of

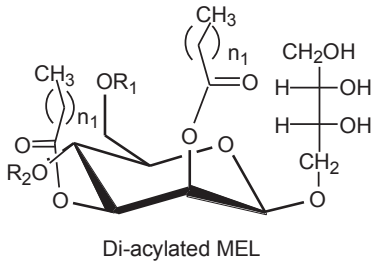


Fig. 1. Chemical structure of di-acylated mannosylerythritol lipids, MEL-A: R_1=Ac, R_2=Ac; MEL-B: R_1=Ac, R_2=H; MEL-C: R_1=H, R_2=Ac; MEL-D: R_1=H, R_2=H. n_1=4–16.

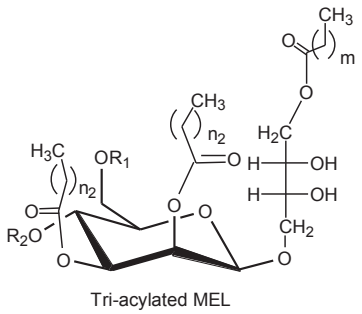


Fig. 2. Chemical structure of tri-acylated mannosylerythritol lipids, MEL-A: R_1=Ac, R_2=Ac; MEL-B: R_1=Ac, R_2=H; MEL-C: R_1=H, R_2=Ac; MEL-D: R_1=H, R_2=H. n_2=6–10, m=12–16.

Candida (*Pseudozyma*) sp in assimilated on n-alkanes. It was found that Strain B-7 extracellularly produce a biosurfactant, of which the acyl residues were analyzed to range from C_7–C_{14} fatty acids and to vary in their proportion with the carbon sources used. *P. antarctica* T-34 secreted MEL-A as major type, which was mainly composed of medium-chain acids, $C_{8:0}$ (27.26%), $C_{10:0}$ (21.38%) and $C_{10:1}$ (27.22%), respectively, from soybean oil.[14,46,47] MEL-A containing $C_{6:0}$ (19.59%), $C_{14:1}$ (42.92%) and $C_{16:1}$ (12.59%) as fatty acids profile was reported to be secreted by *Ustilago maydis* from glucose.[10] In 1980, Schizonellin A and B (similar to MEL-A and MEL-B) were first reported by Deml et al., which were produced by *Schizonella melanogramma* containing $C_{14:0}$, $C_{16:1}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ fatty acids.[48] A novel producer of MEL was identified as a *Kurtzmanomyces* species, strain I-11, which produced MEL-I-11, the fatty acids components were $C_{8:0}$ (36.4%), $C_{12:0}$ (11.9%) and $C_{14:2}$ (25.9%).[49] Table 1 demonstrates that the fatty acid profiles of MELs are in a great diversity with the variation of species (same genus) when one of MELs is produced as a major product. When MEL-A was produced as a major MEL, such as $C_{6:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{14:1}$ from *Candida* (*Pseudozyma*) sp.SY16,[50] $C_{8:0}$, $C_{10:0}$, and $C_{10:1}$ from *P. aphidis* DSM 70725,[40] $C_{8:0}$ (28.09%), $C_{10:0}$ (21.68%), and $C_{10:1}$ (22.94%) from *P. rugulosa* NBRC 10877,[14] and $C_{8:0}$ (34.7%), $C_{10:0}$ (10.7%), $C_{10:1}$ (10.9%), and $C_{12:0}$ (17%) from *P. fusiformata*.[51] MEL-C can also be the major MEL, *P. hubeiensis* KM-59 produced crude MELs containing MEL-C (65%) with $C_{6:0}$ (21.3%), $C_{10:0}$ (9.5%), $C_{12:0}$ (16.3%), and $C_{16:2}$ (30.3%).[13] *P. shanxensis* produced MEL-C as the major type MEL containing $C_{16:0}$, $C_{16:1}$, $C_{16:2}$, and $C_{14:1}$[52] and *P. graminicola* CBS 10092 secreted MEL-C as a higher percentage at 85% containing $C_{6:0}$, $C_{8:0}$, $C_{12:0}$, $C_{12:1}$, $C_{14:0}$, and $C_{14:1}$.[53] MEL-C mixture of monoacetylation and diacetylation were secreted by *P. siamensis* CBS 9960. This MEL-C possessed a short-chain acid (C_2 or C_4) at the C-2′ position, a long-chain acid ($C_{16:0}$ (23.5%), $C_{16:1}$ (12.5%), $C_{16:2}$ (16.6%)) at the C-3′ position of the mannose moiety and contained $C_{14:2}$ (32.6%) as the major fatty acid.[54]

In addition, monoacylated MEL possessed $C_{8:0}$ (11.9%), $C_{10:0}$ (24.6%), $C_{10:1}$ (8.0%), $C_{12:0}$ (19.1%), and $C_{14:0}$ (10.6%),[42] which was produced by *P. rugulos*, similar to those in conventional diacylated MELs as previously reported.[14] Triacylated MEL, which was

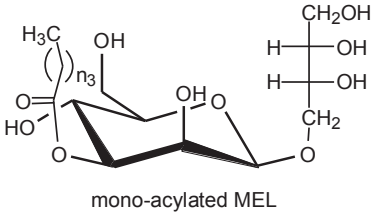


Fig. 3. Chemical structure of mono-acylated mannosylerythritol lipids, n_3=4–14.

