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Characterization of sophorolipids from the yeast *Starmerella bombicola* O-13-1 using waste fried oil and cane molasses as substrates

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ABSTRACT

In order to reduce the cost of sophorolipids (SLs) production, we selected waste fried oil (WFO) and cane molasses as co-carbon sources for biosynthesis of SLs by the strain O-13-1, identified as *Starmerella bombicola*. The SLs production yield reached 49.4 ± 0.7 g/L during shake-flask fermentation period using two carbon sources. The biosurfactant components, identified by liquid chromatography associated with electrospray ionization tandem mass spectrometry, were the mixture homologs of lactonic and acidic SLs of acetylated sophorose binding with octadecanoic, octadecenoic, and octadecadienoic acid moieties. Its critical micelle concentration (36.5 mg/L) and minimum surface tension (34.9 mN/m) were almost similar to those obtained from conventional substrates. The produced SLs possessed high emulsification activity toward various hydrophobic organic compounds and good stability in a wide temperature (10°C–90°C) and pH range (3–14). Cane molasses and WFO were the cheap and easily available feedstock which helped economic production of SLs and reduced the production cost. These SLs indicated good potential in further a wide variety of industrial and environmental applications.

Keywords: Sophorolipids; *Starmerella bombicola*; Cane molasses; Waste fried oil; Emulsification activity

1. Introduction

Biosurfactants have recently attracted increasing attention as promising surfactants because of their advantages over chemical surfactants, such as high emulsification activity, low toxicity, biodegradable nature, and ecological acceptability. Sophorolipids (SLs) are a class of biosurfactants produced by *Starmerella bombicola* as a mixture of lipidized sophoroses with varying lengths of the hydroxy fatty acyl moiety [1]. In addition to their biosurfactant properties, SLs and their derivatives have demonstrated ample potential as therapeutic agents with anticancer, antimicobial, septic shock antagonist, and antialgal functions to control harmful algal blooms [2,3]. The SL biosurfactant has attracted wide attention due to its higher yield and increasing potential applications.

However, high production cost currently impedes the extensive applications of SL biosurfactants. The high yield of SLs by *Candida* usually requires both hydrophilic (glucose) and hydrophobic (vegetable oils) carbon sources, which are important factors that influence the prices of SL products [4]. Thus, the cost of these two types of substrates must be reduced to lower the price of SLs. An alternative strategy is to replace conventional and expensive substrates with inexpensive agricultural by-products or industrial wastes as fermentation feedstock. Recently, studies concerning the utilization of various low-cost raw materials employed as substrates to produce SLs have been gradually conducted [5–7]. Results have shown great potential

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in reducing the cost of SLs production using cheap raw materials as fermentation feedstock [8]. Among these substrates, waste fried oil (WFO) is a better alternative hydrophobic carbon source as a raw material for SL production than other raw materials such as refined vegetable oil and animal fats which are very costly and result in the high cost of SL [9,10]. The WFO generated increasingly from household and industrial sources is growing serious problem worldwide. Approximately five million tons of oil waste are estimated to be generated every year, because of the millions of restaurants and food industries in China [11]. The majority of used oil is generally poured down the drain as waste materials, leading to not only some environmental and ecological problems, but also some human health problems due to integration of WFO into the food chain through animal breeding [12]. Cane molasses, low-cost sustainable raw materials as hydrophilic carbon course, is a by-product of the sugar industry that is responsible for handling 540 million tons of dry sugarcane every year worldwide [13]. Molasses consists of approximately 40%–60% (w/w) total sugars (sucrose, glucose, and fructose), suspended colloids, nitrogenous compounds, and water. Carbon-rich and relatively inexpensive molasses make it a good raw material for the production of a number of industrial important chemicals, such as ethanol [14], polysaccharide [15,16], and organic acid [17]. Previous research focused on the use of one low-cost substrate (hydrophilic or hydrophobic) as a replacement for expensive materials for *Candida* to produce SLs [6,9,10,18]. However, few studies on replacing both lipophilic and hydrophilic carbon sources with cheap and waste materials to produce SLs have been carried out [19]. Recently, both industrial by-products of winterization oil cake and sugar beet molasses were used as co-substrate for the production of SLs by solid-state fermentation [20]. Moreover, although the structure of SLs has been studied in detail, much less information is available concerning the structure and performance of SLs produced using waste raw materials as fermentation substrates [19].

