Use Response Surface Methodology to Study Lipid Production by Thraustochytrium sp

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ABSTRACT

Docosahexaenoic acid is a Polyunsaturated fatty acid that cannot be synthesized in human cell, and therefore must be supplied in the diet .It can help to lower levels of serum cholesterol and triglycerides and to prevent hypertension ; it also has anti- inflammation function. Thraustochytrium sp. was screened for the production of Docosahexaenoic acid.in this study, response surface methodology was used to study the growth of Thraustochytrium sp.,the production of lipid and to develop a numerical model.The method of one-factor-at-a-time was used to investigate the effects of cultivating conditions, including the carbon and nitrogen sources,concentrations of glucose and ammonium chloride, initial pH ,and temperature on the microbial growth and lipid production by Thraustochytrium sp. Then, the central composite design was used to obtain optimal production of lipid. The results by one-factor-at-a-time method showed that Thraustochytrium sp. was best cultivated in a medium with 40 g/L glucose and 1.0 g/L ammonium chloride,under 25°Cand pH 6.0. Based on the resuts by one-factor-at-a-time,a central composite design of three factors, each with two levels,was designed,and 8 factorial,6 compensate and 2 central experiments were conducted. The ranges for the ammonium chloride concentration and the pH were the same in both cultures of Thraustochytrium sp. The concentration of glucose at the central point was 40g/L . The experiments were carried our and results were analyzed with statistical software,STATISTICA. The optimal cultivating condition for Thraustochytrium sp. was as follows: glucose 40.52 g/L,ammonium chloide1.07 g/L, and pH6.19, and the Lipid production 227.69 mg/L(95% of the expected result) , R2＝0.9261. Under the optimal conditions bactch cultivation of Thraustochytrium sp.were conducted in a 5L fermentor. Cultivate Thraustochytrium sp. at 25℃ for 99hr, the biomass was 2.3 g/L , it is 2.7 times more than in a shaker with the optimal conditions; the lipid production is 943 mg/L , it is 4.35 times more than in a shaker with the optimal conditions .

Keywords : Thraustochytrium sp. , one-factor-at-a-time , central composite design, fermentor

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REFERENCES

1.方文峰。1995。利用Mortierella 屬絲狀真菌生產花生四烯酸之研究。國立台灣大學農業化學研究所碩士論文。台北。 2.林嘉銘。2007。ω-6/ω-3脂肪酸比例與心血管疾病之關係。食品工業第39卷第10期:10-13 3.洪哲穎、陳國誠。1992。回應曲面實驗設計法在微生物酵素生產上之應用。化工39 (2):3-18。 4.張洪濤、單雷、畢玉平。2006。n-3和n-6不飽和脂肪酸在人和動物體內的功能關係。山東農業科學。2:115-120。 5.劉清標。1999。海洋微藻 Isochrysis sp. CCMP 1324 超微細結構與不飽和脂肪酸之生成。國立台灣大學農業化學研究所碩士論文。台北。 6. Ahn, T. H., Y. P. Lee and J. S. Rhee. 1997. Investigation of refolding condition for Pseudomonas fluorescences lipase by response surface methodology. J. Biotech. 54, 151-160. 7. Brown, C. M. and Rose, A. H. 1969. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Bibchemistry. 72: 248-254. 8. Carvalho, P. O. Oliveira, J. G. and Pastore, G. M. 1999. Enhancement of gammalinolenic acid production by the fungus Mucor sp LB-54 by growth temperature. Rev. Microbiol. 30 (2): 170-176. 9. Certik,M.and Shimizu,S.1999.Biosynthesis and regulation of microbial Polyunsaturated fatty acid production.J.Bioeng.87:1-14. 10. Chen, H. C. 1996. Optimizing the concentrations of carbon, nitrogen and phosphorus in a citric acid fermentation with response surface method. Food Biotechnol. 10, 13-27. 11. Gellerman, J. L. and Schlenk, K.1979. Methyl directed desaturation of arachidonic acid to eicosapentaenoic acid in the fungus Saprolegnia parasitica. Biochem. Biophys. Acta 573: 23-30. 12. Jiang, Y. and Chen, F. 2000. Effects of medium glucose concentration and pH on docosahexaenoic acid content of heterotrophic Crypthecodinium cohnii. Process Biochem. 35 (10): 1205-1209. 13. Kris-Etherton,P.M.,Harris,W.s. and apple, L.J.2002. Fish consumption fish oil, omega-3 fatty acids,and cardiovascular disease. Circulation. 106:2747-2457 14. Ohta, S. Chang, T. Aozasa, O. Ikegami, N. and Miyata, H. 1993. Alterations in fatty acid composition of marine red alga Porphyridium purpureum by environmental factors. Bot. Mar. 36: 103-107. 15. Ratledge, C. 1994. Yeast, moulds, algae and bacteria as source of lipid, in advances in improved and alternative sources of lipids. Blackie Publishers, London, England, 235-291. 16. Silva, F. L. H., M. I. Rodrigues and F. Maugeri. 1999. Dynamic modeling, simulation and optimization of an extractive continuous alcoholicfermentation process. J. Chem. Technol. Biotechnol. 74, 176-182. 17. Somashekar, D. Venkateshwaran, G. Sambaiah, K. and Lokesh, B. R. 2002. Effect of culture conditions on lipid and amma-linolenic acid production by mucoraceous fungi. Process. biochem. 38: 1719-1724. 18. Sugano, M. and Hiranara, F 2000 . Polyunsaturated fatty acids in the food chain in Japan.Am.J.Clin.Nutr.71(supple):189-196. 19. Tapiero,H.,Ba,G.N.,Couvreur,P.andTew,k.D.2002. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. Biomed.Pharmacother:56:215-222. 20. Yongmanitchai, W. and Ward, O. P. 1989. Omega-3 fatty acids: alternative sources of production. Process Biochem. 24: 117-125. 21. Yamauchi, H., H. Mori, T. Kobayashi and S. Shimizu. 1983. Mass production of lipids by lipomyces starkeyi in microcomputer-aids fedbatch culture. J. Ferment. Technol. 61, 275-280.