Kasetsart J. (Nat. Sci.) 44 : 436 - 445 (2010)

Selection and Optimization for Lipid Production of a Newly Isolated Oleaginous Yeast, *Rhodosporidium toruloides* DMKU3-TK16

Pakawat Kraisintu, Wichien Yongmanitchai and Savitree Limtong*

ABSTRACT

Rhodosporidium toruloides DMKU3-TK16, an oleaginous yeast, was isolated from soil by an enrichment technique in nitrogen-limited medium I. Lipid production by this strain was highest in shaking flask cultivation at 150 rpm and 28°C in nitrogen-limited medium II containing per liter 70 g glucose, 0.75 g yeast extract, 0.55 g $(NH_4)_2SO_4$, 0.4 g KH_2PO_4, 2.0 g $MgSO_4 \bullet 7H_2O$, with a pH of 5.5. Under these optimal conditions, when the C/N ratio of the medium was 140, *R. toruloides* DMKU3-TK16 produced a lipid quantity of 9.26 g/L, which was 71.30 % of the dry biomass (13.33 g/L) after 168 h of cultivation. The major fatty acids of the cellular lipid were oleic acid (41.54%), palmitic acid (22.49%), linoleic acid (15.12%) and steric acid (14.56%).

Keywords: biodiesel, biofuel, lipid, oleaginous yeast, *Rhodosporidium toruloides*

INTRODUCTION

Biodiesel, one of the alternative fuels, has received considerable attention in recent years, as it is renewable, biodegradable and nontoxic. Currently, biodiesel is produced from vegetable oils and animal fats. Although it has been used in many countries, such as Germany, Italy, France, the USA, Australia, Japan, China, Brazil, Argentina, Indonesia and Malaysia (Fukuda *et al*., 2001; Du *et al*., 2008; Zhu *et al*., 2008), manufacturing fuel from vegetable oils is undesirable because this competes with the use of these oils for human consumption and prevents the long-term development and large scale use of biodiesel (Miao and Wu, 2006; Zhu *et al*., 2008). To minimize this, other renewable sources of oils should be considered. Recently, microbial oils have been getting more attention because they, like conventional vegetable oils, are basically triacylglycerols that require a tranesterification process to convert them to biodiesel. From this viewpoint, microbial oils are a potential source for biodiesel production in the future (Li *et al*., 2008).

Oleaginous microorganisms, including bacteria, yeasts and molds, are microorganisms that accumulate lipids that constitute greater than 20% of dry biomass (Ratledge, 1989). In some oleaginous yeasts, such as *Rhodotorula graminis* and *Rhodosporidium toruloides*, the stored lipid content may reach 70% of the dry biomass (Ratledge, 2002). Lipid accumulation usually occurs in cultures with limited nitrogen and excess carbon. In high C/N ratio media, yeasts convert excess carbon into lipid (Ratledge and Wynn, 2002). Environmental conditions, such as temperature and pH, have also been shown to be factors controlling lipid accumulation and

Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fscistl@ku.ac.th

Received date : 11/09/09 Accepted date : 24/12/09

composition (Ratledge, 1989). Until recently, only a few oleaginous yeast species, including *Candida curvatus*, *Lipomyces starkeyi*, *Rhodosporidium toruloides*, *Rhodotorula glutinis* and *Rhodotorula graminis*, have been investigated for lipid production (Ykema *et al*., 1989; Granger *et al*., 1993; Ratledge and Wynn, 2002; Angerbauer *et al*., 2008).

The objective of this study was to isolate a new oleaginous yeast strain capable of high lipid production. Optimization of the medium composition and cultivation conditions for lipid production were also investigated.

