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The Oleaginous *Rhodotorula mucilaginosa* Isolated from Paddy Field Soils and the Profile of Fatty Acid in Its Intracellular Microbial Oils

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Abstract

Oleaginous yeasts are a specific group of yeast species known for their ability to accumulate significant amounts of intracellular microbial oils, also referred to as single-cell oils, which constitute more than 20 % of their dry cell weight. These microbial oils represent a promising resource for various biotechnological and biofuel applications. However, only a few known yeast species have been classified as oleaginous. This study focused on isolating and identifying oleaginous yeasts from the soil in paddy fields across 3 provinces in Thailand. The yeasts that were isolated were assessed for their intracellular lipid accumulation using a quantitative Sudan IV staining and a qualitative weighting method. Among the isolated strains, the one with the highest lipid accumulation was considered for its fatty acid composition using gas chromatography. The results revealed that 6 strains of yeast were isolated from the paddy field soil samples, and these were designated as oleaginous, with intracellular lipid content ranging from 20.41 ± 2.24 to 44.11 ± 3.27 % (w/w). Molecular genetic evaluations indicated that the six strains belonged to the genera *Debaryomyces*, *Meyerozyma*, *Rhodotorula*, and *Starmerella*. Notably, the *R. mucilaginosa* strain RYA07 exhibited the highest lipid accumulation, with its intracellular lipids primarily consisting of palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1n9c). These fatty acids have potential applications in various biotechnological fields, including biodiesel production, food industry, cosmetics, and pharmaceuticals.

Keywords: Fatty acid, Microbial oil, Oleaginous yeast, Paddy field soil, *Rhodotorula mucilaginosa*, Single-cell oil, Soil-yeast oil, Thailand

Introduction

Microbial oil, also known as single-cell oil, refers to the intracellular lipids produced and accumulated by oleaginous microorganisms [1-3]. Various microorganisms that belong to the algae, bacteria, fungi, and yeast, have been identified as oleaginous due to their ability to accumulate significant amounts of lipids [1]. These oleaginous microorganisms can store intracellular oil comprising more than 20 % of their dry cell weight when provided with optimal cultivation conditions [2]. Oleaginous microorganisms are capable of synthesizing a range of fatty acids, from short-chain fatty acids (C6) to long-chain fatty acids (C36). These fatty acids can be categorized as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA) [4]. Currently, microbial oil represents a promising resource for various applications, including biofuels, oleochemicals, medicine, cosmetics, pharmaceuticals, and nutrition [1,3].

Oleaginous microorganisms are increasingly recognized for their potential in producing lipids that can be utilized for biofuel, nutraceutical, and other industrial applications [4]. Oleaginous yeasts are often considered superior for commercial lipid production compared to other oleaginous microorganisms, due to their rapid growth, high lipid content, and high volumetric productivity [5]. In the past decade, there has been a significant increase in efforts to develop yeast lipid technology, with approximately 100 research

papers published each year [5]. This trend reflects the growing demand for microbial oil in various industry segments. As a result, the isolation and characterization of these microorganisms utilize various methods and sources, as highlighted in many recent studies.

Currently, only 8.2 % of the total known yeast species have been classified as oleaginous [6]. This group includes various species within the 32 genera of the phylum Basidiomycota and 27 genera of the phylum Ascomycota [7]. Oleaginous yeasts are commonly isolated from a variety of environments, including fruits, surfaces, and soil [8,9]. Soil is regarded as a reservoir for various yeasts in underground environments [7]. Consequently, many oleaginous yeasts can be isolated from diverse soil environments, such as mountain forests [7], mangroves [9,10], peat bogs [11], and volcanic ash soils [12,13]. These soil-derived oleaginous yeasts include genera such as *Apiotrichum*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Lipomyces*, *Pichia*, and *Trichosporon*, which can accumulate intracellular lipids ranging from 24 % to 74 % of their dry cell weight. Paddy field soil is also a rich environment for various microorganisms, including yeasts. However, recent studies have primarily focused on isolating and characterizing these yeasts for agricultural applications, such as their potential as plant growth-promoting agents or antagonists to soil-borne plant pathogens [14-16]. Consequently, research on oleaginous yeasts isolated from paddy field soil has been limited.

Paddy fields play a vital role in Thailand's agricultural landscape, significantly contributing to the country's economy and food security. Thailand is the world's largest rice exporting country, with nearly 44 % of its agricultural land dedicated to paddy rice cultivation [17]. Thailand has extensive areas of paddy fields that are suitable for sampling sites to isolate oleaginous yeasts. This could result in the discovery of novel or highly effective oleaginous yeasts. This study aimed to isolate and screen oleaginous yeasts from the soil of paddy fields in Thailand. The isolated yeasts were identified using molecular genetics and phylogenetic methods. Among the yeasts studied, the one with the highest lipid accumulation was characterized by its fatty acid composition profile of intracellular lipids, analyzed through gas chromatography (GC). The isolated soil-derived oleaginous yeasts and their accumulated lipids are expected to be promising candidates for biofuels, oleochemicals, and various biotechnological applications.

Materials and methods

Sampling sites and collection of soil samples

The sampling sites are the flooded paddy fields in 3 provinces of Thailand: Rayong Province (12°44'59"N, 101°17'52"E), Chanthaburi Province (12°35'40"N, 101°59'39"E), and Chai Nat Province (15°09'37"N, 100°09'39"E). These fields have been cultivated with rice for over 10 years. Twenty-Four soil samples were collected from Rayong Province, twenty from Chanthaburi Province, and sixteen from Chai Nat Province. The samples were collected from the surface soil layers as yeasts are often found there, but rarely in the deeper soil layers [15]. All samples were collected randomly between July 2020 and February 2021. Soil temperatures were measured using an Extech needle tip thermometer (Extech Instruments, USA) at the sampling sites. The pH values were assessed by suspending 1 g of the soil sample in 10 mL of distilled water, and these measurements were taken with an Ohaus ST20 Starter Pen Meter (Ohaus, USA). All soil samples were stored at 4 °C in sterilized conical polypropylene tubes and transported to the microbiological laboratory for soil yeast isolation within 48 h. The scenery in the sampling sites is shown in **Figure 1**.

Figure 1 Scenery in the sampling sites includes: (A) paddy field in Rayong Province (*n* = 24), (B) paddy field in Chanthaburi Province (*n* = 20), and (C) paddy field in Chai Nat Province (*n* = 16).

