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Studies on biosurfactants from *Pseudozyma* sp. NII 08165 and their potential application as laundry detergent additives

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ABSTRACT

The novel isolate *Pseudozyma* sp. NII 08165 produced glycolipid biosurfactants, which was a combination of all the three mannosylerythritol lipids (MELs) isomers along with some unknown glycolipids. The strain produced 34 g/l MELs in medium containing 8% (w/v) soybean oil as carbon source after nine days of fermentation. The structural characterization of purified MEL revealed the hydrophobic structure of MEL-C consisting of short chain fatty acid (C2 or C4) at the C-2′ position and a long chain fatty acid (C14, C16 or C18) at the C-3′ position of the mannose moiety. The MEL-C showed good surface activity with critical micelle concentration (CMC) of 4.5×10^{-6} M and surface tension of 33 mN/m at CMC. The crude biosurfactants were stable at high temperature and over the alkaline pH range which favour their scope of application as laundry detergent additives. Fabric wash analysis revealed that crude biosurfactants from *Pseudozyma* sp. NII 08165 removed stains efficiently and can be used in laundry detergent formulations.

1. Introduction

Biosurfactants are amphiphilic compounds of microbial origin which exhibit high surface activity and emulsifying activity. Biosurfactants can be potential alternative to chemical surfactants due to their low toxicity, biodegradability, performance under extreme conditions and structural diversity [1,2]. Apart from detergent like activities, biosurfactants display various biochemical activities and are likely to become molecules of the future in areas such as biomedicine and therapeutics [3].

Many potent glycolipid biosurfactants are usually derived from yeast species. Mannosylerythritol lipids (MELs) produced by submerged culture of *Pseudozyma* species and related *Ustilago* species are one of the most promising biosurfactants. Mannosylerythritol lipid (MEL) contains 4-*O*-β-D-mannopyranosyl *meso*-erythritol as the hydrophilic group and a fatty acid and an acetyl group as the hydrophobic moiety (Fig. 1). Based on the number of acetyl group and their order of appearance on thin layer chromatography (TLC), MELs are classified as MEL-A, -B, -C and -D. MEL-A is 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 [4,5]. Some species of *Pseudozyma* also produce structural variants of MELs like triacylated MEL [6]. *Ustilago maydis* and *Pseudozyma flocculosa* were reported to produce more than one biosurfactants in culture medium, i.e. mannosylerythritol lipids and cellobiose lipids [5,7]. Cellobiose lipids from *Cryptococcus humicola* and *Pseudozyma fusiformata* exhibit fungicidal activity [8].

MELs are considered as multifunctional molecules due to their excellent surface activity, biocompatibility and versatile biochemical functions [9,10]. MEL shows antitumor activity against human leukaemia and mouse melanoma cells, and is proposed in treatment of diseases caused by dopamine metabolic dysfunction like schizophrenia [11–13]. They can also be used as vehicle for gene delivery and as a ligand for immunoglobulin purification [14,15]. They can inhibit ice agglomeration in ice slurry system [16] and recently, MEL was also demonstrated to have ceramide like skin care and hair care properties, with potent applications in cosmetic industry [17,18].

Due to the increasing public concern about the environmental hazards of synthetic surfactants, the use of ecofriendly surfactants from natural sources in detergent formulations is under consideration. The high biodegradability, lower toxicity and minimal ecological impact of the microbial surfactants make them environmentally safe material [19]. The crude cyclic lipopeptide (CLP) biosurfactants from *Bacillus subtilis* and rhamnolipids were found to be promising as laundry detergents additives [20,21]. In this

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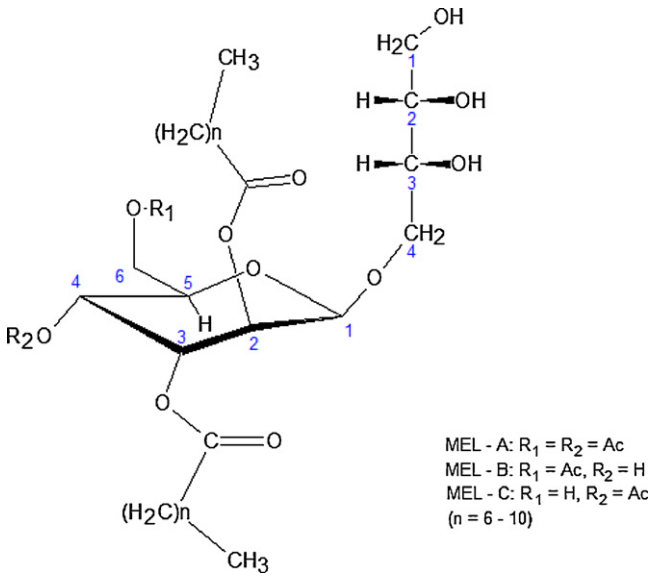

Fig. 1. Chemical structure of mannosylerythritol lipids.

study, a novel strain of *Pseudozyma* sp. NII 08165 was studied for the production of biosurfactants. Detailed structural elucidation of the purified glycolipid was done. The crude biosurfactants were studied for their application as laundry detergent additives.