MATERIALS AND METHODS

Isolation and selection of oleaginous yeast strains

Yeasts were isolated from soil samples collected from natural ecosystems in Thailand by an enrichment technique using nitrogen-limited medium I, consisting of 30 g/L glucose, 1.5 g/L yeast extract, 0.5 g/L NH_4Cl, 7.0 g/L KH_2PO_4, 5.0 g/L $Na_2HPO_4 \cdot 12H_2O$, 1.5 g/L $MgSO_4 \cdot 7H_2O$, 0.08 g/L $FeCl_3 \cdot 6H_2O$, 0.01 g/L $ZnSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 0.1 mg/L $MnSO_4 \cdot 5H_2O$ and 0.1 mg/L $CuSO_4 \cdot 5H_2O$ (Kimura *et al*., 2004), with an adjusted pH of 3.8. Incubation was carried out in a rotary shaker (Bio shaker BR-300 LF, Japan) at 150 rpm and 30°C for 2 d. Enriched cultures were then streaked on agar plates containing the same medium and incubated at 30°C until yeast colonies appeared. Purified yeast cultures were maintained on yeast extract-malt extract (YM) agar (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose and 20 g/L agar) and stored at 8°C.

The newly isolated yeast strains obtained with the method described above and 63 strains of *Rhodotorula* species, consisting of 61 strains of *Rhodotorula mucilaginosa*, one strain of *Rhodotorula glutinis* and one strain of an unknown *Rhodotorula* species, were included in the selection procedure. Three oleaginous yeast strains, namely, *Cryptococcus curvatus* CBS 570, *Lipomyces starkeyi* CBS 1807 and *Rhodosporidium toruloides* CBS 14 obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, were used as references. Inoculum was prepared by transferring one loop full of 24-hour yeast culture grown on YM agar slant to a test tube containing 5 mL nitrogen-limited medium I and incubated on a rotary shaker at 150 rpm and 28°C for 24 h. Oleaginous yeast strains were selected based on their cellular lipid contents by rapid estimation with Nile red staining (Kimura *et al*., 2004). Fully grown culture broth (40-μL) was mixed with 10 μL of Nile red solution (10 μg Nile red in 1 L absolute ethanol). After 5 min, yeast cells were observed with a fluorescence microscope (Olympus BX51, Japan), under which Nile red-stained lipid bodies showed yellow-gold emission. Yeast strains with high cellular lipid content were collected and screened further for their lipid production ability.

Screening of oleaginous yeasts for lipid production

Primary screening in triplicate for high lipid-accumulating yeast strains was conducted using a 250 mL Erlenmeyer flask containing 50 mL of nitrogen-limited medium I with pH adjusted to 5.5 on a rotary shaker at 150 rpm and 30°C for 72 h. Inoculum was prepared as above and transferred at the rate of 5% to the screening medium. For the secondary screening, lipid production was performed using 100 mL nitrogen-limited medium II (30 g/L glucose, 0.1 g/L $(NH_4)_2SO_4$, 0.75 g/L yeast extract, 0.4 g/L KH_2PO_4, 1.5 g/L $MgSO_4 \cdot 7H_2O$, 0.22 g/L $CaCl_2 \cdot 2H_2O$, 0.55 μg/L $ZnSO_4 \cdot 7H_2O$, 24.2 μg/L $MnCl_2 \cdot 4H_2O$ and 25 μg/L $CuSO_4 \cdot 5H_2O$) in which the pH was adjusted to 6.0 in a 500 Erlenmeyer flask. Flasks were incubated in a rotary shaker at 150 rpm and 28°C.

Identification of the selected oleaginous yeast strain

The sequence of the D1/D2 domain of the large subunit (LSU) rRNA gene was determined from PCR products from genomic DNA extracted from yeast cells using the slightly modified method described by Lachance *et al*. (1999). The D1/D2 domain of the LSU rRNA gene was amplified by a PCR with the forward primer NL1 and the reverse primer NL4 (O'Donnell, 1993). The PCR product was checked by agarose gel electrophoresis purified using a QIAquick purification kit (Qiagen, Ontario, USA) and cycle-sequenced using an ABI BigDye terminator cycle sequencing Kit Version 3.1 (Applied Biosystems, California, USA) according to the manufacturer's instructions. The sequences were compared pairwise using the BLASTN homology search program (Altschul *et al*., 1997). Identification was based on the Kurtzman and Robnett (1998) statement that yeast strains showing nucleotide substitutions greater than 1% in the D1/D2 domain of the LSU rRNA gene were usually different species.