Table 1
Fatty acid profiles of MELs in different species (same genus)

Main MEL	Species	C6	C8	C10	C12	C14	C16	C18	References
MEL-A	*Candida* sp.	+			+	+			50
	P. aphidis		+	+					40
	P. rugulosa		+	+					14
	P. fusiformata		+	+	+				51
MEL-C	*P. hubeiensis*	+		+	+		+		13
	P. shanxensis					+	+		52
	P. graminicola	+	+		+	+			53
	P. siamensis					+	+		54
Monoacylated MEL	*P. rugulosa*		+	+	+	+			42
Triacylated MEL	*P. antarctica*		+	+				+	55

+ fatty acid profiles containing Cn.

produced by *P. antarctica* contained $C_{8:0}$ (11.9%), $C_{10:0}$ (12.9%), $C_{10:1}$ (13.1%), $C_{18:0}$ (10.1%), and $C_{18:1}$ (37.5%).[55]

3. The production yield of MELs and their microbial conditions

Pseudozyma sp and *Ustilago* sp were both reported to secrete MELs (Table 2). The former can produce a large amount of MELs from different vegetable oils, even more than 100 g/L of the production yield by fed-batch culture using resting cells in large-scale production with a jar-fermenter.[14,15,40,47,51,56] By far, the highest yield of 165 g/L were produced by strain *P. aphidis* DSM 14930 with additional substrate-feeding (glucose, sodium nitrate, and yeast extract) and a foam-controlled soybean oil supply.[15] The products were mainly MEL-A (di-acetylated MEL) together with MEL-B and MEL-C.[40] When n-Alkanes ranging from C_{12}–C_{18} were converted into MELs by resting cells of *Pseudozyma (Candida) antarctica* T-34, the amount of MEL reached 140 g/L by intermittent feeding of the substrate, in which MEL-A was the main product together with MEL-B and MEL-C.[47] *P. parantarctica* JCM 11752 can also produce MELs (MEL-A (main), MEL-B, and MEL-C) with a concentration of approximately 106.7 g/L on a weight basis to soybean oil supplied.[51,56] *P. rugulosa* NBRC10877 was reported to be another high-yield MEL producer, which provided the yield of MELs (MEL-A (main), MEL-B, and MEL-C) at 142 g/L, using 8% soybean oil (w/v) and the adequate amount of erythritol added.[14] *P. antarctica* JCM 3941 produced a mixture of MELs (MEL-A (main), MEL-B, MEL-C), with the yield of 26 g/L from soybean oil.[18,51] *U. scitaminea* NBRC 32730 selectively produced 25.1 g/L of MEL-B from the juice (19.3% sugars) supplemented with 1 g/L urea in a jar fermenter at 25 °C over 7 days.[57] *P. tsukubaensis*1E5 (JCM 16987) was capable of producing the largest amount of the diastereomer MEL-B in vegetable oils with the maximum yield of 73.1 g/L under the optimal conditions.[58,59] *P. crassa* can produce three glycolipids, i.e. diastereomer MEL-A, MEL-B, and MEL-C, with the total amount of glycolipids approximately as 4.6 g/L in the glucose and oleic medium.[60] So far *P. hubeiensis* KM-59 efficiently produced MEL-C (65%) together with MEL-A and MEL-B, on fed-batch culture for 16 days on soybean oil, and the total yield reached 76.3 g/L.[61] *P. graminicola* CBS 10092 and *P. siamensis* CBS 9960 were also reported to produce mainly MEL-C from soybean medium, the total yield of the former approximately reached 10 g/L, while the latter's reached 18.5 g/L.[53,54] Both *Pseudozyma shanxiensis* CBS 10075 and *Ustilago cynodontis* NBRC 7530 produced selectively MEL-C in relatively minor yield under soybean oil condition.[16,52] *P. antarctica* T34 can also secrete small amounts of MEL-D from soybean oil.[46]

P. antarctica T-34 produced 1.3 g/L of the mono-acylated MEL from 10% (w/w) glucose.[42] Interestingly, the mono-acylated MEL was significantly produced from glucose but not from other carbon sources such as vegetable oils, fatty acid methyl esters, or fatty alcohols under the conditions employed.[42] A new type of MEL, namely tri-acylated MEL, was found and purified from the culture medium of *P. antarctica* (formerly *Candida antarctica*) T-34 as well as *P. rugulosa* NBRC 10877.[55] *P. parantarctica* JCM 11752 can also produce tri-acylated MEL when grew on the medium containing soybean oil with concentration higher than 8% (v/v), and the yield of new type reached 22.7 g/L.[56] A new MEL producer, *P. churashimaensis*, was isolated from sugarcane plant. *P. churashimaensis* OK 96 produced not only MEL-A, but also a novel type of MEL, mono-acylated/tri-acetylated MEL, namely MEL-A2, with the yield of 3.8±0.3 g/L from glucose (Fig. 4).[41] *P. parantarctica* JCM 11752 produces Mannosyl mannitol lipid, mannosyl–arabitol lipids and mannosyl–ribitol lipids in different condition.[11,62] From the manufacturing process of these unconventional MELs in Fig. 4, it is blindingly obvious that different microbials may secret alien MEL even using the same culture medium. In the meantime, identical microbial can produce different MEL while using different carbon source in the same medium. Finally, the carbon chain length and saturation variability of the carbon source affect the number of acylation and the length of unsaturated carbon chain in MEL output. For example, *P. antarctica* and *P. churashimaensis* secrete mono-acylated MEL and mono-acylated/tri-acetylated MEL using glucose ($C_6H_{12}O_6$), respectively. When *P. antarctica*, *P. parantartica* and *P. rugulosa* grow on soybean oil (mainly containing linoleic acid, $C_{18}H_{32}O_2$), they secrete tri-acylated MEL, which has long unsaturated carbon chains and is acylated at most compared to the MEL using glucose.

