納豆激?△硎c與功能研究及腸道定殖之分子檢測技術開發

周可欣、李世傑

E-mail: 9509827@mail.dyu.edu.tw

摘 要

納豆激?＇僭　孝湃猖尷c之胞外酵素，具有強力溶解血栓活性，在酪蛋白分解活性測試中發現納豆菌SJ與F所測得的比活性相差了有52 % 之多，推估這兩株菌所含納豆激?＇b蛋白質的結構上有所差異，而比較其胺基酸序列及蛋白質的二級、三級結構分析結果，推論第53個胺基酸由絲胺酸 (serine) 改為脯胺酸 (proline)可能是結構上造成其活性最主要的原因。本實驗選殖 (clone) 定序了九種從納豆所篩選得到的納豆激?※穑]，同時也從基因庫中搜尋得到納豆菌之16S核醣體基因片段序列，來建立特殊的引子 (primer)，以建立探討納豆菌在小鼠腸胃道定殖效果的分子檢測技術。小鼠定殖（colonization）試驗結果顯示，利用加熱將非耐熱及非產生芽孢之雜菌去除掉，再配合16S rDNA及納豆激?氵牖l可以成功的在餵食耐酸納豆菌 (SJ)、耐鹼納豆菌 (JD11) 以及納豆菌孢子 (SP) 之小鼠糞便檢體中增幅出其特定的16S rDNA及納豆激?氵軉q並鑑定其菌種，推論前述這三株菌在餵食後可以通過胃酸、膽汁的分解而成功的定殖在小鼠腸道中，間接證實該三株菌所產生的納豆激?＇鳥鷴|在腸道被吸收而被小鼠利用。

