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Molecular cloning and expression analysis of cytochrome c oxidase subunit II from *Sitophilus zeamais*

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ABSTRACT: Cytochrome c oxidase subunit II (COX II) containing a dual core CuA active site is one of the core subunits of mitochondrial Cytochrome c oxidase (Cco), which plays a significant role in the physiological process. In this report, the full-length cDNA of COX II gene was cloned from *Sitophilus zeamais*, which had an open reading frame (ORF) of 684 bp encoding 227 amino acids residues. The predicted COX II protein had a molecular mass of 26.2 kDa with pI value of 6.37. Multiple sequence alignment and phylogenetic analysis indicated that *Sitophilus zeamais* COX II had high sequence identity with the COX II of other insect species. The gene was subcloned into the expression vector pET-32a, and induced by isopropyl β-D-thiogalactopyranoside (IPTG) in *E.coli* Transetta (DE3) expression system. Finally the recombinant COX II with 6-His tag was purified using affinity chromatography with Ni²⁺-NTA agarose. Western Blotting (WB) showed the recombinant protein was about 44 kD, and the concentration of fusion protein was 50 μg/mL. UV-spectrophotometer and infrared spectrometer analysis showed that recombinant COX II could catalyze the oxidation of substrate Cytochrome C (Cyt c), and influenced by allyl isothiocyanate (AITC). By using molecular docking method, it was found that a sulfur atom of AITC structure could form a length of 2.9 Å hydrogen bond with Leu-31. These results suggested that tag-free COX II was functional and one of the action sites of AITC, which will be helpful to carry out a point mutation in binding sites for the future research.

Keywords: *Sitophilus zeamais*; Cytochrome c oxidase subunit II; Soluble proteins; Enzyme activity; Allyl isothiocyanate

1. Introduction

Sitophilus zeamais is one of the major pests of stored products in the tropics and subtropics [1]. For a long time, management of the stored product pests has relied on synthetic insecticides and fumigants, such as metal phosphide [2], methyl bromide [3] and sulfuryl fluoride [4]. However, the use of fumigants has led to serious problems like 3R (resistance, resurgence, and residue) *ect* [5], therefore, it is urgent to find a new type of alternative fumigants to

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