ผลของระดับกากน้ำตาลและแอมโมเนียมซัลเฟตต่อการผลิตไขมันในเซลล์ยีสต์ *Trichosporon asahii*

Effect of molasses and ammonium sulfate concentration on intracellular lipid accumulation by *Trichosporon asahii*

อานนท์ ปะเสระกัง[1], วิโรจน์ ภัทรจินดา[1*], คณิต วิชิตพันธุ์[2] และ รัตนภรณ์ ลีสิงห์[3]

Anon Paserakung[1], Virote Pattarajinda[1,*], Kanit Vichitphan[2] and Ratanaporn Leesing[3]

บทคัดย่อ: การทดลองนี้มีวัตถุประสงค์เพื่อทดสอบระดับความเข้มข้นของกากน้ำตาลและแอมโมเนียมซัลเฟตต่อปริมาณการสะสมไขมันในเซลล์ยีสต์ *Trichosporon asahii* โดยใช้แผนการทดลองแบบสุ่มสมบูรณ์ ปัจจัยการทดลองคืออาหารเลี้ยงเชื้อ 9 สูตรที่มีระดับกากน้ำตาล (M,%) และแอมโมเนียมซัลเฟต (N,g/l) แตกต่างกันดังนี้ M8N0.5, M8N1.0, M8N1.5, M12N0.5, M12N1.0, M12N1.5, M16N0.5, M16N1.0 และ M16N1.5. เพาะเลี้ยงเชื้อโดยใช้กล้าเชื้อเริ่มต้น 10% บ่มแบบเขย่าที่อุณหภูมิ 30 องศาเซลเซียส ที่ 150 รอบต่อนาที เป็นเวลา 6 วัน ผลการทดลองพบว่าการใช้สูตรอาหาร M12N0.5 ปริมาณการสะสมไขมันในเซลล์สูงสุดอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ซึ่งความเข้มข้นของกากน้ำตาลและ แอมโมเนียมซัลเฟตระดับนี้จะนำไปใช้เพื่อการผลิตน้ำมันเซลล์เดียวสำหรับเป็นแหล่งไขมันเสริมในอาหารโคนมต่อไป

คำสำคัญ: ยีสต์ไขมัน กากน้ำตาล แอมโมเนียมซัลเฟต น้ำมันเซลล์เดียว

ABSTRACT: The objective of present study was to examine the effect of molasses and ammonium sulfate concentration on intracellular lipid accumulation by *Trichosporon asahii*. Nine culture media with different concentration of molasses (M,%) and ammonium sulfate (N,g/l) includings M8N0.5, M8N1.0, M8N1.5, M12N0.5, M12N1.0, M12N1.5, M16N0.5, M16N1.0 and M16N1.5 were randomly assigned in completely randomized design (CRD). Five milliliter of inoculums was transferred in 45 ml of media and incubated at 30°C with shaking at 160 rpm for 6 days. Results illustrated that lipid content was higher when *T. asahii* was cultured in the medium M12N0.5. This indicated that medium M12N0.5 was suitable for producing single cell oil for use as fat supplement in animal diet.
Keywords: oleaginous yeast, molasses, ammonium sulfate, single cell oil

[1] ภาควิชาสัตวศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น
Department of Animal Science, Faculty of Agriculture, Khon Kaen University

[2] ภาควิชาเทคโนโลยีชีวภาพ คณะเทคโนลียี มหาวิทยาลัยขอนแก่น
Department of Biotechnology, Faculty of Technology, Khon Kaen University

[3] ภาควิชาจุลชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น
Department of Microbiology, Faculty of Science, Khon Kaen University

* Corresponding author: Virote@kku.ac.th

Introduction

Conjugated linoleic acids (CLA) are groups of positional and geometrical isomer of octadecadienoic acid which are synthesized via biohydrogenation of unsaturated fatty acids by rumen microbes as well as desaturation of vaccenic acid (*trans* – 11 C18:1) by delta – 9 – desaturase in the mammary gland (Collomb et al., 2006). *Cis* – 9, *trans* - 11 CLA is a predominant isomer found in milk and dairy product and it has been show benefit effects on human health, including antioxidant, inhibit carcinogenesis and anti - inflammatory (Bhattacharya et al., 2006). Supplementation of unsaturated fatty acid source such as oil seed or vegetable oil is one of methodologies for increasing the proportion of polyunsaturated fats (PUFA) and CLA in cow's milk fat (Daiman et al., 2000; Chouinard et al., 2001). However, using oil seed or vegetable oil may be difficult in the future because it compete with human consumption and biodiesel production.

