BIODIVERSITAS
Volume 19, Number 4, July 2018
Pages: 1266-1272

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190412

Isolation, identification and diversity of oleaginous yeasts from Kuching, Sarawak, Malaysia

MICKY VINCENT♥, HUANG CHAI HUNG, PATRICIA ROWENA MARK BARAN, AFIZUL SAFWAN AZAHARI, DAYANG SALWANI AWANG ADENI

Resource Biotechnology Program, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia. ♥email: vmicky@unimas.my

Manuscript received: 25 May 2018. Revision accepted: 6 June 2018.

Abstract. *Vincent M, Hung MC, Baran PRM, Azahari AS, Adeni DSA. 2018. Isolation, identification and diversity of oleaginous yeasts from Kuching, Sarawak, Malaysia. Biodiversitas 19: 1266-1272.* The present study was performed to isolate, identify and determine the diversity of oleaginous yeasts from various sources in Kuching, Sarawak (Malaysia). Microscopic observations via light and scanning electron microscope (SEM) indicated that the yeast isolates were in sizes ranging from 2-3 µm in width and 4-8 µm in length, typical of most unicellular ascomycotic fungi. Polymerase Chain Reaction (PCR) and molecular identification performed on the yeast isolates, targeting the D1/D2 region of the 26S rDNA, identified 6 yeast species from the 21 isolates, namely *Pichia manshurica* (5/21), *Candida krusei* (8/21), *Candida parapsilosis* (1/21), *Pichia guilliermondii* (2/21), *Clavispora lusitaniae* (1/21) and *Kluyveromyces marxianus* (4/21). All 21 yeast isolates accumulated intracellular lipids when grown in nitrogen-limited medium, as tested via Sudan IV staining. The present study is the first to document the production of lipids bodies in *C. krusei*, *C. parapsilosis*, and *C. lusitaniae*. Further investigations to assess the growth kinetics, lipid production efficiencies and lipids profiles of these oleaginous yeasts may provide insights into the possible utilization of these isolates for a variety of scientific, technical and industrial applications.

Keywords: Fungal lipid, lipid accumulation, oleaginous yeasts, Polymerase Chain Reaction (PCR), Triacylglycerols (TAGs)

Abbreviations: MEGA4: Molecular Evolutionary Genetic Analysis, NCBI: National Center for Biotechnology Information, PCR: Polymerase Chain Reaction, RBDC: Rose-Bengal Dichloran Chloramphenicol, SEM: Scanning Electron Microscope, TAGs: Triacylglycerols, YMB: Yeast Malt Broth

INTRODUCTION

There are growing interests in lipids or triacylglycerols (TAGs) from oleaginous yeasts in recent years (Areesirisuk et al. 2015; Kahr et al. 2015; Wang et al. 2016; Ramírez-Castrillón et al. 2017; Vincent et al. 2018). This fungal group possesses the ability to convert agro and industrial residues into TAGs that are prime alternatives to lipids that are animal and/or plant-based (Pan et al. 2009; Aghbashlo and Demirbas 2016). More importantly, it has been reported that several of these edible lipids are not found in the plant or animal kingdom, especially lipids containing polyunsaturated fatty acids (PUFAs) that are essential fatty acids known to be health promoting (Akpinar-Bayizit 2014). Commonly considered as storage lipids, TAGs are important for the synthesis of carbon skeletons in other biomolecules and as energy during growth and development. The other major TAGs components are sterols, squalene, and terpenes (Akpinar-Bayizit 2014).

In the production of biodiesel, TAGs are indispensable as raw material (Vincent et al. 2014; Hanif et al. 2017). Currently, approximately 70 to 75% of global biodiesel is produced from vegetable oils such as palm oil and soybean oil, animal fat as well as waste cooking oil (Li et al. 2011; Tremblay and Montpetit 2017). Manufacturing biodiesel from vegetable oils, however, is undesirable as this practice competes with the use of these edible oils for human consumption that prevents the long-term development and large-scale production of biodiesel (Li et al. 2011; Aghbashlo and Demirbas 2016). In addition, production of biodiesel is costly when plant oil is used as raw materials (Li et al. 2011). Therefore, TAGs from oleaginous yeasts offer a promising solution, as under optimal conditions, oleaginous yeasts can accumulate up to 70% of their cell weight in the form of TAGs (Pan et al. 2009; Ramírez-Castrillón et al. 2017).

TAGs from oleaginous yeasts are similar to vegetable oils in their fatty acids composition (Ma 2006; Li et al. 2011; Calvey et al. 2016). However, when compared to oil-producing plants, the culturing of oleaginous microorganisms are less laborious and are not affected by common agricultural problems such as land acreage, soil conditions and/or climates (Vincent et al. 2018). Another major advantage of using oleaginous yeasts is their ability to grow in extreme environments such as low temperature and low oxygen availabilities (Calvey et al. 2016). Furthermore, their short life spans enable the rapid production of high-value TAGs that can be used as food, feed, and biodiesel feedstock (Akpinar-Bayizit 2014; Wang et al. 2016).

