

BIOCONVERSION OF PALM OLEIN INTO MANNOSYLERYTHRITOL LIPIDS BY *Pseudozyma antarctica* AND *Ustilago maydis*

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ABSTRACT

Mannosylerythritol lipids (MELs) are glycolipid biosurfactants with versatile applications in green chemistry, personal care and bioremediation, owing to their excellent surface activity and biosustainability. Despite their potential, a direct comparative evaluation of MEL production by *Pseudozyma antarctica* and *Ustilago maydis* using a sustainable carbon source such as palm olein has not been reported. This study addresses this gap by cultivating both strains under optimised submerged fermentation conditions over 10 days to assess species-dependent differences in yield and product characteristics. The MELs were isolated by solvent extraction and further purified by column chromatography. A comprehensive characterisation of the biosurfactants was performed using surface tension measurements, liquid chromatography-mass spectrometry (LC-MS), Fourier transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR). The results showed that *P. antarctica* produced a significantly higher MELs yield (58.37 g/L) compared to *U. maydis* (26.77 g/L). The purified MELs showed a critical micelle concentration (CMC) of 2.8 and 2.6 mg/mL, respectively, confirming their strong surfactant properties. The LC-MS and NMR analyses tentatively confirmed the presence of MEL homologs with fatty acid chains from C6 to C20. These results emphasise the feasibility of using palm olein as an effective and renewable substrate for MELs production and establish their applicability as sustainable, bio-based alternatives to conventional synthetic surfactants.

Keywords: glycolipid biosurfactants, mannosylerythritol lipid, palm olein, yeast.

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INTRODUCTION

Surfactants are amphiphilic molecules with a nonpolar tail and a polar head group and can interact with polar and apolar compounds (Khalfallah, 2023). This unique property enables surfactants to adsorb at various interfaces, lower the surface tension between two phases and create various self-supporting structures, making them valuable for a wide range of applications such as domestic cleaning agents, detergents, personal care, pharmaceuticals, leather processing, paper, textiles, chemicals and food formulations (Nascimento et al., 2022). Surfactants play a

crucial role in various industrial applications. However, most commercial surfactants are derived from petroleum, contributing to environmental pollution and concerns about resource depletion. In response to these challenges, researchers have been exploring alternatives that can replace conventional petroleum-derived products. Biosurfactants produced by living organisms have been identified as promising candidates due to their unique properties and environmental benefits (Lackner et al., 2023).

Biosurfactants, amphiphilic compounds biochemically made by various microorganisms including bacteria, filamentous fungi and yeasts cultivated with diverse carbon substrates (Fardami et al., 2022; Sharma et al., 2025) are emerging as sustainable alternatives to chemically synthesised surfactants. Driven by rising customer demand for sustainable products and environmental concerns

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(Nagtode et al., 2023). Biosurfactants offer broad application potential in various sectors, including pharmaceuticals, cosmetics, food processing, household, agriculture, and metal treatment. Biosurfactants are classified into four main groups: (i) Glycolipids, (ii) lipopeptides, (iii) phospholipids and (iv) polysaccharide-protein complexes. Among these, glycolipids are the most studied. They include three important types of surfactants: (i) Sophorolipids, (ii) rhamnolipids and (iii) mannosylerythritol lipids (MELs).

MELs are nonionic glycolipids produced by certain bacteria and yeasts, such as *Candida* species, as secondary metabolites (Liu et al., 2024). MELs contain a hydrophilic moiety composed of a mannose sugar and an erythritol backbone, linked to fatty acid chains that contribute to their amphiphilic properties (Tiefenbacher et al., 2025). Based on structural variations in their acylation and glycosylation patterns, MELs are classified into four major types: MEL-A, MEL-B, MEL-C and MEL-D. MEL-A and MEL-B contain acetyl groups on the mannose unit, with MEL-A diacetylated and MEL-B monoacetylated, whereas MEL-C and MEL-D lack acetyl groups and differ in their fatty acid compositions (Figure 1). The structure of MELs influences their physical and chemical properties, including their critical micelle concentration (CMC), surface activity and emulsifying ability. The presence of the mannose moiety, for example, improves their biocompatibility and biodegradability, making them suitable for applications in the food and medical industries where safety is paramount. This biodegradability is attributed to the mannose and erythritol components, which are readily metabolised by microorganisms (Pană et al., 2023). MELs have also been shown to have antimicrobial and antifungal properties, making them potential candidates for beauty and care products (De Almeida et al., 2024; Liu et al., 2024).

Traditionally, MELs have been produced using sugar cane or glycerol as feedstock (Beck et al., 2022). However, the growing demand

for sustainable and renewable energy sources has led to a shift towards exploring alternative carbon sources (Zargar & Srivastava, 2024) for example, sunflower oil, rapeseed oil and palm oil (Parveez et al., 2024). These vegetable oils have proven to be a promising substrate as they are abundant, relatively inexpensive and sustainable.

Vegetable oil can be converted into MELs by microbial fermentation, utilising the metabolic capabilities of microorganisms to convert the oil into the desired product. The production of MELs from vegetable oil by microbial fermentation has been reported to be influenced by several factors, including the type of microorganism, fermentation conditions and feedstock composition (Nanda et al., 2023; Nascimento et al., 2022). For example, the fungus *Moesziomyces aphidis* has been reported to produce MELs at concentrations of 64.5 ± 0.7 g/L when grown on vegetable oil (Yu et al., 2022).