Effects of nutrient composition and pH on lipid production

Effects of nutrient composition, including glucose, nitrogen, phosphate and magnesium concentrations were studied in triplicate using the same cultivation procedure as in the secondary screening. Glucose concentrations were varied at 30, 50, 70 and 90 g/L. Combined organic nitrogenous compounds (yeast extract and peptone) at 0.75 g/L and inorganic nitrogenous compounds [$(NH_4)_2SO_4$ or NH_4Cl] at 0.1 g/L were mixed and used as the nitrogen source. The effects of C/N ratios were determined at 65, 90, 115 and 140. KH_2PO_4 at 0.4, 1.6, 2.8 and 4.0 g/L was used to study the requirement for phosphate. $MgSO_4 \cdot 7H_2O$ at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g/L was also added to determine its effect. Optimal pH of the medium was investigated by adjusting it to 5.0, 5.5, 6.0 and 6.5.

Analytical methods

Biomass was determined by measuring the dry weight after drying at 110°C to constant weight. Cellular lipid content was reported on the basis of total fatty acid. To determine total fatty acid, lipids were extracted from cells according to Bligh and Dyer (1959). Pentadecanoic acid was used as an internal standard. Derivation of methyl esters from fatty acids was according to Holub and Skeaff (1987). Fatty acid methyl esters were analyzed with a gas chromatograph equipped with flame ionization detector (Shimazu GC14-A, Shimazu, Japan) using a silica megabore capillary column (30 m × 0.52 mm × 1 μm, Durabond 225, J and W Scientific, USA) and helium as the carrier gas. Fatty acid methyl esters were identified and quantified by comparison of their retention times with authentic standards. Glucose concentration was determined with an HPLC (Waters, Millipore, Water, USA) with an refractive index detector and a sugar column (ULTRON PS-80C, Shinwa Chemical Industries, Japan). The mobile phase was deionized water with a flow rate of 1 mL/min.

RESULTS AND DISCUSSION

Isolation and selection of oleaginous yeast strains

In total, 104 yeast isolates were obtained from soil samples collected from various locations in Thailand. Hence, 170 strains, including 63 strains of *Rhodotorula* spp. and the three reference strains from CBS were subjected to Nile red staining (Kimura *et al*., 2004). Kimura *et al*. (2004) indicated that lipid bodies in oleaginous microorganisms have different shapes depending on species and culture condition. The yeast lipid bodies were believed to form by budding from the endoplasmic reticulum, and this kept most of the enzymes for sterol synthesis and esterification (Kurat *et al*., 2006).

The result of lipid body staining revealed that 48 strains contained many lipid bodies comparable to the reference strains. These were

then selected for further screening. Photomicrographs of strain DMKU3-TK16 and *R. toruloides* CBS 14, the reference oleaginous strain, are shown in Figure 1. The lipid bodies of both strains were spherical in shape. Strain DMKU3-TK16 (Figures 1a and 1b) produced many small lipid bodies of less than 0.5 μm in diameter, while *R. toruloides* CBS 14 (Figures 1c and 1d) showed many larger lipid bodies of 0.5-2 μm in diameter.

Screening of oleaginous yeasts for lipid production

Forty-eight yeast strains from the previous Nile red staining selection were screened for their lipid production. The selected yeast strains were cultivated in nitrogen-limited medium II in a shaking flask at 30°C for 72 h. The results revealed that four stains, DMKU3-TK16, DMKU3-TK17, *Rhodotorula* sp. and *Rhodotorula mucilaginosa* W35 accumulated lipid amounts that were greater than 5% of the dry biomass, with amounts of 12.43, 7.57, 9.94 and 5.49% of dry biomass, respectively (Figure 2). While the three reference oleaginous yeast strains accumulated 2.90-6.69% of dry biomass (Figure 2), the remaining 44 strains accumulated lipid at lower concentrations, between 0.15-4.63% of dry biomass (data not shown).