Oleaginous yeasts refer to yeasts that have a capacity to accumulate intracellular lipid more than 20% of biomass and the lipid are formed similar to vegetable oil (Beopoulos et al., 2009). Feeding the dairy cows with oleaginous yeast is a new approach for increasing the PUFA and CLA in milk fat. In previous study, we isolated oleaginous yeast from soil and feedstuffs for use as unsaturated fatty acid source in dairy cow diet. The result revealed that yeast GSY10 accumulated intracellular lipid up to 24% of dry biomass when use molasses and ammonium sulfate $(NH_4)_2SO_4$ as carbon and nitrogen source. Identification of yeast strain by D1/D2 domain illustrated that yeast GSY10 belonging to *Trichosporon asahii*. However, there are many factors affecting on lipid accumulation. Therefore, the aim of this study was to examine the effect of molasses and $(NH_4)_2SO_4$ concentration on intracellular lipid accumulation.

Materials and Methods

Experimental design and lipid accumulation procedure

The completely randomize design (CRD) was conducted to examined effect of concentration of molasses (M,%) and $(NH_4)_2SO_4$ (N,g/l) on lipid accumulation by *Trichosporon asahii*. Nine treatments consist of M8N0.5, M8N1.0, M8N1.5, M12N0.5, M12N1.0, M12N1.5, M16N0.5, M16N1.0 and M16N1.5. The single colony of *T. asahii* was transferred to 250 ml Erlenmeyer flask containing 50 ml of inoculums medium containing (g/l): glucose 40, yeast 15, peptone 5, with adjusted pH 6 (Dai et al., 2007), and incubated at 30°C, 160 rpm for 24 h. Inoculums (5 ml) was transferred to 45 ml of nitrogen – limited medium containing concentration of molasses and $(NH_4)_2SO_4$ as described above and 0.5 g/l of KH_2PO_4. Cultures were incubated at 30°C with shaking at 160 rpm for 6 days.

Determination of yeast biomass

Portions of 5 ml cultures were harvested by centrifugation at 5000xg for 5 min. Harvested biomass were washed twice with distiller water and then dried at 60°C for 48 h. The biomass was determined gravimetrically.

Determination of lipid content

Lipid content in yeast cells were extracted according to method of Bligh and Dyer (1959) with modification described by Pan et al. (2009). Briefly,

50 ml samples were centrifuged at 5000xg for 5 min, then were washed twice with 50 ml of distiller water, and added into 10 ml of 4 M HCl, incubated at 60°C for 1 h to 2 h. Samples were stirred with 20 ml of chloroform/methanol mixture (1:1 v/v) at room temperature for 2 – 3 h, were followed by centrifugation at 2000xg for 5 min at room temperature to separate the aqueous part and organic lower phase part. The lower phase containing lipid was recovered by Pasteur pipette, and evaporated under reduced pressure for 10 min. The dry lipid was weighed.

Statistical analysis

Data were analyzed by using analysis of variance (ANOVA) according to completely randomized design (CRD). Means were compared with Duncan's multiple range test (DMRT) at $P< 0.05$ by using SAS (1989).

