OOMYCETE MOLECULAR GENETICS NETWORK MEETING

PRESENTATION ABSTRACTS

13-15 March 2011

Pacific Grove, CA

PREFACE

Welcome to the 2011 Oomycete Molecular Genetics Meeting! We are very delighted to have an excellent group of investigators sharing their newest discoveries on oomycete biology. Oomycetes are a diverse phylum of fungus-like microorganisms that include many of the most destructive plant and animal pathogens, as well as several useful model systems. With the rapidly accelerating contributions from genomics, a variety of molecules such as PAMPs and hundreds of putative effectors, all potentially interacting with components of host immune systems, have been identified. The oomycete molecular genetics community now numbers well over one hundred labs, and papers on oomycetes are appearing regularly in the top research journals. The oomycete molecular genetics conference alternates between USA and Eurasia, and returns this year to Asilomar, California. We welcome you to discuss the latest exciting developments in oomycete molecular genetics and genomics, and also to discover some of the many still obscure aspects of their cell biology, evolution and ecological impact.

We thank the Research Coordination Networks in Biology program of the US National Science Foundation for continued support of this conference and of the community.

Brett Tyler

Meeting organizers

Paul F. Morris *Manuel D. Ospina-Giraldo* *Vipaporn Phuntumart*

A note on the abstract book: Presentation abstracts have been organized in alphabetical order by presenter's last name (who may or may not be the first author).

OMGN 2011 Program (Fred Farr Room)

Sunday, March 13, 2011

8:30-9:50	Registration
10:00-10:10	Welcome by Brett Tyler
10:10-12:10	**Effectors I**
	Chair: John McDowell
10:10-10:30	*Brett Tyler*
	Computational and experimental identification of elite effectors in the genome of *Phytophthora sojae*
10:30-10:50	*Stephan Wawra*
	The oomycete RXLR-effectors AVR3a and SpHtp1 show cell type specific import and their RXLR-leaders mediate dimerisation
10:50-11:10	*Mark Banfield*
	Structural biology of *Phytophthora* effectors
11:10-11:30	*Mark Gijzen*
	The *Phytophthora sojae* avirulence genes *Avr5* and *Avr3* are alleles
11:30-11:50	*Eleanor Gilroy*
	Presence/absence polymorphism and differential expression of two diverged forms of *PiAVR2* in *Phytophthora infestans* determine virulence of *R2* plants
11:50-12:10	*Shiv Kale*
	Characterization of oomycete effector entry in plant and animal cells
12:10-2:00	**Lunch**
2:00-3:00	**Keynote address: Gregory Martin**
	Suppression and elicitation of plant immunity by two *Pseudomonas* type III effectors
3:00-3:20	**Break**
3:20-4:20	**Effectors II**
	Chair: Jaime Blair
3:20-3:40	*Rays Jiang*
	Ancient and recently-acquired molecular weaponry in animal pathogenic oomycetes
3:40-4:00	*Isabell Küfner*
	Functional characterization of cytolytic and non-cytolytic members within the NLP effector superfamily
4:00-4:20	*Jens Steinbrenner*
	Elucidating the role of *Hyaloperonospora arabidopsidis* RXLR effector proteins on the suppression of the plant immune system
7:30- 10:00	**Reception and Poster Session I**