Biomass, lipid production and the cellular lipid content of the four strains, DMKU3-

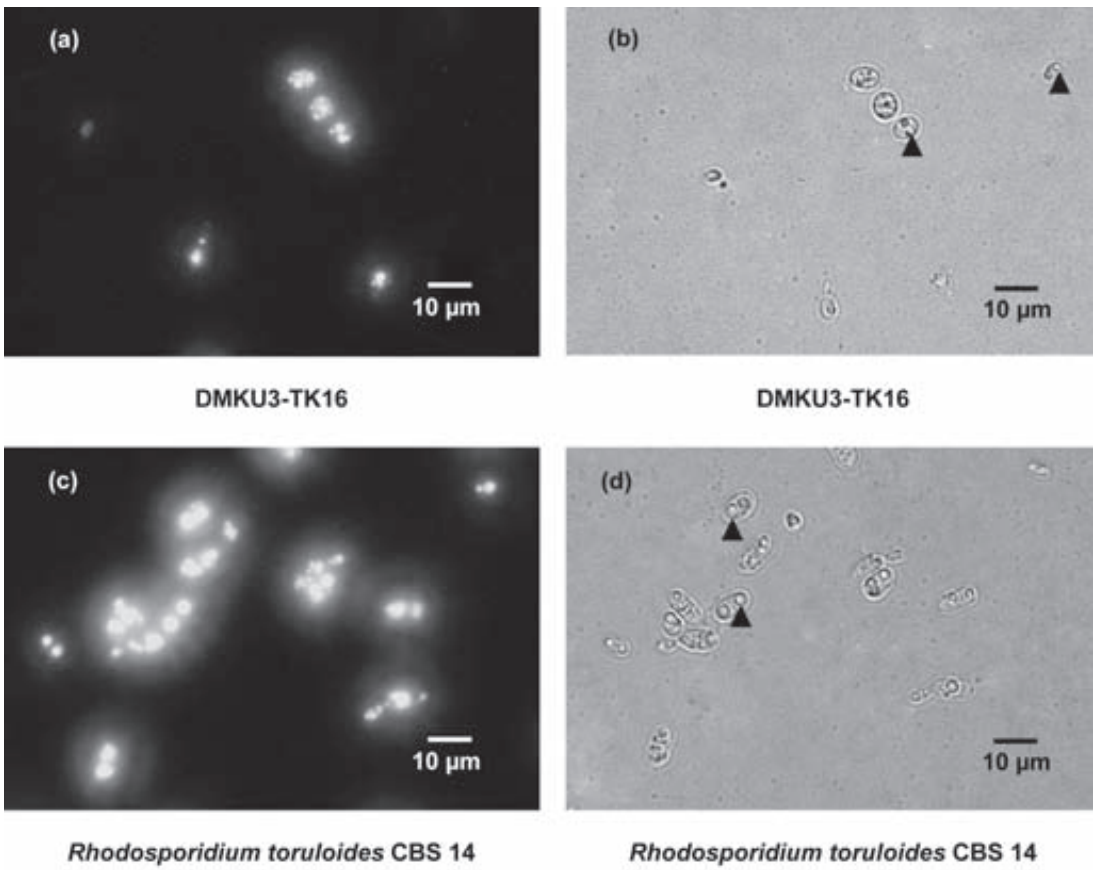


Figure 1 Photomicrographs of Nile red stained cells of strain DMKU3-TK16 and *R. toruloides* CBS 14, under fluorescence microscope (a) and (c), respectively, and light microscope (b) and (d), respectively. Arrows indicate lipid bodies.