A biosurfactant-producing yeast strain, named O-13-1, was screened from oil-contaminated soil samples, which was collected from the Shengli Oil Field in Shangdong, PR China [21]. Previous studies showed that the yeast strain can biosynthesize SL with sugarcane molasses and soybean oil [21]. In the present study, we aimed to investigate the ability of this SL-producing yeast strain to synchronously utilize WFO and molasses as the main raw materials for the production of SLs in shake-flask experiments. Furthermore, the composition of SLs was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with qualitative and quantitative methods. The emulsifying ability of SLs on different hydrophobic organic compounds and effects of temperature and pH on SLs' emulsifying properties were also evaluated.

2. Materials and methods

2.1. Culture maintenance and Seed culture preparation

S. bombicola O-13-1 was isolated from a petroleum-contaminated soil sample collected from Shengli Oilfield, Dongying, Shangdong Province, China. The isolated strain was maintained on YPD (1% yeast extract, 2% peptone, and 2% dextrose) agar. Transfers were made to fresh agar slants to maintain viability each month at 4°C.

Erlenmeyer flasks (250 mL) containing 50 mL of YPD medium as the seed culture were autoclaved at 121°C for 20 min, and then inoculated with a loopful of the microorganism freshly grown in YPD slant. The culture was then incubated for 24 h at 30°C and 200 rpm in a rotating orbital incubator shaker.

2.2. DNA extraction and PCR

Total genomic DNA of the strain O-13-1 was isolated and purified using the methods described by Sambrook et al. [22]. The 18S rDNA and ITS genes in yeasts were amplified by the methods of Wang et al. [23], and the fragments of 18S rDNA and ITS were sequenced by Shanghai Sangon Company.

2.3. Culture conditions, substrates, and different media types

S. bombicola O-13-1 was grown in shake-flask cultures in GYU medium (10% w/v glucose, 1% w/v yeast extract, and 0.1% w/v urea) at 30°C and 200 rpm shaking unless specified otherwise. Cane molasses was collected from Qingdao Sugar Trade Company, and it contained 36.18% (w/w) sucrose, 17.53% (w/w) reducing sugar, 2.43% (w/w) crude protein, 7.61% (w/w) ash, and 5.48% (w/w) metal ions such as sodium, potassium, calcium, magnesium, aluminum, iron and copper. WFO was obtained from local restaurants that deep-fry chips and chicken. The main fatty acid composition of WFO consisted of a mixture of stearic acid (C18:0, 4.35%), oleic acid (C18:1, 46.84%), linoleic acid (C18:2, 30.61%), and palmitic acid (C16:0, 12.54%). The molasses and WFO were sterilized separately in a steam autoclave and added directly to the fermentation broth.

To study SL production using low-cost media based on sugarcane molasses and WFO, batch shake-flask experiments were carried out. The low-cost media, including cane molasses (60 g/L), WFO (60 g/L), yeast extract (5 g/L), and conventional synthetic media (60 g/L glucose + 8 g/L yeast extract + 2 g/L urea + 60 g/L oleic oil), were used to investigate SL production by the yeast O-13-1. Using an initial pH of 5.5 in the production media, experiments were carried out in triplicates in 250 mL Erlenmeyer flasks containing 50 mL of the fermentation medium and incubated for 120 h at 30°C and 200 rpm in a rotating orbital incubator shaker following inoculation with 5% (v/v) of the seed culture mentioned above.

2.4. Biomass determination

The yeast biomass was measured by estimating the cell dry weight after removal of SLs and other hydrophobic substrates in the fermentation broth using previously reported methods [24].

2.5. Extraction and estimation of SLs

SL product was extracted from the fermentation broth and then estimated following the protocol described by Hu and Ju [25]. In brief, 10 mL of fermentation broth was extracted two times with equal volume of ethyl acetate and centrifuged at 10,000 rpm for 15 min at 4°C. The organic phase

was vacuum dried at 40°C to remove the ethyl acetate. The residue was washed with hexane to remove the remaining oil. The crude SLs were obtained after vaporizing the residual hexane at 40°C under vacuum and were used to measure the properties of SLs. The crude SLs were purified according to the method described by Daverey and Pakshirajan [26] and prepared for chemical components analysis.

