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Short Communication: The oleaginous *Candida tropicalis* isolated from mangrove soil in eastern Thailand and the fatty acid composition profile of its intracellular lipids

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Abstract. *Wongchamrearn S, Boontanom P, Ungwiwatkul S, Emnin N, Chantarasiri A. 2023. Short Communication: The oleaginous* Candida tropicalis *isolated from mangrove soil in eastern Thailand and the fatty acid composition profile of its intracellular lipids. Biodiversitas 24: 5088-5095.* Oleaginous yeasts are microorganisms that can accumulate intracellular lipids at a rate of more than 20% of their cell dry weight. They are considered ideal microbes for a sustainable bioeconomy and a promising source for biotechnological applications. Thus, the exploration of efficient oleaginous yeasts from various environments is necessitated. At present, the number of oleaginous yeasts that have been discovered from mangrove environments remains scarce. Therefore, this study isolated and screened for lipid-accumulating yeast strains in mangrove soil samples collected from Rayong Province and Chonburi Province, Thailand. The intracellular lipids of isolated yeasts were extracted and quantitatively analyzed to evaluate the efficient oleaginous strains. Fifteen yeast strains were considered lipid-accumulating yeasts based on the Sudan IV staining method and lipid content determination, with intracellular lipid accumulation ranging from 5.71±1.89% (w/w) to 33.83±1.41% (w/w). The isolated yeast strain MJ13 was designated the most efficient oleaginous strain and subsequently genetically identified as *Candida tropicalis*. The fatty acid composition of its intracellular lipids was analyzed by gas chromatography, which consisted of five saturated fatty acids and five unsaturated fatty acids with 10-22 carbon atoms. The predominant fatty acids were palmitic (C16:0) and stearic (C18:0) acids. Some fatty acids essential to the human body and pharmacological sciences were detected, involving α-linolenic (C18:3n3) and cis-13,16-docosadienoic (C22:2) acids. This oleaginous yeast strain could be applied in lipid-related biofuel and high-value bioeconomy applications.

Keywords: *Candida tropicalis*, mangrove soil, oleaginous yeast, Sudan IV staining method, Thailand

INTRODUCTION

Mangrove forests are coastal intertidal wetland ecosystems composed of halophytic plant species and coastal fauna, with many essential ecosystem services such as carbon storage, coastal protection, fish and aquatic food resources, fuelwood and timber, nutrient cycling, pollution control, and cultural value for local populations (Nguyen 2014; Friess 2016; Friess et al. 2019; Chantarasiri 2021). Several recent studies reported that Southeast Asia is the region with the highest proportion and biodiversity of mangrove forests in the world (Friess 2016; Gerona-Daga and Salmo 2022), accounting for the world's largest area covering 43,767 km^2 (32.2%) and the most diverse mangrove forests (Gerona-Daga and Salmo 2022). The mangrove habitats contain abundant and characteristic microbial resources, which make mangrove ecosystems the hotspots for microbial diversity (Liu et al. 2019). Mangrove forests are extremely rich in yeast species and have been reported to be interesting sampling sites for various novel yeast species (Kunthiphun et al. 2018; Hoondee et al. 2019). Yeast species play a role in the detrital food chain in mangrove ecosystems that are rich in degrading plant matter and a food source for some marine invertebrates and zooplankton (Hoondee et al. 2019). Some mangrove yeasts can accumulate intracellular lipid droplets or single-cell oils (SCOs), characterized as oleaginous yeasts. Oleaginous yeasts can accumulate over 20% of their cell dry weight in intracellular lipids or triacyl glycerides (López et al. 2022).

Today, only 11% of the 1,600 known yeast species have been classified as oleaginous species (Abeln and Chuck 2021; Sapsirisuk et al. 2022). Oleaginous yeast genera have commonly been isolated from different environments, such as fruit products, common surfaces, and soil (Vincent et al. 2018). The soil is considered a reservoir for yeasts in underground environments (Sapsirisuk et al. 2022). They belong to various genera within *Candida*, *Cryptococcus*, *Kluyveromyces*, *Lipomyces*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, and *Yarrowia* (Dey and Maiti 2013; Jiru et al. 2016; Gientka et al. 2017; Patel et al. 2017; Qin et al. 2017; Ayadi et al. 2018; Lopes et al. 2018; Hoondee et al. 2019). The genera *Prototheca*, *Pseudozyma*, *Rhodotorula*, and *Saitozyma* are the oleaginous species typically found in mangrove samples (Kunthiphun et al. 2018; Hoondee et al. 2019). Notably, only some studies have reported on the oleaginous yeasts isolated from mangrove soils in the Southeast Asia region.

