

Cultivation of *Thraustochytrium* sp. to Produce Docosahexaenoic Acid

張榮權、吳淑姿；余世宗

E-mail: 9708299@mail.dyu.edu.tw

ABSTRACT

In the last few years , everyone started paying attention to personal health, and disease prevention due to the improvement of the society. Docosahexaenoic acid (DHA) has physiological effects in heart and circulatory system, inflammatory and dermatitis disease. The study was investigated on batch production of DHA by *Thraustochytrium* sp. using 20 L fermentor. During the batch cultivation, temperature and pH were controlled at 25 °C and 6.5. Agitation and airflow were adjusted to keep the dissolved oxygen above 30 % of Saturation. Samples were taken for measurements of biomass, lipid and DHA contents. The medium contained per liter: 20.0 g glucose, 1.0 g ammonium sulfate. The results of experiment show that after 30 hrs, the nitrogenous source was exhausted, but there was still 11.54 g/L of carbonic source in the broth. However, the biomass and lipid were keeping raising. After 33 hrs, the biomass and lipid increased greatly. Until glucose was exhausted, biomass and lipid was decreasing. Under no carbonic source, microbe would consume the lipid which made by itself so that lipid was just decreasing. At 37 hrs, biomass was 3.48 g/L and lipid was 1.58 g/L. It was close to the stationary phase of microbial growth and lipid. At this time, docosahexaenoic acid was 251.07 mg/L. The lipid was 48 % of biomass and the docosahexaenoic acid was 7.61 % of biomass, respectively. Key Words : *Thraustochytrium* sp. docosahexaenoic acid (DHA)

Keywords : *Thraustochytrium* sp. ; docosahexaenoic acid (DHA)

Table of Contents

封面內頁 簽名頁 授權書iii 中文摘要iv 英文摘要vi 誌謝vii 目錄viii 圖目錄xi 表目錄xii 1. 緒言1 2. 文獻回顧3 2.1 脂肪酸3 2.2 多元不飽和脂肪酸(PUFAs)3 2.2.1 何謂多元不飽和脂肪酸3 2.2.2 PUFAs的生理功能5 2.2.3 PUFAs之生物合成途徑7 2.2.4 DHA的生理作用9 2.2.5 PUFAs之應用13 2.3 -3係多元不飽和脂肪酸之市場需求及生產方式13 2.3.1 -3係PUFAs之市場需求13 2.3.2 -3係PUFAs之生產方式16 3. 材料與方法20 3.1 實驗材料20 3.1.1 菌株20 3.1.2 藥品20 3.1.3 儀器設備21 3.2 菌株培養24 3.2.1 平板培養24 3.2.2 種培養24 3.2.3 預培養27 3.2.4 20L批次發酵培養27 3.3 分析方法29 3.3.1 分析樣品處理流程圖29 3.3.2 生質量之測定29 3.3.3 脂質萃取29 3.3.4 脂肪酸甲酯之製備31 3.3.5 脂肪酸鑑定方法31 3.3.6 碳源測定32 3.3.7 氮源測定33 4. 結果與討論35 4.1 *Thraustochytrium* sp.菌相35 4.2 培養基中碳和氮可利用量對生質量、脂質累積和DHA生成量之影響35 4.3 溶氧量對生質量、脂質和DHA生成量之影響41 4.4 批次發酵之菌體生產速率47 4.5 批次發酵之脂質生產速率51 4.6 每克葡萄糖轉化為脂質的量55 4.7 DHA產量55 5. 結論58 5.1 結論58 5.2 未來展望58 參考文獻59 附錄62 圖目錄 圖2.1 脂肪酸的分類4 圖2.2 典型PUFAs之分子結構式6 圖2.3 PUFAs之生物合成途徑8 圖3.1 實驗架構圖22 圖3.2 樣品分析流程圖30 圖4.1 發酵槽培養*Thraustochytrium* sp.生產DHA38 圖4.2 *Thraustochytrium* sp.搖瓶培養39 圖4.3 光學顯微鏡下觀察*Thraustochytrium* sp.40 圖4.4 轉速及通氣量對生質量之變化42 圖4.5 溶氧量對生質量之變化43 圖4.6 殘碳、氮量對生質量之變化44 圖4.7 殘碳、氮量對脂質及DHA之變化45 圖4.8 溶氧量對脂質及DHA之變化46 圖4.9 培養時間對生質量之平均生產速率之變化49 圖4.10 培養時間對生質量之平均比生長速率之變化50 圖4.11 培養時間對脂質之平均生產速率之變化53 圖4.12 培養時間對脂質之平均比生產速率之變化54 圖4.13 殘碳量對脂質轉化率之變化57 表目錄 表2.1 含PUFAs之脂質的商品在生物醫學及藥物食品上的應用14 表2.2 市場上多元不飽和脂肪酸的商品15 表2.3 多元不飽和脂肪酸之來源17 表2.4 DHA的來源18 表3.1 *Thraustochytrium* sp.的生物學分類20 表3.2 無機鹽類之組成25 表3.3 微量金屬溶液之組成26 表4.1 培養時數對生質量、脂質及DHA生成之影響36 表4.2 培養時數之殘糖、殘氮量對生質量和脂質累積影響37 表4.3 培養時間對生質量之平均生產速率之變化48 表4.4 培養時間對脂質之平均生產速率之變化52 表4.5 碳量對脂質轉化率之變化56