2.6. Characterization of SLs

The purified SLs were characterized by LC-MS as described by previously reported methods [25]. In brief, the individual SL components was separated and identified by an Agilent 1290 HPLC-Bruker Maxis Q-TOF system with UV-Visible diode-array detector and Bruker's Esquire electrospray MS^n analyzer and a Waters ACQUITY UPLC BEH130 C18 column (2.1 mm × 150 mm, 1.7 μm particles). The mobile phase used was water (0.1% formic acid and 1% acetonitrile)/acetonitrile (0.1% formic acid) (80:20) with constant gradient elution. The flow rate was 0.25 mL/min. The effluent was monitored with the UV detector with the wavelength at 207 nm, then passed through the mass spectrometer where they were ionized by electrospray at the positive mode. The molecular ions were collected in an ion trap and mass/charge (m/z) values were scanned from 200 to 900. Capillary voltage was tuned to 4.5 kV. The fragmentation information from mass spectra was used to assess the molecular structure of the SLs.

The minimum surface tension and critical micelle concentration (CMC) of the SL mixture were estimated using a surface tensiometer by the standard Du Nuoy ring method with a platinum ring of 18.7 mm in diameter. Different concentrations (0–250 mg/L) of the partially purified SLs in distilled water were prepared, and the surface tension was measured at 25°C. CMC and minimum surface tension were calculated from the relationship between the SL concentration and corresponding surface tension of distilled water [1].

Emulsification activity of the produced SLs was determined using different organic solvents, namely crude oil (SZ36-1), liquid paraffin, diesel fuel 0#, and toluene using the modified method described by Cooper and Goldenberg [27]. In brief, 6 mL of organic solvent was mixed with 4 mL of the 0.2 mg/mL SLs solution (Tween 80 as control) in a graduated screwcap test tube and the mixture was shaken vigorously in a vortex mixer for 2 min. Emulsion stability was determined after 24 h and the emulsification index was calculated by the following formula:

$$\text{Emulsification index } (E_{24};\ \%) = \frac{\text{The height of the emulsion layer}}{\text{The total height of the mixture}} \times 100\%$$

3. Results and discussion

3.1. Identification of the yeast strain O-13-1

The colony of the yeast strain O-13-1 was white. The vegetative cells of this strain were reproduced by budding (Fig. 1). The yeast strain could ferment glucose, sucrose, and raffinose, but it could not ferment galactose, maltose, lactose, trehalose, and melibiose. However, it could assimilate glucose, galactose, sorbose, sucrose, raffinose, mannitol, trehalose, sorbitol, l-arabinose, and l-rhamnose. It could not liquefy gelatin, but it could grow on medium containing 50% and 60% (w/v) glucose. The results of routine identification of the yeast strain showed that it were closely related to *S. bombicola* [28].

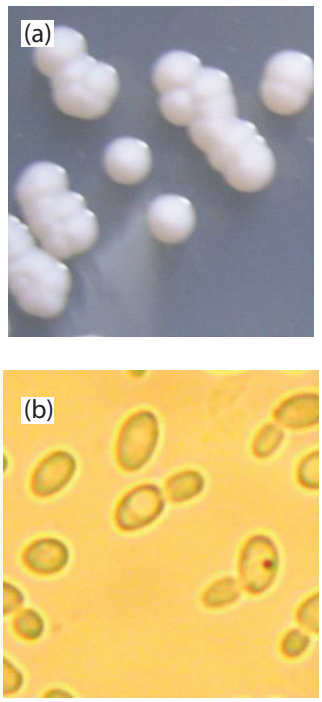

Fig. 1. Microphotographs of colonies (a) and vegetable cells (b) of the yeast strain O-13-1.

Phylogenetic analysis of the 18S rDNA and ITS sequence of the yeast strain O-13-1 with the genus from the NCBI server showed that the similarities between 18S rDNA gene sequences and *S. bombicola* and between ITS sequences and *S. bombicola* were 100% and 99%, respectively (Fig. 2). Therefore, the yeast strain O-13-1 was finally identified as a strain of *S. bombicola* [28].

