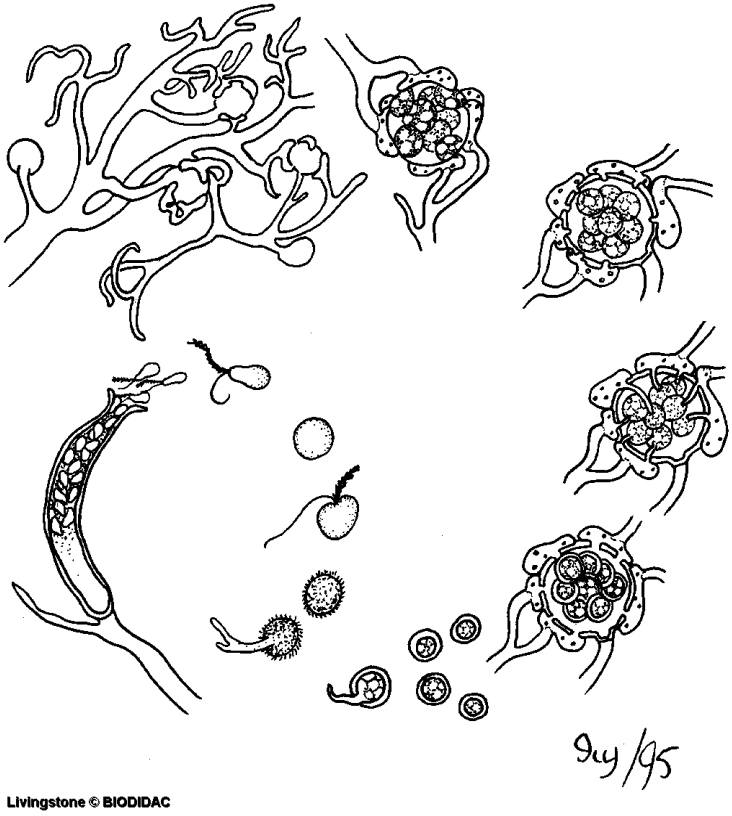
Oomycete Molecular Genetics Network Workshop

Asilomar Conference Center, Pacific Grove, CA, USA
March 18-20, 2007



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SCIENTIFIC PROGRAM

Sessions will be held in "Nautilus"

Abstracts of talks begin on p. 5

Poster abstracts begin on p. 15

Sunday, March 18

5:00	Informal registration begins	
6:00-7:30	Dinner	
Session I	Keynote talk	*chaired by: Howard Judelson*
7:30	Welcome and announcements	
7:45	Elizabeth Winzeler	Systems biology of malaria parasites *Dr. Winzeler is an Associate Professor in the Department of Cell Biology of the Scripps Research Institute in La Jolla, CA USA*
8:45	Mixer	

Monday, March 19

7:30	Breakfast	
Session II	**Disease biology**	*chaired by: Elodie Gaulin*
9:00	Alon Savidor	Announcement: *Phytophthora capsici* genome assembly and annotation
9:10	Dinah Qutob	The *Phytophthora sojae* avirulence gene *Avr*1a, encoding an RxLR effector protein, displays copy number polymorphism
9:35	Jennifer Tedman-Jones	Identification of *Phytophthora sojae Avr3a* by Expression Profiling
10:00	Daolong Dou	Functional Analysis of the *Phytophthora sojae* effector protein Avr1b

10:25	Break	
10:45	Jorunn I.B. Bos	Distinct amino acids of *Phytophthora infestans* AVR3a condition activation of R3a hypersensitivity and suppression of cell death
11:05	Pieter M.J.A. van Poppel	The *Phytophthora infestans* avirulence gene *PiAvr4* encodes an RxLR-DEER effector protein
11:30	Laura Baxter	Allelic diversity of potential RXLR effectors in *Hyaloperonospora parasitica*
12:00	Lunch	
Session III	Gene expression, cellular signalling, and development	*chaired by: John McDowell*
1:40	Yuanchao Wang	Screen and characterization of pathogenicity-related transcription factors based on *Phytophthora sojae* genome
2:05	Audrey Ah Fong	*Cis*-acting elements regulating transcription initiation and sporulation-specific expression of the *PiCdc14* gene of *Phytophthora infestans*
2:30	Stephen C. Whisson	Identifying novel pathogenicity genes by transient gene silencing in *Phytophthora infestans*
2:55	Howard Judelson	Silencing of a zoosporogenesis-specific gene cluster in *Phytophthora infestans*
3:20	Break	
3:55	Emma J. Robertson	EST-mining identifies candidate proteins involved in zoospore development of the fish pathogen *Saprolegnia parasitica*
4:20	Harold J. G. Meijer	Characterization of extracellular phospholipase D activity in *Phytophthora*
Session IV	Population studies and evolution	*chaired by: Anne Dorrance*
4:55	Seogchan Kang and Frank Martin	*Phytophthora* Database: an integrated resource for detecting, monitoring, and managing *Phytophthora* diseases

5:20	Masoomeh Peiman	Molecular phylogenetic and evolutionary characterization of a global collection of *Phytophthora infestans*
6:00	Dinner	
7:30	Posters/Mixer	

Tuesday, March 20

7:30	Breakfast	
Session V	Genome analysis and manipulation	*chaired by: Nik Grunwald*
9:05	Paul Morris	Searching for the Rosetta stones in the multifunctional proteins of the *Phytophthora* genomes
9:30	Michael Zody	Identification and characterization of repeats in genomes of *Phytophthora* Species
9:55	Rays Jiang	Plasticity of the *Phytophthora* genome; amplifications and retrotransposons
10:20	Break	
10:45	Frank Martin	Mitochondrial genomics in the genera *Pythium* and *Phytophthora*
11:10	Pieter van West	Successful transformation of the mycoparasitic oomycete *Pythium oligandrum*
11:35	Liliana Cano	Full-Length cDNA sequences for genome annotation in *Phytophthora infestans*
11:45	Discussion, announcements, etc.	
11:59	Adjournment	
12:00	Lunch	

Abstracts of Oral Presentations (in order of presentation)

The *Phytophthora sojae* avirulence gene *Avr*1a, encoding an RxLR effector protein, displays copy number polymorphism. Dinah Qutob[1], Brett M. Tyler[2] and Mark Gijzen[1]. [1]Agriculture and Agri-Food Canada, London,ON, Canada and [2]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, USA.

We have completed the map-based cloning and identification of the *Phytophthora sojae* avirulence gene *Avr*1a. Mapping of *Avr*1a required more than 1500 F_2 individuals from two separate crosses, illustrating the relatively low recombination frequency of the region. Problems in physical mapping occured even after completion of the *P. sojae* genome sequence, because the *Avr*1a gene does not occur on a large assembly contig. Physical mapping was aided by comparison to a syntenic region in *Phytophthora ramorum*. Eventually, we identified an open reading frame within the *Avr*1a delimited region in *P. sojae* that encodes a secreted RxLR-dEERprotein of 121 amino acids. Transcriptional analysis demonstrated that the gene is expressed during infection, and that differences in the expression level could be correlated to *Avr*1a phenotype. Transient expression of this gene in soybean triggered an *Rps*1a-specific cell death response, confirming that it corresponds to *Avr*1a. Preliminary evidence suggests that multiple copies of *Avr*1a occur as a repetitive unit in certain *P. sojae* strains, including the isolate that was used for whole genome sequencing (P6497), thus accounting for the poor assembly of this region. Overall, our results can be integrated into a model whereby *Avr*1a copy number polymorphisms lead to transcriptional and translational differences that either activate or evade effector-triggered immunity on *Rps*1a soybean plants.

Identification of *Phytophthora sojae Avr3a* by Expression Profiling. Jennifer Tedman-Jones, Jonathan Eckert, and Mark Gijzen. Agriculture and Agri-food Canada, Southern Crop Protection and Food Research Centre, 1391 Sandford Street, London, Ontario, N5V 4T3, Canada

Past studies have shown that some *Phytophthora sojae* isolates have lost expression of a particular avirulence (*Avr*) gene to evade recognition by soybean cultivars carrying the corresponding resistance (*R*) gene. To identify genes whose expression is associated with a particular avirulence trait, two F_2 bulks were generated from an existing *P. sojae* population (Race2: *Avr3a/Avr5/Avr3c* crossed with Race7: *avr3a/avr5/avr3c*) based on the virulence phenotype of individual progeny on cultivars carrying *Rps3a*, *Rps5* or *Rps3c*. The expression profiles of 15,800 transcripts were measured from total RNA, from infected soybean seedlings, for the two bulks and the two parents, using Affymetrix gene chips. The microarray data was mined to identify avirulence-associated transcripts. Transcripts that showed an association were further analyzed by RT-PCR of total RNA from individual F_2 progeny. RT-PCR analysis revealed one transcript that showed 100% association to avirulence, conditioned by *Avr3a* and *Avr5*, among the F_2 progeny and parents. This transcript encodes a small secreted peptide containing RXLR-dEER motif typical of oomycete avirulence genes. The corresponding gene is located in a region of the genome with a high density of genes encoding secreted peptides with the RXLR-dEER motif. Co-bombardment of the avirulence gene candidate with the promoter:reporter gene construct, 35S:beta-glucuronidase (GUS), results in a marked reduction in GUS activity in soybean plants carrying *Rps3a*, but not those carrying *Rps5*. This data shows the protein product of this candidate is specifically recognized by RPS3a in the soybean host, and therefore most likely encodes the *P. sojae* avirulence gene *Avr3a*.

Functional Analysis of the *Phytophthora sojae* effector protein Avr1b. Daolong Dou[1], Rays H.Y. Jiang[1,2], Xia Wang[1], Shiv Kale[1], Felipe Arredondo[1], Sucheta Tripathy[1] and Brett M. Tyler[1]. [1]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA24061. [2] Laboratory of Phytopathology, Wageningen University, NL-6709 PD Wageningen, The Netherlands and Broad Institute, Cambridge, MA02141.

