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The complete genome sequencing of *Xanthomonas oryzae* pv. *oryzae* KACC10331 caused bacterial blight of rice in Korea

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Xanthomonas oryzae* pv. *oryzae* is a plant pathogen that cause rice bacterial blight (BB). This pathogen is the most destructive among bacterial diseases in rice field specially tropical, subtropical and warm template regions. The rice was lost by 30 to 50% of grain yield with bad quality in limited area when the field was infected by the pathogen seriously. We tried to sequence the complete genome of the pathogen to set up platform technology for genomic research of microoganism and find out the pathogenesis on rice. *X. oryzae* pv. *oryzae* KACC10331 isolated from rice field in our country originally was delivered from Plant Pathology Division of NIAST. The culture was showing uniquely virulence against rice cultiva which contained Xa21 resistant gene against the BB pathogen in the world. Shotgun library of the pathogen was constructed and sequenced randomly using ABI3700. Sequencing was performed 12 times of the whole genome in length. The results of sequences were assembled using bioinformatic-tools. About 99.98% of whole genome were contiged with 50gaps and generated 11scaffold. *X. oryzae* pv. *oryzae* KACC10331 has a circular chromosome of 4,495,206 bp in length which is predicted to encode 6,891 genes. One of the most striking finding in the genome is that the pathogen has a very high GC content of 64% and 450 insertion sequence fragments at high level. The several genes including four novel avirulence genes, siderophore, *hrp* genes, exopolysaccharides and extracellular enzymes were found in the genome. Analysis of *hrp* clustering genes will be helpful for more understanding of pathogenesis on rice. Following studies will be carried out for gapfilling, gene annotation, comparative genomics, gene expression profiling to understand the whole genome of *X. oryzae* pv. *oryzae* KACC10331.

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Genomics of phytoplasmal disease

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The identification and classification of phytoplasma were based primarily on such biological properties as the symptoms induced in infected plants, plant host range, and relationships with insect vectors, because it could not be cultured in vitro in cell-free medium. Recent advances in molecular-based biotechology have made it possible to obtain new knowledge about phytoplasmas and to develop accurate detection and classification. From the late 1980s, Phylogenetic analysis of the 16S rRNA gene, 23S rRNA gene, rp operon, tuf gene, two genes encoding membrane protein sequences have great developed taxonomic and evolution of phytoplasmas. Phytoplasma unversal and specific primers developed on the basis of conserved 16S rRNA genes have enabled researchers to use PCR assays to detect a wide array of phytoplasmas associated with plants and insect vectors. Based on RFLP profile of 16S rRNA gene amplified from phytoplasma strains associated with numerous diseasess were proposed a classification scheme that comprised, 14 major groups and 41 sub-groups and combined RFLP analysis of 16S rRNA and ribosomal protain gene sequences, 14 groups and 46 sub-groups recognized. But the phylogenetic analysis of phytopalsma in korea was little know even though that phytoplasmal diseases found from about 30 plants. Among those phytoplasmal plants, the phylogenetic analysis of MD and PaWB phytoplsmas belong to Aster yellow group and JWB phytopalsma belong to Elm yellow group by compleate16S rRNA gene, 16S/23 intergenic space gene sequences. About 1.2 kb, 16S rRNA gene sequences analysis, PaWB, CLL and SuWB phytopalsmas were also belong to Elm yellow groups comparison with other RFLP and phylogenic analysis. But the geographical or symptom different isolates were reveled different diversity by PCR-RFLP and sequence of a portion of 16S rRNA gene from JWB, PaWB, SuWB and CLL phytoplasmas. Other phylogenitic analysis such as rp operon, tuf gene, two genes encoding membrane protein sequences not known completely from phytoplasmal plants in Korea. This review was forcus on recent progress on the understanding of phylogenetic analysis of phytoplasmal diseases.

Proteome Studies of Rice-Rice Blast Fungus Interactions

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A large-scale DNA sequencing of whole genomes and genome-wide analysis of transcriptomes by microarray approaches can provide important clues to understand complex biological processes. Function of gene products is still waiting to be answered due to the complexity of post-translational modifications and protein-protein interactions. Quantitative differential protein display of large numbers of proteins with 2-DE and recent development of micro-quantification by mass spectrometric analysis have spurred up functional genomics through monitoring global changes that occur into protein expression of tissue or organelles, or perturbations by disease. The micro-characterization of a large-scale identification of proteins and differential display proteome with 2-DE/mass spectrometric techniques for plant-microbe interactions could aid to understand cross-talk between plants and microbes. To elaborate subtle differences in protein expression and modifications, we developed prefractionation technique, an important bottle neck for proteome research, such as PEG fractionation, size fractionation and high resolution 2-DE to facilitate display of rare proteins. These techniques were

