

# Cultivation of *Cunninghamella echinulata* and *Bacillus cereus* DYU-Too 12 to Produce Polyunsaturated Fatty Acids

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## ABSTRACT

The aim of this study is to optimize the production of polyunsaturated fatty acids (PUFAs) by *Cunninghamella echinulata* and *Bacillus cereus* DYU-Too 12. The method of one-factor-at-a-time was used to investigate the effect of cultivating conditions, including the carbon and nitrogen sources, concentrations of glucose and ammonium chloride, temperature, and pH on the microbial growth, lipid accumulation, production of  $\alpha$ -linolenic acid (GLA) and eicosapentaenoic acid (EPA) by *Cunninghamella echinulata* and *Bacillus cereus* DYU-Too 12. The central composite design was used to obtain optimal production of PUFAs. After the one-factor-at-a-time method was performed, results showed that *Cunninghamella echinulata* was best to be cultivated in a medium with 35 g/L glucose and 1 g/L ammonium chloride under 25 °C, 100 rpm and pH 7.0, and the highest biomass obtained was 7.05 g/L, the lipid 1.30 g/L and the GLA 602.48 mg/L. Similarly, *Bacillus cereus* DYU-Too 12 was best to be cultivated in a medium containing 20 g/L glucose and 1 g/L ammonium chloride under 30 °C, 100 rpm and pH 7.0, and the highest biomass was 2.65 g/L, the lipid 0.12 g/L and the EPA 35.45 mg/L. A central composite design of three factors, each with two levels, included 16 experiments (8 factorial, 6 compensate and 2 central experiments). The ranges for the ammonium chloride concentration and the pH were the same in both cultures of *C. echinulata* and *Bacillus cereus* DYU-Too 12. The concentration of glucose at the central point was 35 g/L for *C. echinulata* and 20 g/L for *Bacillus cereus* DYU-Too 12. The 16 experiments were carried out randomly, and the results were analyzed with statistical software, STATISTICA. The optimal cultivating condition for *C. echinulata* was as follows: glucose 33.82 g/L, ammonium chloride 1.04 g/L and pH 6.02, and the highest biomass reached 7.45 g/L and GLA 937.32 mg/L (96% of the expected result). For *Bacillus cereus* DYU-Too 12, the optimal condition was: glucose 19.00 g/L, ammonium chloride 0.91 g/L and pH 6.20. The highest biomass obtained was 2.34 g/L and the EPA 29.47 mg/L (96.21% of the expected result).

Keywords : *Cunninghamella echinulata* ; *Bacillus cereus* DYU-Too 12 ;  $\alpha$ -linolenic acid ; eicosapentaenoic acid ; central composite design