Supplementing vegetable oils as a carbon feedstock for MEL production has been found to impact the yield, composition and properties of the resulting biosurfactants (Mohanty et al., 2021). For example, different types of fatty acids in vegetable oil can affect the structure and surface activity of the MELs (Zibek & Soberón-Chávez, 2022). Therefore, investigating the fermentation of MELs from renewable vegetable oils is essential not only for improving process efficiency but also for supporting the circular bioeconomy through enhanced resource utilisation and valorisation of fermentation-derived by-products, including residual lipids and other secondary metabolites.

Contemporary research efforts have been directed toward optimising the production process of MELs from vegetable oils to improve yield, purity and overall efficiency. Strategies such as genetic modification of yeast strains, optimisation of fermentation environments and the use of novel bioreactor systems have been widely investigated to enhance MEL productivity (Zulkifli et al., 2024).

However, these efforts have predominantly examined individual yeast species or isolated

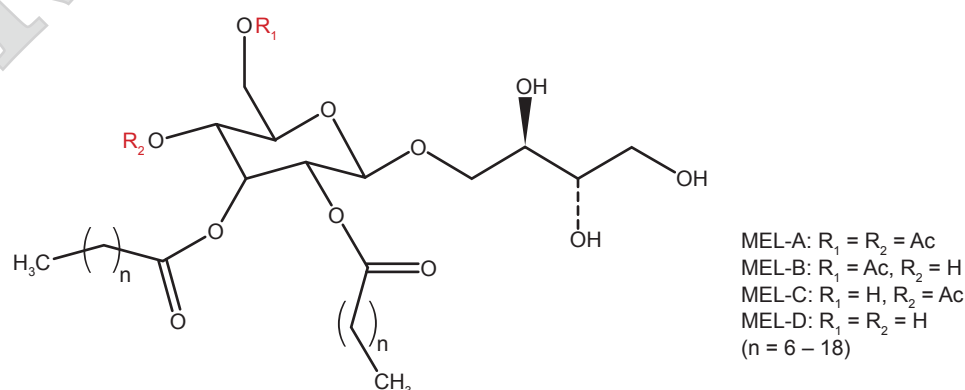


Figure 1. The general chemical structures of mannosylerythritol lipids (MELs), including MEL-A, MEL-B, MEL-C and MEL-D.

process variables, which limits direct comparison of microbial performance across studies. Despite their strong biotechnological relevance, *Pseudozyma antarctica* and *Ustilago maydis* have not been systematically compared for MEL production within a unified experimental framework. As a result, differences in MEL yield and physicochemical characteristics arising under identical cultivation conditions have not been clearly resolved. By addressing this gap, the present study enables a unified evaluation of species-dependent performance on palm olein and provides a basis for more informed selection of microbial platforms for sustainable and cost-efficient MEL production.

MATERIALS AND METHODS

Microorganism

Yeast *P. antarctica* (ATCC 34888) was obtained from the American Type Culture Collection (USA) and *U. maydis* (DSM 4500) was obtained from the European Culture Collections' Organisation (Germany).

Media Preparation and Culture Condition

Pseudozyma antarctica grown on Yeast Malt (YM) Agar (Difco Laboratory, USA) was inoculated into 150 mL Erlenmeyer flasks containing 50 mL of growth medium [4.00% (w/v) glucose, 0.30% (w/v) NaNO_3 , 0.02% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02% (w/v) KH_2PO_4 and 0.10% (w/v) yeast extract] at 28°C on an orbital shaker for 2 days. The 1 L Erlenmeyer flasks containing production media [8.00% (v/v) palm olein, 0.30% (w/v) NaNO_3 , 0.03% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.03% (w/v) KH_2PO_4 and 0.10% (w/v) yeast extract] were incubated for 10 days at 28°C. *U. maydis* stock culture was grown on Potato Dextrose Agar (USA) and cultivated in a growth medium containing 4% (w/v) glucose, 0.30% (w/v) NaNO_3 , 0.03% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.03% (w/v) KH_2PO_4 and 0.10% (w/v) yeast extract for 2 days at 25°C as a seed culture. The seed culture was inoculated with 8.00% (v/v) palm olein and cultured at 25°C for 10 days.

The palm olein utilised was commercially sourced, food-grade refined, bleached and deodorised (RBD) grade procured from a local vendor in Malaysia. Its fatty acid composition, as determined by gas chromatographic analysis of fatty acid methyl esters, consisted of 0.2% C12, 0.9% C14, 39.0% C16:0, 4.2% C18:0, 42.9% C18:1, 11.4% C18:2 and 1.4% others.

To monitor kinetic growth and biosurfactant production, 10 mL samples were collected from each 1 L flask at 12 hr intervals during the active fermentation period. All experiments

were performed in triplicate and independently repeated three times. The 10 mL samples were centrifuged at 4,800 rpm and 4°C for 30 min. The pellet was resuspended in an 85% NaCl solution and centrifuged. The cell-free supernatants were used to measure biosurfactant concentration as detailed below. The pellets were left in the oven at 60°C until a constant weight was reached for cell dry weight (CDW) determination. The cell growth was evaluated by measuring the optical density (OD580 nm) of the culture using a UV-Vis spectrophotometer upon adding NaCl solution. Biosurfactant formation was measured by adding orcinol reagent to the cell-free supernatant. The mixtures were left in the water bath at 80°C for 30 min. All samples were diluted with water before being measured using a UV-Vis spectrophotometer at OD421 nm.