The intracellular lipids of oleaginous yeasts have been studied as a promising feedstock for biodiesel production, as well as human and animal nutrition, oleochemical building block synthesis, and several oil-related biotechnological applications because of their fatty acid compositions, which is similar to vegetable oils (Patel et al. 2017; Ayadi et al. 2018; Abeln and Chuck 2021; Sapsirisuk et al. 2022). The intracellular lipids are nonpolar, primarily C13 to C21 triacylglycerols (TAGs) and steryl esters (SEs) (Abeln and Chuck 2021; Poontawee et al. 2023). The predominant fatty acids in intracellular lipids are myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids (Hoondee et al. 2019). Therefore, they offer alternatives to vegetable oil-based applications without competition for food resources (Sreeharsha and Mohan 2020). Oleaginous yeasts seem to be the most suitable oleaginous microorganisms for lipid production due to their rapid growth, high lipid content, and volumetric productivity (Sawangkeaw and Ngamprasertsith 2013; Abeln and Chuck 2021). Various low-cost substrates can be utilized for their growth and lipid production (Poontawee et al. 2023). Thus, oleaginous yeasts are now used for waste management (Sreeharsha and Mohan 2020).

The key hindrance to the prospects for oleaginous yeasts is discovering novel, effective species from various environments. Moreover, this study aimed to isolate, screen, and genetically identify the oleaginous yeasts from mangrove soil samples in eastern Thailand. Ultimately, the intracellular lipids extracted from the most effective oleaginous yeast were identified by gas chromatography. The isolated oleaginous yeast and its intracellular lipids were expected to be a feedstock for bio-oil-related applications in the future.

MATERIALS AND METHODS

Study area

The study areas included the Phra Chedi Klang Nam Mangrove Forest (12°39'55.5" N, 101°14'50.1" E) in Mueang Rayong district, Rayong Province and the Khao Ma Cho Mangrove Forest (12°36'8.7" N, 100°57'1.7" E) in Sattahip district, Chonburi Province. These provinces are located in the eastern region of Thailand. The Phra Chedi Klang Nam Mangrove Forest is a riverine mangrove forest of the Rayong River with an estuary that connects to the Gulf of Thailand. The Khao Ma Cho Mangrove Forest is a small fringe mangrove forest along the coast of the Gulf of Thailand. The ecological characteristics of the study areas are described in Bamrungpanichtavorn et al. (2023) and Bulan et al. (2018), respectively. The scenery in the study areas is shown in Figure 1.

Procedures

Soil sample collection and yeast isolation

Thirty-two soil samples were collected from the study areas in August 2020. The soil temperatures were determined by a needle probe thermometer (Extech Instruments, USA) at the sampling sites. The pH and salinity values were determined from 1 g of soil sample suspended in 10 mL of distilled water by an Ohaus ST20 Starter Pen Meter (Ohaus, USA). All the soil samples were kept at a temperature of 4°C in sterilized plastic containers and taken to the laboratory for yeast isolation within 24 h of collection.

Next, 1 gram of each soil sample was suspended in 10 mL of sterilized deionized water and serially 10-fold diluted to obtain 1:1,000,000 dilutions. Afterward, 100 µL of the diluted sample was spread-plated on Dichloran Rose-Bengal Chloramphenicol (DRBC) agar (HiMedia, India). All agar plates were incubated at 29°C (the average soil temperature) in an incubator cabinet for 72 h. The yeast isolates were selected based on their dissimilar colony morphology and then colony purified by the streak plate method on Yeast Malt (YM) agar (HiMedia, India). Each pure isolate from the Phra Chedi Klang Nam Mangrove Forest was given the code PN, while the isolated yeast from the Khao Ma Cho Mangrove Forest was given the code MJ. All culture media's pH and salinity values were adjusted to 7.25 (the average pH of the collected soil samples) and 0.22 ppt (the average salinity of the collected soil samples), respectively.

Figure 1. Scenery in the study areas includes: A. The Phra Chedi Klang Nam Mangrove Forest in Rayong Province and B. The Khao Ma Cho Mangrove Forest in Chonburi Province

Accumulation and extraction of intracellular lipids from the isolated yeasts

All isolated yeasts were pre-cultured in YM broth (HiMedia, India) at 29°C for 48 h and subsequently inoculated in a Glycerol Yeast Peptone (GYP) medium for accumulation of intracellular lipids. The composition of the GYP medium was mentioned previously in a study by Planonth and Chantarasiri (2022). The GYP medium contained 4% (w/v) of glycerol, 1% (w/v) of yeast extract, 1% of peptone (w/v), and distilled water. Next, 10% (v/v) of each pre-cultured yeast was inoculated in GYP medium and incubated at 29°C for 120 h with orbital shaking at 150 rpm. As mentioned previously, the pH and salinity values of the culture media were adjusted to 7.25 and 0.22 ppt.