Isolation of soil yeasts from the collected samples

The method for isolating soil yeasts was based on the previous study by Wongchamrearn *et al.* [9], with minor modifications. One g of each collected soil sample was suspended in 10 mL of sterilized distilled water and serially 10-fold diluted to achieve a final dilution of 1:1,000,000. Subsequently, 100 µL of the diluted sample was spread-plated on a selective medium, Dichloran Rose Bengal Chloramphenicol (DRBC) agar (HiMedia, India). The pH and temperature conditions necessary for yeast growth were obtained from the data collected at sampling sites in this study. All DRBC agar plates were incubated at 29.1 °C, which is the average soil temperature, in a KB720 incubator cabinet (Binder, Germany) for 72 h. Yeast isolates were selected based on their variations in colony morphology and were then colony purified using the streak plate method on Yeast Malt (YM) agar (HiMedia, India). The pH value of all culture media used in this experiment was adjusted to 7.0, reflecting the average pH of the paddy field soil samples, prior to use. Each pure isolate was given the code followed: RYA for Rayong Province, CTB for Chanthaburi Province, and CNC for Chai Nat Province.

Accumulation, screening, and extraction of intracellular lipids from the isolated soil yeasts

Each isolated soil yeast was first pre-cultured in a yeast cultivation medium, YM broth (HiMedia, India), at 29.1 °C for 48 h. They were then inoculated into a Glycerol Yeast Peptone (GYP) medium to promote the accumulation of intracellular lipids. The composition of the GYP medium is detailed in a previous study by Planonth and Chantarasiri [2]. Subsequently, 10 % (v/v) of each pre-cultured yeast was inoculated into the GYP medium and incubated at 29.1 °C for 120 h, with a MS-NOR-30 orbital shaker at 150 rpm (Major Science, Taiwan). As mentioned previously, all culture media was adjusted to the neutral pH (7.0).

The Sudan IV staining method was used to assess the intracellular lipid accumulation in the isolated yeasts [8,9]. To prepare for staining, 300 µL of each yeast culture grown in GYP medium was mixed with 300 µL of Sudan IV solution (Sigma-Aldrich, Germany). This mixture was then incubated in a dark cabinet at 30 °C, for 1 h. After the incubation period, the stained yeast cells were examined under an ECLIPSE-E200 light microscope (Nikon, Japan) to check for the presence of dark red intracellular lipid droplets within their cells.

Centrifugation method was performed to harvest lipid-accumulating yeast cells at 3,500 × g for 15 min by a refrigerated centrifuge Digicen 21R (OrtoAlresa, Spain). The collected yeast cells were washed with 0.85 % (w/v) sterilized NaCl solution to remove any residual GYP medium. The harvested yeast cells were dried to a constant weight in a FD240 hot-air oven (Binder, Germany) at 105 °C, after which they were weighed. The extraction of intracellular lipids from the dried yeast was performed following the previously described methodology of Planonth and Chantarasiri [2]. One g of dried yeast was suspended in 9 mL of *n*-hexane and sonicated using a VCX 130PB Vibra-Cell ultrasonic liquid processor (Sonics, USA) at 90 % amplitude for 10 min. The sonicated mixture was then centrifuged at 3,500 × g for 15 min. The supernatant (*n*-hexane layer) was collected and dried using a Hei-VAP rotary evaporator (Heidolph Instruments, Germany) at 69 °C to obtain the extracted intracellular lipids. Yeast isolates that contained over 20 % (w/w) intracellular lipids

relative to their dry cell weight were classified as oleaginous yeasts [2]. The positive control strain used was the known oleaginous yeast, *Yarrowia lipolytica* strain TISTR 5212 (Thailand Institute of Scientific and Technological Research, Thailand), while the negative control strain was the baker's yeast, *Saccharomyces cerevisiae*. The experiments were conducted in triplicate.

Molecular genetics and phylogenetic identification of the isolated oleaginous yeasts

Genomic DNA was extracted from yeast cells using a genomic DNA isolation kit (Bio-Helix, Taiwan), following the manufacturer's instructions. The extracted genomic DNA was then used as a template for amplifying the internal transcribed spacer (ITS) regions through polymerase chain reaction (PCR). The amplification was performed with a OnePCR reaction mixture (Bio-Helix, Taiwan) using the universal ITS1 and ITS4 primer set. Thermal cycling comprised 35 amplification cycles on an Eppendorf Mastercycler Nexus Gradient (Eppendorf, Germany). The PCR cycling conditions adhered to the methodology outlined by Wongchamrearn *et al.* [9]. The PCR products were electrophoresed on a 1.5 % (w/v) OmniPur agarose gel (Calbiochem, Germany) and visualized with Novel Juice stain (Bio-Helix, Taiwan).

The PCR products were sequenced using the services of Macrogen Inc. (South Korea). The nucleotide sequences were aligned for genetic identification using the BlastN program, which is based on the core nucleotide databases (core_nt) from the National Center for Biotechnology Information (NCBI). A phylogenetic tree of the isolated oleaginous yeasts was constructed using Seaview software version 5.0.1, developed by the Laboratoire de Biométrie et Biologie Evolutive at Université de Lyon (France) and FigTree software version 1.4.4 from the Institute of Evolutionary Biology at the University of Edinburgh (UK). This analysis utilized the BIONJ algorithm with 10,000 bootstrap replicates. The ITS regions of the isolated oleaginous yeast were submitted in the NCBI GenBank database under accession numbers PQ637150, PQ637151, PQ637152, PQ637153, PQ637154, and PQ637155.

Analysis of the fatty acid composition profile of intracellular lipids from the *Rhodotorula mucilaginosa* strain RYA07

The isolated yeast, *R. mucilaginosa* strain RYA07, was identified as the highest lipid-accumulating yeast in this study. The RYA07 yeast was cultured in GYP medium, and intracellular lipids were extracted following the aforementioned methodology. These extracted lipids were chemically converted into a mixture of fatty acid methyl esters (FAMEs) through a process called transesterification. The resulting FAMEs were analyzed using a 7890A gas chromatograph (Agilent Technologies, USA), equipped with a flame-ionized detector (FID) and a CP-Sil 88 column (Agilent Technologies, USA). The preparation of FAMEs and the gas chromatography conditions were performed according to the methodology outlined by Chantarasiri and Ungwiwatkul [18].

Statistical analysis

The statistical analyses were conducted using R software version 4.4.1 (R Foundation for Statistical Computing, Austria). The normality of the data and the homogeneity of variances were assessed using the Shapiro-Wilk normality test and Bartlett's K-squared test, respectively. One-way ANOVA was employed for multiple comparison analyses, followed by Tukey's test with a 95 % confidence interval ($p < 0.05$).

Results and discussion

Properties of soil samples

Sixty soil samples were collected from the surface layers of flooded paddy fields in 3 provinces of Thailand. All the samples were primarily classified as clay. The properties of these samples were measured, including soil temperature and pH levels. The average soil temperature recorded at the sampling sites was 29.10 ± 0.27 °C, while the average soil pH was 7.03 ± 0.02. This study utilized all soil properties to assess the conditions for yeast cultivation, focusing on the growth and accumulation of intracellular lipids.

