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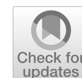
ORIGINAL PAPER



Occurrence of oleaginous yeast from mangrove forest in Thailand

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Abstract
A total of 191 yeasts were isolated from 197 samples collected from eight estuarine mangrove forests along four different coastlines of Thailand (Andaman Sea and the East, North and West coasts of the Gulf of Thailand). Of these, 178 isolates were identified as 32 species in 16 genera of Ascomycota, 12 species in nine genera of Basidiomycota, and 13 isolates as potential new species, respectively. Mangroves located along the Andaman Sea coastline had a higher yeast diversity at the species and genera levels than those along the Gulf of Thailand. *Kluyveromyces siamensis* was the most frequently isolated species, whilst *Candida tropicalis* was the only species isolated at all eight sites. Screening isolated yeast strains belonging to genera previously reported as oleaginous yeast plus the 13 potential new species, revealed two oleaginous strains, *Rhodotorula sphaerocarpa* 11-14.4 and *Saitozyma podzolica* 11-11.3.1. Both of these strains were isolated from the same mangrove forest on the Andaman Sea coastline. They could accumulate lipid when suspended in glucose solution without any supplementation, while the fatty acid composition and oil profile of *Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11.3.1 were similar to vegetable oil and cocoa butter, respectively.

Keywords Yeast oil · Intracellular lipid · Biodiesel · Cocoa butter · *Rhodotorula sphaerocarpa* · *Saitozyma podzolica*

Introduction

Mangroves, an ecotone ecosystems, that are transitional between terrestrial and marine habitats (Alongi et al. 2001) in estuaries of tropical and sub-tropical climate regions (Kathiresan and Bingham 2001). Mangroves are unusually rich in microbial species including yeasts. The yeasts are involved in detrital food chain in this ecosystem that is rich in degrading plant materials (Meyers et al. 1971) and a food source for some marine invertebrates and zooplanktons (Araujo et al. 1995; Kutty and Phili 2008; Fell et al. 2011).

In particular, some yeasts can accumulate intracellular lipids at high levels in the form of triacylglycerols as oil droplets (yeast oil) when they are grown under an excess carbon but limited nitrogen condition (Thanh 2006). The lower nitrogen condition causes an increased adenosine monophosphate (AMP) deaminase activity, which leads to decreased AMP and isocitrate dehydrogenase activity, and an accumulation of citrate in the TCA cycle. The citrate subsequently enters the fatty acid synthesis pathway upon conversion to acetyl CoA. Yeasts that accumulate intracellular lipid above 20% (w/w) by dry cell weight (DCW) are defined as oleaginous (Ratledge 1989).

Yeast oils have already been developed as an alternative source of biofuels and high-valued oils for oleochemical industries (Papanikolaou et al. 2001; Sitepu et al. 2014; Bandhu et al. 2018). The major fatty acids in most yeast oils are myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids, which is similar to those of plant oils that are used as feedstock for biodiesel production (Beopoulos and Nicaud 2012). Oils of several oleaginous yeast strains, such as *Yarrowia lipolytica*, *Rhodosporidium toruloides* and *Cryptococcus curvatus*, are mainly composed of steric,

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oleic and palmitic acids, which is similar to cocoa butter, a high value natural fat extracted from cocoa beans (Hassan et al. 1994, 1995; Papanikolaou et al. 2001, 2003; Wu et al. 2011). Oils of *Rhodotorula mucilaginosa* IIPL32 have a fatty acid composition, physicochemical and tribophysical properties suitable as a renewable base oil for biolubricant production (Bandhu et al. 2018). Oils of *Pichia segobiensis* SSOH12 had a considerable amount (16%) of palmitoleic acid, an omega 7, and may be suitable for medical applications (Schulze et al. 2014).

The usage of yeast for oil production provides better benefits over plants or other microorganisms, as yeasts grow fast without effects from changing climatic or seasonal conditions, not requiring land space for plantation compared with plant cultivation and easier for production expansion versus single cell algae, ability to use diverse carbon sources (Sitepu et al. 2014). The component of oil produced from yeasts will be different dependent on the carbon sources used (Ageitos et al. 2011). Therefore, yeast oil can be used in a variety of industries.

There are around 2400 km^2 of mangrove forests in Thailand (Pumijumnong 2014), which are situated across four distinctively separate coastal regions along the Andaman Sea and the Gulf of Thailand (GOT) (Vibulsresth et al. 1975) namely: (i) the Andaman Sea coastline on the west of Thailand, which covers the five provinces of Ranong, Phuket, Krabi, Trang and Satul; (ii) the northern GOT coastline, which covers the four provinces of Samut Prakarn, Samut Sakorn, Samut Songkram and Bangkok (iii) the eastern GOT coastline, which covers the five provinces of Trat, Chantaburi, Rayong, Cholburi and Chachoengsao; and (iv) the western GOT coastline, which covers the eight provinces of Petchaburi, Prachuap Khirikhan, Chumporn, Surathani, Nakorn Sri Thamarat, Songkhla, Pattani and Naratiwat (Fig. 1).

Oleaginous yeasts have been found in various habitats, such as soil and the surface of flowers, fruits or plant leaves (Schulze et al. 2014; Jiru et al. 2016; Maina et al. 2017). Mangrove forests are known to have a high yeast diversity, and as such there were several reports on new yeast species isolated from different mangrove forests and coastlines in Thailand (Limtong et al. 2004, 2007; Limtong and Yongmanitchai 2010; Am-In et al. 2008, 2011). Lipid accumulation by yeasts is somewhat strain dependent, and not species or genus dependent (Polburee et al. 2015). There was a high success rate in obtaining oleaginous yeast strain by screening from those capable to grow in nitrogen-limited medium (Kraisintu et al. 2010; Sitepu et al. 2013). The high yeast diversity in mangrove forests increases the chance to isolate oleaginous yeast strain including new species of oleaginous yeast. Moreover, as the fatty acid composition of yeast oil is species dependent, the new species of oleaginous yeast isolated increases chance to obtain new high value yeast oil.

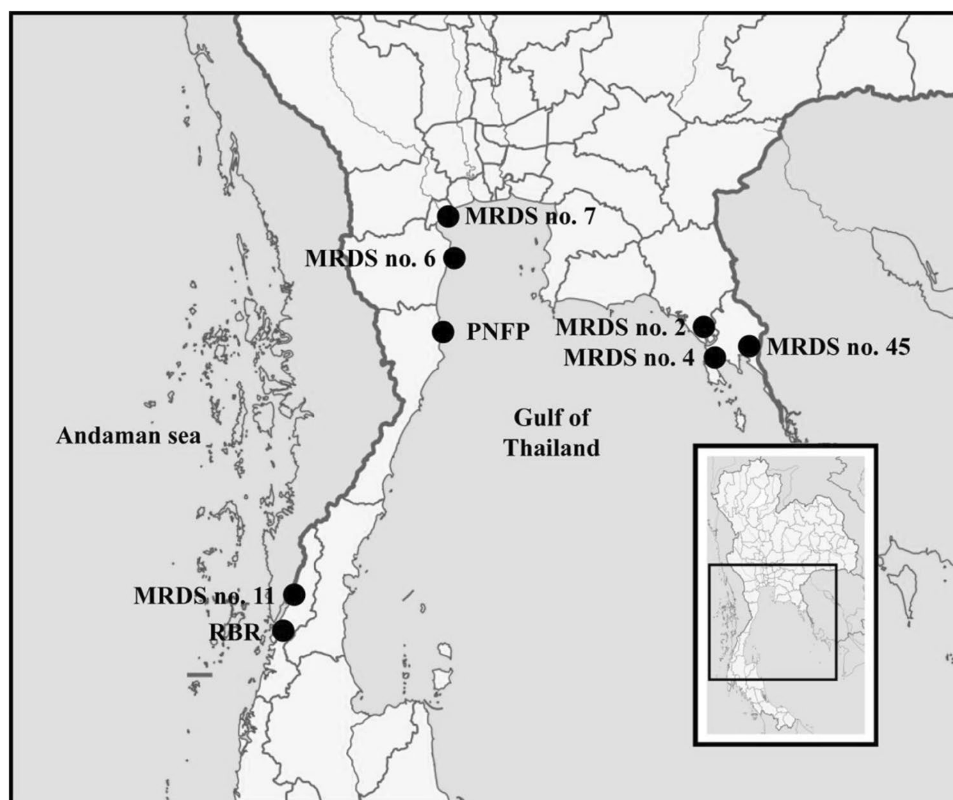

Fig. 1 Map of sampling sites

The objective of this study was to isolate diverse yeasts using nitrogen-limited medium from eight mangrove forests located in six provinces across the four different coastlines of Thailand, identifying the diversity of isolated yeasts, screening them for oleaginous yeast strains and characterizing their lipid production.

