

CHAPTER 7

Conclusions

The oleaginous red yeast, *Sporobolomyces pararoseus* TISTR5213 showed a great potential for bioconversion of crude glycerol to high valuable chemicals of microbial lipids and carotenoids. This oleaginous red yeast produced maximum dry cell weight (DCW), lipids, β -carotene and total carotenoids production of 3.49 ± 0.39 g/L, 2.05 ± 0.02 g/L, 1.17 ± 0.01 mg/L and 1.77 ± 0.01 mg/L, respectively, when cultivated in the basal medium supplemented with crude glycerol (20 g/L) as a carbon source. Methanol, an impurity represented in crude glycerol showed strong effect on the growth, lipids and carotenoids production of strain TISTR5213. Hence, the highest DCW, lipids, β -carotene and total carotenoids of 9.40 ± 0.12 g/L, 2.92 ± 0.03 g/L, 25.76 ± 0.85 mg/L and 33.67 ± 1.28 mg/L, respectively, were obtained, with the basal medium supplemented with 34.0 g/L of demethanolized crude glycerol. The CCD model suggested the optimal conditions for DCW, β -carotene, total carotenoids and lipids production by strain TISTR5213 that cultivation in the basal medium supplemented with demethanolized crude glycerol 55.04 g/L, initial pH 5.63 at 24.01°C . Under these conditions, the highest DCW, lipids, β -carotene and total carotenoids productions were 8.83 ± 0.05 g/L, 4.00 ± 0.06 g/L, 27.41 mg/L and 53.70 ± 0.48 mg/L, respectively.

Sporobolomyces pararoseus TISTR5213 was reclassified as *Sporidiobolus pararoseus* KM281507 based on nucleotide sequences of the 26S rRNA gene. The effect of additive agents on lipids and carotenoids productions by this strain was investigated. The results showed that olive oil showed high potential as an activator for enhancing growth and lipids and carotenoids accumulation. Olive oil at concentration of 2.0% (w/v) enhanced DCW to 14.96 ± 0.19 g/L, while 1.5% supported the lipids production to 6.40 ± 0.09 g/L and 0.5% enhanced carotenoids production to 70.93 ± 0.51 mg/L. Moreover, the fatty acid composition of *Sporidiobolus pararoseus* KM281507

was dominated by oleic acid (C18:1) at $75.08\pm 3.54\%$ under optimal conditions and olive oil 1.5% was used as the additive agent.

The enhancement of lipids and carotenoids productions of *Sporidiobolus pararoseus* KM281507 by scaling up and process developments using an internal loop airlift bioreactor was investigated. The combination effect of light irradiation 10,000 Lux plus pure oxygen with constant DO level ($60\pm 5.0\%$) enhanced the DCW, lipids, β -carotene and total carotenoids productivities of 5.12 ± 0.01 g/L/d, 0.94 ± 0.04 g/L/d, 15.68 ± 0.40 mg/L/d and 21.56 ± 0.20 mg/L/d, respectively when uncontrolled pH regime with initial pH 5.63 was used. The lipids obtained from strain KM281507 showed high content of oleic acid (81%) indicated the best choice for the third biodiesel feedstock. Under the optimal condition, the carotenoids production of 151.00 mg/L with β -carotene of 147.49 mg/L were obtained, indicating successful to develop the fermentation process for lipids and carotenoids production using a low cost biodiesel derived-crude glycerol.

The ultraviolet (UV), ethyl methane sulfonate (EMS) and 5-bromouracil (5BU) mutagenesis were investigated to improve DCW, carotenoids and lipids production from the wild-type strain of *Sporidiobolus pararoseus* KM281507 in the optimal medium supplemented with demethanolized crude glycerol. A mutant B151 obtained by 5BU mutagenesis produced the highest level of DCW (9.82 ± 0.06 g/L), total carotenoids (74.01 ± 0.17 mg/L) and total lipids (5.28 ± 0.06 g/L), which were 11.46, 54.28 and 27.85% higher than wild-type, respectively. Hence, this result indicated that B151 showed high potential on bioconversion of crude glycerol to high value-added products of carotenoids and lipids.

From this study, it could be concluded that oleaginous red yeast, *Sporidiobolus pararoseus* KM281507 was a great potential microorganism that could be used as the effective microorganism for bioconversion of demethanolized crude glycerol into high valuable lipids, β -carotene and carotenoids according to the view of efficient renewable resource utilization. Crude lipids obtained from this strain was getting attention as the third generation for biodiesel feedstock by reusing crude glycerol obtained from biodiesel production plant as a carbon source and convert it to the

KM281705's lipids, which might reduce total cost of biodiesel production. Moreover, *Sporidiobolus pararoseus* KM281507 was also considered as a source of carotenoids which would be used as the natural food colorant or other applications.



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