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## REFERENCES

- 參考文獻 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. 李秀、賴滋漢。1992。食品分析與檢驗。第173-174頁。精華出版社。台北。台灣。 3. 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。 4. 林登禎、李河水、華傑。2002。機能性油脂在加工食品的應用研究。第22-30頁。財團法人食品工業發展研究所。新竹。台灣。 5. 邱淑媛。2000。利用植物細胞培養及微生物發酵生產多元不飽和脂肪酸之研究。國立台灣大學農業化學研究所博士論文。台北。 6. 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。 7. 段盛秀、楊海明。2002。食品化學實驗修定版。第20-32頁。藝軒圖書出版社。台北。台灣。 8. 陳俊興。1995。利用海洋微藻生產  $\omega$ -3系列之多元不飽和脂肪酸。大葉大學碩士論文。彰化。 9. 陳清泉。1993。最適化實驗設計在食品工業產品開發上的應用。食品工業25 (2):50-62。 10. 陳鴻章、張志嘉。1994。多元不飽和脂肪酸生產菌之篩選。中國農業化學會誌32 (1):33-46。 11. 黃偉勳。1998。EPA的功能、應用及利用 *Shewanella putrefaciens* 來生產。食品工業30 (3):30-37。 12. 楊世民、林讚峰。1994。簡介利用回應曲面實驗設計法決定工業微生物的最佳培養基。製酒科技專論彙編 (16):135-150。 13. 蕭鳳岐。1992。DHA提高腦神經機能。食品資訊 (6):19-25。 14. 蘇惠美。1999。飼料生物之培養與利用。台灣省水產試驗所。台北。台灣。 15. Alonso, D. L. and Maroto F. G. 2000. Plants as 'chemical factories' for the production of polyunsaturated fatty acids. *Biotechnol. Adv.* 18: 481-497. 16. Bajpai, P. Bajpai, P. K. and Ward, O. P. 1991. Optimization of production of docosahexaenoic acid (DHA) by *Thraustochytrium aureum* ATCC 4304. *J. Am. Oil Chem. Soc.* 68: 509-514. 17. Bajpai, P. K. and Bajpai, P. 1992. Arachidonic acid production by microorganisms. *Biotechnol. Appl. Biochem.* 15: 1-10. 18. 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, 5,130,242. 19. Bowles, R. D. Hunt, A. E. Bremer, G. B. Duchars, M. G. and Eaton, R. A. 1999. Long chain n-3 polyunsaturated fatty acid production by members of the marine protistan group the Thraustochytrids: screening of isolates and optimization of docosahexaenoic acid production. *J. Biotechnol.* 70: 193-202. 20. Carter, J. P. 1988. Gamma-linolenic acid as a nutrient. *Food Technol.* 41(6): 72-82. 21. Carvalho, P. O. Oliveira, J. G. and Pastore, G. M. 1999. Enhancement of gamma-linolenic acid production by the fungus *Mucor* sp LB-54 by growth temperature. *Rev. Microbiol.* 30 (2): 170-176. 22. Certik, M. and Shimizu, S. 1999. Biosynthesis and regulation of microbial polyunsaturated fatty acid production. *J. Biosci. Bioeng.* 87 (1): 1-14. 23. Certik, M., Balteszova, L. and Sajbidor, J. 1997. Lipid formation and  $\omega$ -linolenic acid production by *Mucorales* fungi grown on sunflower oil. *Lett. Appl. Microbiol.* 25: 101-105. 24. Chen, H. C. and Chang, C. C. 1996. Production of  $\omega$ -linolenic acid by the fungus *Cunninghamella echinulata* CCRC 31840. *Biotechnol. Prog.* 12: 338-341. 25. Cohen, Z. and Cohen, S. 1991. Preparation of eicosapentaenoic acid (EPA) concentrate from *Porphyridium creurentum*. *J. Am. Oil Chem. Soc.* 68 (1): 16-19. 26. Conti, E. Stredansky, M. Stredanska, S. and Zanetti, F. 2001.  $\omega$ -linolenic acid production by solid-state fermentation of *Mucorales* strains on cereals. *Bioresour. Technol.* 76(3): 283-286. 27. Dunstan G. A., Volkman, J. K. Barrett, S. M. and Garland C. D. 1993. Changes in the lipid composition and maximization of the polyunsaturated fatty acid content of three microalgae grown in mass culture. *J. Appl. Phycol.* 5: 71-83. 28. Gema, H., Kavadia, A., Dimou, D. and Tsagou, V. 2002. Production of  $\omega$ -linolenic acid by *Cunninghamella echinulata* cultivated on glucose and orange peel. *Appl. Microbiol. Biotechnol.* 58: 303-307. 29. Goodnight, S. H., Harris, W. S., Conner, W. E. and Illingworth, D. R. 1982. Polyunsaturated fatty acid, hyperlipidemia, and thrombosis. *Arteriosclerosis.* 2 (2): 87-113. 30. Horrobin, D. F. 1987. Low prevalences of coronary heart disease (CHD), psoriasis, asthma and rheumatoid arthritis in Eskimos: are they caused by high dietary intake of eicosapentaenoic acid (EPA), a genetic variation of essential fatty acid (EFA) metabolism or a combination of both? *Med. Hypotheses.* 22 (4): 421-428. 31. Iida, I. Nakahara, T. Yokochi, T. Kamisaka, Y. Yagi, H. Yamaoka, M. and Suzuki, O. 1996. Improvement of docosahexaenoic acid production in a culture of *Thraustochytrium aureum* by medium optimization. *J. Fermentation Bioeng.* 81 (1): 76-78. 32. Jang, H. D. Lin, Y. Y. and Yang, S. S. 2000. Polyunsaturated fatty acid production with *Mortierella alpine* by solid substrate fermentation. *Bot. Bull. Acad. Sin.* 41: 41-48. 33. Jiang, Y. and Chen, F. 2000. Effects of medium glucose concentration and pH on docosahexaenoic acid content of heterotrophic *Cryptocodinium cohnii*. *Process Biochem.* 35 (10): 1205-1209. 34. Kamlangdee, N. and Fan, K. W. 2003. Polyunsaturated fatty acids production by *Schizochytrium* sp. isolated from mangrove. *Songklanakarin J. Sci. Technol.* 25 (5): 643-650. 35. Karmali, R. A., Marsh, J. and Fuchs, C. 1984. Effect of omega-3 fatty acid on growth of a rat mammary tumor. *J. Natl. Cancer Inst.* 73 (2): 457-461. 36. Kavadia, A., Komaitis, M., Chevalot, I., Blanchard, F., Marc, I. and Aggelis, G. 2001. Lipid and gamma-linolenic acid accumulation in strains of *Zygomycetes* growing on glucose. *J. Am. Oil Chem. Soc.* 78 (4): 341-345. 37. Kendrick, A. and Ratledge, C. 1992. Lipids of selected molds grown for production of n-3 and n-6 polyunsaturated fatty acids. *Lipids.* 27 (1): 15-20. 38. Kinsella, J. E. 1987. Effects of polyunsaturated fatty acid on factors related to cardiovascular

