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關鍵字(中)	納豆激酶 啟動子 抽出物 酵母菌 自由態 生理學
關鍵字(英)	nattokinase promoter already future system Japan
摘要(中)	納豆激?(nattokinase)是一些枯草桿菌發酵大豆而生成的一種蛋白質。1986年日本生理學教授須見洋行 (Hiroyuki Sumi) 博士從 200 多種食品中發現納豆含有天然的血栓溶解酵素，並命名之。目前已知道與納豆激?基因極為相似之 aprE 基因表現受到複雜的調控機制，包括 σ37 因子及對應的 RNA 聚合酵素，此外 DegS-DegU 雙蛋白調控系統，Spo0A、ScoC、SinR、SinI、Hpr 等蛋白均會影響啟動子的表現。目前對於納豆激?的研究主要在基因的調控以及食用生理反應上，本研究希望了解如何從培養基成份來看納豆激?基因啟動子的生化特性分析。研究結果顯示枯草桿菌菌株 ZU011、BCRC 14718 均具有血纖維溶解酵素存在，而大腸桿菌則

	沒有；序列比對顯示 B. subtilis ZU011、B. subtilis BCRC 14718 及 B. subtilis str 168 之納豆激?基因上游含啟動子片段約 1.8kb 有 97%的高相似度。以 TS 培養基培養含重組質體 pMT7-1、pMT7-2、pMNP011-1、pMNP011-2、pMNP718 之轉形菌株，發現 pMNP011-1 冷光表現量最高；以 LB 培養基培養則 pMNP718 冷光表現量最高，惟仍低於 TS 培養基；以 MFB 培養基培養則 pMNP011 表現量最高；以 MFBS 培養基培養則仍是 pMNP011 表現量最高；從上述培養基可以了解酵母菌抽出物（yeast extract）具有有效的讓啟動子表現活性的成份，分析成分後以不同自由態胺基酸添加入沒有酵母菌抽出物之 MFB 培養基，發現納豆激?啟動子表現均不顯著，且細菌生長情況不佳，如果將 20 種自由態胺基酸一起添加，雖然細菌的生長可以回復到一般培養基的生長速度，然納豆激?基因啟動子的表現仍不強。酵母菌抽出物是不是其中的有效成分可以讓大腸桿菌代謝產生可以誘導納豆激?基因啟動子表現的相關蛋白，未來可以透過如 HPLC 或蛋白質電泳來分出不同的多?或其他成分，來做更進一步的析研究。
摘要(英)	Nattokinase is a kind of protein that some withered fermented soybeans of Bacillus subtilis are produced . The physiologic professor of Japan Hiroyuki Sumi found that natto contain the natural thrombus-dissolving enzyme in 1986, and named it.Now it has already known that aprE gene is very similar to nattokinase gene and displays the complicated regulation and control mechanism, including sigma 37 factor and corresponding RNA polymerase. In addition one pair of albumens of DegS-DegU controls the system, the albumens , such as Spo0A , ScoC , SinR , SinI , Hpr ,etc. will influence promoter expression.On the foretime research mainly at the gene expression and physiological reaction. It is a wish to understand the nattokinase gene promoter expression from culture medium composition in this research. The results show that B. subtilis ZU011and BCRC 14718 have thrombus-dissolving enzyme.The sequence alignment displays B. subtilis ZU011、B. subtilis BCRC 14718 and B. subtilis str.168, have 97% high comparability with each other in nattokinase gene and upstream sequence. In the culture of transformed TOP10 with recombined plasmid pMT7-1、 pMT7-2、pMNP011-1、pMNP011-2 and pMNP718,it is clear the pMNP011-1has the highest luminescence expression in TS medium. In other mediums the pMNP011 almost has the highest luminescence expression.In other words the yeast extract has effective components to express promoter activity. Does the yeast extract take the effective composition that E.coli can produce related proteins to enhance nattokinase promoter expression.In the future it can do further study by means of HPLC or PAGE.