2. Materials and methods

2.1. Microorganism and screening for biosurfactants production

The strain *Pseudozyma* sp. NII 08165 was isolated from an aerial sampling on lipase screening plate at biotechnology lab of NIIST. Stock culture was prepared in PDA slants and stored at 4 °C and subcultured at every four weeks.

The organism was screened for biosurfactants production by classical biosurfactants screening methods like drop collapse assay and haemolytic activity. In drop collapse assay, 25 μl of two days old culture supernatant of *Pseudozyma sp*. NII 08165 was added to surface of parafilm after adding bromophenol blue for staining. The distilled water was taken as a negative control and 1% SDS was taken as positive control. The dye bromophenol blue has no influence on the shape of the droplets. Haemolytic activity was analyzed by spotting 10 μl of an overnight culture on blood agar plates (24 g/l potato dextrose, 20 g/l agar, 8 g/l NaCl, and 5% goat blood). Hemolysis was monitored after incubation of plates for three days at 30 °C [22].

2.2. Phylogenetic analysis

DNA was extracted using yeast genomic DNA isolation protocol [23]. The ITS region was amplified using primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [24]. The PCR amplicon was sequenced and BLAST analysis was performed. The sequences were aligned using Clustal W software [25]. The phylogenetic analysis was performed with MEGA4 software [26]. The sequence was submitted in genbank with accession no. JN969989.

2.3. Media preparation and culture conditions

Seed culture was prepared by inoculating *Pseudozyma* into growth medium [4% (w/w) glucose, 0.3% $NaNO_3$, 0.03% $MgSO_4{\cdot}7H_2O$, 0.03% KH_2PO_4, 0.1% yeast extract (pH 6.0)] and incubated at 30 °C, 200 rpm for two days. The seed culture (4%, v/v) was inoculated to 50 ml production medium (8% [w/v] soybean oil, 0.3% $NaNO_3$, 0.03% $MgSO_4{\cdot}7H_2O$, 0.03% KH_2PO_4, 0.1% yeast extract [pH 6.0]) in 500 ml Erlenmeyer flasks and fermentation was carried out at 30 °C, 200 rpm for nine days-modified protocol [27].

2.4. Isolation and analysis of glycolipids

The glycolipids were extracted from the culture medium with equal amount of ethyl acetate. The ethyl acetate was evaporated and the crude extract was dissolved in chloroform. The crude extract was analyzed by thin layer chromatography (TLC) on silica plates (Silica gel 60F; Merck) using the solvent system – chloroform/methanol/7 N ammonium hydroxide (65:15:2, v/v) [27]. The spots were visualized by charring the plates after Orcinol spray (0.2% Orcinol in 20% H_2SO_4 solution).

2.5. Quantification of glycolipids

The glycolipids produced were quantified by normal phase HPLC on silica gel column using a low temperature evaporative light scattering detector (ELSD). Agilent-1200 series HPLC was used with Merck LiChrosorb Si-60 column (5 μm, 2 cm × 3.0 mm). Here a gradient solvent programme of chloroform and methanol (from 100:0 to 0:100) was set at a flow rate of 1 ml/min [28]. The HPLC analysis was based on the standard curve using purified MEL.

2.6. Purification of glycolipids

The purification of glycolipids was done by silica gel column chromatography with silica gel of mesh size 200–400 (Merck) using a gradient elution of chloroform:acetone (10:0–0:10, v/v) mixtures as solvent systems [4].

2.7. Structural characterization of purified glycolipid

2.7.1. NMR spectroscopy

The purified glycolipid was dissolved in $CdCl_3$ and ^1H and ^{13}C NMR analysis was carried out using a Bruker Avance II-500 spectrometer.

2.7.2. GC–MS analysis

GC–MS analysis was performed to determine the fatty acid composition of purified MEL. The methyl esters of fatty acid were prepared by incubating purified MEL (10 mg) with 5% HCl–methanol at 60 °C for 5 h [29]. The fatty acid methyl esters were extracted by hexane and analyzed by GC–MS. The GC–MS detection was performed with Agilent 6890N Gas Chromatograph connected to Agilent 5973 Mass Selective Detector in the EI mode with a HP-1 ms capillary column (30 m × 0.25 mm i.d. × 0.25 μM film thickness). The oven temperature was kept at 60 °C for 2 min and programmed from 60 °C to 170 °C at 10 °C/min, kept for 2 min, and finally raised to 300 °C at 15 °C/min.

2.7.3. Mass spectrometry

The molecular weight of purified MEL was analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) using Axima CFR$^+$ spectrometer. Here α-cyano-4-hydroxycinnamic acid was used as matrix.

2.8. Determination of surface tension

The surface tension of aqueous solution of purified MEL-C was determined at different concentrations by Wilhelmy plate method using Wilhelmy type automatic tensionmeter (Dataphysics DCAT

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21) at 25 °C. Critical micelle concentration (CMC) was then determined from the break point of the surface tension versus log of bulk concentration curve.

2.9. Evaluation of crude biosurfactants from Pseudozyma sp. NII 08165 for the application as laundry detergent additives

2.9.1. Preparation of crude biosurfactants for wash analysis

The crude biosurfactants was prepared by washing the ethyl acetate extract with hexane for three times to remove the residual oil.

