

Species identification of stranded seaweeds on eastern seashores of Thailand and utilization as a sole carbon source for single cell oils synthesized by oleaginous yeasts

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Abstract. Chantarasiri A. 2020. *Species identification of stranded seaweeds on eastern seashores of Thailand and utilization as a sole carbon source for single-cell oils synthesized by oleaginous yeasts.* Biodiversitas 21: 2353-2361. Many stranded seaweeds are left to decompose on the seashore. This study aimed to identify and utilize the stranded seaweeds collected from seashores in Rayong Province on the east coast of Thailand. Seaweeds were identified as *Dictyota dichotoma* strain A and *Padina australis* strain B based on their distinctive morphology and 18S rDNA sequence analyses. The most abundant seaweed, *D. dichotoma* strain A, was analyzed for chemical compositions following AOAC standard methods. This seaweed was considered a low-cost carbon source for the cultivation of three oleaginous yeasts without any other nutrient supplements. All oleaginous yeasts utilized the carbon source from *Dictyota* medium for growth and accumulation of single-cell oils (SCOs). The oleaginous yeast, *Cryptococcus albidus* strain TISTR 5103 accumulated SCOs at 51% (w/w). The resulting SCOs from oleaginous yeasts comprise many fatty acids with 10 to 22 carbon atoms. This study is the first to identify stranded seaweeds in Rayong Province using morphological and genetic techniques. Results showed the potential application of stranded seaweeds as a low-cost carbon source for SCO biosynthesis of oleaginous yeasts.

Keywords: *Cryptococcus albidus*, *Dictyota dichotoma*, oleaginous yeast, seaweed, single cell oil

INTRODUCTION

Seaweeds or marine macroalgae are autotrophic organisms consisting of a wide variety of species that grow in marine environments. Seaweeds can be split into three taxonomic groups that have different pigment compositions as Chlorophyta (green algae), Rhodophyta (red algae), and Ochrophyta (brown algae) (Mantri et al. 2020). Seaweeds are commonly valued as animal feed, human food and also used in industrial applications such as biofuels, chemicals, cosmetics, fertilizers, gels and stabilizers, papers, and medicines (Buschmann et al. 2017; Kim et al. 2017). Five genera of seaweeds are globally cultivated including *Saccharina* (brown alga), *Undaria* (brown alga), *Porphyra* (red alga), *Euclima/Kappaphycus* (red alga) and *Gracilaria* (red alga) (Buschmann et al. 2017). In their natural environment, seaweeds are usually attached to rocks and reefs with their holdfast structure. Strong storms, herbivores, and ocean waves cause seaweeds to detach and some species with high buoyancy remain afloat (López et al. 2018). Detached floating seaweeds are eventually washed up on seashores. Stranded seaweeds that are considered as edible may be harvested for domestic purposes, while the remainder negatively impacts coastal ecosystems due to their foul-smelling decay processes.

Thailand is situated at the center of the Indochinese Peninsula, bordered to the east and south by the Gulf of Thailand and to the west and south by the Andaman Sea. Seaweed beds are distributed widely along the Thai coastline as one of the country's marine fishery resources

(Petsut et al. 2012). Studies of seaweeds in Thailand and the Indo-Pacific region have examined the cultivation and nutrition of some edible seaweeds such as *Gracilaria* and *Caulerpa* (green alga) (Prathep et al. 2011; Gaillande et al. 2017). Previous investigations have focused on biodiversity hotspots along the Gulf of Thailand and the Andaman Sea in Southern Thailand (Prathep et al. 2011; Chankaew et al. 2016; Seangkaew et al. 2016), with scant research on non-edible and low-value stranded seaweeds in Eastern Thailand.

Seaweeds can be used as a potential feedstock for many biorefineries. The carbohydrate content of seaweeds can be converted into high-value organic chemicals and also utilized as an organic carbon source for lipid bioproduction by oleaginous yeasts. These biosynthesized lipids can be converted into biodiesel by the transesterification process (Sadhukhan et al. 2019). Oleaginous yeasts comprise a small group of microorganisms that can utilize organic carbon and accumulate large amounts of oil droplets, mainly in the form of triacylglycerol, in their cellular compartments (Fakas 2017; Patel et al. 2017). Species that are considered as oleaginous are able to accumulate more than 20% of their dry biomass as intracellular lipids, and biosynthesis of microbiological lipids is defined as single-cell oils (SCOs) (Gientka et al. 2017). Effective oleaginous yeasts belong to the genera *Candida*, *Cryptococcus*, *Lipomyces*, *Rhodospiridium*, *Rhodotorula*, *Trichosporon*, and *Yarrowia* (Patel et al. 2017; Qin et al. 2017). Several seaweed species have been studied as potential feedstock for SCO production by various oleaginous yeasts such as

Laminaria japonica (*S. japonica*) and *S. latissimi* (Xu et al. 2015; Zhang et al. 2016; Abeln et al. 2019). However, previous studies have only focused on edible high-value seaweeds in genera of *Laminaria* (*Saccharina*). The utilization of non-edible stranded seaweeds as a low-cost feedstock in biorefinery applications is challenging.