Monday, March 14, 2011

9:00-10:20	**Microbe Interaction**
	Chair: Rays Jiang
9:00-9:20	*Bernard Dumas*
	Perception and role of *Aphanomyces euteiches* effectors on *Medicago truncatula* roots: Is there molecular crosstalk between symbiotic and pathogenic signals?
9:20-9:40	*Susan Breen*
	Host target of Avr2, an RXLR effector from *Phytophthora infestans*
9:40-10:00	*Sandra Goritsching*
	An *Arabidopsis thaliana* CC-NBS-LRR resistance gene recognizes a novel conserved effector from *Hyaloperonospora arabidopsidis*
10:00-10:20	*Mathieu Larroque*
	Natural variations in the perception of the oomycetal effector CBEL in Arabidopsis
10:20-10:40	**Break**
10:40-12:00	**Genomics**
	Chair: Manuel D. Ospina-Giraldo
10:40-11:00	*Frank Martin*
	Mitochondrial haplotype analysis for differentiation of isolates of *Phytophthora cinnamomi*
11:00-11:20	*Liliana Cano*
	Genome analysis of an aggressive clonal lineage blue 13 of *Phytophthora infestans* reveals significant genetic and expression polymorphisms in effector genes
11:20-11:40	*Laura Greenville-Briggs*
	A molecular insight into algal-oomycete warfare: cDNA analysis of *Ectocarpus siliculosus* infected with the basal oomycete *Eurychasma dicksonii*
11:40-12:00	*Daniel Gobena*
	Finding the best set of needles in the *Phytophthora capsici* SNP haystack
12:00-1:00	**Lunch**
1:00-2:00	**Keynote address: Bihua Cai. Title: TBA**
2:00-3:40	**Evolution and Population Genetics**
	Chair: Harold Meijer
2:00-2:20	*Ian Misner*
	Understanding oomycete gene evolution using evolutionary gene networks
2:20-2:40	*Michael Seidl*
	Genome reconstruction of oomycete pathogens
2:40-3:00	*Guillaume Bilodeau*
	Multiplex assay for genus and species-specific detection of *Phytophthora* based on differences in mitochondrial gene order

3:00-3:20	**Break**
3:20-3:40	*Jean Ristaino* Deployment of rapid diagnostic tools for *Phytophthora* on horticultural crops in Central America
3:40-4:00	*Theo van der Lee* Meet the Dutch; population genetics of *Phytophthora infestans* in the Netherlands in the last decade
6:00-7:30	**Banquet**
8:00- 10:00	**Reception and Poster Session II**

Tuesday, March 15, 2011

9:00-11:40	**Oomycete Biology** ***Chair: Bernard Dumas***
9:00-9:20	*Howard Judelson* The composition and development of flagellated oomycete spores revealed through cell biology and comparative genomics approaches
9:20-9:40	*Manuel Garavito* Biochemical characterization of recombinant dihydroorotate dehydrogenase from the plant pathogenic oomycete *Phytophthora infestans*
9:40-10:00	*Harold Meijer* The aspartic proteinase family of three *Phytophthora* Species
10:00-10:20	*Takao Kasuga* Host induced epigenetic alteration in *Phytophthora ramorum*
10:20-10:40	**Break**
10:40-11:00	*Vipaporn Phuntumart* Transcriptional gene regulation in the oomycetes
11:00-11:20	*James Wong* MicroRNA regulation in soybean during *Phytophthora sojae* infection
11:20-11:40	*Qijun Xiang* Regulation of primary and secondary sporulation by Myb transcription factors in the oomycete *Phytophthora infestans*
11:40-12:00	Closing Remarks

ORAL PRESENTATIONS

Structural biology of *Phytophthora* effectors

Laurence S Boutemy, Stuart RF King, Richard K Hughes, Joe Win, Sophien Kamoun & Mark J Banfield

John Innes Centre & The Sainsbury Laboratory, Norwich, UK

Oomycete effectors of the RxLR class share little sequence homology with other proteins, making functional annotation of virulence activities and an in-depth understanding of effector triggered immunity a significant challenge. Structural biology offers an opportunity to determine whether evolutionary relationships that may define function in the host cell are recognizable in three dimensional structure when they are not in sequence. We established a pipeline of bioinformatics, heterologous expression, crystallization and structure determination of RxLR effectors from plant pathogenic *Phytophthora*. In this talk I will outline our approach to designing constructs suitable for expression of RxLR effectors for structure determination, give an overview of our *E. coli* expression studies and present the structures we have determined. Finally, I will discuss how we are using these structures to enlighten RxLR effector biology.