applied to analyze differential proteome during appressorium development of rice blast fungus, *Magnaporthe grisea*. New or enhanced protein spots were identified through pulsed/continuous 35S methionine labeled proteins under the inductive or non-inductive condition of appressorium formation. Four prominent proteins induced during appressorium formation were internally sequenced and identified from protein database. One of the identified proteins, á-subunit of 20S proteasome, was further investigated to understand its function. Treatment of proteasome inhibitors MG132, proteasome inhibitor I, and II, provoked 4 to 6hr delay of appressorium formation. This lag time can also be observed on the rice leaf surface. The expression level of á-subunit of 20S proteasome identified by Western blot under time course of appressorium formation was also enhanced more than that of germination. Treatment of proteasome inhibitors alleviates or protects disease development on detached leaves through hypersensitive reaction. These data indicated that 20S proteasome actively takes part in appressorium formation of *M. grisea*.

Molecular analysis of the trichothecene biosynthesis gene clusters from the cereal scab fungus *Gibberella zeae*

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Gibberella zeae, a major cause of cereal scab, may be divided into two chemotypes based on production of the trichothecenes deoxynivalenol (DON) and nivalenol (NIV). We cloned and sequenced the gene cluster for trichothecene biosynthesis from each chemotype. *G. zeae* H-11 is a DON producer isolated from corn, and *G. zeae* 88-1 is a NIV producer from barley. We sequenced a 27-kb gene cluster from H-11 and a 30-kb cluster from 88-1, along with the unlinked *Tri101* genes. Each gene cluster contained 12 *Tri* gene homologues in the same order and transcriptional directions as those of *Fusarium sporotrichioides*. Between H-11 and 88-1 all of the *Tri* homologues except *Tri7* and *Tri13* were conserved, with identities ranging from 88 to 98% and 82 to 99% at the nucleotide and amino acid levels, respectively. The *Tri7* sequences were only 80% identical at the nucleotide level. We aligned the *Tri7* genes and found that the *Tri7* open reading frame of H-11 carried several mutations and an insertion containing 10 copies of an 11-bp tandem repeat. The *Tri7* gene from 88-1 carried neither the repeat nor the mutations. The *Tri13* genes from 88-1 and *F. sporotrichioides* are 78 and 80% identical at the nucleotide and amino acid levels, respectively, whereas H-11 carries a *Tri13* homolog that is strikingly different from the *Tri13* homologs of 88-1 and *F. sporotrichioides*. The H-11 *Tri13* gene is only 65 and 61% identical to the *Tri13* genes from *G. zeae* 88-1 and *F. sporotrichioides*, respectively. In addition, this gene appears to have incurred several substitutions, insertions, and deletions. We assayed 100 *G. zeae* isolates of both chemotypes by PCR amplification with primer pairs derived from the *Tri7* and *Tri13* genes, and could differentiate the chemotypes by polyacrylamide or agarose gel electrophoresis. The PCR-based method developed in this study should provide a simple and reliable diagnostic tool for differentiating the two chemotypes of *G. zeae*. To confirm the roles of the *Tri13* and *Tri7* genes in trichothecene production by *G. zeae*, we genetically altered toxin production in 88-1 and H-11. In transgenic strains, the targeted deletion of *Tri13* from the genome of 88-1 caused production of DON rather than NIV. Heterologous expression of the 88-1 *Tri13* gene alone or in combination with the 88-1 *Tri7* gene conferred on H-11 the ability to synthesize NIV; in the latter case, 4-acetylnivalenol (4-ANIV) also was produced. These results suggest that *Tri13* and *Tri7* are required for oxygenation and acetylation of the oxygen at C-4 during synthesis of NIV and 4-ANIV in *G. zeae*. These functional analyses of the *Tri13* and *Tri7* genes provide the first clear evidence for the genetic basis of the DON/NIV chemotypes in *G. zeae*.