A large variety of oleaginous yeast genera can be found in soil, common surfaces and fruit products. Of the

estimated 600 different yeast species, only 25 or so are reported to be able to accumulate more than 20% lipid (Ratledge and Wynn 2002). These oleaginous yeast members are part of a larger group of oleaginous fungi such as *Candida utilis*, *Lipomyces starkeyi*, *Trichosporon cutaneum*, and *Rhodotorula minuta* (Pan et al. 2009; Brandenburg et al. 2016, Vincent et al. 2018).

Although, many studies have been conducted on the lipid accumulation in oleaginous yeast, these studies have been confined to the optimization of lipid production using established species such as *Lipomyces starkeyi*, *Candida lipolytica* and *Yarrowia lipolytica* (Li et al. 2011, Kahr et al. 2015; Brandenburg et al. 2016; Wang et al. 2016). Limited studies have been done to isolate other oleaginous microorganisms from the natural environment. Therefore, it is vital to screen for more lipid accumulating yeasts, as there is abundance of oil-producing yeasts that may possess potential novel industrial applications that are yet to be discovered and exploited (Pan et al. 2009; Li et al. 2011).

In this study, oleaginous yeasts were isolated from various food-related sources such as fruit surfaces, fruit stalls, sago plant effluent, sugar cane juice and agricultural soil samples. After microscopic examination of isolated yeasts under light and scanning electron microscope, lipid assay via Sudan IV staining was carried out to screen for their ability to produce lipids. Once their lipid accumulation abilities have been confirmed, the identities of the yeast isolates were established via DNA sequence analyses.

MATERIALS AND METHODS

Isolation of oleaginous yeasts

Samples from fruit surfaces, tabletop swabs, sugar cane juice, sago effluent and agricultural soils were collected from the Kuching, Sarawak (Malaysia) area. Fruit surfaces and table tops were sampled by swabbing using sterile wet cotton buds before placing in universal bottles containing 10 mL of Yeast Malt Broth (YMB) (Sigma, USA) supplemented with 100 µL of 40 mg mL^{-1} chloramphenicol (Duchefa, Netherlands). Samples from the sugar cane juice and sago effluent were prepared by diluting 10 mL of samples into enrichment medium which consisted of 90 mL YMB (1:10 dilution) and 1 mL of 40 mg mL^{-1} chloramphenicol. For the soil samples, 10 g of soil sample was mixed into 90 mL YMB supplemented with 1 mL of 40 mg mL^{-1} chloramphenicol. The samples collected were incubated in an incubator shaker (InnovaFM 4000, New Jersey, USA) at 28ºC for 48 h with shaking at 150 rpm.

Upon enrichment, serial dilutions were made from the enriched-culture broths. In brief, 1 mL of the culture was serially diluted from 10^{-1} to 10^{-6} folds. Next, 300 µL of broth from each dilution was then plated onto Rose-Bengal Dichloran (RBD) agar (Oxoid, England) supplemented with chloramphenicol, by using a sterile 'hockey stick' glass rod. The plates were then incubated at 30ºC for 48 h. Subculture of chosen colonies was then carried out on the agar plates containing the same medium and incubated at 30ºC for another 48 h. Distinct single colonies were selected and grown in liquid medium containing 10 mL YMB and 100 µL chloramphenicol. The purified yeast cultures were then added with 20% glycerol (Daejung, Korea) and stored in a -20ºC freezer.

Morphological examination of the yeast isolates

Microscopic observations were performed to observe the morphological and size characteristics of the yeast isolates under a light microscope (Olympus BX51, Japan). This was supplemented with scanning electron microscopy (SEM) visualization.

Lipid accumulation in the yeast isolates

To perform the lipid accumulation study, each yeast isolate was transferred into a conical flask containing double strength YMB. After an incubation period of 72 h, an equal volume of 10% (w/v) glucose (Daejung, Korea) solution was added to provide excessive carbon source in the medium to induce intracellular lipogenesis. This process was conducted for another 72 h.

Detection of lipid via Sudan IV staining

To test the yeast isolates for lipid bodies, 300 µL of culture was mixed with 300 µL of Sudan IV solution. After 1 h of staining, the culture was fixed onto a glass slide and observed under a light microscope for the presence of bright red colored fat globules within the yeast cells.