論文目次	

參考文獻

吳佩儀。1998 柑橘潰瘍病菌 recA 基因表現的調控。天主教輔仁大學生命科學研究所碩士論文。 邱子玲。2004 自然環境及市售納豆產品中 Bacillus 分離株之特性分析及種類鑑定。天主教輔仁大學生命科學研究所碩士論文。 施英隆、范宜琮。2001 納豆~神奇之保健食品。生物資源生物技術第四卷第四期，37-45。 Adinarayana, K., and P. Ellaiah. 2002. Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated Bacillus sp. J Pharm Pharm Sci 5:272-8. Arai, A., E. Kawachi, M. Hata, M. Ogura, and T. Tanaka. 2003. Inhibition of Bacillus subtilis aprE expression by lincomycin at the posttranscriptional level through inhibition of ppGpp synthesis. J Biochem (Tokyo) 134:691-7. Arai, A., E. Kawachi, M. Hata, M. Ogura, and T. Tanaka. 2003. Inhibition of Bacillus subtilis aprE expression by lincomycin at the posttranscriptional level through inhibition of ppGpp synthesis. J Biochem (Tokyo) 134:691-7. Baev, M. V., D. Baev, A. J. Radek, and J. W. Campbell. 2006. Growth of Escherichia coli MG1655 on LB medium: monitoring utilization of sugars, alcohols, and organic acids with transcriptional microarrays. Appl Microbiol Biotechnol 71:310-6. Baev, M. V., D. Baev, A. Jansco Radek, and J. W. Campbell. 2006. Growth of Escherichia coli MG1655 on LB medium: monitoring utilization of amino acids, peptides, and nucleotides with transcriptional microarrays. Appl Microbiol Biotechnol. Bai, U., I. Mandic-Mulec, and I. Smith. 1993. SinI modulates the activity of SinR, a developmental switch protein of Bacillus subtilis, by protein-protein interaction. Genes Dev 7:139-48. Carmona, M., M. J. Rodriguez, O. Martinez-Costa, and V. De Lorenzo. 2000. In vivo and in vitro effects of (p)ppGpp on the sigma(54) promoter Pu of the TOL plasmid of Pseudomonas putida. J Bacteriol 182:4711-8. Carter, H. L., 3rd, and C. P. Moran, Jr. 1986. New RNA polymerase sigma factor under spo0 control in Bacillus subtilis. Proc Natl Acad Sci U S A 83:9438-42. Cases, I., and V. de Lorenzo. 1998. Expression systems and physiological control of promoter activity in bacteria. Curr Opin Microbiol 1:303-10. Cases, I., and V. de Lorenzo. 2000. Genetic evidence of distinct physiological regulation mechanisms in the sigma(54) Pu promoter of Pseudomonas putida. J Bacteriol 182:956-60. Chang, C. T., M. H. Fan, F. C. Kuo, and H. Y. Sung. 2000. Potent fibrinolytic enzyme from a mutant of Bacillus subtilis IMR-NK1. J Agric Food Chem 48:3210-6. Chiang, C. J., H. C. Chen, Y. P. Chao, and J. T. Tzen. 2005. Efficient system of artificial oil bodies for functional expression and purification of recombinant nattokinase in Escherichia coli. J Agric Food Chem 53:4799-804. Choi, N. S., K. H. Yoo, K. S. Yoon, P. J. Maeng, and S. H. Kim. 2004. Nano-scale proteomics approach using

two-dimensional fibrin zymography combined with fluorescent SYPRO ruby dye. J Biochem Mol Biol 37:298-303. Dahl, M. K., T. Msadek, F. Kunst, and G. Rapoport. 1991. Mutational analysis of the Bacillus subtilis DegU regulator and its phosphorylation by the DegS protein kinase. J Bacteriol 173:2539-47. Dahl, M. K., T. Msadek, F. Kunst, and G. Rapoport. 1992. The phosphorylation state of the DegU response regulator acts as a molecular switch allowing either degradative enzyme synthesis or expression of genetic competence in Bacillus subtilis. J Biol Chem 267:14509-14. Dartois, V., M. Debarbouille, F. Kunst, and G. Rapoport. 