The temperature used for yeast cultivation in this study was aligned with the optimal growth temperatures for several oleaginous yeast strains, which typically range from 25 to 30 °C [19]. However, the neutral pH level of the culture medium may slightly affect lipid accumulation in yeasts. Some strains of oleaginous

yeasts thrive at slightly acidic pH levels, which can enhance lipid yield [19]. Interestingly, a pH level range of 4 to 7 did not significantly affect lipid synthesis in the oleaginous yeast *Rhodotorula glutinis* [19,20].

Soil yeasts isolated from the collected soil samples

Soil yeasts were isolated from paddy soil samples using DRBC selective agar, and the colonies were purified using YM agar. The results indicated that yeast colonies could be successfully isolated from the collected soil samples. A total of six yeast isolates, showing distinct colony morphology, were purified. These isolates included 3 from Rayong Province (coded RYA), 2 from Chanthaburi Province (coded CTB), and one from Chai Nat Province (coded CNC). All isolated soil yeasts exhibited a similar pattern of colony morphology, characterized by opaque, circular shapes with smooth edges and convex elevations. However, their morphology varied primarily in terms of colony pigmentation and diameter. The yeast colonies measured between 1.17 and 3.94 mm in diameter after being cultured on YM agar for 72 h. The colony morphology of the isolated soil yeasts is illustrated in **Figure 2**.

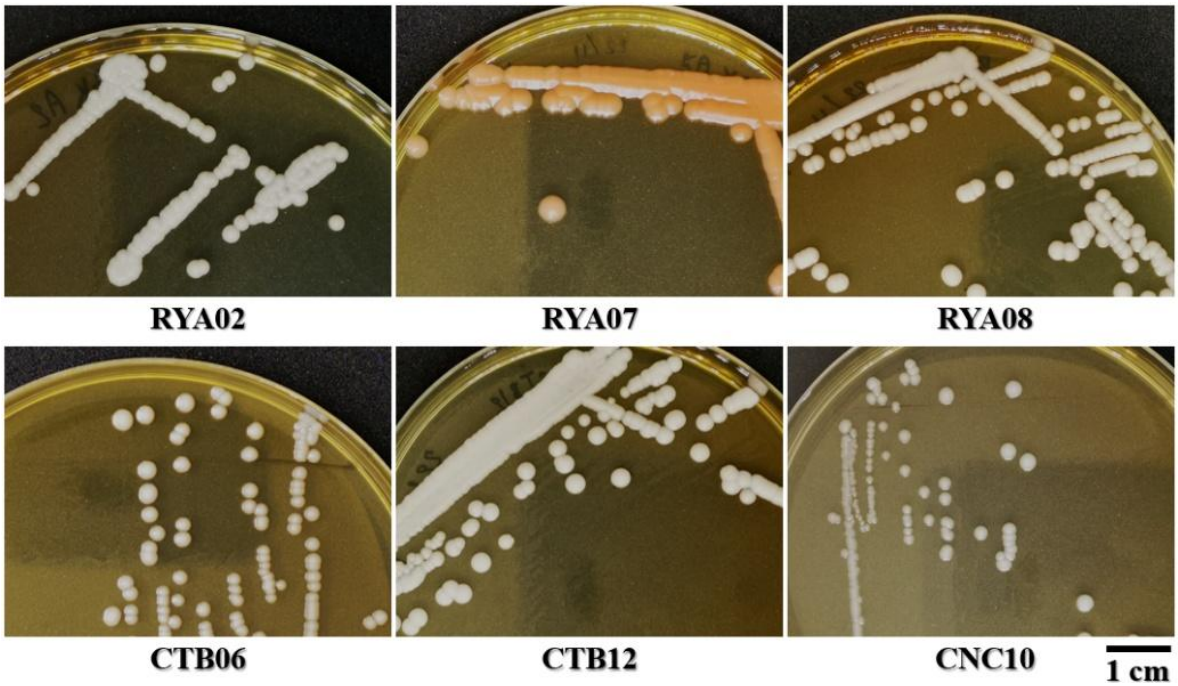

Figure 2 Colony morphologies of six isolated soil yeasts grown on YM agar at 29.1 °C for 72 h.

Accumulation, screening, and extraction of intracellular lipids from the isolated soil yeasts

All soil yeast isolates were cultured in GYP medium to promote the accumulation of intracellular lipids. This medium is rich in glucose and low in nitrogen, which encourages the formation of lipid droplets within the cells [2]. The presence of intracellular lipids in the isolated yeasts was determined using the Sudan IV staining method, which confirmed that all isolates tested positive for Sudan IV. Sudan IV is a lipid-soluble dye commonly used to stain intracellular lipids in yeast cells, resulting in lipid droplets appearing as deep red bodies within the cells. The Sudan IV-stained lipid droplets from the isolated yeasts are illustrated in **Figure 3**.

All yeast isolates were collected, dried, and processed to extract intracellular lipids. They could accumulate intracellular lipids ranging from 20.41 ± 2.24 % (w/w) in isolate CTB06 to 44.11 ± 3.27 % (w/w) in isolate RYA07. Oleaginous yeasts are microorganisms capable of accumulating significant amounts of lipids, typically over 20 % of their biomass, under specific growth conditions [21]. Consequently, all isolated soil yeasts were classified as oleaginous yeasts. A recent study on oleaginous yeast from mountain forest soil in Thailand found that only 15 out of 127 yeast

strains were able to accumulate lipid content exceeding 40 % of their dry biomass [7].

Isolate RYA07 showed a significantly higher intracellular lipid content than the other isolates ($p < 0.05$), leading to its selection for further study. Notably, the lipid composition of RYA07 was slightly different from that of the well-known oleaginous yeast, *Yarrowia lipolytica*. A previous report indicated that some wild-type strains of *Y. lipolytica* can accumulate up to 40 % (w/w) lipids when grown on volatile fatty acids and 58.5 % (w/w) lipids when grown on sugar cane bagasse hydrolysate [22]. The intracellular lipid contents (%, w/w) of the isolated yeasts are presented in **Table 1**. An oleaginous *Y. lipolytica* strain TISTR 5212 served as the positive control, while *S. cerevisiae* was employed as the negative control.