關鍵詞 : 納豆菌 ; 納豆激? ; 定殖 ; 結構與功能 ; 分子檢測

目錄

參考文獻

參考文獻 1. 江曉、董明盛和江漢湖。2002。一種食源性纖溶? (納豆激?) ?學性質的研究。中國釀造 117: 23-25。 2. 董明盛、江曉和劉誠。2001。納豆激?穩定性的研究。食品與發酵工業 27: 13-15。 3. 鄒文雄與黃明經。1997。蛋白質結構之電腦預測。中國化學會55: 101-109。 4. 謝秋玲、郭勇和林劍。2001。納豆激?的合成機制。暨南大學學報 (自然科學版) 22: 110-114。 5. Addison, J. M., Burston, D. and Matthews, D. M. 1972. Evidence for active transport of the dipeptide glycylsarcosine by hamster jejunum in vitro. Clin. Sci. 43 (6): 907-911. 6. Aoyama, M., Toma, C., Yasud, M. and Iwanaga, M. 2000. Sequence of the gene encoding an alkaline serine proteinase of Bacillus pumilus TYO-67. Microbiol. Immunol. 44 (5): 389-393. 7. Chiang,C. J. H., Chen, C., Chao, Y. P. and Tzen, J. T. 2005. Efficient system of artificial oil bodies for functional expression and purification of recombinant nattokinase in Escherichia coli. J. Agric. Food Chem. 53 (12): 4799-4804. 8. Chang,C. T., Fan, M. H., Kuo,F. C. and Sung, H. Y. 2000. Potent fibrinolytic enzyme from a mutant of Bacillus subtilis IMR-NK1. J. Agric. Food Chem. 48 (8): 3210-3216. 9. Chowdhury, P. and Soulsby, M. 2002. Lipid peroxidation in rat brain is increased by simulated weightlessness and decreased by a soy-protein diet. Ann. Clin. Lab Sci. 32 (2): 188-192. 10. Fei,Y. J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F. H., Romero, M. F., Singh, S. K., Boron, W. F. and Hediger, M. A. 1994. Expression cloning of a mammalian proton-coupled oligopeptide transporter. Nature 368 (6471): 563-566. 11. Fujita,M., Nomura, K., Hong, K., Ito, Y., Asada, A. and Nishimur -o, S. 1993. Purification and characterization of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheese natto, a popular soybean fermented food in Japan. Biochem. Biophys. Res. Commun. 197 (3): 1340-1347. 12. Fujita, M., Hong, K., Ito, Y,. Fujii, R., Kariya, K. and Nishimuro, S. 1995. Thrombolytic effect of nattokinase on a chemically induced thrombosis model in rat. Biol.Pharm.Bull. 18 (10): 1387- 1391. 13. Fujita, M., Hong, K., Ito, Y., Misawa, S., Takeuchi, N., Kariya, K . and Nishimuro, S. 1995. Transport of nattokinase across the rat intestinal tract. Biol.Pharm.Bull. 18 (9): 1194-1196. 14. Guex, N., Diemand, A. and Peitsch, M. C. Protein modelling for all. 1999. Trends Biochem.Sci. 24 (9): 364-367. 15. Guo, J., Sun, Y. and Su., Y. Preparation of natto and its function in health care. 2002. Zhong.Yao Cai. 25 (1): 61-64. 16. Isezaki, M., Hosoya, S., Takeuchi, M. and Sato, T. 2001. A putati -ve ATP-binding cassette transporter YbdA involved in sporulati -on of Bacillus subtilis. FEMS Microbiol.Lett. 204 (2): 239-245. 17. Jacobs, M. F. 1995. Expression of the subtilisin carlsberg-encod -ing gene in Bacillus licheniformis and Bacillus subtilis. Gene 152 (1): 69-74. 18. Ko, J. H., Yan, J. P., Zhu, L. and Qi, Y. P. 2004. Identification of two novel fibrinolytic enzymes from Bacillus subtilis QK02. Comp Biochem. Physiol C.Toxicol.Pharmacol. 137 (1): 65-74. 19. Liu, B. Y. and Song, H. Y. 2002. Molecular cloning and expressio -n of Nattokinase gene in Bacillus subtilis. Sheng Wu Hua Xue.Yu Sheng Wu Wu Li Xue.Bao. (Shanghai) 34 (3): 338-340. 20. Liang, R., Fei, Y. J., Prasad, P. D., Ramamoorthy, S., Han, H., Yang-Feng, T. L., Hediger, M. A., Ganapathy, V. and Leibach, F. H. 1995. Human intestinal H+/peptide cotransporter. cloning, functional expression, and chromosomal localization. J.Biol.Chem. 270 (12): 6456-6463. 21. Lucchini, F., Kmet, V., Cesena, C., Coppi, L., Bottazzi, V. and Morelli, L. 1998. Specific detection of a probiotic Lactobacillus strain in faecal samples by using multiplex PCR. FEMS Microbiol. Lett. 158 (2): 273-278. 21. Markland, F. S. and Smith, E. L. 1967. Subtilisin BPN. VII. Isolat -ion of cyanogen bromide peptides and the complete amino acid sequence. J. Biol. Chem. 242 (22): 5198-5211. 22. Messina, M. 1999. Soy, soy phytoestrogens (isoflavones), and breast cancer. Am. J. Clin. Nutr. 70 (4): 574-575. 23. Nakamura, T., Yamagata, Y. and Ichishima, E. 1992. Nucleotide sequence of the subtilisin NAT gene, aprN, of Bacillus subtilis (natto). Biosci. Biotechnol. Biochem. 56 (11): 1869-1871. 24. Pace, N. R., Olsen, G. J. and Woese, C. R. 1986. Ribosomal RNA phylogeny and the primary lines of evolutionary descent. Cell 45 (3): 325-326. 25. Patterton, H. G. and Graves, S. 2000. DNAssist: the integrated editing and analysis of molecular biology sequences in windows. Bioinformatics. 16 (7): 652-653. 26. Peng, Y., Huang, Q., Zhang, R. H. and Zhang, Y. Z. 2003. Purific -ation and characterization of a fibrinolytic enzyme produced by Bacillus amyloliquefaciens DC-4 screened from douchi, a traditional Chinese soybean food. Comp Biochem. Physiol B Biochem. Mol. Biol. 134 (1):45-52. 27. Samanya, M. and Yamauchi, K. E. 2002. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto. Comp Biochem.Physiol A Mol.Integr.Physiol 133 (1): 95-104. 28. Sato, T., Yamada, Y., Ohtani, Y., Mitsui, N., Murasawa, H. and Araki, S. 2001. Production of menaquinone (vitamin K2)-7 by Bacillus subtilis. J.Biosci.Bioeng. 91 (1): 16-20. 29. Sato, T., Yamada, Y., Ohtani, Y., Mitsui, N., Murasawa, H. and Araki, S. 2001. Efficient production of menaquinone (vitamin K2) by a menadione-resistant mutant of Bacillus subtilis. J. Ind. Microbiol. Biotechnol. 26 (3): 115-120. 30. Stackebrandt, E., Liesack, W. and Witt, D. 1992. Ribosomal RNA and rDNA sequence analyses. Gene 115 (1-2): 255-260. 31. Sumi, H., Hamada,

