

Cloning, Structure Prediction, Heterologous Expression and Biological Role of a Class III Chitinase Paralogous Gene from Coffee (*Coffea arabica*)

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A Class III Chitinase paralogous gene from *Coffea arabica* (PCIIIChitCa), expressed in coffee floral and fruit parts as well as in stems infected with *X. fastidiosa*, was isolated from leaves (UFV) and cloned into pGAPZ $\alpha$ -B vector for expression in *Pichia pastoris*. The amino acid sequence, which predicts a ( $\beta/\alpha$ )<sub>8</sub> barrel topology, shares 53% identity with Hevamine, a model Class III Chitinase from *Hevea brasiliensis*; and 42% identity with Concanavalin B, a known natural inactivated chitinase from *Canavalia ensiformes*. According to the modeling prediction, PCIIIChitCa lacks the  $\alpha$ 5 helix, and the conserved catalytic domain of Class III Chitinases (DXDXE) is presented as "DFHIQ". The substitution of Glutamic acid by Glutamine in the catalytic domain, also found in Concanavalin B, has been considered to be the cause of the lack of chitinolytic activity in such enzymes. An enzymatic assay performed with the purified recombinant protein showed no chitinolytic activity. We suggest that this molecule belongs to a class of naturally inactive chitinases that have evolved to act in plant cell defense as glycoside hydrolase inhibitors.

Keywords: *Coffea arabica*, Class III Chitinase, Xylanase inhibitor  
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