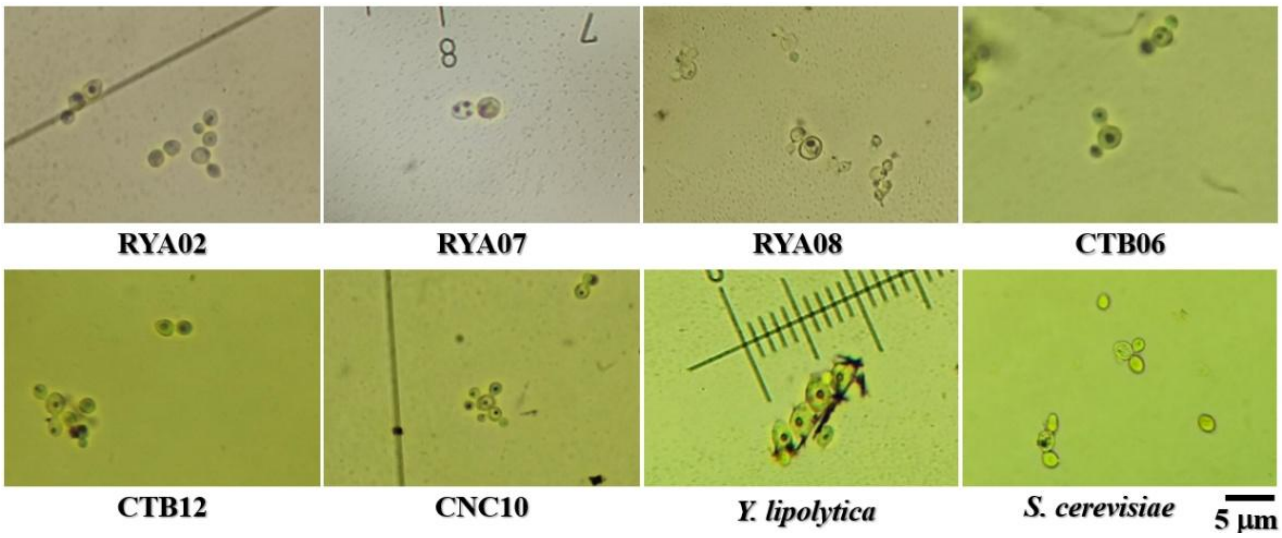

Figure 3 Light microscopic examination and Sudan IV staining revealed lipid bodies within the cells of the isolated soil yeasts. The control strains utilized included the oleaginous *Y. lipolytica* strain TISTR 5212 as the positive control and *S. cerevisiae* as the negative control.

Table 1 The intracellular lipid content of six isolated soil yeasts and 2 control yeast species.

Isolated soil yeasts	Intracellular lipid content (%, w/w)
RYA02	32.45 ± 0.17^{de}
RYA07	44.11 ± 3.27^{f}
RYA08	27.02 ± 2.06^{be}
CTB06	20.41 ± 2.24^{a}
CTB12	24.00 ± 2.56^{ab}
CNC10	20.49 ± 1.83^{a}
Y. lipolytica strain TISTR 5212 (positive control)	36.36 ± 3.13^{d}
S. cerevisiae (negative control)	0.95 ± 0.05^{c}

Notes: All data showed normal distribution, as assessed by the Shapiro-Wilk normality test. Additionally, all groups demonstrated homogeneity of variance, evaluated using the Bartlett's K-squared test. Mean values that share the same superscript letter indicate no significant differences among the %(w/w) intracellular lipids of yeast strains, as determined by Tukey's test. Statistical analyses were conducted with a 95 % confidence interval ($p < 0.05$). All experiments were performed in triplicate.

Identification of the isolated oleaginous yeasts by molecular genetics and phylogenetic methods

Each oleaginous yeast strain was extracted for genomic DNA, and then PCR amplified for their ITS regions. The PCR products were then purified, sequenced, and aligned for molecular genetic identification. The nucleotide alignment results, obtained using the BlastN suite based on the core nucleotide databases (core_nt) from NCBI, indicated that the oleaginous yeasts isolated from paddy soil samples belonged to 4 genera: *Debaryomyces*, *Meyerozyma*, *Rhodotorula*, and *Starmerella*. These yeasts showed 98 - 99 % identity and 97 - 100 % query coverage. All E-values resulting from the BLASTn alignment were recorded as zero. The identity percentages and the corresponding GenBank accession numbers of the isolated oleaginous yeasts are presented in **Table 2**.

Table 2 Identity percentages of oleaginous yeasts isolated from soils in paddy fields based on the alignment of ITS regions.

Oleaginous yeast isolate	Closely related yeast	GenBank accession number (NCBI database)	Query coverage (%)	Identity (%)	GenBank accession number (Submitted)
RYA02	*Debaryomyces hansenii* strain AC2-3	MW895058.1	100	99.66	PQ637152
RYA07	*Rhodotorula mucilaginosa* isolate 2	MG020687.1	97	99.66	PQ637154
RYA08	*Meyerozyma caribbica* isolate B-WHX-12-26	KC544483.1	98	99.65	PQ637155
CTB06	*Starmerella bacillaris* strain CZ12	KP281428.1	100	98.70	PQ637153
CTB12	*Debaryomyces nepalensis* strain AUMC 11208	KY495776.1	99	99.07	PQ637151
CNC10	*Starmerella bacillaris* strain BZL-128	MN371903.1	98	99.76	PQ637150

Note: The identity results were analyzed using the BlastN program on November 24, 2024.

A circular phylogenetic tree of the isolated oleaginous yeasts was constructed using the BIONJ algorithm, supported by 10,000 bootstrap replications. The tree identified 2 main phylogenetic clades: Ascomycota and Basidiomycota. The bootstrap values for the representative yeast strains sourced from GenBank within these clades ranged from 42 to 100. The isolate RYA07 was grouped with *Rhodotorula* within the Basidiomycota clade. In the Ascomycota clade, the subclades included *Debaryomyces*, *Meyerozyma*, and *Starmerella*. The isolates RYA02 and CTB12 were classified under the *Debaryomyces* subclade, while CTB06 and CNC10 were placed in the *Starmerella* subclade. Finally, the isolate RYA08 was assigned to the *Meyerozyma* subclade. The circular phylogenetic tree depicting the oleaginous yeast isolates is shown as **Figure 4**.

The isolated oleaginous yeasts were identified as closely related based on the BLASTn alignment results of their ITS nucleotide sequences and the bootstrap values obtained from the phylogenetic tree. All nucleotide sequences of the ITS genes have been submitted in the GenBank database at NCBI, under the accession numbers PQ637150, PQ637151, PQ637152, PQ637153, PQ637154, and PQ637155. A summary of the deposited GenBank accession numbers for the isolated oleaginous yeasts is presented in **Table 2**.