The Sudan IV staining method determined the isolated yeasts for their intracellular lipid accumulation (Vincent et al. 2018). Next, 300 µL of each yeast culture was mixed with 300 µL of Sudan IV solution (Sigma-Aldrich, Germany). The mixture was incubated under dark conditions at room temperature for 1 h. The stained yeast cells were observed under an ECLIPSE E200 light microscope (Nikon, Japan) for intense red-colored intracellular lipid droplets within the cells.

The centrifugation at 3,500 × g for 15 minutes was used to harvest yeast cells and washed three times with 0.85% (w/v) of sterilized NaCl to remove the residual culture medium. The harvested cells were dried to a constant weight in a hot-air oven at 105°C and then weighed. The extraction of intracellular lipids from dried yeasts was conducted according to the methodology of Planonth and Chantarasiri (2022). One gram of dried yeast was suspended in six milliliters of *n*-hexane, then sonicated by a VCX 130PB Vibra-Cell ultrasonic liquid processor (Sonics, USA) with 90% amplitude for 10 min at room temperature. The sonicated mixture was centrifuged at 3,500 × g for 15 min. The supernatant was harvested and dried by a Hei-VAP rotary evaporator (Heidolph Instruments, Germany) at 69°C to obtain the extracted intracellular lipids. Any yeast isolates capable of intracellular lipids over 20% (w/w) on their dry cell weight were defined as oleaginous yeasts. The positive control strain was a known oleaginous yeast, *Yarrowia lipolytica* strain TISTR 5212 (Thailand Institute of Scientific and Technological Research, Thailand). The negative control strain was a baker yeast, *Saccharomyces cerevisiae*. All experiments were performed in triplicate.

Genetic identification of the isolated oleaginous yeast

Yeast genomic DNA was extracted and purified by a genomic DNA isolation kit (Bio-Helix, Taiwan) according to the manufacturer's standard protocol. The extracted genomic DNA was used as the polymerase chain reaction (PCR) DNA template for the internal transcribed spacer (ITS) regions. Amplification was done using a OnePCR reaction mixture (Bio-Helix, Taiwan) with the universal ITS1 and ITS4 primers. Thermal cycling was performed for 35 amplification cycles in an Eppendorf Mastercycler nexus gradient (Eppendorf, Germany). The PCR cycling conditions were: initialization at 95°C for 5 min, then denaturation at 95°C for 1 min 30 s, and annealing at 55°C for 2 min, as well as an extension at 72°C for 3 min and then a final extension at 72°C for 5 min. Next, the PCR products were electrophoresed on a 1.5% (w/v) OmniPur agarose gel (Calbiochem, Germany) and subsequently visualized by Novel Juice (Bio-Helix, Taiwan).

The PCR products were nucleotide sequenced using the services of Macrogen Inc. (South Korea). The nucleotide sequences were aligned for genetic identification by the BlastN program based on the experimental taxonomic nt databases (Eukaryota nt databases) of the National Center for Biotechnology Information (NCBI). The phylogenetic tree of the isolated oleaginous yeast was generated using Seaview software version 5.0.1 (Gouy 2010) and FigTree software version 1.4.4 (http://tree.bio.ed.ac.uk) with the BIONJ algorithm for 100,000 bootstraps. The resulting ITS regions of the isolated oleaginous yeast were deposited in the GenBank database of the NCBI under accession number MW786656.

Fatty acid composition profile of the intracellular lipids from oleaginous Candida tropicalis *strain MJ13*

Candida tropicalis strain MJ13 was designated as the highest lipid-accumulating yeast in this study. It was cultured in the GYP medium, cell harvested, and extracted to obtain the previously-mentioned intracellular lipids. The gas chromatography (GC) was used to determine the fatty acid composition of intracellular lipids. The extracted intracellular lipids were converted into a mixture of fatty acid methyl esters (FAMEs) by transesterification following the method of Lin and Lin (2017). The resultant FAMEs were analyzed by a gas chromatograph 7890A (Agilent, USA) equipped with an SP-2560 capillary column (Sigma-Aldrich, USA) and a flame-ionized detector (FID) system. The GC conditions were conducted according to the methodology of Leesing and Nontaso (2011).