Isolation of Mannosylerythritol Lipids (MELs)

The culture broth was extracted three times using an equivalent amount of ethyl acetate following submerged fermentation. After vacuum evaporation, 0.5 g of crude MELs were diluted and isolated on a glass column containing 40 g silica gel. The MEL extraction was performed using an elution technique adapted from the methods described by Jing et al. (2022). The extracts were analysed using thin-layer chromatography (TLC) on a silica plate. Following separation, the individual compounds were identified by spraying an orcinol reagent and sulphuric acid and charring at 100°C for 10 min.

Characterisation of Mannosylerythritol Lipids (MELs)

Surface tension. Surface tensions against water were determined at 25°C using a Kruss tensiometer K100 (Germany), using the Du Noüy ring method. A total of 30 mL of sample in a 50 mL beaker was required for each test. The distance between the immersed platinum-iridium ring and the liquid surface was positioned at 4.5 mm. The ring was left in contact with the sample solution for 10 min to allow equilibration. The CMC was determined based on the concentration over surface tension.

Liquid chromatography-mass spectrometry (LC-MS). The purified compounds were analysed using LCMS. Thermo Scientific Dionex Ultimate 3000 (USA) was connected to an MS spectrometer (Bruker MicroTOF-Q, USA). A Gemini C18, 2 x 150 mm, 5 µm Phenomenex (USA) was used. The LCMS method was adopted from Becker et al. (2022) and Valkenburg et al. (2024). An injection volume of 5 µL was employed throughout the analysis.

Nuclear magnetic resonance (NMR). Purified MELs were structurally determined by ^1H - and ^{13}C -NMR (JEOL, Japan) analysis, dissolved in deuterated (d) chloroform- d /methanol- d_4 (1:1 v/v) at 800 MHz. Comprehensive structural insights were obtained through two-dimensional NMR methodologies, ^1H - ^1H correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HMQC), and long-range heteronuclear correlation (HMBC) recorded on an 800 MHz JEOL ECA-800.

RESULTS AND DISCUSSION

Product formation was detected in the fermentation of *P. antarctica* and *U. maydis* after day 1, but the onset of the stationary phase occurred at different times. The stationary phase was reached after 48 hr for *P. antarctica* and after 60 hr for *U. maydis*. A short lag phase was observed in the culture of *P. antarctica*, whereas no lag phase was observed in the profile of *U. maydis*. This could indicate that both microorganisms adapted easily to the environment. Microbial growth continued for up to 10 days after fermentation. The stationary phase was extended to 204 and 216 hr for

P. antarctica and *U. maydis*, respectively. *P. antarctica* had a higher maximum CDW (5.66 g) compared to *U. maydis* (4.89 g). The pattern of growth profiles was similar for both microorganisms, and the maximum CDW was reached almost at the same time. This is because *U. maydis* is taxonomically related to *P. antarctica* (Kidd et al., 2023). The growth profile shows that the cell started to decay almost at the end of the 10-day fermentation, i.e., after 216 hr for *P. antarctica* and 228 hr for *U. maydis*. The growth profile for both microorganisms was measured using UV-Vis spectroscopy at 580 nm and plotted against time, as shown in Figure 2.

P. antarctica and *U. maydis* are described as MEL producers, though significant variation in secretion levels was observed. *P. antarctica* secreted a substantial MEL yield of 58.37 g/L at 168 hr during the stationary phase, which was more than double the yield produced by *U. maydis* (26.77 g/L at 216 hr), as illustrated in Figure 3. This lower yield in *U. maydis* was accompanied by the secretion of cellobiose lipids as a by-product, a phenomenon previously documented by Becker et al. (2021); Münßinger et al. (2024) and Valkenburg et al. (2025).

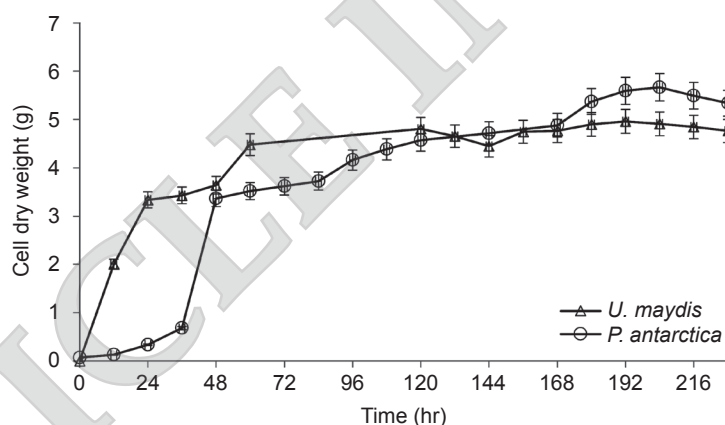


Figure 2. Growth profiles of *Pseudozyma antarctica* (circle) and *Ustilago maydis* (triangle) during a 10-day monitoring period.

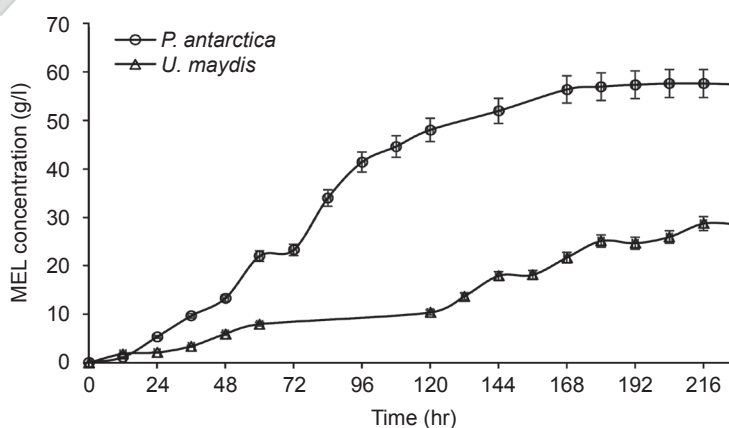


Figure 3. Time course of mannosylerythritol lipids (MELs) production by *Pseudozyma antarctica* (circle) and *Ustilago maydis* (triangle) during the feeding of palm olein. The strain was incubated in 1 L of the fermentation medium.