Materials and methods

Sample collection and yeast isolation

A total of 197 samples, comprised of soil, water and decayed biological matter, were collected from eight mangrove forests at four different coastlines (Tables 1 and 2). The sampled sites were (i) Ranong Biosphere Reserve (RBR; Ranong province) and (ii) Mangrove Resource Development station (MRDS) no. 11 (Ranong province) on the Andaman Sea coastline; (iii) MRDS no. 7 (Samut Songkhram province) on the northern GOT coastline; (iv) MRDS no. 2 (Chanthaburi province) (v) MRDS no. 4 (Trat province) and (vi) MRDS no. 45 (Trat province) on the eastern GOT coastline; and (vii) Pranburi National Forest Park (PNFP; Prachuap Khiri Khan province) and (viii) MRDS no. 6 (Phetchaburi province) on the western GOT coastline (Fig. 1). The latitude and longitude of these sampled mangrove forests are shown in Table 1.

Table 1 Location of the eight mangrove forest sampling sites in Thailand

Sampling site	Province	Latitude, longitude
Andaman Sea		
RBR	Ranong	9.878145, 98.602366
MRDS no. 11	Ranong	10.173566, 98.710563
Gulf of Thailand (a) North		
MRDS no. 7	Samut Songkhram	13.184048, 100.020182
Gulf of Thailand (b) East		
MRDS no. 2	Chantaburi	12.381264, 102.357817
MRDS no. 4	Trat	12.169602, 102.406304
MRDS no. 45	Trat	12.208180, 102.552114
Gulf of Thailand (c) West		
PNFP	Prachuap Khiri Khan	12.412191, 99.984147
MRDS no. 6	Phetchaburi	13.327734, 99.988013

Sampling sites: RBR (Ranong Biosphere Reserve); MRDS (Mangrove Resource Development station); PNFP (Pranburi National Forest Park)

Sources: *S* soil, *W* water, *D* decayed biological matter

Each sample (1 g or 1 ml) was inoculated into 10 ml of nitrogen-depleted medium (NDM; 20 g/l glucose, 0.85 g/l KH_2PO_4, 0.15 g/l $K_2HPO_4·3H_2O$, 0.5 g/l $MgSO_4·7H_2O$, 0.1 g/l NaCl, 0.1 g/l $CaCl_2·6H_2O$, 0.5 mg/l H_3BO_3, 0.04 mg/l $CuSO_4·5H_2O$, 0.1 mg/l KI, 0.2 mg/l $FeCl_3·3H_2O$, 0.4 mg/l $MnSO_4·H_2O$, 0.2 mg/l $Na_2MOO_4·2H_2O$, 0.4 mg/l $ZnSO_4·7H_2O$, pH 5.5) and supplemented with 100 mg/l chloramphenicol and incubated at 30 ºC, 200 rpm for 2 d (Thanh 2006). The resultant culture was streaked on NDM-agar and incubated at 30 ºC for 5 d. Purification to clonality of the culture was performed by the streak plate method on YM agar (3 g/l yeast extract, 3 g/l malt extract, 5 g/l peptone, 10 g/l glucose, 20 g/l agar, pH 5.5). Each clonal culture was kept on a YM agar slant at 4 ºC for further study.

Molecular identification of yeast isolates

Genomic DNA extraction

Yeast cells were lysed in 200 µl lysis solution (60% (w/v) Yatalase, 6% (v/v) Yatalase buffer, 6% (w/v) RNase, 4.65% (w/v) NaCl, 10 mM K_2HPO_4, 100 mM EDTA, pH 8) at 37 °C for 1–2 h, vortex mixed with glass beads (diameter 0.8 mm; Sigma-Aldrich Co., LLC., U.S.A.) in 8% (w/v) sodium dodecyl sulphate (67 µl) for 1.5 min and incubated at 60 °C for 10 min. The cell lysate was further mixed with 87 µl of 3 M sodium acetate (pH 5) on ice, centrifuged at 20,600×*g*, 4 °C for 5 min, and the supernatant was transferred into Acroprep 96 multi-well filter plate (PALL®, Pall corporation, U.S.A.) containing 110 µl isopropanol in each well. The DNA pellet obtained after centrifugation (1580×*g*, 16 °C for 5 min) was washed twice with 200 µl of 70% (v/v) ethanol, dissolved in 60 µl TE buffer and kept at −20 °C until used.

(GTG)$_5$ fingerprinting pattern

The extracted genomic DNA (1–20 ng/µl) was PCR amplified in a 10 µl reaction volume comprised of 1 µl DNA template, 0.2 µl of 10 mM $(GTG)_5$ primer (5′-GTGGTGGTG GTGGTG-3′) (Meyer et al. 1993), 5 µl 2× Go Tag green and 3.8 µl distilled water. The thermal cycling was performed at 95 °C for 5 min followed by 40 cycles of 95 °C for 45 s, 50 °C for 1 min and 72 °C for 1 min, and then a final 72 °C for 6 min. The PCR products were resolved by 1.5% (w/v) agarose gel electrophoresis and visualized after ethidium bromide staining. The resolved pattern was defined as the $(GTG)_5$ fingerprint pattern. The $(GTG)_5$ fingerprint patterns were grouped according to their respective relevant similarity.

Table 2 Phylogenetic placement and related genera of the yeasts isolated from mangrove forests, number and proportion of the strains and sources

Phylogenetic placement, related genus	Closest related species	Strains	Representative strains (GenBank accession no.)	Source		
				S	W	D
Basidiomycota						
Pucciniomycotina						
Microbotryomycetes						
Rhodotorula	*Rhodotorula paludigena*	1-4W.1[a], 11-2W.2[b], 11-14.7[b] WW1.2[d], BW7.3[e], BW8.1[e], BW1.3[e], BW9.1[e], BW7.1[e], BW8.5[e], MTW11.1[f], MTW9.2[f],	BW7.3 (LC435579)			
	Rhodotorula sphaerocarpa	11-14.4[b]	11-14.4 (LC335750)			
	Rhodotorula toruloides	1-1.2[a]	1-1.2 (LC435580)			
	Rhodotorula mucilaginosa	11-1W.1.1[b], 11-2W.1[b]	11-1W.1.1 (LC435581)			
Cystobasidiomycetes						
Sakaguchia	*Sakaguchia* aff. *lamellibrachiae*	MTW10.1[f]	MTW10.1 (LC435582)			
Agaricomycotina						
Tremellomycetes						
Saitozyma	*Saitozyma podzolica*	1-5W.5.1[a], 11-15.5.1[b], 11-11.3.1[b]	11-11.3.1 (LC335751)			
Papiliotrema	*Papiliotrema flavescens*	11-10W.3[b], BW1.2[e]	11-10W.3 (LC435583)			
	Papiliotrema laurentii	1-3W.1[a], MTT4.1[f]	1-3W.1 (LC435584)			
Naganishia	*Naganishia liquefaciens*	11-2W.7[b]	11-2W.7 (LC435585)			
	Naganishia albida	11-1W.1[b]	11-1W.1 (LC435586)			
Kwoniella	*Kwoniella dejecticola*	11-12.2[b],11-15.7.1[b]	11-12.2 (LC435587)			
Heterocephalacria	*Heterocephalacria* aff. *arrabidensis*	1-7W.1[a]	1-7W.1 (LC270815)			
Hannaella	*Hannaella phetchabunensis*	11-14.6[b]	11-14.6 (LC435588)			
Goffeauzyma	*Goffeauzyma* aff. *gilvescens*	11-8.2[b]	11-8.2 (LC435590)			
Ustilaginomycotina						
Ustilaginomycetes						
Pseudozyma	*Pseudozyma hubeiensis*	BW3.3.1[e], BW3.4[e], BW6.2[e]	BW3.3.1 (LC435589)			
Ascomycota						
Saccharomycotina						
Saccharomycetes						
Lodderomyces/Spathaspora	*Candida tropicalis*	1-6.4[a], 11-17.2[b], NSK5-1[c], NSK 6-1[c], NSK7-1[c], NSK15-2[c], NSK 17-1[c], NWK4-1[c], NWK5-2[c], WW 8.2[d], WS7.7. 2[d], BS3.1[e], BW5.2[e], BW7.4[e], BW9.6[e], MTW3.1.2[f], MTW4.3[f], MTT5.1. 2[f], NSP10-1[g], NSP23-1[g] NWB3-1[h], NWB7-1[h]	WS7.7.2 (LC435591)			
	Candida viswanathii	NSP22-2[g]	NSP22-2 (LC435592)			
	Candida maltosa	11-1.1[b], 11-3W.4[b]	11-3W.4 (LC435593)			
	Candida orthopsilosis	BW8.4[e]	BW8.4 (LC435594)			
Suhomyces	*Suhomyces atakaporum*	1-3W.6.4[a], 1-5W.5.2[a]	1-3W.6.4 (LC435595)			
Wickerhamomyces	*Wickerhamomyces anomalus*	NWB2-3[h]	NWB2-3 (LC435596)			
	Candida aff. *quercuum*	NSK9-2[c], NSK13-2[c]	NSK9-2 (LC335754)			
Debaryomyces	*Debaryomyces nepalensis*	MTW3.1.3[f], NSB6-1[h], NSB7-1[h]	NSB6-1 (LC435597)			
Schwanniomyces	*Schwanniomyces polymorphus*	BW4.2[e]	BW4.2 (LC435598)			
	Schwanniomyces vanrijiae var.	11-12.1[b], 11-14.1[b], 11-16.1[b], 11-	11-12.1 (LC435599)			