1998. Characterization of a novel member of the DegS-DegU regulon affected by salt stress in Bacillus subtilis. J Bacteriol 180:1855-61. Doi, R. H. 1982. Multiple RNA polymerase holoenzymes exert transcriptional specificity in Bacillus subtilis. Arch Biochem Biophys 214:772-81. Farrell, M. J., and S. E. Finkel. 2003. The growth advantage in stationary-phase phenotype conferred by rpoS mutations is dependent on the pH and nutrient environment. J Bacteriol 185:7044-52. Fayek, K. I., and S. T. El-Sayed. 1980. Fibrinolytic activity of an enzyme produced by Bacillus subtilis. Z Ernahrungswiss 19:21-3. Fayek, K. I., and S. T. El-Sayed. 1980. Purification and properties of a fibrinolytic enzyme from Bacillus subtilis. Z Allg Mikrobiol 20:375-82. Fayek, K. I., and S. T. El-Sayed. 1980. Some properties of two purified fibinolytic enzymes from Bacillus subtilis and B. polymyxa. Z Allg Mikrobiol 20:383-7. Fujita, M., K. Hong, Y. Ito, R. Fujii, K. Kariya, and S. Nishimuro. 1995. Thrombolytic effect of nattokinase on a chemically induced thrombosis model in rat. Biol Pharm Bull 18:1387-91. Fujita, M., K. Nomura, K. Hong, Y. Ito, A. Asada, and S. Nishimuro. 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 197:1340-7. Guo, J., Y. Sun, and Y. Su. 2002. [Preparation of natto and its function in health care]. Zhong Yao Cai 25:61-4. Haldenwang, W. G., and R. Losick. 1980. Novel RNA polymerase sigma factor from Bacillus subtilis. Proc Natl Acad Sci U S A 77:7000-4. Haldenwang, W. G., N. Lang, and R. Losick. 1981. A sporulation-induced sigma-like regulatory protein from B. subtilis. Cell 23:615-24. Hambraeus, G., K. Karhumaa, and B. Rutberg. 2002. A 5' stem-loop and ribosome binding but not translation are important for the stability of Bacillus subtilis aprE leader mRNA. Microbiology 148:1795-803. Hambraeus, G., M. Persson, and B. Rutberg. 2000. The aprE leader is a determinant of extreme mRNA stability in Bacillus subtilis. Microbiology 146 Pt 12:3051-9. Harwood, Colin R. 1989. Bacillus. Plenum Press, New York.. Hata, M., M. Ogura, and T. Tanaka. 2001. Involvement of stringent factor RelA in expression of the alkaline protease gene aprE in Bacillus subtilis. J Bacteriol 183:4648-51. Hata, M., M. Ogura, and T. Tanaka. 2001. Involvement of stringent factor RelA in expression of the alkaline protease gene aprE in Bacillus subtilis. J Bacteriol 183:4648-51. Hirsch, M., and T. Elliott. 2002. Role of ppGpp in rpoS stationary-phase regulation in Escherichia coli. J Bacteriol 184:5077-87. Igo, M., M. Lampe, C. Ray, W. Schafer, C. P. Moran, Jr., and R. Losick. 1987. Genetic studies of a secondary RNA polymerase sigma factor in Bacillus subtilis. J Bacteriol