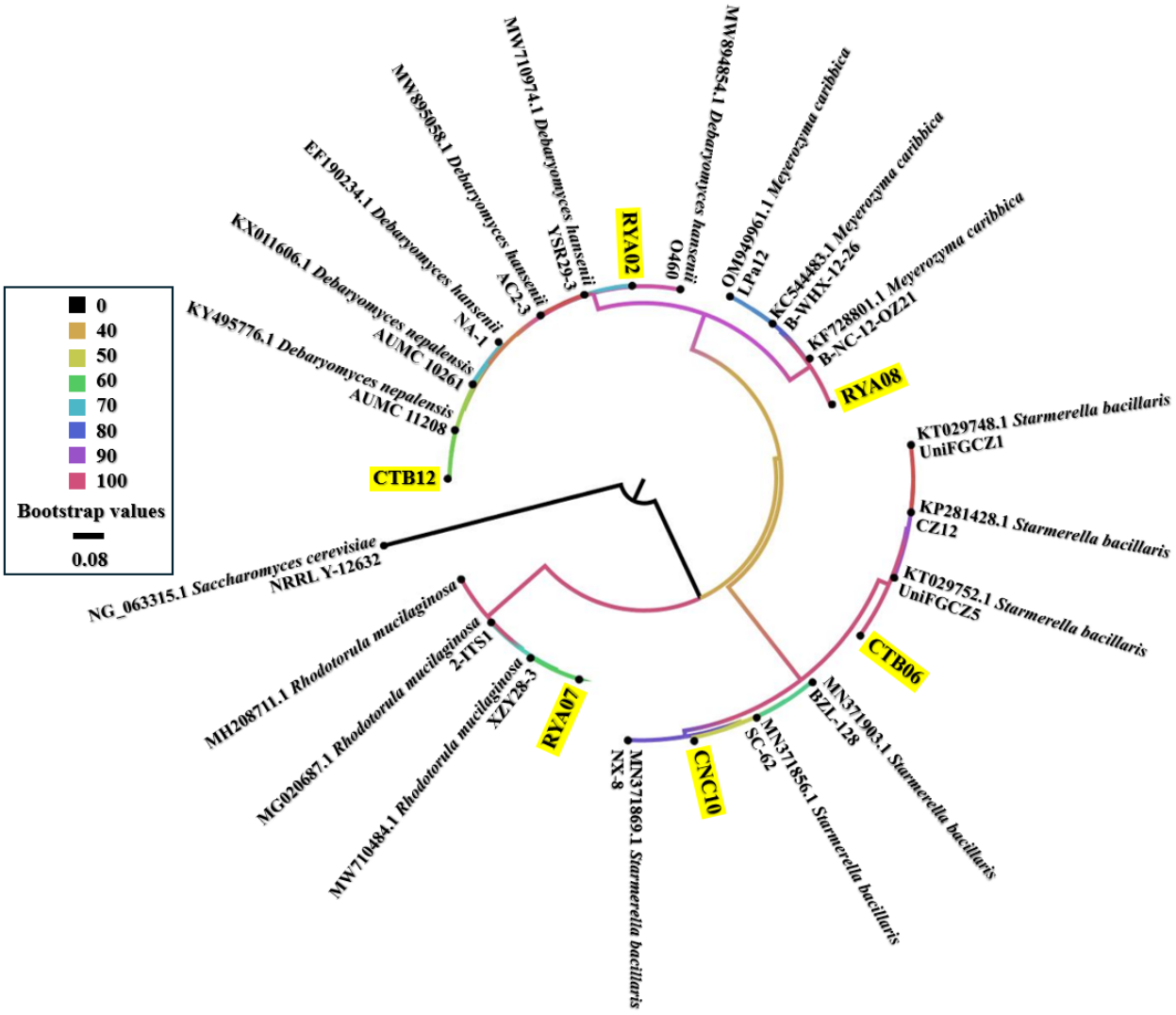

Figure 4 Circular phylogenetic tree of isolated soil-derived oleaginous yeasts created using the algorithm of BIONJ with 10,000 bootstraps. The analysis of the phylogenetic tree was conducted using Seaview and FigTree software.

The isolate RYA07 was identified as *Rhodotorula mucilaginosa* strain RYA07, with the GenBank accession number PQ637154. *R. mucilaginosa* is a species of red yeast in the Basidiomycota phylum and is commonly found in various environments, including the air, animals, plants, sea, and soil [23,24]. This yeast is known to produce carotenoid pigments such as β-carotene, torulene, and torularhodin, which give yeast colonies their orange, pinkish, or red color [25]. Moreover, *R. mucilaginosa* is known for its oleaginous characteristics, allowing it to produce substantial amounts of microbial oils or single-cell oils. A study on oleaginous yeast isolated from traditional fermented foods and beverages showed that the *R. mucilaginosa* strain R2 exhibited a maximum lipid content of 21.63 % (w/w) after 96 h of growth [26]. Consequently, the lipids produced by *R. mucilaginosa* hold potential for applications in biodiesel production [27].

The isolates RYA02 and CTB12 have been designated as *Debaryomyces hansenii* strain RYA02

and *D. nepalensis* strain CTB12, with the GenBank accession numbers PQ637152 and PQ637151, respectively. *Debaryomyces* is a genus of yeasts belonging to the Ascomycota phylum and is commonly found in natural substrates [28]. The *D. hansenii* yeast is a promising target for both basic and applied biotechnological research [29]. *D. nepalensis* has been isolated from various environments, including rotten apples [30]. This species has been studied for its ability to produce valuable metabolites such as ethanol and arabitol [30]. A recent study on soil-derived oleaginous yeasts reported that *Debaryomyces* sp. strain PP1 is a promising candidate for the production of microbial oils [13].

The isolate RYA08 was identified as the *Meyerozyma caribbica* strain RYA08, with the GenBank accession numbers PQ637155. It is a yeast species that belongs to the Ascomycota phylum and is part of the *M. guilliermondii* species complex [31]. This species has been isolated from various environments, including soil samples [32]. *M. caribbica* has been utilized in biorefineries for the production of ethanol and xylitol [32]. Several strains of *M. caribbica* are known to accumulate intracellular storage lipids and are considered oleaginous species [2,33,34].

The isolates CTB06 and CNC10 have been identified as strains of *Starmerella bacillaris*, designated as *S. bacillaris* strain CTB06 and *S. bacillaris* strain CNC10, with GenBank accession numbers PQ637153 and PQ637150, respectively. *S. bacillaris* is a non-*Saccharomyces* yeast classified within the phylum Ascomycota. This yeast is found in the microbial community on grape surfaces and in oenological environments [35]. It has also been isolated from fruits, fruit-associated insects, and soil samples [35]. *S. bacillaris* has been considered a potential co-starter yeast species for industrial mixed fermentations [36]. In this study, the 2 isolated strains, CTB06 and CNC10, are regarded as oleaginous yeasts, exhibiting intracellular lipid contents of 20.41 and 20.49 % (w/w), respectively. Although no direct evidence from other studies indicates that *S. bacillaris* strains can accumulate intracellular lipid levels exceeding 20 % of their dry weight. In addition, other species of *Starmerella* yeast, such as *S. bombicola*, have been classified as oleaginous [37].

