Aspergillus oryzae Leucine Aminopeptidase基因轉殖番茄之育成

鄭仁君、游志文

E-mail: 9417453@mail.dyu.edu.tw

摘 要

白胺酸N端切位?（Leucine aminopeptidase；LAP）可水解大分子量食物蛋白質，特異性（specific）切除胜?兩端的疏水性胺基酸，除去苦味，使食物呈現正面的風味。本研究以食品發酵工業常使用的菌種Aspergillus oryzae的LAP基因，構築於pMON530表現載體上，並轉型至番茄植株以建立含Aspergillus oryzae LAP基因的轉基因株系。?此，藉內含LAP基因之質體pQE30-lap為模板，設計引子進行聚合?連鎖反應（PCR），以擴增含LAP基因的DNA片段。所增殖之LAP基因5’-端另包含6個histidine殘基，以方便LAP蛋白純化分析。將PCR擴增之DNA片段接合於pMON530載體，利用質體上之CaMV35S promoter為啟動子，建構成pMON530-LAP質體。再藉農桿菌轉殖方式，將Aspergillus oryzae LAP基因轉型至番茄植株中，並藉由genomic PCR鑑定LAP基因確已嵌插至轉殖植株之染色體中。經馴化後各轉基因株系隨即出瓶轉移至溫室培養。

關鍵詞 : 番茄、組織培養、基因轉殖、再生、農桿菌、白胺酸N端切位?、麴菌

目錄

參考文獻

Development of transgenic tomato of Aspergillus oryzae Leucine Aminopeptidase 指導教授: 游志文 指導教授(英文姓名): Chih-Wen Yu 學位類別: 碩士 校院名稱: 大葉大學　系所名稱: 分子生物科技學系碩士班 學號: R9160017 學年度: 93 語文別: 中文 論文頁數: 61 關鍵詞: 番茄、組織培養、基因轉殖、再生、農桿菌、白胺酸N端切位?、麴菌 英文關鍵詞: Agrobacterium, Aspergillus oryzae, Leucine aminopeptidase (LAP), regeneration, tissue culture, tomato, transformation 被引用次數: 0 [摘要] 白胺酸N端切位?（Leucine aminopeptidase；LAP）可水解大分子量食物蛋白質，特異性（specific）切除胜?兩端的疏水性胺基酸，除去苦味，使食物呈現正面的風味。本研究以食品發酵工業常使用的菌種Aspergillus oryzae的LAP基因，構築於pMON530表現載體上，並轉型至番茄植株以建立含Aspergillus oryzae LAP基因的轉基因株系。?此，藉內含LAP基因之質體pQE30-lap為模板，設計引子進行聚合?連鎖反應（PCR），以擴增含LAP基因的DNA片段。所增殖之LAP基因5’-端另包含6個histidine殘基，以方便LAP蛋白純化分析。將PCR擴增之DNA片段接合於pMON530載體，利用質體上之CaMV35S promoter為啟動子，建構成pMON530-LAP質體。再藉農桿菌轉殖方式，將Aspergillus oryzae LAP基因轉型至番茄植株中，並藉由genomic PCR鑑定LAP基因確已嵌插至轉殖植株之染色體中。經馴化後各轉基因株系隨即出瓶轉移至溫室培養。
[英文摘要] Aspergillus oryzae leucine aminopeptidase (LAP) specifically and effectively catalyzes the hydrolytic digestion of the hydrophobic amino acid residues in the N-terminus of the peptides of different origin. For the purpose of reducing the production cost of LAP, the LAP gene was amplified by polymerase chain reaction (PCR), and cloned into plant expression vector pMON530. A hexa-histidine affinity tag was fused to the N-terminus of the LAP gene for protein purification by affinity chromatography. With leaf-disc method, the recombinant plasmid was introduced into tomato cells via Agrobacterium tumefaciens-mediated transformation to overexpress LAP gene in plants. LAP gene was already transformation in tomato plant, and determine by genomic PCR. The transgenic tomato of Aspergillus oryzae Leucine Aminopeptidase also domestication and growing in green house.