It has already been reported that MELs can be produced from various vegetable oils, including soybean, sunflower and olive. The published concentration of MEL was 5.6 g/L by *P. antarctica* T34 (Fukuoka et al., 2021), ~16 g/L by *P. antarctica* JCM 10317T on glycerol, ~75 g/L by *P. aphidis* DSM 70725 on soybean oil and ~25 g/L by *P. tsukubaensis* JCM 10324 on olive oil (Valkenburg et al., 2024). The wide range of MEL concentrations is probably associated with the differences in substrate type and utilisation efficiency among closely related *Pseudozyma* species. Nevertheless, the integration of palm oil as a renewable substrate in MEL biosurfactant fermentation aligns with the principles of the circular bioeconomy, highlighting its potential as a resource-efficient and environmentally sustainable bioprocess within circular manufacturing systems. Currently, there are no data on the production of MELs with palm oil as substrate by *Ustilago* sp. and *Pseudozyma* sp. which are taxonomically similar to *Moesziomyces* sp. (Beck, 2023; Nasanit et al., 2016). Therefore, this study may be the first report of MEL production by *U. maydis* and *P. antarctica*.

Surface Activity Properties

As depicted in Figure 4, a pronounced decrease in surface tension was observed with increasing MEL concentrations, following a logarithmic trend. The surface tension of water (72 mN/m) was substantially reduced to approximately 30 mN/m upon the introduction of MELs. This value is lower than that typically reported for the synthetic surfactant sodium dodecyl sulfate (SDS: ~37 mN/m) (Chai et al., 2019; Li et al., 2024). In comparison, the minimum surface tension achieved by MELs is comparable to values reported for well-established microbial glycolipid biosurfactants, including rhamnolipids (~35 mN/m; Mahamad et al. (2025) and sophorolipids (~33 mN/m; Kaga et al. (2024), suggesting a similar level of surface-active performance.

The CMC of crude MELs was 2.8 and 2.6 mg/mL for *P. antarctica* and *U. maydis*, respectively. The low CMC value indicates excellent surface activity at low concentrations. This behaviour may be attributed to the presence of ester and glycoside linkages in the MELs structure. Moreover, the presence of two alkyl chains linked to the mannose ring by an ester group has conferred amazing properties to MELs (De Almeida et al., 2024). It is well known that a short distance between neighbouring positions contributes to the best surfactant properties. The extended alkyl chains in MELs contribute to substantial disruption of the hydrogen-bonding network within the aqueous phase. Compared to conventional surfactants, the tendency of the biosurfactant molecule to absorb and form micelles is directly increased. Nevertheless, the water solubility is improved with the help of erythritol groups that balance the two alkyl groups.

Identification of Functional Group

The analysis was continued with Fourier transform infrared spectroscopy (FTIR) to tentatively identify the functional groups present in the concentrated brown liquid. The FTIR spectrum of MELs showed transmittance peaks at 3,377, 2,916, 2,849, 1,701 and 1,093 cm^{-1} corresponding to hydroxyl group (-OH), methylene group (-CH₂), methyl group (-CH₃), carbonyl group (C=O), and ether linkage (C-O-C), respectively (Figure 5). The observation of these functional groups was the first tentative identification of purified MELs, which was also found by Pal et al. (2022) and Wu et al. (2022). The broad band at 3,377 cm^{-1} was attributed to the -OH stretching vibrations of the carboxyl groups. The sharp bands at 2,916 cm^{-1} and 2,849 cm^{-1} indicate the presence of C-H stretching vibrations indicative of methylene (-CH₂) and methyl (-CH₃) functionalities within the aliphatic backbone. The sharp peak at 1,701 cm^{-1} was assigned to carbonyl stretching in esters and acids.

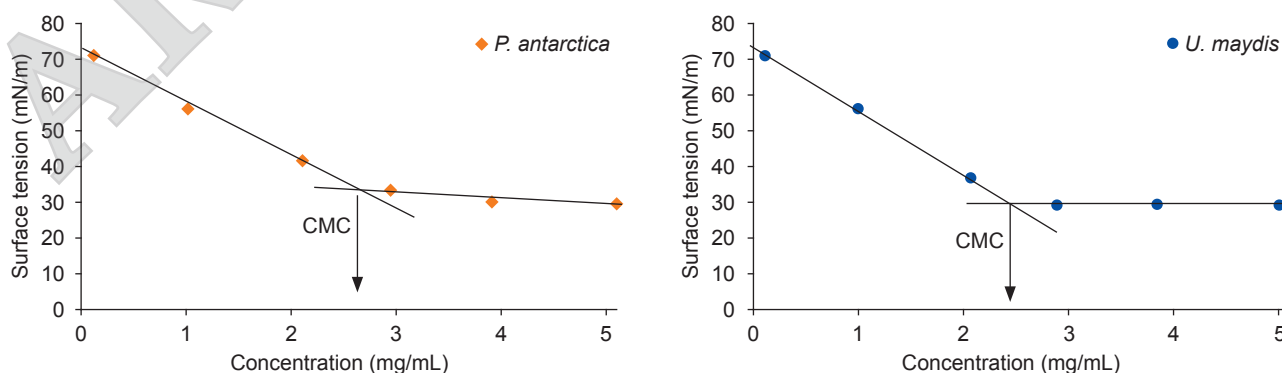


Figure 4. The plot of surface tension versus mannosylerythritol lipid (MEL) concentration of (a) *Pseudozyma antarctica* and (b) *Ustilago maydis* show the critical micelle concentration (CMC) values.