The genome sequences of several oomycete plant pathogens, including the soybean pathogen *Phytophthora sojae*, reveal that each of these genomes encodes several hundred proteins with sequence similarity to cloned oomycete avirulence genes. We have identified conserved motifs present in large numbers of these genes, including the *P. sojae* avirulence gene *Avr1b* -1. We have tested the function of the motifs in *Avr1b* -1 using *P. sojae* stable transformants and soybean transient expression assays. The RXLR motif, found near the N terminus of most of the effectors, is required for *Avr1b*-1 to confer avirulence when expressed in *P. sojae*, but not when expressed inside soybean cells, supporting the hypothesis that RXLR is required for Avr1b-like proteins to transit into plant cells. The sequences surrounding the RXLR motif are also required, but there is only a weak requirement for the adjacent dEER motif. Avr1b contains two C terminal motifs that occur as a pair in approximately half of all the *P. sojae* effectors, and both are required to confer avirulence. Overexpression of *Avr1b*-1 increases the virulence of *P. sojae* transformants on susceptible cultivars and we are currently determining the role of the conserved motifs in conferring increased virulence.

Distinct amino acids of *Phytophthora infestans* AVR3a condition activation of R3a hypersensitivity and suppression of cell death. Jorunn I. B. Bos, Angela Chaparro-Garcia, Lina M. Quesada, Sophien Kamoun. Dept. of Plant Pathology, Ohio State University-OARDC, Wooster, OH, USA

The AVR3a effector of *Phytophthora infestans* is a member of the RXLR family of cytoplasmic effectors that exhibits dual effector functions. AVR3a induces hypersensitivity mediated by the resistance protein R3a and suppresses cell death induced by *P. infestans* INF1 elicitin. Two polymorphic forms of AVR3a that differ in two amino acids in the mature region occur in *P. infestans*. While AVR3a^{KI} can mediate both R3a activation and cell death suppression, no effector function has been identified for AVR3a^{EM}. To gain insights in the molecular basis of AVR3a activities, we performed structure-function analyses of both forms of AVR3a. We used near saturation high-throughput random mutagenesis screens based on functional expression in *Nicotiana benthamiana* to identify *Avr3a* mutants with altered activation of R3a hypersensitivity. Out of more than 6500 AVR3a^{EM} mutants tested, we identified about 150 gain-of-function mutants that induced R3a hypersensitivity. About 10 residues, mostly charged amino acids, were frequently substituted in these mutants. These AVR3a^{EM} gain of function mutants did not always gained the ability to suppress cell death suggesting that distinct residues condition R3a hypersensitivity and cell death suppression. A similar loss of function screen of 4500 AVR3a^{KI} mutants resulted in smaller number of mutants with altered activity. These results point to a model that involves the interaction of AVR3a with a host protein and is not consistent with the recognition of AVR3a through an enzymatic activity.

The *Phytophthora infestans* avirulence gene *PiAvr4* encodes an RxLR-DEER effector protein. Pieter M.J.A. van Poppel[1], Jun Guo[1,2], Peter J.I. van de Vondervoort[1], and Francine Govers[1]. [1]Laboratory of Phytopathology, Wageningen University, The Netherlands and [2]IVF-CAAS Beijing and Northwest A & F University, Yangling Shaanxi, China

The oomycete pathogen *Phytophthora infestans* causes late blight, an important disease in potato world wide. *P. infestans* secretes numerous effector molecules, some of which are recognized by host plants carrying resistance (*R*) genes. These effectors then act as avirulence (*Avr*) factors and elicit a hypersensitive response (HR) that arrests growth of the pathogen. Through a combined approach of genetic mapping and transcriptional profiling (cDNA-AFLP) we isolated an *Avr* gene that shows a gene-for-gene interaction with *R4*. This gene, named *PiAvr*4, is highly expressed in germinated cysts and encodes a 287 amino acid protein with a putative signal peptide and an RxLR-DEER motif. The Avr4 protein belongs to a large family of *P. infestans* effector proteins that are highly divergent but share the RxLR-DEER motif. This motif is thought to play a role in delivery of effectors into the host cell. Transformation of *PiAvr*4 into *P. infestans* isolates virulent on *R4* plants, resulted in complementation, i.e., the transformants elicited HR and thus became avirulent on plants carrying *R4*. *In planta* expression of *PiAvr*4 using agroinfection-based Potato Virus X (PVX) expression vectors caused an HR specifically on *R4* plants but not on *r0* plants. However, the HR was only observed when a signal peptide sequence was included in the construct and not when the mature protein was produced. Apparently, ER modification and/or secretion of Avr4 are required for recognition of Avr4 as avirulence factor. Direct virus inoculation of plants with PVX expressing *PiAvr*4 with and without signal peptide sequence gave the same results as the agroinfection inoculation.

Allelic diversity of potential RXLR effectors in *Hyaloperonospora parasitica*. L. Baxter, P. Bittner-Eddy, M. Coates, S. Hall, L. Hughes, J. Beynon. Warwick HRI, University of Warwick, Wellesbourne, CV35 9EF, UK.

Hyaloperonospora parasitica is a biotrophic oomycete pathogen of *Arabidopsis thaliana*, from which two functional avirulence genes, *ATR1* and *ATR13*, were previously identified (Allen *et al*., 2004[1], Rehmany *et al.*, 2005[2]). Each encodes a small protein containing a signal peptide sequence and an RXLR motif, and display very high levels of allelic diversity. We used a bioinformatics approach to mine the *H. parasitica* (isolate *Emoy2*) genome sequence for other members of this class of cytoplasmic effector molecule. We then assessed allelic diversity for 140 of those genes from corresponding loci of six other isolates of *H. parasitica*. A broad spectrum of diversity was observed, with 80% showing some degree of polymorphism, caused both by point mutations and indels of varying lengths. In the most highly diverse sequences, the calculated Ka/Ks ratios suggest that these genes are under diversifying selection. This would fit with the model of a coevolutionary "arms race" involving reciprocal plant genes. By contrast, 20% were conserved at the protein level. Gene expression data, obtained from cDNA and EST libraries, showed that >70% are present as mRNA in asexual spores from the *Emoy2* isolate. Downstream functional and structural analyses (including mapping, biolistics, Y2H and protein purification) of selected candidate effectors will help to elucidate their role in modulating host defences.

[1]Allen, R. L., Bittner-Eddy, P. D., Grenville-Briggs, L. J., Meitz, J. C., Rehmany, A. P., Rose, L. E., and Beynon J. L. 2004. Host-parasite coevolutionary conflict between Arabidopsis and downy mildew. Science 306:1957-1960.

[2]Rehmany A. P., Gordon, A., Rose, L. E., Allen, R. L., Armstrong, M. R., Whisson, S. C., Kamoun, S., Tyler, B. M., Birch, P. R. J., and Beynon, J. L. 2005. Differential recognition of highly divergent downy mildew avirulence gene alleles by RPP1 resistance genes from two Arabidopsis lines. Plant Cell 17:1839-1850.

Identifying novel pathogenicity genes by transient gene silencing in *Phytophthora infestans.* Stephen Whisson[1], Laura Grenville-Briggs[2], Pieter van West[2], Paul Birch[2], Anna Avrova[1]. [1]Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom, and [2]Aberdeen Oomycete Group, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, United Kingdom.

Late blight of potato and tomato, caused by *Phytophthora infestans*, is a re-emerging plant disease and occurs in most locations where these host plants are grown. *P. infestans* expresses many genes specific to each of its lifecycle stages. Genes specifically upregulated in germinating cysts, appressoria, and during infection were selected to determine their involvement in pathogenesis using transient gene silencing initiated by double stranded RNA homologous to each candidate gene. The majority of highly upregulated genes in appressorial and infection stages were predicted to encode secreted proteins. Phenotypes observed in transient gene silencing assays have included aberrant appressorial development, burst appressoria, reduced penetration, and attenuated pathogenicity; many candidate pathogenicity genes yielded no obvious phenotype. Progress in screening and characterising further genes will be presented.

Screen and characterization of pathogenicity-related transcription factors based on *Phytophthora sojae* genome. Dong Suomeng, Wang Yonglin, Zheng Xiaobo and Wang Yuanchao. Plant Pathology Department of Nanjing Agricultural University, Nanjing 210095, China. E-mail: wangyc@njau.edu.cn

Phytophthora sojae is a hemi-biotrophic plant pathogen that cause root and stem rot in soybean resulting severe agricultural losses world widely. The regulation pattern of pathogenicity and development of this pathogen are still limited despite the exciting release of *P. sojae* and other oomycete genome sequence. Based on a series of bioinformatics resources, we collected 838 annotated and new predicted transcription factors from 20 families. Genome level analysis indicated different gene evolution speed and distribution pattern. We made RT-PCR analysis of 63 putative transcription factors from families including CCHC-type, FYVE-type, C2H2-type zinc finger protein, Homeobox, myb and bZIP factors, finding 4 genes up-regulated during pathogen-soybean interaction and one in zoospore stage. Among 5 candidate genes, one gene (*PsC2HZ2*) contained two putative zinc finger domains of the Cys2His2 class, suggesting it is a putative C2H2 transcription factor. Blast results indicated that *PsC2HZ2* shared a high homolog with *Saccharomyces cerevisiae* gene *ScCRZ1*, which plays an important role in regulating yeast calcium signal pathway. Coupled with previous reports that calcium pathway is critical for life stages in *Phytophthora* spp., we cloned *PsC2HZ2* and obtained PsC2HZ2-deficient mutants by homology dependent gene silencing. The latest studies showed that the pathogenicity of mutants was decreased significantly. Furthermore, the growth rate and colony morphology of mutants on V8 plate had a clear discriminations compared with wild type and vector transformed alone strain. Recently, we are trying to get mutants of other putative transcription factors by similar methods and the analysis of the mechanism of decreased pathogenicity in *PsC2HZ2* mutant is still undergoing. Lastly, the transcriptional regulation patterns of important pathogenicity related transcription factors will be uncovered and be set as targets for new drug designing in agricultural productions.

