米麴菌白胺酸N端切位?※稭]轉殖番茄之鑑定與特性分析

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摘 要

白胺酸N端切位?（Leucine aminopeptidase；LAP）可切除胜?兩端的疏水性胺基酸，去除苦味。本研究以Aspergillus oryzae (米麴菌) LAP基因轉殖番茄植株為材料，探討米麴菌LAP 基因在番茄體內的異源表現。因番茄本身有兩種異構型酵素LAP-A、LAP-N的存在，但胺基酸序列比對結果顯示，米麴菌LAP與番茄LAP，在序列上並無明顯的相關性。轉殖殖株之鑑定，首先先分析各植株之LAP 酵素活性，確定轉殖植株酵素活性後，再分別以PCR和RT-PCR進行DNA、RNA的分析工作。然於酵素活性分析時，除了發現活性比wild type高之植株外，也發現活性和wild type相當或較wild type低者。這可能是基因插入的位置不同，造成表現上的差異。但是，為何連番茄本身內在的LAP活性也受到影響，則須進一步釐清。而於酵素特性分析方面，發現溫度在50到70℃、pH在 8.5 到 10.5之間，以及16% NaCl高鹽存在之下，酵素有較佳的活性表現。特性分析結果顯示，異源表現之LAP，較原始來源之LAP更為耐熱、耐鹼以及耐鹽。此結果暗示，米麴菌LAP 酵素，經番茄異源性表現後，可能經過某種後修飾作用，造成此酵素性質發生改變。

關鍵詞 : 番茄、白胺酸N端切位?、苦味、米麴菌

目錄

參考文獻

1.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。2.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。3.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。4.鄭仁君 (2005) 基因轉殖番茄之育成碩士論文，分子生物學研究所，大葉大學，彰化。5.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。6.Bachmair A, Finley D, Varshavsky A (1986) In Vivo Half-Life of a Protein is a Function of Its Amino-Terminal Residue. Science. 234: 179-186.7.Chen H, Gonzales-Vigil E, Wilkerson CG, Howe GA (2007) Stability of Plant Defense Proteins in the Gut of Insect Herbivores. Plant Physiol. 143: 1954-1967.8.Chien H-CR, Lin L-L, Chao S-H, Chen C-C, Wang W-C, Shaw C-Y, Tsai Y-C, Hu H-Y, Hsu W-H (2002) Purification, Characterization, and Genetic Analysis of a Leucine Aminopeptidase from Aspergillus Sojae. Biochim. Biophys. Acta. 1576: 119-126.9.Damle MV, Jamdar SN, Harikumar P (2006) Modification of Protein Hydrolysates By Chicken Intestinal Aminopeptidase. Food Technol Division, BARC.10.Gamborg OL, Miller RA, Ojima K (1968) Nutrient Requirements of Suspension Cultures of Soybean Root Cells. Exp. Cell Res. 50: 151-158.11.Ge S, Zhang L (1996) The Immobilized Porcine Pancreatic Exopeptidases and Its Application in Casein Hydrolysates Debittering. Appl. Biochem. Biotechnol. 59: 159-165.12.Gu Y-Q, Holzer FM, Walling LL (1999) Overexpression, Purification and Biochemical Characterization of the Wound-induced Leucine Aminopeptidase of Tomato. Eur. J. Biochem. 263: 726-735.13.Gu Y-Q, Pautot V, Holzer FM, Walling LL (1996) A Complex Array of Proteins Related to the Multimeric Leucine Aminopeptidase of Tomato. Plant Physiol. 110: 1257-1266.14.Habibi-Najafi M, Lee B (1996) Bitterness in Cheese: A Review. Crit. Rev. Food Sci. Nutr. 36: 397-411.15.Ishibashi N, Kubo T, Chino M, Fukui H, Shinoda I, Kikuchi E, OKAi H, Fukui S (1988) Taste of Proline-Containing Peptides. Agric. Biol. Chem. 52: 95-98.16.Iwase A, Nomura S, Mizutani S (2001) Characterization of A Secretase Activity for Placental Leucine Aminopeptidase. Arch. Biochem. Biophys. 393: 163-169.17.Kanno T, Maekawa M, Kanda S, Kohno H, Sudo K (1984) Evaluation of Cytosolic Aminopeptidase in Human Sera. Evaluation in Hepatic Disorders. Am. J. Clin. Pathol. 82: 700-705.18.Lahl W, Braun S (1994) Enzymatic Production of Protein Hydrolysates for Food Use. Food Technol. 48: 68-71.19.Lehrach H, Diamond D, Wozney J, Boedtker H (1977) RNA Molecular Weight Determinations by Gel Electrophoresis under Denaturing Conditions: A Critical Reexaminat. Biochemistry 16: 4743-4751.20.Machida M, Yamada O, Gomi K (2008) Genomics of Aspergillus oryzae: Learning from the History of Koji Mold and Exploration of Its Future. DNA Res. 15: 173-183.21.Mow B, Blajeski A, Chandra J, Kaufmann S (2001) Apoptosis and the Response to Anticancer Therapy. Curr. Opin. Oncol. 13: 453-462.22.Murashige T, Skoog F (1962) A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture. Physiol. Plant. 15: 473-497.23.Nishiwaki T, Yoshimzu S, Furuta M, Hayashi K (2002) Debittering of Enzymatic Hydrolysates Using an Aminopeptidase from the Edible Basidiomycete Gribola frondosa. J. Biosci.

Bioeng. 93: 60-63.24.Saha BC, Hayashi K (2001) Debittering of Protein Hydrolyzates. Biotechnol. Adv. 19: 355-370.25.Sanderink G, Artur Y, Siest G (1988) Human Aminopeptidases: A Review of the Literature. J. Clin. Chem. Clin. Biochem. 26: 795-807.26.Strater N, Lipscomb W (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: 14792-14800.27.Taylor A, Daims M, Lee J, 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. 12: 47-56.