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Note

A basidiomycetous yeast, *Pseudozyma tsukubaensis*, efficiently produces a novel glycolipid biosurfactant. The identification of a new diastereomer of mannosylerythritol lipid-B

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Abstract—Mannosylerythritol lipids (MELs) are glycolipid biosurfactants produced by the yeast strains of the genus *Pseudozyma*. These compounds show not only excellent surface-active properties but also versatile biochemical activities. In the course of MEL production by *Pseudozyma tsukubaensis*, we found an unusual MEL that had a different carbohydrate structure from that of conventional MELs. The carbohydrate structure was identified as 1-*O*-β-D-mannopyranosyl-D-erythritol, and the MEL was confirmed to be 1-*O*-β-(2′,3′-di-*O*-alka(e)noyl-6′-*O*-acetyl-D-mannopyranosyl)-D-erythritol. Interestingly, the configuration of the erythritol moiety in the present MEL was opposite to that of the known MEL-B, 4-*O*-β-(2′,3′-di-*O*-alka(e)noyl-6′-*O*-acetyl-D-mannopyranosyl)-D-erythritol, and to that of all MELs hitherto reported. The present MEL should thus provide different interfacial and biochemical properties compared to conventional MELs.

Keywords: Biosurfactant; Glycolipid; Mannosylerythritol lipid; *Pseudozyma* yeasts; *Pseudozyma tsukubaensis*

Mannosylerythritol lipids (MELs, Fig. 1) are one of the most promising biosurfactants,[1–3] which are surface-active compounds secreted by various microorganisms. MELs are produced in large amounts (over 100 g/L) from vegetable oils by the yeast strains belonging to the genus *Pseudozyma*.[4–6] These compounds exhibit not only excellent surface-active properties,[7] but also versatile biochemical activity,[2,8–14] including the induction of cell-differentiation of different mammalian cells[11] as well as affinity binding toward different immunoglobulins.[8,13,14] MELs have thus great potential as environmentally friendly and advanced materials that can be manufactured from renewable resources.

All hitherto known MELs, namely MEL-A, -B, and -C, consist of 4-*O*-β-D-mannopyranosyl-D-erythritol as the hydrophilic part and two fatty acyl groups as the hydrophobic part (Fig. 1). Each homolog has one or two acetyl groups at C-4′ and/or C-6′ in the mannose moiety. Interestingly, these compounds show specific phase behavior and different self-assembled structures

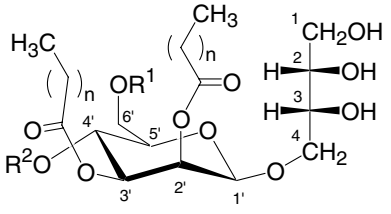

Figure 1. Chemical structures of conventional mannosylerythritol lipids.

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in aqueous solution,[15–18] although the difference in the chemical structure is very small. For instance, MEL-A spontaneously forms an L_3 (sponge) phase in a wide range of concentrations, while MEL-B forms L_α (lamellar) and myelin structures.

As described above, even a slight difference in chemical structure brings about drastic changes in the interfacial properties of MELs. We thus have focused our attention on the identification of structural variants of MELs, and have studied the products of different strains of the genus *Pseudozyma*. Very recently, we found novel types of MELs, namely the mono-acylated[19] and tri-acylated MEL,[20] which have different hydrophobic structures and show different hydrophilicities compared to the conventional di-acylated MELs. Moreover, we have reported that newly isolated *Pseudozyma* strains selectively produce each MEL homolog, MEL-A, -B, and -C, respectively.[21] These studies will facilitate the research and development of MELs because selective production will make the downstream process more simple and cost-effective.

Among MEL-producing yeasts, we have concentrated on *Pseudozyma tsukubaensis*[21,22] due to its unique production features. Interestingly, it efficiently produces only a glycolipid similar to MEL-B, while many other *Pseudozyma* strains produce a mixture of different MEL homologs. MEL-B shows higher hydrophilicity and critical micelle concentration than MEL-A, and thus is advantageous for the use as water-in-oil type emulsifiers and/or washing detergents.[17] Therefore, we investigated the production conditions and structure of the glycolipid produced by *P. tsukubaensis*. Surprisingly, the structure of the carbohydrate moiety was different from that of the hitherto known MELs. Here we describe for the first time a new diastereomer of the known MEL-B.