disease. *Am. J. Cardiol.* 60 (12): 23-32. 39. Krauss, R. M. Eckel, R. H. Howard, B. Appel, L. J. Daniels, S. R. Deckelbaum, R. J. Erdman, J. W. Etherton, P. K. Goldberg, I. J. Kotchen, T. A. Lichtenstein, A. H. Mitch, W. E. Mullis, R. Robinson, K. Rosett, J. W. Jeor, S. S. Suttie, J. Tribble, D. L. and Bazzarre, T. L. 2000. AHA Dietary Guidelines. AHA Scientific Statement, USA. 102 (18):2284-2299. 40. Kristofikova, L. Rosenberg, M. Vlnova, A. Sajbidor, J. and Certik, M. 1991. Selection of *Rhizopus* strains for L(+)-lactic acid and gamma-linolenic acid production. *Folia Microbiol.* 36 (5): 451-455. 41. 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. 42. Patnayak, S. and Sree, A. 2005. Screening of bacterial associates of marine sponges for single cell oil and PUFA. *Lett. Appl. Microbiol.* 40 (5):358-363. 43. Picciano, M. F. 2001. Nutrient composition of human milk. *Pediatr. Clin. North Am.* 48 (1): 53-67. 44. 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. 45. Schalin-Karrila, M. Mattila, L. Jansen, C. T. and Uotila, P. 1987. Evening primrose oil in the treatment of atopic eczema: effect on clinical status. Plasma phospholipid fatty acids and circulating blood prostaglandins. *Br. J. Dermatol.* 117 (1): 11-19. 46. Singh, A. and Ward, O. P. 1997. Production of high yields of arachidonic acid in a fed-batch system by *Mortierella alpina* ATCC 32222. *Appl. Microbiol. Biotechnol.* 48 (1): 1-5. 47. Somashekar, D. Venkateshwaran, G. Sambaiah, K. and Lokesh, B. R. 2002. Effect of culture conditions on lipid and gamma-linolenic acid production by mucoraceous fungi. *Process. biochem.* 38: 1719-1724. 48. Stinson, E. E. Kwoczak, R. Kurantz, M. 1991. Effect of culture conditions on production of eicosapentaenoic acid by *Pythium irregulare*. *J. Ind. Microbiol.* 8 (3): 171-178. 49. Stredanska, S. and Sajbidor, J. 1992. Oligounsaturated fatty acid production by selected strains of micromycetes. *Folia Microbiol.* 37 (5): 357-359. 50. Uauy-Dagach, R. and Valenzuela, A. 2000. Marine Oils: The health benefits of omega-3 fatty acids. *Nutrition Reviews.* 16 (7-8): 680-684. 51. Vazhappilly, R. and Chen, F. 1998a. Eicosapentaenoic acid and docosahexaenoic acid production potential of microalgae and their heterotrophic growth. *J. Am. Oil Chem. Soc.* 75: 393-397. 52. Vazhappilly, R. and Chen, F. 1998b. Heterotrophic production potential of omega-3 polyunsaturated fatty acids by microalgae and algae-like microorganisms. *Bot. Mar.* 41: 553-558. 53. Weitzman, I. 1984. The case for *Cunninghamella elegans*, *C. bertholletiae*, and *C. echinulata* as separate species. *Trans. Br. Mycol. Soc.* 83:527-528. 54. Wen, Z. Y. and Chen, F. 2000. Heterotrophic production of eicosapentaenoic acid by the diatom *Nitzschia laevis*: effects of silicate and glucose. *J. Ind. Microbiol. Biotechnol.* 25: 218-224. 55. Wen, Z. Y. and Chen, F. 2001. Optimization of nitrogen sources for heterotrophic production eicosapentaenoic acid by the diatom *Nitzschia laevis*. *Enzyme Microb. Technol.* 29: 341-347. 56. Xu, X. Q. and Beardall, J. 1997. Effect of salinity on the fatty acid composition of a green microalga from an Antarctic hypersaline lake. *Phytochemistry.* 45: 655-658. 57. Yongmanitchai, W. and Ward, O. P. 1989. Omega-3 fatty acids: alternative sources of production. *Process Biochem.* 24: 117-125. 58. Yongmanitchai, W. and Ward, O. P. 1991. Growth of and omega-3 fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. *Appl. Environ. Microbiol.* 57: 419-425.