2.9.2. Effect of temperature and pH on crude biosurfactants

Aqueous solution of crude glycolipids was prepared at a concentration of 0.05%. The effect of temperature was studied by incubating the glycolipid solution at 60 °C and 80 °C for 2 h followed by measuring the surface tension of the solution. The pH stability was studied over a pH range of 8.0–12.0. The stability was studied by measuring the surface tension by Wilhelmy plate method using Wilhelmy type automatic tensionmeter (Dataphysics DCAT 21) at 25 °C.

2.9.3. Fabric wash analysis

Clean white cotton clothes (5 cm^2) were stained with goat blood, ketchup and chocolate sauce: one ml goat blood, ketchup and chocolate sauce was applied onto cloth and cloth was kept for drying overnight. The stained clothes were subjected to wash analysis with commercial detergent (Surf excel), glycolipids solution and a mixture of commercial detergent with glycolipids solution. Briefly, the stained cloth was put into separate flask; flask with tap water only; flask with tap water and commercial detergent at a final concentration of 10 mg/ml, flask with tap water and glycolipids at a final concentration of 10 mg/ml and flask with tap water with a mixture of commercial detergent and glycolipids at a final concentration of 5 mg/ml and 5 mg/ml, respectively. In all flasks, final volume is 10 ml. Then flasks were kept for agitation at 200 rpm at room temperature. After incubation, cloth pieces were taken out, rinsed with water and dried. The stain removal was determined by measuring lightness of cotton clothes using COLORTOUCH Brightnessmeter ISO model (Technidyne Corp., USA) and the percentage of stain removal was calculated.

Here, percentage stain removal was calculated by the following equation

$$\%\ \text{stain removal} = \frac{\text{lightness of the stained and washed cloth}}{\text{lightness of the clean and unstained cloth}} \times 100 \quad (1)$$

All washing experiments were done in triplicates and mean value was taken.

3. Results and discussion

3.1. Microorganism

Because of ability to grow on media containing lipid as carbon source and the peculiar media characteristics, the organism was suspected to produce biosurfactants. The strain was found to be positive for both screening methods, which indicated that strain could produce some surface active compounds in submerged culture. The drop collapse assay is based on the collapse or spreading of the droplets on the hydrophobic surface because of the biosurfactants [30]. The culture supernatant gave a collapsed droplet on parafilm when compared to negative control (Fig. 2a). On blood agar plate, a prominent clear lytic zone was observed around the growing cells (Fig. 2b). It was reported that the production of surface active compounds like mannosylerythritol lipids in the culture medium caused a significant collapse of the droplet and hemolytic activity by *Ustilago maydis* [22].

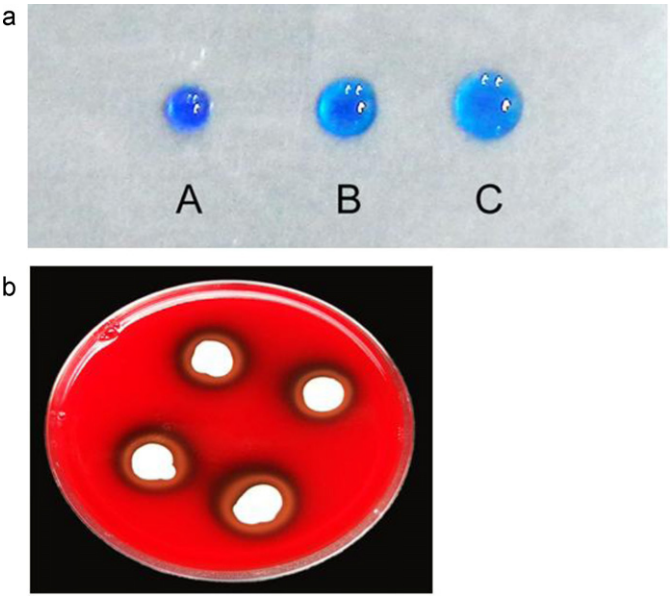

Fig. 2. (a) Drop collapse assay. The culture supernatant of *Pseudozyma* sp. NII 08165 (C) gave collapsed droplet on parafilm just like SDS solution (B). Here distilled water was taken as negative control (A). (b) Hemolytic activity of *Pseudozyma* sp. NII 08165. Picture of *Pseudozyma* sp. NII 08165 growing on blood agar plates. Clear lytic zone was observed around the colonies.

3.2. Phylogenetic analysis

To identify the organism, region of ITS1, 5.8S rRNA and ITS2 gene was sequenced and phylogenetic analysis was performed. Fig. 3 shows the phylogenetic tree constructed with ITS sequences. On phylogenetic tree, the isolate was positioned near to *Pseudozyma siamensis*, which is a predominant producer of MEL-C [31]. The strain was deposited in NII culture collection and designated as *Pseudozyma* sp. NII 08165.