Here, seaweeds that detached from their beds and washed up on the shores of Rayong Province in Eastern Thailand were collected and identified. The Rayong Province seashore is ecologically important, but knowledge of seaweed diversity remains limited. The most abundant seaweed, *Dictyota dichotoma* strain A, was considered a non-edible seaweed. The seaweed composition was analyzed and used as the sole carbon source for the cultivation of three different oleaginous yeasts with no added nutrient supplements. Contents and fatty acid composition of the SCO produced from the oleaginous yeasts were determined.

MATERIALS AND METHODS

Study area

The study area was located at the adjoining seashores of two Districts in Rayong Province in the east of Thailand. The study area was located at the adjoining seashores of two Districts in Rayong Province in the east of Thailand comprising Hadd Nam Rin Beach (12° 40' 31" N, 101° 5' 32" E) in Banchang District and Haad Son Krasip Beach in Mueang Rayong District (12° 40' 42" N, 101° 5' 59" E). The beaches are not in the marine reserve area of the Gulf

of Thailand, and nearby Map Ta Phut Industrial Port (Figure 1). The shores comprise sandy beaches alternated with a few rocky reefs. Coastal plants are predominantly beach morning glory (*Ipomoea pes-caprae*), ironwood horsetail (*Casuarina junghuhniana*), and sea almond (*Terminalia catappa*). Some areas of the study seashores have been converted by the local communities into street stalls.

Procedures

Sampling of seaweeds

The stranded seaweeds were randomly collected from the study area during the winter season in February 2018. Strong winds and ocean waves during winter often detach the seaweeds from their beds. Samples were taken along the coastlines of Hadd Nam Rin Beach and Haad Son Krasip Beach every 100 meters using a sampling quadrat (50 × 50 cm). The sampling distance was two kilometers as the length of the two beaches. The stranded seaweeds lying on the study shores are shown in Figure 2. The collected seaweeds were rinsed with water and stored in sterilized plastic bags. They were primarily categorized and identified by their dominant morphology and pigmentation based on seaweed pictures in the reference book, *Seaweeds and Seagrasses of the Southern Andaman Sea coast of Thailand* (Coppejans et al. 2017). Seaweed blades and stipes were cleaned using sterilized water several times to remove epiphytes, fauna, sand particles, and plastic debris. They were then sun-dried for 72 hours before drying in an oven at 60°C for 48 hours. Dried seaweeds were weighed and comminuted to fine powder of 500 µm particle size.

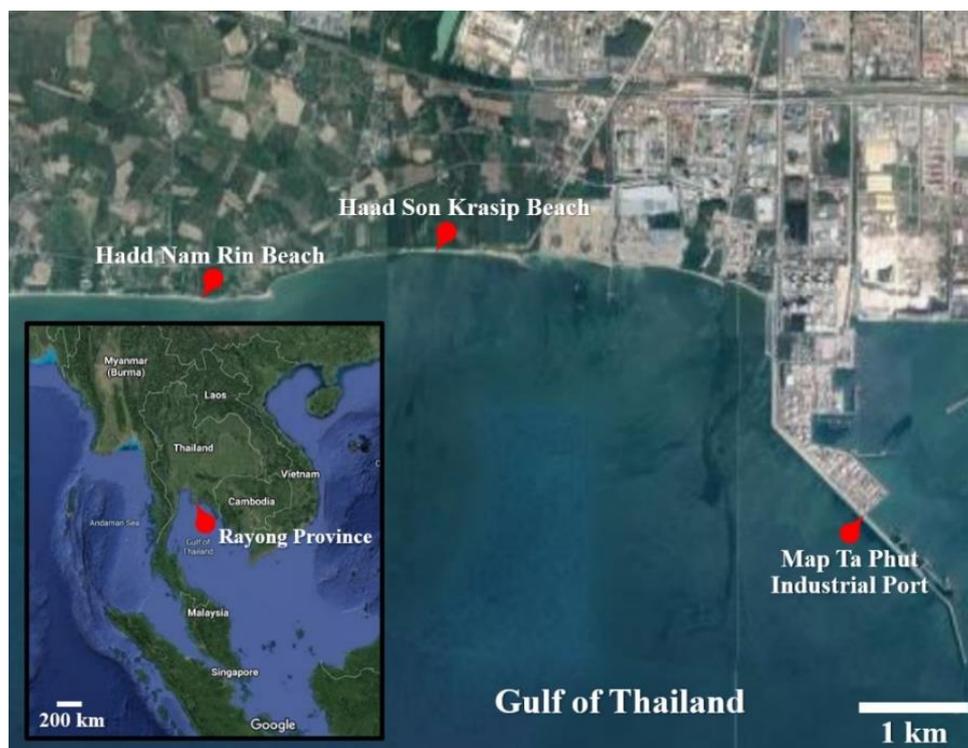


Figure 1. Location of Hadd Nam Rin Beach and Haad Son Krasip Beach in Rayong Province, Thailand generated from Google Maps