TK16, DMKU3-TK 17, *Rhodotorula* sp. and *R. mucilaginosa* W35 were determined by shaking cultivation in nitrogen-limited medium II at 28°C for 120 h. The biomass of strains DMKU3-TK16, *Rhodotorula* sp. and *R. mucilaginosa* W35 was comparable, being 8.23, 8.49 and 8.25 g/L, respectively, while strain DMKU3-TK17 produced slightly less at 7.38 g/L. However, lipid production by DMKU3-TK16 was the highest at 4.56 g/L, while DMKU3-TK17 and *R. mucilaginosa* W35 produced extremely low amounts of lipid, with 0.64 and 0.51 g/L, respectively; these results reflected their cellular lipid content of 56.76, 8.99 and 6.52 % of dry biomass, respectively. Therefore, the strain DMKU3-TK16 was selected as the potential lipid producer.

Effects of nutrient composition and pH on lipid production

Cultivation in a medium with an excess of carbon and a limited amount of nitrogen has significant influence on cell growth and lipid accumulation in oleaginous yeasts (Ratledge, 2004). The effects of medium composition on biomass, lipid production and the cellular lipid content of *R. toruloides* DMKU3-TK16 were determined by shaking flask cultivation at 150 rpm, 28°C; the results are shown in Table 1.

The effect of combined organic nitrogen (yeast extract or peptone at 0.75 g/L) and inorganic [$(NH_4)_2SO_4$ or NH_4Cl at 0.1 g/L] nitrogen was determined in the nitrogen-limited medium II composed of 30 g/L glucose and adjusted to pH 6.0. Huang *et al.* (1998) reported that organic nitrogenous compounds are good for lipid accumulation, but not for cell growth; on the other hand, inorganic nitrogenous compounds are favorable for cell growth, but not for lipid accumulation. Results from the current study showed that using yeast extract with ammonium salt yielded higher biomass, but lipid production was increased by using peptone with ammonium salt. The highest biomass (8.71 g/L) was obtained when yeast extract with $(NH_4)_2SO_4$ was used, while a higher cellular lipid content of 53.71 and 53.10% of dry biomass was produced, when peptone was used with NH_4Cl and with $(NH_4)_2SO_4$, respectively (Table 1). Hence, yeast extract with $(NH_4)_2SO_4$ was chosen for further study.

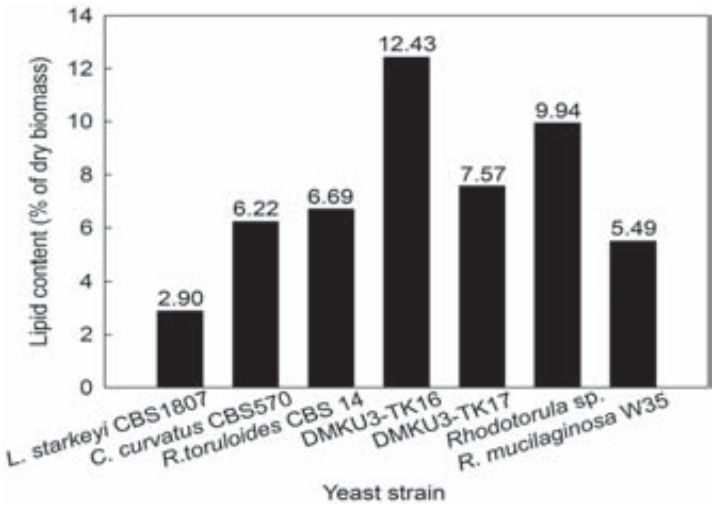


Figure 2 Lipid content of the four selected strains cultivated in nitrogen-limited medium II on a rotary shaker at 150 rpm and 28°C in comparison with the reference oleaginous yeast strains.

Table 1 Biomass, lipid production and cellular lipid content of DMKU3-TK16 cultivated in nitrogen-limited medium II with various nutrients and pH levels on a rotary shaker at 150 rpm and 28°C.