H., Nakanishi, K. and Hiratani, H. 1990. Enha -ncement of the fibrinolytic activity in plasma by oral administration of nattokinase. Acta Haematol. 84 (3): 139-143. 32. Sumi, H., Hamada, H., Tsushima, H., Mihara, H. and Muraki, H. 1987. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. Experientia 43 (10): 1110-1111. 33. Stahl, M. L. and Ferrari, E. 1984. Replacement of the Bacillus subtilis subtilisin structural gene with an In vitro-derived deletion mutation. J.Bacteriol. 158 (2): 411-418. 34. Suzuki, K., Koike, H., Matsui, H., Ono, Y., Hasumi, M., Nakazat -o, H., Okugi, H., Sekine, Y., Oki, K., Ito, K., Yamamoto, T., Fukabori, Y., Kurokawa, K. and Yamanaka, H. 2002. Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3. Int.J.Cancer 99 (6): 846-852. 35. Suzuki, Y., Kondo, K., Ichise, H., Tsukamoto, Y., Urano, T. and Umemura, K. 2003. Dietary supplementation with fermented soy beans suppresses intimal thickening. Nutrition 19 (3): 261-264. 36. Tai, M. W. and Sweet, B. V. 2006. Nattokinase for prevention of thrombosis. Am.J.Health Syst.Pharm. 63 (12): 1121-1123. 37. Suzuki, Y., Kondo, K., Matsumoto, Y. Zhao, B. Q., Otsuguro, K., Maeda, T., Tsukamoto, Y., Urano, T. and Umemura, K. 2003. Dietary supplementation of fermented soybean, natto, suppresses intimal thickening and modulates the lysis of mural thrombi after endothelial injury in rat femoral artery. Life Sci. 73 (10): 1289-1298. 38. Takano, A., Hirata, A., Ogasawara, K., Sagara, N., Inomata, Y., Kawaji, T. and Tanihara, H. 2006. Posterior vitreous detachment induced by nattokinase (subtilisin NAT): a novel enzyme for pharmacologic vitreolysis. Invest Ophthalmol. Vis. Sci. 47 (5): 2075-2079. 39. Urano, T., Ihara, H., Umemura, K., Suzuki, Y., Oike, M., Akita, S., Tsukamoto, Y., Suzuki, I. and Takada., A. 2001. The profibrinolytic enzyme subtilisin NAT purified from Bacillus subtilis cleaves and inactivates plasminogen activator inhibitor type 1. J.Biol.Chem. 276 (27):24690-24696. 40. Vasantha, N., Thompson, L. D., Rhodes, C., Banner, C., Nagle, J. and Filpula, D. 1984. Genes for alkaline protease and neutral protease from Bacillus amyloliquefaciens contain a large open reading frame between the regions coding for signal sequence and mature protein. J.Bacteriol. 159 (3): 811-819. 41. Van de, Peer Y., Chapelle, S. and De, W. R. 1996. A quantitative map of nucleotide substitution rates in bacterial rRNA. Nucleic Acids Res. 24 (17): 3381-3391. 42. Van de, Peer Y., Jansen, J., De, R. P. and De, W. R. 1997. Database on the structure of small ribosomal subunit RNA. Nucleic Acids Res. 25 (1): 111-116. 43. Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18 (22): 6531-6535. 44. Welsh, J., Rampino, N., McClelland, M. and Perucho, M. 1995. Nucleic acid fingerprinting by PCR-based methods: applications to problems in aging and mutagenesis. Mutat. Res. 338 (1-6): 215-229. 45. Wattiau, P., Renard, M. E., Ledent, P., Debois, V., Blackman, G. and Agathos, S. N. 2001. A PCR test to identify Bacillus subtilis and closely related species and its application to the monitoring of wastewater biotreatment. Appl. Microbiol. Biotechnol. 56(5-6) : 816-819. 46. Yamaguchi, M., Kakuda, H., Gao, Y. H. and Tsukamoto, Y. 2000. Prolonged intake of fermented soybean (natto) diets containing vitamin K2 (menaquinone-7) prevents bone loss in ovariectomiz -ed rats. J.Bone Miner. Metab 18 (2): 71-76. 47. Zheng, Z. L., Ye, M. Q., Zuo, Z. Y., Liu, Z. G., Tai, K. C. and Zou, G. L. 2006. Probing the importance of hydrogen bonds in the active site of the subtilisin nattokinase by site-directed mutagenesis and molecular dynamics simulation. Biochem.J. 395 (3): 509-515. 48. Zheng, Z. L., Zuo, Z. Y., Liu, Z. G., Tsai, K. C., Liu, A. F. and Zou, G. L. 2005. Construction of a 3D model of nattokinase, a novel fibrinolytic enzyme from Bacillus natto. A novel nucleophilic catalytic mechanism for nattokinase. J. Mol. Graph. Model. 23 (4): 373-380.