169:3464-9. Ikemura, H., and M. Inouye. 1988. In vitro processing of pro-subtilisin produced in Escherichia coli. J Biol Chem 263:12959-63. Ikemura, H., H. Takagi, and M. Inouye. 1987. Requirement of pro-sequence for the production of active subtilisin E in Escherichia coli. J Biol Chem 262:7859-64. Jan, J., F. Valle, F. Bolivar, and E. Merino. 2000. Characterization of the 5' subtilisin (aprE) regulatory region from Bacillus subtilis. FEMS Microbiol Lett 183:9-14. Kallio, P. T., J. E. Fagelson, J. A. Hoch, and M. A. Strauch. 1991. The transition state regulator Hpr of Bacillus subtilis is a DNA-binding protein. J Biol Chem 266:13411-7. Kim, W., K. Choi, Y. Kim, H. Park, J. Choi, Y. Lee, H. Oh, I. Kwon, and S. Lee. 1996. Purification and characterization of a fibrinolytic enzyme produced from Bacillus sp. strain CK 11-4 screened from Chungkook-Jang. Appl Environ Microbiol 62:2482-8. Ko, J. H., J. P. Yan, L. Zhu, and Y. P. Qi. 2004. Identification of two novel fibrinolytic enzymes from Bacillus subtilis QK02. Comp Biochem Physiol C Toxicol Pharmacol 137:65-74. Kunst, F., and G. Rapoport. 1995. Salt stress is an environmental signal affecting degradative enzyme synthesis in Bacillus subtilis. J Bacteriol 177:2403-7. Kunst, F., T. Msadek, J. Bignon, and G. Rapoport. 1994. The DegS/DegU and ComP/ComA two-component systems are part of a network controlling degradative enzyme synthesis and competence in Bacillus subtilis. Res Microbiol 145:393-402. Liu, B. Y., and H. Y. Song. 2002. [Molecular cloning and expression of Nattokinase gene in Bacillus subtilis]. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 34:338-40. Losick, R., and J. Pero. 1981. Cascades of Sigma factors. Cell 25:582-4. Msadek, T., F. Kunst, A. Klier, and G. Rapoport. 1991. DegS-DegU and ComP-ComA modulator-effector pairs control expression of the Bacillus subtilis pleiotropic regulatory gene degQ. J Bacteriol 173:2366-77. Msadek, T., F. Kunst, D. Henner, A. Klier, G. Rapoport, and R. Dedonder. 1990. Signal transduction pathway controlling synthesis of a class of degradative enzymes in Bacillus subtilis: expression of the regulatory genes and analysis of mutations in degS and degU. J Bacteriol 172:824-34. Mukai, K., M. Kawata, and T. Tanaka. 1990. Isolation and phosphorylation of the Bacillus subtilis degS and degU gene products. J Biol Chem 265:20000-6. Mukai, K., M. Kawata-Mukai, and T. Tanaka. 1992. Stabilization of phosphorylated Bacillus subtilis DegU by DegR. J Bacteriol 174:7954-62. Ogura, M., A. Matsuzawa, H. Yoshikawa, and T. Tanaka. 2004. Bacillus subtilis SalA (YbaL) negatively regulates expression of scoC, which encodes the repressor for the alkaline exoprotease gene, aprE. J Bacteriol 186:3056-64. Ogura, M., K. Shimane, K. Asai, N. Ogasawara, and T. Tanaka. 2003. Binding of response regulator DegU to the aprE promoter is inhibited by RapG, which is counteracted by extracellular PhrG in Bacillus subtilis. Mol Microbiol 49:1685-97. Ogura, M., K. Shimane, K. Asai, N. Ogasawara, and T. Tanaka. 2003. Binding of response regulator DegU to the aprE promoter is inhibited by RapG, which is counteracted by extracellular PhrG in Bacillus subtilis. Mol Microbiol 49:1685-97. Olmos, J., R. de Anda, E. Ferrari, F. Bolivar, and F. Valle. 1997. Effects of the sinR and degU32 (Hy) mutations on the regulation of the aprE gene in Bacillus subtilis. Mol Gen Genet 253:562-7.