Table 2
The production yield of MELs and their microbial conditions in conventional MELs

Microorganism	Yield (g/L)	Carbon source	MEL-A	MEL-B	MEL-C	MEL-D	References
P. aphidis	165	Soybean oil	++	+	+		15
P. rugulosa	142	Soybean oil	++	+	+		14
P. antarctica	140	n-Alkanes	++	+	+		47
P. parantarctica	106.7	Soybean oil	++	+	+		51,56
P. hubeiensis	76.3	Soybean oil	+	+	++		61
P. tsukubaensis	73.1	Vegetable oil		+++			58
P. antarctica	26	Soybean oil	++	+	+	+	18
U. scitaminea	25.1	Juice		+++			57
P. siamensis	18.5	Soybean oil			+++		54
P. graminicola	10	Soybean oil			+++		53

+ minor product.
++ main product.
+++ selective product.

4. MELs: biosynthesis backgrounds and their enzymatic modification

There are three main biopathways to explain the synthesis of MEL, chain-shortening pathway based on *Candida (Pseudozyma)*

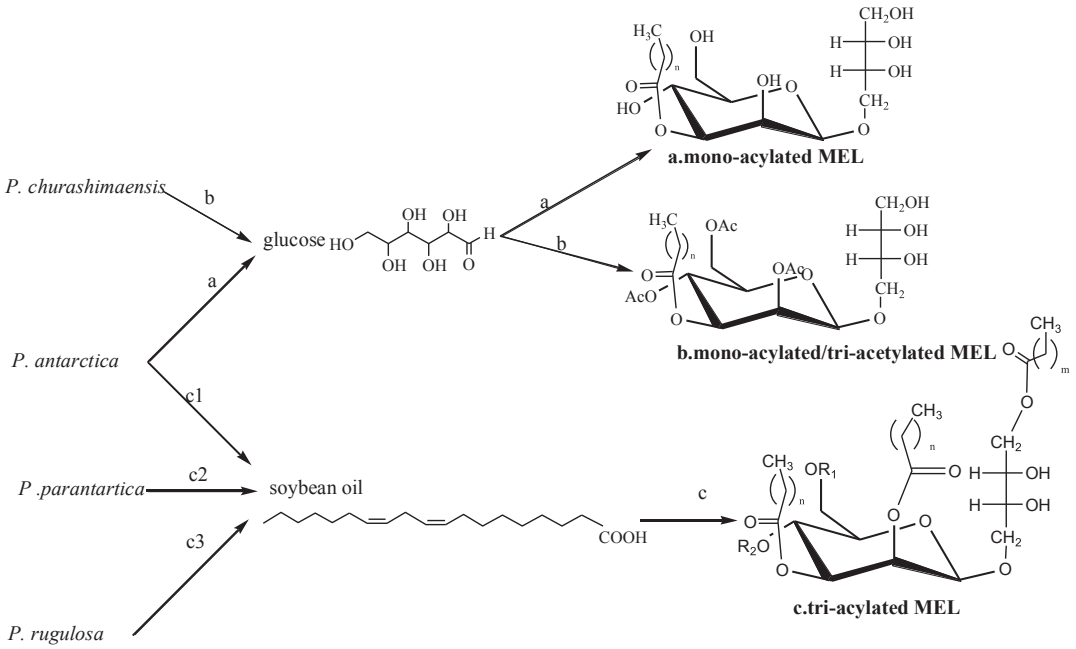


Fig. 4. Manufacturing process of unconventional MELs. a, *P. antarctica* secretes mono-acylated MEL in glucose medium. b, *P. churashimaensis* secretes mono-acylated/tri-acetylated MEL in glucose medium. c1, c2, and c3, *P. antarctica*, *P. parantartica* and *P. rugulosa* secrete tri-acylated MEL.

antarctica, gene synthesis route based on *U. maydis* and *P. antarctica*, and enzymatic modification based on Lipase-catalyzed reactions (deacetylation and acylation).

4.1. Chain-shortening pathway

There are three biosynthetic pathways of fatty acids in alkane or fatty acid utilizable microorganism, (I) *denovo* synthesis pathway followed by β-oxidation, (II) chain elongation pathway, and (III) intact incorporation pathway (Scheme 1).[63] When fatty alcohols or acids of chain length of C_n were used, the products of MELs were formed with the chain length of C_{n-2}, C_{n-4} and C_{n-6}. So it is concluded that the products were the β-oxidation intermediates of the substrates supplied.[17] Since most of the fatty acids are β-oxidation intermediates of the substrate from pathway (II). Pathway (III) certainly have little relation to MEL synthesis. By the way, Kitamota et al. examined the effect of cerulenin, a strong inhibitor of pathway (I), on MEL synthesis, and it demonstrated that pathway (I) has little contribution to MEL synthesis.[17,63] Therefore it is assumed that the chain-shortening pathway, which is distinct from these known pathways and 'complete boxidation', participates in MEL synthesis.[63] However, the detailed mechanism of the 'Chain-shortening pathway' is still unknown.