Table 2 (continued)

	vanrijiae	15.3[b]				
Meyerozyma	*Meyerozyma guilliermondii*	BW5.6[e]	BW5.6 (LC435600)			
Yamadazyma	*Yamadazyma* aff. *mexicana*	NWB2-1[h]	NWB2-1 (LC435601)			
	Yamadazyma Mexicana	NWK2-1[c]	NWK2-1 (LC435602)			
	Candida andamanensis	NSP11-1[g]	NSP11-1 (LC435603)			
	Candida amphicis	WT1.3[d], WT5.1[d]	WT1.3 (LC435604)			
	Candida aaseri	1-3.1[a], 1-8W.2[a] ,1-3W.8[a]	1-3.1 (LC435605)			
	Candida insectorum	1-3W.5.3[a], NSK3-1[c], NSK12-1[c], NSK17-3[c], MTT3.1.4[f]	NSK12-1 (LC435606)			
Kodamaea	*Kodamaea ohmeri*	1-6.3[a], BW4.1[e], BW5.4[e], MTS4.4[f]	BW5.4 (LC435607)			
Clavispora	*Candida intermedia*	1-12.1[a], 1-2W.1[a]	1-12.1 (LC435608)			
	Candida aff. *ecuadorensis*	NSK10-1[c]	NSK10-1 (LC259007)			
Metahyphopichia	*Metahyphopichia laotica*	11-2.5[b], 11-2W.4[b]	11-2.5 (LC435609)			
Nakazawaea	*Candida nonsorbophila*	BW4.5.2[e]	BW4.5.2 (LC435611)			
Kluyveromyces	*Kluyveromyces siamensis*	1-3.3[a], 11-2.2[b], 11-3.1[b], 11-6.1.1[b], 11-3W.2[b], NWK8-1[c], WW2.1[d], WS2.1[d], WS3.2[d], WS6.1[d], WS7.5[d], BS1.1[e], BS5.1[e], BS9.3[e], BW4.5.1[e], BW6.4[e], BS8.3[e], NSP1-1[g], NSP2-1[g], NSP6-1[g], NSP8-1[g], NSP9-1[g], NSP11-2[g], NSP12-1[g], NSP14-1[g], NSP15-1[g], NSP16-3[g], NSP18-1[g], NSP19-1[g], NSP20-2[g], NSP21-1[g], NSP22-3[g], NSP24-1[g], NWP5-2[g], NWP7-1[g], NSB1-1[h], NSB2-1[h], NSB3-1[h], NSB6-2[h], NSB8-1[h], NWB6-4[h],	NSP14-1 (LC435612)			
	Kluyveromyces aestuarii	NSB1-2[h], NSB2-2[h], NSB9-1[h], NSB10-1[h]	NSB1-2 (LC435613)			
Pichia	*Pichia kudriavzevii*	NWB4-1[h], NWB5-1[h], NWB6-1[h]	NWB5-1 (LC435614)			
	Candida pseudolambica	1-4.3[a], 11-4.1[b], 11-7.2[b], WS7.3[d], WW3.1[d], MTW8.1[f]	1-4.3 (LC435615)			
	Candida thaimueangensis	1-3W.6.3[a], 1-7W.8[a], 1-8W.1[a], 11-6.1.2[b], NSK3-2[c], NSK14-1[c], NWK4-2[c], BW9.3[e], MTW6.1.1[f], NSP16-1[g], NSP20-1[g], NSP22-1[g], NWP2-1[g], NWP4-1[g], NWP5-1[g], NWP7-2[g], NWP8-1[g], NWP10-1[g], NSP11-3[g], NSP15-2[g], NSP24-2[g], NWB1-1[h], NWB2-2[h]	NWP2-1 (LC435616)			
Pichia (continued)	*Candida* aff. *californica*	BW6.1[e], MTT3.2[f], MTW10.3[f]	MTT3.2 (LC435617)			
	Pichia chibodasensis	BW6.8[e], MTT3.1[f]	MTT3.1 (LC435618)			
	Pichia occidentalis	1-7W.2[a]	1-7W.2 (LC435619)			
	Pichia sporocuriosa	1-3W.2[a]	1-3W.2 (LC435620)			
Ogataea	*Candida sithepensis*	MTW10.4[f]	MTW10.4 (LC435621)			
	Candida cylindracea	1-6.9[a], MTS1.1.2[f]	MTS1.1.2 (LC435622)			
Ambrosiozyma	*Ambrosiozyma monospora*	NWK2-3[c]	NWK2-3 (LC435623)			
Candida	*Candida* aff. *sorboxylosa*	1-6.6[a]	1-6.6 (LC435624)			
	Candida silvanorum	11-18.2[b], 11-14.2[b]	11-18.2 (LC435610)			
	Candida aff. *silvanorum*	11-14.2[b], 11-15.8[b]	11-14.2 (LC435625)			
Myxomycota						
Myxomycocetes						
Prototheca	*Prototheca* aff. *wickerhamii*	NSP10-2[g]	NSP10-2 (LC435626)			

Sampling sites: [a]RBR, [b]MFDS no. 11, [c]MFDS no. 7, [d]MFDS no. 2 , [e]MFDS no. 4, [f]MFDS no. 45, [g]PNFP, [h]MFDS no. 6.
Sources: *S* (soil), *W* (water), *D* (decayed biological matter)

Sequencing of the 26S rRNA gene (D1/D2 domain)

The 26S rRNA gene at the D1/D2 domain (LSU D1/D2 domain) was PCR amplified using the NL1 (5′-GCATAT CAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCG TGTTTCA AGACGG-3′) primers (O'Donnell and Gray 1995). The PCR was performed in a 20 µl reaction volume containing 2 µl DNA template, 0.4 µl of each primer (10 pmol/µl), 10 µl 2× Go Tag green and 7.2 µl distilled water, and was thermocycled at 94 °C for 3 min, followed by 36 cycles of 94 °C, 52 °C and 72 °C, each for 30 s, and then a final 72 °C for 5 min. The PCR product was purified by washing with sterile deionized water, filtered through a MinElute® multi-well filter plate (QIAGEN sciences, U.S.A.) and then used as the DNA template for the thermal cycle sequencing reaction. Primers used were NL1, NL4, NL3A (5′-GAGACCGATAGCGAACAAG-3′) and NL2A (5′-CTTGTTCG CTATCGGTCTC) (Kurtzman and Robnett 1998). Cycle sequencing was performed in a 10 µl reaction volume using a BigDye® Terminator V3.1 Cycle Sequencing kit (Applied Biosystems, U.S.A.). Each reaction contained 1–2 µl DNA template and 1.5 µl of the respective primer (1 pmol/µl). Samples were thermocycled at 96 °C for 10 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min, followed by a final 60 °C for 10 min. The PCR products were purified and sequence analyzed using an Auto-sequencer ABI Prism 3130xl Genetic analyzer (Applied Biosystems, USA) following the manufacturer's instructions.

The DNA sequence was manually edited using the BioEdit program version 7.2.5 (Hall 1999) and compared to those in the online database available in the NCBI GenBank using the BLASTn program.

Biodiversity analysis

Yeast diversity in each sampling sites was analyzed using Shannon–Wiener index (H'), $H' = -\sum_{i=0}^{s} Pi(\ln Pi)$ where Pi is the proportion of the number of yeast strains in each species to the total number of yeast strains in that sampling site (Shannon 1948; Spellerberg and Fedor 2003). Yeast species evenness in sampling sites was determined using equitability (E_H), $E_H = \frac{H'}{\ln S}$, where S is total number of yeast species in each sampling site (Pielou 1975). Evenness indices range from 0 to 1, where a value close to 1 means there is complete evenness among all species in that area (Heip et al. 1998).