Multiplex assay for genus and species-specific detection of *Phytophthora* based on differences in mitochondrial gene order

Guillaume J. Bilodeau[1], Frank N. Martin[1], Michael D. Coffey[2] and Cheryl Blomquist[3]

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More than one hundred *Phytophthora* species are described and cause disease in agriculture and forest ecosystems worldwide. Having rapid diagnostic tools are essential to be able to detect, manage and control spread of these pathogens. However, markers usually identify at a specific species level and don't tell about other *Phytophthora* species that may be present. A range of markers have been developed for this genus, usually based on polymorphisms at primer annealing sites that rely on accurate control of annealing temperature for specificity. A different approach for enhanced specificity is to design markers based on differences in the location of annealing sites. We have looked at gene order differences in the mitochondrial genome of *Phytophthora* compared to *Pythium* and plants for developing a single amplification assay for genus as well as species specific detection (TaqMan probes for genus and species-specific ID in the same reaction). On the three conserved gene order differences identified, two of these should allow for design for genus specific detection and species-specific probes for more than 65 species. The amplification primers and genus specific probe were effective when evaluated against a wide range of isolates representing most all *Phytophthora* spp. (>94) known as well as a number of *Pythium* spp. and plants. Multiplex amplifications with species-specific probe combinations were evaluated for 14 different species-specific probes including *P. ramorum, P. kernoviae, P. fragariae, P.rubi, P. cactorum.* The different markers systems developed for genus and specific-species probes multiplexed with a plant probe were validated with field samples, including samples from the *P. ramorum* national survey.

Host target of Avr2, an RXLR effector from *Phytophthora infestans*

Susan Breen[1,2], Eleanor M. Gilroy[1], Diane Saunders[3], Miles R. Armstrong[2], Juan G. Morales[1], Ingo Hein[1], Petra C. Boevink[1], Eva Randall[1], Anna O. Avrova[1], Leighton Pritchard[1], Stephen C. Whisson[1], Sophien Kamoun[3], Frederic Brunner[4] & Paul R. J. Birch[1,2]

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3. The Sainsbury Laboratory, John Innes Centre, Norwich, NR4 7UH
4. ZMBP Eberhard-Karls-Universität, Tübingen D-72076, Germany

An important research goal in the fight against potato late blight, caused by the Oomycete *Phytophthora infestans,* is to identify and characterise pathogen effector proteins likely to be delivered into host cells where they manipulate host metabolism and defence responses. Avr3a, the first effector characterized from *P. infestans*, was found to contain an N-terminal RxLR and dEER motif required for transport across the host plasma membrane, where it interacts with and modifies the activity of a host defence protein, CMPG1. We have identified the RxLR-dEER effector gene *Avr2* from the sequenced isolate t30-4, which is recognised by *R2-like* genes. Cloning *Avr2* from virulent isolates collected around the world has revealed an additional, variant form that evades recognition by *R2-like* genes. *Avr2* alleles were used to screen for an interacting plant host protein using the Yeast 2-Hybrid system. The Y2H library was generated from pathogen challenged resistant and susceptible potato cultivars. The interacting protein identified is called BSU1-like 3 (BSL3) which is a Ser/Thr Phosphatase from the brassinosteriod hormone signal transduction pathway. This gene is part of a family of 4 from *Arabidopsis* which are known to cause the de-phosporylation of transcription factors key to this pathway. In addition to BSL3, we have also observed that AVR2 interacts with another family member, BSL1. A range of techniques are being used to further examine the interactions between AVR2 and the host proteins BSL1 and BSL3 and their implications to both infection and disease resistance.

Genome analysis of an aggressive clonal lineage blue 13 of *Phytophthora infestans* reveals significant genetic and expression polymorphisms in effector genes

Liliana M. Cano[1], Sylvain Raffaele[1], Ricardo Oliva[1], David Cooke[2], Paul Birch[2] and Sophien Kamoun[1]

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[2] *SCRI, Invergowrie, Dundee. DD25DA, Scotland, UK*

In 2005, a clonal lineage of the potato late blight pathogen *Phytophthora infestans,* genotype blue 13, was identified in the UK and now this genotype has become the most prevalent in the country. *P. infestans* blue 13 strains are characterized by an increased aggressiveness and virulence on several resistant potato varieties. Genome analysis of *P. infestans* blue 13 06_3928 strain revealed regions containing effectors with copy number variation (CNV). In addition, using whole-genome microarray analysis we detected that majority of induced genes in 06_3928 show an unusual sustained induction pattern during the biotrophic phase of infection on potato. Our findings suggest that *P. infestans* blue 13 exhibit both genetic and expression polymorphisms. A better understanding of these variations will help to gain insights into the evolution of virulence of this genotype and will also provide clues for the management of this epidemic disease.