Data analysis

The statistical analyses were performed using R software version 4.2.1 (R Foundation for Statistical Computing, Austria) in this study. The multiple comparison analyses were determined by one-way ANOVA and then followed by Tukey's test with a 95% confidence interval ($p < 0.05$).

RESULTS AND DISCUSSION

Characteristics of the collected soil samples

Furthermore, 32 soil samples were collected from the Phra Chedi Klang Nam Mangrove Forest in Rayong Province and the Khao Ma Cho Mangrove Forest in Chonburi Province. The characteristics of the samples were measured involving temperatures, pH values, and salinity values. The average soil temperature measured at the sampling sites was 29.30±0.59°C. The average soil pH and salinity values were 7.25±0.52 and 0.22±0.03 ppt, respectively. This study used all soil characteristics as the growth conditions for yeast cultivation.

Isolation of yeast from the soil samples

The mangrove yeasts were subsequently isolated from collected soil samples by DRBC agar and colony purified using YM agar. The result revealed that 15 yeast isolates with dissimilar colony morphology were isolated and colony purified from the collected soil samples. Several soil samples could not isolate any yeast colonies. These isolated yeasts were obtained from 10 isolates from the Phra Chedi Klang Nam Mangrove Forest (with the code name PN) and 5 isolates from the Khao Ma Cho Mangrove Forest (with the code name MJ). All isolated yeasts had a similar pattern of colony morphology involving opaque, circular shape, entire margin, and convex elevation. Apart from that, their morphology differed mainly in terms of the colony pigmentation and diameter of the colony. The colonies were cream and white pigmentation, with diameters ranged from 1.07 to 2.36 mm after being cultured on YM agar for 72 h.

Accumulation and extraction of intracellular lipids from the isolated yeasts

All mangrove yeast isolates were cultured in the GYP medium for intracellular lipid accumulation. This culture medium was defined as a glucose-rich and nitrogen-limiting medium that promotes lipid accumulation as droplets within the cells (Planonth and Chantarasiri 2022). The determination of intracellular lipids from the isolated yeasts by the Sudan IV staining method revealed that all 15 isolated yeasts were Sudan IV positive. Under microscopic observation, the isolated yeasts had a similar shape and size to the individual cells. The shapes were oval to ellipsoidal with sizes of 2-3 µm in width and 4-6 µm in length. They were shown to harbor noticeable intracellular lipid droplets. The stained lipid droplets appeared as intense red-colored bodies within the yeast cells. The Sudan IV-stained lipid droplets within the cells of the isolated yeasts are shown in Figure 2. The positive control was *Y. lipolytica* strain TISTR 5212, and the negative control was *S. cerevisiae*.

All yeast isolates were harvested, dried, and extracted to obtain intracellular lipids. They could accumulate intracellular lipids ranging from 5.71±1.89% (w/w) (isolate PN07) to 33.83±1.41% (w/w) (isolate MJ13). Only isolate MJ13 could accumulate intracellular lipids greater than 20% (w/w) of their dry biomass. Therefore, it was considered an oleaginous yeast and was selected for further study. Interestingly, its intracellular lipid content slightly differed from a well-known oleaginous yeast, *Y. lipolytica* strain TISTR 5212. The intracellular lipid contents of the fifteen isolated yeasts are shown in Table 1.

