Title	CLONING OF ALPHA-AMYLASE GENES FROM RED FLOUR BEETLE (TRIBOLIUM CASTANEUM)
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ABSTRACT

Tribolium castaneum (Coleoptera: Tenebrionidae), commonly known as red flour beetles, causes extensive damage of more than 30% loss of stored rice grains. The damage is from the utilization of alpha-amylases of the insects to digest starch in rice grains as their nutritious source. Understanding the insect alpha-amylase biochemical characteristic could be important to control growth and development of these insect pests. The goal of this study is to clone *Tribolium castaneum* alpha-amylase genes, and to study their biochemical properties. The beetles were reared on 95% wheat flour with 5% yeast powder in a plastic box and covered with cheesecloth for ventilation. The culture was maintained in laboratory at 28±2 °C for 50-60 days. About 200 g of wheat flour with 5% yeast powder and 200 g Jasmine rice (*KDML 105*) powder were transferred separately into each plastic box. Thirty mg of adults *Tribolium castaneum* were collected at 1, 2, 3, 4, 6 and 8 week for RNA extraction and cDNA synthesis. Three clones of full coding sequence of *Tribolium cataneum* α-amylase gene were obtained. Each clone contains 1,470 bp open reading frame encoding a predicted protein of 490 amino acids starting from the first ATG. The amino acid sequences of these clones show almost 99% identities to the *Tribolium castaneum* α-amylase when compared to NCBI nucleotide database (NCBI Reference Sequence: NM_001114376.1). The first seventeen amino acids represent the signal peptide. The catalytic site residues of *Tribolium cataneum* α-amylase are Asp204, Glu241 and Asp303. In addition, the thirteen recombinant clones of 1,650 bp cDNA containing *Tribolium castaneum* α-amylase sequences were also obtained. However, when compared to the NCBI nucleotide database (NCBI Reference Sequence:

NM_001114376.1), these clones contain four extra regions of, 50 bp, 38 bp, 46 bp and 47 bp along with an abnormal stop codon within, assuming that they probably are pseudo-genes. The recombinant protein expressed in *E.coli* showed an apparent mass of 72 kDa (TcAm: 53.3 kDa + Thioredoxin/Hitidine tag from vector: 18.7 kDa). The enzyme was purified using nickel affinity chromatography. The activity of *Tribolium castaneum* alpha-amylase was determined by Zymogram, 2-Chloro-4-nitrophenyl-alpha-D-maltotrioside substrate. Purified recombinant TcAm exhibited a relatively high activity of 4338.41 U/mg specific activities. The optimum condition of TcAm is 50 °C, pH 7. It also demonstrated stability in a wide range of temperatures. The results showed that the enzyme activity was significantly enhanced with 10 mM of $CaCl_2$, $MgCl_2$, $MnCl_2$ and Imidazole under optimal condition. On the other hand, in the reaction without $CaCl_2$, the enzyme activity relatively decreased in the negative control (no ion), NaCl, EDTA, and KCl. Nevertheless, only the KDML 105 α-amylase inhibitor (AI) showed significant inhibition about 25 % of α-amylase activity.