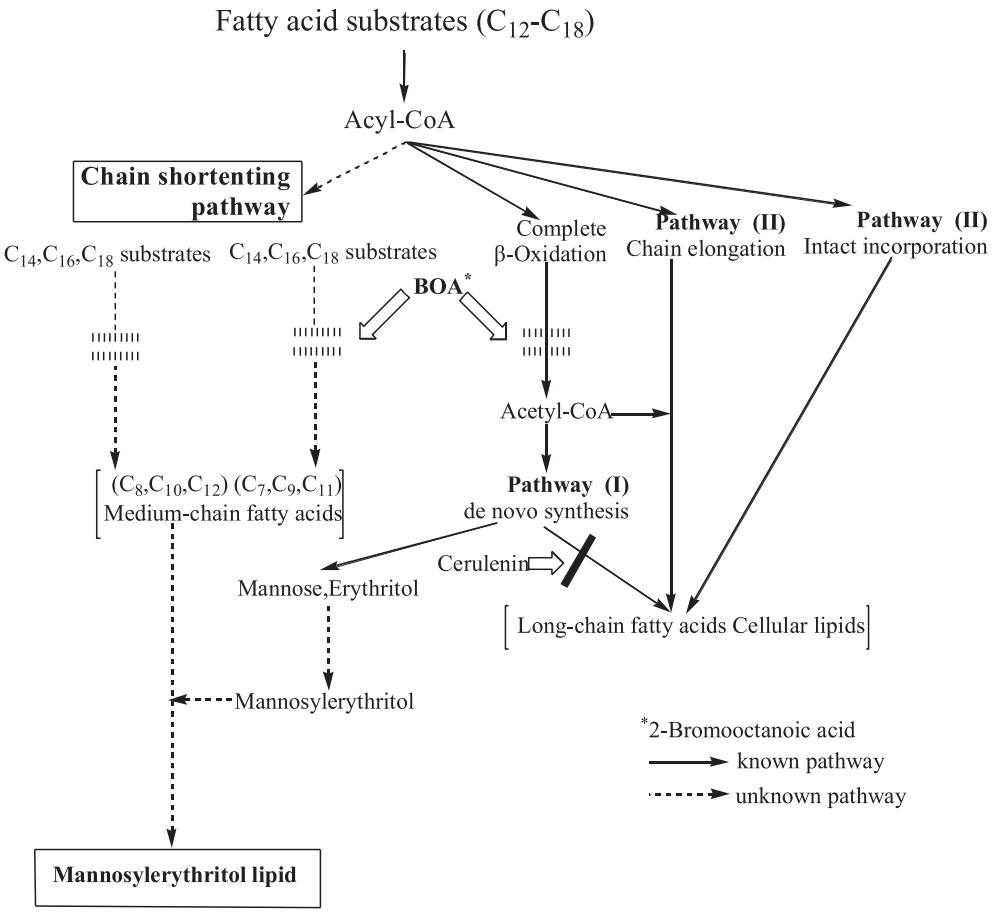
4.2. Gene synthesis route

Because MELs were first isolated from the dimorphic fungus *U. maydis* as extracellular oil with a higher density than water,[2] the genes responsible for MEL biosynthesis were initially identified on a dimorphic basidiomycet *U. maydis*,[3] which produces large amounts of MELs under condition of nitrogen starvation.[64] Hewald et al. reported the first identification of the gene, *emt1*, which is essential for production of fungal extracelluar MEL,[64] and it is part of a gene cluster comprising five open reading frames.[65] There are three identified proteins called Mac1, Mac2, and Mat1, containing short sequence motifs characteristic for acyl- and acetyltransferases. The biosynthetic of MELs may be the three steps (Fig. 5).[65] First, Emt1 catalyzes the synthesis of mannosyl-D-erythritol by transfer of GDP-mannose. Then, Mac1 and Mac2 are proposed to transfer short- and medium-chain fatty acids to positions C-2 and C-3 of mannose. Finally, acetylation of deacetylated MEL at positions C-4′ and C-6′ is catalyzed by a single enzyme, Mat1.[65]

Another bioproducer of MELs, *P. antarctica* shows the similarity genotype, according to expressed sequence tag (EST) analysis and estimated genes expressing under MEL production conditions.[67] A contiguous sequence of 938 bp, PA_004, showed high sequence identity (72%) to the gene *emt1* from *U. maydis*, namely *PaEMT1*.[66,67] The obtained △*PaEMT1* (using hygromycin B resistance) strain failed to produce MELs, while its growth was the same as that of the parental strain.[66] A gene homologous with a mitochondrial ADP/ATP carrier was dominantly expressed in *P. antarctica* under MEL-producing conditions on the basis of previous gene expression analysis, namely *PaAAC1*, it is suggested that *PaAAC1* encoding a mitochondrial ADP/ATP carrier should be involved in MEL biosynthesis in the yeast.[67] While *PaAAC1* only contributes to ATP transport activity, the relationship between *PaAAC1* and *PaEMT1* is still unclear.