The fatty acid profile of intracellular lipids (microbial oils) from the oleaginous *R. mucilaginosa* strain RYA07

Rhodotorula mucilaginosa strain RYA07 was identified as the oleaginous yeast with the highest intracellular lipid content in this study. This strain was cultured in GYP medium to promote lipid accumulation, after which the lipids were extracted. The extracted intracellular lipids were converted into fatty acid methyl esters (FAMEs) and analyzed for their fatty acid profiles using gas chromatography with flame ionization detection (GC-FID). The GC-FID analysis revealed that the intracellular lipids consisted of 11 fatty acids, ranging from C10 to C23. These fatty acids were classified into 6 saturated types and 5 unsaturated types. The identified saturated fatty acids included C10:0, C14:0, C15:0, C16:0, C18:0, and C23. The unsaturated fatty acids noted were C16:1, C18:1n9c, C18:2n6c, C18:3n3, and C22:2. Among the intracellular lipids of *R. mucilaginosa* strain RYA07, the primary fatty acids were palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1n9c), with respective percentages of 41.304, 37.348, and 10.422 %. The GC-FID chromatogram and the FAME profile for the oleaginous *R. mucilaginosa* strain RYA07 are presented in **Figure 5** and **Table 3**, respectively.

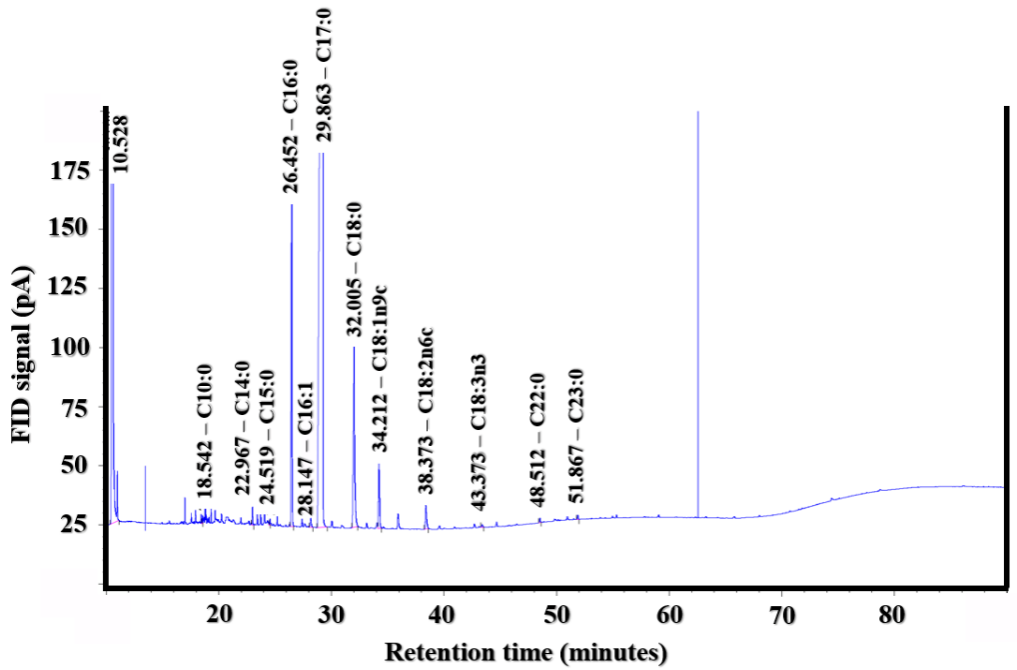

Figure 5 The GC-FID chromatogram displays the fatty acid composition of intracellular lipids extracted from the *R. mucilaginosa* strain RYA07. Heptadecanoic acid methyl ester (C17:0) was utilized as the internal standard, measured at a retention time of 29.863 min in this experiment.

Table 3 The profile of fatty acid methyl esters (FAME) obtained from intracellular lipids extracted from the *R. mucilaginosa* strain RYA07.

FAMEs	Retention time (min)	Peak area (pA*s)	Percentage of FAMEs in intracellular lipids (%) of *R. mucilaginosa* strain RYA07
Capric AME (C10:0)	18.542	9.370	0.551
Myristic AME (C14:0)	22.967	26.590	1.461
Pentadecanoic AME (C15:0)	24.519	10.780	0.588
Palmitic AME (C16:0)	26.452	752.445	41.304
Palmitoleic AME (C16:1)	28.147	25.340	1.404
Stearic AME (C18:0)	32.005	630.333	37.348
Oleic AME (C18:1n9c)	34.212	181.431	10.422
Linoleic AME (C18:2n6c)	38.373	80.134	4.732
α-Linolenic AME (C18:3n3)	43.373	7.115	0.443
Cis-13,16-docosadienoic AME (C22:2)	48.512	9.861	0.835
Tricosanoic AME (C23:0)	51.867	9.331	0.912
Total			100.000

R. mucilaginosa is recognized for its biotechnological significance due to its ability to produce substantial amounts of lipids. The lipids synthesized by *R. mucilaginosa* mainly consist of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) [25]. A previous study on cultivating oleaginous *R. mucilaginosa* in an airlift bioreactor found that the 3 major components in its fatty acid profile were palmitic acid (C16:0), oleic acid

(C18:1), and linoleic acid (C18:2) [38]. This finding relates to another study on the cultivation of the oleaginous *R. mucilaginosa* strain IIPL32 in a split-column airlift reactor, which reported that the major fatty acid components of this strain were palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), and linoleic acid (C18:2) [39]. It is important to note that differences in fatty acid profiles may exist among certain strains, influenced by various culture conditions.

Microbial lipids produced by the *R. mucilaginosa* strain RYA07 have potential applications in biofuels, oleochemicals, and various other industries. The primary fatty acids identified in this strain include palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1n9c). Both palmitic acid (C16:0) and stearic acid (C18:0) are commonly found in various vegetable oils [40] and are recommended for biodiesel production [41]. Carta *et al.* [42] have reported that palmitic acid is a significant component of palm oil, comprising 44 % of its total fats. It also occurs in significant amounts in meat and dairy products, where it makes up 50 - 60 % of the total fats. Other sources include cocoa butter, which contains 26 % palmitic acid, and olive oil, which ranges from 8 to 20 %. Notably, the amount of palmitic acid accumulated in the *R. mucilaginosa* strain RYA07 exceeds that found in these commonly reported sources. Stearic acid is produced through the hydrolysis of animal fat or by hydrogenating vegetable oil. It is an inexpensive fatty acid widely used in various industrial food and non-food applications [43]. Oleic acid (C18:1n9c) is an essential component in foods, cosmetics, and pharmaceuticals. It has been reported to possess anti-inflammatory properties and plays a role in activating various immune cell pathways [44]. Additionally, the R. *mucilaginosa* strain RYA07 accumulates linoleic acid (C18:2n6c) at a level of 4.732 %. This fatty acid is essential; the human body cannot synthesize it and must obtain it through diet. A deficiency in linoleic acid can result in scaly skin lesions, growth retardation, and thrombocytopenia [45].