***Cis*-acting elements regulating transcription initiation and sporulation-specific expression of the *PiCdc14* gene of *Phytophthora infestans*.** Audrey M. V. Ah Fong and Howard S. Judelson. Department of Plant Pathology, University of California, Riverside, California, USA.

Asexual spores are central to the disease cycle of *Phytophthora infestans,* the cause of tomato and potato late blight. To understand transcriptional processes involved in sporulation, functional regions were identified within the promoter of *PiCdc14*, a gene normally expressed only during asexual spore development. These were defined by testing truncated promoters or site-directed mutants in transformants of *P. infestans,* using the GUS reporter. Two functional classes of motifs were identified, one directing sporulation-specific expression and others regulating the position of transcription initiation. Two apparently redundant binding sites for transcription factors directing expression during sporulation were localized just upstream of nt -66 (relative to transcription start site), within a region showing high conservation in four *Phytophthora* spp. The site of transcription initiation was controlled both by sequences flanking the normal major start site and the motif CTCAAC, which was present four times between -74 and -138. Promoters in which either the normal start site or all four CTCAAC motifs were eliminated still exhibited sporulation-specific expression, but initiation commonly occurred at nt -109. Deletions of single CTCAAC motifs had intermediate effects. Although the region containing the major start site of *PiCdc14* lacks the Inr-like consensus described previously for many other *Phytophthora* genes*,* it does not appear to represent a sporulation-related determinant since exchanging it with the Inr-like region of the *PiExo1* gene did not impair *PiCdc14* expression. Experiments to better define the biochemical and genetic interactions between these sites and their cognate transcription factors are underway.

Characterization of extracellular phospholipase D activity in *Phytophthora*. Harold J.G. Meijer and Francine Govers. Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, Binnenhaven 5, NL-6709 PD, Wageningen, The Netherlands

Phospholipase D (PLD) catalyzes the hydrolysis of structural phospholipids, such as phosphatidylcholine, leading to the production of phosphatidic acid and a free headgroup. In comparison to other eukaryotes, *Phytophthora* spp. have a more complex and diverse set of PLD genes. Mammals have only one type of PLD called PXPH-PLD. Plants also have PXPH-PLDs but in addition they have a large family of C2-PLDs. In these proteins, PX, PH and C2 are the lipid-binding domains that precede the catalytic PLD domain. *Phytophthora* has one PXPH-PLD, lacks C2-PLDs, but has three additional novel PLD sub-families (Meijer and Govers 2006), one of which is a large family of 12 members that all have a signal peptide and are probably secreted. The substrates for these secreted PLDs could be phospholipids that reside in plant membranes, one of the barriers faced by the pathogen when it enters the host. To test the hypothesis that the secreted PLDs play a role in the infection process, we first analyzed PLD activity in the pool of extracellular proteins secreted by *Phytophthora.* Here we present the first biochemical characterization of PLD activity present in extracellular fluid and of the substrate specificity.

Meijer, H.J.G., and Govers, F. 2006. Genomewide analysis of phospholipid signalling genes in *Phytophthora* spp.: novelties and a missing link. Mol. Plant Microb. Inter. 19:1337-1347.

EST-mining identifies candidate proteins involved in zoospore development of the fish pathogen *Saprolegnia parasitica.* Emma J. Robertson[a], Vicky L. Anderson[a,b], Andrew J. Phillips[a,b], Chris J. Secombes[b], and Pieter van West[a. a]The Aberdeen Oomycete Group, College of Life Sciences and Medicine, University of Aberdeen, Foresterhill, Aberdeen, Scotland, UK. E-mail: p.vanwest@abdn.ac.uk; [b]Scottish Fish Immunology Research Centre, Aberdeen, Scotland,UK.

The oomycete *Saprolegnia parasitica* causes Saprolegniosis, a serious fish disease whereby mycelium grows on incubating eggs, or into the fins and body of freshwater fish. Severe infection results in death of the host, due to an imbalance in osmoregulation. Very little is known about the molecular biology of *S. parasitica*, and pathogenicity is largely undetermined. To gain more information about which genes are expressed at the onset of an infection and during pathogen development, we have constructed three cDNA libraries; to date 1000 clones have been sequenced from the pre-infectious stages of the *S. parasitica* life cycle, namely zoospores, cysts and germinating cysts. An additional 6000 clones are being sequenced from cDNA libraries that were made from mRNA isolated from a fish cell line that was infected with *S. parasitica* (8 hours post infection); and mRNA isolated from an infected fish (*Salmo salar*) that was challenged with *S. parasitica*. From the initial 1000 ESTs, we have identified a number of interesting candidate genes that may be virulence factors. In addition, several genes were identified that are thought to play a role in developmental processes. Two of these genes are thought to be involved in vesicle transport and protein secretion. These have been selected for further characterization. Here we present our latest findings.

Successful transformation of the mycoparasitic oomycete *Pythium oligandrum*. Neil Horner and Pieter van West. The Aberdeen Oomycete Group, College of Life Sciences and Medicine, University of Aberdeen, Foresterhill, Aberdeen, Scotland, UK. E-mail: p.vanwest@abdn.ac.uk

The mycoparasitic oomycete, *Pythium oligandrum*, can suppress various economically important soil-borne oomycete and fungal plant pathogens. Very little is known about the molecular processes that take place during the mycoparasitic interaction in the rhizosphere. In order to investigate the interaction at the cellular and molecular level, we decided to develop a transformation system that would allow us to generate transgenic *P. oligandrum* strains with integrated reporter genes and constructs that may induce silencing of endogenous genes. A Ca^{2+}-PEG-protoplast-based method was tested and found to be very effective. Up to 70 transformants were obtained in each round of transformation. As selection markers, both hygromycin (pHAMT34H) and geneticin (pTH209, pTor) were used successfully. A green fluorescent protein (GFP) construct (pVW2) was also transformed into *P. oligandrum* and about 50% of all generated strains were expressing GFP to detectable levels employing fluorescence microscopy. Furthermore we generated strains expressing an antisense construct of a gene with unknown function. We are currently analysing whether silencing is induced in the transformed lines. Here we present our latest findings.

***Phytophthora* Database: An integrated resource for detecting, monitoring, and managing *Phytophthora* diseases.** Seogchan Kang[1], Jaime Blair[1], Dave Geiser[1], Izabela Makalowska[1], Sook-Young Park[1], Bongsoo Park[1], Mike Coffey[2], Kelly Ivors[3], Yong-Hwan Lee[4], Jong-Seon Park[4], Kerry O'Donnell[5] and Frank Martin[6]. [1]Penn State, University Park, PA; [2]UC, Riverside, CA; [3]NC State Univ., Raleigh, NC; [4]Seoul National Univ., Seoul, Korea; [5]USDA-ARS, Peoria, IL; [6]USDA-ARS, Salinas, CA USA.

The ability to accurately and rapidly identify *Phytophthora* spp. is crucial for developing effective regulatory and management strategies against *Phytophthora* diseases. The *Phytophthora* Database project (www.phytophthoradb.org) aims to enhance our capability of rapid detection and diagnosis of *Phytophthora* spp. by archiving known genotypic and phenotypic diversity in a highly integrative database. To compliment the species morphological descriptions and serve as a molecular reference for isolate identification, ~2000 accessions representing 71 morphological species maintained in the WPC Genetic Resource Collection (UC-Riverside) and a few other facilities have been genetically characterized. A seven-locus phylogeny of the whole genus supports the division of *Phytophthora* into approximately eight major groups. In addition to these nuclear genes, four mitochondrially-encoded genes are currently being sequenced to construct a mitochondrially based phylogenetic framework. This project is on-going; sequence and phenotypic data are continually being deposited, a section on molecular diagnostics will include detailed procedures for different techniques and sequence alignments that were used in their development, and new data analysis and visualization tools are being developed to increase utility and breadth of this database. An overview of database functions will be presented.

Molecular phylogenetic and evolutionary characterization of a global collection of *Phytophthora infestans* M. Peiman and M.D. Coffey, Department of Plant Pathology, University of California, Riverside, CA 92521 USA

Without doubt the most important of the *Phytophthora* species is still the Late Blight fungus *P. infestans*, the cause of the Irish Potato Famine. Many new aggressive strains of this pathogen have emerged in the last few decades and seriously threaten potato and tomato production in many parts of the world. The World Phytophthora Collection has a large (~2000 isolates) and geographically diverse collection of this pathogen. In a preliminary assessment of the inherent variation within the species 165 isolates selected from diverse geographical regions were examined by RAPD, RG-57 RFLP and mtDNA haplotype analysis. Phylogenetic relationships among these isolates and sympatric species such as *P. mirabilis* and *P. phaseoli* were also inferred from sequence analysis of rDNA ITS, Cox I and β-Tubulin regions. The interpretation of the results will focus on inferences with regards population biology including the possibility of sexual recombination in different geographical regions, and evidence for their evolutionary divergence.

Searching for the Rosetta stones in the multifunctional proteins of the *Phytophthora* genomes. Tom Wittenschlaeger[1], Ryan Austin[2], Nicholas Provart[2], Paul F Morris[1]. [1]Department of Biological Sciences, Bowling Green State University, Bowling Green OH 43403, and [2]Cell and Systems Biology University of Toronto, Toronto ON M5S 3G5.