3.2. SL production using different media

The time course of SL production during yeast fermentation was investigated (Fig. 3). The results indicated that SL production decreased when conventional synthetic medium was replaced with the inexpensive medium. More precisely, the yeast O-13-1 was found to produce 80.55 ± 1.85 g/L SLs when grown on synthetic medium compared with 49.4 ± 0.7 g/L SLs when grown on the low-cost medium containing cane molasses, WFO, and some yeast extract. These results matched well with the literature, in which glucose was replaced with other inexpensive fermentative carbon sources, such as sucrose [29] or deproteinized whey [5], yielding a low amount of SLs by the yeast. With respect to yeast biomass growth, a minimal difference in biomass was observed between the conventional synthetic medium and low-cost low-medium during the fermentation period (Fig. 3).

Table 1 exhibits some low-cost substrates, such as soy molasses, cane molasses, and WFO, which could be

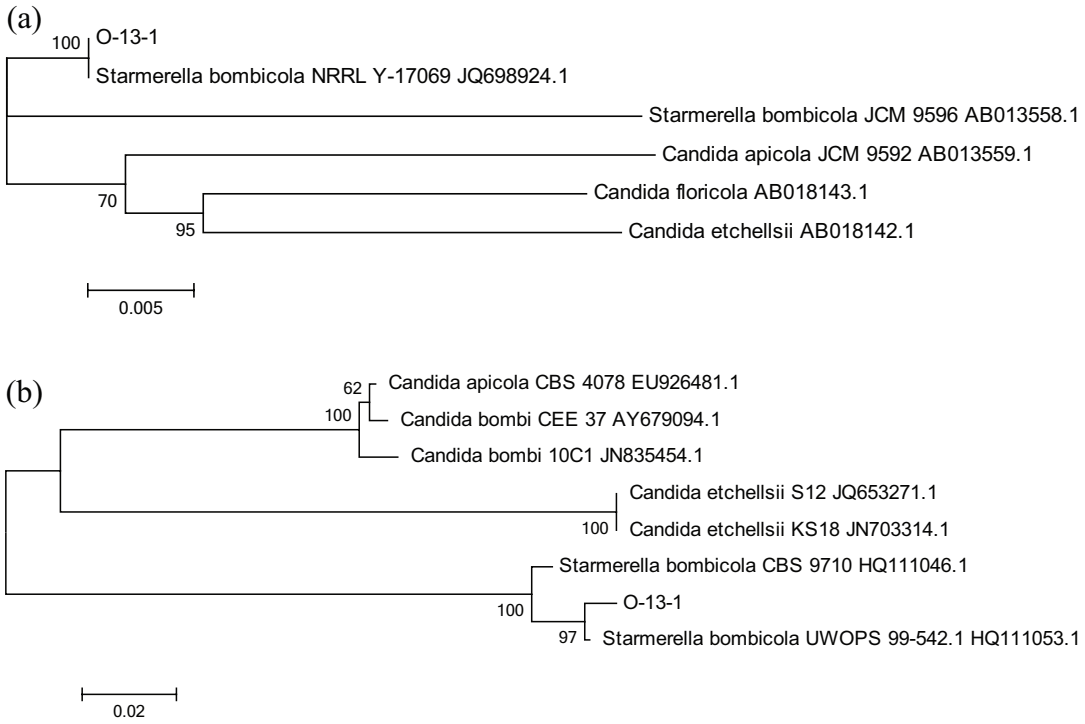

Fig. 2. The phylogenetic tree based on 18S rDNA (a) and ITS (b) sequence of the strain O-13-1 and other yeast strains.

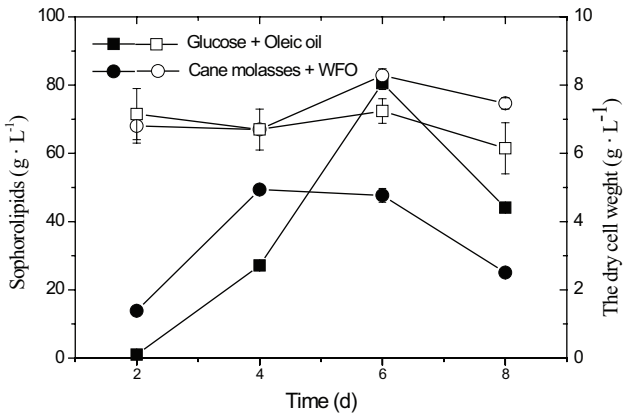

Fig. 3. Time profile of sophorolipids production and biomass grown by the yeast strain O-13-1 employing different media (-●- and -■- SLs, -○- and -□- DCW).

