

Identification and Characterization of Transgenic Tomato Plants Expressing an *Aspergillus oryzae* Leucine Aminopeptidase

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ABSTRACT

Leucine aminopeptidase (LAP) is widely applied in food industry to reduce the bitterness of protein hydrolysate by liberating the hydrophobic residues in the N terminus of peptides. For the overproduction of the enzyme, transgenic tomato plants had been developed in our previous work. The transgene, *Aspergillus oryzae* LAP, as well as the NPT II marker gene were introduced into tomato via *Agrobacterium*-mediated transformation. After antibiotic selection, approximately 100 independent lines were obtained. Although two main LAP isoforms, LAP-A and LAP-N, are present in tomato, sequence comparison showed no apparent homology between the heterologous LAP and the endogenous LAPs. In this study, these transgenic plants were further identified and characterized. The enzyme activities were assayed firstly, and then, according to the level of activity, genomic PCR and RT-PCR were performed to confirm the presence of NPT II gene and *Aspergillus* LAP mRNA respectively. As expected, several transgenic plants showed higher enzyme activity than wild type, especially line 38; meanwhile, some showed no significant difference compared to wild type, such as lines 29, 34, and 67. Surprisingly, some lines were found to possess lower activity than wild type. Moreover, the characteristic analysis of the heterologous LAP showed some variations in enzymatic properties such as optimal temperature, optimal pH, and salt requirement. These results indicated that the *Aspergillus* LAP, expressed heterologously in tomato, differs from the original form, which is isolated from its natural source.

Keywords : *Aspergillus oryzae*, bitterness, leucine aminopeptidase, tomato

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