4.3. Enzymic modification of MELs

Though MEL-D can be produced by a bioproducer *P. antarctica* T34, commonly it is synthesized by lipase-catalyzed hydrolysis of acetyl groups from a known MEL.[8,58] An immobilized lipase, Novozym 435, served as a catalyst and tried to selectively hydrolyze the acetyl groups of MEL-A and MEL-B. In the reaction using MEL-A (Fig. 6a), more hydrophilic glycolipid compared to the starting MEL-A was partially obtained.[8] The product was MEL-C containing one acetyl group at the C4-position, not MEL-D. When MEL-B with one acetyl group at C6-position was used as a starting material (Fig. 6b), the starting MEL-B almost disappeared and the MEL-D was produced at a yield of 99.1%.[8] In further study, diastereomer MEL-D was synthesized by lipase-catalyzed hydrolysis from diastereomer MEL-B (Fig. 6c).[58]



Scheme 1. Presumptive chain-shortening pathway of MELs.[63]

Conventional di-acetylated MEL-A and MEL-B can also be modified by lipase-catalysis using uncommon fatty acids from other microbial glycolipids, such as 3-hydroxydecanoic acid from rhamnolipids and 17-hydroxyoctadecanoic acid from classical sophorolipids.[4] The reaction was performed in organic solvents and yielded functionalized products at the C-1 position of the erythritol (Fig. 7). Except for the differential physicochemical characterization, the novel compounds inhibited the growth of gram-positive bacteria and showed a potential for anti-tumor-promoting activity.[4]

5. Physicochemical aspects of MELs

5.1. Interfacial properties

MEL-A, MEL-B and MEL-C show excellent surface tension-lowering ability and low critical micelle concentrations (CMC) (Table 3).[18,53,54,61] Those of MEL-A produced by *P. antarctica* T-34 were 2.7×10^{-6} M and 28.4 mN/m, respectively, while those of MEL-B were 4.5×10^{-6} M and 28.2 mN/m, respectively.[18] However, MEL-C shows different critical micelle concentration and surface tension-lowering actions. The estimated critical micelle concentration (CMC) and surface tension at CMC(γCMC) of MEL-C purified from *P. hubeiensis* KM-59 were 6.0×10^{-6} M and 25.1 mN/m, respectively.[61] The CMC and the surface-tension at CMC of MEL-C, which was produced by *P. graminicola* CBS 10092 were 4.0×10^{-6} M and 24.2 mN/m, respectively.[53] As for *P. siamensis* CBS 9960, the estimated critical micelle concentration (CMC) and surface tension at CMC (γCMC) of the present MEL-C were 4.5×10^{-6} M and 30.7 mN/m, respectively.[54] MEL-D showed a higher critical aggregation concentration (CAC) as 1.2×10^{-5} M and hydrophilicity compared to known MELs, retaining an excellent surface tension lowering activity.[8] CAC and γCMC are different between S- and R-MEL-D (Table 4).[58] In conclusion, when C-4′ or (and) C-6′ in mannopyranosyl is (are) substituted by OH, MEL exhibits higher hydrophilic character, which leads to an increase of the CMC and to decrease of the corresponding γCMC. Simultaneously, these parameters are also related to fatty acids (hydrophobic group).

In contrast to Tween 80 (polyoxyethylene sorbitan monooleate), the emulsifying activity of MEL-A towards soybean oil and n-tetradecane is much higher.[1] At the air–water interface experience, the molecular occupation area of MEL-A is approximately 60 $Å^2$/molecule.[1,33] Therefore it shows an excellent packing property despite the bulky structure. In addition, MELs provide the rheological characteristics of flour products like bread, and its carbohydrate backbone, mannosylerythritol, has a moisturizing effect.[33]

In the past, applying W/O microemulsion in various fields, the cosurfactant such as alcohol had to be added into surfactant to generate microemulsions except a few surfactants such as sodium bis (2-ethylhexyl) sulfosuccinate (AOT) or soybean lecithin.[68,69] It is reported that the formation of water-in-oil (W/O) microemulsion based on the single component of MEL-A was confirmed using dynamic light scattering (DLS) and freeze fracture electron microscopy (FF-EM).[68–70] The diameter of the microemulsion range from 20 to 60 nm.[69,70] The maximum W/O value was found to be 20, which is as high as that of soybean lecithin.[70] When n-decane was used as an oil phase, diacetylated MEL-A formed single-phase W/O microemulsion in a remarkably large region without any other

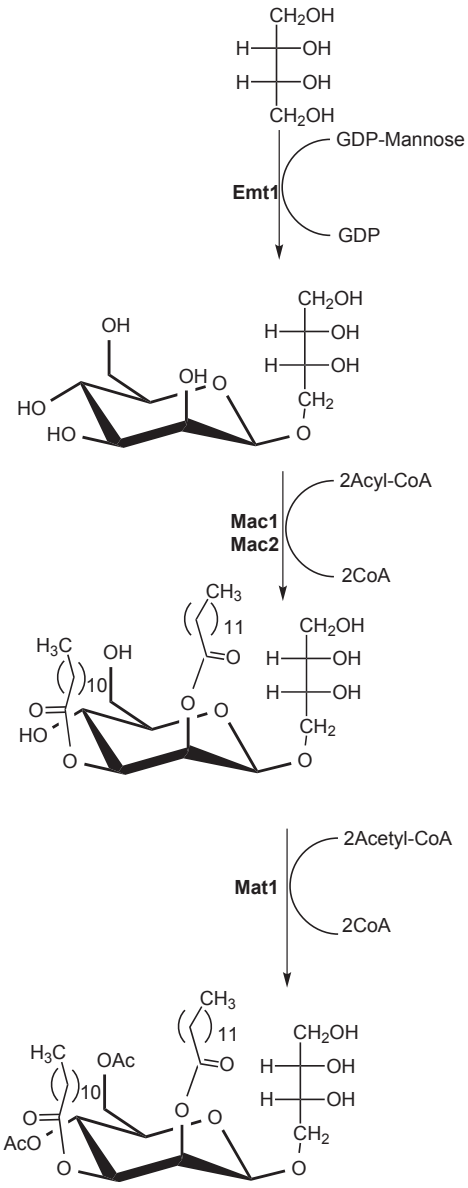


Fig. 5. Tentative biosynthetic pathway of mannosylerythritol lipids in *Ustilago maydis*.[65]