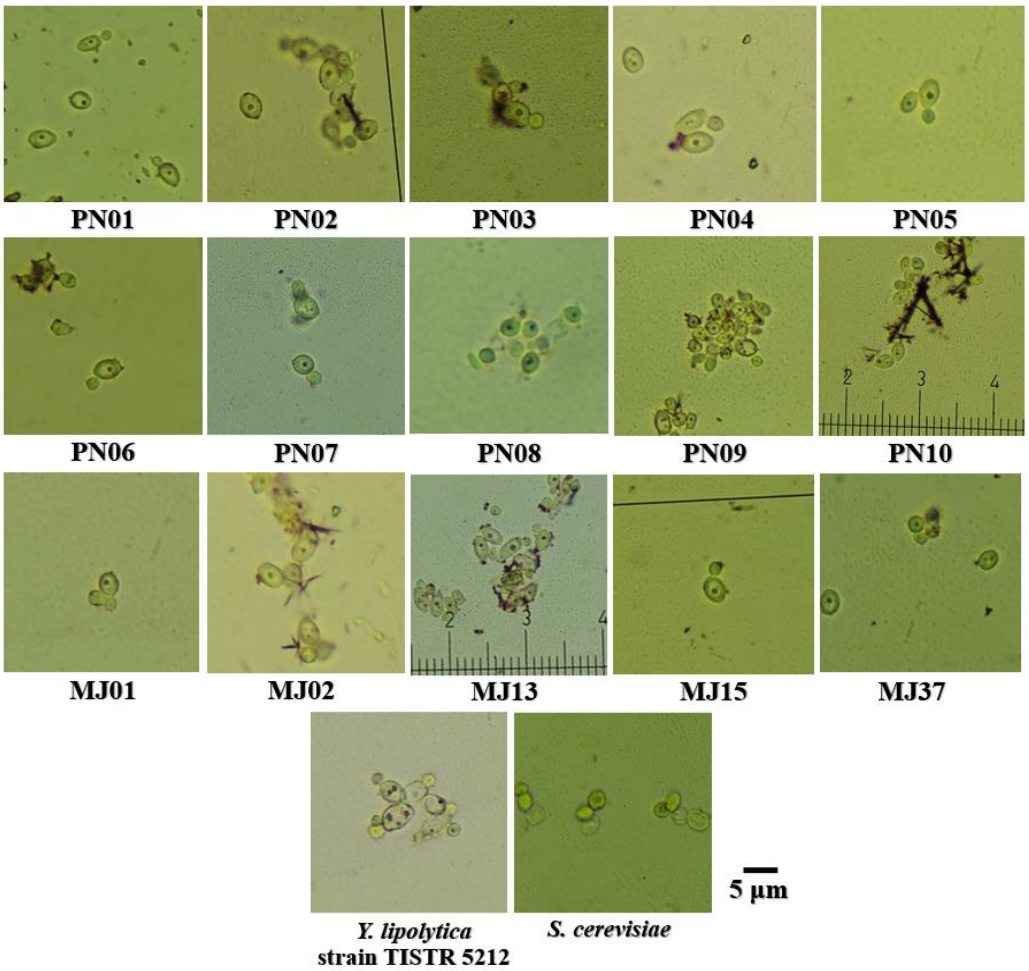

Figure 2. Microscopic morphologies and Sudan IV-stained lipid droplets within the cells of the fifteen isolated yeast samples. The control strains were *Yarrowia lipolytica* strain TISTR 5212 (positive control) and *Saccharomyces cerevisiae* (negative control)

Genetic identification of the isolated oleaginous yeast

Yeast isolate MJ13 was considered oleaginous according to the results of the previous experiment. It was extracted for genomic DNA, and then PCR amplified for ITS regions. The PCR products were purified, nucleotide sequenced, and nucleotide aligned for genetic identification. The alignment results from the BlastN program based on the Eukaryota nt databases of the NCBI revealed that the isolate MJ13 was closely related to *C. tropicalis* isolate SY1-2 clone SY1-2A (accession number KY963098.1) with a 94% query coverage and 96.72% identity. The resulting E value obtained from the nucleotide alignment was zero. The circular phylogenetic tree was generated by Seaview software with the BIONJ algorithm for 100,000 bootstraps. It demonstrated that the isolate MJ13 was phylogenetically grouped in the clade of *C. tropicalis* with a bootstrap value of 100. The bootstrap values among representative *C. tropicalis* strains downloaded from GenBank in the clade were ranged from 64-80. Therefore, yeast isolate MJ13 was designated as *C. tropicalis* strain MJ13. The resulting ITS nucleotide sequence was deposited in the GenBank database of the NCBI under accession number MW786656. The phylogenetic tree is shown in Figure 3.