Figure 2. Stranded seaweeds on the study shores in February 2018

Species identification of seaweeds by 18S ribosomal DNA amplification

Total DNA was extracted from the dried seaweed powder by DNA Trap I kit (DNA TEC, Kasetsart University, Thailand) following the protocol described by DNA TEC. Polymerase chain reaction (PCR) amplification of the 18S rRNA region was performed using the OnePCR™ reaction mixture (Bio-Helix, Taiwan). Primers used for the amplification included a forward primer MA1 (5'-CGGGATCCGTAGTCATATGCTTGTCTC-3') and a reverse primer MA2 (5'-CGGAATTCCTTCTGCAGG TTCACC-3') (Sigma-Aldrich, Singapore). Amplification conditions followed the method of Feng et al. (2016) with slight modifications. Conditions involved a preheating step at 95°C for 5 minutes, denaturation step at 95°C for 1 minute, annealing step at 55°C for 45 seconds, extension step at 72°C for 1 minute and a final extension step at 72°C for 5 minutes. Amplification was performed for 30 cycles in a Mastercycler® Nexus (Eppendorf, Germany). Approximately 1,000-bp PCR products were analyzed using 1.5% (w/v) OmniPur® agarose gel (Calbiochem, Germany) and visualized by Novel Juice (Bio-Helix, Taiwan). The PCR ladder used in this study was 1 kbp DNA ladder RTU (Bio-Helix, Taiwan). The 18S rDNA PCR-products were purified and sequenced by the nucleotide sequencing service of Macrogen Inc. (Seoul, Korea). Sequence similarity analysis of the 18S rDNA PCR-products was aligned using the BLASTn program (National Center for Biotechnology Information, NCBI). The phylogenetic tree was analyzed by SeaView software version 4.6.4 (Laboratoire de Biométrie et Biologie Evolutive, Université de Lyon, France) and visualized by FigTree software version 1.4.3 (Institute of Evolutionary Biology, University of Edinburgh, UK). The phylogenetic tree was generated by the neighbor-joining (NJ) method with 100,000 bootstraps. All 18S rDNA sequences were deposited in the GenBank database of NCBI under the accession numbers MT291824 and MT291865.

Chemical composition analysis of the model seaweed (Dictyota dichotoma strain A)

The representative seaweed, *D. dichotoma* strain A was considered as the model seaweed and selected for chemical composition analysis due to its abundance in the study area. Chemical composition analyses of dried seaweed powder were conducted following the standard methods of AOAC International (AOAC, 2016). The analyses comprised moisture content by the oven drying method (AOAC method No. 952.08), total protein by the Kjeldahl method ($N \times 6.25$) (AOAC method No. 992.23), total fat by the Soxhlet method (AOAC method No. 948.15 and 945.16), total carbohydrate and total sugars by the liquid chromatographic method (AOAC method No. 982.14) and ash content by the incineration method (AOAC method No. 930.30 and 945.14). All analyses were conducted using the services of the Institute of Nutrition (Mahidol University, Thailand) (service No. SFC 885/2561). All experiments were performed in triplicate.

Preparation of inoculums and culture conditions of oleaginous yeasts

The oleaginous yeasts used in this experiment were *C. albidus* strain TISTR 5103, *R. toruloides* strain TISTR 5186, and *Y. lipolytica* strain TISTR 5212 obtained from the Thailand Institute of Scientific and Technological Research (TISTR). All yeast inoculums were pre-cultured in YM broth (HiMedia, India) at 30°C for 24 hours in a rotary shaker at 150 rpm. The culture medium was prepared from dried seaweed powder of *D. dichotoma* strain A following the method of Xu et al. (2014) with slight modifications. Thirty grams of dried seaweed powder were mixed in one liter of tap water. The pH of the seaweed mixture was adjusted to 3.0 with acetic acid, stirred for 6 hours and then left unstirred for 24 hours at room temperature. The seaweed mixture was centrifuged at 3,500 ×g for 15 minutes to remove any solid substances. The supernatant, with no other nutrients supplements, was

adjusted to pH 5.5 and autoclaved at 121°C for 15 minutes. This was considered as the culture medium. A baffled flask containing 1 liter of culture medium was seeded with 10% (v/v) of each inoculum. The culture flasks were incubated at 30°C for 120 hours in a rotary shaker at 150 rpm. One milliliter of culture suspension was collected to measure yeast growth using optical density at 600 nm (OD₆₀₀) every 24 hours. All experiments were performed in triplicate.