Nile red staining

Yeast isolates that were designated as likely to belong to genera known to contain oleaginous yeasts, plus the potential new species, were grown on modified YM agar (20× diluted nitrogen source) at 30 °C for 5 days. The resultant cells were suspended in Nile red solution (50 µg/ml in acetone diluted 100× with 25% (v/v) dimethyl sulfoxide) and incubated at 30 °C for 10 min. The intracellular lipid accumulation was preliminary examined under a fluorescence microscope (Olympus BX51, USA) using a U-WNB2 filter with excitation and emission wavelengths of 470–490 and 520 nm, respectively (Greenspan et al. 1985). The known oleaginous yeast, *Lipomyces starkeyi* JCM 5995, was used as a positive control.

Analysis of the intracellular lipid content

Nile red stained yeasts that showed an oil droplet bigger than 2/3 of the cell size were selected for analysis of their intracellular lipid content. One loopful of yeast grown on YM agar was transferred into YM broth (50 ml) in a 250-ml flask and incubated at 30 °C, 200 rpm for 24 h. A 15-ml aliquot was then inoculated into YM broth (150 ml) in a 500-ml flask and incubated at 30 °C, 200 rpm for 48 h. The resultant culture was collected by centrifugation (9803×*g*, 4 °C, 15 min) and the cell pellet was washed with lipid production medium (LPM; modified from Galafassi et al. (2012) to contain 50 g/l glucose, 1 g/l yeast extract, 0.05 g/l $MgSO_4 \cdot 7H_2O$, 1 g/l KH_2PO_4, 1 g/l $(NH_4)_2SO_4$, 0.01 g/l NaCl, 0.01 g/l $CaCl_2 \cdot 2H_2O$, pH 5.5). The washed cells were inoculated into 150 ml LPM in a 500-ml flask and incubated at 30 °C, 200 rpm for 6 days. Cells were then harvested by centrifugation (as above), washed with distilled water and lyophilized. The intracellular lipid level of the lyophilized cells was determined as described previously (Folch et al. 1957). The lyophilized cells (1 g DCW) were suspended in 20 ml of 2:1 (v/v) chloroform: methanol, sonicated at 37 kHz, room temperature for 30 min (Elmasonic, E60H model, Germany) and centrifuged at 9803×*g*, 4 °C for 40 min. The supernatant was harvested, dried by evaporation at room temperature and the weight of the lipid residue was measured.

Analysis of the fatty acid composition

Lipid was extracted and converted to fatty acid methyl esters (FAMEs) as reported (Anamnart et al. 1998). In brief, 1 g of wet cells was saponified with 0.8 ml of 10% (w/v) potassium hydroxide in methanol at 80 °C for 2 h. After cooling down to room temperature, 1 ml of petroleum ether was added to remove the unsaponified materials in the reaction mixture. The obtained aqueous phase was acidified by 0.3 ml of 6N hydrochloric acid and extracted with diethyl ether to recover the fatty acids. The fatty acid fraction was evaporated to dryness under nitrogen gas and derivatized to FAMEs using BF_3/MeOH, and then the FAMEs were extracted in hexane and further analyzed on a flame ionization detector system using gas chromatography (GC; Agilent Technologies

6890N, USA) equipped with an INNOWAX capillary column (30 m × 0.3 mm, 0.2 µm film thickness) as reported (Limsuwatthanathamrong et al. 2012). Helium was used as the carrier gas at a flow rate of 2.3 ml/min. The temperature program started at 150 °C and was then increased to 180 °C, 200 °C and 205 °C at a rate of 10 °C/min, 5 °C/min and 0.5 °C/min, respectively. The temperature was then held at 205 °C for 2 min before increasing to 250 °C at 5 °C/min and maintained for 5 min. The FAMEs were identified by comparison with reference standards.

Phenotypic characterization of the oleaginous yeast isolates

To confirm the molecular identification results of the isolated oleaginous yeasts, morphological and physiological characteristics of the isolated oleaginous yeasts were determined and then compared to the description of type strains in Kurtzman et al. (2011). Cell morphology was observed under light microscopy, while the colony morphology, including the texture, color, surface, elevation and margin were recorded visually. Carbon assimilation tests were conducted using an ID 32 C kit (BioMerieu, France) following the manufacturer's instructions. Formation of pseudomycelium and true mycelium were examined by the Dalmau slide culture method at 25 °C for up to 14 days (Kurtzman et al. 2011).

Determination of lipid accumulation in glucose solution

Capability of the isolated oleaginous yeasts to accumulate oil in the absence of a nitrogen source was determined (Lin et al. 2011). One loopful of oleaginous yeasts grown on YM agar was transferred into YM broth (50 ml) in a 250-ml flask and incubated at 30 °C, 200 rpm for 24 h. A 15-ml aliquot was then inoculated into YM broth (150 ml) in a 500-ml flask and incubated at 30 °C, 200 rpm for 48 h. The cells were then collected by centrifugation (4 °C, 9803×*g*, 10 min), washed with sterile distilled water twice, and suspended in 150 ml glucose solution (40 g/l) in a 500-ml flask and incubated at 30 °C with agitation at 200 rpm for 60 h. Every 12 h, cells (three independent culture flasks) were harvested by centrifugation, washed with sterile distilled water and lyophilized. The DCW, lipid content (% (w/w, DCW)) and lipid yield were determined.

Results

Isolation and molecular identification of yeast isolates

A total of 191 yeasts were isolated and categorized by PCR $(GTG)_5$ fingerprinting. The obtained $(GTG)_5$ fingerprint patterns were manually grouped into 94 distinct patterns. One representative strain from each of the 94 patterns was then selected for molecular operational taxonomic unit (MOTU) identification and species inference by comparing their LSU D1/D2 domain sequence (500–600 bp) with those deposited in the NCBI GenBank database using the BLASTn program. Yeast strains with six or more nucleotide substitutions (> 1%) were likely to be different species, while strains with 0–3 nucleotide substitutions were assigned as conspecific or sister species (Kurtzman and Robnett 1998). In this study, yeast strains with less than 1% nucleotide substitution were assigned as the same species, and the isolates with the same $(GTG)_5$ fingerprint pattern were assumed to be the same. The term 'aff.' (species affinis) was used to indicate a potentially new species that affiliated to but not identical to its closest known species. As a result, the 191 isolated yeast strains were classified to ascomycetous yeasts (156), basidiomycetous yeasts (34) and yeast-like algae (1). From the 156 yeast strains belonging to the Ascomycota, 147 strains were classified to 32 species in 16 genera. Another nine strains were assigned as five new species within the genera *Wickerhamomyces* sp. (two strains), *Yamadazyma* sp. (one strain), *Clavispora* sp. (one strain), *Pichia* sp. (three strains) and *Candida* sp. (two strains). Thirty one out of the 34 strains belonging to the Basidiomycota were classified to 12 species in nine genera, while the three remaining strains were assigned as potential new species in the genera *Sakaguchia*, *Heterocephalacria* and *Goffeauzamy*. One other strain was classified as a potential new species in the genus *Prototheca*, a yeast-like non-photosynthetic algae with yeast-like cells and colonies. The number of each species of yeasts isolated, including the accession number of the LSU D1/D2 domain sequence of each representative yeast species submitted to the GenBank database are shown in Table 2.

Yeast diversity and community

Andaman Sea coastline

At RBR, a total of 24 yeast strains were isolated from 20 collected samples. Nineteen and four strains belonged to Ascomycota (thirteen species in eight genera) and

Basidiomycota (four species in three genera), respectively. The remaining basidiomycetous yeast was ascribed as new species closely related to *Heterophalacria arrabidensis* with 2.44% nucleotide substitutions. The most frequently isolated species were *Candida aaseri* and *C. thaimuengensis* with a fairly even distribution. The species isolated only at this site were *Candida aaseri*, *C. intermedia*, *C. sorboxylosa*, *Pichia occidentalis*, *P. sporocuriosa*, *Rhodotorula sphaerocarpa*, *Suhomyces atakaporum* and the new species (Tables 2 and 3). The Shannon diversity index (*H'*) and community evenness (E_H) of the isolated yeasts were 2.79 and 0.96, respectively (Table 3).

For MRDS no. 11, 34 yeast strains were isolated from 23 collected samples. Eighteen strains were ascomycetous yeasts (eight species in six genera) and 13 strains were basiodiomycetous yeasts (nine species in five genera). The remaining three strains were ascribed as new species closely related to *Goffeauzyma gilvescens* (one strain) and *Candida silvanorum* (two strains) with 8.15% and 12.79% nucleotide substitutions, respectively. The most frequently isolated species were *Kluyveromyces siamensis* and *Schwanniomyces vanrijiae*. The species isolated at this site only were *Candida maltose*, *C. silvanorum*, *Hannaella phetchabunensis*, *Kwoniella dejecticola*, *Metahyphopichia laotica*, *Naganishia liquefaciens*, *Nag. albida*, *Rhodotorula toruloides*, *R. mucilaginosa*, *Schwanniomyces vanrijiae* and the two new species (Tables 2 and 3). The *H'* and E_H of the isolated yeasts were 2.83 and 0.96, respectively (Table 3).