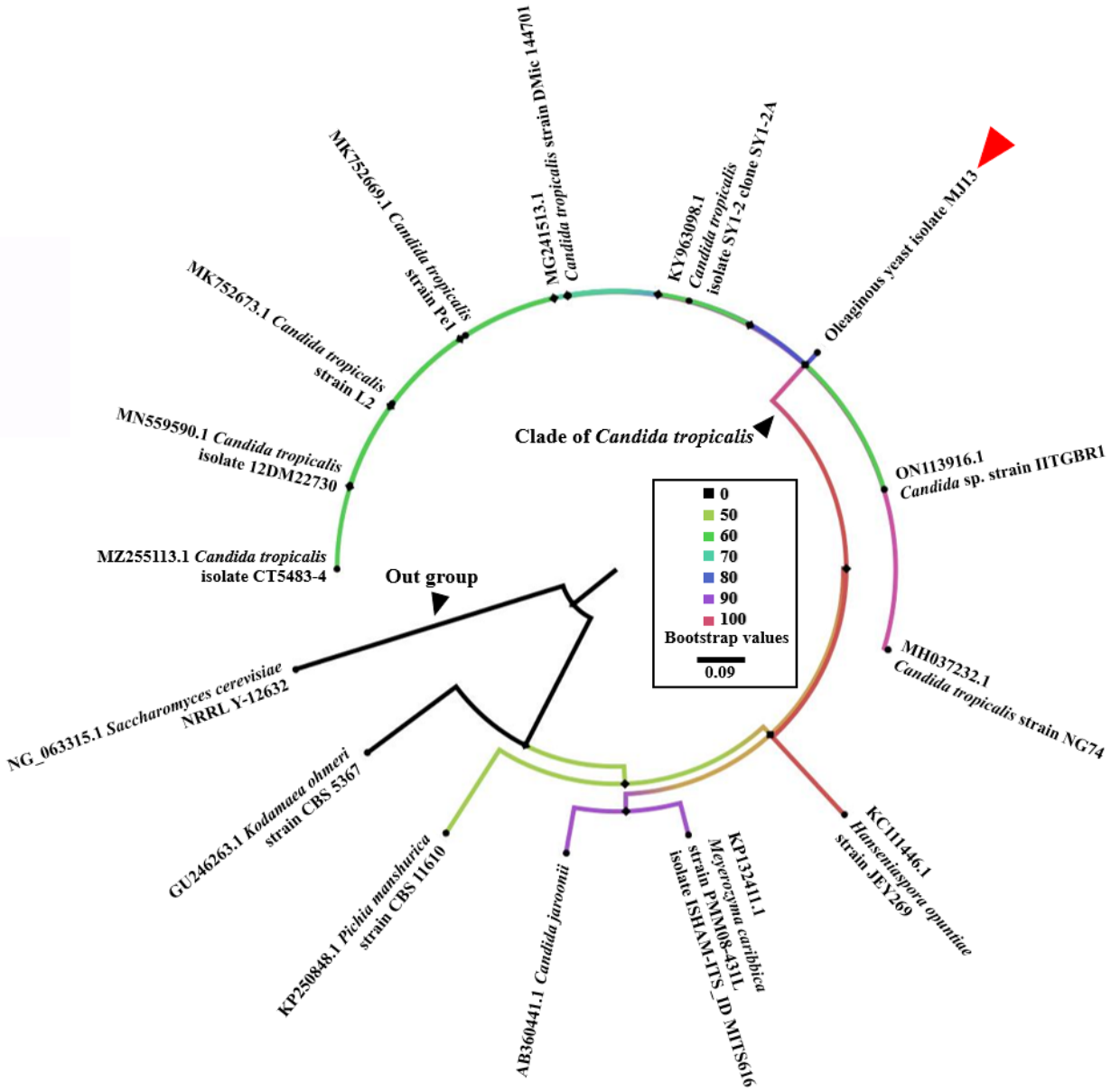

Figure 3. Circular phylogenetic tree of the oleaginous yeast isolate MJ13 shown using the BIONJ algorithm for 100,000 bootstraps. The phylogenetic tree was analyzed by Seaview and FigTree software

Fatty acid composition profile of the intracellular lipid from oleaginous *C. tropicalis* strain MJ13

Candida tropicalis strain MJ13 was declared oleaginous yeast with an intracellular lipid content of 33.83% (w/w) (Tabel 1). The extracted intracellular lipids were chemically converted into FAMEs and analyzed for fatty acid composition by the GC-FID. The resulting FAME profile from GC-FID analysis showed that the intracellular lipids comprised five saturated fatty acids and five unsaturated fatty acids. The saturated fatty acids included C10, C12, C14, C16, and C18-fatty acids, while the unsaturated fatty acids included C14:1, C18:1n9c, C18:2n6c, C18:3n3, and C22:2-fatty acids. The *C. tropicalis* strain MJ13 mainly contained saturated long-chain fatty acids such as stearic acid (C18:0) and palmitic acid (C16:0) at 25.672% and 36.699%, respectively. The GC-FID chromatogram and FAME profile for the oleaginous *C. tropicalis* strain MJ13 are shown in Figure 4 and Table 2, respectively.