Determination of dry cell weight of oleaginous yeasts

Yeast cells were collected after 120 hours of incubation by centrifugation at 3,500 ×g for 15 minutes and washed with sterilized 0.85% NaCl to remove the culture medium. Cell pellets were re-suspended in sterilized 0.85% NaCl and filtered through a pre-weighed 0.45-µm nitrocellulose filter (Millipore, USA) using a vacuum pump. Samples on nitrocellulose filters were dried to constant weight at 105°C in an oven and then weighed. All experiments were performed in triplicate.

Extraction of SCOs and determination of fatty acid composition

Extraction of total SCOs was conducted according to Byreddy et al. (2015) by sonication-assisted solvent extraction. Fifty milligrams of dried yeast were suspended in three milliliters of chloroform/methanol (2:1, v/v) solution. The sample was sonicated at 20 kHz with 40% amplitude using a Vibra-Cell™ ultrasonic liquid processor (Sonics, USA), and the mixture was centrifuged at 3,500 ×g for 15 minutes to partition the SCOs into the solvent. The solvent was then evaporated using nitrogen gas. The SCO concentration was defined as the amount of extracted SCOs per liter working volume (g/L). The SCO content (lipid content) was represented as a percentage of dry cell weight (% w/w). All experiments were performed in triplicate.

Fatty acid compositions of extracted SCOs produced by each oleaginous yeast were determined using a gas chromatograph 7890A (Agilent, USA) equipped with a

flame ionized detector (FID) and a SP-2560 column (Sigma-Aldrich, USA). The SCO samples were converted to fatty acid methyl esters (FAMES) by transesterification reaction before analysis following the method of Lin and Lin (2017). All gas chromatography analyses were conducted using the services of the Center for Scientific and Technological Equipment (Suranaree University of Technology, Thailand).

Data analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's test with a 95% confidence interval using R software version 3.6.1 (The R Foundation, Austria).

RESULTS AND DISCUSSION

Sampling of seaweeds

Stranded seaweeds from the coastlines of Hadd Nam Rin Beach and Haad Son Krasip Beach were primarily categorized and identified based on their morphology and pigmentation. Only two seaweeds were found during the winter study season in February and identified as brown algae belonging to genera *Dictyota* and *Padina* in Phylum Ochrophyta. They were named *Dictyota* sp. strain A (Figure 3A) and *Padina* sp. strain B (Figure 3B). Some specimens of seaweed blades and stipes were preserved at the Faculty of Science, Energy and Environment, King Mongkut's University of Technology North Bangkok, Thailand. The seaweeds were subsequently dried, weighed, and ground to a fine powder. Results showed that *Dictyota* sp. strain A was the most abundant seaweed in Hadd Nam Rin Beach and Haad Son Krasip Beach, with dry weight at 91.08% of the total collected seaweeds. The other seaweed, *Padina* sp. strain B sparsely appeared on the study shores with dry weight at 8.92% of the total collected seaweeds.

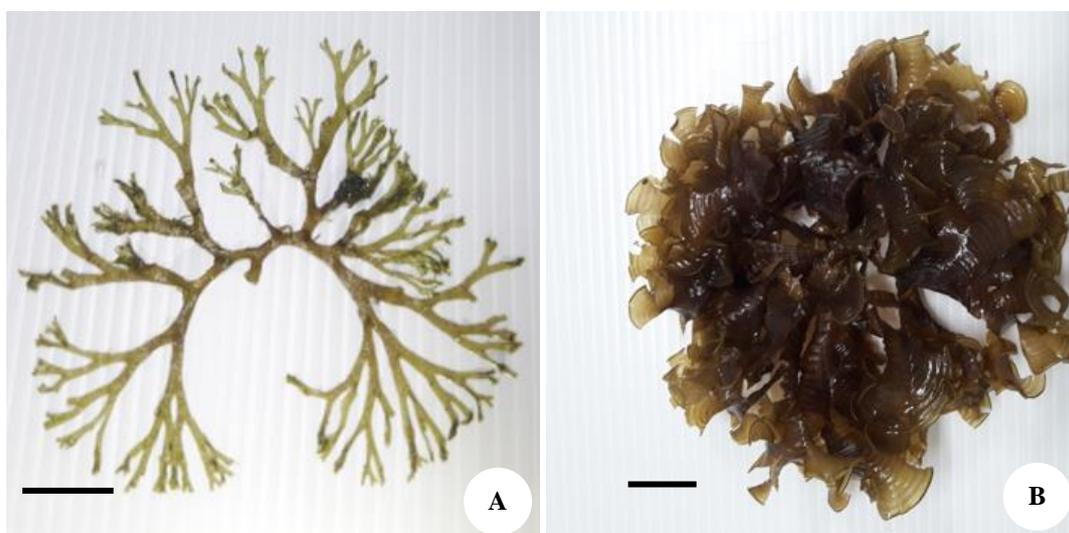


Figure 3. Primary identification of stranded seaweeds based on dominant morphology and pigmentation. A. *Dictyota* sp. strain A, B. *Padina* sp. strain B. Bar = 2 cm