Plant Virus Genomics

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Virus is a nano-scaled submicroscopic obligate parasite. Virology as a science was established more than 100 years ago with the publication by Martinus Beijerinick (1898) of a paper describing his pioneering experiments with diseased tobacco plants. He confirmed and extended Iwanowski's results on *Tobacco mosaic virus* (TMV) and was the first to develop the modern idea of the virus, which he referred to as "Contagium vivum fluidum ('soluble living germ')". The Age of Genomics dawned only for plant viruses. It was 1982 when the genome of TMV was published, only 20 years from now on which was the first record for RNA genome. TMV is a first recognized virus and the best characterized one in virology world. Today, the International Committee on Taxonomy of Viruses (ICTV) recognizes more than 3,600 virus species. The current ICTV report (2000) contains 951 species of plant viruses. Plant viruses are responsible for many major agricultural problems. Therefore, studying various viruses and their interaction with hosts is a prerequisite for finding remedies against viral diseases and understanding principles of the organization of life. With development of modern recombinant technology, we have lived in the postgenomic era, and this affected on plant virology gradually. You can find 147,779 accessions for virus as a keyword search in the GenBank database and 303 accessions for plant viruses are being tracked in the GenBank database and the pace has quickened. The GenBank Entrez Genomes currently contains 912 reference sequences for 763 viral genomes. Full genomic sequences of over 140 plant viruses have been completed so far, and this is over 15% of the known plant viruses. These sequences have been informative for the biology of the individual viruses, i.e., for evolution and pathogenicity. Recent plant virus genomics have focused on elucidating host genes involved in resistance mechanisms for development of new virus protection system. This includes development of functional full-length cDNA clone of plant virus, microarray techniques, transposition-based in vitro insertional mutagenesis, and other various modern strategies. The methodology used in plant virology is applicable to a detailed functional analysis of any viral nucleic acid cloned as DNA and can be used to address many different processes during viral infection cycles. Plant Virus GenBank (http://www.virusbank.org) is a nonprofit organization, one of the National Support Program for Special Materials Banks by the Ministry of Science & Technology and by the Korea Science & Engineering Foundation dedicated to the collection, identification, research development, distribution and deposition of plant virus research biomaterials established since 1999. Its role and prospects of virus genomics will be discussed.

Toward Functional Genomics of Plant Defense: Generation and Sequence Analysis of Expressed Sequence Tags from Hot Pepper (*Capsicum annuum* L.) in Relation to Disease Resistance

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Large-scale single-pass sequencing of cDNA has proven to be a useful tool for discovery of new genes and understanding of biological mechanisms. As a first step to understand the complexity of plant defense mechanism, expressed sequence tags (EST) were generated from hot pepper leaf cDNA library constructed from

combined leaves prepared at different time after inoculation with soybean pustule pathogen (*Xanthomonas campestris* pv. *glycines*). For further extension of gene diversity, ESTs were also generated from cDNA libraries constructed from anther, and flower buds using dye termination method. Among total of 10,061 generated ESTs, 9434 had good quality to be analyzed and clustering analysis revealed that 50% of total ESTs (4685) were unique. BLASTX analysis revealed that 74% of ESTs had significant sequence similarities with known proteins present in NCBI nr database. In addition, 1,265 of tentative full-length cDNAs were also identified from ESTs analysis. Functional classification of ESTs derived from pathogen-infected pepper leaves revealed that about 25% of the ESTs represented disease- or defense-related genes. Here, we describe detailed sequence analysis data. Although we focused on the genes related to plant defense response, our data are the useful depository for further comparative studies or other purposes.

[The sequence data in this paper are available at http://plant.pdrc.re.kr]

Genomics and Patent

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Recently, the number and complexity of nucleic acid molecule-related inventions have been increasing and changing with the progress of research and development projects such as the Human Genome Project. As a result, genomics and the availability of high-speed automated sequencing capability pose a new generation of questions for patent law. The standards for granting patents require that the invention be new, nonobvious, and useful, and that the patent adequately describe the invention and how to practice it. Of these standards, it is the utility doctrine that currently provides the leading approach to deciding which kinds of genomic patents should be granted. The Korean Industrial Property Office's Revised Biotechnology Patent Examination Guidelines, published in January 2001, suggest that a SNP or an EST associated with a well-defined function is patentable, whereas a more abstract or less well-understood SNP or EST is not patentable. The following items are confirmed by the Trilateral Patent Offices through discussion at the Trilateral Technical Meeting in June, 2000. 1. All nucleic acid molecule-related inventions, including full-length cDNAs and SNPs, without indication of function or specific, substantial and credible utility, do not satisfy industrial applicability, enablement or written description requirements. 2. Isolated and purified nucleic acid molecule-related inventions, including full-length cDNAs and SNPs, of which function or specific, substantial and credible utility is disclosed, which satisfy industrial applicability, enablement, definiteness and written description requirements would be patentable as long as there is no prior art (novelty and inventive step) or other reasons for rejection (such as, where appropriate, best mode [US] or ethical grounds [EPC/JP]).