The pronounced absorption peak at $1,093\text{ cm}^{-1}$ indicated the existence of ether bonds between aromatic and fatty acid chains.

Identification Using LC-MS Analysis

This analysis was conducted at the Centre for Research and Instrumentation Management (CRIM), National University of Malaysia (UKM). Positive ion peaks and predicted MELs in the associated retention time were summarised. The LC-MS eluents were identified as MEL-A, -B, -C and -D. MELs were detected as adduct ions with sodium $[M + Na]^+$ at a mass-to-charge ratio (m/z) of 559–741 and a potassium $[M + K]^+$ adduct at m/z 578. Several isomers appeared at a shorter retention

time. In addition, a characteristic pattern of the mass spectrum was observed. The ions detected for MELs were $[M + H]^+$ with a mass-to-charge ratio of 635, 747, 761, 787, 789, 803 and 871. Potential fatty acids ranging from C6 to C20 with an unsaturated bond were detected, and peaks with higher masses were identified. A summary of the molecular ion data can be found in Table 1. Some molecular ion data showed that only adducts with sodium or potassium were detected, but no protonated molecules $[M + H]^+$ were observed. This finding was similarly noted by Fan et al. (2016). Therefore, the tentative composition of the purified MEL-A, -B, -C and -D was determined in this work. Part of the mass spectra of the individual MEL homologs can be seen in Figure 6.

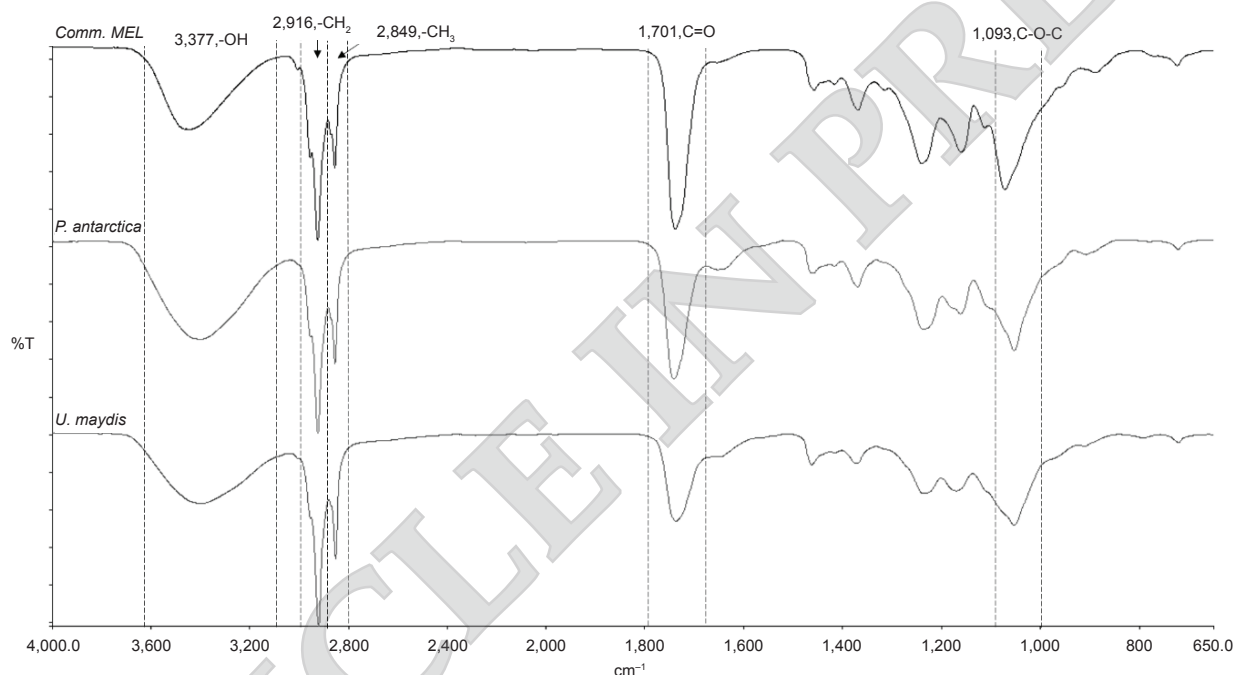


Figure 5. Fourier transform infrared spectroscopy (FTIR) spectrum of commercial mannosylerythritol lipids (MELs), MELs produced by *Pseudozyma antarctica* and *Ustilago maydis*.