Gulf of Thailand

At MRDS no. 7, 16 ascomycetous yeasts (six species in five genera) plus two new species closely related to *Candida quercuum* (two strains) and *Candida ecuadorensis* (one strain) with 5.13% and 6.78% nucleotide substitutions, respectively, were isolated from 25 collected samples. *Candida tropicalis* was the most prevalent species, while *Ambrosiozyma monospora, Yamadazyma mexicana* and the two new species were unique species to this site (Tables 2 and 3). The *H'* and E_H of the isolated yeasts were 1.81 and 0.87, respectively (Table 3).

For MRDS no. 2, 12 yeast strains including four species in four genera of Ascomycota (11 strains) and one species of Basidiomycota (one strain) were isolated from 22 collected samples. *Candida amphicis* was only isolated from this site, while *Kluyveromyces siamensis* was by far the most common species isolated (Tables 2 and 3). The *H'* and E_H of the yeast isolated were 1.47 and 0.91, respectively (Table 3).

For MRDS no. 4, 29 yeast strains were isolated from the 26 collected samples and contained nine species in seven genera of Ascomycota (18 strains) and three species in three genera of Basidiomycota (10 strains) plus one new species closely related to *Candida californica* with 2.23% nucleotide substitutions. *Kluyveromyces siamensis* and *Rhodotorula paludigena* were the most prevalent species. *Candida nonsorbophila, C. orthopsilosis*, *Meyerozyma guilliermondii*, *Pseudozyma huberensis* and *Schwanniomyces polymorphus* were unique species to this site (Tables 2 and 3). The *H'* and E_H of the isolated yeasts were 2.27 and 0.89, respectively (Table 3).

Seventeen yeasts isolated from 25 samples collected at MRDS no. 45 were composed of 11 strains of ascomycetous yeasts (nine species in six genera), three strains of basidiomycetous yeasts (two species in two genera) plus two new species that were closely related to *Sakaguchia lamellibrachiae* (one strain) and *Candida californica* (two strains) with 2.44% and 2.22% nucleotide substitutions, respectively. The most frequently isolated species was *Candida tropicalis*. The new species (one strain) and *Candida sithepensis* were only isolated at this site (Tables 2 and 3). The *H'* and E_H of the isolated yeasts were 2.48 and 0.97, respectively (Table 3).

At PNFP, 35 yeast strains were isolated from 34 collected samples. Thirty four strains were ascribed to five species in four genera of Ascomycota plus one new species closely related to *Prototheca wickerhamii* with 9.14% nucleotide substitutions. The most common species isolated was *Kluyveromyces siamensis*. In addition to the new species, *Candida viswanathii* and *C. andamanensis* were unique to this site (Tables 2 and 3). The *H'* and E_H of the isolated yeasts were 1.18 and 0.66, respectively (Table 3).

Finally, at MRDS no. 6, 21 yeasts strains were isolated from 22 samples and were comprised of 20 strains of

Table 3 Numbers of samples, yeast strains isolated, yeast species, yeast diversity and community evenness indices of yeasts isolated from the eight different mangrove forests

Diversity index	Sampling site							
	RBR	MRDS no. 11	MRDS no. 7	MRDS no. 2	MRDS no. 4	MRDS no. 45	PNFP	MRDS no. 6
Total number of collected samples	20	23	25	22	26	25	34	22
Total number of yeast strains	24	34	14	12	29	17	35	26
Total number of yeast species (S)	18	19	8	5	13	13	6	8
Shannon–Weiner index (*H'*)	2.79	2.83	1.81	1.47	2.27	2.48	1.18	1.91
Equitability index (E_H)	0.96	0.96	0.87	0.91	0.89	0.97	0.66	0.92

ascomycetous yeasts (seven species in five genera) plus one new species closely related to *Yamadazyma mexicana* with 2.64% nucleotide substitutions. The new species plus *Wickerhamomyces anomalus*, *Kluyveromyces aestuarii* and *Pichia kudriavzevii* were only isolated from this site, while the most common species was *Kluyveromyces siamensis* (Tables 2 and 3). The *H'* and E_H of the isolated yeast were 1.91 and 0.92, respectively (Table 3). The relative abundance of the yeast species among the different sampling sites is shown in Fig. 2.

Nile red staining

Of the 191 isolated yeast strains, 30 were designated to belong to a genera that has been reported to contain oleaginous yeasts; *Rh. paludigena*, *Rh. sphaerocarpa*, *Rh.*

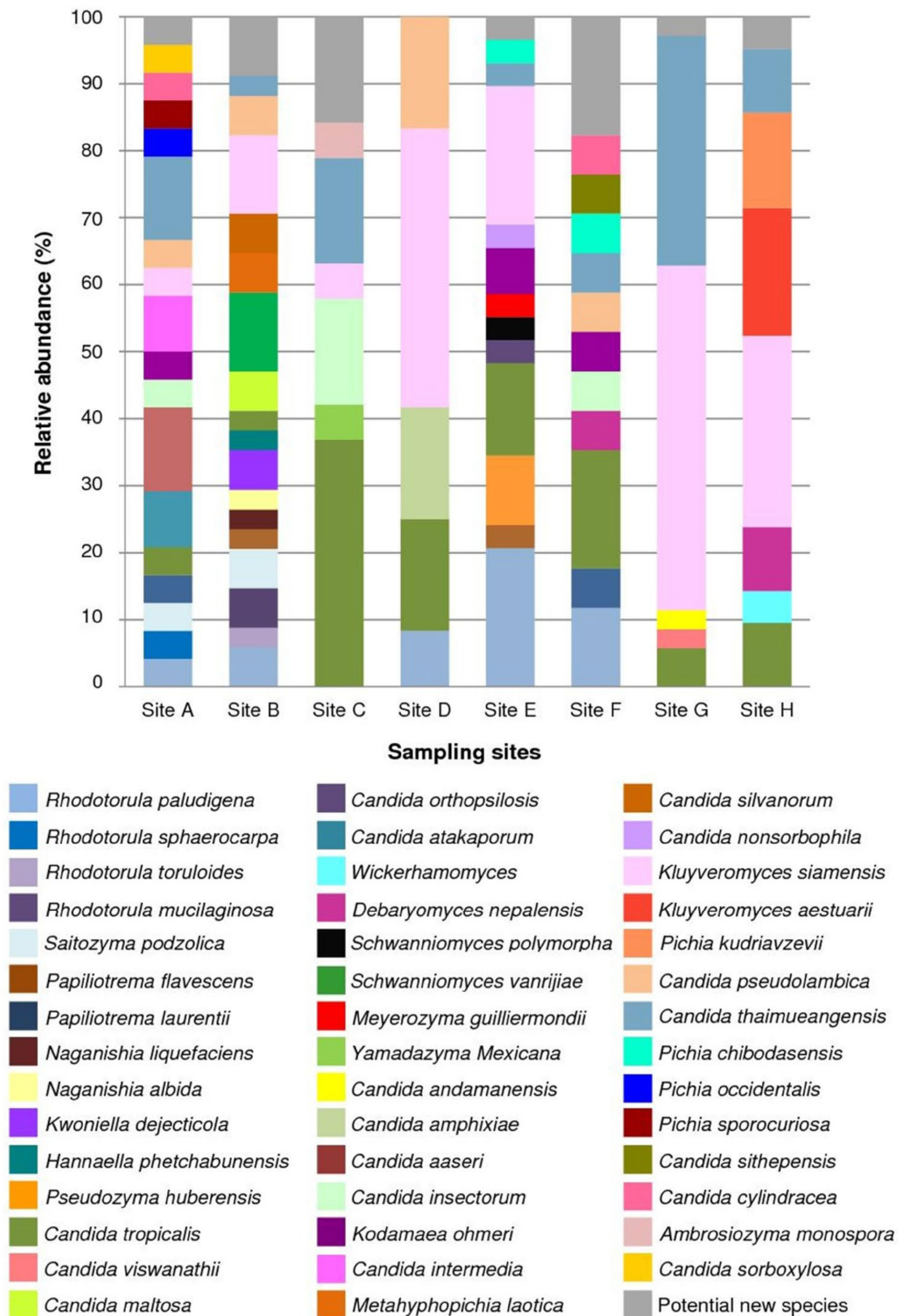

Fig. 2 Relative abundance of species isolated from each sampling site. RBR (Site A), MRDS no.11 (Site B), MRDS no.7 (Site C), MRDS no. 2 (Site D), MRDS no. 4 (Site E), MRDS no. 45 (Site F), PNFP (Site G) and MRDS no. 6 (Site H)

