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Mutation of Virulence Candidate Genes of *Ralstonia solanacearum*

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Ralstonia solanacearum is a gram-negative plant pathogenic bacterium that causes bacterial wilt in the most of the solanaceous plants and other crops. In order to study virulence mechanism of this bacterium, we selected two genes and conducted marker-exchange mutagenesis. *dam* encoding DNA adenine methylase is one candidate which is known as a virulence regulator in animal pathogenic bacteria and *rpoS* encoding sigma S is the other which is a regulator of bacteria survival. Both *dam* and *rpoS* were amplified from *R. solanacearum* GMI 1000 and antibiotic resistance gene cassettes were inserted in the middle of each gene for marker exchange mutagenesis. We were only successful to obtain *dam*- mutant and the expected mutation was confirmed by Southern hybridization. Bacterial growth and extrapolysaccharide production between wild type strain and *dam*- mutant were not different, indicating the cell cycle of the bacteria was not affected by the mutation. Bacterial virulence are being investigated between wild type and *dam*- mutant in different host plants such as tobacco, tomato and Arabidopsis.

Key words: *dam*, *Ralstonia solanacearum*, virulence

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Heat Stable Antifungal Component of *Burkholderia cepacia* CH-67

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A potential biocontrol bacterium CH-67 was isolated from forest soil by a fungal bating method. The CH-67 strain produced chitinase, cellulase, protease, amylase and some antifungal component. The strain was identified as *Burkholderia cepacia* CH-67 based on microscopy, biochemical test and 16S rDNA sequence analysis. The strain CH-67 exhibited the strong antifungal activity against several plant pathogenic fungus including *Fulvia fulva* causing tomato leaf mold. Although the antifungal activity of the CH-67 culture fluids was thought to be both from the chitinase activity and from antifungal component, boiled culture fluids of CH-67 maintained the antifungal activity while chitinase activity was disppeared. This result indicated that major antifungal activity of the CH-67 strain against *F. fulva* results from the heat stable antifungal component. Subsequent fractionation effort of the antifungal activity from *B. cepacia* CH-67 was performed by serial extraction process with various organic solvents such as hexane, ethylacetate, and butanol. However, any organic fractions did not exhibit the antifungal activity against *F. fulva*.

Key words: Antifungal activity, *Burkholderia cepacia*, heat-stable component