 [參考文獻] 陳俊彰（2002）Aspergillus oryzae與Aspergillus sojae leucine aminopeptidase 基因的調控、特性與表現，碩士論文，分子生物學研究所，中興大學，台中。 程竹青（1996）蛋白水解液苦味之探討，食品工業，28（10）:32-39。 楊芳鏘，陳志雄，楊明哲（2001）以酵素水解法生產黃豆胜?之研究:去除苦味方法之探討，東海學報，42:67-77。 管宜家（2003）Aspergillus oryzae leucine aminopeptidase 基因的調控與表現，碩士論文，分子生物學研究所，中興大學，台中。 趙秀慧（2000）Aspergillus oryzae Leucine Aminopeptidase 基因的選殖，碩士論文，分子生物學研究所，中興大學，台中。 鄭靜桂（1997）蛋白質之水解與水解液之利用，食品工業月刊，29:10-17。 Bachmair A, Finley D, and Varshavsky A (1986) In vivo half-life of a protein is a function of its amino-terminal residue. Science. 234:179-186. Bartling D and Weiler EW (1992) Leucine aminopeptidase from Arabidopsis thaliana. Molecular evidence for a phylogenetically conserved enzyme of protein turnover in higher plants. Eur. J. Biochem. 205:425-431. Benedetti E, Gavuzzo E, Santini A, Kent DR, Zhu YF, Zhu Q, Mahr C, and Goodman M (1995) Sweet and bitter taste: structure and conformations of two aspartame dipeptide analogues. J. Pept. Sci. 1:349-359. Carr JW, Lougheed TC, and Baker BE (1956) Studies on protein hydrolysis. IV. Futher observation on the taste of enzymic protein hydrolysates. J. Sci. Food Agric. 7:629-637. Cerovsky V and Bordusa F (2000) Protease-catalyzed fragment condensation via substrate mimetic strategy: a useful combination of solid-phase peptide synthesis with enzymatic methods. J. Pept. Res. 55:325-329. Chevrier B, Schalk C, D'Orchymont H, Rondeau JM, Moras D, and Tarnus C (1994) Crystal structure of Aeromonas proteolytica aminopeptidase: a prototypical member of the co-catalytic zinc enzyme family. Structure. 2:283-291. Chien H-CR, Lin LL, Chao SH, Chen CC, Wang WC, Shaw CY, Tsai YC, Hu HY, and Hsu WH (2002) Purification, characterization, and genetic analysis of a leucine aminopeptidase from Aspergillus sojae. Biochim. Biophys. Acta. 1576:119-126. Esser K and Lemke PA (1995) THE MYCOTA. Springer-Verlag, New York. Fujimaki M, Yamashita M, Okazawa Y, and Arai S (1968) Diffusable bitter peptides inpeptic hydrolyzate of soyben protein. Agric. Biol. Chem. 32:794-795. Gamborg OL, Miller RA, and Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158. Ge SJ, and Zhang LX (1996) The immobilized porcine pancreatic exopeptidases and its application in casein hydrolysates debittering. Appl. Biochem. Biotechnol. 59:159-165. Gu YQ, Pautot V, Holzer FM, and Walling LL (1996) A Complex Array of Proteins Related to the Multimeric Leucine Aminopeptidase of Tomato. Plant Physiol. 110:1257-1266. Habibi-Najafi MB, and Lee BH (1996) Bitterness in cheese: a review. Crit. Rev. Food Sci. Nutr. 36:397-411. Hanahan D (1983) Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. 166:557-580. Hoekema A, Hirsch PR, Hooykaas PJJ, and Schilperoort RA (1983) Binary vector strategy based on separation of vir- and T-region of the Agrobacterium tumefaciens Ti-plasmid. Nature. 303:179-180. Ishibashi N, Kubo T, Chino M, Fukui H, Shinoda I, KiKuchi E, and OkaiH (1988a) Taste of proline-containing peptides. Agric. Biol. Chem. 52:95-98. Ishibashi N, Ono I, Kato K, Shigenaga T, Shinoda I, Okai H, and Fukui S (1988b) Role of hydrophobic amino acid residue in the bitterness of peptides. Agric. Biol. Chem. 52:91-94. Ishizaki T, Tosaka A, Nara T, Aoshima N, Namekawa S, Watanabe K, Hamada F, Omori A, and Sakaguchi K (2002) Leucine aminopeptidase during meiotic development. Eur. J. Biochem. 269:826-32. Ivanova NM, Vaganova TI, Strongin AI, and Stepanov VM (1977) Isolation and properties of leucine aminopeptidase from Aspergillus oryzae. Biokhimiia. 42: 843-849 Iwase A, Nomura S, and Mizutani S (2001) Characterization of a secretase activity for placental leucine aminopeptidase. Arch. Biochem. Biophys. 393:163-169. Kanno T, Maekawa M, Kanda S, Kohno H, and Sudo K (1984) Evaluation of cytosolic aminopeptidase in human sera. Evaluation in hepatic disorders. Am. J. Clin. Pathol. 82:700-705. Ku HM and Tsay HS (1994a) Effect of medium composition on the vitrification of carnation plantlets cultured in vitro. Jour. Agric. Res. China. 43:51-62. Ku HM and Tsay HS (1994b) Influence of subculture generation on the vitrification of carnation plantlets cultured in vitro. Jour. Agric. Res. China. 43:308-319. Lahl WJ.and Braun SD (1994) Enzymatic production of protein hydrolysates for food use. Food Technol. 48:68-71. Lalasidis G (1978) Four new methods of debittering protein hydrolysates and a fraction of hydrolysates with high content of essential amino acids. Ann. Nutr. Aliment. 32:709-723. Mc Donnell M, Fitzgerald R, Fhaolain IN, Jennings PV, and O'Cuinn G (1997) Purification and characterization of aminopeptidase P from Lactococcus lactis subsp. cremoris. J. Dairy Res. 64:399-407. Mersereau M, Pazour GJ, and Das A (1990) Efficient transformation of Agrobacterium tumefaciens by electroporation. Gene. 90:149-51. Minamiura N, Matsumura Y, and Yamamoto T (1972) Bitter peptides in the casein digests with bacterial proteinase. II. A bitter peptide consisting of tryptophan and leucine. J. Biochem. 72:841-848. Mow BM, Blajeski AL, Chandra J, and Kaufmann SH (2001) Apoptosis and the response to anticancer therapy. Curr. Opin. Oncol. 13:453-462. Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497 Nakata T, Takahashi M, Nakatani M, Kuramitsu R,