Molecular identification of the yeast isolates

DNA extraction was performed using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratory, USA) according to the manufacturer instruction. All DNA samples were then subjected to polymerase chain reaction (PCR) amplification using a pair of universal fungi primers, 26S-D1F (5'-GCATATCAAAAGCGGAGGAAAAG-3') and 26S-D2R (5'-GGTCCGTGTTTCAAGACGG-3') to generate amplicons of approximately 600 bp in sizes. Briefly, the diluted DNA (3.0 µL of 10 ng μL^{-1}) was mixed with 22.0 µL of PCR master mix, consisting of 2.5 µL of 10X PCR buffer, 1.0 µL of 10 mM dNTPs, 1.5 µL of 50 mM $MgCl_2$, 1.0 µL 5 pmol μL^{-1} of each primer, 0.3 µL Taq DNA polymerase and 14.7 µL sterile distilled water. The PCR amplification was performed in a LabCycler System (Sensoquest, Germany) as followed: initial denaturation at 95ºC for 7 min, followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 55ºC for 1 min and extension of DNA at 72ºC for 1 min; and a final elongation at 72ºC for 10 minutes.

After the PCR amplification, the products were then loaded into 1.5% agarose gel, electrophoresed and placed onto a UV transilluminator for visualization. Upon confirmation of amplicon presence, the PCR products were purified using UltraClean® PCR Clean-Up Kit (Mo Bio Laboratory, USA) and sent for DNA sequencing. The sequence data received were then analyzed by using the BLASTN algorithm and cross-referenced to the NCBI database. All sequences were deposited in the NCBI GenBank.

Phylogenetic analysis

A BlastN search of the 26S rRNA nucleotide sequences of the 21 isolates was conducted using the NCBI database for homology identification. Sequences were edited using the BioEdit and CAP3 sequence editor to trim the multiple sequences within the conserved region (Huang and Madan 1999). The compiled contig sequences were later aligned using ClustalW to construct a phylogenetic tree using the Molecular Evolutionary Genetic Analysis (version 4.0) (MEGA4) software via the neighbour-joining method (Tamura et al. 2007).

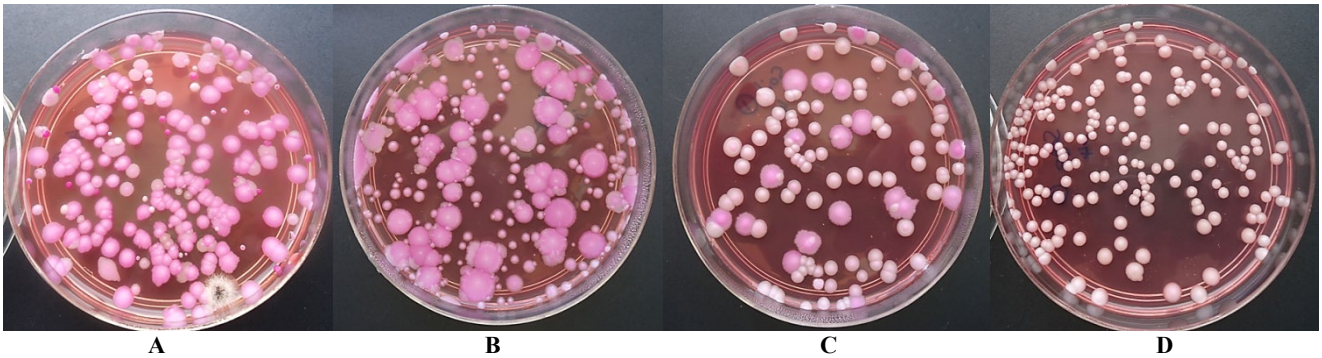
RESULTS AND DISCUSSION

In the initial stage of this study, 21 presumptive yeast colonies were selected; 9 from fruit surfaces, 8 from table tops, 1 from sugar cane juice, 1 from sago effluent and 2 from soil samples. Table 1 shows the detailed list of the yeast isolates. On Rose-Bengal Dichloran Chloramphenicol (RBDC) agar (Figure 1), the yeast colonies appeared slightly pinkish in color, consisting of smooth circular, irregular and rhizoid elevated morphologies, typical of yeast features. RBDC agar was used during initial isolation as chloramphenicol in this medium acts as a bacteriostatic agent to suppress a wide spectrum of bacterial species. According to Broadbent and Terry (1985), chloramphenicol does not negatively affect the growth of most fungi, while dichloran and rose bengal retard the growth of filamentous molds. Hence, isolation of yeasts can be made accurately because of the absent of mixed colonies of bacteria and filamentous fungi.

Table 1. Results for the identification of the yeast isolates and their lipid production assays

