TITLE: BACTERIAL EXPRESSION OF A 17.98 KDA PROTEIN from MONILIOPHTHORA PERNICIOSA, POTENTIAL EFFECTOR IN INTERACTION WITH THEOBROMA CACAO

AUTHORS: FARIAS, S.F; PIROVANI, P.C; GRAMACHO, P.K; REZENDE, P.R

INSTITUTION: UNIVERSIDADE ESTADUAL DE SANTA CRUZ-UESC (BR-415 Km 16, Bairro Salobrinho CEP 45662-900. - Ilhéus/BA)

ABSTRACT:

The hemibiotrophic fungus *Maniliophthora perniciosa* is the causative agent of witches broom, one of the main diseases that affects *Theobroma cacao*. Throughout the infection process the pathogen secretes protein effectors that manipulate host cell physiology. A recent genomic study of subpopulations of *M. perniciosa* isolated from different hosts, was carried out with the participation of our group and resulted in the identification of innumerable genes/potential effectors (Barbosa et al., 2018). The characterization of these potential effectors may contribute to a strategy to control the disease, with the identification of eventual receptor proteins of the cocoa plant. Thus, the objective of this work was to characterize an effector potential of the fungus, through bioinformatics analysis and expression in a heterologous system. The results showed that the protein Mp4145-3305 has characteristics common to other effectors described in the literature: low molecular weight 17.98 kDa, with 162 amino acid residues, presence of signal peptide secretion, absence of conserved domain and no homology with proteins characterized in the databases. The *E. coli* strain, *Rosetta* (DE3), was transformed with pET-28a vector containing a synthetic ORF optimized for expression, under the control of the T7 RNA Polymerase promoter and the lac Operator. The protein was accumulated in the soluble and insoluble fractions of bacterial extract. The recombinant protein was purified by affinity chromatography from the soluble fraction and used as an immunogen to produce polyclonal antibodies in mouse. These antibodies are being used in analysis of protein accumulation in vivo. Circular dichroism (CD) spectra at 25 ° C and 95 ° C showed that the protein is stable at high temperatures. The complete characterization of this protein can contribute to the understanding of its role in the pathogen colonization process in host cells and the development of new fungal control strategies.

Keywords: effector protein, plant-pathogen, polyclonal antibody, witch's broom

Development Agency: FAPESB