Species identification of seaweeds by 18S rDNA amplification

Dictyota sp. strain A and *Padina* sp. strain B were genetically identified by analysis of 18S rDNA. Alignment results using the BLASTn program showed that *Dictyota* sp. strain A was closely related to *D. dichotoma* strain SZKIZ047 with 99.45% identity, while *Padina* sp. strain B was closely related to *P. australis* strain GUMTG035 at 98.20% identity (Table 1). A phylogenetic tree using the neighbor-joining (NJ) method with 100,000 bootstrap replications is shown in Figure 4. All 18S rDNA sequences from this study were deposited in the GenBank database of NCBI under the accession numbers MT291824 and MT291865, as previously mentioned. Therefore, *Dictyota* sp. strain A was confidently designated as *D. dichotoma* strain A, and *Padina* sp. strain B was assigned to *P. australis* strain B.

Chemical composition analysis of *D. dichotoma* strain A

Dictyota dichotoma strain A was considered as the representative seaweed for the analysis of chemical composition since it was abundant on Hadd Nam Rin Beach and Haad Son Krasip Beach. Moisture content of *D. dichotoma* strain A was 7.57 ± 0.05 g per 100 g of dried seaweed. It was not a good nitrogen source as the protein content by the Kjeldahl method ($N \times 6.25$) was found to be 14.66 ± 0.05 g per 100 g of dried seaweed. A small amount of fat was detected in this seaweed at 1.65 ± 0.01 g per 100 g of dried seaweed. Results suggested that *D. dichotoma* strain A was a satisfying carbon source because total carbohydrate recorded a maximum chemical composition of 52.47 ± 0.05 g per 100 g of dried seaweed. The contents of five sugars comprising glucose, fructose, lactose, maltose, and sucrose were considered as total sugar of 1.21 ± 0.01 g per 100 g of dried seaweed, while the ash content was 23.64 ± 0.04 g per 100 g of dried seaweed.

Table 1. Percentage of 18S rDNA sequences for the two stranded seaweeds

| Seaweed | Closely related seaweed | GenBank accession No. (Database) | Identity (%)* | GenBank accession No. (Deposited) |
|------------------------------|---|----------------------------------|---------------|-----------------------------------|
| <i>Dictyota</i> sp. strain A | <i>Dictyota dichotoma</i> strain SZKIZ047 | AB087107.1 | 99.45 | MT291824 |
| <i>Padina</i> sp. strain B | <i>Padina australis</i> strain GUMTG035 | AB087124.1 | 98.20 | MT291865 |

Note: * Identity results were analyzed on April 6th, 2020.

