Cloning and Expression of the *Cucumis melo aco1* Gene cDNA

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Ethylene is a phytohormone which triggers fruit ripening and consequently reduces fruit firmness. In *Cucumis melo* (melon) *cantalupensis*, ripening is related to the increase of ACC Oxidase 1 (ACO1) expression. This work aims to isolate and clone the *C. melo cantalupensis aco1* gene (*cm-aco1*) cDNA, which codes for the CM-ACO1 enzyme. The *cm-aco1* gene contains a coding region interrupted by three introns. Therefore, the total RNA of *C. melo* was isolated from wounded melon leaves and used as a template in a RT-PCR reaction with 5´and 3´ specific primers flanked by *Eco*RI and *Hind*III restriction sites, respectively. After checking the cDNA authenticity and integrity by sequencing, it was subcloned into the pGEM-T easy vector, generating the recombinant plasmid pMG1. Then, the *Eco*RI/*Hind*III insert of pMG1 was cloned into the expression vector pET28-b and inserted into *E. coli* DH5α cells by thermal shock transformation. After selecting the recombinant clone, the over-expression of CM-ACO1 protein was induced in *E. coli* BL21 λDE3. The purification of the protein of interest and consequently, the characterization of its activity and structure will contribute to a better understanding of its function in plant metabolism, senescence, and the biotic and abiotic responses.

Palavras chaves: ACC oxidase protein, *Cucumis melo*, protein purification, RT-PCR
Supported by: Fundação Araucária, CNPq/MCT