TABLE 1. MASSES DETECTED BY LC-MS IN THE FRACTIONS COLLECTED FROM COLUMN CHROMATOGRAPHY

MELs homologues	m/z	Positive ion	Molecular mass	Possible fatty acid chain combination
MEL-A	643	$[M + Na]^+$	620	C6-C8 (A/D)
	787	$[M + H]^+$	786	C10-C16:1 (A/D)
	871	$[M + H]^+$	870	C16:0-C16:1
MEL-B/C	617	$[M + K]^+$	578	C6-C8
	635	$[M + H]^+$	634	C8-C10 / C6-C12
	685	$[M + Na]^+$	662	C10-C10 / C6-C14 / C8-C12
	713	$[M + Na]^+$	690	C6-C16:0 / C10-C12
	741	$[M + Na]^+$	718	C10-C14
	747	$[M+H]^+$	746	C10-C16:0
	803	$[M+H]^+$	802	C14-C16:0 / C12-C18:0
MEL-D	559	$[M+Na]^+$	536	C6-C8
	587	$[M+Na]^+$	564	C6-C10
	761	$[M+H]^+$	760	C14-C16:0
	789	$[M+H]^+$	788	C16:0-C16:0 (A/D)

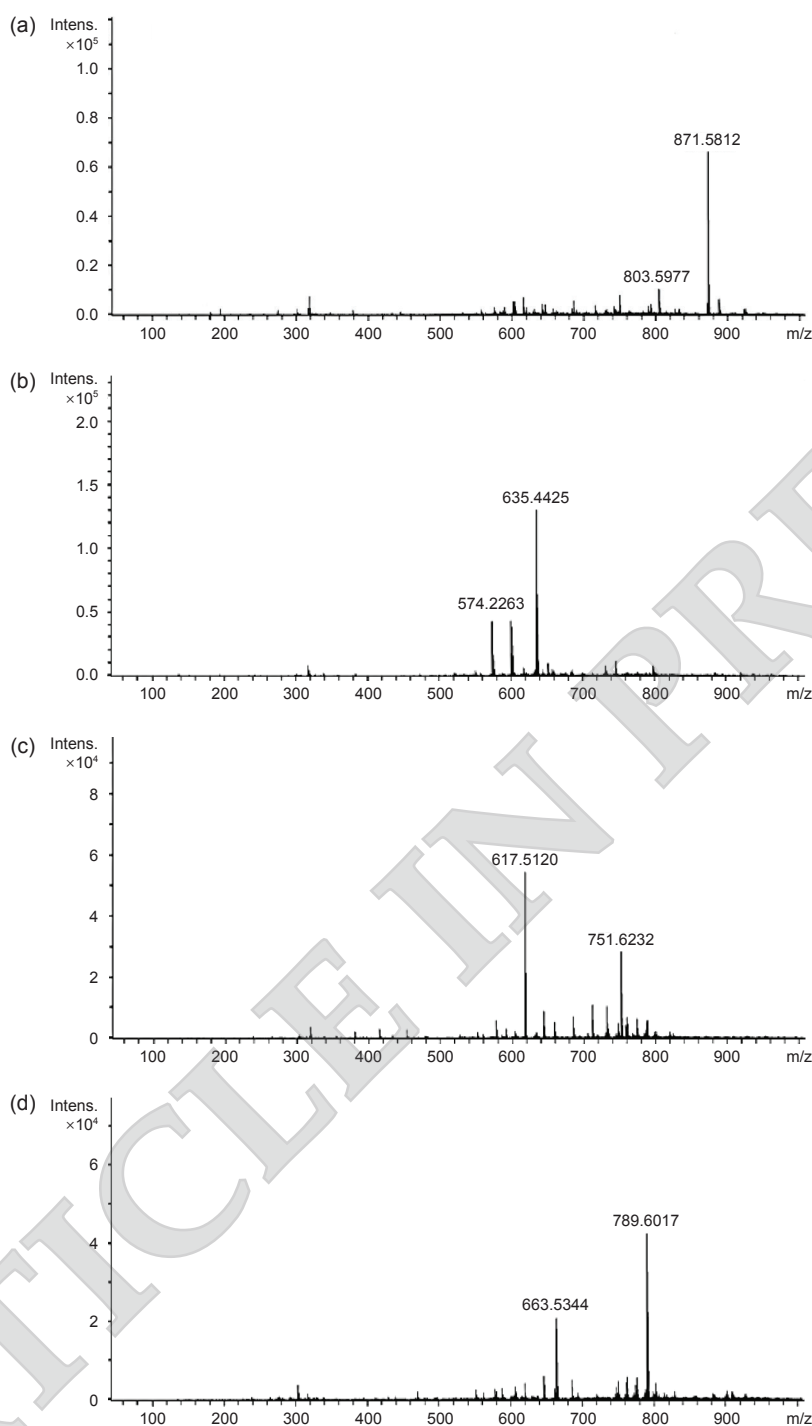


Figure 6. Positive electrospray ionisation-mass spectra of mannosylerythritol lipids (MELs) analysed by liquid chromatography (LC). The mass spectra are for chromatographic peaks of (a) MEL-A (m/z 871), (b) MEL-B/C (m/z 635), (c) MEL-B/C (m/z 617) and (d) MEL-D (m/z 789).

The results showed that MEL-B and MEL-C were dominant compounds produced by both strains. Based on the TLC chromatogram, the MEL-B stain was visible compared to MEL-C and could be distinguished as the main compound. MEL-B exhibits the capacity to self-assemble into a lamellar phase across broad concentration and temperature ranges and is capable of forming comparatively large vesicular structures even at low concentrations (Kondapi, 2021). These properties are highly sought

after for many applications such as drug and gene delivery (Wu et al., 2022). In another study by Aziz et al. (2021), MEL-B can suppress perspiration for 2 hr by increasing the water content of the skin. MELs are known for their skin moisturising effect; therefore, limiting perspiration is beneficial for the advancement of the personal care industry. In addition, MEL-B has a high hydrophilicity and CMC value, which is beneficial for use in oil-in-water emulsions and/or household care.

