Functional Pathogenomics of *Burkholderia glumae* - The Causative Agent of Bacterial Grain Rot of Rice

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Bacterial grain rot of rice is caused by *Burkholderia glumae* and is recently becoming serious disease in Korea, Japan, and other Asian countries. This disease is highly dependent upon weather conditions at the flowering stage. Infected seeds are a primary source of inoculum, and secondary infections occur at the flowering stage under hot temperature and high moisture conditions (Fig. 1). Recently, condition during the summer in Korea is very similar to sub-tropical weather, which is favorable for bacterial diseases in rice. In 1997, the disease occurrence reached 35% in certain areas in Korea.

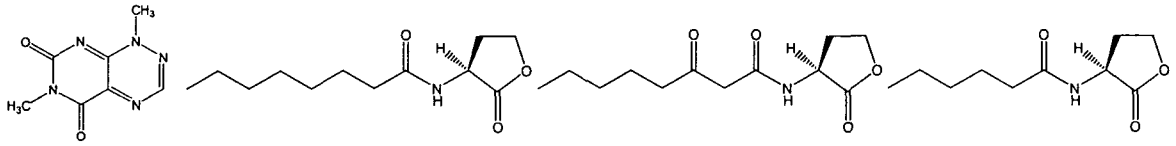
The aim of this study is to characterize the interactions of rice and *B. glumae*, the causal agent of bacterial grain rot of rice, at molecular levels using whole genomic sequences and identifying genes important for pathogenicity including toxin and enzyme biosynthetic genes and symptom development. We also determine functions and regulation of those genes and subsequently find useful genes that may confer disease resistance for rice. To do these, we isolated transposon-tagged mutants using various transposons and screened for nonpathogenic mutants, toxin nonproducers. Toxoflavin is known as a key pathogenicity factor in *B. glumae* (Fig. 2). However, the biosynthetic gene locus and metabolic pathway is not reported previously. We have cloned and sequenced gene locus for toxoflavin biosynthesis and identified ORFs in the cluster. Transporter systems of the toxoflavin are separated from the biosynthetic gene cluster. We found that quorum sensing regulates toxoflavin biosynthesis. Quorum sensing also called autoinduction is conserved among diverse gram-negative bacteria, and the regulation of gene expression is mediated by *N*-acylhomoserine lactone (acyl-HSL). *B. glumae* produces three kinds of acyl-HSL, *N*-octanoyl-L-homoserine lactone, *N*-hexanoyl-L-homoserine lactone, and *N*-(3-oxooctanoyl)-L-homoserine lactone (Fig. 2). We have cloned and sequenced the gene responsible for acyl-HSL biosynthesis, called *tofI*. An acyl-HSL receptor gene, *tofR*, was found upstream of *tofI*. TofI and TofR are homologs of LuxI and LuxR, respectively. Mutagenesis and complementation results clearly indicated that toxoflavin biosynthesis and pathogenicity are regulated by quorum sensing.

We have isolated the Hrp pathogenicity island (PAI) of *B. glumae* and partially characterized by sequencing and mutagenesis. We identified six *hrp*, nine *hrc*, and *hpaB* genes from the region. The *hrp* cluster resembled most the putative Type III secretion systems of *B. pseudomallei*, which is the causative agent of melioidosis, a serious disease of man and animals. However, the upstream region of *hrcC* and downstream region of *hrcS* were very different between two pathogens. Features of *B.*

glumae Hrp PAI were mosaic. The Hrp PAI core region showed high similarity to that of *Ralstonia solanacearum* and *Xanthomonas campestris*, however some aspects were dissimilar. Interestingly, we found a *hrpK* homolog of *Pseudomonas syringae* pv. *syringae* even though its role in pathogenicity remains to be answered. This mosaic nature of *B. glumae* Hrp PAI indicates horizontal transfer of Hrp PAI and unstability in the genome. Pathogenicity related factors often secret out of the cells when interacting with host cells.

Studying pathogenicity genes of *B. glumae* will lead to develop a new way of disease control. We also believe that it is possible to find useful novel genes conferring disease resistance or tolerance. With genomic studies of *B. glumae*, functional pathogenomics on rice-*B. glumae* interactions will provide a new model system to under plant-microbe interactions at molecular levels.

Fig. 1. Typical symptoms of bacterial grain rot caused by *B. glumae* in the field.



Toxoflavin *N*-octanoyl-L-HSL *N*-(3-oxooctanoyl)-L-HSL *N*-hexanoyl-L-HSL

Fig. 2. The structure of toxoflavin and autoinducer molecules.