3.3. Glycolipid production

After carrying out fermentation for nine days, glycolipid production was analyzed by TLC using the solvent system – chloroform:methanol:7 N ammonium hydroxide (65:15:2), four prominent glycolipid spots were observed (Fig. 4). Three spots were having R_f value 0.72. 0.69 and 0.67 which represent MEL-A, MEL-B and MEL-C, respectively. *Pseudozyma antarctica* T-34 was reported to give four anthrone positive spots in TLC solvent system of chloroform:methanol:water (65:15:2), which were designated as MEL-A (R_f 0.77), MEL-B (R_f 0.63), MEL-C (R_f 0.58) and MEL-D (R_f 0.52) [4]. The spot with lower R_f value (0.06) might represent cellobiose lipid, but detailed structural examination is needed to confirm this. The faint spots of glycolipids observed above the MELs spots on TLC plate might be tri-acylated MEL [6]. Some trace unknown glycolipid spots were also observed below the MELs spots and on solvent front along with neutral lipids.

3.4. Quantification of glycolipids

To quantify the glycolipids, HPLC analysis of crude ethyl acetate extract was performed (Fig. 5). The quantification of MELs was carried out based on the standard curve using purified MEL. Purified

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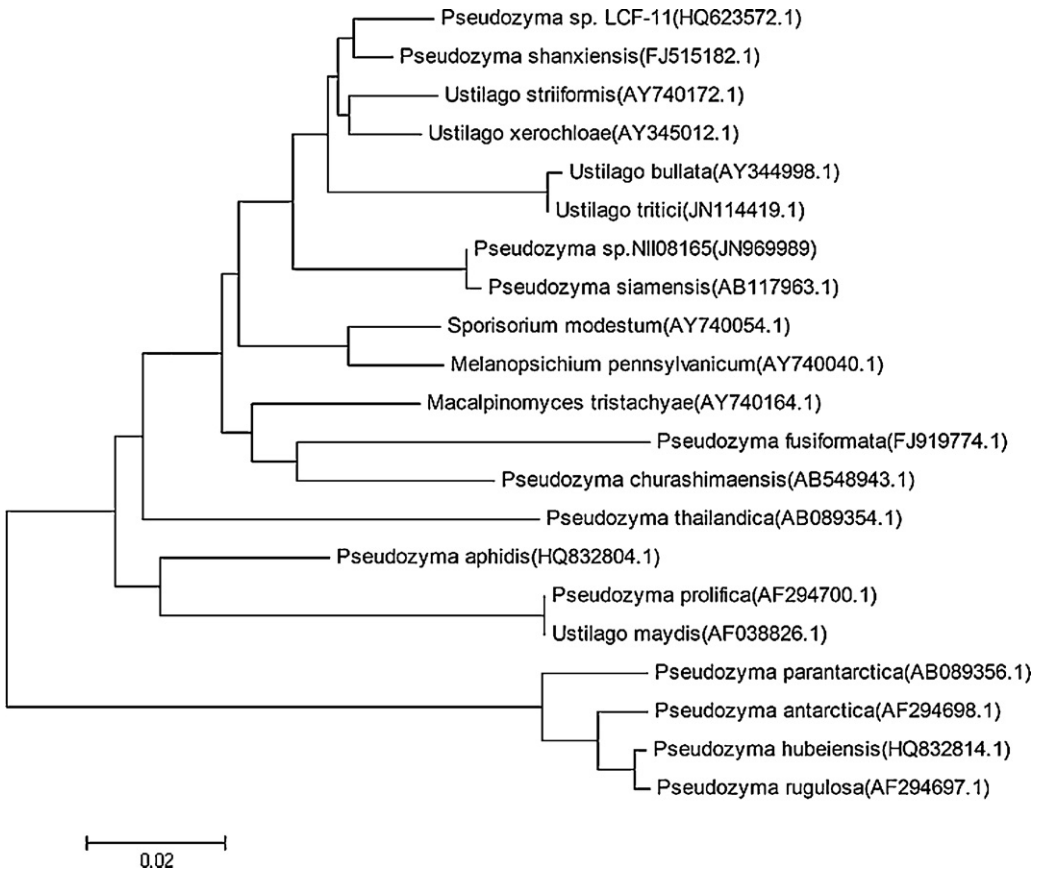

Fig. 3. Phylogenetic tree constructed with ITS1, 5.8S rRNA and ITS4 gene sequences of related species. Number in bracket indicates accession number.