Eukaryotic genomes have in common a large number of multifunctional proteins. A global survey of the oomycete *Phytophthora sojae* genome, identified 274 novel multifunctional proteins using strict criteria that excluded multi-exonic gene models without EST support. These *P. sojae* proteins have significant BLAST hits to two or more different proteins. Such proteins have been posited as Rosetta stones, since their association in one genome has been used to infer the association of orthologous proteins in other genomes. In our analysis, we adopted the reciprocal smallest distance algorithm (Wall et al 2003) to identify potential orthologs in 34 sequenced genomes. Surprisingly, this approach identified only seven potential Rosetta stones, where each domain of a multifunctional protein had an ortholog in the same organism. A separate phylogenetic analysis has identified several examples where each half of a multifunctional protein, clusters in a node with homologs from separate kingdoms. The evolutionary history of oomycetes involved the endosymbiotic acquisition of a red algae, and subsequent transfer of nuclear and plastid genes to the host nucleus. We postulate that this endosymbiotic event (genome acquisition and recombination) has enabled the ancestral genome to develop metabolic and regulatory pathways that are distinct from those of the animal, fungal and plant genomes. Oomycete pathways that include genes from plant and animal ancestral genomes may have metabolic and regulatory efficiencies that are not present in other organisms. Our observations suggest that the evolutionary strategy of genome acquisition and recombination should also be assessed in other members of the Chromalveolates.

Plasticity of the *Phytophthora* genome - amplifications and retrotransposons. Rays H.Y. Jiang[1,2], Rob Weide[1], Michael C. Zody[2], Chad Nusbaum[2] and Francine Govers[1]. [1]Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, NL-6709 PD Wageningen, The Netherlands. [2]Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, Massachusetts 02141, USA.

The destructive late blight pathogen *Phytophthora infestans* is notorious for its rapid adaptation to avoid recognition by plant resistance (R) genes. Gene amplifications are found in the genome by comparative genomic hybridization on microarrays as well as by analyzing the draft genome sequences. In particular, one gene amplification at an avirulence locus is shown to be correlated with changes in virulence phenotypes (Jiang et al., 2006, Genome Res.). The genome of *P. infestans* is larger than that of other *Phytophthora* species. A large fraction is estimated to consist of high copy repeats (>100 copies). The majority of identified repeats belong to the LTR (Long Terminal Repeat) - retrotransposons. Numerous retrotransposition events of several characterized mobile elements have substantially contributed to the large genome size of *P. infestans*. When comparing two species, *P. sojae* and *P. ramorum*, the predominant LTR-retrotransposons appear to occur more frequently in the genomic regions containing genes with no unambiguous ortholog than in the regions containing genes having 1:1 orthologs. The genomic regions containing RXLR-dEER effectors also show a higher frequency of these retrotransposons than those containing other genes. These results indicate that these abundant retrotransposons are associated with increased genome fluidity.

Identification and Characterization of Repeats in Genomes of Phytophthora Species
Michael C. Zody[1], Manfred Grabherr[1], Rays H.Y. Jiang[1,2], and Chad Nusbaum[1]. [1]Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge MA 02453, and [2]Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, NL-6709 PD Wageningen, The Netherlands.

The *Phytophthora infestans* genome (240 Mb) is substantially (2.5-5-fold) larger than those of other Phytophthora species. With the recent availability of a draft genome sequence for *P. infestans*, we can begin to explain this phenomenon. Preliminary characterization of the genome prior to sequencing suggested that an unusually large fraction (for a microbe) of the genome, somewhere between 50 and 75%, might be high copy repeat resulting from transposition. Using the draft sequence of the genome, we have identified potential elements by a procedure we call "depth marking". A small amount (0.1x in this case) of random shotgun sequence is aligned to the genome, with all alignments of any quality maintained. Each position in the genome is then marked by the number of times it is hit by random reads. Potential mobile elements are identified as regions of much higher than expected coverage with discrete boundaries. Based on this, 81 Mb of the 190 Mb *P. infestan*s assembly appears to be high copy (>100) repeat. In addition, we estimate as much as 50 Mb is still unassembled and may all be repeat, giving a repeat content of 42-55%. Further, by filtering and clustering such elements, we have come up with a set of putative repeat families which explains over 75% of the repeat regions identified by depth marking. These consist of over 20 distinct families thus far characterized, of which more than 75% are novel. The majority are LTRs with a small number of apparent non-autonomous DNA transposons. Application of identical methodologies to the genomes of *P. sojae* (95 Mb) and *P. ramorum* (65 Mb) reveals a smaller number of easily characterized families (11 in *P. sojae*, 8 of which are novel, and 5 in *P. ramorum*, 4 novel). Again, 65-75% of the repeat elements in these genomes show similarity to one of these families. Preliminary analysis of the nature of the repeat relics suggests that adjacent elements have actively recombined, possibly with significant impact on genome and gene remodeling.

Transgene-induced silencing of the zoosporogenesis-specific *PiNIFC* gene cluster of the oomycete *Phytophthora infestans* involves chromatin alterations. Howard S. Judelson and Shuji Tani. University of California, Riverside, California, USA.

Transformation-based methods for silencing genes in oomycetes are still in their infancy and the underlying cellular mechanisms are poorly understood. Transformants of the phytopathogen *Phytophthora infestans* in which the *PiNIF* family of transcriptional regulators were silenced were therefore generated and analyzed to better understand the process. *PiNIFC1, PiNIFC2,* and *PiNIFC3* are zoosporogenesis-induced and clustered within 4 kb, and 20 kb away resides a sporulation-induced form*, PiNIFS*. Hairpin constructs of *PiNIFC1* or *PiNIFC2* triggered silencing of the cognate gene in about one-third of transformants and frequently all three *PiNIFC* genes became silenced coordinately. This resulted in a defect in the germination of zoospore cysts. *PiNIFS* was not silenced by the *PiNIFC* transgenes, even though all genes are closely related in DNA sequence. Silencing of the *PiNIFC* cluster was transcriptional based on nuclear run-on assays, and associated with tighter chromatin packing based on nuclease accessibility experiments involving DNA blotting and qPCR. The chromatin alterations extended a few hundred nucleotides beyond the boundaries of the transcribed region of the *PiNIFC* cluster, and were not associated with increased DNA methylation. Silencing of the different members of the *PiNIFC* family by a spreading heterochromatin domain, as opposed to a diffusible signal, may explain why *PiNIFS* escaped silencing.

Mitochondrial Genomics in the Genera *Pythium* and *Phytophthora*. Frank N. Martin[1] and Paul Richardson[2]. [1]USDA-ARS, Salinas, CA and [2] Joint Genomics Institute, Walnut Creek, CA USA.

The mitochondrial genomes of the related genera *Pythium* and *Phytophthora* encode a similar suite of genes but they differ from each other by the presence of a large inverted repeat (IR) that is found in *Pythium* (it can represent approximately 80% of the genome size). In an effort to gain a better understanding of the evolutionary forces responsible for sequence divergence in genomes with and without an IR, as well as to clarify the phylogenetic relationships within the individual genera, the mitochondrial genomes of 15 *Pythium* and 10 *Phytophthora* species were sequenced (multiple isolates for several species were also sequenced to assess intraspecific variation). Comparative genomics among species within a genus indicated that certain regions of the genome were more polymorphic than others. In *Pythium*, the most polymorphic region was the small unique region and adjacent IR sequences. In *Phytophthora* genomic inversions were observed with many of the rearrangements corresponding to phylogenetic groupings. Two closely related species (*P. ramorum* and *P. hibernalis*) also were found to have a small IR (1.1-1.5 kb in size) that encoded a unique ORF. While the IR in *Pythium* appeared to stabilize the genome from rearrangements, the data suggests that the rate of evolutionary divergence was more dependent on the specific gene rather than its location within the IR.

Full-Length cDNA Sequences for Genome Annotation in *Phytophthora infestans*
William Morgan[1], Joe Win[1], Liliana Cano[1], Michael C. Zody[2], Chad Nusbaum[2], Sophien Kamoun[1]. [1]Department of Plant Pathology, The Ohio State University, Ohio Agriculture Research and Development Center, Wooster, OH 44691 USA. [2]The Broad Institute of MIT and Harvard, Cambridge, MA 02142 USA.

Phytophthora infestans is a devastating phytopathogenic oomycete that causes late blight on tomato and potato. Recent genome sequencing of *P. infestans* and other *Phytophthora* species resulted in vast amounts of sequence data providing opportunities to unlock the complex nature of pathogenesis. However, accurate annotation of *Phytophthora* genomes is a significant challenge. Full-length cDNA (FLcDNA) sequences are essential for the correct annotation of genomic sequences and for downstream functional analyses. We initiated three separate approaches to generate novel FLcDNA resources for *P. infestans*. First, bioinformatics analysis of 31592 *P. infestans* 5' ESTs identified 4943 putative FLcDNAs predicted to contain a complete open reading frame. Of these, we sequenced the inserts of 480 clones corresponding to unique sequences. Second, we constructed and sequenced normalized FLcDNA libraries using mRNA isolated from various developmental stages of *P. infestans* strain T30-4 to acquire additional FLcDNA sequences. Finally, we constructed new cDNA libraries that are adapted for high throughput sequencing of 5' mRNA ends using 454 sequencing. The accurate picture of *P. infestans* gene structure that emerges will help us develop more precise gene predictions algorithms. In addition, the FLcDNA sequences will allow us to gauge the degree of sequence conservation between *P. infestans* genes and those of other oomycetes, and identify patterns of gene conservation between *P. infestans* and various eukaryotes.