used as carbon sources to produce SLs by the yeast. Some SL-producing strains, the volumetric yield of SLs, and production efficiency from fermentation runs using different combinations of co-substrates are also shown in Table 1. Compared with this study, both SL yield and productivity of previous studies were comparative or lower, even though at least one costly hydrophilic or lipophilic substrate was used as a carbon source in the fermentation medium (Table 1). The SL yields reported by Fleurackers [9] were similar to the results of this research using WFO as a hydrophobic carbon source, but their incubation period was longer (14 d). Although Solaiman et al. [6] acquired higher SL yields; they used costly oleic acid as lipophilic carbon sources. High SL production efficiency and productivity in the presence of WFO compared with oleic acid could be attributed to the presence of high C18 fatty acids [24], which are the major chain length fatty acids (81.8%) in WFO in this study. The similar results also were obtained by Bezergianni et al. [30], who used waste cooking oil with a C18 fatty acid content of 91.6%.

The fermentation carbon resources have a great contribution to the price of the SL products [4]. The prices of WFO, soybean oil, and oleic acid were about 4.5, 8, and 12 CNY/kg, respectively. The prices of cane molasses (containing 53.71% total sugar) and glucose were about 1.4 and 3.6 CNY/kg, respectively. Without considering the cost of downstream purification, the cost of lipophilic raw material for producing SLs from WFO, oleic oil, or soybean oil was estimated at 5.5, 11.6, or 8.8 CNY/kg, respectively, under similar high production efficiencies (Table 1). Therefore, simplified media containing cane molasses and WFO could very well replace the costly conventional synthetic medium for better SLs yields by the yeast [31].

3.3. Structures of SLs produced from the strain O-13-1 by LC-MS analysis

The LC/Q-TOF/MS chromatogram elution profile of the fermented product from O-13-1 with cane molasses and WFO as fermentation substrates showed the presence of a mixture of SLs with varying molecular weights (Fig. 4). The chromatogram revealed various peaks showing corresponding anions with different *m/z* ratios in mass spectra (Table 2). The major peak at 4.294 min confirmed the presence of cations with *m/z* ratios of 711.36, 689.37, 671.36, and 653.35. These ions corresponded to the ionized molecules $[M + Na]^+$ of the diacetylated lactonic SL with molecular weight of 688

Table 1
The yield of sophorolipid produced from various by-products as fermentation substrates

Strain	Substrates		Price of oil (RMB/kg)[a]	SLs yield (g/L)	Oil residue (%)[b]	SLs production efficiency (g product/g substrate)	Volumetric productivity (g/L d)	References
	Carbohydrate	Lipid						
Starmerella bombicola O-13-1	Cane molasses	Waste frying oil	4.5	49.4	–	0.41	12.35	This study
Candida bombicola ATCC 22214	Soy molasses	Oleic acid	12	75.0	–	0.44	10.71	[6]
Candida bombicola	Glucose	Waste frying oil	4.5	49.0	12	0.34	3.5	[9]
Candida bombicola	Glucose	Distilled biodiesel	6.5	23.0	42	0.16	1.64	[9]
Candida bombicola ATCC 22214	Glucose	Restaurant waste oil	4.5	30.0	–	0.21	3.0	[10]
Candida bombicola ATCC 22214	Sugar beet molasses	Winterization oil cake	–	–	–	0.25	–	[20]
Candida bombicola ATCC 22214	Glucose	Stearic fatty acid residues	–	52.3	–	0.23	6.54	[24]

[a]The data from http://www.oils.net.cn/.
[b]"–" represents "no data".