Table 1. Intracellular lipid accumulation of fifteen isolated mangrove yeasts and two control yeasts

Yeast strains	Intracellular lipid content (%, w/w)
PN01	12.03±2.21[a]
PN02	10.63±0.45[ac]
PN03	9.45±0.87[abc]
PN04	10.15±1.28[ac]
PN05	9.20±2.27[abc]
PN06	11.71±1.83[a]
PN07	5.71±1.89[be]
PN08	8.26±2.03[abc]
PN09	11.55±0.88[a]
PN10	6.40±0.32[ce]
MJ01	12.22±0.77[a]
MJ02	9.24±0.97[abc]
MJ13	33.83±1.41[d]
MJ15	8.97±0.43[abc]
MJ37	9.75±0.17[abc]
Y. lipolytica strain TISTR 5212 (positive control)	31.71±2.55[d]
S. cerevisiae (negative control)	3.25±0.45[e]

Note: The mean values followed by the same letter were not significantly different according to Tukey's test ($p < 0.05$) among the yeast strains. All experiments were performed in triplicate

Table 2. Fatty acid methyl ester (FAME) profile of intracellular lipids extracted from *Candida tropicalis* strain MJ13

FAME profile	Retention time (minutes)	Peak area (pA*s)	Percentage of FAMEs in intracellular lipids (%) of *C. tropicalis* strain MJ13
Capric AME (C10:0)	19.040	25.789	2.157
Lauric AME (C12:0)	20.936	12.319	0.970
Myristic AME (C14:0)	23.366	89.984	7.127
Myristoleic AME (C14:1)	25.131	5.354	0.423
Palmitic AME (C16:0)	27.211	437.355	36.699
Stearic AME (C18:0)	33.159	258.429	25.672
Oleic AME (C18:1n9c)	35.612	74.348	6.959
Linoleic AME (C18:2n6c)	40.124	105.628	10.181
α-Linolenic AME (C18:3n3)	44.726	71.184	7.938
Cis-13,16-docosadienoic AME (C22:2)	53.970	10.389	1.874
Total			100.00

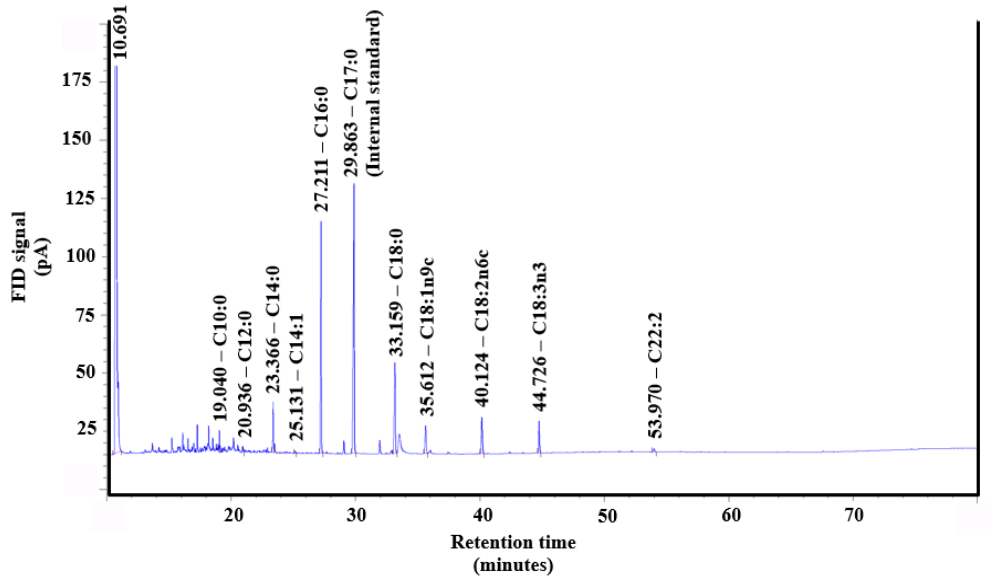

Figure 4. GC-FID chromatogram of fatty acid composition in the intracellular lipids extracted from *Candida tropicalis* strain MJ13. Heptadecanoic AME (C17:0) was the internal standard used in this experiment

Discussion

Only a few mangrove oleaginous yeast species have been isolated and reported on their fatty acid compositions. A recent study reported that the mangrove forests have been a source of several novel yeast species (Kunthiphun et al. 2018). Thailand is one of 20 mangrove-holding nations with 1,876 km^2 based on the Landsat-based mangrove database (Hamilton and Casey 2016). Therefore, it is an interesting sampling source for isolating novel oleaginous yeast strains. For the first time, this study reported on the isolation, screening, and genetic identification of effective oleaginous yeast from mangrove soil in eastern Thailand (Rayong and Chonburi Provinces). In addition, the results showed that the mangrove *C. tropicalis* yeast strain MJ13 was considered the most effective oleaginous yeast with the accumulation of intracellular lipids at 33.83% (w/w) in a GYP medium. Its intracellular lipid content resembled a well-known oleaginous yeast, *Y. lipolytica.* Only 5% of oleaginous yeasts have been reported to accumulate intracellular lipids, more than 25% (w/w) (Ageitos et al. 2011; Hoondee et al. 2019). Thus, the isolated *C. tropicalis* strain MJ13 is believed to be an attractive strain for further applications.