Abstracts of Poster Presentations (alphabetical by first author)

Characterizing Conserved Effector Proteins from *Hyaloperonospora parasitica*. R. G. Anderson[1], R. H. Y. Jiang[2,3], D. Dou[2], X. Wang[2], B. M. Tyler[2], and J. M. McDowell[1]. [1]Department of Plant Pathology, Physiology and Weed Science, [2]Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061, [3] Laboratory of Phytopathology, Wageningen University, NL-6709 PD Wageningen, The Netherlands and Broad Institute, Cambridge, MA 02141 USA.

Many types of plant pathogens utilize effector proteins that are secreted to the inside of the host cells, where they interact with host targets to promote disease. Oömycete effectors carry a host targeting (HT) sequence that is required for translocation into the host cell. This HT is functionally conserved in the malarial pathogen *Plasmodium*. We are using the interaction between the model plant *Arabidopsis* and its downy mildew pathogen *Hyaloperonospora parasitica (Hpa*) to begin investigating how oömycete effectors manipulate plant cells. We have recently assembled a draft sequence of the *Hpa* genome. Bioinformatic analyses have revealed over 180 candidate effector genes with a secretory leader, RXLR, and dEER motifs. We are focusing on a small subset of these genes that have moderately conserved homologs in the soybean pathogen *Phytophthora sojae*. Conserved secreted effectors between *Hpa* and *Phytophthora* may have important functions in oömycete pathogenicity. We have determined *in planta* expression for each candidate effector during the course of the *Hpa* interaction with *Arabidopsis*. In addition, we determined that several effectors contain a functional nuclear localization sequence, suggesting that these effectors modulate host gene expression. Finally, we have shown that an RXLR motif from an *Hpa* effector can functionally replace the RXLR motif of the *Phytophthora sojae* effector, Avr1b.

The Description and Phylogenetic Placement of Two Putative New Species of *Pythium*. K.D. Broders and A.E. Dorrance. The Department of Plant Pathology, The Ohio State University, Wooster, OH USA.

During a survey of corn and soybean fields in Ohio for seed and seedling pathogens, several species of *Pythium* were recovered and identified, as well as two distinct morphological groups. These two distinct morphological groups were evaluated for their pathogenicity on corn and soybean and sensitivity to fungicides used as seed treatments for corn and soybean. In addition, the sequence of the ITS1, 5.8s, and ITS2 genes of the ribosomal DNA was used to determine the phylogenetic position of these two putative new species within the genus *Pythium*. Both species were found to be pathogenic on both corn and soybean, and were insensitive to both mefenoxam and azoxystrobin *in vitro*. The two new species possess internally proliferating sporangia which produce zoospores in a vesicle placing them in the genus *Pythium* by morphology. Based on the morphological and sequence data these are two new species, grouped with several other species of *Pythium* with proliferating sporangia, which appear to be more closely related to several *Phytophthora* species than to species of *Pythium* which possess globose or filamentous sporangia. The identification and placement of these two new species has added to the knowledge base of this group of species which belong to the genus *Pythium* but are phylogenetically close to *Phytophthora.* Additionally, both of these species appear to be important pathogens of corn and soybean, and may be selected for their insensitivity to seed treatment fungicides.

Virus-Like dsRNAs in *Phytophthora infestans.* Guohong Cai and William E. Fry, Plant Pathology Department, Cornell University, Ithaca NY 14853.

Little is known about virus-like double-strand RNAs (dsRNAs) in *P. infestans*. dsRNAs have been reported, but not sequenced. We searched 22 isolates of *P. infestans* for dsRNAs and found five in four distinct patterns. We report here the characteristics of two dsRNAs that occurred together. RNA1 was 3,160 bp plus a poly $(A)_{36\text{-}60}$ tail and RNA2 was 2,776 bp plus a poly $(A)_{48\text{-}120}$ tail.. A large ORF was found on the positive strand of each RNA. The ORF on RNA1 (ORF1) could encode a polyprotein of 977aa. The only region of ORF1 that shares similarity to sequences in public databases was a region that encodes an RNA-directed RNA polymerase (pfam00680). When ORF1 was used in BLASTP search of GenBank, the best hit was Tobacco Etch Virus, a potyvirus. The ORF on RNA2 (ORF2) could encode a polyprotein of 847 aa. ORF2 lacks significant similarity to known sequences, but profile hidden Markov Model identified a region likely to be a trypsin serine proteinase. RNA1 and RNA2 lack sequence similarity except in the 5' and 3' nontranslated region. Neither dsRNA was detected in BLASTN searches of the *P. infestans, P. ramorum, or P. sojae* genomes.

Classification of resistance genes from wild *Solanum* species based on responses to RXLR effectors of *Phytophthora infestans*. Nicolas Champouret[1], Carolyn Young[2], Minkyoung Lee[2], Sophien Kamoun[2], Evert Jacobsen[1], Richard Visser[1] and Vivianne Vleeshouwers[1]. [1]Department of Plant Science – Laboratory of Plant Breeding, Wageningen, The Netherlands. [2]Ohio State University, OARDC, Department of Plant Pathology, Wooster, OH, USA.

The causal agent of potato late blight *Phytophthora infestans* possesses numerous genes encoding extracellular effector proteins. One class of these secreted proteins contains a host cell targeting (HCT) motif centered on an RXLR core, which is a well-studied motif in effectors of the human malaria parasite *Plasmodium falciparum*. RXLR effectors are thought to be translocated inside the plant cell as they are inside the erythrocyte cell. In the plant cell they can interact with the intracellular NBS-LRR Resistance proteins, the most common class of R proteins effective to oomycetes. In addition to the known isolated R genes from *S. demissum* and *S. bulbocastanum*, other R genes are identified in various other wild *Solanum* species. In this study, we test some of these plants in a transient expression system based on *Agrobacterium tumefaciens* and potato virus X (PVX) expressing cDNAs of RXLR candidate effectors of *P. infestans*. A number of specific responses to various RXLR effectors were identified in these wild and cultivated *Solanum*. Classification of different resistances could be made based on the recognition pattern of the *P. infestans* RXLR effectors. The aim is to comprise a catalogue of RXLR effectors useful for functional profiling of other R-genes in wild *Solanum*.

Ortholog detection and transfer of Gene Ontology annotations in related oomycete genomes. M.C. Chibucos, T. Torto-Alalibo, S. Tripathy, A. Dickerman, and B.M. Tyler. Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24060 USA.

The VBI Microbial Database (http://phytophthora.vbi.vt.edu) houses the complete genome sequences of the plant-pathogenic oomycetes Phytophthora sojae and P. ramorum. At VBI we are manually annotating predicted genes putatively involved in pathogenesis with Gene Ontology (GO) terms. GO annotation facilitates comparative genomic analyses of myriad organisms by providing a controlled vocabulary to describe the biological processes, molecular functions and cellular locations of gene products. Current work involves creating and validating an automated procedure for transferring relevant GO annotations from Phytophthora genes to putative orthologous Hyaloperonospora parasitica genes. Techniques employed for ortholog determination include a combination of phylogenetic tree building using multiple oomycete sequences and reciprocal best BLAST between related organisms. A special emphasis of the annotations includes roughly 500 GO terms generated by the Plant-Associated Microbe Gene Ontology (PAMGO) consortium, a multi-institutional collaborative special interest group of the GO Consortium, which focuses on specific processes involved in the interactions between microbes (prokaryote and eukaryote) and their hosts (plant and animal) in relationships ranging from mutualism to pathogenesis. PAMGO members are annotating the genomes of diverse plant pathogens including the bacteria Agrobacterium tumefaciens, Pseudomonas syringae pathovars, and Erwinia chrysanthemi; the oomycetes Phytophthora sojae and P. ramorum; the fungus Magnaporthe grisea; and the nematode Meloidogyne hapla. By describing similar events across a range of interactions, insights about the mechanisms underlying those interactions may be gained.

Simple sequence repeats for the soybean pathogen, *Phytophthora sojae*. A.E. Dorrance[1], P. Roongsattham[1] and N. Grünwald[2]. [1]Dept. of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691 [2]USDA-ARS, Horticulture Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330-5014 USA.

P. sojae is a diploid oomycete which is homothallic and is limited to primarily, one host, soybean. There are a limited number of population genetic studies with *P. sojae* and none from a narrow geographic region. Recently, 72 different pathotypes were identified in a collection of *P. sojae* isolates from Ohio with as many as 54 pathotypes identified in one field. The overall objective of this study is to determine if these large numbers of pathotypes identified in Ohio are due to mutation, random genetic drift, gene and genotype flow, or through widespread outcrossing. SSRs were identified in the genome, and repeats $\geq$ 15 in size were selected. Primers, 18 to 20 bp in length, were designed flanking SSRs and evaluated on *P. sojae* races 1, 2, 3, 4, 7, and 25. Thirty-six SSR primer pairs are polymorphic on these isolates. However, only Race 1 and 7 could readily be distinguished from the remaining five isolates. Further analysis of isolates from one field will also be presented to examine the diversity that exists within a field and compare this to the diversity among a set of standard isolates.

Expressed Sequence Tags from the legume pathogen *Aphanomyces euteiches* reveal new oomycete putative virulence factors. E. Gaulin, A. Madoui, A. Bottin, C. Jacquet, B. Dumas. UMR CNRS-UPS5546, Toulouse III-24 Chemin de Borde-Rouge, BP42617 Auzeville 31326 Castanet-Tolosan, France. gaulin@scsv.ups-tlse.fr

Aphanomyces sp. (*Saprolegniaceae*) houses plant, animal and pathogens found in both terrestrial and aquatic habitats. *Aphanomyces euteiches* Drechs. causes seedling and root rot diseases on many legumes and is the most pea (*Pisum sativum*) serious disease in several countries. Because the strictly soil borne pathogen may survive for long periods in the soil (more than 10 years), the only existing control is to avoid cultivating peas in infested field for many years. While a huge genomic research effort were devoted to *Phytophthora*, *Aphanomyces* received little attention and the mechanism by which it infects its hosts is largely unknown. *A. euteiches* is an interesting model to study plant-microbe interaction since it display differential specificity of interaction with different lines of the legume model *Medicago truncatula*. To gain a first insight into the transcriptome of *A. euteiches*, a total of 20000 EST were generated. 7 000 consensus sequences were assembled and will be available through a public database. First analyses revealed the presence of putative virulence effectors distinct from *Phytophthora sp*., indicating that *Aphanomyces euteiches* as evolved original mechanisms of pathogenicity.