MEL fraction was prepared by silica gel column chromatography. Since the polar column is being used, peaks of neutral lipids are followed by that of polar lipids. Peaks of triglycerides and free fatty acids were observed. Separation of the main types of MELs into four individual peaks has already been demonstrated by Rau et al. [28]. Along with fatty acid peak, four peaks of glycolipids were observed. The three peaks with retention time 5.902, 6.169 and 6.489 corresponded to MEL-A, -B and -C, respectively. The other peak with retention time 5.530 could be some derivative of MEL since the MEL-A spot on TLC was found to be merged. The peaks of MEL isomers were comparable to the retention times proposed by Morita et al. [32]. The pure MEL fraction of *P. antarctica* gave three peaks with retention times of 5.89, 6.14 and 6.49 which corresponded to MEL-A, MEL-B and MEL-C [32]. HPLC analysis revealed that *Pseudozyma* sp. NII 08165 was capable of producing MELs at a concentration of 34 g/l from 8% (w/v) soybean oil. The major glycolipids, MEL-B and MEL-C comprised of 35.7% and 59.6% of all glycolipids. HPLC analysis revealed that soybean oil was consumed and some residual oil was present in the medium after nine days of fermentation. The phlyogenetically related organism, *Pseudozyma siamensis* produced 19 g/l MELs in medium supplemented with 4% safflower oil, which comprised 84.6% of MEL-C [31]. The *Pseudozyma* sp. NII 08165 was a good producer of MELs and the productivity could further be improved by optimizing the substrate concentration and media conditions. Production of MELs by certain species of *Pseudozyma* can reach above 100 g/l in fed-batch fermentation [33,34] and the type of MEL produced by different *Pseudozyma* could be different; the feature which could even be used as an important taxonomic index in the identification of these yeasts [35]. *Pseudozyma tsukubaensis* produces a novel diastereomer of MEL-B as the predominant MEL while a strain closely related to *P. hubeiensis*, mainly produces MEL-C [36,37].

3.5. Structural characterization of purified glycolipid

The purified compound, which gave single spot on TLC, was subjected to structural determination in detail.

3.5.1. NMR spectroscopy

Based on NMR analysis, the purified compound was confirmed to be 4-*O*-[4′-*O*-acetyl-2′, 3′-di-*O*-alka (e) noil-β-D-mannopyranosyl]-D-erythritol which is called MEL-C (Fig. 6). The chemical shifts of the compound are summarized in Table 1. The chemical shift of the compound matched with those of the previously reported MEL-C of *P. siamensis*. On proton NMR spectra, a peak at 2.03 ppm indicated the presence of acetyl group (—CH_3) at C-4′ position. A broad peak at 0.88 ppm represented fatty acid group at C-3′ position. Based on a sharp peak at 2.17 ppm and triplet peak at 0.982 ppm, the present MEL-C was determined to have C2 or C4 acids at the C-2′ position of the mannose moiety. In the ^{13}C NMR spectrum, the peak at 170.2 ppm was assigned to carbonyl groups (—C=O) bound to C-4′ position on the mannose sugar.

3.5.2. GC–MS analysis

The fatty acid composition of MEL-C was analyzed by GC–MS (Table 2). It was found that MEL-C mainly comprised of long chain fatty acids, mainly C-14 to C-18 acids. The major fatty acid of MEL produced from soybean oil by *Pseudozyma* sp. NII 08165 was C16 fatty acid (57.1%). The NMR analysis and fatty acid profiling reveals that MEL-C from *Pseudozyma* sp. NII 08165 contains a unique hydrophobic structure proposed by Morita et al. [31] which is shown in Fig. 7.

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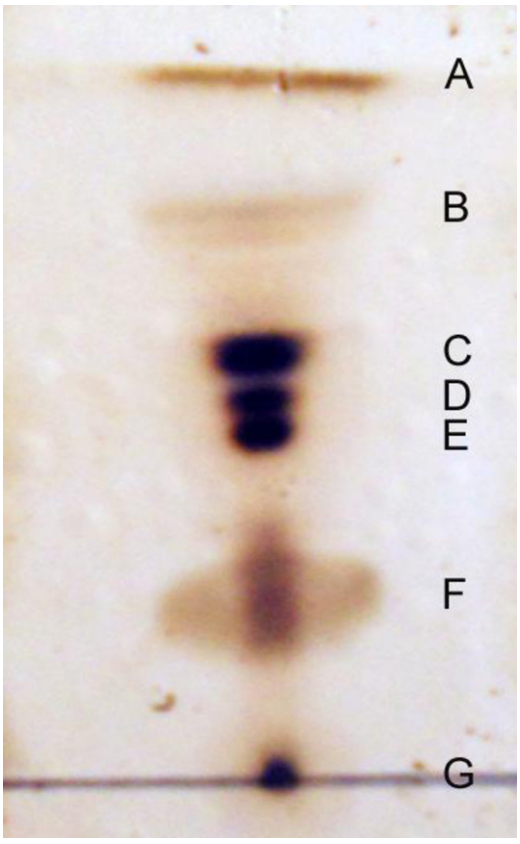

Fig. 4. TLC picture of glycolipids. Here C, D and E represented MEL-A, MEL-B and MEL-C, respectively. Spots B and G could be triacylated MEL and cellobiose lipids, respectively. Detailed structural elucidation is needed to prove the structure. Spots of some unknown glycolipids (A and F) were also observed.