Conclusions

This study successfully isolated and screened six strains of soil-derived oleaginous yeasts from paddy fields in 3 provinces of Thailand. These yeasts were capable of accumulating intracellular lipids ranging from 20.41 ± 2.24 to 44.11 ± 3.27 % (w/w) in GYP medium after 72 h of incubation. They were molecular genetically identified and phylogenetically analyzed, belonging to 4 genera: *Debaryomyces*, *Meyerozyma*, *Rhodotorula*, and *Starmerella*. Among the six strains, *R. mucilaginosa* strain RYA07 demonstrated the highest lipid accumulation, with intracellular lipid content of 44.11 % (w/w). Its fatty acid profile showed predominant fatty acids, including palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1n9c). These fatty acids are similar to those found in various vegetable oils. Therefore, the fatty acids accumulated by *R. mucilaginosa* strain RYA07 could be utilized in biofuel and oleochemical production. Further studies could focus on optimizing culture conditions to enhance microbial oil production. Additionally, the seasonal impact on the diversity of oleaginous yeasts and their fatty acid profiles should be investigated.

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References

[1] S Maina, C Pateraki, N Kopsahelis, S Paramithiotis, EH Drosinos, S Papanikolaou and A Koutinas. Microbial oil production from various carbon sources by newly isolated oleaginous yeasts. *Engineering in Life Sciences* 2017; **17(3)**, 333-344.

[2] S Planonth and A Chantarasiri. The oleaginous yeast *Pichia manshurica* isolated from *Lansium domesticum* fruit in Thailand and its fatty acid composition of single cell oil. *Biodiversitas* 2022; **23(2)**, 801-809.

[3] S Ugur, B Zieniuk and A Fabiszewska. Nutritional and medicinal properties of microbial oil. *Applied Sciences* 2024; **14(10)**, 4232.

[4] A Patel, D Karageorgou, E Rova, P Katapodis, U Rova, P Christakopoulos and L Matsakas. An overview of potential oleaginous microorganisms and their role in biodiesel and omega-3 fatty acid-based industries. *Microorganisms* 2020; **8(3)**, 434.

[5] F Abeln and CJ Chuck. The history, state of the art and future prospects for oleaginous yeast research. *Microbial Cell Factories* 2021; **20**, 221.

[6] R Poontawee, W Lorliam, P Polburee and S Limtong. Oleaginous yeasts: Biodiversity and cultivation. *Fungal Biology Reviews* 2023; **44**, 100295.

[7] S Sapsirisuk, P Polburee, W Lorliam and S Limtong. Discovery of oleaginous yeast from mountain forest soil in Thailand. *Journal of Fungi* 2022; **8(10)**, 1100.

[8] M Vincent, MC Hung, PRM Baran, AS Azahari and DSA Adeni. Isolation, identification and diversity of oleaginous yeasts from Kuching, Sarawak, Malaysia. *Biodiversitas* 2018; **19(4)**, 1266-1272.

[9] S Wongchamrearn, P Boontanom, S Ungwiwatkul, N Emnin and A Chantarasiri. Short communication: The oleaginous *Candida tropicalis* isolated from mangrove soil in eastern Thailand and the fatty acid composition profile of its intracellular lipids. *Biodiversitas* 2023; **24(9)**, 5088-5095.

[10] S Kunthiphun, P Chokreansukchai, P Hondee, S Tanasupawat and A Savarajara. Diversity and characterization of cultivable oleaginous yeasts isolated from mangrove forests. *World Journal of Microbiology and Biotechnology* 2018; **34**, 125.

[11] I Schulze, S Hansen, S Großhans, T Rudszuck, K Ochsenreither, C Syldatk and A Neumann. Characterization of newly isolated oleaginous yeasts-*Cryptococcus podzolicus*, *Trichosporon porosum* and *Pichia segobiensis*. *AMB Express* 2014; **4**, 24.

[12] PE Diaz, C Aranda, O Martínez, R Godoy, A Gonzales and E Valenzuela. Characterization of yeast in hapludands soil with biotechnological potential. *Journal of Soil Science and Plant Nutrition* 2017; **17(4)**, 948-965.

[13] P Diaz-Navarrete, L Marileo, H Madrid, C Belezaca-Pinargote and P Dantagnan. Lipid production from native oleaginous yeasts isolated from southern Chilean soil cultivated in industrial vinasse residues. *Microorganisms* 2023; **11(10)**, 2516.

[14] K Amprayn, MT Rose, M Kecskes, L Pereg, HT Nguyen and IR Kennedy. Plant growth promoting characteristics of soil yeast (*Candida tropicalis* HY) and its effectiveness for promoting rice growth. *Applied Soil Ecology* 2012; **61**, 295-299.

[15] AM Yurkov. Yeasts of the soil-obscure but precious. *Yeast* 2018; **35(5)**, 369-378.

[16] C Sripodok, A Thammasittirong and SN Thammasittiron. Antifungal activity of soil yeast (*Lachancea kluyveri* sp132) against rice pathogenic fungi and its plant growth promoting activity. *Journal of the International Society for Southeast Asian Agricultural Sciences* 2019; **25(1)**, 55-65.

[17] L Xu, H Zhang, C Wang, S Wei, B Zhang, F Wu and Y Tang. Paddy rice mapping in Thailand using time-series Sentinel-1 data and deep learning model. *Remote Sensing* 2021; **13(19)**, 3994.

[18] A Chantarasiri and S Ungwiwatkul. Effects of CO_2 aeration and light supply on the growth and lipid production of a locally isolated microalga, *Chlorella variabilis* RSM09. *Applied Sciences* 2024; **14(22)**, 10512.

[19] A Caporusso, A Capece and ID Bari. Oleaginous yeasts as cell factories for the sustainable production of microbial lipids by the valorization of agri-food wastes. *Fermentation* 2021; **7(2)**, 50.

[20] AM Kot, S Błażejak, A Kurcz, J Brys, I Gientka, A Bzducha-Wróbel, M Maliszewska and L Reczek. Effect of initial pH of medium with potato wastewater and glycerol on protein, lipid and carotenoid biosynthesis by *Rhodotorula glutinis*. *Electronic Journal of Biotechnology* 2017; **27**, 25-31.