Tamura M, and Okai H (1995) Role of basic and acidic fragments in delicious peptides (Lys-Gly-Asp- Glu-Glu-Ser-Leu-Ala) and the taste behavior of sodium and potassium salts in acidic oligopeptides. Biosci. Biotechnol. Biochem. 59:689-693. 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. 18:6169. Oliveira SM, Freitas JO Jr, and Alves KB (1999) Rabbit kidney aminopeptidases: purification and some properties. Immunopharmacology. 45:215-221. Pautot V, Holzer FM, Chaufaux J, and Walling LL (2001) The induction of tomato leucine aminopeptidase genes (LapA) after Pseudomonas syringae pv. tomato infection is primarily a wound response triggered by coronatine. Mol. Plant Microbe. Interact. 14:214-224. Pautot V, Holzer FM, Reisch B, Walling LL (1993) Leucine aminopeptidase: an inducible component of the defense response in Lycopersicon esculentum (tomato). Proc. Natl. Acad. Sci. U.S.A. 90:9906-9910. Pulido-Cejudo G, Conway B, Proulx P, Brown R, and Izaguirre CA (1997) Bestatin-mediated inhibition of leucine aminopeptidase may hinder HIV infection. Antiviral Res. 36:167-177. Reusch RN, Hiske TW, and Sadoff HL (1986) Poly-beta-hydroybutyrate membrane structure and its relationship to genetic transformability in Escherichia coli. J. Bacteriol. 168:553-562 Rouseff RL (1990) Bitterness in Foods and Beverages, Elsevier Science Pub. Co., New York 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. Sambrook J and Russell DW (2001) Molecular Cloning: a laboratory manual, third edition. Cold Spring Harbor Laboratory Press, New York. Sanderink GJ, Artur Y, and Siest G (1988) Human aminopeptidases: a review of the literature. J. Clin. Chem. Clin. Biochem. 26:795-807. Stevenson DE, Ofman DJ, and Fenton GA(1998) Protease catalysed Concondensation Oligomerisation of Hydrophobic Peptides as a Means of Flavour Modification. J. Mol. Catal., B Enzym. 5:39-44. Strater N and Lipscomb WN (1995) Two-metal ion mechanism of bovine lens leucine aminopeptidase: active site solvent structure and binding mode of L-leucinal, a gem-diolate transition state analogue, by X-ray crystallography. Biochemistry. 34:14792-14800. Tadanobu N, Seiichi N, and Nobuyoshi I (1973a) Purification and properties of leucine aminopeptidase I from Aspergillus oryzae. Agr. Biol. Chem. 37:757-765. Tadanobu N, Seiichi N, and Nobuyoshi I (1973b) Purification and properties of leucine aminopeptidase II from Aspergillus oryzae. Agr. Biol. Chem. 37:767-774. Tadanobu N, Seiichi N, and Nobuyoshi I (1973c) Purification and properties of leucine aminopeptidase III from Aspergillus oryzae. Agr. Biol. Chem. 37:775-782. Tan PS, van Kessel TA, van de Veerdonk FL, Zuurendonk PF, Bruins AP, and Konings WN (1993) Degradation and debittering of a tryptic digest from beta-casein by aminopeptidase N from Lactococcus lactis subsp. cremoris Wg2. Appl Environ Microbiol. 59:1430-1436. Taylor A, Daims M, Lee J, and Surgenor T (1982) Identification and quantification of leucine aminopeptidase in aged normal and cataractous human lenses and ability of bovine lens LAP to cleave bovine crystallins. Curr. Eye Res. 2:47-56. Tzfira T and Citovsky V (2002) Partners-in-infection: host proteins involved in the transformation of plant cells by Agrobacterium. Trends Cell Biol.12:121-129. Uh Y, Son JS, Jang IH, Yoon KJ, and Hong SK (2002) Penicillin-resistant Aerococcus viridans Bacteremia Associated with Granulocytopenia. J. Korean. Med. Sci. 17:113-115. Vandenmoortele JL (1999) A procedure to prevent hyperhydrcicty in cauliflower axillary shoots. Plant Cell Tiss. Org. Cult. 56:85-88. Watanabe M, Shimizu J, and Arai S (1990) Debittering of tryptic hydrolysate of casein by incubating with the ice nucleationactive basterium, Erwinia ananas, and its aminopeptidase at low temperature. Agric. Biol. Chem. 54:3351-3353. Watson AJ, Fuller LJ, Jeenes DJ, and Archer DB (1999) Homologs of aflatoxin biosynthesis genes and sequence of aflR in Aspergillus oryzae and Aspergillus sojae. Appl. Environ. Microbiol. 65:307-10.