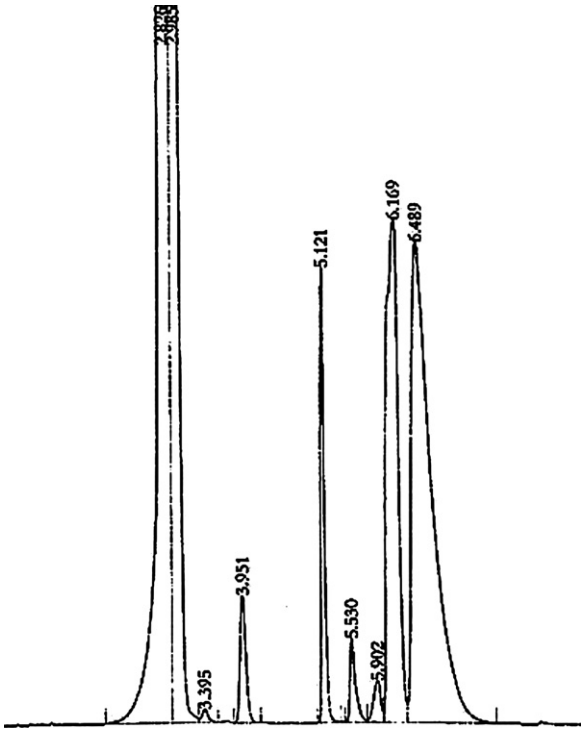

Fig. 5. HPLC chromatogram of ethyl acetate crude extract of *Pseudozyma* sp. NII 08165.

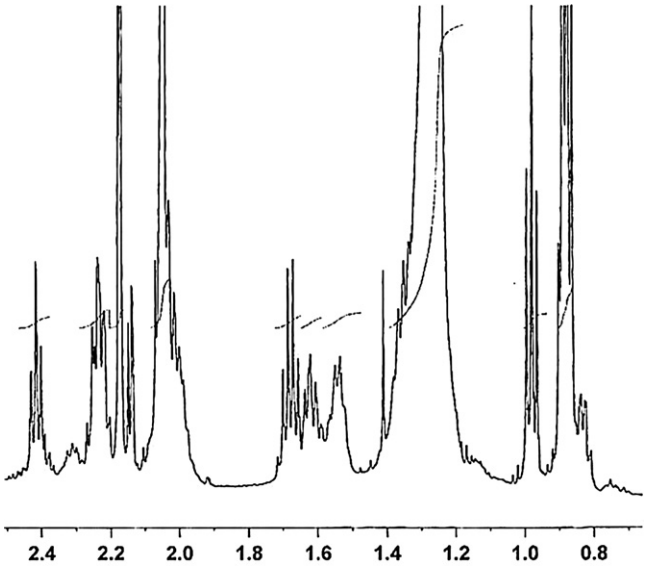

Fig. 6. ^{1}H NMR spectrum of purified MEL-C.

3.5.3. Mass spectrometry

The molecular weight of MEL-C was determined by MALDI-TOF/MS. In MALDI-TOF spectrum, 3 peaks were observed at 607.42, 634.57 and 660.57 which were estimated to be MEL-C with a short chain fatty acid C4 and a long chain fatty acids – C14:0, C16:0 and C18:1, respectively. Here the MALDI-TOF spectrum was found to be consistent with structure elucidated by NMR and GC–MS.

The MEL-C produced by NII 08165 was determined to have a unique hydrophobic structure similar to *P.*

Table 1
NMR data for MEL-C (chloroform-d, 500 MHz).

Functional group	^{13}C NMR (ppm)	Functional group	^{1}H NMR (ppm)
D-Mannose			
C-1′	99.05	H-1′	4.79 s
C-2′	69.03	H-2′	5.50 dd
C-3′	71.03	H-3′	5.16 t
C-4′	66.22	H-4′	5.11 dd
C-5′	74.88	H-5′	3.56 m
C-6′	60.43	H-6′	3.6–3.7 m
meso-Erythritol			
C-1	63.40	H-1	3.6–3.7 m
C-2	71.00	H-2	3.6–3.7 m
C-3	72.1	H-3	3.6–3.7 m
C-4	71.89	H-4a	3.77 dd
		H-4b	4.03 dd
Acetyl groups			
—C=O (C-2′)	170.20		
—C=O (C-4′)	170.81		
—CH_3 (C-2′)	20.73		2.05
—CH_3 (C-4′)	20.84		2.13
Acyl groups			
—C=O (C-2′)	173.43		
—C=O (C-3′)	171.22		
—CO—CH_2— (C-2′)	35.97		2.41 m
—CO—CH_2— (C-3′)	33.83		2.23 m
CO—CH_2—CH_2— (C-2′)	18.54		1.68 m
CO—CH_2—CH_2— (C-3′)	24.7–25.6		1.53–1.62 m
$(CH_2)_n$	22.5–31.9		1.25 b
—CH=CH—	127.5–130.5		5.3 m
—CH=CH—CH_2—CH=CH	26.42		2.75 t
—C=CH—CH_2—	26–27		2.05 b
—CH_3 (C-2′)	13.58		0.98 t
—CH_3 (C-3′)	14.12		0.86–0.89 m

s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; b, broad.

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Table 2
Fatty acid composition of MEL-C from *Pseudozyma* sp. NII 08165.

Fatty acid	wt. (%)
14:0	8.1
14:1	12.7
14:2	8.7
16:0	35.7
16:1	18.9
16:2	6.1
18:0	2.5
18:1	1.9
18:2	3.1
18:3	1.8
Unknown	0.5

siamensis and *P. shanxiensis* in having a shorter chain like C2 or C4 at the C-2′ position and a long chain like C14, C16 or C18 at C-3′ position of the mannose moiety [29,31]. Conventional MELs, on the other hand have a medium chain acid like C8–C14 at C-2′ and C-3′ positions. This unique hydrophobic structure of MEL-C contributes to higher water solubility, the property that makes it highly attractive for industrial applications.