In the known high-level MEL producers such as *Pseudozyma antarctica*,[4] *Pseudozyma aphidis*,[5] and *Pseudozyma rugulosa*,[6] MEL-A is produced in the largest amount and comprises more than 70% of the total MELs. It is thus difficult to isolate MEL-B in abundance from the culture broth of these yeasts, because the polarity of MEL-B is intermediate among the three MEL homologs and is present only in very small amount. In contrast, *P. tsukubaensis* JCM10324^T and *Pseudozyma* sp. KM-160 selectively produce only a single glycolipid product, which corresponds to MEL-B, in high yield (more than 25 g/L from 4% (w/v) of soybean oil).[21,22] These strains should thus be potential MEL-B producers.

In our previous study, the glycolipid produced by *P. tsukubaensis* was tentatively identified as MEL-B, based on ^{1}H and ^{13}C NMR data that showed very similar spectra to those of purified MEL-B prepared from soybean oil by *P. antarctica*. However, when the spectra were compared more in detail, the glycolipid had different peak patterns in the part of the carbohydrate moiety (Fig. 2). For example, the two resonances arising from the H-4a and H-4b in the erythritol (~3.8–4.1 ppm) were widely separated in the product produced from

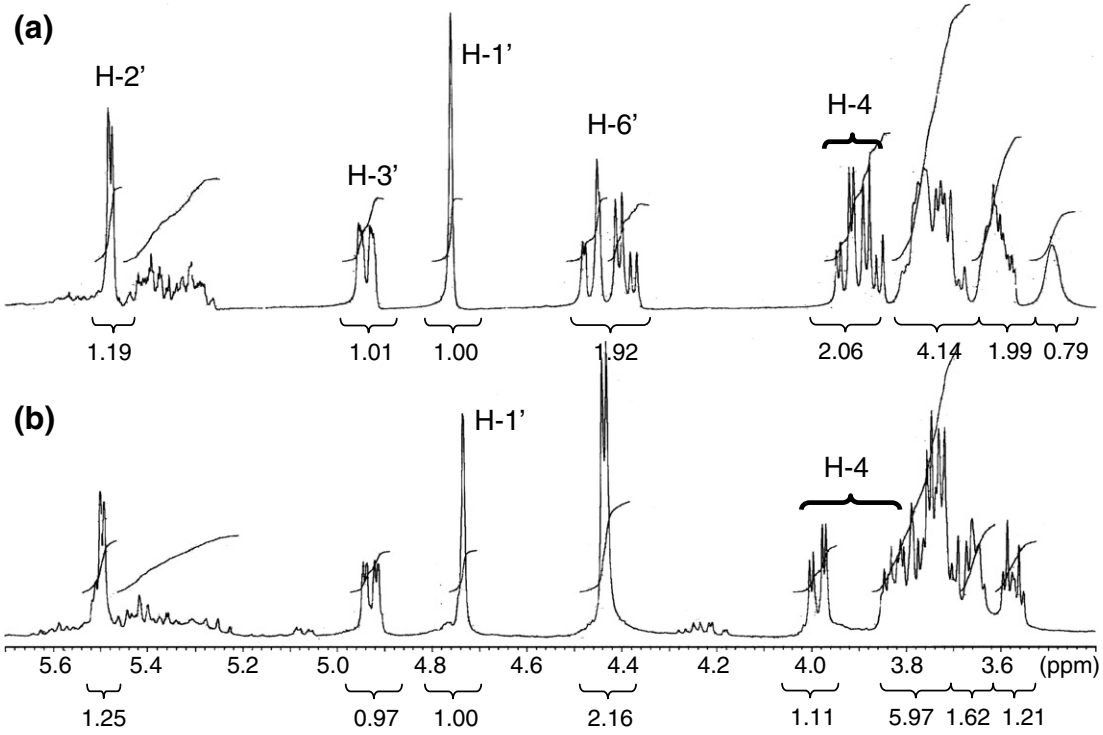

Figure 2. Partial ^{1}H NMR spectra of: (a) the glycolipid produced by *P. tsukubaensis*, and (b) MEL-B produced by *P. antarctica*.