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Lipid production by *Lipomyces starkeyi* using sap squeezed from felled old oil palm trunks

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The ability of oleaginous yeast *Lipomyces starkeyi* to efficiently produce lipids when cultivated on sap extracted from felled oil palm trunk (OPT) as a novel inexpensive renewable carbon source was evaluated. OPT sap was found to contain approximately 98 g/L glucose and 32 g/L fructose. Batch fermentations were performed using three different OPT sap medium conditions: regular sap, enriched sap, and enriched sap at pH 5.0. Under all sap medium conditions, the cell biomass and lipid production achieved were approximately 30 g/L and 60% (w/w), respectively. *L. starkeyi* tolerated acidified medium (initial pH ≈ 3) and produced considerable amounts of ethanol as well as xylitol as by-products. The fatty acid profile of *L. starkeyi* was remarkably similar to that of palm oil, one of the most common vegetable oil feedstock used in biodiesel production with oleic acid as the major fatty acid followed by palmitic, stearic and linoleic acids.

[**Key words:** Oleaginous yeast; *Lipomyces starkeyi*; Oil palm trunk; Sap; Single cell-oil]

Under the condition of nutrient stress (particularly nitrogen-limited) and in the presence of excess carbon source, oleaginous yeasts accumulate single cells-oils (SCOs) as intracellular carbon and energy reserves. The SCOs or microbial lipids have attracted much attention due to their similar fatty acid profile to that of oils from agricultural oil crops, which make them generally suitable for processing into several other products in addition to biodiesel, such as fungicides, bactericides, emulsifier, plasticizers, surfactant and lubricants (1–3). The use of microbial lipids for such applications has many advantages over agricultural oil crops, since their production life-cycles are short, do not require of large land areas for production, independence from a climatic condition and require comparatively less manpower (2–4). However, techno-economics evaluations of such processes (i.e., biodiesel from single cell oil) show that this production route also depends on the cost of feedstock which is estimated to be about 80% of the total medium cost (4–6). It has been shown that the cost of carbon source is the major factor that contributes to the production cost of SCOs. Hence, the utilization of cheap renewable carbon source for SCOs production would have a positive influence to make the processes economically feasible (7–9).

Oil palm (*Elaeis guineensis*) is widely planted for its edible oil in tropical countries such as Indonesia and Malaysia. The Indonesian palm oil industry has thrived in recent decades and Indonesia has emerged as one of the world's leading palm oil-producing countries. Together with neighboring Malaysia, it accounts for approximately 88% of the world palm oil production according to FAOSTAT database (http://faostat.fao.org/site/567/default.aspx; accessed March 5, 2018). In order to maintain the palm oil productivity, the palm oil needs to be replanted at an interval of 20–25 years.

When replanting, old palm trees are cut and most of them are discarded or burnt at the plantation site (used for mulching). Therefore, appropriate ways for utilizing oil palm trunks is necessary for the ideal and sustainable palm oil industry (10). According to Kosugi et al. (11), replanting of palm oil trees would generate on an average of 64–80 million palm trees every year as lignocellulosic residues in Indonesia and Malaysia for the next 25 years. Due to the high moisture contents (70–80% on the basis of the total mass), palm trunks are not appropriate as lumber which leads to large warping after drying and only a small percentage of trunks, especially the outer part of the trunk are utilized as plywood and particleboard industries. In the plywood production process, the inner part is discarded in large amounts due to its extremely weak physical properties. In the meantime, it has been traditionally practiced to produce palm sugar and palm wine using sap obtained by tapping the inflorescence of various species of palm (10,11).

Previous study by Kosugi et al. (11) was able to obtain 0.65 g of sap from 1 g of the trunk (inner part) using a laboratory-scale press.