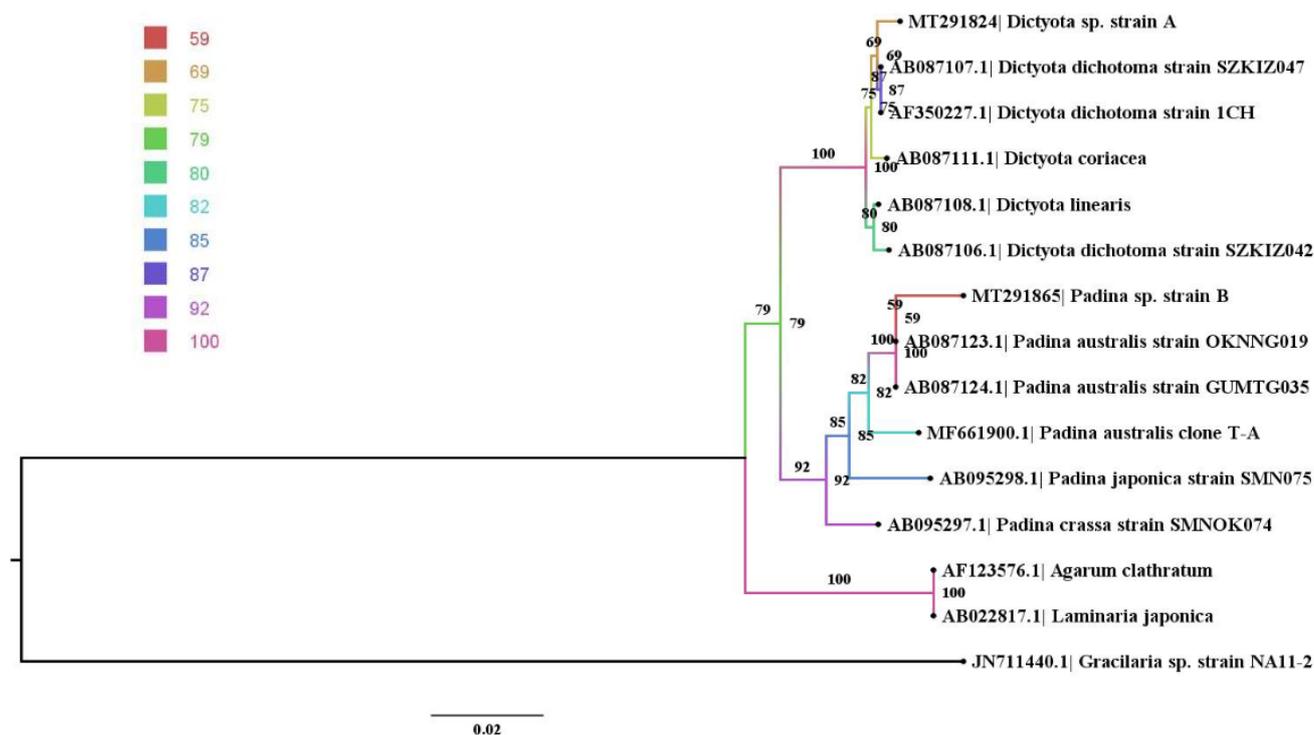


Figure 4. Phylogenetic tree of 18S rDNA for the two stranded seaweeds using the neighbor-joining (NJ) method with 100,000 bootstrap replications. The phylogenetic tree was generated by the SeaView program version 4.6.4

Culture and growth of oleaginous yeasts in medium prepared from *D. dichotoma* strain A

Three oleaginous yeasts were cultured in medium prepared from *D. dichotoma* strain A without supplementing any other nutrients. Their growths were monitored every 24 hours for 120 hours by measurement of OD₆₀₀. Results showed that all oleaginous yeasts thrived well in this medium, indicating that the carbon source and other nutrients extracted from *D. dichotoma* strain A were sufficient for the growth and survival of oleaginous yeasts. Their growths entered the log phase during 24-72 hours of incubation and the stationary phase after 96 hours of incubation. *C. albidus* strain TISTR 5103 exhibited significantly better growth than the other yeasts during the log phase. Growth phases of all oleaginous yeasts are shown in Figure 5.

Determination of dry cell weight and SCO content of oleaginous yeasts

Cells of the three oleaginous yeasts were measured for dry weight and SCO content after 120 hours of incubation. *C. albidus* strain TISTR 5103 recorded higher dry weight than the other yeasts that directly correlated to its growth obtained from OD₆₀₀. Dry weight was achieved at 3.24 ± 0.13 g/L. *R. toruloides* strain TISTR 5186 and *Y. lipolytica* strain TISTR 5212 showed less dry weight than *C. albidus* strain TISTR 5103 by approximately 50%. Dry cell weights of the three oleaginous yeasts are reported in Table 2. The SCO concentration and SCO content indicated intracellular lipid accumulation in oleaginous yeasts. The SCO concentration recorded the weight of SCOs

accumulated in the oleaginous yeast per liter of culture volume, whereas SCO content was the percentage of SCOs per dry weight of yeast cells. *C. albidus* strain TISTR 5103 was the most SCO accumulating yeast with SCO concentration of 1.66 ± 0.04 g/L and significantly higher SCO content of 51.26 ± 2.99%, while *Y. lipolytica* strain TISTR 5212 showed reduced SCO production. SCO concentration and SCO contents of the three studied oleaginous yeasts are shown in Table 2.

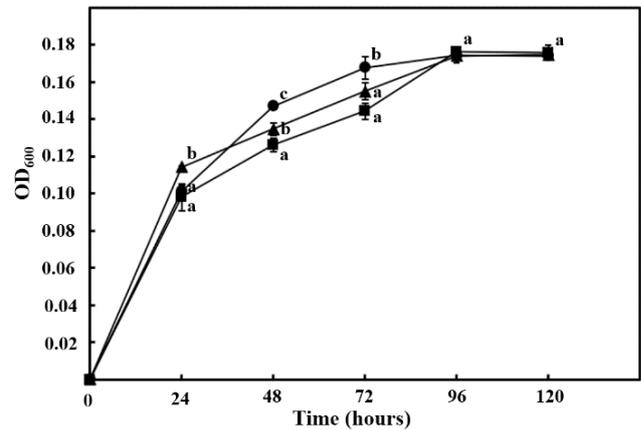


Figure 5. Growth phases for the three oleaginous yeasts cultured in medium prepared from *D. dichotoma* strain A. *C. albidus* strain TISTR 5103 (●), *R. toruloides* strain TISTR 5186 (▲) and *Y. lipolytica* strain TISTR 5212 (■). Error bars represent the standard deviation of three replicates. Mean values for the same incubation time followed by the same letter were not significantly different according to Tukey's test ($p < 0.05$)

Table 2. Dry cell weight and SCO production for the three oleaginous yeasts

| Oleaginous yeast | Dry cell weight (g/L) | SCO concentration (g/L) | SCO content (or lipid content) (% w/w) |
|--|--------------------------|--------------------------|--|
| <i>C. albidus</i> strain TISTR 5103 | 3.24 ± 0.13 ^b | 1.66 ± 0.04 ^c | 51.26 ± 2.99 ^c |
| <i>R. toruloides</i> strain TISTR 5186 | 1.63 ± 0.07 ^a | 0.62 ± 0.06 ^b | 37.76 ± 3.50 ^b |
| <i>Y. lipolytica</i> strain TISTR 5212 | 1.58 ± 0.16 ^a | 0.39 ± 0.02 ^a | 24.85 ± 1.91 ^a |

Note: Mean values in the same column followed by the same letter were not significantly different according to Tukey's test ($p < 0.05$).