toruloides, *Rh. mucilaginosa*, *Sait. podzolica*, *Pap. flavescens*, *Pap. laurentii*, *Nag. liquefaciens*, *Nag. albida*, *Kwoniella dejecticola* and *Debaryomyces nepalensis* (Li et al. 2008; Rossi et al. 2009; Ageitos et al. 2011; Kurtzman et al. 2011; Sitepu et al. 2012). These 30 yeast strains plus the 13 strains ascribed as potential new species were selected for preliminary determination of their intracellular lipid accumulation by Nile red staining. Of these 43 selected yeast strains, 11 (*Rh. sphaerocarpa* 11-14.4, *Rh. paludigena* BW7.3, *Rh. paludigena* 11-14.7, *Rh. paludigena* MTW11.1, *Rh. paludigena* BW1.3, *Rh. paludigena* BW8.1, *Rh. paludigena* WW1.2, *Sait. podzolica* 11-11.3.1, *Pap. laurentii* MTT4.1, *Prototheca* sp. NSP10-2 and *Debaromyces nepalensis* NSB6-1) showed an oil droplet bigger than 2/3 of the cell size and so were selected for further analysis of their lipid content. The oil droplet stained with Nile red of *Rh. sphaerocarpa* 11-14.4, *Sait. podzolica* 11-11.3.1 and *Lipomyces starkeyi* JCM 5995 (positive control) are showed in Fig. 3.

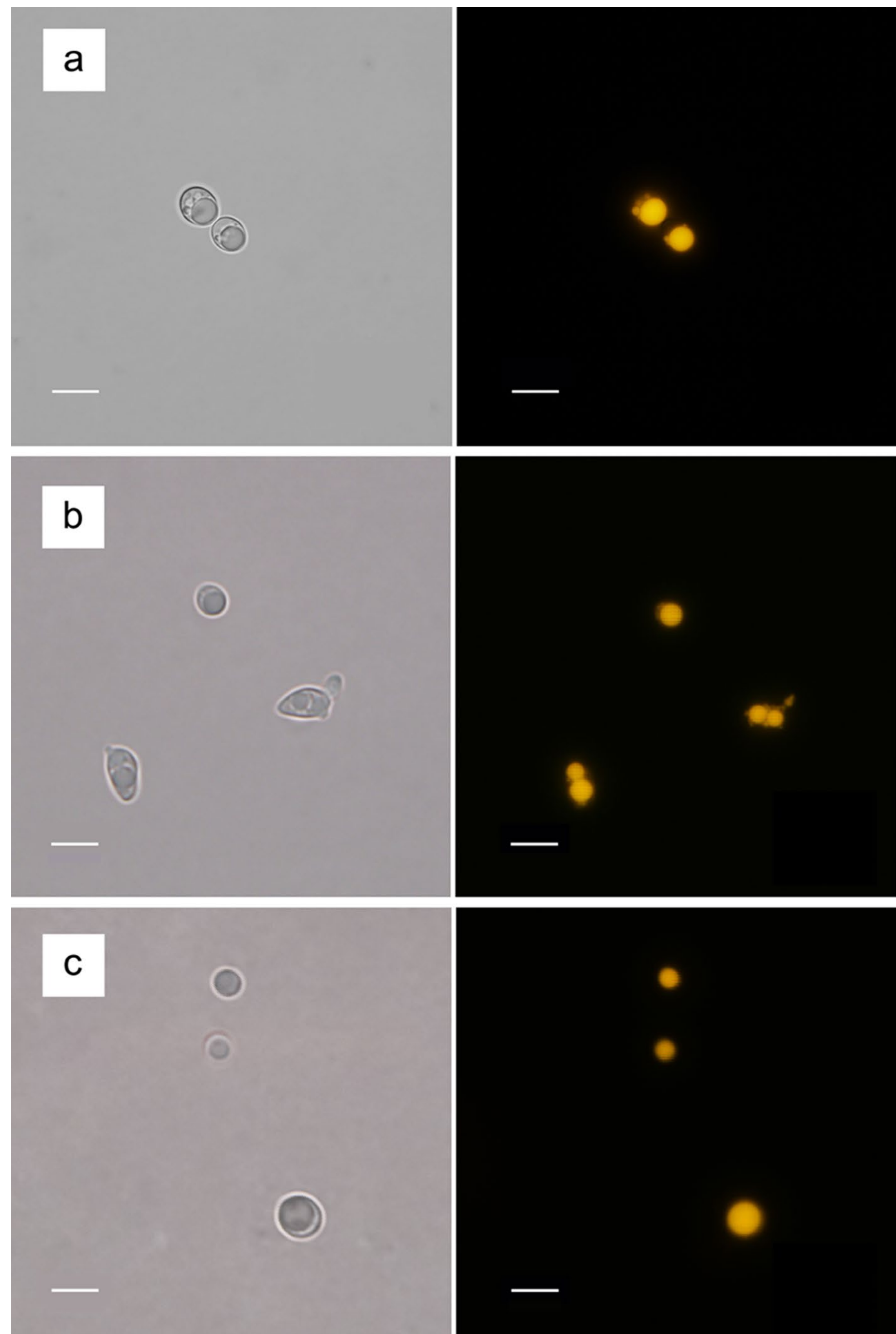

Fig. 3 Nile red staining of **a** *Rhodotorula sphaerocarpa* 11-14.4, **b** *Saitozyma podzolica* 11-11-3.1 and **c** *Lipomyces starkeyi* JCM 5995 grown on modified YM agar (20 × diluted nitrogen source) at 30 °C for 5 days. Bright field (left) and fluorescent (right) images at × 100 magnification. Scale bar = 10 µm. Each image is a representative of at least three such fields of view per sample and two independent samples

Analysis of lipid accumulation

Analysis of the intracellular lipid content of the 11 selected yeast strains revealed that *Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11.3.1 had an intracellular lipid content in excess of 20% (w/w, DCW), at 27.3 ± 0.006 and 24.0 ± 0.010% (w/w, DCW), respectively, and so were designated as oleaginous strains (Fig. 4) and selected for further fatty acid composition analysis. Of interest, both these oleaginous strains (*Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11.3.1) were isolated from the same site (MRDS no. 11 (Ranong province) on the Andaman Sea coastline).

Fatty acid composition

The fatty acid composition of the two oleaginous strains is presented in Table 4. The major fatty acids were oleic, steric, palmitic and linoleic acids. The oil from *Rh. sphaerocarpa* 11-14.4 was comprised of oleic, palmitic and linoleic acids at 55.7, 18.9 and 12.4%, respectively, while myristic, linolenic and palmitoleic acids were present in lesser amounts. For *Sait. podzolica* 11-11.3.1, the three main fatty acids were steric (28.7%), oleic (28.7%) and palmitic (25.9%) acids, with linoleic and myristic acids as minor and trace amounts, respectively.

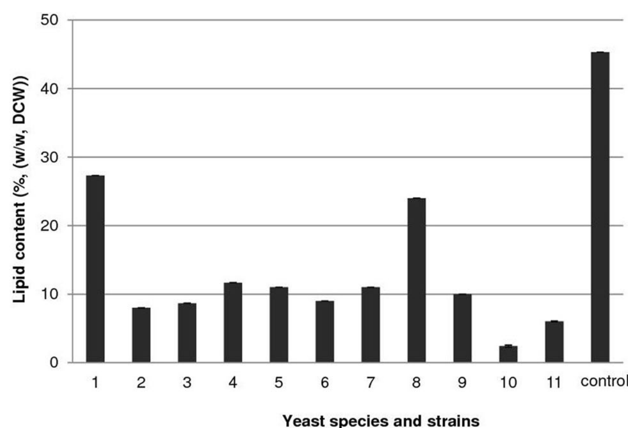

Fig. 4 Intracellular lipid accumulation of the 11 yeast strains having an oil droplet bigger than 2/3 of their cell size. (1) *Rh. sphaerocarpa* 11-14.4 (2) *Rh. paludigena* BW7.3 (3) *Rh. paludigena* 11-14.7 (4) *Rh. paludigena* MTW11.1 (5) *Rh. paludigena* BW1.3 (6) *Rh. paludigena* BW8.1 (7) *Rh. paludigena* WW1.2 (8) *Sait. podzolica* 11-11.3.1 (9) *Pap. laurentii* MTT4.1 (10) *Prototheca* sp. NSP10-2 (11) *Debaromyces nepalensis* NSB6-1 and (control) *Lipomyces starkeyi* JCM 5995. Data are shown as the mean ± 1SD, derived from three independent trials

Phenotypic characteristics of the oleaginous yeast isolates

Morphological and physiological characteristics of the oleaginous strains, *Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11-3, were the same as their type strains, *Rh. sphaerocarpum* CBS5939^T and *Sait. podzolicus* CBS 6819^T, respectively. The *Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11-3 were deposited in the Microbe Division/ Japan Collection of Microorganisms, RIKEN BioResource Center, Tsukuba, Japan under numbers JCM 32,652 and JCM32653, respectively.