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OPT sap is rich in sugars, amino acids, organic acids, mineral and vitamins (11). Sugars form the major constituent of sap and it has been reported to contain 8.3% w/v of total sugars in a freshly logged trunk (10). Glucose was found to be the dominant sugar in OPT sap accounting for approximately 85% of the total free sugars. In addition to glucose, a significant amount of fructose was also contained in the sap (11). To date, nutrient-rich sap from OPT has not yet been commercially exploited and has high potential to be utilized as an oil palm industry waste to produce value-added products like biofuels (11,12). Another advantage of OPT sap is that it contains all the macro and micronutrients which are required for microbes growth, and it can potentially be exploited as a yeast growth medium.

Escorted by cheap renewable carbon sources available, complete utilization of these resources are requisite to realize the cost effective value-added bioconversion products. Hereof, employing a microbial strain with efficiently utilizing these resources is urgently needed. Fewer than 30 of the about 600 yeast species are known to be oleaginous (13,14). Oleaginous yeasts are typically found but are not limited to genera such as *Candida*, *Crytococcus*, *Rhodotorula*, *Rhizopus*, *Trichosporon*, *Lipomyces*, and *Yarrowiya* (15). *Lipomyces starkeyi* is a well-known strain and promising candidate to produce SCOs. This strain has the capability to accumulate over 70% (w/w) lipid under defined culture conditions and can produce lipid on various carbon sources including from wastes such as sewage sludge, fish meal wastewater and MSG wastewater (16,17). In our previous study (18), *L. starkeyi* was used as a platform to evaluate the SCOs production in synthetically nitrogen-limited mineral media and lipid production was achieved at higher than 80% (w/w). Several studies (19–22) also have shown that *L. starkeyi* could produce a considerable amount of SCOs in hemicellulose hydrolysate under the presence of inhibitory chemical compounds such as aldehyde, alcohol and organic acid derivatives. In regards of these findings, *L. starkeyi* can be considered a promising oleaginous yeast platform for industrial scale production of lipid. A combined approach has been attempted for the first time to evaluate the potential of OPT sap as a novel inexpensive renewable carbon feedstock for SCOs production.

MATERIALS AND METHODS

Yeast strain The yeast strain *L. starkeyi* NBRC10381 used in this study was selected from the NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, Japan. The yeast strain was preserved in 20% (w/w) glycerol at −80°C and was revived by streaking onto a potato dextrose agar (PDA) plate. Afterwards, the yeast strain was grown on a yeast extract-malt extract-peptone-glucose (YMPG) agar plate (yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, glucose 10 g/L and agar 16 g/L).

Seed preparation One colony from a YMPG agar plate was inoculated into pre-culture medium which contained 12 mL of YMPG broth (yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, glucose 10 g/L) in a 100 mL Erlenmeyer flask and incubated in an orbital shaker incubator (BioShaker BR-43FH MR, Taitec Corp., Tokyo, Japan) at 190 rpm for 24 h at 30°C. Then, these seeds culture were transferred to 12 mL OPT sap medium for lipid production.

Extraction of OPT sap Oil palm trunks (*E. guineensis* var. Tenera) approximately 25 years old, were obtained from the oil palm plantations of Pahang, Malaysia. The palm trees were felled and cut approximately 10 cm thick disk and kept at −20°C prior to use. The disks were cut into smaller sections and the central moist region was retained while the harder peripheral bark region was disposed. Sap was collected by pressing the disks using a laboratory-scale press at 80 MPa. The extracted sap was centrifuged at 10,000 × *g* for 5 min and the supernatant was at −20°C before use.

