Development of transgenic tomato of Aspergillus oryzae Leucine Aminopeptidase

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ABSTRACT

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.

Keywords : Agrobacterium, Aspergillus oryzae, Leucine aminopeptidase (LAP), regeneration, tissue culture, tomato, transformation

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REFERENCES

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.

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