Perception and role of *Aphanomyces euteiches* effectors on *Medicago truncatula* roots: Is there molecular crosstalk between symbiotic and pathogenic signals?

Bernard Dumas, Amaury Nars, Diana Ramirez, Thomas Rey, Claude Lafitte, Arnaud Bottin, Elodie Gaulin, Christophe Jacquet

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Plant cells are able to perceive molecular signals produced by symbiotic and pathogenic microorganisms and to develop an appropriate response to these signals. In legumes, symbiotic bacterial signals, such as the lipochitooligo saccharides Nod factors, produced by produced by soil bacteria called rhizobia, are essential for the establishment of root symbiosis. Recently, LysM receptors proteins (NFP and LYK3 in the legume model *Medicago truncatula*) have been proposed to be involved in Nod factor perception. Intriguingly, related fungal signals, chitosaccharides, also interact with LysM receptors, raising the question of how roots can distinguish between symbiotic and pathogenic molecules. Additionally, pathogenic microorganisms produced apoplastic and cytoplasmic effectors which interfere with responses developed upon perception of microbial elicitors, but activity of these effectors on the symbiotic signalling pathway is not known. To address these issues, we have focussed our work on the interaction between *M. truncatula* and the oomycete root pathogen *Aphanomyces euteiches*. Biochemical analyses of *A. euteiches* cell wall revealed the presence of high levels of chitin-like materials which have been shown to elicit defence responses on *M. truncatula* roots. Work is now on progress to identify LysM receptors involved in the perception of these signals. Interestingly, a LysM mutant (*nfp*) has been shown to be more susceptible to *A. euteiches* than the wild type plant suggesting that this receptor is not only involved in the perception of symbiotic signals but also in pathogenic interactions. Mining of an *A. euteiches* ESTs database revealed the presence of putative translocated effectors belonging to the Crinkle family but not to the RXLR family. Transgenic *M. truncatula* roots expressing these effectors are being produced and effects towards pathogenic and symbiotic signals will be evaluated. Together, our results show that i) pathogenic and symbiotic microorganisms produced related signalling molecules and ii) signalling pathways involved in response to these signals are closely intertwined.

Biochemical characterization of recombinant Dihydroorotate dehydrogenase from the plant pathogenic Oomycete *Phytophthora infestans*

Garavito, M.F. [1,2], **García Bayona, L.**[1,2], **Vasquez J.J.**[1,2], **Lozano G. L, Bernal, A.** [1], **Loffler, M.**[3], **Zimmermann, B.H.** [2], **Restrepo, S**[2].

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Phytophthora infestans the causal agent of late blight, causes tremendous losses in the highlands dedicated to potato production. Despite its economic importance, current control strategies with antifungal agents are increasingly ineffective and new ones are urgently needed. We propose that as with the human parasite *Plasmodium falciparum*, a possible a new control strategy based on the inhibition of the pyrimidine metabolism might work as well in *P. infestans*. In this study we investigated the pathogen's pyrimidine enzymes using relative expression profiles evaluated by qRT-PCR during its infection of *Solanum phureja*. Our preliminary results suggest the importance of *de novo* pyrimidine synthesis during the fast replicative early infection stages. In order to test the pirimidine enzymes as potential control target, a truncated dihydroorotase dehydrogenase DHODase of *P. infestans* was recombinantly overexpresed in *E. coli*, purified, and characterized for kinetics and substrate specificity. A DHODase inhibitor screening with 26 selected compounds was performed. Four of the compounds tested Redoxal, DCL, A77-1726 and 5,8 Dihidroxy 1-4 Naphtoquinone, which are potent inhibitors of animal DHODases, markedly decreased the *P. infestans* DHODase activity (IC_{50}= 126 uM). This study provides a good background for the development of potent *P. infestans* selective pyrimidine inhibitory compounds that could be helpful controlling Oomycete pathogens.