additives. Meanwhile, monoacetylated MEL-B with an almost zero spontaneous curvature gave single-phase bicontinuous microemulsion. Moreover, Worakitkanchanakul et al. have succeeded in preparation of O/LC emulsion in the biphasic Lα+O region of the MEL-B/water/n-decane system.[70,71] The obtained gel-like emulsion was stable for at least 1 month. In conclusion these MELs would be quite distinctive from conventional biosurfactants hitherto reported, and would have great potential or the preparation of microemulsion and LC-based emulsion.[71]

5.2. Self-assembling properties

In particular, conventional MEL-B, a monoacetyl derivative of MELs produced by *P. antarctica* at fairly low yield of product, was found to spontaneously form giant vesicles with a diameter of 1–20 μm upon mixing with water at remarkably low concentration.[72,73] The new diastereomer MEL-B was found to self-assemble into a lamellar (L_α) phase over remarkably wide concentration and temperature ranges.[74] Furthermore, Worakitkanchanakul et al. found the relatively large vesicles (1–5 μm) at the low MEL-B concentration with two-phased region using CLSM (confocal laser scanning microscopy) observation.[74] MEL-C can also spontaneously form giant unilamellar vesicles of diameter larger than 10 μm.[1] In contrast, MEL-D was found to form reverse vesicles without co-surfactants and co-solvent in various oily solutions, such as n-alkanes, cyclohexane, squalane, squalene, and silicone oils at a concentration below 10 mM.[75]

Generally, coacervates, including 'simple coacervates'[76] and 'complex coacervates',[77] are prepared from complicated multicomponent systems such as surfactants with salt/cosolvent or two oppositely charged polyelectrolytes.[78] This makes their structural characterization difficult. Interestingly, self-assembling products of MEL-A were described for the first time as simple coacervate formation from a single 'natural' glycolipid biosurfactant.[73] Moreover MEL-A was found to selfassemble into a variety of distinctive lyotropic liquid crystals including L_3, bicontinuous V_2, and L_α phases over a wide concentration range, especially.[72] The MEL-A L_3 region is spread considerably over a wide temperature range (20–65 °C) compared to L_3 of those relatively hydrophobic poly (oxyethylene) or fluorinated surfactants.[72] This may also demonstrate that only a slight decrease in spontaneous curvature resulting from the absence of the 4′-O-acetyl group induces a drastic morphological change in the self-assembled structure from coacervates to vesicles, ordered bilayer membranes.[75]

6. Environmental potential of MELs

Biosurfactants application in environmental industries are promising due to their biodegrability, low toxicity and effectiveness in enhancing biodegradation and solubilization of low solubility compounds.[79] MELs show not only excellent interfacial properties but also versatile biochemical actions, which can be used for cold thermal storage soil remediation,[79] cell cycle regulation,[33,80] lectin binding,[29] immunoglobulin sensing,[27,28,30] gene delivery,[35,37,38,81,82] and skin care.[3] However, the enviornmental potential of MELs was rarely reported. In this paper, the environmental benefits of MELs were discussed including the following aspects, which were not confined to applications alone.

6.1. Application of MELs on petroleum hydrocarbons

The various components of petroleum hydrocarbons are alkanes, cycloalkanes, aromatics, polycyclic aromatic hydrocarbons, asphaltenes, and resins. Although alkanes, represented by the formula C_nH_{2n+2} (where n is the number of carbons and $2n+2$ is the number of hydrogens), can have many isomers as the number of carbons increase, relatively few exist in petroleum. They are sometimes referred to as aliphatic compounds. It was reported that low molecular weight alkanes are the most easily degraded by microorganisms.[79] Previous studies have investigated the effect of rhamnolipids on biodegradation of organic contaminants. Beal and Betts[83] showed that the cell surface hydrophobicity increased by the biosurfactant strain more than a non-biosurfactant producing strain during growth on hexadecane. The rhamnolipids also increased the solubility of the hexadecane from 1.8 to 22.8 mg/L. There are two possible mechanisms for enhancing biodegradation, increasing solubility of the substrate for the microbial cells, and interactions with the cell surface, which increase the hydrophobicity of the surface allowing hydrophobic substrates to associate more easily.[84–86] MELs have the similar amphiphilic structure to that of rhamnolipids. Moreover, MELs show lower critical micelle concentration and higher production, which may demonstrate more efficient utilization. It was reported *Candida antarctica* T-34