Table 3. Fatty acid methyl ester (FAME) profile for the three oleaginous yeasts

| FAME profile | Percentage of FAME in SCOs (%) of three oleaginous yeasts | | |
|---|---|--|--|
| | <i>C. albidus</i> strain TISTR 5103 | <i>R. toruloides</i> strain TISTR 5186 | <i>Y. lipolytica</i> strain TISTR 5212 |
| Capric AME (C10:0) | 0.12 | - | 0.15 |
| Undecanoic AME (C11:0) | - | - | 0.23 |
| Lauric AME (C12:0) | 0.09 | - | 0.38 |
| Myristic AME (C14:0) | 1.12 | 0.68 | 1.27 |
| Pentadecenoic AME (C15:0) | 0.17 | 0.17 | 0.48 |
| Pentadecenoic (<i>cis</i> -10) AME (C15:1) | 0.36 | 0.35 | 0.42 |
| Palmitic AME (C16:0) | 36.46 | 14.96 | 35.12 |
| Palmitoleic AME (C16:1) | 0.56 | 1.54 | 2.08 |
| Heptadecenoic AME (C17:1) | - | 0.34 | 0.69 |
| Stearic AME (C18:0) | 38.66 | 6.43 | 35.02 |
| Oleic AME (C18:1n-9c) | 12.99 | 49.56 | 10.91 |
| Linoleic AME (C18:2n-6t) | 0.75 | - | 10.67 |
| Linoleic AME (C18:2n-6c) | 5.38 | 23.48 | - |
| Linolenic AME (C18:3n-6) | 0.69 | 0.51 | 0.57 |
| Eicosenoic AME (C20:1) | - | 1.21 | - |
| Henicosanoic AME (C21:0) | - | 0.47 | - |
| Erucic AME (C22:1n-9) | 2.65 | 0.29 | 2.01 |

Determination of fatty acid composition in SCOs

Fatty acids in SCOs obtained from the three oleaginous yeasts were converted to fatty acid methyl esters (FAMES) by transesterification and analyzed for FAME profiles by gas chromatography. Results showed that all oleaginous yeasts yielded different FAME profiles with C10-fatty acid to C22-fatty acid. They mainly produced long-chain fatty acids with 16 and 18 carbon atoms. *C. albidus* strain TISTR 5103 and *Y. lipolytica* strain TISTR 5212 produced the most saturated fatty acids comprising palmitic acid (C16:0) and stearic acid (C18:0) ranging 35.0-38.7%. Interestingly, *R. toruloides* strain TISTR 5186 produced different dominant fatty acids as unsaturated C18-fatty acids comprising oleic acid (C18:1n-9c) of 49.5% and linoleic acid (C18:2n-6c) of 23.5%. FAME profiles for the three oleaginous yeasts are shown in Table 3.

Discussion

Marine benthic algae found in the Gulf of Thailand belong to four Phyla of Chlorophyta, Cyanobacteria, Ochrophyta, and Rhodophyta (Titioatchasai et al. 2019). Here, the brown seaweeds stranded on the coastlines of Hadd Nam Rin Beach and Haad Son Krasip Beach in Eastern Thailand were designated as *D. dichotoma* strain A and *P. australis* strain B of Phylum Ochrophyta. Dominant characteristics of *Dictyota* species are dichotomously branched, flattened, and brown in color (Baweja et al. 2016). *D. dichotoma* plays a particularly important role in marine productivity (Mantiri et al. 2019). *Padina* species are calcified brown seaweeds with fan-shaped fronds light brown and whitish in color due to light calcification (Baweja et al. 2016; Mantiri et al. 2019). These two seaweeds are the common seaweeds found in coastal Thailand. *D. dichotoma* and *P. australis* were reported as commonly found from January to March in Trat Province on the east coast of Thailand (Petsut et al. 2012). Phang et al. (2016) reported that there were eight species of *Dictyota* and ten species of *Padina* in the Gulf of Thailand and the Andaman Sea. *P. australis* is widely distributed in several Southeast Asian countries including Thailand, The Philippines, Singapore, Vietnam, Indonesia, and Malaysia, while *D. dichotoma* is also found in Southeast Asia except for Indonesia and Malaysia.

