Symposium Session

Symposium Session I : Functional Genomics in Plant Pathology

SI-1

Functional genomics of *Phytophthora infestans* interactions with solanaceous plants

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The Irish famine pathogen, *Phytophthora infestans*, causes late blight, a worldwide devastating disease of potato and tomato. Although a pathogen of great economic importance, little is known about the molecular mechanisms involved in pathogenicity and host specificity of *P. infestans* since only a handful of genes have been implicated in host interaction.

P. infestans is an oomycete, a diverse group of eukaryotic microorganisms that includes pathogens of plants and animals, as well as saprophytic species (water molds). The position of the oomycetes as a unique lineage of stramenopiles (or heterokonts), unrelated to true fungi but closely related to brown algae and diatoms, has been well established using molecular phylogenies based on ribosomal RNA sequences, compiled amino acid data for mitochondrial proteins, and several protein encoding chromosomal genes. It is evident from these analyses that oomycetes evolved the ability to infect plants independently from other eukaryotic plant pathogens, such as "true" fungi, and are likely to have unique mechanisms to do so.

Similar to other plant pathogens, *P. infestans* has the remarkable ability to manipulate biochemical, physiological and morphological processes in its host plants through a diverse array of virulence or avirulence molecules, defined here as effectors. In susceptible plants, these effectors promote infection by suppressing defense responses, enhancing susceptibility, or inducing disease symptoms. Alternatively, in resistant plants, effectors are recognized by the products of plant resistance genes resulting in the hypersensitive response and effective defense responses. The complex interplay between these effectors and their plant targets is thought to determine the outcome of a particular interaction. Therefore, one central objective in studying the molecular basis of pathogenicity of *P. infestans* is to identify and functionally characterize effector genes.

Structural genomic studies of *P. infestans* are well under way within the framework of a variety of consortia, as well as efforts in individual laboratories. Genomics is having a significant impact on our understanding of *P. infestans* biology and pathology. The main applications of genomics are:

- Understanding the molecular basis of *P. infestans* pathogenicity through the identification of genes that contribute to the infection process.
- Identifying *P. infestans* targets for genetic control, specifically avirulence genes that function at host and nonhost level.
- Understanding *P. infestans* population structure and evolution, through improved molecular markers and markers that determine phenotypes.

P. infestans genomics has already facilitated the discovery of candidate effector genes. The challenge in the post-genome era is to link candidate effector sequences to phenotypes. Here we provide an overview of ongoing functional genomic studies on *P. infestans*. These studies have allowed us to unravel a battery of novel *P. infestans* effector genes that trigger a variety of cellular and molecular responses in plant cells and to establish functional connections between *P. infestans* genes and plant processes.

To link sequences to phenotypes, we applied the functional genomics paradigm for the discovery of novel effector genes in *P. infestans*. First, we developed computational tools for mining the sequence data, then we applied robust low- and high-throughput functional assays to validate the predicted function of the candidate genes.

To select candidate genes from sequence databases, we used the following selection criteria:

- *Genes that encode degradative enzymes.* These genes are predicted to encode putative virulence factor involved in host tissue penetration and degradation.
- *Genes that encode extracellular proteins.* These are more likely to be involved in cross-talk with host plant.
- *Genes that are up-regulated during infection.* These encode putative virulence or pathogenicity factor and could serve as fungicide targets.
- *Genes that are polymorphic and under diversifying selection.* These are more likely to be involved in co-evolutionary arms race with host plants.

A number of computational tools were developed or implemented either at Ohio State University or in collaboration with a bioinformatics team at the National Center for Genome Research. Several of these tools have been incorporated into the *Phytophthora* Functional Genomics Database (www.pfgd.org), a publicly available resource.

Examples of candidate genes identified using the various data mining strategies includes genes predicted to function in adhesion to host cells during appressorial formation, genes encoding degradative enzymes that may facilitate penetration of plant tissue and formation of haustoria, as well as genes that function in infection, such as putative virulence and avirulence genes. The recent report that *P. infestans* secretes serine protease inhibitors that target host proteases is an example of a a discovery that directly resulted from the sequence data.

To validate the various candidate genes and to identify novel

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