Origin	Designation	Species	Accession number	Lipid droplets
Table top swabs	T1A1	*Candida krusei*	KM279367	+
	T1B1	*Candida krusei*	KM279368	+
	S1A1	*Candida krusei*	KM279369	+
	S1A2	*Candida krusei*	KM279370	+
	U-MF 4	*Candida parapsilosis*	KM279373	+
	TKB5	*Pichia guilliermondii*	KM279364	+
	TKP4	*Clavispora lusitaniae*	KM279374	+
	TKW4	*Candida krusei*	KM279366	+
Soil sample	Y1S2	*Candida krusei*	KM279372	+
	MS4	*Pichia manshurica*	KM279358	+
Sago effluent	SE6	*Candida krusei*	KM279365	+
Fruit swabs (Rambutan)	U-MF 6	*Kluyveromyces marxianus*	KM279375	+
	U-MF 7	*Kluyveromyces marxianus*	KM279376	+
	U-MF 8	*Pichia manshurica*	KM279360	+
	U-MF 9	*Pichia manshurica*	KM279361	+
	U-MF 10	*Kluyveromyces marxianus*	KM279377	+
	U-MF 11	*Kluyveromyces marxianus*	KM279378	+
	U-MF 14	*Pichia manshurica*	KM279362	+
Fruit swabs (Lemon)	L5	*Pichia guilliermondii*	KM279363	+
Fruit swabs (Pineapple)	N6	*Pichia manshurica*	KM279359	+
Sugar cane juice	C1S1	*Candida krusei*	KM279371	+

Note: "+": present; "-": absent



A B C D

Figure 1. Presumptive yeast colonies on RBDC agar of samples from: A. Table top swabs, B. Sugar cane juice, C. Soil sample, D. Sago effluent

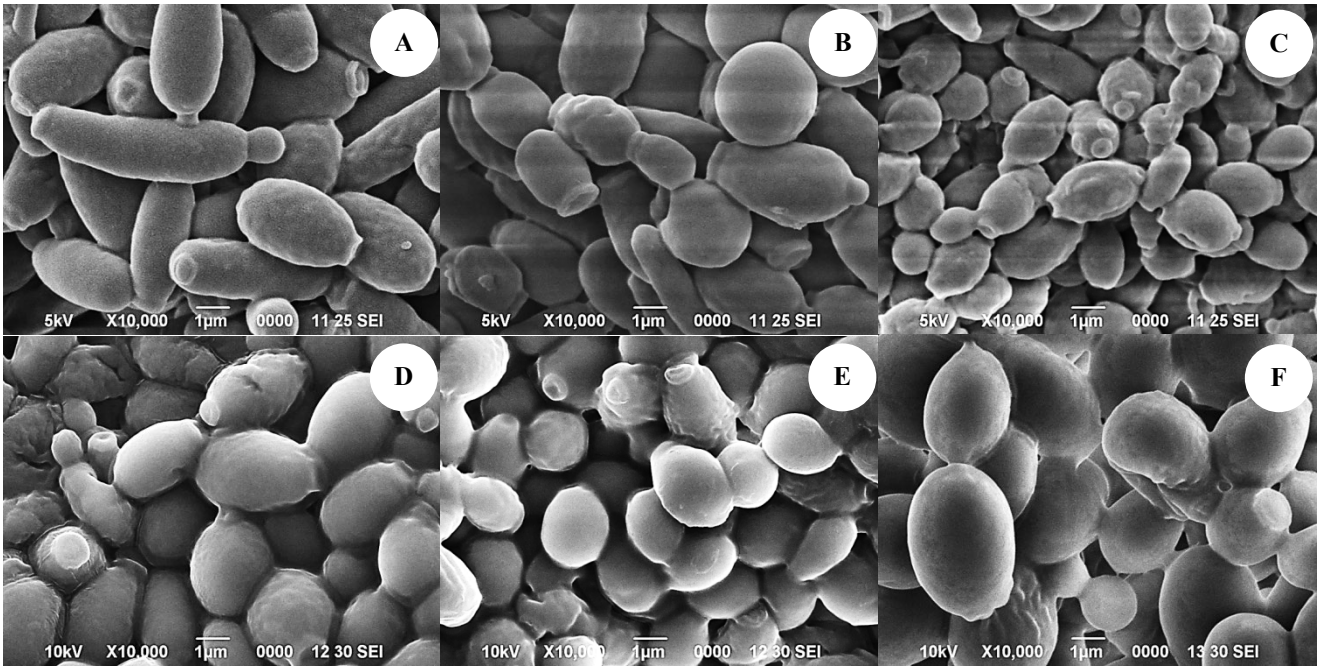


Figure 2. Scanning electron Microscope (SEM) of selected yeast cells: A. *Candida krusei*, B. *Candida parapsilosis*, C. *Candida lusitaniae*, D. *Pichia guilliermondii*, E. *Pichia manshurica*, F. *Kluyveromyces marxianus*