REFERENCES

- 王中奇。1996。魚油中多元不飽和脂肪酸在人體內的代謝及生理機能。食品工業月刊10:8-15。
- 王致誠。1997。DHA生理保健新發現。藥物與人10(6):32-33。
- 王萍。1998。DHA可造就天才。國外科技動態6:47。
- 王萍。1998。DHA可治癒阿茲海默氏病。國外科技動態6:48。
- 江孟桀、張淑美。1995。膳食魚油大白鼠的血漿脂蛋白及血漿濃度之影響。中華營養會志20:201-214。
- 吳淑姿。2002。海洋單細胞真菌 *Schizochytrium* sp. S31生產多元不飽和脂肪酸-DHA。國立台灣大學農業化學研究所博士論文。台北。
- 吳平、應如冰。1995。DHA、EPA在乳制品中的應用。中國乳品工業23(6):280-282。
- 吳葆杰。1997。各種脂肪酸與冠心病猝死關係的研究進展。中國生化藥物染志18(6):317-320。
- 范文洵。1988。-亞麻酸及其代謝產物EPA和DHA。生理科學進展19(2):110-113。
- 祝向紅。1986。

二十碳五烯酸的抗缺血性疾病作用。生理科學進展17(2):166-168。 11.洪濱、劉會洲。1996。國內EPA及DHA研究現狀和發展趨勢。化工冶金17(1):80-85。 12.翁鵬傑、彭俊明、陳玉燕、許碧蘭、黃東裕、盧虹佑。2000。哈伯氏生物化學(冊)。第 277-286頁。藝軒圖書出版社。台北。台灣。 13.劉清標。1999。海洋微藻 *Isochrysis* sp. CCMP 1324 超微細結構與多元不飽和脂肪酸之生成。國立台灣大學農業化學研究所博士論文。台北。 14.劉兆平。1984。二十碳五烯酸代謝和生理作用。海洋藥物12(4):9-14。 15.Alonso, D. L. and Maroto, F. G. 2000. Plant as chemical factories for the production polyunsaturated fatty acid. Biotechnol. Adv. 18, 481-497. 16.Bajpai, K. and Bajpai, P. K. 1993. Eicosapentaenoic acid(EPA)production from microorganisms. Biotechnol. Appl. Biochem. 30,161-183. 17.Barclay, W. R. and Boulder, C. 1992. Process for the heterotrophic production of microbial products with high concentrations of omega-3 highly unsaturated fatty acids. US Patent, 5130242. 18.Barclay, W. R., Meager, K. M. and Abril, J. R. 1994. Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. J. Appl. Phycol.6, 123-129. 19.Belarbi, E. H., Molina, E. and Chisti, Y. 2000. A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. Enzyme Microbiol. Technol. 26, 516-529. 20.Bimbo, A. P. 1987. The imerging marine oil industry. J. Am. Oil. Chem. Soc.64,706-715. 21.Dyerberg, J. 1986. Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. Nutri. Rev. 44, 125-134. 22.Gill, I. and Valivety, R. 1997. Polyunsaturated fatty acids, part 1: occurrence, biological activities and applications. Trends Biotechnol. 15, 401-409. 23.Granger, L. M., Perlot, P., Goma, G. and Pareilleux, A. 1993. Effect of various nutrient limitations on fatty acid production by *Rodotorula glutinis*. App. Micr obiol Biotechnol. 38, 784-789. 24.Horrobin, D. F. 1981. Loss of delta-6-desaturase as a key factor in aging. Med. hypothesis, Vol. 7, pp. 1211-1220. 25.Jareokitmongkol, S., Sakuradani, E. and Schimizu, S. 1993. A novel 5-desaturase-defective mutant of *Mortierella alpina* 1S-4 and its dihomo- -linole nic acid production. App. Environ. Microbiol. 59, 4300-4304. 26.Kris-Etherton, P. M., Hecker, K. D. and Binkoski, A. E. 2004. Polyunsaturated fatty acids and cardiovascular health. Nutr: Rev. 62: 414-426. 27.Leaf, A., Kang, J. X., Xiao, Y. F. and Billman, G. E. 1999. n-3 fatty acids in the prevention of cardiac arrhythmias, Lipids, Vol. 34, pp. S187-S189. 28.Sardesai, V. S. 1992. Nutritional role of polyunsaturated fatty acids, J. Nutr. biochem, Vol.3, pp. 154-166. 29.Sayanova, O., Smith, M. A., Lapinskas, P., Stobart, A. K., Dobson, G., Christie, W. W.,Shewry, P. R. and Napier, A. J. 1997. Expression of a borage desaturase cDNA containing an N-terminal cytochrome b5 domain results in the accumulation of high levels of 6-desaturated fatty acids in transgenic tobacco. Proc. Natl. Acad. Sci. USA. 94, 4211-4216. 30.Shirasala, N. and Shimizu, S. 1995. Production of eicosapentaenoic acid by *Saprolegnia* sp. 28YTF-1. J. Am. Oil. Chem. Soc. 72, 1545-1549. 31.Singh, A., and Ward, O. P. 1997. Microbial production of docosahexaenoic acid (DHA, C22:6). Adv. App. Microbial. 45, 271- 312. 32.Yamauchi, H., Mori, H., Kobayashi, T. and Shimizu, S. 1983. Mass production of lipids by *Lipomyces starkeyi* in microcomputer-aids fedbatch culture. J. Ferment. Technol. 61, 275-280. 33.Yongmanitchai, W. and Ward, O.P.1989. Omega-3 fatty acids: alternative sources of production. Process Biochem. 21, 117-125.