

富含降血脂機能性胜?忖妊J白質開發

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摘要

醫學報導指出，造成心血管疾病及肥胖的主要原因是血脂肪(serum triglyceride level)太高，其次才是膽固醇。造成高血脂症的主要原因大都是因為脂肪代謝不正常，高血脂症好發於中年人身上，主要會引起心臟冠狀動脈硬化，因此，如何預防高血脂症將是首要。故日本Hankyu-kyoei Bussan公司從豬的血清蛋白水解液中，分離出具有降低血脂肪功能的寡勝?Val-Val-Tyr-Pro (VVYP)，並證實VVYP勝?能抑制腸道中脂肪的吸收，同時增強肝臟中三酸甘油脂脂解? (hepatic triglyceride lipase) 的活性，以達到排泄體內原有過多的脂肪。為了建立有效的VVYP勝?生產方式，利用Aspergillus oryzae alpha-amylase作為生產VVYP勝?的攜帶者。因此，本研究在不影響A. oryzae alpha-amylase的立體結構及活性之下，將多套VVYP轉成的DNA序列置換到A. oryzae alpha-amylase基因中，並定序確認。回收VVYP-amylase蛋白?，經由trypsin與carboxypeptidase B水解後的液體，利用LC-MS-MS分析VVYP產生的情形，確認VVYP-amylase的蛋白?水解液中含有VVYP勝?。實驗結果顯示，將含有四套VVYP-amylase重組基因的A. oryzae (pD3456H)培養20天時，其alpha-amylase活性及VVYP-amylase蛋白分泌量均最高，並估算1公升的A. oryzae (含有VVYP-amylase重組基因的pD3456H) 培養後的上清液可生產1.25克VVYP- amylase蛋白。

關鍵詞：降血脂機能性胜?；澱粉?；絲狀真菌

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參考文獻

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2. 行政院衛生署。2004。國人血清總膽固醇的平均值、標準差、過高比率依性別、年齡層之比較。第16-20頁。台北，台灣。
3. 行政院衛生署。2004。93年國人主要死因與主要癌症統計結果。21-24頁。台北，台灣。
4. Arai S, Osawa T, Ohigashi H, Yoshikawa M, Kaminogawa S, Watanabe M, Ogawa T, Okubo K, Watanabe S, Nishino H, Shinohara K, Esashi T, and Hirahara T. 2001. A mainstay of

functional food science in Japan--history, present status, and future outlook. Biosci Biotechnol Biochem 65(1):1-13. 5. Brantl V, Teschemacher H, Henschen A, and Lottspeich F. 1979. Novel opioid peptides derived from casein (beta-casomorphins). I. Isolation from bovine casein peptone. Hoppe Seylers Z Physiol Chem 360(9):1211-1216. 6. Braunitzer G, Schrank B, Stangl A, and Scheithauer U. 1978. Hemoglobins, XXI: sequence analysis of porcine hemoglobin. Hoppe Seylers Z Physiol Chem. 359(2):137-146. 7. Chen HM, Muramoto K, Yamauchi F, Fujimoto K, and Nokihara K. 1998. Antioxidative Properties of Histidine - Containing Peptides Designed from Peptide Fragments Found in the Digests of a Soybean Protein. J Agric Food Chem 46(1):49-53. 8. Curr SJ, Unkles SE, and Kinghorn JR. 1987. The structure and organization of nuclear gene of filamentous fungi. Oxford University press, New York. 9. Fang TY, Lin LL, and Hsu WH. 1994. Recovery of isoamylase from Pseudomonas amyloferosa by adsorption-elution on raw starch. Enzyme Microb. Technol. 16:247-252. 10. Frick MH, Elo O, Haapa K, Heinonen OP, Heinsalmi P, and Helo P. 1987. Helsinki Heart Study : primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. N. Engl. J. Med. 317(20):1237-1245. 11. Gill I, Lopez-Fandino R, Jorba X, and Vulson EN. 1996. Biologically active peptides and enzymatic approaches to their production. Enzyme Microb Technol 18(3):163-183. 12. Hoek KS, Milne JM, Grieve PA, Dionysius DA, and Smith R. 1997. Antibacterial activity in bovine lactoferrin-derived peptides. Antimicrob Agents Chemother 41(1):54-59. 13. James R, and Sowers MD. 2004. Treatment of Hypertension in patients with diabetes. Arch Intern Med.164:1850-1857. 14. Kaminogawa S. 1996. Food allergy, oral tolerance and immunomodulation--their molecular and cellular mechanisms. Biosci Biotechnol Biochem 60(11):1749-56. 15. Kagawa K , Matsutaka H , Fukuhama C , Watanabe Y , and Fujino H . 1996. Globin digest,acidic protease hydrolysate,inhibits dietary hypertriglyceridemia and Val-Val-Tyr-Pro,one of its constituents, possesses most superior effect. Life Sci. 58:1745-1755. 16. Kagawa K , Matsutaka H , Fukuhama C , Fujino H , and Okuda H . 1998.Suppressive effect of globin digest on postprandial hyperlipidemia in male volunteers. J Nutr. 128:56-60. 17. Laemmli UK. 1970. Cleavage of structural protein during the assembly of the head of bacterophage T4. Nature 227:680-685. 18. Lee KA, and Kim SH. 2005. SSGE and DEE, new peptides isolated from a soy protein hydrolysate that inhibit platelet aggregation. Food Chemistry 90: 389-393. 19. Manley CH, and Ahmed S. 1995. The development of process flavors.Trends Food Sci Technol 6(2):46-51. 20. Matoba N, Usui H, Fujita H, and Yoshikawa M. 1999. A novel anti- hypertensive peptide derived from ovalbumin induces nitric oxide- mediated vasorelaxation in an isolated SHR mesenteric artery. FEBS Lett 452(3):181-184. 21. Nakamura Y, Yamamoto N, Sakai K, and Takano T. 1995. Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme. J Dairy Sci 78(6):1253-1257. 22. Nishimura T, and Kato H. 1980. Test of free amino acid and peptides. Food Rev.Int.4(2):175-194. 23. Nishimura A, Morita M, Nishimura Y, and Sugino Y. 1990. A rapid and highly efficient method for preparation of competent Escherichia coli cells. Nucl. Acids Res. 18:61-69. 24. Raeder U, and Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1:17-20. 25. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, and Erlich HA. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science 239:487-491. 26. Sato R, Noguchi T, and Naito H. 1986. Casein phosphopeptide (CPP) enhances calcium absorption from the ligated segment of rat small intestine. J Nutr Sci Vitaminol (Tokyo) 32(1):67-76. 27. Sambrook J, and Russell DW. 2001. Molecular Cloning: a laboratory manual, third edition. Cold Spring Harbor Laboratory Press, New York. 28. Takahashi M, Fukunaga H, Kaneto H, Fukudome S, and Yoshikawa M. 2000. Behavioral and pharmacological studies on gluten exorphin A5, a newly isolated bioactive food protein fragment, in mice. Jpn J Pharmacol 84(3):259-265. 29. Tsukagoshi N , Furukawa M , Nagaba H , Kirita N , Tsuboi A ,and Udaka S. 1989. Isolation of a cDNA encoding Aspergillus oryzae Taka-amylase A:evidence for multiple related genes. Gene 84:319- 327. 30. Villalobos A, Ness JE, Gustafsson C, Minshull J, and Govindarajan S. 2006. Gene Designer: A synthetic biology tool for constructing artificial DNA segments. BMC Bioinformatics. 7 (1):1-17.