

**MOLECULAR BIOLOGICAL CHARACTERIZATION OF THE
VACUOLATING CYTOTOXIN A (VacA) FROM THAI
CLINICAL ISOLATE *HELICOBACTER PYLORI***



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ABSTRACT

H. pylori vacuolating cytotoxin A (VacA) is an exotoxin that represents one of the most important virulence factors produced by *H. pylori*. Activities of VacA include formation of large cytoplasmic vacuoles in the host gastric epithelial cells as well as release of cytochrome c (Cyt c) from mitochondria resulting in cell apoptosis. VacA is present in all *H. pylori* strains, but its cytotoxic activity has been shown to occur in only 50% of *H. pylori* strains. Structurally, the mature VacA is an 88-kDa monomer that consists of two domains: p33, responsible for pore formation on plasma membrane and p55, which has an important role in binding to target host cells following its internalization into the cytosolic compartment. We aimed to study the sequence of Thai isolate VacA and compare it with model strains (60190) as well as to characterize the biological activities of this toxin on intestinal (T84) and kidney (MDCK) epithelial cell lines. We established molecular methods including PCR, gel electrophoresis, SDS-PAGE, Western blot analysis for cloning, sequencing, and expression of VacA in *E. coli*. Nuclear staining using DAPI was employed to detect apoptosis induced by purified VacA. Results showed that the Thai isolate protein is structurally similar to the *H. pylori* isolate s1m2 VacA strains: whereas homology to the 60190 model strain was found to be lower than expected due to the presence of extra amino acids in the mid region (m region) of the Thai isolate VacA protein. The m region has been found to be linked to the binding specificity of VacA to specific epithelial cells that are induced to undergo vacuolization. DAPI staining showed lower apoptotic effects of Thai isolate VacA on T84 cells while it has a higher apoptotic effect on MDCK cells. These findings suggest that the Thai isolate VacA could have biological activities on the host cells that may differ from those of the model strains.

KEY WORDS: *HELICOBACTER PYLORI*/ VACA/ APOPTOSIS/ CLONING

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