Preparation of extracted OPT sap as the fermentation medium To evaluate the capability of OPT sap as the fermentation medium, the OPT sap was divided into three categories i.e., regular sap, enriched sap, and enriched sap with adjusted pH. Enriched sap was supplemented with mineral medium (MM), consisting of the following compounds dissolved in deionized water: $(NH_4)_2SO_4$ (0.25 g/L); $MgSO_4 \cdot 7H_2O$ (1 g/L); KH_2PO_4 (1.5 g/L); $Na_2HPO_4 \cdot 2H_2O$ (5 g/L). The trace element solution consisting of $FeSO_4 \cdot 7H_2O$ 0.08 g/L, $ZnSO_4 \cdot 7H_2O$ 0.01 g/L, $CaCl_2 \cdot 2H_2O$ 0.1 g/L, $MnSO_4 \cdot 4H_2O$ 0.1 g/L, $CuSO_4 \cdot 5H_2O$ 0.002 g/L, $CoCl_2 \cdot 6H_2O$ 0.002 g/L was also included in MM. The enriched sap with adjusted pH was prepared in the same way with enriched sap and the initial pH was adjusted to 5 with 2 N NaOH. Whereas, the regular sap was prepared with neither of additional mineral medium nor adjusted pH, where the initial pH of regular sap was 3.2. In order to obtain an equal concentration of sugars at the initial stage a certain amount of deionized water was added to the regular sap medium and the dilution effects are negligible. Hereinafter, the three types of OPT sap used in this study are: regular sap (Reg-Sap), enriched sap (Sap+MM) and enriched sap with adjusted pH (Sap+MM pH 5). The OPT sap medium was sterilized by the addition of both ampicillin (100 μg/mL) and kanamycin (20 μg/mL) to prevent contamination.

Cultivation conditions The seed volume of the pre-culture in YMPG broth was adjusted to an initial optical density (OD_{600}) of 14–16 for obtaining an appropriate active cell fraction which increases the capability of cells to utilize carbon sources. Afterward, the seed culture from the YMPG broth was transferred to regular-sap, enriched-sap, and enriched-sap with adjusted pH media for cultivation with 12 mL working volume. These cultures were incubated in an orbital shaker incubator that was operated at 190 rpm and 30°C until all of the carbon sources were completely exhausted. Growth was analyzed gravimetrically by measuring the DCW of biomass. Following inoculation, samples for the measuring of DCW were taken on the initial day of cultivation and every 24 h thereafter until all the carbon sources were consumed. The determinations of DCW, lipid quantity and fatty acid composition were conducted according to the method as previously described (18,22).

Sugar analysis The concentrations of sugars during fermentation were analyzed using high-performance liquid chromatography (HPLC) (LC-20AB, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) equipped with a Shimpack SPR-Pb column (Shimadzu) operated at a column temperature of 80°C. The mobile phase was water at a rate of 0.6 mL/min. Any possibilities of alcohol and polyols contents in the OPT sap during or prior the fermentation were analyzed on the same HPLC system. ICSep Coregel-87H column (7.8 mm I.D. × 300 mm Transgenomic) was used for this purpose and operated at the same conditions with 5 mM H_2SO_4 as the mobile phase. All samples were centrifuged to remove the cell mass and other water-insoluble substances and then filtered through a 0.22 μm filter before analysis.

Determination of total lipid quantity by gravimetric analysis To measure total lipid content, a gravimetric analysis was used as previously described (18,23). Briefly, triplicate 15 mg samples of freeze-dried cells were transferred to a 2 mL polypropylene microvial with an O-ring sealed cap containing 0.5 mm zirconia beads and 1.5 mL of Folch solvent (2:1 of $CHCl_3$: MeOH, v/v). Cells were pulverized using a Shake Master Neo ver. 1.0 (BMS-M10N21, BMS, Tokyo, Japan) at 1500 rpm for 15 min. Afterward, cells were centrifuged at 14,000 × *g* for 5 min, the supernatant was removed, and the pellets were washed with 1.5 mL of deionized water, then pulverized a second time, centrifuged, and the remaining water was then removed. The cell pellets were dried at 60°C to a constant weight. The lipid content was determined by the weight difference and expressed as a percent of dry cell weight.