The *C. tropicalis* has been identified as a pathogenic yeast species of the candida-non-albicans (CNA) group (Kothavade et al. 2010). It has been considered an osmotolerant yeast that can survive in high salt concentrations (Zuza-Alves et al. 2017). Moreover, it utilizes various carbon sources to produce appreciable biomass with high lipid yields (Mishra et al. 2016). This characteristic explains the potential use of *C. tropicalis* in biotechnological processes (Zuza-Alves et al. 2017). Two previous studies revealed that *C. tropicalis* was the most prevalent species found in mangrove soil samples from six provinces in Thailand (Chantaburi, Prachuap Khiri Khan, Phetchaburi, Ranong, Samut Songkhram, and Trat) (Kunthiphun et al. 2018; Hoondee et al. 2019). However, the isolated *C. tropicalis* yeasts from these studies were not considered oleaginous (Kunthiphun et al. 2018; Hoondee et al. 2019). A recent study reported that *C. tropicalis* strain ATCC 750 was a promising oleaginous yeast for olive mill wastewater (OMW) bioconversion in Portugal (Dias et al. 2021). According to the results of a study on the isolation of oleaginous yeast strains from sago processing wastewater (SWW) in India, it was found that *C. tropicalis* strain ASY2 was designated as an effective oleaginous yeast (Thangavelu et al. 2020). Genome-scale metabolic modeling and *in silico* analysis are used to understand and improve the characteristics of oleaginous yeasts. There have been attempts to reconstruct the genome-scale metabolic model of *C. tropicalis* to investigate lipid accumulation characteristics (Mishra et al. 2016).

The major fatty acids of *C. tropicalis* strain MJ13 were palmitic acid (C16:0) at 36.699% and stearic acid (C18:0) at 25.672%. Other fatty acids were detected at lower concentrations. Its fatty acid profile was closely resembled with a previous study on the soil *C. tropicalis* that predominantly contained palmitic and stearic acids (Dey and Maiti 2013). Palmitic and stearic acids are commonly found in vegetable oils (Giakoumis 2018) and have been recommended for biodiesel production (Areesirisuk et al. 2015). Interestingly, many high-value polyunsaturated fatty acids have been identified in the intracellular lipids of *C. tropicalis* strain MJ13, such as linoleic (C18:2n6c), α-linolenic (C18:3n3), and cis-13,16-docosadienoic (C22:2) acids. Linoleic acid is a precursor of bioactive oxidized linoleic acid metabolites and arachidonic acid (Choque et al. 2014). Arachidonic acid is a precursor of pro-inflammatory eicosanoids and endocannabinoids (Choque et al. 2014). Noticeably, *C. tropicalis* strain MJ13 accumulated this fatty acid at 10.181%. The α-linolenic acid has a wide range of metabolic and pharmacological effects. The human body cannot synthesize it; it can only be acquired from food and common edible oils (Yuan et al. 2021). The results showed that *C. tropicalis* strain MJ13 yielded α-linolenic acid at 7.938%. It was more than various reported common edible oils (corn, olive, and sunflower seed oils) with α-linolenic acid content by 1.0% (Yuan et al. 2021). Cis-13,16-docosadienoic acid was detected in the intracellular lipid of *C. tropicalis* strain MJ13 by 1.874%. This fatty acid could reduce the severity of inflammation in Alzheimer's disease (AD) as part of anti-inflammatory mechanisms (Nasaruddin et al. 2016).

In conclusion, mangrove forests in eastern Thailand are a potential source for isolating efficient oleaginous yeasts. This study successfully isolated, screened, and laboratory-cultured 15 lipid-accumulating yeasts from Rayong and Chonburi Provinces mangrove soil samples. The lipid-accumulating yeast isolate MJ13 was considered oleaginous with an intracellular lipid content of 33.83% (w/w). It was genetically identified and phylogenetically analyzed as *C. tropicalis* strain MJ13. The extracted intracellular lipids were then GC-analyzed to obtain the fatty acid profile. The intracellular lipids of *C. tropicalis* strain MJ13 consisted of palmitic, stearic, and several high-value polyunsaturated fatty acids; they are essential and precursor fatty acids in various bioeconomy and biotechnological applications. Further studies could be conducted to optimize the culture conditions to increase intracellular lipid production and investigate the culture conditions using low-cost raw waste materials.

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