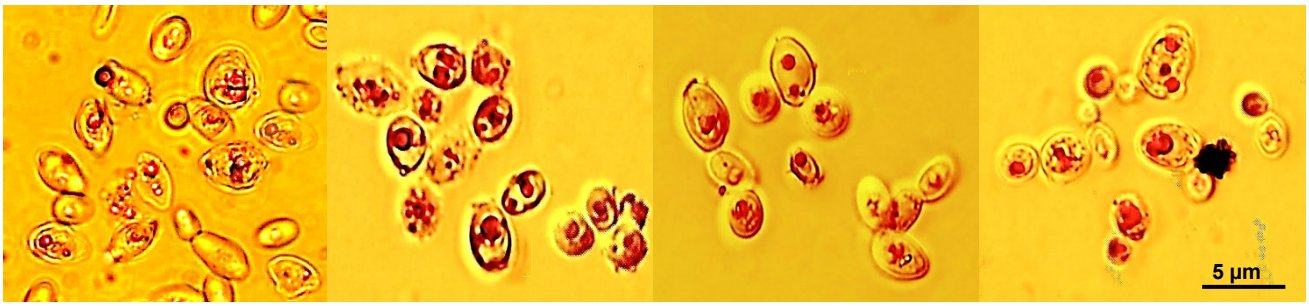


Figure 3. Lipid droplets (bright red bodies shown by arrows) in selected oleaginous yeasts cells as seen under the light microscope: A. *Candida krusei*, B. *Candida parapsilosis*, C. *Candida lusitaniae*, D. *Pichia guilliermondii*, E. *Pichia manshurica*, F. *Kluyveromyces marxianus*

When observed under the microscope, the shapes and sizes of the individual cell varied slightly, with most cells seen as ellipsoidal to oval in shape. The sizes of the yeasts were approximately 2-3 µm in width and 4-8 µm in length. Further examination under the scanning electron microscope (SEM) showed cells that are curved cylindrically at the point-end, as shown in Figure 2. The 21 isolated fungal species can also be seen projecting holoblastic budding bodies and naked asci that are phenotypic characteristics of yeasts under the phylum Ascomycota (Suh et al. 2006).

After a 72 h incubation period in a glucose-rich, nitrogen-limited medium, the yeast cells were examined for intracellular lipid production via Sudan IV staining. According to Pan et al. (2009), this procedure offers partial information on the lipid accumulation abilities of the tested yeast isolates. Sudan IV stained lipid bodies appear as reddish-orange globules within the yeast cells when observed under the light microscope. All 21 yeast isolates were shown to harbor noticeable intracellular lipid bodies, as seen in Figure 3.

Polymerase chain reaction (PCR) targeting the conserved D1/D2 regions of 26S rDNA performed on the lipid producing yeast isolates were successful in producing the desired amplicons of approximately 600 bp in sizes (Figure 4) in all the presumptive isolates. This particular locus was selected, as according to Sugita and Nishikawa (2003), conspecific fungi strains have more than 99% nucleotide similarity in the D1/D2 regions of 26S rDNA sequences, making this genetic marker highly species specific to confirm the identities of different fungi strains (Maoura et al. 2005). DNA sequences data from fungal isolates that have not been registered in data libraries can be cross-referenced against the fungal 26S rDNA database in National Center for Biotechnology Information (NCBI) by using the BLASTN algorithms. This method has been

applied in identifying 666 out of 685 fungi species (Sugita and Nishikawa 2003). BlastN analyses of PCR product sequences were cross-referenced to the NCBI database, revealing that the 21 isolates belonged to 6 yeast species; *Pichia manshurica*, *Candida krusei*, *Candida parapsilosis*, *Pichia guilliermondii*, *Clavispora lusitaniae* and *Kluyveromyces marxianus*, as presented in Table 1. From the pool of the identified yeast isolates, 16 of the 21 isolates belonged to the genera Candida (9/21) and Pichia (7/21). These two yeast groups are frequently isolated in the soil obtained from orchards in China and South Korea as reported by Pan et al. (2009) and Kang et al. (2005). Our findings are also in agreement with previous studies on the associations of *Pichia* spp. and *Kluyveromyces* spp. with fruit surfaces (Chanchaichaovivat et al. 2007; Gana et al. 2014) as well as in fruit juices (Covadonga et al. 2002).

The yeast DNA sequences obtained in this study have been deposited in the GenBank database under unique accession numbers as recorded in Table 1. These sequences were then used to perform phylogenetic analysis to study the relationship among the yeast isolates via the construction of a dendrogram as depicted in Figure 5. The dendrogram generated using the MEGA4 software (Tamura et al. 2007) showed a high degree of heterogeneity among the species. From the phylogenetic tree, 42.9% of isolates displayed >99.0% genus-level sequence homology to species belonging to the genera Candida. Majority of the *Candida* spp. belonged to *C. krusei*. Only one isolate was identified as *Candida parapsilosis*. 33.3% of all isolates had 98.0% sequence homology to *Pichia* spp. 71.4% and 28.6% of the *Pichia* spp. were *P. manshurica* and *P. guilliermondii,* respectively. The other 5 isolates were divided into two different phylogenetic groups. Four of the isolates showed 98.0% sequence homology belonging to *K. marxianus* while the remaining isolate was identified as *C. lusitaniae* with 99.0% sequence homology. *C. krusei* and *P. manshurica* clades strongly supported the bootstrap value of 100.0% as in Figure 5.