Determination and quantification of total fatty acid methyl esters via gas chromatography To determine the fatty acid methyl esters (FAMEs) composition, derivatization of methyl esters from fatty acids by transmethylation was performed following the protocol stipulated in the fatty acid methylation kit (Nacalai tesque, Inc., Japan). Then the fatty acid methyl esters were analyzed with a gas chromatography–mass spectroscopy (GC-MS QP 2010 Ultra, Shimadzu) instrument, equipped with a DB-23 capillary column 0.25 mm × 30 m (Agilent J&W Scientific, Santa Clara, CA, USA) and helium as the carrier gas. Operating conditions were as follows: the flow rate for helium gas at 0.8 mL/min and with a split ratio 1:5, the injection temperature was 250°C. The primary GC oven temperature was programmed at 50°C for 1 min, then increased at 25°C/min up to 190°C and finally increased at 5°C/min up to 235°C for 4 min. The temperatures of the ion source and the interface of the mass spectroscopy (MS) detector were set at 230 and 250°C, respectively. A full scan (at mass range m/z 46-500) and SIM methods were applied using an MS detector. The volume of injection was one microliter. Fatty acids were identified and quantified by comparison of their retention times and peak areas with a standard mixture of fatty acids using Supelco 37 component FAME mixture (Sigma–Aldrich). Caprylic acid (C8:0) was included in each sample as the internal standard. The percentage of total fatty acid content was calculated as the ratio of the individual FAME peak area to the sum of all FAME peak areas, excluding the internal standard. The weight of each fatty acid was calculated by noting the amount of internal standard (IS) that had been added, and then multiplying that amount by the ratio of the FAME area to the IS peaks.

RESULTS AND DISCUSSION

Fermentation profiles The sugar components in OPT sap was analyzed by HPLC prior to its fermentation. Glucose and fructose were found as sugar component in sap at concentrations approximately 98 and 32 g/L, respectively. To obtain an equal concentration of sugars, a certain amount of deionized water was added prior to

the fermentation process. In each OPT sap medium conditions, the concentration of glucose and fructose were set up at 85 and 25 g/L, respectively, to initiate the fermentation process for lipid production. The HPLC analysis also confirmed that polyol or alcohol derivatives were not detected before the fermentation begin.

The time courses for the carbon source consumption and the cell growth of *L. starkeyi* in different OPT sap medium conditions are shown in Fig. 1. In general, the yeast rapidly utilized glucose and fructose due to its complete carbon source consumption and produced approximately 30 g DCW/L biomass within 4 d under different sap medium conditions. Under all condition tested, glucose was most quickly consumed followed by fructose. To attain a clearer perception into the ability of *L. starkeyi* to utilize carbon source and grow, the carbon source consumption rates (Fig. 2A) and growth rates (Fig. 2B) were evaluated. The carbon source consumption rates and growth rates were either determined in the first 24 h (q_i) of fermentation process or when the carbon sources were completely consumed (q_t).

L. starkeyi achieved the highest q_i for glucose at 2.3 g/L·h in the Sap+MM (pH 5) medium, following 2.2 and 1.5 g/L·h in Sap+MM and Reg-Sap media, respectively (Fig. 2A). A similar pattern was also observed for fructose, where high q_i was achieved at 0.5 g/L·h in Sap+MM pH 5 and Sap+MM mediums, following 0.3 g/L·h in Reg-Sap medium. Enrichment of the OPT sap with mineral medium and adjustment of the initial pH into suitable fermentation condition seemed to instigate the ability of yeast cells to consume carbon sources with higher rates at the first 24 h. Nevertheless, the consumption rates in the entire cultivation process (q_t) were identical for all medium conditions and achieved approximately at 0.9 g/L·h for glucose and 0.3 g/L·h for fructose. This condition indicated that although in the absence of mineral medium and without adjusting the initial pH, the fermentation process in Reg-Sap medium could achieve the carbon source consumption at the similar rate to Sap+MM and Sap+MM (pH 5) media. This could be attributed to the fact that the OPT sap was a complex mixture with various sugars, organic acids, vitamins, minerals and amino acids (11,12) which indicating that OPT sap contains sufficient nutrients to support microbial fermentation, especially by *L. starkeyi*. Additionally, high level of initial inoculum concentration was applied in this study (at OD_{600} 14 to 16) to initiate the fermentation process. A higher level of inoculum concentration leads to an increase in the active cell fraction. Consequently, it will lead to a higher cell/carbon source ratio and to an increase in the carbon-source consumption rate (18).