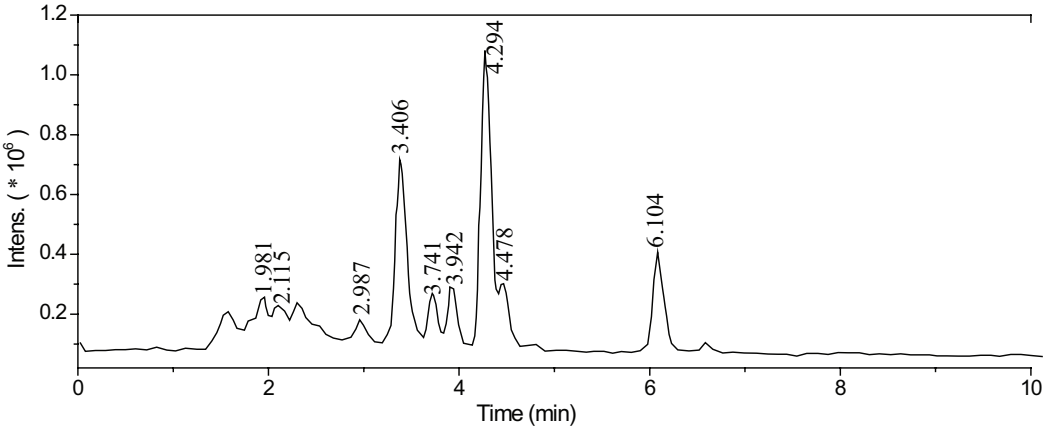

Fig. 4. Total current ion chromatogram of sophorolipids produced by the yeast strain O-13-1 with waste fried oil and cane molasses as co-carbon sources.

(SL-1, C18:1) (Fig. 5; Table 2). A characteristic ion at *m/z* 485.3 and 467.3 could be contributed to the loss of a hexose ring from the protonated molecular [M + H^+] and the sequential loss of a H_2O molecule, respectively (Fig. 5; Table 2) [32].

According the results of LC-MS analysis, the identified components of SLs produced by O-13-1 using cane molasses and WFO as the co-substrates was a mixture of both lactonic (C18:0, C18:1, and C18:2) and acidic SLs (C18:1 and C18:2; Table 2). The compositional analysis showed that most of the SLs molecules (more than 76%) existed in the lactone form, in consistent with the previous studies [20], which indicated SLs be naturally synthesized with a preference for the latonic form. In fact, the medium compositions as well as the incubation conditions, such as temperature and aeration, have influence on the ratio of the acidic form to lactone. It was found that more than 95% of the analogs are lactonic SLs when growing the yeast in the presence of glucose and oleic acid (or soybean oil) under higher aeration [33,34].

The hydroxy fatty acids of the SLs from cane molasses and WFO fermentation yielded hydroxyoleic acid (C18:1) as the major fatty acid component, followed by hydroxy-linoleic acid (C18:2) and hydroxy-octadecanoic acid (C18:0; Table 3). The results matched well with the results of other authors who used soybean oil as a lipid carbon source to produce SLs [6,18], indicating that the composition of the SLs was highly dependent on the fatty acid composition of the lipidic carbon source. On the other hand, the findings of the present study had some difference with the components of Sopholiance (commercially available SL), which was derived from oleic acid as a lipid co-substrate and contained the high C18:1 (81.3%) content (Table 3) [6]. It is noteworthy that C16

Table 2
Structure and relative abundance of the sophorolipid homologues produced by the strain O-13-1

Sophorolipid	Retention time (min)	Glycolipids type	Molecular ion	Mass fraction (%)	MS (*m*/*z*)
17-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecadienoic acid-6′,6″-diacetate	1.981	Acid	705.3692	5.48	727.35, 705.37, 587.23, 501.31
17-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecenoic acid-6′,6″-diacetate	2.115	Acid	707.3856	6.43	729.37, 707.39, 589.25, 503.32
17-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecadienoic acid-1′,4″-lactone-6′-acetate	2.987	Lactonic	647.3647	4.49	669.35, 647.36, 485.31, 467.30
17-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecadienoic acid-1′,4″-lactone-6′,6″-diacetate	3.406	Lactonic	687.3595	22.40	709.34, 687.36, 669.35, 651.33
17-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecenoic acid-6″-acetate	3.741	Acid	663.3595	5.49	685.34, 663.36, 645.35, 627.34
18-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecenoic acid-6″-acetate	3.942	Acid	663.3595	6.58	685.34, 663.36, 645.35, 627.34
17-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecenoic acid-1′,4″-lactone-6′,6″-diacetate	4.294	Lactonic	689.3747	31.71	711.36, 689.37, 671.36, 653.35
18-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecenoic acid-1′,4″-lactone-6′,6″-diacetate	4.478	Lactonic	689.3747	6.90	711.36, 689.37, 671.36, 653.35
17-L-[(2′-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)-*O*-]-octadecanoic acid-1′,4″-lactone-6′,6″-diacetate	6.104	Lactonic	691.3907	10.50	713.37, 691.39, 673.38, 655.37

saturated or unsaturated hydroxy fatty acids were not identified in this study (Table 3). It was likely due to the ability of the strain O-13-1 to convert non-oleic fatty acids (C12–C16) into primary C18 fatty acids [35].

