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Construction of the isogenic collagenase mutant of *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus has been known as a cause of acute gastroenteritis. Foodborne outbreaks and sporadic cases by this marine bacterium occur worldwide and are usually associated with the consumption of contaminate seafood. Numerous secreted and cell-associated virulence factors have been proposed to account for the fulminating and destructive nature of *V. parahaemolyticus* infection. Among the virulence factors is a collagenase. Previously, we cloned and sequenced a collagenase gene (*vppC*) from *V. parahaemolyticus* 04. The function of this enzyme for bacterial virulence is assessed by the construction the insertional knockout mutant of *vppC*.

For the construction of the allelic exchange mutant, *vppC* was inserted *in vitro* by insertion of *nptI* isolated from pUC4K. The 3.6 kb *vppC::nptI* cartridge was ligated into the suicide vector pCVD442 to form pCM03. The pCM03 was transformed into *E. coli* SM10 λ pir and conjugated with the recipient strain *V. parahaemolyticus* 04. The transconjugant was selected on the TCBS medium supplemented with 5% sucrose that colicin B was spread. Transconjugant formed by homologous recombination was selected by kanamycin-resistant.