The growth rate of *L. starkeyi* at the first 24 h (g_i) and over the whole fermentation process (g_t) in different condition of sap mediums are shown in Fig. 2B. Following the similar patterns of consumption rates, the growth rates of *L. starkeyi* in Reg-Sap medium at the first 24 h was lower (0.1 g/L·h) compared with two other medium conditions (0.3 and 0.2 g/L·h, for Sap+MM and Sap+MM pH 5 media, respectively). However, the growth rates of *L. starkeyi* over the whole fermentation process (g_t) were found at similar level, approximately at 0.2 g/L·h for all conditions tested. Hence, the OPT sap containing almost all the macro and micronutrients, therefore its suitable for enhancing the cell growth of *L. starkeyi* even without mineral medium supplementation. Moreover, the maximum growth rate was also strongly influenced by the inoculum size (18).

Ethanol and xylitol as by-products Pentose fermenting yeasts have been reported to accumulate ethanol and a variety of polyols, including xylitol, ribitol, arabitol, glycerol, acetoin and 2,3-butanediol (24). In the group of the oleaginous yeasts, only *Yarrowiya lipolytica* is known to be a significant producer of ethanol and other polyols (25). However, recent studies by Calvey et al. (20) found that *L. starkeyi* produces a significant amount of ethanol and other polyol compounds under lipogenic conditions. In this study, a considerable amount of ethanol and xylitol were also produced as by-products during *L. starkeyi* fermentation under all different sap medium conditions. The time courses for ethanol and xylitol production under different sap medium conditions are shown in Fig. 3.

The concentrations of ethanol and xylitol increased overtime in all condition tested. Additionally, the catabolism of accumulated alcohol only attempted after carbon sources exhausted. The high sugar consumption rate during the first 24 h of fermentation (Fig. 2A) leads to the high utilization of glucose and fructose contained in Sap + MM medium (pH 5), resulting in complete assimilation of ethanol and xylitol (Fig. 3). Fermentation with Regular sap and Sap+MM media were unable to metabolize the remaining ethanol in the time allotted (Fig. 3). Lower initial pH might be responsible for this condition which reduce the number of active cells fraction to metabolize the remaining ethanol and xylitol. As a result, the pH was decreased over the course of fermentation process which could decelerate cell growth and escort premature death (data not shown). The optimal initial pH for lipid production in *L. starkeyi* depends upon the carbon and nitrogen sources present in the media (17). In our preliminary experiments, it was observed an optimum SCOs production could be achieved by adjusting initial pH media approximately at pH 5 (data not shown). However, *L. starkeyi* has been characterized as a yeast tolerant to low pH and grows at pH 3.5 or even lower (26,27).

Ethanol and xylitol productions were observed under all condition tested (Fig. 3). The accretion of alcohols (ethanol and xylitol) began at the first 24 h, with the higher ethanol accumulation was observed during 2 d fermentation in Reg-Sap medium with concentration achieved approximately 25 g/L. Higher xylitol accumulation was also observed in the fermentation with Reg-Sap medium with concentration approximately 10 g/L during 4 d fermentation. In general, the concentrations of each alcohol increased progressively. After the exhaustion of carbon sources, some alcohols were consumed by cells and probably used for production of cell mass