3.4. Properties of the produced SL

3.4.1. Surface tension and CMC

A low CMC indicates that less surfactant is needed to saturate the interface between either air–liquid or liquid–liquid, as well as to form micelles. CMC and minimum surface tension in water caused by the SL products from O-13-1 were determined to be 36.5 mg/L and 34.9 mN/m, respectively. These values were comparable with those obtained with the commercially available Sopholiance [6] and SLs produced by the stain *S. bombicola* NRRL Y-17069 with sugarcane as the main carbon source [8], while the value of CMC was almost three times lower than that of SLs produced by the yeast using deproteinized whey and rapeseed oil as carbon sources [5], which could be attributed to the low ratio of acyl moieties in SLs produced with rapeseed oil as lipidic substrate and thus reduction of the hydrophobia interaction (Traube's rule) [36]. Compared with these literature-reported values of CMC and surface tension for the SLs, the results obtained in this study suggested that the SLs produced by the yeast *S. bombicola* O-13-1 provided excellent properties in terms of the reduction in surface tension and low CMC.

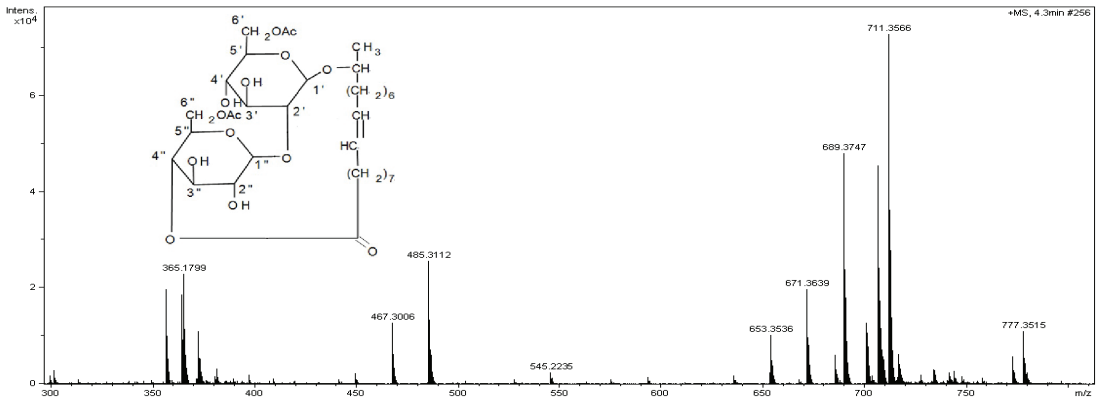

Fig. 5. ESI mass spectra of the peak with the retention time at 4.294 min. The compounds were synthesized on waste fried oil and cane molasses as co-carbon sources (lactonic sophorolipid (LS-1, $C_{18:1}$); molecular weight = 688).

Table 3
Distribution of sophorolipid according to substrates and hydroxy fatty content

Substrate		Fatty acyl group on sophorolipid (% relative abundance)				Reference
Carbohydrate	Lipid	C16:1	C18:0	C18:1	C18:2	
Cane molasses	Waste frying oil	n.d.	10.50	57.11	32.37	This study
Soy molasses	Oleic acid	3.9	6.1	81.3	5.2	[6]
Glucose	Soybean oil	6.1	13.8	42.3	36.5	[6]
Glucose, Cane molasses	Soybean oil	n.d.	13.61	40.23	35.72	[18]

n.d., not detected.