3.6. Determination of surface tension

The surface activity of MEL-C produced by *Pseudozyma* sp. NII 08165 was studied by Wilhelmy method (Fig. 8). The surfactant concentration at which micelle formation begins is known as critical micelle concentration. A graph of surface tension versus log of MEL concentration was plotted. Two linear plots were made on the graph which represented the two phases. In the first phase, increasing surfactant concentration decreases the surface tension and another phase where no further change in surface tension was made when the surface became fully loaded with surfactant. The CMC is the point at which these two lines intersect that represents the break point of surface tensions. The CMC of MEL-C was found to be 4.5×10^{-6} M and the surface tension at the CMC of the MEL is 33 mN/m. The purified MEL had good surface activity with foaming property. This observation was similar to that of MEL-C from *P siamensis*, which reported to have CMC value of 4.5×10^{-6} with γCMC is 30.7 mN/m [31]. MEL-A from *P. antarctica* were reported to have CMC value of 2.7×10^{-6} with γCMC of 28.4 mN/m [38]. CMC of MEL-C was higher than that of MEL-A and MEL-B due to its hydrophilicity. The applicability of MEL varies with type of MEL. The most hydrophobic MEL, MEL-A was reported to be biologically active and can be used in biomedical and therapeutic applications while the least hydrophobic MEL, MEL-C can be used in oil-in-water type emulsifier and detergent formulation [31].

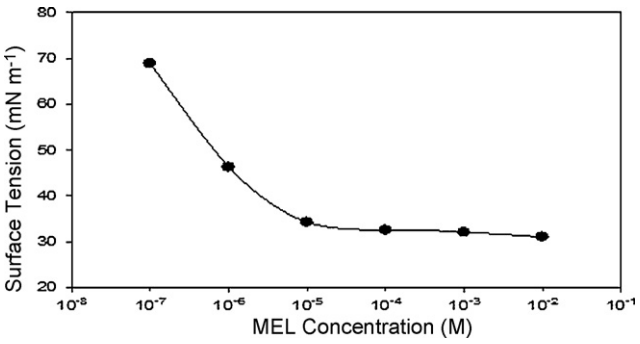

Fig. 8. CMC determination of MEL-C by surface tension versus concentration plot.

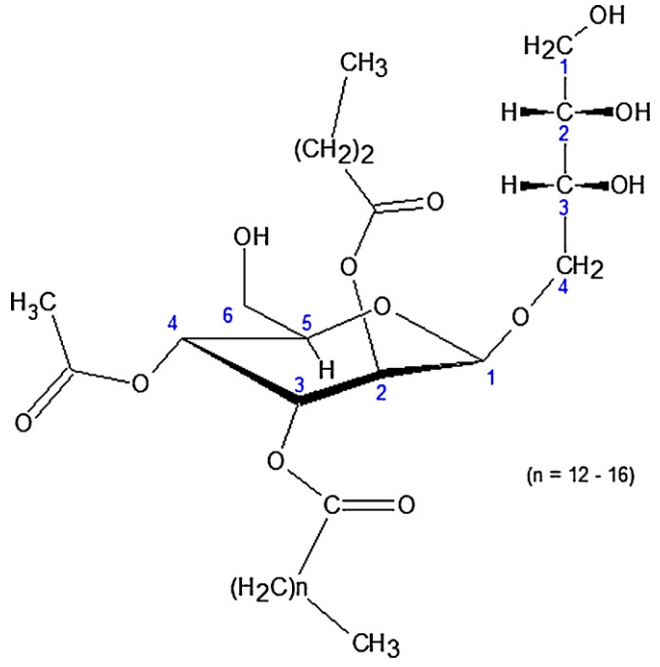

Fig. 7. The chemical structure of MEL-C from *Pseudozyma siamensis*. The MEL-C was having a unique hydrophobic structure of short chain fatty acid at the C-2′ position and long chain fatty acid at the C-3′ position, different from the conventional MEL-C which possessed two medium chain fatty acids.

3.7. Evaluation of crude biosurfactants from Pseudozyma sp. NII 08165 for the application as laundry detergents additives

3.7.1. Effect of temperature and pH on crude biosurfactants

The crude biosurfactants from *Pseudozyma* sp. NII 08165 were considered to be stable at 60 °C and 80 °C as incubation at these respective temperatures for 2 h did not result in any loss of surface activity. Temperature stability is attractive characteristic for a compound to be used as laundry detergent additives as high temperature washing result in better cleaning. The crude biosurfactants were stable over the pH range 8.0–12.0 which favour the scope of application in laundry detergent formulation because pH of laundry detergent is usually in the range of 9.0–12.0 (data not shown). Crude lipopeptide biosurfactants from *Bacillus subtilis* were stable over the pH range of 7.0–12.0, heating them at 80 °C did not result in loss of their surface active properties [20].