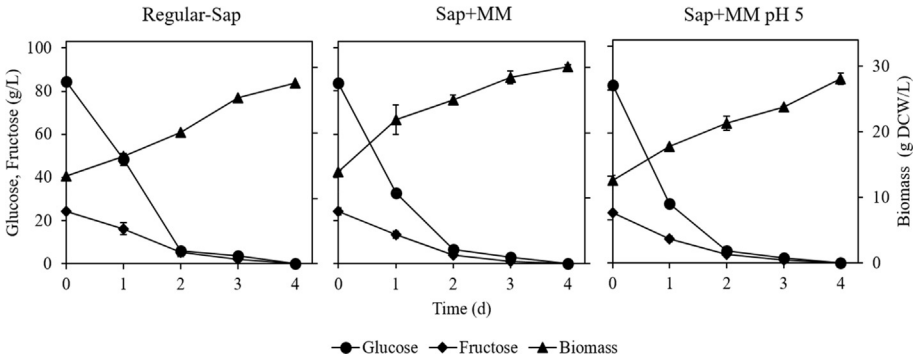

FIG. 1. Time courses for carbon source consumption and cell growth of *L. starkeyi* in different sap media. Circles represent glucose consumption, diamonds refer to fructose consumption, and triangles chart for the cell growth. The error bars indicate the standard deviation from three independent experiments.

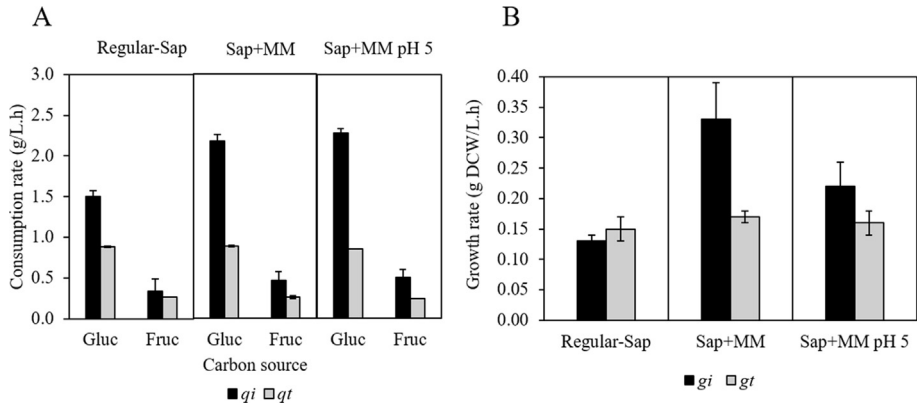

FIG. 2. Carbon consumption rates and growth rates of *L. starkeyi* in different sap media. (A) Carbon source consumption rate of *L. starkeyi* at the first 24 h (q_i: closed bars) and over the whole fermentation process (q_t: shaded bars) in the different sap media. (B) Growth rate of *L. starkeyi* at the first 24 h (g_i: closed bars) and over the whole fermentation process (g_t: shaded bars) in the different sap media. The error bars indicate the standard deviation from three independent experiments.

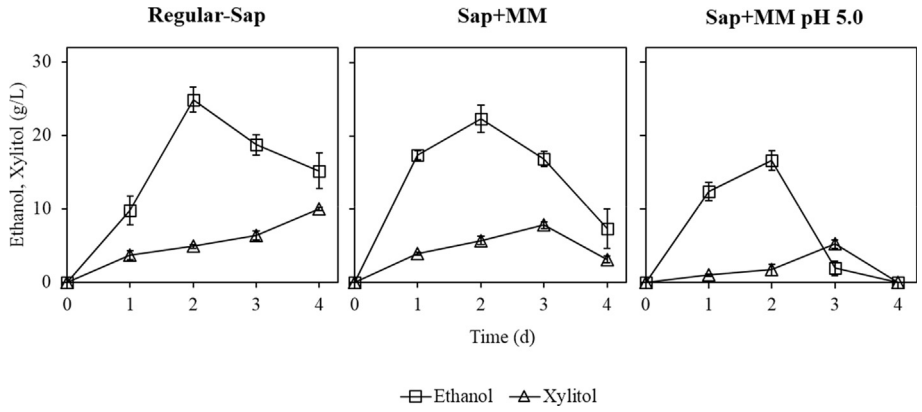

FIG. 3. Time course for ethanol and xylitol as by-products from fermentation of *L. starkeyi* under different sap media conditions. Circles represent ethanol production; triangles refer to xylitol production. The error bars indicate the standard deviation from three independent experiments.