Olmos, J., V. Bolanos, S. Causey, E. Ferrari, F. Bollvar, and F. Valle. 1996. A functional Spo0A is required for maximal aprE expression in Bacillus subtilis. FEBS Lett 381:29-31. Palva, I. 1982. Molecular cloning of alpha-amylase gene from Bacillus amyloliquefaciens and its expression in B. subtilis. Gene 19:81-7. Park, S. S., S. L. Wong, L. F. Wang, and R. H. Doi. 1989. Bacillus subtilis subtilisin gene (aprE) is expressed from a sigma A (sigma 43) promoter in vitro and in vivo. J Bacteriol 171:2657-65. Peng, Y., Q. Huang, R. H. Zhang, and Y. Z. Zhang. 2003. Purification 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 134:45-52. Peng, Y., X. J. Yang, L. Xiao, and Y. Z. Zhang. 2004. Cloning and expression of a fibrinolytic enzyme (subtilisin DFE) gene from Bacillus amyloliquefaciens DC-4 in Bacillus subtilis. Res Microbiol 155:167-73. Powell, B. S., and D. L. Court. 1998. Control of ftsZ expression, cell division, and glutamine metabolism in Luria-Bertani medium by the alarmone ppGpp in Escherichia coli. J Bacteriol 180:1053-62. Prakasham, R. S., S. Rao Ch, and P. N. Sarma. 2006. Green gram husk-an inexpensive substrate for alkaline protease production by Bacillus sp. in solid-state fermentation. Bioresour Technol 97:1449-54. Sanchez, A., and J. Olmos. 2004. Bacillus subtilis transcriptional regulators interaction. Biotechnol Lett 26:403-7. Schallmey, M., A. Singh, and O. P. Ward. 2004. Developments in the use of Bacillus species for industrial production. Can J Microbiol 50:1-17. Shimane, K., and M. Ogura. 2004. Mutational analysis of the helix-turn-helix region of Bacillus subtilis response regulator DegU, and identification of cis-acting sequences for DegU in the aprE and comK promoters. J Biochem (Tokyo) 136:387-97. Stephens, M. A., S. A. Ortlepp, J. F. Ollington, and D. J. McConnell. 1984. Nucleotide sequence of the 5' region of the Bacillus licheniformis alpha-amylase gene: comparison with the B. amyloliquefaciens gene. J Bacteriol 158:369-72. Stragier, P., and R. Losick. 1990. Cascades of sigma factors revisited. Mol Microbiol 4:1801-6. Sumi, H., H. Hamada, H. Tsushima, H. Mihara, and H. Muraki. 1987. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. Experientia 43:1110-1. Sumi, H., H. Hamada, K. Nakanishi, and H. Hiratani. 1990. Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinase. Acta Haematol 84:139-43. Tai, M. W., and B. V. Sweet. 2006. Nattokinase for prevention of thrombosis. Am J Health Syst Pharm 63:1121-3. Takano, A., A. Hirata, K. Ogasawara, N. Sagara, Y. Inomata, T. Kawaji, and H. Tanihara. 2006. Posterior vitreous detachment induced by nattokinase (subtilisin NAT): a novel enzyme for pharmacologic vitreolysis. Invest Ophthalmol Vis Sci 47:2075-9. Tover, A., E. L. Ojangu, and M. Kivisaar. 2001. Growth medium composition-determined regulatory mechanisms are superimposed on CatR-mediated transcription from the pheBA and catBCA promoters in Pseudomonas putida. Microbiology 147:2149-56. Urano, T., H. Ihara, K. Umemura, Y. Suzuki, M. Oike, S. Akita, Y. Tsukamoto, I. Suzuki, and A. Takada. 2001. The profibrinolytic enzyme subtilisin NAT purified from Bacillus subtilis Cleaves and

	inactivates plasminogen activator inhibitor type 1. J Biol Chem 276:24690-6. Wang, C. T., B. P. Ji, B. Li, R. Nout, P. L. Li, H. Ji, and L. F. Chen. 2006. Purification and characterization of a fibrinolytic enzyme of Bacillus subtilis DC33, isolated from Chinese traditional Douchi. J Ind Microbiol Biotechnol. Wiggs, J. L., M. Z. Gilman, and M. J. Chamberlin. 1981. Heterogeneity of RNA polymerase in Bacillus subtilis: evidence for an additional sigma factor in vegetative cells. Proc Natl Acad Sci U S A 78:2762-6. Wong, S. L., and R. H. Doi. 1984. Utilization of a Bacillus subtilis sigma 37 promoter by Escherichia coli RNA polymerase in vivo. J Biol Chem 259:9762-7. Wong, S. L., C. W. Price, D. S. Goldfarb, and R. H. Doi. 1984. The subtilisin E gene of Bacillus subtilis is transcribed from a sigma 37 promoter in vivo. Proc Natl Acad Sci U S A 81:1184-8. Zhao, K., M. Liu, and R. R. Burgess. 2005. The global transcriptional response of Escherichia coli to induced sigma 32 protein involves sigma 32 regulon activation followed by inactivation and degradation of sigma 32 in vivo. J Biol Chem 280:17758-68. Zheng, Z. L., Z. Y. Zuo, Z. G. Liu, K. C. Tsai, A. F. Liu, and G. L. Zou. 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 23:373-80.
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