Results and Discussion

Effect of molasses and ammonium sulfate concentration

In order to increase the lipid production by *T. asahii,* concentration of molasses and $(NH_4)_2SO_4$ were optimized by increasing molasses concentration at levels 8, 12 and 16 % and $(NH_4)_2SO_4$ at levels 0.5, 1.0 and 1.5 g/l. Result illustrated that *T. asahii* showed maximum lipid contents in media containing 12% molasses and 0.5 g/l $(NH_4)_2SO_4$ ($P < 0.05$). The lipid content was 31.4% of biomass (**Figure 1**). Karatay and Dönmez (2010) reported that the concentration of molasses at a level 8% was optimum for lipid accumulation by *Candida lipolytica*, *C. tropicalis* and *Rhodotorula mucilaginosa*. Wu et al. (2011) found that a level of 10% molasses concentration, *Trichosporon capitatum* gave the maximum biomass and lipid content. It showed that the optimum concentration of molasses for lipid accumulation depend upon oleaginous yeast strain. However, an increasing molasses concentration up to 16% trend to reduced lipid accumulation by *T. asahii*. Karatay and Dönmez (2010) suggested that increasing molasses concentration reduced lipid accumulation because of the toxic effects of molasses on the cell growth and lipid accumulation.

In case of nitrogen concentration, an increasing concentration of $(NH_4)_2SO_4$ showed the reduction of intracellular lipid accumulation. Oleaginous yeast begins accumulate lipid when they have an excess carbon but limits other nutrient, usually this is nitrogen. This condition activates the activity of enzyme adenosine monophosphate (AMP) deaminase in order to converts AMP to inosine $5'$ - monophosphate (IMP) and ammonia (NH_3). Decreasing in concentration of AMP is attributable to slowing of activity of isocitrate dehydrogenase (ICDH) because this enzyme in oleaginous yeast has an absolute requirement for AMP (Ratledge, 2004). The inactivity of ICDH increases the accumulation of citrate in the mitochondria. The citrate is transported out of the mitochondria and cleavage by ATP:citrate lyase on to acetyl – coA, precursor for fatty acid synthesis (Ratledge, 2004). These indicated that media containing molasses and $(NH_4)_2SO_4$ at a level 12% and 0.5 g/l was suitable for single cell oil production by *T. asahii*. Although, this condition gave dry biomass (10.6 g/l) lower than media M16N1.0 and M16N1.5 ($P < 0.05$) but lipid yield was higher than media M16N1.0 and M16N1.5 (**Figure 2**).

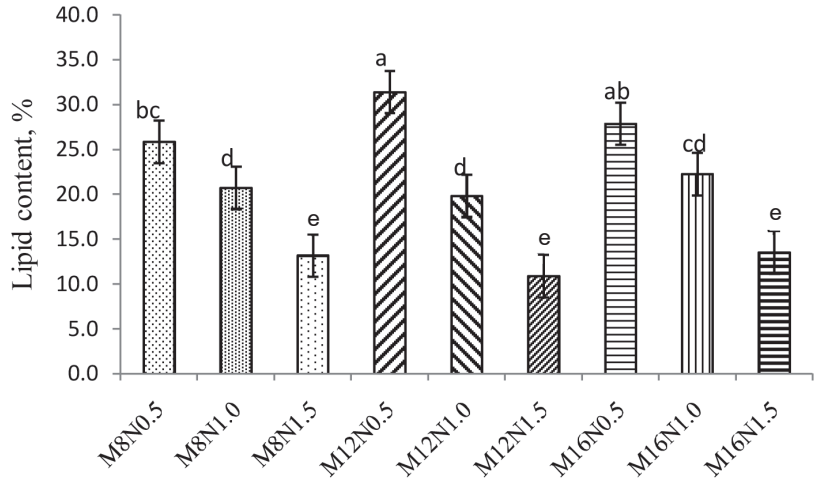

Figure 1 Effect of molasses and ammonium sulfate concentration on lipid content of *Tricosporon asahii*. Each column represents the mean (± SE) for lipid content.

[a, b, c, d]A common letter show significantly difference at $P < 0.05$.