and more lipids. Catabolism of alcohols followed a similar pattern in all conditions tested, except for xylitol in Reg-Sap medium. Supplementation of OPT sap with mineral medium and adjusted initial pH to the optimum fermentation condition for *L. starkeyi* led to more rapid catabolism of ethanol and xylitol after carbon sources completely consumed. As noted in *Y. lipolytica* by Workman et al. (25), the production of alcohols (polyols) as by-products in oleaginous yeast is occur due to cofactor imbalances occurred after the cells reached oxygen limitation, particularly an excess of NADPH, which are restored by the reduction of sugars into alcohols or by the reduction of pyruvate into ethanol or butanediol. Additionally, in the SCOs production process, high sugar concentration in the medium may also result in the secondary metabolite formation (8), since the total sugar concentration in OPT sap was higher than 100 g/L.

SCOs production and fatty acid profiles of intracellular lipid Table 1 shows the SCOs production of *L. starkeyi* grown on different sap medium conditions. The SCOs production was expressed as the lipid content when the carbon sources were completely consumed during fermentation process. According to Table 1, the SCOs productions from approximately 110 g/L total carbon sources (glucose+fructose) generally reached more than 50% (w/w) of lipid. The highest lipid content of 64.4 ± 1.9% (w/w) was achieved from the fermentation of *L. starkeyi* in Sap+MM medium with a lipid yield of 0.19 ± 0.02 (w/w), followed by fermentation in Sap+MM pH 5.0 medium which produced 63.1 ± 1.6% (w/w) lipid content with lipid yield of 0.17 ± 0.00 (w/w). For the fermentation in Reg-Sap medium, the lipid content achieved was 55.2 ± 1.9% (w/w) with lipid yield of 0.15 ± 0.01 (w/w). The high lipid accumulation in fermentation process with Sap+MM and Sap+MM (pH 5) media was probably due to complete assimilation of alcohols (ethanol and xylitol) as by-products, which led to high biomass (DCW) and lipid production. Additionally, the supplementation of OPT sap with mineral medium and adjusting initial pH could provide beneficial nutrition and suitable medium environment for continuous cell growth. However, the use of Reg-Sap as the fermentation medium also produced good lipid content of higher than 50% (w/w) with no requirement for any additional mineral medium or adjusting initial pH, which is beneficial for the process economics.

The pH of oleaginous yeast cultures is an important factor, as lipid production is significantly reduced at low pH values in some

TABLE 1. SCOs production comparison by *L. starkeyi* in the different sap media conditions[a].

Medium type	Biomass (g DCW/L)	Biomass yield (w/w, %)	Lipid content (w/w %)	Lipid yield (w/w)	Cultivation time (d)
Regular-Sap	27.7 ± 0.7	25.4 ± 0.6	55.2 ± 1.9	0.15 ± 0.01	4
Sap+MM	30.1 ± 0.4	27.9 ± 0.5	64.4 ± 1.9	0.19 ± 0.02	4
Sap+MM pH 5.0	28.1 ± 0.8	26.7 ± 0.3	63.1 ± 1.6	0.17 ± 0.00	4

Biomass yield: gram of dry cell biomass per gram of sugar consumed ×100%. Lipid yield: milligram of lipid per milligram of sugar consumed.

[a] The values are given as the mean ± SD of triplicate determinations.

