CitB is required for full virulence of *Xanthomonas oryzae* pv. *oryzae*

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Received: 2 May 2015 / Accepted: 29 July 2015 / Published online: 7 August 2015
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**Abstract**

To identify novel virulence associated genes in *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), a *Xoo* isolate (*Xoo*IR42), obtained from north of Iran, was selected to generate a mini-Tn5 transposon mutation library. One mutant (*Xoo*M176) that indicated reduced virulence on rice plants, while grew similar to wild type was selected. This mutant had an insertion in a coding region with 96% amino acid identity to a response regulator of *Xoo* KACC10331, citB (*Xoo_RS12710*). Genome analysis of *Xoo* KACC10331 indicated several genes including a flagelin protein (FlgL) and a chemotaxis protein (*Xoo_RS12720*) which were identified as virulence genes 4297 and 1403 nucleotides from the *citB*, respectively. The swarming motility, resistance to hydrogen peroxide, induced a hypersensitive response, *in planta* growth and pathogenicity were reduced in *Xoo*M176 mutant compared to that of wild-type. A plasmid containing the full *citB* gene of *Xoo* KACC10331 was sufficient to complement the *Xoo*M176 mutant for lesion formation and resistance to hydrogen peroxide. We therefore propose that *Xoo* requires CitB for full pathogenicity in rice plants and also for protection against oxidative stress.

**Keywords**

Bacterial blight • Oxidative stress • Pathogenicity • Rice

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**Introduction**

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the casual agent of bacterial blight (Shen and Ronald 2002), is one of the most important rice pathogens in both temperate and tropical regions especially in Asia. It causes wilting of seedlings and yellowing and drying of leaves. When the disease occurs on seedlings or susceptible varieties grown in environments favorable to the disease, the yield loss can be more than 70%. When plants are infected at booting stage, bacterial blight cause poor quality grains and a high proportion of broken kernels. The pathogen has been identified in Africa and in low land area of the south Asia, India, United state and Australia (Mew et al. 1993). The *Xoo* has been used as a model organism in plant-pathogen interactions, bacterial race differentiations and the evolutionary studies on plant pathogens (Nino-Lio et al. 2006).

The first studies on virulence character of *Xoo* indicated that the pathogen follow the gene-for-gene model in race-specific interactions with rice (Jacobs et al. 2003). Almost thirty different rice genes (called *Xa* genes) which confer resistance against various races and pathotypes of *Xoo* have been characterized (Nino-Liu et al. 2006). Therefore, identification of the virulence associated genes in *Xoo* is essential for selecting resistance genes that might be used in a breeding program. Construction of a mutant library by transposable elements would facilitate the comprehensive identification of genes required for pathogenesis (Voelker and Dybvig 1998). Using a transposon mutant library several pathogenicity related genes in *Xoo* have been already identified. One group of these genes involved in biosynthesis of extracellular polysaccharides (EPS), lipopolysaccharides, adhesins, and harpins (Böttner and Bonas 2010). The second group are the genes encoding for nutrient acquisition proteins such as phytase like proteins,