Gene and structural characterisation of pea cDNA library for pullulanase

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Abstract

Pullulanases belong to a discrete class of debranching enzymes that are capable of hydrolysing the α-1,6-branches in starch. This work described the screening, identification and characterization of pullulanase gene from pea cDNA library. The screening process used degenerate primers based on the alignment of pullulanase genes from four plant species that has been previously identified. Initially, PCR using these primers for pullulanase on pea genomic DNA produced bands of about 600 bp, which was of approximately the expected size when compared to the sequence of pullulanase from spinach. Using this fragment as probe, screening of the pea cDNA library resulted in a full-length identification of a pullulanase gene with the size of 3.2 kb and encoded for 952 amino acids. Further analysis of the isolated clone, named PSPUL, from the screening process showed that it consists of 93 nucleotides in the 5'-untranslated region and 266 nucleotides in the 3'-untranslated region. The 3'-untranslated region contained three putative polyadenylation signals (AATAAT/A) located at 110 bp, 212 bp and 220 bp upstream of the last polyadenylation site. The predicted size for mature PSPUL peptides was 888 amino acids and calculation by PEPTIDESORT gave predicted molecular mass of 93 kDa. Three dimensional features of these enzymes showed that the pullulanase from pea contains eight regions of β-strand followed by eight regions of α-helix, which confirmed that it has the (β/α)8 characteristic of the α-amylase super family.