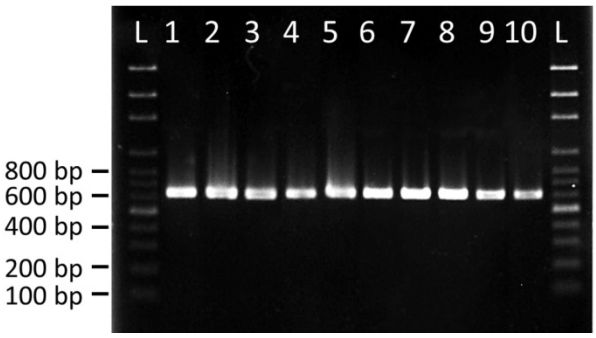


Figure 4. PCR products (approximately 600 bp) electrophoresed on 1.5% agarose gel: 1. *Candida krusei*, 2. *Candida krusei*, 3. *Candida parapsilosis*, 4. *Pichia guilliermondii*, 5. *Pichia guilliermondii*, 6. *Candida lusitaniae*, 7. *Kluyveromyces marxianus*, 8. *Kluyveromyces marxianus*, 9. *Pichia manshurica*, 10. *P. manshurica*, (L) 100 bp ladder (Fermentas, Germany)

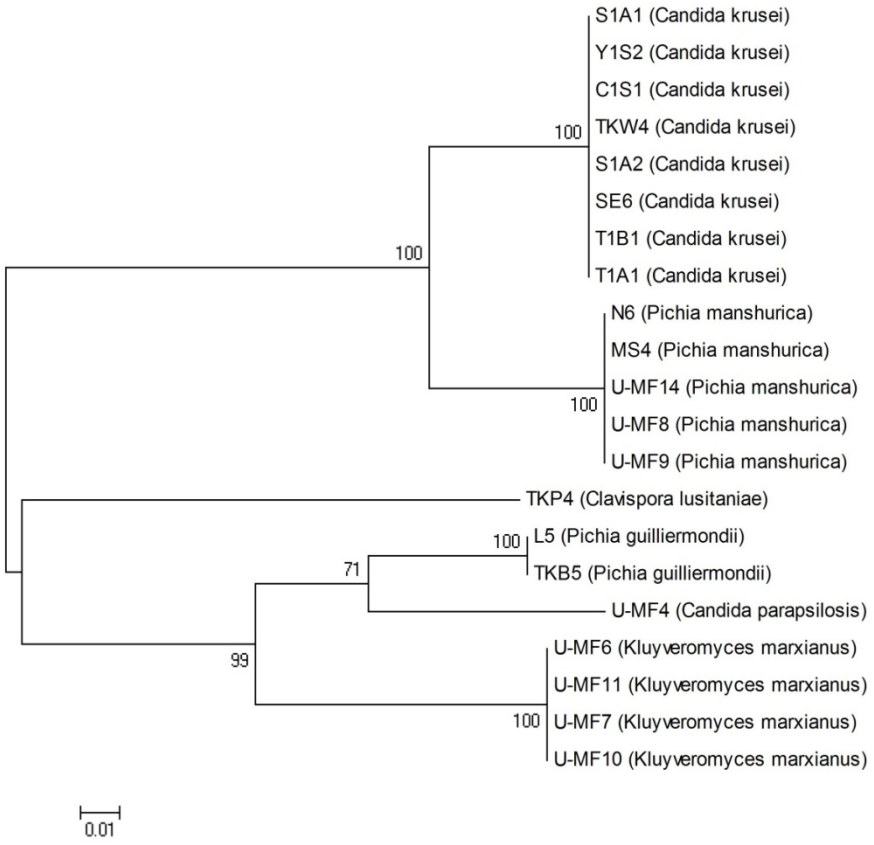


Figure 5. Phylogenetic analysis of the 21 yeast isolates analyzed using MEGA4 depicting the relationships of partial 26S rRNA sequence with close-related sequences retrieved from the NCBI database

Storage lipids are usually found within specialized organelles known as lipid particles or lipid bodies. In yeast, lipid bodies accumulate during stationary phase and can constitute up to the total lipid content of the cell (Zweytick et al. 2000; Zhao et al. 2008). Although there are limited literature that have reported on the intracellular lipid production in *P. manshurica* (Areesirisuk et al. 2015), *P. guilliermondii* (Wang et al. 2012; Ramírez-Castrillón et al. 2017) and *K. marxianus* (Tampitak et al. 2015), no solid references can be found on lipogenesis in *C. parapsilosis*, *C. krusei* and *C. lusitaniae*. Therefore, this study is the first to report lipid bodies accumulation in these three species.

