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Screening of endophytic fungi for biofuel feedstock production using palm oil mill effluent as a carbon source

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ABSTRACT

Aims: Palm oil mill effluent (POME) is a major agricultural waste product of Malaysia. The aim of this study was to identify endophytic fungi capable of producing biofuel feedstock utilizing POME.
Methodology and results: Endophytes were isolated from the Nipah palm tree, *Nypa fruticans*, and exposed to different POME concentrations (25%, 50% and 75%), with and without the addition of nutrients. The utilization of glucose was measured using the Dinitrosalicylic Acid assay whereas the lipid content in the fungal cells was extracted using the Bligh and Dyer method with slight modifications. Three endophytic fungi that displayed the highest growth on POME were identified using ITS 1 and 4 primers and found to be related to *Pestalotiopsis* sp., *Lasiodiplodia theobromae* and *Rhizoctonia bataticola*. Nutrient addition caused an average increase of 8 times in biomass, indicating nitrogen requirement for cell proliferation. The highest POME concentration (75%) resulted in lower biomass yield. Furthermore, all fungal samples in high POME concentration and nutrient conditions showed a decrease in lipids accumulated per milligram of biomass whereby lipid synthesis was enhanced under nitrogen limitation (25% without nutrients).
Conclusion, significance and impact of study: In conclusion, all fungal samples can be classified as oleaginous microorganisms with *Pestalotipsis* sp. being the most efficient (up to 70% of its biomass). This is to our knowledge the first study that shows the potential use of *Pestalotiopsis* sp., *L. theobromae* and *R. bataticola* for the utilisation of POME as biofuel feedstock and could in the future potentially provide an alternative approach to the treatment of POME with value-added effect.

Keywords: biofuel feedstock, endophytic fungi, oleaginous fungi, *Nypa fruticans,* palm oil mill effluent

INTRODUCTION

Since the mid-1950s, petroleum has been the world's major source of energy. However, due to the fact that fossil fuels are limited and also the impacts of global warming (Fargione *et al.*, 2008), it is crucial to find alternative cleaner energy sources. In an effort to produce biodiesel, it was shown that endophytic fungi have the ability to accumulate high lipid content of more than 70% (Certik *et al.*, 1999) with a high ratio of monounsaturated fatty acids to polyunsaturated fatty acids (Dey *et al.*, 2011). This is generally preferable as low amounts of polyunsaturated fatty acids (PUFAs) contribute to oxidative stability and also operability at low temperatures (Liu *et al.*, 2013). In this study, endophytic fungi were isolated from the Nipah palm which can be found in mangrove wetlands. According to Khot *et al.* (2012), fungi and mangrove plays an important role in decomposition, nutrient cycling and energy flow of the marine web which justifies the usage of endophytic fungi for the degradation of POME. Also, endophytic fungi possess enzymes such as cellulases (Peng and Chen, 2007) which are able to degrade wastes such as sugarcane (Robl *et al.*, 2013) and would be essential for degrading the components of the POME.

Current trends of producing biofuels using fungi include using wastewaters from the treatment of animal fat and olive oil mill as their alternative carbon source and have shown to produce promising results (Rossi *et al.*, 2011). Furthermore, other oleaginous microorganisms such as microalgae, yeast, fungi and bacteria were also studied for their lipid accumulating capabilities (Meng *et al.*, 2009) where microalgae have been grown in POME (Putri *et al.*, 2011). POME was used in this study as the fermentable media for the endophytic fungi isolated because the discharge of POME into rivers and the

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