TABLE 2. Comparison of fatty acid profiles of *L. starkeyi* at different sap medium[a].

Medium type	FAME composition										
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C24:0
Regular-Sap	0.0 ± 0.0	0.4 ± 0.1	30.3 ± 1.1	1.6 ± 0.6	6.3 ± 1.4	53.7 ± 1.8	5.7 ± 0.6	0.3 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.5 ± 0.1
Sap+MM	0.0 ± 0.0	0.1 ± 0.0	34.6 ± 0.8	1.22 ± 1.0	8.2 ± 0.7	49.3 ± 2.4	3.0 ± 0.9	0.7 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.0
Sap+MM pH 5.0	0.0 ± 0.0	0.2 ± 0.0	33.3 ± 0.4	2.8 ± 0.9	4.8 ± 1.4	52.0 ± 1.9	3.6 ± 0.7	0.4 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.3 ± 0.1
Average	0.0	0.2	32.7	1.9	6.4	51.8	4.1	0.5	0.1	0.0	0.3
Palm oil[b]	0.1	0.7	36.7	0.1	6.6	46.1	8.6	0.3	0.4	0.1	0.0

[a] The values are given as the mean ± SD of triplicate determinations.
[b] Palm oil fatty acid profile as reported by Ramos et al. (37).

species (7). The SCOs production results in the media Reg-Sap and Sap+MM indicated that *L. starkeyi* is capable of thriving and accumulating lipids under acidic conditions (pH at the initial fermentation process was 3.2). This also may be beneficial in consideration of biodiesel economics, as pH control adds to the production cost of any fermentation process (28). Furthermore, acidic pH can help to suppress the growth of bacterial contaminants, which is the major concern in industrial microbiology (29).

A summary of the fatty acid methyl ester profiles obtained from these experiments is shown in Table 2. On average, the profile of total accumulated lipids was comprised of approximately 33% palmitic acid (C16:0), 2% palmitoleic acid (C16:1), 6% stearic acid (C18:0), 52% oleic acid (C18:1), and 4% linoleic acid (C18:2). These results are similar with lipid profiles obtained previously by other researchers (20,30–32), although inappreciable differences were observed which can be explained by discrepancy between *L. starkeyi* strains and culture conditions (28). The fatty acid profile of *L. starkeyi* was remarkably similar to that of palm oil, which is one of the most familiar vegetable oil feedstock used in biodiesel production (Table 2). This observation suggests that biodiesel produced from lipids extracted from this species would have desirable fuel properties and would be compatible with existing diesel engine technology. This is not the case in most oleaginous fungi and algae, which have elevated levels of polyunsaturated fatty acids which are undesirable in biodiesel fuels (20,32,33). Moreover, there has been considerable interest in high oleic oils for biofuels with improved oxidative stability and cold-flow properties, and as input for many renewable polymers, lubricants, elastomers, and other oleochemicals (34–36).

For the first time, we have successfully demonstrated the potential of OPT sap as a novel inexpensive renewable carbon feedstock for SCOs production by *L. starkeyi* NBRC10381. High lipid content at around 55% (w/w) could be achieved in 4 d, even when the raw material (Reg-Sap) was used directly as the medium. Addition of some minerals (MM) could enhance the lipid content up to around 66% (w/w). Moreover, the profile of fatty acids indicated that the quality of lipid produced by the strain was considered as a material not only for biodiesel production but also for high-valued fatty acid production.

This work has demonstrated that *L. starkeyi* could grow well in each condition of OPT sap medium and efficiently accumulate lipids. The OPT sap represents almost all the macro and micronutrients required for good cell growth. Moreover, the supplementation of OPT sap with MM and adjusting initial pH prior to the fermentation process was not necessary, which is beneficial for the process economics. Results from this study clearly demonstrated the potential of *L. starkeyi* as a microbial lipid producer as well as OPT sap as an important renewable carbon feedstock for SCOs production.

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