Identification Using NMR Analysis

Structural features of MELs were inferred from the spectral signatures obtained by ^1H - and ^{13}C - NMR spectroscopy. The NMR spectra of *P. antarctica* cultivated in palm olein were consistent with those reported previously (Matosinhos et al., 2023; Mawani et al., 2021; Yu et al., 2024). NMR spectra for MELs produced by *U. maydis* were not included in this study because the crude product consisted of a complex mixture of MELs and cellobiose lipids, resulting in extensive signal overlap that precluded reliable spectral interpretation. The separation of MELs from cellobiose lipids requires an extensive and dedicated purification strategy, which represents a distinct analytical effort beyond the scope of the present study.

The observation of mannose peaks could be detected in the ^{13}C -NMR spectrum as shown in Figure 7. Analysis revealed two distinct resonances corresponding to the carbonyl carbons of acetyl moieties at 169.9 and 171.2 ppm, each correlating with the H-4' and H-6' protons, respectively. Additionally, three separate carbonyl carbon signals attributed to acyl groups were detected at 174.5, 173.6 and 172.9 ppm, exhibiting correlations with the H-1', H-2' and H-3' protons, accordingly

(Figure 8). The collective pattern of these correlations was consistent with a mixture of MELs, which are summarised in Table 2. Consequently, *P. antarctica* was tentatively confirmed to produce MELs using palm olein.

CONCLUSION

This study presents the first systematic comparative evaluation of *P. antarctica* and *U. maydis* for MEL production using palm olein as a lipid-based carbon source, revealing pronounced species-dependent differences in both productivity and substrate conversion efficiency. Under identical cultivation conditions, *P. antarctica* exhibited substantially higher MEL yields than *U. maydis*, demonstrating a previously unreported disparity in metabolic suitability toward palm olein utilisation. Comprehensive structural characterisation by LC-MS and NMR confirmed the simultaneous presence of all four major MEL homologs (MEL-A, -B, -C and -D) with acyl chain lengths spanning C6–C20, directly linking palm-derived substrates to structurally diverse MEL profiles. Together, these findings offer new insight into substrate-microbe compatibility and positioning palm olein as a viable renewable feedstock for tailored MEL production.

TABLE 2. ^1H - AND ^{13}C -NMR DATA FOR THE MELs (CD_3OD , 600 MHz)

Functional groups	^{13}C -NMR, δ (ppm)		^1H -NMR, δ (ppm)	
D-Mannose	C-1'	99.2	H-1'	4.15 s
	C-2'	68.9	H-2'	5.47 d
	C-3'	71.2	H-3'	5.05 dd
	C-4'	66.1	H-4'	5.23 t
	C-5'	73.4	H-5'	3.72 m
	C-6'	62.4	H-6'	4.11 m
meso-Erythritol	C-1	65.0	H-1	4.87 dd
	C-2	69.9	H-2	4.10 m
	C-3	72.1	H-3	5.21 m
	C-4	70.7	H-4	3.69 dd
Acetyl groups				
-CH ₃ (C-4')	20.2		1.99 s	
(C-6')	20.2		2.10 s	
-C=O (C-4')	169.9			
(C-6')	171.2			
Acyl groups				
-CH ₃	13.6–13.8		0.81–0.86 t	
-(CH ₂) _n -	22.4–31.9		1.19–1.33 b	
-CH=CH-	127.8–130.0		5.27–5.32 b	
-CH=CH-CH ₂ -	28.9–29.7		1.95–2.06 b	
-CO-CH ₂ -CH ₂ -	24.6–25.1		1.47–1.65 b	
-CO-CH ₂ - (C-2')	34.2		2.30 m	
(C-3')	34.0		2.19 m	
-C=O (C-2')	173.6			
(C-3')	172.9			

Note: s - singlet; d - doublet; t - triplet; dd - double doublet; m - multiplet; b - broad.