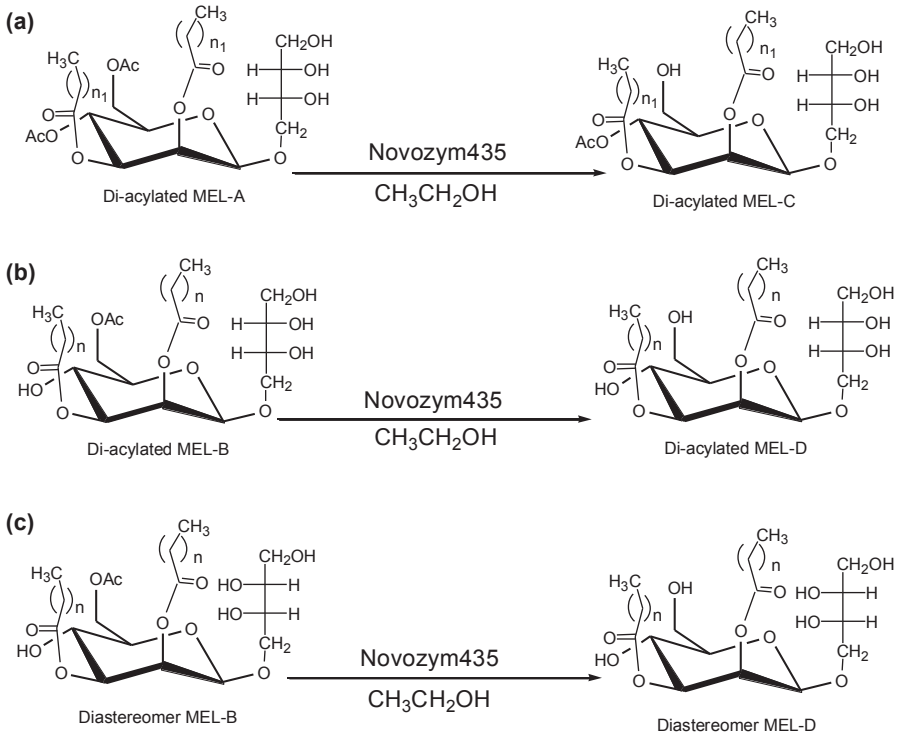


Fig. 6. Lipase-catalyzed processes by Novozym 435. (a) MEL-A, (b) MEL-B and (c) Diastereomer MEL-B.

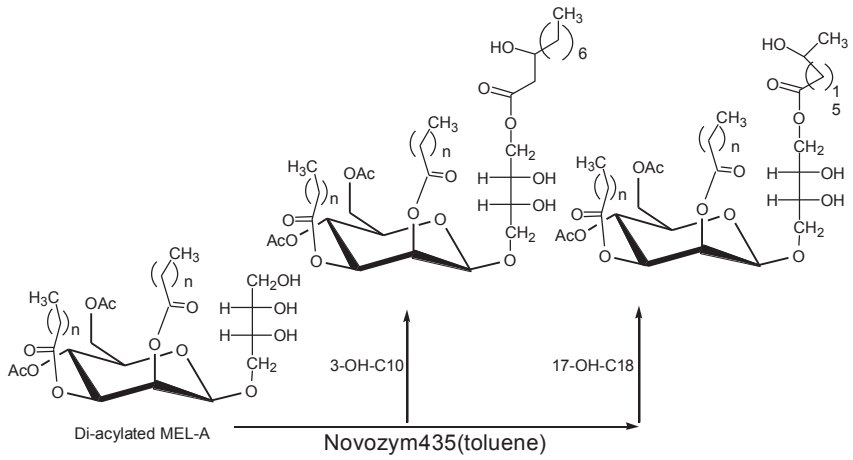


Fig. 7. Lipase-catalyzed acylation of MEL-A. (a) 3-OH-decanoic acid and (b) 17-OH-stearic acid. n=6–12 (7 dominant).

could produce extracellular biosurfactant mannosylerythritol lipids (MELs) when it was cultured in vegetable oil or *n*-undecane ($C_{11}H_{24}$).[47] The highest degradation rate of kerosene by addition of MEL and BS-UC reached 87% and 90% at 15 h, respectively, at the concentration of crude oil as 8%.[39] On the other hand, MEL in the process of biotreatment could also enhance the emulsification of hydrocarbon in water.[39] It is suggested that MELs could be used for the degradation of petroleum compounds instead of chemical synthetic surfactants, thus will reduce the environmental pollution. At present, MELs can be produced with yields >100 g/L.[87] Thus MELs have potential commercial advantages.

Table 3
Surface tension values of MEL-A, MEL-B, and MEL-C and corresponding microorganisms

Type of MEL	Microorganism	CMC (M)	γCMC (mN/m)	References
MEL-A	*P. antarctica*	2.7×10^{-6}	28.4	18
MEL-B	*P. antarctica*	4.5×10^{-6}	28.2	18
MEL-C	*P. hubeiensis*	6.0×10^{-6}	25.1	61
	P. graminicola	4.0×10^{-6}	24.2	53
	P. siamensis	4.5×10^{-6}	30.7	54

Table 4
Surface tension values and fatty acid profiles of MELs[58]

	CAC(M)	γCAC (mN/m)	C8:0 (%)	C12:0 (%)	C12:1 (%)	C14:1 (%)
S-MEL-D	7.1×10^{-6}	24.2	29.4	13.1	18.8	16.9
R-MEL-D	1.2×10^{-5}	24.6	33.5	12.5	16.4	18.9