Condition	Biomass (g/L)	Lipid production (g/L)	Lipid content (% of dry biomass)
Nitrogen source [a]			
Yeast extract and $(NH_4)_2SO_4$	8.71 (108 h)	4.07 (84 h)	48.89 (84 h)
Yeast extract and NH_4Cl	9.19 (108 h)	3.53 (84 h)	42.83 (72 h)
Peptone and $(NH_4)_2SO_4$	5.91 (108 h)	3.05 (120 h)	53.10 (120 h)
Peptone and NH_4Cl	5.99 (108 h)	3.03 (120 h)	53.71 (120 h)
C/N ratio			
65	10.52 (72 h)	2.71 (60 h)	25.84 (60 h)
90	9.77 (84 h)	3.24 (84 h)	33.16 (84 h)
115	8.23 (156 h)	4.13 (96 h)	50.41 (96 h)
140	6.79 (120 h)	4.23 (120 h)	62.30 (120 h)
Glucose (g/L)			
30	9.22 (120 h)	4.78 (108 h)	52.01 (108 h)
50	10.48 (156 h)	6.67 (156 h)	63.65 (156 h)
70	13.56 (156 h)	8.11 (168 h)	64.43 (180 h)
90	13.14 (120 h)	7.95 (180 h)	62.11 (180 h)
KH_2PO_4 (g/L)			
0.4	12.51 (156 h)	8.85 (168 h)	65.19 (156 h)
1.6	12.96 (168 h)	8.61 (168 h)	66.44 (168 h)
2.8	13.62 (144 h)	8.03 (168 h)	63.91 (168 h)
4.0	13.26 (180 h)	7.96 (156 h)	64.34 (156 h)
$MgSO_4 \bullet 7H_2O$ (g/L)			
0.5	13.82 (180 h)	7.14 (180 h)	58.24 (168 h)
1.0	13.34 (180 h)	8.15 (180 h)	63.65 (144 h)
1.5	13.19 (180 h)	8.20 (180 h)	62.17 (180 h)
2.0	13.36 (180 h)	8.79 (168 h)	69.78 (168 h)
2.5	13.39 (168 h)	8.58 (168 h)	64.08 (168 h)
3.0	13.36 (168 h)	8.08 (180 h)	61.17 (180 h)
pH			
5.0	12.83 (156 h)	8.34 (180 h)	66.70 (168 h)
5.5	13.33 (156 h)	9.26 (168 h)	71.30 (168 h)
6.0	12.88 (156 h)	8.68 (168 h)	67.64 (168 h)
6.5	12.87 (156 h)	8.48 (168 h)	65.66 (180 h)

[a] Organic nitrogen (yeast extract and peptone) and inorganic nitrogen [$(NH_4)_2SO_4$ and NH_4Cl] were used at a concentration of 0.75 and 0.1 g/L, respectively.

The influence of the C/N ratio on biomass and lipid production was studied by varying C/N ratios (65, 90, 115 and 140) of the nitrogen-limited medium II composed of 30 g/L glucose, 0.75 g/L yeast extract with varying $(NH_4)_2SO_4$ concentrations and adjusted to pH 6.0. The C/N ratio was found to be a very important factor for lipid accumulation (Angerbauer *et al*., 2008). The results in Table 1 revealed that the cellular lipid content increased with the increase in the C/N ratio. The increase in cellular lipid content reflected the increase in lipid production, though a decrease in biomass was obtained when the C/N ratio was increased. The highest cellular lipid content (62.30% of dry biomass) and 4.23 g/L of lipid production) was attained at the C/N ratio of 140 [30 g/L glucose, 0.75 g/L yeast extract and 0.014 g/L $(NH_4)_2SO_4$], but with the lowest biomass (6.79 g/L). However, the lowest C/N ratio (65) produced the highest biomass (10.52 g/L) and the lowest lipid production (2.7 g/L).

The effect of carbon concentration was studied using nitrogen-limited medium II composed of glucose at 30, 50, 70 and 90 g/L and yeast extract with $(NH_4)_2SO_4$ at a C/N ratio of 140 and a pH of 6.0. The result revealed that the higher the glucose concentration, the higher the final biomass and lipid production, but only up to 70 g/L glucose (Table 1). Any further increase in the glucose concentration led to a decrease in the biomass and lipid production. The highest biomass (13.56 g/L), lipid production (8.11 g/L) and cellular lipid content (64.43% of dry biomass) were achieved when 70 g/L glucose was used.