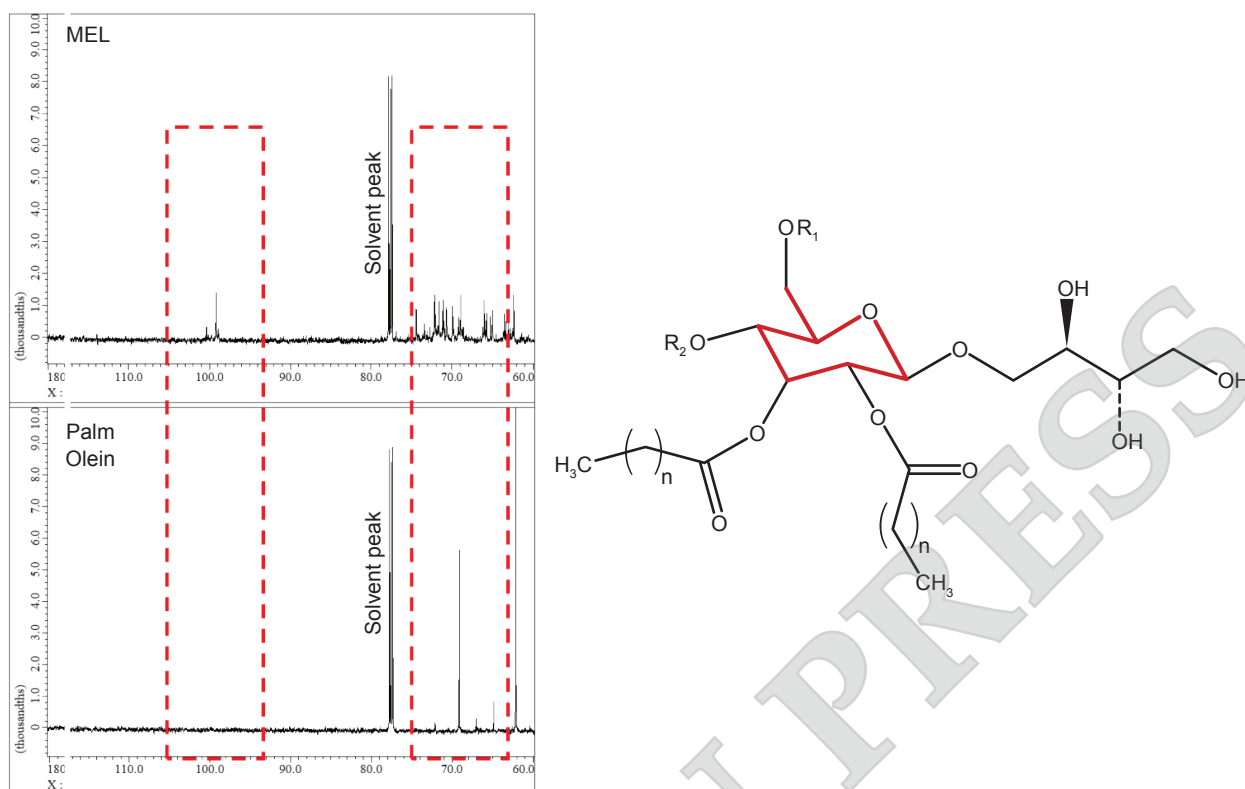


Figure 7. Partial 800 MHz ^{13}C nuclear magnetic resonance spectroscopy (NMR) spectra of mannosylerythritol lipids (MELs) produced by *Pseudozyma antarctica* and palm olein as feedstock.

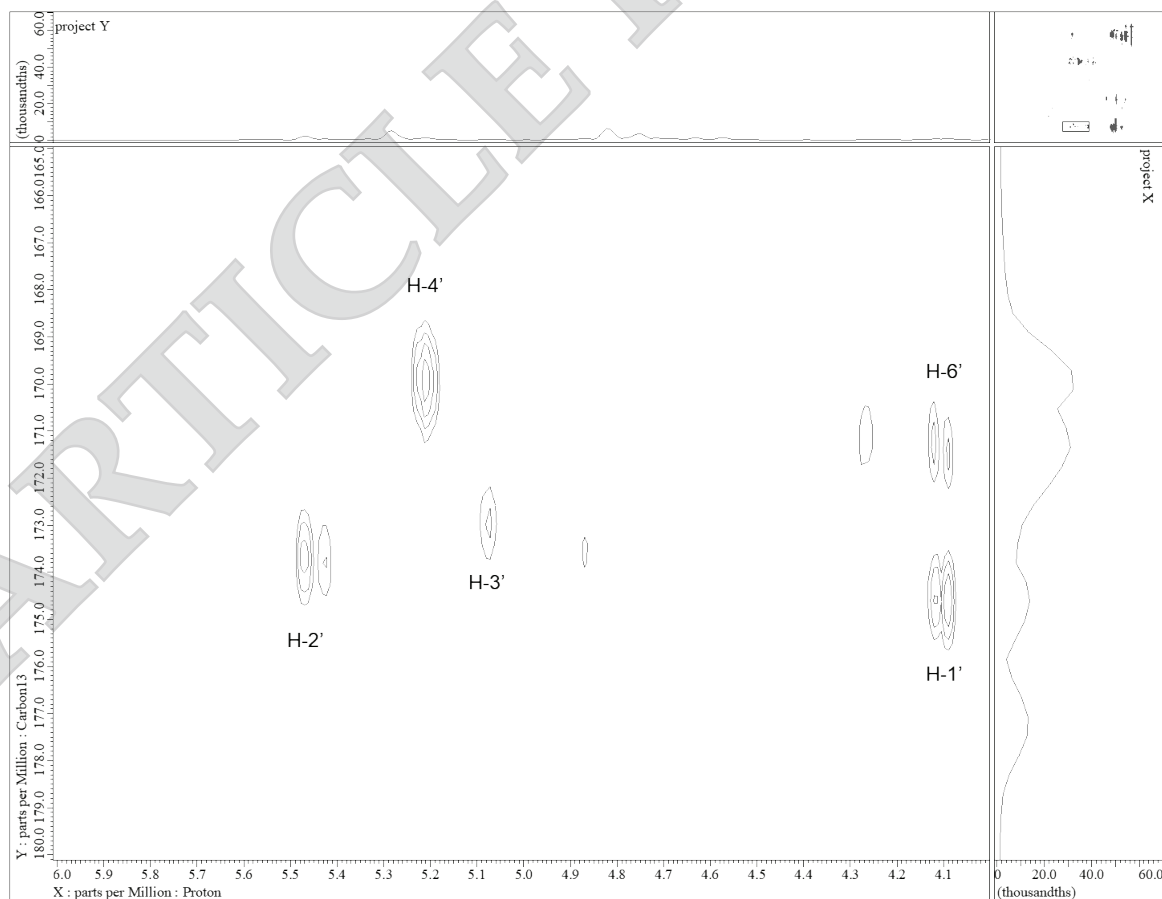


Figure 8. Heteronuclear multiple-bond correlation (HMBC) spectrum of mannosylerythritol lipids (MELs) produced by *Pseudozyma antarctica*.

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