Molecular Cloning, Expression and Purification of Tex Protein from
*Xanthomonas citri* subsp. *citri*, the Causal Agent of Citrus Canker

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**INTRODUCTION:** *Xanthomonas citri* subsp. *citri* (Xac) is the causal agent of citrus canker in citrus species. A gene knockout approach has been widely used in functional analysis of genes involved in Xac:citrus pathosystem. In a previous study, the disruption of XAC2053 ORF from Xac (gene tex) elicited a strongly hypersensitive response in citrus, in contrast to the wild type strain, which led to the development of the disease. Thus, the present study aimed to clone, express in *E.coli* and purify the Tex protein of Xac for biochemical characterization. **MATERIAL AND METHODS:** The tex gene was obtained by PCR, cloned into the pETSUMO expression vector and transformed into *E.coli* DH10B chemically competent cells. The correct constructs were used to transform *E.coli* BL21 (DE3) pLysS chemically competent cells. After screening, large-scale expression of HIS-SUMO-Tex fusion recombinant protein was made in LB medium at 20 °C using 0.2 mM IPTG for 8 hours, condition which show higher concentrations of the recombinant protein in the soluble form. The recombinant protein was purified in a Ni-Sepharose affinity column. **RESULTS AND DISCUSSION:** The primers used amplified fragments with expected size, confirmed by gel electrophoresis and sequencing. After confirmation by sequencing, the plasmid vector was transformed into *E. coli* BL21 (DE3) pLysS cells and Tex protein was successfully expressed as a fusion protein. After two runs of purification in the Ni-column, followed by precipitation with 40% ammonium sulfate, Sumo Protease digestion and another step of purification in the Ni-column, highly purified Tex recombinant native protein was obtained. **CONCLUSIONS:** The results showed that it is possible to express in *E. coli* the Tex protein from Xac in a soluble form and get enough amount of highly purified for future structural and functional studies, such as crystal X-ray diffraction for three-dimensional structure determination.

Keywords: *Xanthomonas citri* subsp. *citri*, recombinant expression, Tex protein

Acknowledgment: CAPES