The basic mechanisms of lipid accumulation in microorganisms are well documented (Zweytick et al. 2000; Pan et al. 2009). In culture medium that is high in initial C/N ratio, excess of sugars or saccharides is converted into triglycerides in lipid bodies. This occurs when nicotinamide adenine dinucleotide isocitrate dehydrogenase activities decrease from the mitochondria, causing the repression of the tricarboxylic acid cycle that is largely due to low nitrogen in the culture medium (Rossi et al. 2011). To induce lipid accumulation in yeast cells, batch fermentation system is usually performed in two stages; the propagation/growth stage and the lipogenic stage. During the propagation stage, lipid-free biomass is produced at high concentration rapidly as there is positive carbon flow toward biomass generation. This is followed by lipid accumulation in the yeast cells during the lipogenic phase when the concentration of nitrogen in the culture medium becomes limited while carbon sources are increased (Li et al. 2007; Rossi et al. 2011). Unlike other oleaginous organisms, yeasts are not only efficient lipid producers, but many studies have reported their abilities to generate lipids from various carbon sources, even from lipids present in the culture media (Iassonova et al. 2008; Zhao et al. 2008). The fatty acid contents from yeast lipids include stearic, oleic, linoleic and palmitic acids that are similar to those of plants, which mean that yeast lipids could be used as edible oils, animal feed and as biodiesel feedstock (Xin et al. 2009).

This study has successfully isolated and identified 21 yeast isolates from various food-related sources in Kuching, Sarawak (Malaysia). All the yeast isolates were found to produce intracellular lipid bodies when grown in a glucose-rich medium, making them potentially applicable in the production of edible lipids, animal feeds and as biodiesel feedstocks. Further studies can be conducted to examine their growth kinetics, lipid production efficiencies and lipids profiles may provide insights into the possible utilization of the yeast isolates for a host of scientific, technical and industrial applications. In addition, the lipids produced can be characterized from each isolate to determine the possible presence of rare and novel triglycerides which can be utilized for other more specific applications.

ACKNOWLEDGEMENTS

This study was supported by the Malaysian Ministry of Higher Education (MOHE) Fundamental Research Grant Scheme grant [FRGS/SG (01)/1286/2015 (03)] and the Tun Openg Chair grants [F07-05-ORC26/2015 (13) and F07-TOC-1518-2016].

REFERENCES

Ageitos JM, Vallejo, JA, Veiga-Crespo, P, Villa, TG. 2011. Oily yeasts as oleaginous cell factories. Appl Microbiol Biotechnol 90: 1219-1227.

Aghbashlo M, Demirbas A. 2016. Biodiesel; hopes and dreads. Biofuel Res J 10: 379.

Akpinar-Bayizit A. 2014. Fungal lipids; the biochemistry of lipid accumulation. Int J Chem Eng Appl 5 (5): 409-414.

Areesirisuk A, Chiu CH, Yen TB, Liu CH, Guo JH. 2015. A novel oleaginous yeast strain with high lipid productivity and its application to alternative biodiesel production. Appl Biochem Microbiol 51 (4): 387-94.

Brandenburg J, Blomqvist J, Pickova J, Bonturi N, Sandgren M, Passoth V. 2016. Lipid production from hemicellulose with *Lipomyces starkeyi* in a pH-regulated fed-batch cultivation. Yeast 33 (8): 451-462.

Broadbent D, Terry DA. 1985. Effect of chloramphenicol on a fungus. Nature 182: 1107-1108.

Calvey CH, Su YK, Willis LB, McGee M, Jeffries TW. 2016. Nitrogen limitation, oxygen limitation, and lipid accumulation in *Lipomyces starkeyi*. Bioresour Technol 200: 780-788.

Chanchaichaovivat A, Ruenwongsa P, Panijpan B. 2007. Screening and identification of yeast strains from fruits and vegetables; potential for biological control of postharvest chili anthracnose (*Colletotrichum capsici*). Biological Control 42: 326-335.

Covadonga RA, Jacqueline KB, Lorrie MF, Renee MG, Mickey EP. 2002. Yeast species associated with orange juice; evaluation of different identification methods. J Appl Environ Microbiol 68: 1955-1961.

Gana NHT, Mendoza BC, Monsalud RG. 2014. Isolation, screening and characterization of yeasts with amylolytic, lipolytic, and proteolytic activities from the surface of Philippine bananas (*Musa* spp.). Philippines J Sci 143 (1): 81-87.

Hanif M, Mahlia TMI, Aditiya HB, Abu Bakar MS. 2017. Energy and environmental assessments of bioethanol production from Sri Kanji 1 cassava in Malaysia. Biofuel Res J 4 (1): 537-544.

Huang X, Madan A. 1999. CAP3; a DNA sequence assembly program. Genome Res 9: 868-877.

Iassonova DR, Hammond EG, Beatie SE. 2008. Oxidative stability of polyunsaturated triacylglycerols encapsulated in oleaginous yeast. J Am Oil Chem Soc 85: 711-716.

Kahr H, Pointner M, Krennhuber K, Wallner B, Jäger A. 2015. Lipid production from diverse oleaginous yeasts from steam exploded corn cobs. Agron Res 13 (2): 318-327.

Kang HY, Kim YS, Kim GJ, Seo JH, Ryu YW. 2005. Screening and characterization of flocculent yeast *Candida* spp. HY200 for the production of xylitol from D-Xylose. J Microbiol Biotechnol 15: 362-367.