Evolution of an avirulence homolog (Avh) gene subfamily in *Phytophthora ramorum.* Erica M. Goss, Caroline M. Press, and Niklaus J. Grunwald. Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR USA

Pathogen effectors can serve a virulence function on behalf of the pathogen or trigger a rapid defense response in resistant hosts. Sequencing of the *Phytophthora ramorum* genome and subsequent analysis identified a diverse superfamily of approximately 350 genes that share two protein motifs (RXLR and dEER) with the four known effectors in plant pathogenic oomycetes. These have been termed Avh (avirulence homolog) genes. While as a whole the genes in this superfamily share modest sequence similarity, small groups of closely related genes can be identified. We have investigated the molecular evolution of one such group of seven Avh genes. Microarray data suggests that four of these genes are expressed in isolate Pr-102. We sequenced the full coding region (approximately 400 bp) and flanking noncoding regions of each gene in the three *P. ramorum* lineages. The number of polymorphic sites within *P. ramorum* genes ranges from 1 to 12, suggesting different evolutionary pressures among genes. Analysis indicates that these genes contain both amino acids under purifying selection (e.g. in the signal peptide and RXLR and dEER motifs) and under positive selection. We have also been able to obtain the sequence of homologous Avh genes in the sister taxa *P. hibernalis*and *P. lateralis*, allowing for examination of the evolution of these genes across species.

Orthosearch: Comparative gene prediction in *Phytophthora infestans*. Bob Handsaker[1], Michael C. Zody[1], Chad Nusbaum[1]. [1]The Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA.
The draft genome sequence of the oomycete *Phytophthora infestans* is available and annotation of the genome is underway. In support of this effort, we are developing Orthosearch, a computational gene prediction algorithm. Developed specifically in the context of oomycetes, Orthosearch compares a target genome to the genomes of one or more informant species at intermediate genetic distances from the target genome. To annotate *P. infestans*, we are using the published genomes of *P. sojae* and *P. ramorum* as informants.
The Orthosearch algorithm make gene predictions by searching for structurally valid transcripts that are supported by valid orthologous transcripts in one or more informant genomes and maximizing the conservation of predicted protein products within these constraints. Orthosearch detects orthology based on DNA alignments between the genomes without using a database of known proteins. This makes Orthosearch highly complementary to other gene prediction methods that use either protein databases, statistical models (such as HMMs), or evidence-based prediction (e.g. EST alignments).
In the annotation of the *P. infestans* genome, we are using Orthosearch plus several other gene prediction algorithms to arrive at a set of consensus predictions. Different algorithms have different biases. By combining multiple algorithms with different methodologies, including some developed specifically for use with oomycetes, we hope to arrive at the best possible set of gene predictions.
Orthosearch has performed well in initial trials on the *P. infestans* draft genome assembly. On a set of 536 training models based on EST alignments, Orthosearch made predictions for 503 of the models (93.8%). Within these, Orthosearch correctly predicted 61.6% of the start codons, 81.5% of the stop codons and 86.2% of the splice junctions. Actual start codon accuracy is likely higher, since some of the EST-based training models are not full length. We expect these benchmark results to improve based on refinements to the algorithm and additional curation of the training models.

The effector secretome of *Phytophthora infestans*: Structure and function. Sophien Kamoun[1], Joe Win[1], William Morgan[1], Sang-Keun Oh[1], Carolyn Young[1], Rays Jiang[2, 3], Francine Govers[3], Michael C. Zody[2], Chad Nusbaum[2]. [1]Department of Plant Pathology. The Ohio State University, OARDC, Wooster, OH, USA. [2]The Broad Institute of MIT and Harvard, Cambridge, MA, USA. [3]Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, The Netherlands.

The oomycete *Phytophthora infestans* causes late blight of potato and tomato and is arguably the most destructive pathogen of solanaceous crops. *P. infestans* establishes parasitic colonization of plants by modulating host cell defenses through an array of disease effector proteins. The biology of *P. infestans* effectors is poorly understood, but tremendous progress has been made recently. Two classes of effectors target distinct sites in the host plant: apoplastic effectors are secreted into the plant extracellular space, while cytoplasmic effectors are translocated inside the plant cell, where they target different subcellular compartments. Of particular interest are the RXLR effectors that are characterized by a conserved motif following the signal peptide. The RXLR domain is functionally interchangeable with a malaria host targeting domain and appears to function in delivery into host cells. The recent completion of the genome sequence of *P. infestans* enables genome-wide cataloguing of the effector secretome. Using computational analyses, we identified up to 700 candidate RXLR effector genes. These were frequently organized in clusters of paralogous genes, many of which exhibit hallmarks of positive selection probably as a result of a coevolutionary arms race with host factors. Predictably, effector genes are typically expressed and often up-regulated during infection. We also utilized the discovered RXLR effectors in high-throughput *in planta* expression assays to screen for alteration of plant defense response and gain an insight into their function.

A highly conserved heptameric motif within proteins of the NEP1-like protein superfamily is crucial for triggering cell death in plants. Isabell Küfner[1], Dinah Qutob[2], Mark Gijzen[2], Minna Pirhonen[3], Frédéric Brunner[1] and Thorsten Nürnberger[1]. [1]ZMBP Plant Biochemistry, Auf der Morgenstelle 5, 72076 Tübingen, Germany; [2]Agriculture and Agri-food Canada, London ON N5V 4T3, Canada; [3]Department of Applied Biology, University of Helsinki, PO Box 27, FIN 00014 Finland

NPP1 (Necrosis-inducing Phytophthora protein 1) from the oomycete *Phytophthora parasitica* is a member of the Nep1-like Protein (NLP) superfamily, named after its first identified member from *Fusarium oxysporum*, Nep1 (Necrosis and Ethylene incucing Peptide 1). Proteins of this family have been found in oomycetes, fungi and bacteria, but no homologous proteins are present in animals or plants. Since the expression of NPP1 increases strongly during the transition from the biotrophic to the nectrotrophic phase in the pathogen's life cycle, a function as a toxin-like virulence factor is assumed. However, NPP1 triggers various immune responses in dicotyledonous plants (e. g. the activation of MAP kinases, the production of nitric oxide and ethylene and a hypersensitive-like cell death), which are comparable to those of well-studied pathogen associated molecular patterns (PAMPs). Similar responses in monocotyledonous plants or other organisms could not be observed. Activity of NPP1 was also observed in protoplasts and thus does not require an intact plant cell wall. Furthermore, the protein acts on the extracytoplasmic side of the plasma membrane. Structure analysis of NPP1 didn't reveal the presence of any characterized functional domain. Single amino acid replacement within the heptameric motif "GHRHDWE", highly conserved among members of the NEP1-like protein superfamily, allowed to identify residues essential for the necosis-inducing activity. A conditional lethal screen to identify Arabidopsis mutants insensitive to NPP1 failed to identify putative targets *in planta*. Yet, the molecular mechanism underlying the mode of action of NPP1 remains to be elucidated.

Simple sequence repeat (SSR) markers in *Pythium aphanidermatum*, *Pythium irregulare*, and *Pythium cryptoirregulare*. Seonghee Lee and Gary W. Moorman. Department of Plant Pathology, The Pennsylvania State University, 16803, University Park, USA. Email: sul22@psu.edu

Six microsatellite-enriched genome libraries from three *Pythium* species, *P. aphanidermatum, P. irregulare*, and *P. cryptoirregulare* were constructed to develop simple sequence repeat (SSR) markers. Four synthetic di-, $(AG)_{12}$, $(AC)_{12}$, $(GT)_{12}$, and $(CT)_{12}$, and three trinucleotides repeats, $(GGT)_6$, $(AAG)_8$, and $(AAC)_6$, were used to screen microsatellite loci in the three *Pythium* species. Approximately 600 positive recombinant clones for each *Pythium* species were selected and sequenced. About 35 % in *P. aphanidermatum*, 17 % in *P. irregulare*, and 25 % clone sequences in *P. cryptoirregulare* contained the unique simple sequence repeats (> 4 repeats). One hundred and ten SSR primer pairs for *P. aphanidermatum*, 73 for *P. cryptoirregulare*, and 82 for *P. irregulare* were developed and tested for amplifications and polymorphisms on four isolates of each *Pythium* species. Twenty three polymorphic SSRs in *P. aphanidermatum*, 39 in *P. irregulare*, and 41 in *P. cryptoirregulare* were found. After screening the polymorphic SSR markers to 8 isolates of each species, the most polymorphic and reproducible SSR markers were developed for each *Pythium* species; 9 in *P. aphanidermatum*, 18 in *P. irregulare*, and 23 in *P.cryptoirregulare*. These newly developed SSR markers can be useful for monitoring the movement of isolates and in population genetic studies.

