Dual enzymatic activity of the pathogenesis-related protein TcPR-4 from *Theobroma cacao*: ribonuclease and Ca$^{+2}$ and Mg$^{+2}$ dependent deoxyribonuclease activities

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The class 4 pathogenesis-related proteins (PR4) are classified as chitinases and contain a conserved Barwin domain. The TcPR-4b cDNA identified from a library of *Theobroma cacao* L. pod (genotype TSH1188) infected by *Moniliophthora perniciosa* also presents the Barwin domain with six conserved cysteine residues, but lacks the chitin-binding site and for this reason was classified as class II PR4. The TcPR-4b gene was cloned into pET28a and the resulting in-frame fusion plasmid was used to transform *Escherichia coli* Roseta (DE3) for protein expression. The expression of the TcPR-4b recombinant protein was induced by 0.4 mM isopropyl-β-D-thio-galactoside and purified by immobilized metal affinity chromatography with TALON Metal Affinity Resin. To determine the DNase activity of the purified recombinant TcPR-4b protein, 1 µg of purified pGEM-T Easy Vector DNA was incubated with different protein amounts (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg) in the presence or absence of 10 mM of MgCl$_2$ or 1 mM of CaCl$_2$ overnight at room temperature. RNase activity of recombinant TcPR-4b was performed using different protein amounts (5, 10, 15, 20 and 25 µg) incubated for 30 min with 5 µg of RNA extracted from *Solanum lycopersicum* leaves. The reaction products were analyzed in 1.5% agarose electrophoresis gel. The TcPR-4b protein recombinant showed both DNase and RNase activity. DNase activity was observed only in the presence of Mg$^{+2}$ and Ca$^{+2}$ ions. The results of this study suggest that TcPR-4b may act as nuclease during the infection of cacao plants with *M. perniciosa*. Financial Support: CNPq, BNB, FINEP/RENORBIO, CAPES.