Li Q, Du W, Liu D. 2008. Perspectives of microbial oils for biodiesel production. Appl Microbiol Biotechnol 80: 749-756.

Li SL, Feng SL, Li ZT, Xu H, Yu YP, Qiao DR, Cao Y. 2011. Isolation, identification and characterization of oleaginous fungi from the soil of Qinghai Plateau that utilize D-xylose. Afr J Microbiol Res 5: 2075-2081.

Li Y, Zhao Z, Bai F. 2007. High-density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture. Enzyme Microb Technol 41: 312-317.

Lin L, Zhou C, Saritporn V, Shen X, Dong M. 2011. Opportunities and challenges for biodiesel fuel. Appl Energy 88: 1020-1031.

Ma YL. 2006. Microbial oils and its research advance. Chin J Bioprocess Eng 4 (4): 7-11.

Maoura N, Mbaiguinam M, Nguyen HV, Gaillardin C, Pourquie J. 2005. Identification and typing of the yeast strains isolated from bili bili, a traditional sorghum beer of Chad. Afr J Biotechnol 4 (7): 646-656.

Pan L, Yang D, Shao L, Li W, Chen G, Liang Z. 2009. Isolation of the oleaginous yeasts from the soil and studies of their lipid-producing capacities. Food Technol Biotechnol 47 (2): 215-220.

Ramírez-Castrillón M, Jaramillo-Garcia VP, Rosa PD, Landell MF, Vu D, Fabricio MF, Ayub MAZ, Robert V, Henriques JAP, Valente P. 2017.

The oleaginous yeast *Meyerozyma guilliermondii* BI281A as a new potential biodiesel feedstock; Selection and lipid production optimization. Frontiers Microbiol 8: 1776.

Ratledge C, Wynn JP. 2002. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Adv Appl Microbiol 51 (1): 1-44.

Rossi M, Amaretti A, Raimondi S, Leonardi A. 2011. Getting lipids for biodiesel production from oleaginous fungi. In Stoytcheva M (eds). Biodiesel - Feedstocks and processing technology. InTech, Rijeka, Croatia.

Sugita T, Nishikawa A. 2003. Fungal identification method based on DNA sequence analysis; Reassessment of the methods of the pharmaceutical society of Japan and the Japanese pharmacopoeia. J Health Sci 49 (6): 531-533.

Suh SO, Blackwell M, Kurtzman CP, Lachance MA. 2006. Phylogenetic of *Saccharomycetales*, the ascomycete yeasts. Mycological Soc Am 98 (6): 1006-1017.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4; molecular evolutionary genetic analysis (MEGA) software version 4.0. Mol Biol Evol 24 (8): 1596-1599.

Tampitak S, Louhasakul Y, Cheirsilp B, Prasertsan P. 2015. Lipid production from hemicellulose and holocellulose hydrolysate of palm empty fruit bunches by newly isolated oleaginous yeasts. Appl Biochem Biotechnol 176 (6): 1801-1814.

Tremblay AY, Montpetit A. 2017. The in-process removal of sterol glycosides by ultrafiltration in biodiesel production. Biofuel Res J 13: 559-564.

Vincent M, Suali L, Azahari, AS, Baran, PRM, Nillian E, Bilung, LM. 2018. Growth kinetics of ethidium bromide mutagenized *Lipomyces starkeyi* strains. J Adv Res Appl Sci Eng Technol 11 (1): 47-54.

Vincent M, Pometto III AL, van Leeuwen J (Hans). 2014. Ethanol production via simultaneous saccharification and fermentation of sodium hydroxide treated corn stover using *Phanerochaete chrysosporium* and *Gloeophyllum trabeum*. Bioresour Technol 158: 1-6.

Wang GY, Chi Z, Song B, Wang ZP, Chi ZM. 2012. High-level lipid production by a novel inulinase-producing yeast *Pichia guilliermondii* Pcla22. Bioresour Technol 124: 77-82.

Wang W, Wei H, Knoshaug E, van Wychen S, Xu Q, Himmel ME, Zhang M. 2016. Fatty alcohol production in *Lipomyces starkeyi* and *Yarrowia lipolytica*. Biotechnol Biofuels 9: 227-239.

Wang XM, Zhang J, Wu LH, Zhao YL, Li T, Li JQ, Wang YZ, Liu HG. 2014. A mini-review of chemical composition and nutritional value of edible wild-grown mushroom from China. Food Chem 151: 279-285.

Zhao X, Kong X, Hua Y, Feng B, Zhao ZK. 2008. Medium optimization for lipid production through co-fermentation of glucose and xylose by the oleaginous yeast *Lipomyces starkeyi*. European J Lipid Sci Technol 110: 405-412.

Zweytick D, Athenstaedt K, Daum G. 2000. Intracellular lipid production of eukaryotic cells. Biochimica et Biophysica Acta 1469 (2): 101-112.