3.7.2. Fabric wash analysis

Since lightness is a dimension of the colour of an object, by which the object appears to reflect more or less of the incident light, stain intensity on cloth can be measured by lightness. For determining the efficiency of the detergent comprising alpha-amylase variants from alkaliphilic *Bacillus* species, stain removal on clothes was measured by reflectometry using CIE $L^*a^*b^*$ colour space where L is the lightness [39].

Fig. 9 shows percentage stain removal calculated for all the clothes stained with blood, ketchup and chocolate sauce and washed with tap water, commercial detergent, crude biosurfactants and commercial detergents mixed with crude biosurfactants in the proportion of 1:1 (w/w). Studies on cHAL (compost humic acid-like matter) in detergent formulation revealed a proportion of 1:1 (w/w) biosurfactants-commercial surfactants gave significant synergy on wash performance [40]. In this study, commercial detergent mixed with crude biosurfactants cleaned clothes better than washing with commercial detergent or crude biosurfactants alone. Stain removal by crude biosurfactants alone was efficient and comparable to that of commercial detergent, although it lacks additives

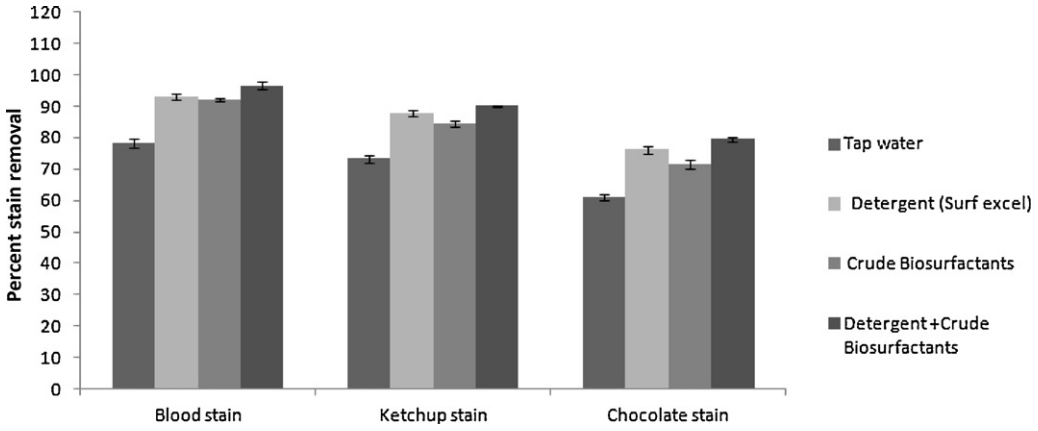

Fig. 9. Fabric wash analysis. Percent stain removal of stains such as blood, ketchup and chocolate from cotton clothes by commercial detergent, crude biosurfactants from *Pseudozyma* sp. NII 08165. and mixture of commercial detergent and crude biosurfactants from *Pseudozyma* sp. NII 08165. Here tap water was taken as control.

present in commercial detergents. The result shows that crude biosurfactants can be used as laundry additive as it improves wash performance of the detergent. The bio-washing powder which contains biosurfactants in the presence of chemical surfactants is environmentally friendly, require less post wash rinsing as glycolipids are non toxic to skin. The biosurfactants are very good at loosening fat due to structural diversity which results in better cleaning [20]. Because of all these properties, washing with biosurfactants could result in reduced consumption of energy and water. A laundry detergent comprising rhamnolipids and alkyl benzene sulphonate was efficient in removing fatty soil from cotton clothes [21]. The wash performance of laundry detergent was improved in presence of crude lipopeptide biosurfactants, which was evident from the enhanced removal of oil and blood stain from the cotton fabrics [39].

Biosurfactants such as cHAL (compost humic acid-like matter) obtained from the ground green waste or aerobically digested compost can be used in detergent formulations. Drawbacks of cHAL biosurfactants such as sensitivity to water hardness and fabric yellowing are minimized or not critically evident when biosurfactants are used together with commercial surfactants in detergent formulation [40]. Our study revealed the potential application of biosurfactants from *Pseudozma* sp. NII 08165 as laundry detergent additives. This could add up to development of sustainable technology for the formulation of laundry detergent using biosurfactants.

4. Conclusion

Pseudozyma sp. NII 08165 could be considered as a potential source of glycolipid biosurfactants as it produced mixture of MEL-A, -B and -C and some unknown glycolipids when grown on vegetable oil as carbon source. Total production of MELs reached 34 g/l and MEL-C was produced in higher quantity than all other MELs. The structure of MEL-C was elucidated in detail. The MEL-C was found to have a unique hydrophobic structure as reported from *P. siamensis*. MEL-C exhibited good surface activity with CMC 4.5×10^{-6} M and its γCMC was 33 mN/m. Due to high production of MEL-C with good surface activity and presence of more than one type of glycolipids, crude glycolipids biosurfactants from *Pseudozyma* sp. NII 08165 was studied for their application as laundry detergents additives. The temperature and pH stability of crude biosurfactants favoured their scope of application as laundry additives. Crude biosurfactants from *Pseudozyma* sp. NII 08165 removed stains efficiently and could be used in laundry detergent formulations.

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