Lipid accumulation of the oleaginous yeasts in glucose solution

The *Rh. sphaerocarpa* 11-14.4 suspended in 40 g/l glucose solution had the highest lipid content at 32.1% (w/w, DCW) and lipid yield (3.04 g/l) at 48 h, while the cell biomass was highest (14.1 g/l) at 36 h (Fig. 5a). For *Sait. podzolica* 11-11.3.1 under the same conditions, the highest lipid content, lipid yield and cell biomass 25.8% (w/w DCW), 3.46 g/l and 13.40 g/l, respectively, were all found at 36 h (Fig. 5b).

Discussion

Based on the isolation condition performed in this study, ascomycetous yeasts were dominant and were found at all the sampling sites, whereas basidiomycetous yeasts were less prevalent and were not found at three sites (MRDS no.7, PNFP and MRDS no.6). Chi et al. (2012) used YPD as the isolation medium at 25 °C reported that ascomycetes yeasts were the most abundant yeasts in mangrove ecosystems

Table 4 Relative fatty acid content of the two oleaginous yeasts cultivated in lipid production medium (LPM)

Strains	Relative content of fatty acids (% (w/w))							
	Myristic C14:0	Palmitic C16:0	Palmitoleic C16:1	Stearic C18:0	Oleic C18:1 n9	Linoleic C18:2 n6	Linolenic C18:3 n3	Others
Rh. sphaerocarpa 11-14.4	1.17 ± 0.19	18.86 ± 0.58	0.68 ± 0.25	5.69 ± 0.40	55.68 ± 2.29	12.37 ± 1.01	1.15 ± 0.32	4.40
Sait. podzolica 11-11.3.1	0.48 ± 0.06	25.91 ± 1.32	ND	29.86 ± 1.95	28.69 ± 1.19	7.33 ± 0.45	ND	7.74

ND not determined (the peak was not detected or the peak area was too small to determine)

Data are shown as the mean ± 1SD, derived from three replications

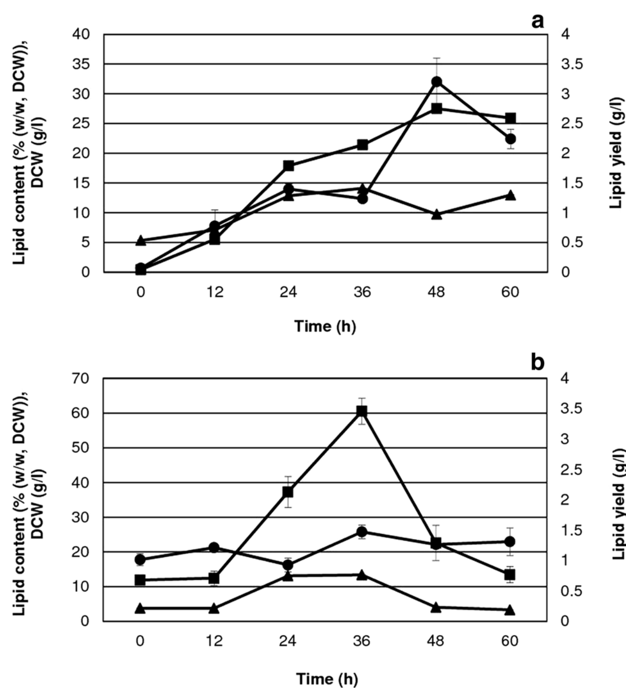

Fig. 5 Lipid accumulation in glucose solution of **a Rh. sphaerocarpa** 11-14.4 and **b Sait. podzolica** 11-11.3.1. Symbols used are: lipid content (filled circle), lipid yield (filled square) and dry cell weight (DCW) (filled triangle). The data are displayed as the mean ± SD, and are derived from triplicate experiments

in China, where they represented 95.2% of the 269 yeasts isolated from the sediment, water and various plant parts. Culture-independent studies on fungal diversity in mangrove forests of New Caledonia revealed that ascomycetes yeasts were dominant, and comprised 82% of the sequence reads (Arfi et al. 2012). The distribution and population of yeasts were depended on the type and concentration of organic materials (Kutty and Philip 2008).

Among the 44 known species isolated in this study, 33 species have been reported from a mangrove environment (Araujo et al. 1995; Fell et al. 2011; Chi et al. 2012; Kunthiphun et al. 2018). Many of these yeast species were likely to have been introduced from terrestrial environments and the marine water. Terrestrial and human-associated yeasts are introduced into mangrove forests by rain, rivers and human activity, while marine yeasts can influx into this convergence area from marine waters by tidal action, natural drainage and human activities (Kutty and Philip 2008; Fell 2012; Libkind et al. 2017; Hagler et al. 2017).

The most prevalent yeast species isolated in this study was *Kluyveromyces siamensis* (21.5%) followed by *Candida thaimueangensis* (12.0%) and *Candida tropicalis* (11.5%), while the remaining species were represented by only 1–6 isolated strains (0.5–3.1%). *Kluyveromyces siamensis* was the most frequently isolated species (5/8 sampling sites; MRDS no. 11, MRDS no. 2, MRDS no. 4, PNFP and MRDS no. 6). This species was first isolated from mangrove water in Thailand (Am-In et al. 2008) and then subsequently reported from mangrove forests in China (Chi et al. 2012) and Thailand (Kunthiphun et al. 2018). The high prevalence of occurrence of *K. siamensis* in this study may suggest that mangroves are its natural habitat.

Candida thaimueangensis was described by Limtong et al. (2007) from mangrove water in Thailand and then in mangroves in the USA (Fell et al. 2011), China (Chi et al. 2012) and Thailand (Kunthiphun et al. 2018). However, Bautista-Gallego et al. (2011) reported finding *C. thaimueangensis* in fermented olives in Spain. Nevertheless, the high prevalence of occurrence of *C. thaimueangensis* in this study of Thai mangroves could suggest that mangroves are its primary habitat.

Candida tropicalis, a pathogenic yeast of human and marine invertebrates (Moore and Strom 2003; Wang et al. 2007), was the most widely distributed species, being isolated from all the sampling sites in this survey. Moreover, *C. tropicalis* was reported to be the most typical yeast species in mangroves (Hagler et al. 2017) and was found in all mangrove ecosystems surveyed in China (Chi et al. 2012). However, this species is widely distributed, being isolated from clinical samples and considered as a human-associated species but is also common in a variety of natural sources, such as plants, fruits, flower, soil, water and food products (Kvasnikov et al. 1975; Lachance et al. 2011). Thus, the ecological association of this species is hard to ascertain. Other opportunistic pathogen yeast species found in this survey were *C. viswanathii*, *C. orthopsilosis*, *C. aaseri*, *C. intermedia*, *Wickerhamomyces anomalus*, *Meyerozyma guilliermondii* and *Pichia kudriavzevii*. They are often observed in feces of warm-blooded animals. However, there are few mammals in mangroves but lots of birds so a fecal origin from birds for such species is very likely.

Kluveromyces aestuarii is marine adapted species which typically found in mangroves habitat (Kutty and Philip 2008; Fell 2012; Hagler et al. 2017). *K. aestuarii* was first described by Fell (1961) from estuaries in Florida (USA) and subsequently in mangroves in Brazil (Araujo et al. 1995; Araujo and Hagler 2011) China (Chi et al. 2012) and Thailand (Kunthiphun et al. 2018). It was found in detritus feeding crabs and shipworms (mollusk) (Araujo et al. 1995). *Kluveromyces aestuarii* has been used as an indicator species for natural well-preserved mangrove ecosystems (Araujo and Hagler 2011). In this study, *K. aestuarii* was only isolated from one site, at MRDS no.6, located in Bang Khunsai, which contains the largest cockle breeding and cultivating grounds in Thailand (Pumijumnong 2014). Accordingly, the occurrence of *K. aestuarii* at MRDS no.6 may suggest that this site is situated in a good practice mangrove community, or it may simply reflect its proximity to the large cockle bed.