[21] TM Jiru, D Abate, N Kiggundu, C Pohl and Ma Groenewald. Oleaginous yeasts from Ethiopia. *AMB Express* 2016; **6**, 78.

[22] SE Kantar, A Khelfa, E Vorobiev and M Koubaa. Strategies for increasing lipid accumulation and recovery from *Y. lipolytica*: A review. *Oilseeds & Fats Crops and Lipids* 2021; **28**, 51.

[23] MR Capoor, S Aggarwal, C Raghvan, DK Gupta, AK Jain and R Chaudhary. Clinical and microbiological characteristics of *Rhodotorula mucilaginosa* infections in a tertiary-care facility. *Indian Journal of Medical Microbiology* 2014; **32(3)**, 304-309.

[24] P Hu, J Mao, Y Zeng, Z Sun, H Deng, C Chen, W Sun and Z Tang. Isolation, identification, and function of *Rhodotorula mucilaginosa* TZR2014 and its effects on the growth and health of weaned

piglets. *Frontiers in Microbiology* 2022; **13**, 922136.

[25] Z Li, C Li, P Cheng and G Yu. *Rhodotorula mucilaginosa*-alternative sources of natural carotenoids, lipids, and enzymes for industrial use. *Heliyon* 2022; **8(11)**, e11505.

[26] P Bardhan, K Gupta, S Kishor, P Chattopadhyay, C Chaliha, E Kalita, VV Goud and M Mandal. Oleaginous yeasts isolated from traditional fermented foods and beverages of Manipur and Mizoram, India, as a potent source of microbial lipids for biodiesel production. *Annals of Microbiology* 2020; **70**, 27.

[27] S Tsai, H Yu and C Lin. The Potential of the oil-producing oleaginous yeast *Rhodotorula mucilaginosa* for sustainable production of bio-oil energy. *Processes* 2022; **10(2)**, 336.

[28] M Desnos-Ollivier, M Ragon, V Robert, D Raoux, JC Gantier and F Dromer. *Debaryomyces hansenii* (*Candida famata*), a rare human fungal pathogen often misidentified as *Pichia guilliermondii* (*Candida guilliermondii*). *Journal of Clinical Microbiology* 2008; **46(10)**, 3237-3242.

[29] L Ramos-Moreno, F Ruiz-Perez, E Rodriguez-Castro and J Ramos. *Debaryomyces hansenii* is a real tool to improve a diversity of characteristics in sausages and dry-meat products. *Microorganisms* 2021; **9(7)**, 1512.

[30] H Kumdam, SN Murthy and SN Gummadi. Production of ethanol and arabitol by *Debaryomyces nepalensis*: Influence of process parameters. *AMB Express* 2013; **3**, 23.

[31] L De Marco, S Epis, A Capone, E Martin, J Bozic, E Crotti, I Ricci and D Sassera. The genomes of four *Meyerozyma caribbica* isolates and novel insights into the *Meyerozyma guilliermondii* species complex. *Genes Genomes Genetics* 2018; **8(3)**, 755-759.

[32] BRA Alencar, RAA de Freitas, VEP Guimaaes, RK Silva, C Elsztein, SPD Silva, ED Dutra, MADM Junior and RBD Souza. *Meyerozyma caribbica* isolated from vinasse-irrigated sugarcane plantation soil: A promising yeast for ethanol and xylitol production in biorefineries. *Journal of Fungi* 2023; **9(8)**, 789.

[33] P Polburee, W Yongmanitchai, N Lertwattanasakul, T Ohashi, K Fujiyama and S Limtong. Characterization of oleaginous yeasts accumulating high levels of lipid when cultivated in glycerol and their potential for lipid production from biodiesel-derived crude glycerol. *Fungal Biology* 2015; **119(12)**, 1194-1204.

[34] H Chebbi, D Leiva-Candia, M Carmona-Cabello, A Jaouani and MP Dorado. Biodiesel production from microbial oil provided by oleaginous yeasts from olive oil mill wastewater growing on industrial glycerol. *Industrial Crops and Products* 2019; **139**, 111535.

[35] C Nadai, VDS Duarte, J Sica, S Vincenzi, M Carlot, A Giacomini and V Corich. *Starmerella bacillaris* released in vineyards at different concentrations influences wine glycerol content depending on the vinification protocols. *Foods* 2023; **12(1)**, 3.

[36] MLR Eder and AL Rosa. Genetic, physiological, and industrial aspects of the fructophilic non-*Saccharomyces* yeast species, *Starmerella bacillaris*. *Fermentation* 2021; **7(2)**, 87.

[37] JMS Lopez, S Jezierska, AE Kocabey, J Lee, R Schneiter and INAV Bogaert. The oleaginous yeast *Starmerella bombicola* reveals limitations of *Saccharomyces cerevisiae* as a model for fatty acid transport studies. *FEMS Yeast Research* 2022; **22(1)**, foac054.

[38] HW Y, YT Liao and YX Liu. Cultivation of oleaginous *Rhodotorula mucilaginosa* in airlift bioreactor by using seawater. *Journal of Bioscience and Bioengineering* 2016; **121(2)**, 209-212.

[39] D Dasgupta, T Sharma, A Bhatt, S Bandhu and D Ghosh. Cultivation of oleaginous yeast *Rhodotorula mucilaginosa* IIPL32 in split column airlift reactor and its influence on fuel properties. *Biocatalysis and Agricultural Biotechnology* 2017; **10**, 308-316.

[40] EG Giakoumis. Analysis of 22 vegetable oils' physicochemical properties and fatty acid composition on a statistical basis, and correlation with the degree of unsaturation. *Renewable Energy* 2018; **126**, 403-419.

[41] A Areesirisuk, CH Chiu, TB Yen, CH Liu and JH Guo. A novel oleaginous yeast strain with high lipid productivity and its application to alternative

biodiesel production. *Applied Biochemistry and Microbiology* 2015; **51**, 411-418.

[42] G Carta, E Murru, S Banni and C Manca. Palmitic acid: Physiological role, metabolism and nutritional implications. *Frontiers in Physiology* 2017; **8**, 902.

[43] P Hiremath, K Nuguru and V Agrahari. *Chapter 8-Material attributes and their impact on wet granulation process performance*. Academic Press, Massachusetts, 2019.

[44] C Carrillo, MDM Cavia and S Alonso-Torre. Role of oleic acid in immune system; mechanism of action; a review. *Nutricion Hospitalaria* 2012; **27(4)**, 978-990.

[45] J Whelan and K Fritsche. Linoleic acid. *Advances in Nutrition* 2013; **4(3)**, 311-312.