6.2. Efficient microbial conversion of chemical wastes to MELs

The amount of waste has been increasing year by year through the increasing production of biodiesel and other oleochemicals.[88] Thus the utilization of waste is becoming very important for environmental conservation, especially waste glycerol, which is successfully used as the water soluble carbon source for different microbial productions.[89–91] The previous studies manifested that soybean oil is the best substrate for MEL production.[47] However, in the case of soybean oil, the complicated separation of MELs is inevitable, because of coexisting by-products such as free fatty acids and mono- or diacylglycerols. The use of glycerol would thus be considerably advantageous for improving the large-scale production of MELs, compared with the use of vegetable oils.[92] *P. antarctica*, *P. parantarctica* and *P. rugulosaare* were high-level MEL producers when grown on soybean oil.[14,51] *P. antarctica* JCM 10317^T significantly produced MEL when grown on glycerol, with the yield of 16.3 g/L by intermittent feeding condition.[92] Although the yield is still relatively low, it can be improved using a large-scale fermentor with a larger number of resting cells. The application of economic technologies based on utilization of waste substrates for MELs production may significantly contribute to cost reduction[93] and easing pressure on the environment. Further studies of genomic analytical methods and the development of gene expression systems for the genus *Pseudozyma* should thus help to improve MELs production from glycerol.[92] On the other hand, we should expand sorts of wastes substrates for biosurfactant production.

6.3. Potential enhanced bioremediation of MELs for amphiphilic toxicants

Bioremediation, which uses biological systems to catalyze degradation or transformation of these recalcitrant molecules to less toxic or nontoxic compounds is attracting wide attention to purify the environment.[94] This technique is environment friendly and sustainable.[101,102] The common approaches to bioremediation are basic, (1) intrinsic bioremediation, (2) biostimulation, and (3) bioaugmentation.[103–105] With it, microbes endowed with inherent abilities to live, metabolize, thrive, and colonize. Several bioremediation technologies were developed: (i) Enhanced natural attenuation, (ii) Biopiles, (iii) Composting, and (iv) Sequential A/O treatment. Among those, composting technology has broad application prospect because of effectively reducing the environmental pollution of organic solid waste, which is seriously polluted by organic wastes from factories, agriculture and garbage from cities. However, the presence of an amount of amphiphilic toxicants may do harm to microorganisms. Typically, phenolic pollutants are a potential threat to human health as well as microorganisms.[95] They, kind of recalcitrant compounds, exert toxicity to microorganisms during biological treatment and lead to failure of the whole composting treatment if the microbial flora is not adaptable to phenol because their toxic nature can disrupt the cell membrane and enzyme system.[96] The previous studies indicated that pretreatments with rhamnolipid increased adsorption of phenol by *P. simplicissimum*.[97] In addition, on the degradation of phenol by *C. tropicalis* in aqueous solution, mono-rhamnolipid not only diminished the cell toxicity of phenol, but also improved the cell growth and the removal of phenol.[98] Recently, it was reported that MELs have the similar amphiphilic structure to that of ceramide-3,[3] which is an essential component of the intracellular lipids of stratum corneum, and efficiently form various lyotropic liquid crystals including the lamellar phase.[1] Thus MELs can be used to repair the damaged cell membranes, which are exposed to SDS,[99] phenol and any other toxicants. On the other hand, because of the high yield and excellent interfacial properties of MELs compared to rhamnolipids, it is likely more efficient for MELs to enhance adsorption of phenol by microoragnisms[100] and combine with phenolic compounds. So it indicates the potential for MELs in the application of composting bioremediation as restorative agents for microbes and enhanced additives in the application of biodegradation.

7. Recommendations for future research

More than a half century, the three most promising microbial surfactants have been reported, sophorolipids produced by *Candida* yeasts, mannosylerythritol lipids (MELs) produced by *Pseudozyma* yeasts, and rhamnolipids produced by *Pseudomonas*.[87] Especially over the past two decade, the researchers expanded the structure and functional variety of MELs based on the advanced microbial screening methods, which makes the environmental use and commercial application of MELs possible.

MELs own the excellent interfacial and biochemical properties and restorative function for cells. These make MELs become new biosurfactant substituting for rhamnolipids on environmental protection if the cost of the separation and purification can be reduced even further. In future research, the breakthrough of MELs metabolite governed by three basic factors can be pursued: (i) initial cheap substrates; (ii) fast, efficient and cheap product recovery of downstream processing: and (iii) novel strains from isolation. So that MELs might work on a large scale in polluted water and contaminated soil.

Interestingly, MELs show strong activity against gram positive bacteria, weak activity against gram negative bacteria and no activity against fungi,[18,48] which makes MELs be a double-edged sword. One side, MELs may inhibit the growth and metabolizing of the heterogeneous population whereas the target microorganisms will fully utilize resources in the whole biodegradation process. From another point of view, the additive MELs can also affect the instrumental microbe mass or other enzymes, and the influence of subsequent processing is still unknown. So it should be done to determine the role of cell wall components (*Pseudozyma* sp.) to understand how they tolerate high levels of MELs and establish mechanisms of antimicrobial and antitumor activities of MELs.

In addition, the application of MEL as a transporter for drug and gene delivery was rarely reported. This may constrain the development of the genetic engineering microbials.

8. Conclusion

MEL is considered as a promising biosurfactant because of its excellent surface tension properties, wide applications, biocompatibility and biodegradability. It is also used as an important taxonomic index to identify *Pseudozyma* yeast. MELs have various homologs and versatile characteristics, which can easily facilitate biodegradation by influencing the bioavailability of the contaminant. Their unique properties differ them from conventionally implemented biosurfactants like rhamnolipids and sophorolipids. Though the mechanism of their biochemical function, structure–activity relationship, synthesis pathway are still unknown or unclear that do not seem to be any major impediments to the utilization of MELs in a wide range of productions and applications within the next few years.

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