Rhodotorula paludigena and *Rh. sphaerocarpa* are frequently found in marine habitats but not exclusively. *Rh. paludigena* was found in plant nectars (Canto et al. 2017) and *Rh. Sphaerocarpa* was found in freshwater habitats (Brandão et al. 2017). The two remaining *Rhodotorula* species observed in this study, namely *Rh. toruloides* and *Rh. mucilaginosa*, are found in a wide range of natural habitats such as phyllophane (Khunnamwong et al. 2018) soil, decayed plants (Sampaio 2011) and marine habitats (Kurtzman et al. 2011).

Heavy rain with enhanced river flow and floods can wash out terrestrial and freshwater microbial populations and carry them to mangrove forests (Araujo et al. 1998). In this study, four yeast species that are commonly found in soil (*Saitozyma podzolica*, *Candida sithepensis*, *Pichia terricola* and *P. chibodoensis*) were isolated. And three strains of the *Sait. podzolica* isolated were from different kind of samples, one strain from water at RBR and the other two strains from soil and decaying moss at MRDS no. 11. *Saitozyma podzolica* has previously been reported as a typical soil-borne yeast, since it is frequently isolated from various kinds of soil, such as podzolic and sod-podzolic soils in the taiga zone (Babjeva and Rheshetova. 1975), peat soil (Golubev 1991), spruce forest soil (Yurkov et al. 2012a) and forest and grassland soils (Yurkov et al. 2012b). Moreover, *Sait. podzolica* has been reported to occur in other habitats that contained high organic matter and high moisture content, such as rotten wood in a beech forest (Middelhoven et al. 2006), decayed biomaterial in a mangrove forest (Kunthiphun et al. 2018), litters from the temperate forests (Mašínová et al. 2017) and from the spruce frorest (Štursová et al. 2012), *Sphagnum* moss (Kachalkin et al. 2008), and water from tropical lake (Brandão et al. 2017). The oil from the oleaginous *Sait. podzolica* CPOH4, isolated from peat bog soil in the black forest of Germany, had a fatty acid profile suitable for biodiesel production (Schulze et al. 2014).

The highest diversity of yeasts was found at MRDS no. 11 (11 genera and 17 species) followed by RBR (10 genera and 16 species), which are both located in Ranong province on the Andaman Sea coastline. Shannon diversity index (*H'*) of MRDS no. 11 and RBR were 2.83 and 2.79, respectively. Mangrove forests in the Ranong province were the least invaded by human activities compared to the other provinces in this study (Department of Marine and Coastal Resources 2014). The RBR has been declared a UNESCO Biosphere Reserve area (Pumijumnong 2014). The MRDS no. 11 and RBR are mature mangrove forests (Pumijumnong 2014) which have *Rhizophora apiculata* as their dominant plant species (Mangrove Conservation Office 2018). The *R. apiculata* is the highest biomass-mangrove plant species (Meepol 2010). Carbon sequestration in the MRDS no. 11 and RBR was higher than the other surveyed mangrove forests (Mangrove Conservation Office 2018). So they are potentially richer in nutrients for yeasts. Additionally, *Candida tropicalis* was the only human-associated species isolated at MRDS no. 11. This low level of human-associated yeast species indicates the lower level of human influence (Hagler et al. 2017; Libkind et al. 2017).

The MRDS2 and PNFP sites, located in Chanthaburi and Prachuap Khiri Khan provinces, respectively, have the lowest yeast diversity. More than 50% of the mangrove area in these provinces had been invaded for agriculture and aquaculture (Department of Marine and Coastal Resources 2014). The distribution of yeasts is influenced by the type and concentration of available nutrients (Kutty and Philip 2008), plant vegetation (Yurkov et al. 2012b; Hagler et al. 2017) and anthropogenic activities (Yurkov et al. 2012b; Yurkov 2017).

Among the 30 yeast strains designated to belong to a genera that has been reported to contain oleaginous yeast species plus the 13 strains ascribed as potential new species, only two strains (6.67%; *Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11-3.1) were found to be oleaginous yeasts. This result confirmed that the oleaginicity was species and genus independent. Interestingly, both of them were isolated from the same site (MRDS no. 11, Andaman Sea coastline), where there was the highest yeast diversity and so the potential chance to isolate such. Screening of oleaginous yeast from yeasts inherent in genus reported to contain oleaginous yeast provided the chance of finding more oleaginous yeast (Sitepu et al. 2013).

The *Rh. sphaerocarpa* 11-14.4 had an intracellular lipid content of 27% (w/w, DCW). Only 5% of oleaginous yeasts have been reported to accumulate intracellular lipid more than 25% (w/w, DCW) (Agetios et al. 2011). *L. starkeyi* (Angerbauer et al. 2008), *Rhodotorula glutinis* (Beopoulos et al. 2009) and *Rhodotorula toruloides* (Zhao et al. 2008) were reported to accumulate oil up to 70% (w/w, DCW). The lipid content of yeasts is markedly influenced by the growth conditions, such as the carbon source, C/N ratio, other nutrients, oxygen level, pH and temperature (Sitepu et al. 2013). Lipid content of *Rhodotorula sphaerocarpa* UCDFST 68–43 was increased from 25.95 to 36.6% (w/w, DCW) when nitrogen was removed from medium (Sitepu et al. 2013). *Saitozyma podzolica* CPOH4 grown in modified YM medium containing 50 g/l glucose at 25 °C, 130 rpm for 120 h had an intracellular lipid content of 34.6% (w/w, DCW) (Schulze et al. 2014). The intracellular lipid content of *Sait. podzolica* DMKU-CPC19 (1) grown in nitrogen-limited medium at 28 °C, 150 rpm for 120 h was 30.1% (w/w, DCW) (Polburee et al. 2015). This makes it impossible to compare our results to others (Ageitos et al. 2011).

The major fatty acids of *Rh. sphaerocarpa* 11-14.4 oil were oleic and palmitic acids, which is similar to vegetable oils, such as rapeseed (Hoekman et al. 2012), that are generally used as a raw material for biodiesel production. The

oil from *Rh. sphaerocarpa* 11-14.4 could, therefore, serve as a raw material for biodiesel production. The major fatty acids of *Sait. podzolica* 11-11-3.1 oil were steric, oleic and palmitic acids, similar to those of cocoa butter, a high value natural fat extracted from cocoa beans (Zarringhalami et al. 2012). Cocoa butter has several applications in the food, medicine and cosmetic industries, such as providing the texture and structure of chocolate (Wang and Maleky 2018), increasing the HDL cholesterol and improvement of UV-induced erythema resistance (Marsu et al. 2004; Heinrich et al. 2006) and inhibition of lipid peroxidation (Marsu et al. 2004)). Cocoa butter like-lipids (CBLs) have been reported to be accumulated in several oleaginous yeasts, such as *Cutaneotrichosporon* (*Cu.*) *curvatus* ATCC 2059 (Hassan et al. 1994), *Yarrowia lipolytica* LGAM S(7)1 (Papanikolaou et al. 2001) and *Yarrowia lipolytica* ACA-DC 50109 (Papanikolaou et al. 2003) and *Cu. oleaginosus* DSM11815 (Wei et al. 2017), while *Rh. toluroids* Y4 produced CBLs under sulfate-limited conditions (Wu et al. 2011). The fatty acid composition and profile of the yeast oil depends on the culture medium and condition (Sitepu et al. 2014). The oil of *Sait. podzplica* CPOH4 grown in medium containing glucose as the sole carbon source had oleic (59.4%) and palmitic (18.4%) acids as the major fatty acids (Schulze et al. 2014), which is different from that for *Sait. podzolica* 11-11-3.1 in this study.

When grown in LPM medium, *Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11.3.1 had a lipid content of 27.3 and 24% (w/w, DCW), respectively, after 6 days. But when suspended in glucose solution, the lipid content of *Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11.3.1 increased to 32.1 and 25.8% (w/w, DCW), respectively, after 48 h and 36 h, respectively. This indicated that the cell growth and lipid accumulation of *Rh. sphaerocarpa* 11-14.4 and *Sait. podzolica* 11-11.3.1 could occur separately. This characteristic makes the time to reach the maximum lipid content level shorter. Likewise, the cell propagation and lipid accumulation of *Lipomyces starkeyi* AS 2.1560 has also been reported to be temporally separated (Lin et al. 2011).

Conclusions

Ascomycetous yeasts (thirty-two species in sixteen genera), Basidomycetous yeasts (twelve species in nine genera), and 13 potential new species were distributed in the 8 mangrove forests across 4 different coastlines of Thailand. Occurrence frequency of oleaginous yeast was 3.82% (two out of one hundred ninety one yeast isolates). Oil of the *Rhodotorula sphaerocarpa* 11-14.4 had oleic acid and palmitic acid as major fatty acids and so it was suitable to be raw material for biodiesel and biolubricant productions. Oil of the *Saitozyma podzolica* 11-11.3.1 was similar to cocoa butter used in food industry in that it contained high saturated fatty acids.

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