ones, high throughput assays need to be implemented. At this stage, there is still a need for improvement and adaptation to large scale analyses of the gene knockout and complementation assays currently available for *P. infestans* and other *Phytophthora* species. However, ectopic expression of pathogen genes in plant cells can be performed at a remarkable high throughput rate using potato virus X (PVX) and *Agrobacterium tumefaciens*-based vectors. Therefore, we have been using virus-mediated gene expression to carry out high throughput functional screens of *Phytophthora* genes in plants. Preliminary PVX based functional screens unraveled a battery of novel *Phytophthora* effector genes that trigger hypersensitive-like necrosis in *Nicotiana* and tomato and alter the interaction between *Phytophthora* and plants.

In summary, *P. infestans* genomics has already generated numerous candidate genes, several of which are being validated using various functional assays. This research is allowing us to understand the molecular basis of pathogenicity, to identify targets for genetic control, and to understand *P. infestans* population structure and evolution.

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SI-2

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.

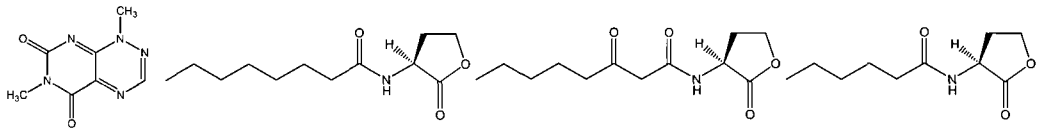
The aim of this study is to characterize the interactions of rice and *B. glumae*, the causal agent of bacterial grain rot of rice, at molecular levels using whole genomic sequences and identifying genes important for pathogenicity including toxin and enzyme biosynthetic genes and symptom development. 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. Studying functional genomic aspects of *B. glumae*, functional pathogenomics on rice-*B. glumae* interactions will provide a new model system to understand 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 structures of toxoflavin and autoinducer molecules.

SI-3

Functional genomics of the plant-probiotic bacterium, *Paenibacillus polymyxa* E681

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Paenibacillis polymyxa, frequently isolated from soil, especially from rhizosphere is a spore-forming low-G+C Gram-positive rod. It can fix atmospheric nitrogen and secrete diverse hydrolyzing enzymes. As the type species of the genus *Paenibacilli* and the representative of a phylogenetic group distinct from those containing species of *Bacillus* or *Clostridium*, *P. polymyxa* may serve as a model for studying metabolic processes and evolution of low-G+C Gram-positives. Further, some strains in the species can enhance the growth of plants and antagonize harmful soil microorganisms that inflict plants through its ability to synthesize plant hormones and to produce antimicrobial compounds.

A plant-growth-promoting rhizobacterial strain capable of suppressing plant diseases, *P. polymyxa* E681, was isolated from the rhizosphere of winter barley grown in South Jeolla Province, Korea. The sequence of the ~5.6 Mb genome was determined through the whole-genome shotgun sequencing strategy. More than 72,000 reads generated from both ends of genomic library clones (plasmids, fosmids, and BACs) and 2,600 finishing reads were assembled into 48 contigs (> 2 kb) by phrap. Sum of the contig lengths was around 5.54 Mb with a G+C content of 46%. 4,839 open reading frames (ORFs) were predicted using Glimmer