3.4.2. Emulsification activity of produced SLs

The emulsification activity with the produced SLs from O-13-1 using various organic reagents and crude oil as the substrate is shown in Fig. 6. Among the different non-aqueous phase liquids tested, all the substrates showed that the SLs exhibited higher emulsification activity than Tween 80. SLs produced by the yeast *Candida bombicola* are generally reported to be poor emulsifying agents [37]. The results of Cooper and Paddock [38] showed that SLs are unable to stabilize emulsions containing water and hydrocarbons or vegetable oils. However, SLs produced by the yeast O-13-1 exhibited better emulsification activity when grown on cane molasses as a hydrophilic carbon source alternative to costly glucose.

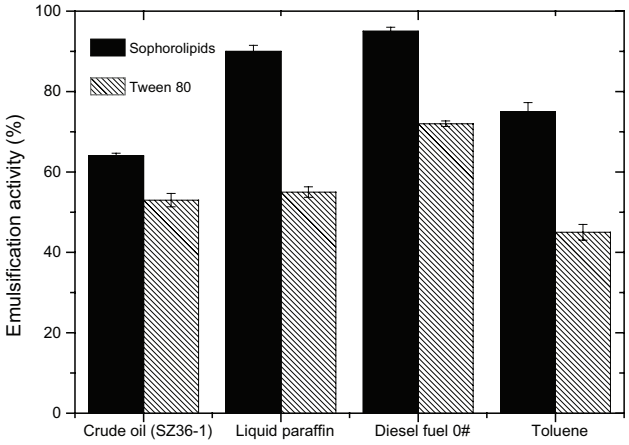

Fig. 6. Emulsification activity of the cheaply produced SLs and Tween 80 towards different oils.

3.4.3. Effects of pH and temperature on the surface tension of produced SLs

Temperature and pH are the most important environmental factors that influence the performance of a surfactant. The surface tension of the SLs was found to be nearly similar (34.5–38.5 mN/m) at the pH range of 3–14, with the lowest surface tension shown at pH 9.0 (Fig. 7). By contrast, the surface tension of liposan (another biosurfactant) was found to be better only in a narrow pH range of 2.0–5.0 [39]. Fig. 7 also illustrates that SLs could maintain low surface tension at a wide range from 10°C to 90°C. A similar trend was also reported by Mahanty et al. [40].

4. Conclusion

In this study, conventional synthetic media were replaced with cheaper alternative media containing cane molasses and WFO as the main carbon sources for producing SLs by *S. bombicola* O-13-1. LC-MS spectra confirmed that the structure and composition of SLs using WFO as a co-carbon resource were similar to those mentioned previously. The properties of the produced SLs, including CMC and minimum surface tension in water, emulsification activity against several organic solvents and crude oil, and good stability in a

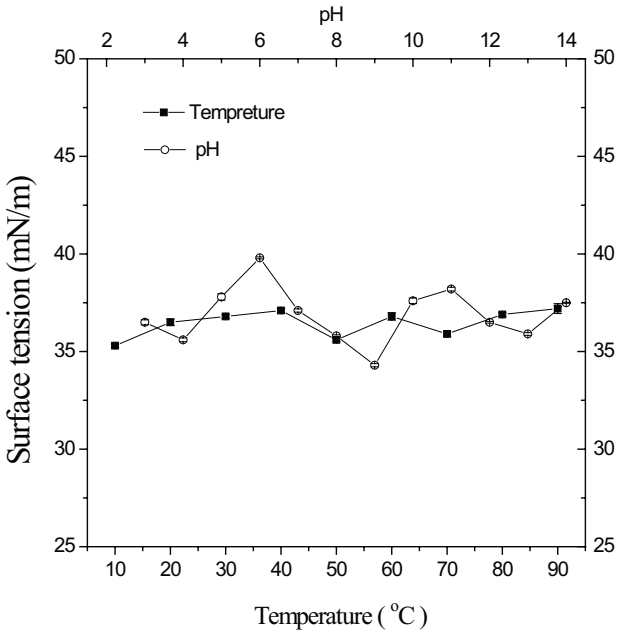

Fig. 7. Influence of pH and temperature on the surface tension of the solutions containing sophorolipids produced by the yeast strain O-13-1.

broad range of pH and temperatures, indicated the very high potential of cheaply produced SLs for further environmental applications.

Since the two major substrates cane molasses and WFO are two major industrial wastes and which are a big threat to the environment, the government has spent much budget to deal with them every year. Here, we recycled these two waste and succeeded to produce a more valuable biosurfactants. This might also help to relief the environmental burden to some extent.

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