Morphological traits and high phenotypic plasticity have become the main bottlenecks in seaweed identification. Moreover, identification based on morphology is almost impossible especially when distinctive characteristics are absent (Herrero et al. 2010). The identification of *Dictyota* species is challenging due to their phenotypic plasticity and poor species delineation (Lopes-Filho et al. 2017). Likewise, *Padina* species are also taxonomically challenging due to their phenotypic plasticity coupled with a poor understanding of reliable morphological characters (Win et al. 2018). Therefore, genetic analysis based on rDNA could help to clarify taxonomy and differentiation among the seaweeds (Elsaied et al. 2015). Two stranded seaweeds in this study were genetically identified by sequence analysis of their 18S rDNA and taxonomic results were clarified. The 18S rDNA

obtained were deposited in the GenBank database of NCBI under the approved accession numbers.

The most abundant chemical composition of *D. dichotoma* strain A was carbohydrates, indicating a satisfying carbon source, and comprised several common sugars such as glucose, fructose, lactose, maltose, and sucrose based on total sugar analysis. The main carbohydrate compounds in brown seaweed, except for the common sugars were alginate, fucoidan, laminaran, and mannitol (Xu et al. 2014; Malyarenko et al. 2019). Carbohydrates were reported as the most abundant component of macroalgae based on the proximate composition analysis (Omer et al. 2013; Radha 2018). Protein content of seaweeds varied due to many factors such as species, seasons, and geographic area (Radha 2018). The little amount of fat detected in *D. dichotoma* strain A was supported by Radha (2018) that revealed the crude fat content of most seaweeds was generally less than 3.0%. Composition of fatty acids in *D. dichotoma* extracts showed that palmitic acid (C16:0) was the most abundant (Bakar et al. 2017). Seaweed usually contains high ash contents of approximately 20% (Radha, 2018).

Recently, several seaweeds have been studied as a feedstock for SCO production. Xu et al. (2015) selected *L. japonica* (*S. japonica*) as model marine biomass for the cultivation of four different oleaginous yeasts to produce SCOs by the repeated batch system. Results showed that the oleaginous yeast, *C. curvatus* strain ATCC 20509, yielded the highest SCO content at 61%. Laminaria residue hydrolysates from the kelp industry were used for SCO production by two species of oleaginous yeasts, *Rhodospiridium toruloides* strain Y4 and *Rhodotorula glutinis* strain AS 2.107. Results showed that *R. toruloides* strain Y4 yielded 37.6% and *R. glutinis* strain AS 2.107 yielded 22.2% of SCO contents (Zhang et al. 2016). The *S. latissima* seaweed was hydrolyzed and cultured by oleaginous yeast, *Metschnikowia pulcherrima* in a stirred tank reactor fermentation and 37.2% SCO was achieved (Abeln et al. 2019).

Utilization of *D. dichotoma* strain A was prepared as a growth medium without supplementing any other nutrients for the culture of three oleaginous yeasts. Results showed that OD₆₀₀ increased over time, indicating that oleaginous yeasts utilized the carbon source and other nutrients in the prepared medium for growth. Acetyl-CoA molecules in cytosol of yeast cells serve as fatty acid precursors (Fakas, 2017). Biosynthesis of fatty acids is initiated when growth media are starved of nitrogen (Kumar et al. 2017). Fortunately, *D. dichotoma* strain A was evaluated as a deficient source of nitrogen. Oleaginous yeasts are capable of biosynthesis and can accumulate more than 20% of their cell mass as intracellular lipids. In this study, all oleaginous yeasts accumulated SCOs at more than 20% of their dry weight. *C. albidus* strain TISTR 5103 was the most effective oleaginous yeast cultured in the seaweed medium yielding 51.2% of SCO content. Fatty acid compositions of SCOs produced from the three oleaginous yeasts were analyzed. Results showed palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9c), and linoleic acid

(C18:2n-6c) as the highest fatty acids contents. These fatty acids are commonly found in various vegetable oils. Giakoumis (2018) studied the physicochemical properties and fatty acid composition of 22 vegetable oils. Findings indicated that saturated fatty acids comprising palmitic acid and stearic acid were mostly found from coconut, palm, and mahua oils. Unsaturated fatty acids including oleic acid and linoleic acid are highly presented in olive, rapeseed/canola, sunflower, corn, and soybean oils. Many previous studies reported that SCOs produced by yeasts had similar fatty acid profiles to various vegetable oils (Xu et al. 2014; Gientka et al. 2017; Saran et al. 2017; Cho and Park, 2018). This characteristic of SCOs promotes their use as edible oil, and an appropriate alternative for second-generation fuel production such as biodiesel.

In conclusion, the stranded seaweeds were identified as *D. dichotoma* strain A and *P. australis* strain B based on their dominant morphology and 18S rDNA sequence analyses. The most abundant seaweed, *D. dichotoma* strain A, was considered the sole carbon source for the cultivation of three different oleaginous yeasts. The oleaginous yeasts accumulated SCOs in a medium prepared from *Dictyota* seaweed. *D. dichotoma* strain A showed potential for yeast cultivation at low-cost with a high-value carbon source. The SCOs produced from the oleaginous yeasts revealed fatty acid profiles similar to vegetable oils, with potential for use in various industrial applications such as biofuels.

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