Characterization of a polyamine transporter of *Phytophthora sojae.* G. R. V. Mulangi[1], V. Phuntumart[1], M. C. Chibucos[2] and P. F. Morris[1*] [1]Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403 [2]Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Polyamines, principally putrescine, spermidine and spermine are the most abundant organic cations in all cells, and are essential for cell growth and development. While transport into cells is thought to be an essential component of polyamine homeostasis, only three high affinity polyamine transporters have been identified in eukaryotes. In prior work, we showed that swimming zoospores of the soybean pathogen, *Phytophthora sojae* expressed one or more polyamine transporters. These functional assays showed that zoospores are capable of scavenging both putrescine and spermidine that is released from decaying organic matter and roots. POT1 was identified as a candidate polyamine transporter from the *P. sojae* genome because it is expressed by zoospores and is homologous to recently identified polyamine transporters from the human pathogens, *Leishmania major* and *Trypanosoma cruzi*. RT-PCR of mRNA from swimming zoospores was used to amplify a full length cDNA of this gene. To facilitate the expression of the *P. sojae* gene as a plasma membrane-localized transporter in yeast, POT1 was fused with the C-terminal region of the yeast membrane transporter, GAP1 to create pPOT1-GAP1. Using Gateway enabling technology, the pPOT-GAP1 was transferred to the yeast expression plasmid pDEST 52 to create pEXP-POT1-GAP1. Transformation of this plasmid into yeast mutants that are deficient in polyamine transport, results in enhanced uptake of 14C spermidine. Kinetic characterization of this gene is presently underway.

Characterization of genes expressed during sexual reproduction in *Phytophthora*. Xiaofan Niu, Waraporn Prakob, and Howard S. Judelson, Department of Plant Pathology, University of California, Riverside CA 92521 USA.

Sexual reproduction serves an important role in the life and disease cycles of *Phytophthora* since the sexual spores (oospores) result in new genotypes and are an important means of long-term survival. In order to understand the mechanism of sexual development in the genus, *P. infestans,* the causal agent of potato and tomato late blight, is being used as a model in our study. Microarrays representing much of the P. infestans transcriptome were used to profile gene expression during mating. Approximately 87 genes induced more than ten-fold were identified using these arrays and validated by semi-quantitative RT-PCR. A majority of these genes are induced exclusively during mating, but some are also up-regulated during asexual spore development. Most are expressed during oosporogenesis in the homothallic species P. phaseoli. To understand the temporal and spatial patterns of expression of the genes, the activity of ten genes transcribed only during mating are being tested in P. infestans transformants by fusing their promoters with the GUS reporter gene. Deletions, site-directed mutagenesis, and electrophoresis mobility gel shift assays will be used to define the cis-elements regulating the genes. In addition, the function of selected mating-induced proteins are being tested, such as a RNA-binding protein belonging to the Puf family. Affinity strategies are identifying mRNAs bound and presumably regulated by Puf.

Structural and functional analysis of the glycosyl hydrolase family 12 genes from *Phytophthora sojae* and *P. ramorum.* M.D. Ospina-Giraldo (4), S. Costanzo (3), R. W. Hammond (2), K. L. Deahl (1), R. W. Jones (1) (1) USDA/ARS, Vegetable Laboratory, Beltsville, MD, USA; (2) USDA/ ARS/PSI/MPPL, Beltsville, MD, USA; (3) University of Maryland, Dept. NRSL, College Park, MD, USA; (4) Biology Department, Lafayette College, Easton, PA 18042 USA.

A total of eighteen paralogs of xyloglucan-specific endoglucanases (EGLs) from the glycosyl hydrolase family 12 in *Phytophthora sojae* and *P. ramorum* were identified and characterized. The overall gene copy number and relative organization appeared well conserved between *P. sojae* and *P. ramorum*, with apparent synteny in this region of their respective genomes. These genes encode predicted extracellular enzymes, with sizes ranging from 189 to 435 amino acid residues. RT-PCR experiments with gene specific-primers using total RNA extracted from two-week-old mycelium revealed functional expression of all eighteen copies of EGL genes. To assess a potential role for this cell wall modifying enzyme during pathogenesis, a selected gene (sEGL12-B) was introduced into a *Potato virus* X (PVX) vector, and *Nicotiana benthamiana* plants were inoculated with the modified vector. EGL expression (verified by Western blot analysis) caused expanding circular lesions at the site of inoculation. Additional lesions formed on non-inoculated leaves and on stems after systemic movement of PVX. This study demonstrates the destructive nature of a single type of endoglucanase, and suggests a role for the *P. sojae* EGL 12 protein in facilitating the colonization of host tissues during pathogenesis.

Survey and analysis of microsatellites from transcript sequences in Phytophthora species: frequency, distribution, and potential as markers for the genus. Diana P Garnica, Andrés M Pinzón, Lina M Quesada-Ocampo, Adriana J Bernal, Emiliano Barreto, Niklaus J Grünwald, and Silvia Restrepo. Laboratorio de Micología y Fitopatología Uniandes (LAMFU). Universidad de Los Andes. Bogotá, Colombia.

Background: Members of the genus Phytophthora are notorious pathogens with world-wide distribution. The most devastating species include *P. infestans, P. ramorum* and *P. sojae.* In order to develop molecular methods for routinely characterizing their populations and to gain a better insight into the organization and evolution of their genomes, we used an in silico approach to survey and compare simple sequence repeats (SSRs) in transcript sequences from these three species. We compared the occurrence, relative abundance, relative density and cross-species transferability of the SSRs in these oomycetes. *Results:* The number of SSRs in oomycetes transcribed sequences is low and long SSRs are rare. The in silico transferability of SSRs among the Phytophthora species was analyzed for all sets generated, and primers were selected on the basis of similarity as possible candidates for transferability to other Phytophthora species. Sequences encoding putative pathogenicity factors from all three Phytophthora species were also surveyed for presence of SSRs. However, no correlation between gene function and SSR abundance was observed. The SSR survey results, and the primer pairs designed for all SSRs from the three species, were deposited in a public database. *Conclusion:* In all cases the most common SSRs were trinucleotide repeat units with low repeat numbers. A proportion (7.5%) of primers could be transferred with 90% similarity between at least two species of Phytophthora. This information represents a valuable source of molecular markers for use in population genetics, genetic mapping and strain fingerprinting studies of oomycetes, and illustrates how genomic databases can be exploited to generate data-mining filters for SSRs before experimental validation.

Global Gene Expression Profiles of *Phytophthora ramorum* strain Pr102 in response to plant host and tissue differentiation. Caroline M. Press and Niklaus J. Grunwald. Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR 97330 USA

The release of the draft genome sequence of *P. ramorum* strain Pr102, enabled the construction of an oligonucleotide microarray of the entire genome of Pr102. The array contains 344,680 features (oligos) that represent the transcriptome of Pr102. *P. ramorum* RNA was extracted from mycelium and sporangia and used to compare gene expression across tissue types and in the presence of the host (*Rhododendron sp.*). The purpose of the experiment was to identify genes whose expression was responsive to tissue types and upon exposure to the host plant for further study. Gene expression studies were performed using a Nimblegen microarray. Genes were determined to be differentially expressed between tissue types if they were statistically significant ($P = 0.05$ after false discovery rate correction) and resulted in a greater than 20-fold change in gene expression selecting only those genes with the greatest response to tissue changes or host influence. In the comparison between mycelia and sporangial tissues, 263 genes demonstrated a greater than 20 fold change in gene expression. Of those genes, 52 genes were significantly downregulated in sporangia as compared to mycelium and 214 genes were significantly upregulated in sporangia as compared to mycelium. Several of the differentially expressed genes appear to be of the same type as those in a similar study in *Phytophthora infestans* by Kim and Judelson (2003) and include genes involved in cell wall restructuring, cell division and signaling functions. Not surprisingly, several genes involved in energy production, electron transport chains and growth are reduced in sporangia. 50% of the genes with differential expression have not yet been classified as to function in the current annotation. Further experimentation and analysis is ongoing.

Molecular studies of the *Saprolegnia*-fish interaction. Andrew J. Phillips[a,b], Emma J. Robertson[a], Vicky L. Anderson[a,b], Chris J. Secombes[b], and Pieter van West[a]. [a]The Aberdeen Oomycete Group, College of Life Sciences and Medicine, University of Aberdeen, Foresterhill, Aberdeen, Scotland, UK. E-mail: p.vanwest@abdn.ac.uk; [b]Scottish Fish Immunology Research Centre, Aberdeen, Scotland,UK.

Oomycetes of the genus *Saprolegnia* are responsible for devastating infections of fish. The disease (Saprolegniosis) is characterized by visible white or grey patches of filamentous mycelium on the body or fins of freshwater fish and is of particular problem to aqua-cultural businesses. We are investigating the molecular mechanisms which enable *Saprolegnia* to successfully infect fish, the molecular processes that suppress host defenses during infection, and the nature of the pathogen/host interaction. To enable us to study the fish-pathogen interaction we have developed an *in-vitro* infection model. In this model system a cultured-monolayer of a primary fish cell-line (RTG-2) is infected with cysts of *S. parasitica*. This model has enabled us to harvested material from several stages of the interaction between fish and *Saprolegnia*, allowing us to investigate the kinetics of the infection using a range of molecular, microscopic and biochemical techniques. We are particularly interested in the early time-points of the interaction, and are studying the mechanisms which allow *Saprolegnia* to establish an infection, and the defensive mechanisms employed by the host. We are currently addressing the latter by conducting microarray studies to detect changes in the host-transcriptome in response to infection by *S. parasitica*. Our latest findings will be presented.

