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Development of *Rhodotorula mucilaginosa* strain via random mutagenesis for improved lipid production

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ABSTRACT

Aims: Oleaginous yeasts are widely used for the production of biodiesel feedstocks because of their high lipid content. This research was aimed to conduct random mutagenesis of *Rhodotorula mucilaginosa* using ethyl methane sulfonate (EMS) and identify the mutants with improved lipid production.
Methodology and results: A total of twenty-two mutant isolates prescreened with cerulenin were produced and further characterized via M13 PCR fingerprinting to determine their polymorphism and genetic distances. Eight strains, namely M1, M2, M3, M4, M7, M10, M11 and M18, were chosen based on their genetic distances from the parental strain for biomass production. Six mutants (M1, M2, M3, M4, M7 and M18) showing the highest dry cell weights were further selected for evaluation of lipid production in a laboratory-scale bioreactor using glucose as a carbon source. Results indicated that parental strain exhibited lipid content of 1.83 g/L, while strains M1, M2, M3, M7 and M18 generated 2.37 g/L, 2.27 g/L, 2.27 g/L, 3.10 g/L and 3.83 g/L of intracellular lipid, respectively. These five mutants were identified to have significant increase in lipid production compared to the parental strain.
Conclusion, significance and impact of study: This study demonstrated enhanced lipid production in *R. mucilaginosa* by random mutagenesis. New generated strains had higher lipid productivity compared to parental strain and application of these strains in industry may reduce the overall cost of biodiesel production.

Keywords: Oleaginous yeasts, *Rhodotorula mucilaginosa*, random mutagenesis, cerulenin, lipid productivity

INTRODUCTION

The limited petroleum reserves and serious environmental pollutions have triggered the necessity to exploit alternative renewable energy sources, such as bioethanol and biodiesel (Huang *et al*., 2018). Biodiesel is an outstanding renewable fuel that can supplement petroleum fuel (Vincent *et al*., 2018). It is produced from the transesterification of triacylglycerides (TAGs) and alcohol in the presence of a catalyst such as NaOH or KOH (Moser *et al.*, 2009). Biodiesel is highly degradable, non-toxic and could reduce the emission of harmful gases (Sitepu *et al.*, 2013; Vincent *et al*., 2018). Most currently produced biodiesel is made from plant oils such as sunflower, rapeseed, peanut and palm oils (Atabani *et al.*, 2012; Demirbas *et al.*, 2016). Besides the high feedstock cost, the usage of edible plant oils for biodiesel production causes the issue of food-fuel competition, therefore a cheaper raw material with high oil productivity is crucial to enhance biodiesel production (Chhetri *et al.*, 2008; Atabani *et al.*, 2012; Shikha and Rita, 2012). Third-generation biodiesel produced from microbial oils has the potential as an excellent alternative source for biodiesel production (Soccol *et al.*, 2017; Vincent *et al*., 2018).

Microbial oil, also known as single cell oil (SCO), is produced by oleaginous microorganisms. Oleaginous microorganisms are microbes that are able to accumulate more than 20% of its dry biomass as storage lipid (Ochsenreither *et al.*, 2016). There are different types of oleaginous microorganisms that include yeasts, microalgae, bacteria and fungi (Steensels *et al.*, 2014). Compared to plant oils, microbial oil production does not use large arable lands, have higher lipid yield and production rate, and is independent of climate and geographical locations (Gientka *et al.*, 2017). Despite the extensive use of oleaginous microorganisms in industrial fermentation, further improvements are needed to enhance the metabolic capacity through genetic modifications and optimization of fermentation conditions.

Genetic engineering is frequently practiced to generate novel strains with improved productivity and better utilization of low-cost substrates in the development of biodiesel (Ochsenreither *et al.*, 2016). It involves the insertion of foreign genes into another species to modify the fatty acid profile or increase the lipid content

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