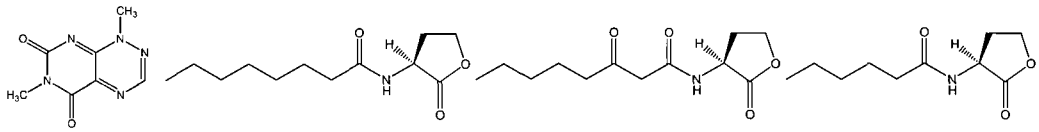
indicated that toxoflavin biosynthesis and pathogenicity are regulated by quorum sensing.

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

and their starting positions were adjusted using RBSfinder. The ORFs were then subjected to an in-house semi-automatic genome annotation system, which is based on pairwise/HMM searches against public databases. Of the putative protein-coding genes, 1,621 (33.1%) could be functionally assigned by COG, and 2,193 (45.3%) showed significant matches to characterized protein sequences in the databases. 1,025 ORFs (21.6%) have no significant similarities to proteins in the databases.

The genome sequence contains at least 15 sigma factors. Ten of them belong to the ECF family of sigma factors. 108 genes possibly involved in the sporulation process were identified based on their amino-acid sequence identities of more than 30% to known sporulation genes of other bacilli and clostridia. Other interesting groups of genes present in the E681 genome are those for production of polyketides, lantibiotics or non-ribosomal peptides, which may function as siderophores or antimicrobials. E681 appears to produce at least five kinds, since polyketide synthase genes are found in one supercontig, lantibiotic genes in one supercontig and non-ribosomal peptide synthetase genes are in four supercontigs.

Approaches of functional genomics including systematic mutagenesis, DNA microarray and proteome analysis are being applied to E681 to understand the regulation of developmental processes such as sporulation, metabolic processes and the nature of the 'plant-probiotic' property. Random mutagenesis of the E681 strain using mini-Tn*10* transposon was carried out successfully and more than 15,000 mutants have been generated. We obtained 180 sporulation-deficient mutants and 23 of them were analyzed; the flanking region of mini-Tn*10* transposition of each mutant was recovered by plasmid rescue and the nucleotide sequence was determined. The mutations were found on the homologues of *sigE*, *spoIIIAH*, *asnO*, and some unknown protein genes, respectively. We analyzed 86 mini-Tn*10* mutants lost antifungal activities against *R. solani* and/or *F. oxysporum*, and mutations were found on the homologues of a polykitide synthase gene and other 18 different genes. We also obtained 17 mutants showing changed auxin activities and analyzed the mutation sites. Two mutant strains carrying mutations on a homologue of a transcriptional regulator gene and an unknown gene, respectively, were analyzed to produce 15-times and 17-times higher auxin than mother strain, respectively.Also, for the monitoring of transcriptome profiles of E681 we selected candidate genes which seem to be involved in developmental processes such as sporulation, global regulatory system, synthesis of antimicrobial compounds, and built a 0.5K-scale experimental oligonucleotide microarray chip. Using this chip we are analyzing the transcriptome of E681 cells grown together with a fungal plant pathogen. 2-D gel electrophoresis and MALDI-TOF analysis are being set up to identify proteins produced during interaction with plants. We also found that *P. polymyxa* E681 produces antifungal antibiotics, fusaricidins A and B, by HPLC and LC/MS analysis.

In another effort, we isolated forty *Paenibacillus* spp. closely related to the *P. polymyxa* E681 strain from rhizosphere. The genome sizes of forty *Paenibacillus* isolates were measured with pulsed-field gel electrophoresis using the genomic DNA digested with *Not*I, *Pme*I and *Sfi*I. The estimated genome sizes were ca. 5.2-5.5 Mb. Five distinct groups were identified based on large-restriction-fragment (LRF) polymorphism of *Not*I- and *Pme*I-digested isolates. About half of the isolates were in a single group. The presence of absence of *nifH*, plasmid profiles, and results of box PCR further supported this grouping.

SI-4

Systems biology initiatives in the rice blast fungus, *Magnaporthe grisea*

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Rice blast, caused by *Magnaporthe grisea*, has been considered as a model plant disease to study plant-fungi interactions. This is due to not only economic significance of this disease worldwide but genetic and molecular tractability of the fungal pathogen. These features include genetic crossing with two different mating types, extensive genetic maps, developments of transformation and gene knock-out technologies. Extensive research has been conducted to understand infection mechanisms by the pathogen and defense mechanisms of the host at cellular and molecular biology levels during the last decade. Through an elegant series of research, we know environmental cues and related signaling systems involved in infection of host plant by the fungus. More than a dozen of genes are identified as pathogenicity determinants through insertional mutagenesis using REMI (restriction enzyme mediated integration) or reverse genetic strategy. However, the precise mechanisms to complete the disease cycle remain to be understood. Recent advances on genomics research are making much progress to approach the ultimate understanding of pathogenesis at biochemical and molecular biological levels. In 2002, both whole genome drafts of rice and *M. grisea* were obtained and all information is available in public. Currently much effort is being focused on accurate annotation of genes in both organisms.

Agrobacterium tumefaciens-mediated transformation (ATMT) has long been used to transfer genes to a wide variety of plants and has also been used extensively as a tool for insertional mutagenesis in *Arabidopsis thaliana*. More recently, several fungi have been transformed using *A. tumefaciens*. For