Survey and Analysis of Microsatellites in Oomycete and Fungal Genomes. L.M. Quesada-Ocampo[1,3], D.R. Matute[2], N. Castro[3] and S. Restrepo[3]. [1]Department of Plant Pathology, Michigan State University, East Lansing, MI 48824; [2]Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637; and [3]Laboratorio de Micología y Fitopatología Uniandes (LAMFU). Universidad de Los Andes. Bogotá, Colombia. Email: srestrep@uniandes.edu.co

Microsatellites or simple sequence repeats (SSR) are short tandem repeated sequence motifs consisting of one to eight nucleotides. SSRs have attracted considerable attention as molecular markers due to their high polymorphism and abundance on both prokaryotic and eukaryotic genomes. In this study we used an *in silico* approach to examine and compare SSRs in completely sequenced fungal and oomycete genomes and sequencing projects in progress. We analyzed and compared the occurrence, relative abundance, most common, and longest SSRs in 38 taxonomically different fungal and oomycete species. These analyses revealed that, in all of the genomes studied, the occurrence and abundance of SSRs varied and was not influenced by the genome sizes. In most genomes, mononucleotide, dinucleotide, and trinucleotide repeats were more abundant than the longer repeated SSRs. Generally, for each organism, the occurrence and relative abundance of SSRs decreased as the repeat unit increased. Furthermore, each organism had its own common and longest SSRs. Statistical analysis were completed to determine if there were differences in SSR relative abundance between taxonomic groups, plant pathogens vs. human pathogens, parasite vs. free living organisms and haploid vs. diploid organisms. Our analysis showed that longer SSRs in fungi and oomycetes are rare. In addition to providing new information concerning the abundance of SSRs for each of these fungi, the results provide a general source of molecular markers that could be useful for a variety of applications such as population genetics, genetic mapping and strain fingerprinting of fungal and oomycete organisms.

The effectors Avr2 and EPIC2B secreted by two unrelated pathogens target the tomato defense protease Rcr3. Jing Song, Joe Win, Miaoying Tian, Hsin-Yen Liu and Sophien Kamoun. Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH 44691 USA. song.189@osu.edu

Current models of plant-pathogen interactions stipulate that pathogens secrete effector proteins that disable plant defense components known as virulence targets. Occasionally, the perturbations caused by these effectors trigger innate immunity via plant disease resistance proteins as described by the “guard model”. This model is nicely illustrated by the interaction between the fungus *Cladosporium fulvum* and tomato. *C. fulvum* secretes a protease inhibitor Avr2 that targets the tomato cysteine protease Rcr3. In plants that carry the resistance protein Cf2, Rcr3 is required for resistance to *C. fulvum* strains expressing Avr2, thus fulfilling one of the predictions of the guard model. The model has two other predictions that have not been tested. First, Rcr3 could be disabled by different pathogens. Second, Rcr3 may directly contribute to basal defense. In this study we tested these two predictions using a different pathogen of tomato, the oomycete *Phytophthora infestans*. This pathogen secretes an array of protease inhibitors, one of which, EPIC2B, was shown to inhibit tomato cysteine proteases. Here, we showed that, similar to Avr2, EPIC2B binds and inhibits Rcr3 using co-immunoprecipitation and activity profiling assays. We also found that the *rcr3-3* mutant of tomato that carries a premature stop codon in *Rcr3* exhibits enhanced susceptibility to *P. infestans* suggesting a role for Rcr3 in basal defense. In conclusion, our findings fulfill the predictions of the guard model and suggest that the effectors Avr2 and EPIC2B secreted by two unrelated pathogens of tomato target the same defense protease Rcr3.

Cross-species proteomics reveals processes in early infection of *Phytophthora*. A. Savidor[1,2], R. Donahoo[1], O. Hurtado-Gonzales[1], W. H. McDonald[2] and K. Lamour[1]. [1]Department of Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN 37996. [2]Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831 USA.

Phytophthora ramorum and *Phytophthora sojae* are destructive plant pathogens. *P. sojae* has a narrow host range, infecting only soybean, while *P. ramorum* has a wide host range, capable of infecting many different trees and shrubs. A proteomic comparison of the mycelium and germinating cyst life stages of *P. sojae* and *P. ramorum* was carried out in order to identify candidate proteins for involvement in host range capabilities of these pathogens, as well as candidates for involvement in early infection (germination of the cyst) and vegetative growth (growth of the mycelium). 82 candidates for early infection were identified, 35 candidates for vegetative growth were identified, and hundreds of proteins were suggested as candidates for host range capability. In addition, proteomic trends in cellular processes between life stages were observed. In the mycelium, proteins involved in transport and metabolism amino acids, carbohydrates and other small molecules were up-regulated. In the germinating cyst, up-regulated proteins were involved in lipid transport and metabolism, cystoskeleton and protein synthesis. Based on the results, a model is proposed where the germinating cyst catabolizes lipid reserves through the □-oxidation pathway as an energy source to drive extensive protein synthesis necessary for generation of the germ tube and initiation of infection. Once inside the host, the pathogen switches to vegetative growth, where energy is derived from glycolysis and utilized for synthesis of amino acids and proteins that assist survival in the plant tissue.

Plant-Associated Microbe Gene Ontology (PAMGO): A community resource of gene ontology terms describing gene products involved in microbe-host interactions. Trudy Torto-Alalibo[1], Bryan Biehl[2], David Bird[2], Marcus Chibucos[1], Alan Collmer[3], Candace Collmer[3,4], Ralph Dean[2], Michelle Gwinn Giglio[5], Jeremy D. Glasner[6], Amelia[7] Ireland, Magdalen Lindeberg[3], Jane Lomax[7], Thomas K. Mitchell[2], Nicole Perna[6], Joao Setubal[1], Brett M. Tyler[1] and Owen White[5]. [1]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, USA; [2]North Carolina State University, Raleigh, NC, USA; [3]Cornell University, Ithaca, NY, USA; [4]Wells College, Aurora, NY, USA; [5]The Institute for Genome Research, Rockville, MD, USA; [6]University of Wisconsin, Madison, WI, USA; [7]European Bioinformatics Institute, Hinxton, UK.

The PAMGO interest group was formed to develop new gene ontology (GO) terms describing various processes, functions and cellular components related to microbe-host interactions, primarily terms describing the microbial side of the interaction. Plant-associated microbes have evolved similar mechanisms to evade, neutralize or suppress defense systems of their plant hosts and obtain nutrients. For these functional similarities to be maximally useful for comparative genomics, it is important that a controlled vocabulary is set in place to describe them. In a multi-institutional collaborative effort, we are currently working on developing new GO terms and relationships for gene products implicated in plant interactions in four major groups of microbial pathogens: bacteria, fungi, oomycetes and nematodes. Our goal is to develop simple, yet robust and extensible controlled vocabularies that accurately reflect the biology of the microbes during interaction with plant hosts in relationships ranging from mutualism to parasitism. Many of the terms should be useful for animal-associated microbes also. Most of the terms currently developed (113) are housed under the “interaction between organisms” (IBO) node. A recent ontology development meeting resulted in the generation of 190 more terms, which are currently being processed for integration into the GO. Annotations are being done concurrently with ontology development. In the future, we hope to develop and evaluate an automated system to transfer PAMGO terms from the current reference species to genomes of related host-associated microbes. Researchers willing to contribute to the ontology development and other discussions can subscribe to the PAMGO mailing list at (http://pamgo.vbi.vt.edu/). This project is supported by grants from the CSREES/NRI # 2005-35600-16370 and the NSF # EF-0523736.

PhESTDB: An Integrated resource for Phytophthora and Soybean EST sequences.
Sucheta Tripathy and Brett M. Tyler. Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA

PhESTDB is an unique integrated EST data resource, containing 33,350 *P.sojae* ESTs, 99,319 *P.infestans* ESTs and 37,465 public Soybean unigenes. This database is the most complete resource for *P.sojae* EST sequences to date. The *P.sojae* sequences were quality trimmed followed by vector/ adaptor, poly A/T trimming. A total of 3769 Soybean EST sequences were separated from the *P.sojae* ESTs from an infection library using in silico methods. Subsequently, the sequences were clustered and assembled using a wrapper perl script TGICL.The clustering and assembly produced 7863 unigenes of *P.sojae*, 2292 soybean unigenes from the infection library, and 14754 unigenes of *P.infestans*. The unigenes were primarily annotated using NCBI- BLASTX against NR database accelerated with a Timelogic board.The unigenes were then passed through a modified algorithm of loglikelihood(McLachlan,1984) to separate the UTRs from the ORFs. The protein sequences were annotated for domains, motifs, profiles and fingerprints using InterproScan. Sequences were submitted to signalp and TMHMM servers to get secretory and trans-membrane domains. The resulting outputs were parsed and stored in the database. The database was designed in mysql and the interfacing was done with PHP and perl CGI. We have created an easy-to-use Perl GD-based browser which is distinct from Gbrowse. In the browser the genome to unigene alignments are shown in greater detail which also indicates any overlaps between the unigenes. Each EST unigene is made clickable to view detailed information about assembly and their primary annotation including various other related information such as NCBI links and links to gene models. Through the gene models this database is linked to the VBI microbial database. This database has a very powerful query page, that can retrieve any kind of information the user may need. Data can also be downloaded from the database in text format. The database is publicly available at http://phytophthora.vbi.vt.edu/EST.

Identifying cis- and trans-factors involved in spore-specific gene expression in the oomycete *Phytophthora infestans.* Qijun Xiang, Audrey Ah Fong, Kyoung Su Kim, Howard S. Judelson, Department of Plant Pathology, University of California, Riverside, CA 92521 USA

Using microarray analysis, we have identified a large number of genes induced during sporulation and zoospore formation in *Phytophthora infestans,* the cause of the potato late blight disease. The promoters of several of these genes are being analyzed to define the sequences that confer sporulation or zoospore-specific patterns of gene expression, using a GUS reporter system to test deleted or mutagenized versions of the promoters in *P. infestans* transformants. This approach has been used to identify a 7-nt "cold box" in the promoters of several genes induced during zoospore formation, and to define the binding sites of development-associated transcription factors in the promoters of other genes such as those encoding a sporulation-specific serine-threonine kinase and a protein phosphatase. DNA fragments from the promoters are being used to purify the relevant transcription factors using DNA affinity approaches. EMSA (electrophoretic mobility shift assay) is being used to examine the DNA binding activities of the proteins.