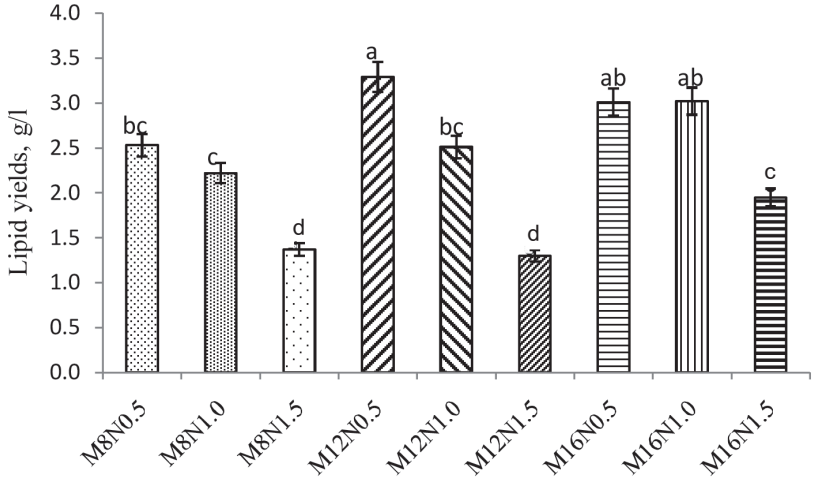

Figure 2 Effect of molasses and ammonium sulfate concentration on lipid yields of *Tricosporon asahii*. Each column represents the mean (± SE) for lipid yield.

[a, b, c, d]A common letter show significantly difference at $P < 0.05$.

Conclusions

Concentration of carbon and nitrogen source was main effect on lipid accumulation by oleaginous yeast. In case of *T. asahii*, it could assimilate molasses and $(NH_4)_2SO_4$ for lipid production which an optimal level of molasses and $(NH_4)_2SO_4$ were 12% and 0.5 g/l, respectively. These indicating that it is possible to produce single cell oil by *T. asahii* for use as unsaturated fatty acid source in dairy cow diet.

Acknowledgments

Authors acknowledge the Thailand Research Fund through the Royal Golden Jubilee Ph.D. program for financial support. The center of Excellence and Research Development office, Office of Higher Education Commission, Ministry of Education (AG – BIO/PERDO – CE) and Agricultural Biotechnology Research Center for Sustainable Economy, Khon Kaen University for partially support. Department of Animal Science, faculty of Agriculture, Khon Kaen University and the Thermo-tolerant Dairy Cattle Research Group of Khon Kaen University for research facility support.

References

Beopoulos, A., J. Cescut, R. Haddouche, J. L. Uribelarrea. C. M. Jouve and J. M. Nicaud. 2009. *Yarrowia lipolytica* as a model for bio – oil production. Prog Lipid Res. 48: 375 – 387.

Bhattacharya, A., J. Banu, M, Rahman, J. Causey and G. Fernandes. 2006. Biological effects of conjugated linoleic acids in health and disease. J. Nutr. Biochem. 17: 789 – 810.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method to total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Chouinard, P. Y., L. Corneau, W. R. Butler, Y. Chillard, J. K. Drackley and D. E. Bauman. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. J. Dairy Sci. 84: 680 – 690.

Collomb, M., A. Schmid., R. Sieber., D. Wechsler. and E. L. Ryhänen. 2006. Conjugated linoleic acids in milk fat: variation and physiological effects. Int. Dairy J. 16: 1347 – 1361.

Dai, C. C., J. Tao, F. Xie, Y. J. Dai and M. Zhao. 2007. Biodiesel generation from Oleaginous yeast *Rhodotorula glutinis* with xylose assimilating capacity. Afr. J. Biotechnol. 6(18): 2130 – 2134.

Dhiman, T. D., L. D. Satter., M. W. Pariza., M. P. Galli., K. Albright. and M. X. Tolosa. 2000. Conjugated linoleoic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic. J. Dairy Sci. 83:1016 – 1027.

Karatay, S. E. and G. Dönmez. 2010. Improving the lipid accumulation properties of the yeast cells for biodiesel production using molasses. Bioresour. Technol. 101: 7988-7990.

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

Ratledge, C. 2004. Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie. 86: 807 – 815.

SAS. 1989. SAS User's Guide: Statistics, version 6 Edition. SAS inst., Inc., Cary, NC.

Wu, H., Y. Li, L. Chen and M. Zong. 2011. Production of microbial oil with high oleic acid content by *Trichosporon capitatum*. Appl. Energ. 88: 138 – 142.