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

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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