The effect of phosphate was studied by supplementation of KH_2PO_4 at 0.4-4 g/L to the nitrogen-limited medium II composed of 70 g/L glucose, 0.75 g/L yeast extract, 0.55 g/L $(NH_4)_2SO_4$ and pH 6.0. The highest lipid production of 8.85 g/L was obtained when the medium was supplemented with 0.4 g/L KH_2PO_4, but the lipid production and cellular lipid content obtained from various concentrations of KH_2PO_4 were only slightly different (Table 1). Thus, KH_2PO_4 at 0.4 g/L was chosen for further experiments.

The effect of magnesium was investigated using $MgSO_4 \bullet 7H_2O$ at concentrations of 0.5-3.0 g/L in a nitrogen-limited medium II composed of 70 g/L glucose, 0.75 g/L yeast extract, 0.55 g/L $(NH_4)_2SO_4$, 0.4 g/L KH_2PO_4 and pH 6.0. The highest lipid production (8.79 g/L) and cellular lipid content (69.78% of dry biomass) were obtained when the medium was supplemented with 2.0 g/L $MgSO_4 \bullet 7H_2O$. Any further increase in the magnesium concentration beyond 2.0 g/L led to a slight decrease in lipid production (Table 1).

The influence of the initial pH of the medium on lipid production was studied, using pH values ranging from 5.0 to 6.5. It was found that the highest cellular lipid content (71.30% of dry biomass) and lipid production (9.26 g/L) were obtained when the pH of the medium was adjusted to 5.5. Cultivation at pH 5.0, 6.0 and 6.5 resulted in slight differences in the cellular lipid content and production. Many investigators have reported that the pH of the medium had an effect on lipid production and seemed to depend on carbon sources (Angerbauer *et al*., 2008). An optimal pH of 4.0 for lipid production by *Lipomyces starkeyi* was found when ethanol was used as a carbon source (Yamauchi *et al*., 1983). Holdsworth and Ratledge (1988) reported the optimum pH of *L. starkeyi* at 5.5 with glucose, while Angerbauer *et al*. (2008) obtained the highest lipid content when *L. starkeyi* was cultivated at pH 5.0.

In conclusion, lipid production of the strain DMKU3-TK16 by shaking flask cultivation (150 rpm) in a nitrogen-limited medium II was optimum when the medium was composed of 70 g/L glucose, 0.75 g/L yeast extract, 0.55 g/L $(NH_4)_2SO_4$, 0.4 g/L KH_2PO_4, 2.0 g/L $MgSO_4 \bullet 7H_2O$, with a pH of 5.5 at 28°C. Under these optimal nutrient and pH conditions, this strain gave maximal lipid production of 9.26 g/L lipid and a cellular lipid content of 71.30 % of dry

biomass after 168 h of cultivation (Figure 3). Biomass determined gravimetrically was 13.33 g/L at 156 h.

Fatty acid profiles

The fatty acid profiles of oleaginous yeasts depend on the species and the growth conditions. Environmental conditions, such as temperature, pH, substrate, C/N ratio and oxygen influence the efficiency with which lipids are accumulated (Jacob, 1993). The fatty acid profile of the strain DMKU3-TK16 under optimal nutrient and pH conditions in terms of the percentage of total fatty acid was oleic acid (41.54 %), palmitic acid (22.49 %), linoleic acid (15.12 %), stearic acid (14.56 %), linolenic acid (4.51 %), myristic acid (0.96 %) and palmitoleic acid (0.82 %) as shown in Table 2. It can be seen clearly that the fatty acid composition was similar to vegetable oils, with the four major fatty acids being oleic, palmitic, linoleic and stearic acid (Table 2).

Identification of the selected oleaginous yeast strain

Identification of the strain DMKU3-TK16 was carried out using a molecular taxonomic

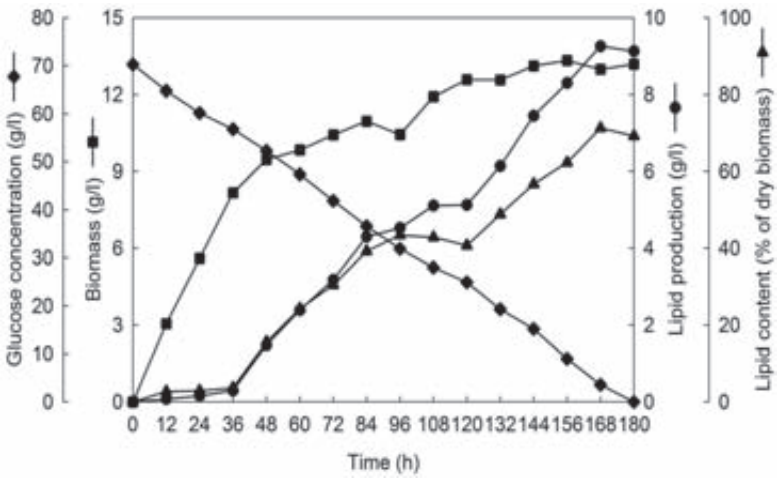


Figure 3 Time course of biomass (■), lipid production (●), cellular lipid content (▲) and glucose concentration (◆) by DMKU3-TK16 in a nitrogen-limited medium II composed of 70 g/L glucose, 0.75 g/L yeast extract, 0.55 g/L $(NH_4)_2SO_4$, 0.4 g/L KH_2PO_4 and 0.2 g/L $MgSO_4 \cdot 7H_2O$ with pH of 5.5 using shaking flask cultivation at 150 rpm and 28°C.

Table 2 Comparison of fatty acid profiles of *R. toruloides* DMKU3-TK16 and the other vegetable oils.

	Major fatty acid residue (%w/w)						
	14:0	16:0	16:1	18:0	18:1	18:2	18:3
R. toruloides DMKU3-TK16	0.96	22.49	0.82	14.56	41.54	15.12	4.51
Soybean [a]	nd	12	nd	3	23	56	6
Palm [a]	nd	43	nd	5	41	10	nd
Cotton seed [a]	nd	20	nd	3	19	55	nd
Corn [a]	nd	12	nd	2	25	61	nd
Sun flower [a]	nd	6	nd	3	17	74	nd

[a] Data from Ma and Hanna (1999); nd = no data

approach. Molecular taxonomic studies based on analysis of nucleotide sequences of the D1/D2 domain of the LSU rRNA gene showed that the sequences of the strain DMKU3-TK16 (559 nucleotides) and the type strain of *Rhodosporidium toruloides*, *R. toruloides* CBS 14^T, were identical. Therefore, the strain DMKU3-TK16 was identified as *Rhodosporidium toruloides*. The D1/D2 domain of the LSU rRNA gene sequence of strain DMKU3-TK16 was deposited in GenBank under the accession number AB507798.

CONCLUSION

The results of this study revealed that the newly isolated oleaginous yeast *R. toruloides* strain DMKU3-TK16, has potential to be a lipid producer for biodiesel production. It yielded 9.26 g/L of lipid, with a cellular lipid content of 71.30 % of dry biomass at 168 h, when cultivated in nitrogen-limited medium composed of 70 g/L glucose, 0.75 g/L yeast extract, 0.55 g/L $(NH_4)_2SO_4$, 0.4 g/L KH_2PO_4, 2 g/L $MgSO_4 \bullet 7H_2O$, with a pH of 5.5 using shaking flask cultivation at 150 rpm and 28°C. The fatty acid profiles of strain DMKU3-TK16 revealed that its lipid content was similar to that of vegetable oil and that it could be used for biodiesel production.

ACKNOWLEDGEMENTS

The authors would like to thank the Faculty of Science, Kasetsart University, Thailand and the Thailand Research Fund for their partial financial support of this research work. The authors thank Ms Rungluk Kaewwichian for laboratory assistance in yeast identification.

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