The *Phytophthora sojae* Avirulence Genes *Avr5* and *Avr3a* are Alleles

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The perception of *Phytophthora sojae* avirulence (*Avr*) gene products by corresponding soybean resistance (*Rps*) gene products causes effector triggered immunity. Past studies have shown that the *Avr5* and *Avr3a* genes of *P.sojae* are genetically linked, and the *Avr3a* gene encoding a secreted RxLR effector protein was recently identified. We now provide evidence that *Avr5* and *Avr3a* are allelic. Genetic mapping data from F_2 progeny indicates that *Avr5* and *Avr3a* co-segregate, and haplotype analysis of *P. sojae* strain collections reveal sequence and transcriptional polymorphisms that are consistent with a single genetic locus encoding *Avr3a/5*. Transformation of *P. sojae* and transient expression in soybean were performed to test how *Avr3a/5* alleles interact with soybean *Rps3a* and *Rps5*. Over-expression of *Avr3a/5* in *P. sojae* results in avirulence in a strain that is normally virulent on *Rps3a* and *Rps5*; whereas silencing of *Avr3a/5* causes gain of virulence in a *P. sojae* strain that is normally avirulent on *Rps5* and *Rps3a* soybean lines. Transient expression and co-bombardment with a reporter gene confirms that *Avr3a/5* triggers cell death in *Rps5* soybean leaves in an appropriate allele specific manner. Sequence analysis of the *Avr3a/5* locus points to crucial residues in the effector domain that are under positive selection pressure and that distinguish recognition by *Rps3a* and *Rps5*.

Presence/absence polymorphism and differential expression of two diverged forms of *PiAVR2* in *Phytophthora infestans* determine virulence on *R2* plants

Eleanor M Gilroy[1], Susan Breen[1,2], Stephen C Whisson[1], Julie Squires[1], Ingo Hein[3], Anoma Lokossou[4], Petra C Boevink[1], Juan Morales[1,7], Anna O Avrova[1], Leighton Pritchard[1], Dionne Turnbull[1], Maciej Kaczmarek[1], Liliana Cano[5], Eva Randall[1], Francine Govers[4], Pieter van West[6], Sophien Kamoun[5], Vivianne Vleeshouwers[4], David EL Cooke[1], Paul R J Birch[1,2]

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A detailed molecular understanding of how oomycete plant pathogens evade disease resistance is essential to inform the deployment of resistance (*R*) genes that will be durable. Map-based cloning, transient expression *in planta*, pathogen transformation and DNA sequence diversity across diverse isolates were used to identify and characterize *PiAVR2* from the potato late blight pathogen *Phytophthora infestans*. PiAVR2 is an RXLR-EER effector that is up-regulated during biotrophy, accumulates at the site of haustoria formation, and is recognized inside host cells by the potato R2 protein during infection. Transformation of a virulent *P. infestans* isolate to express *PiAVR2* conveys a gain-of-avirulence phenotype, indicating this is a dominant gene triggering R2-dependent disease resistance. Both presence/absence polymorphism and transcriptional differences explain virulence on *R2* plants. Isolates that infect *R2* plants express Piavr2, which evades recognition by R2. *PiAVR2* and *Piavr2* encode proteins that differ in 13 amino acids, 8 of which reside in the C-terminal effector domain; one or more of these specifies recognition by R2. Nevertheless, few polymorphisms were observed within each gene in pathogen isolates, perhaps indicating that *PiAVR2* and *Piavr2* are fixed within the population. Our results direct a search for *R* genes that recognize *Piavr2* which, deployed in combination with *R2*, may exert a strong selection pressure against the *P. infestans* population.

Finding the best set of needles in the *Phytophthora capsici* SNP haystack

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Phytophthora capsici is an important pathogen of cucurbit and Solanaceous vegetables. Populations can be genetically highly diverse although the contribution of sexual vs. asexual reproduction varies dramatically at locations worldwide. Our goal is to develop an optimized panel of between 20 and 100 Single Nucleotide Polymorphism (SNP) markers useful for characterizing diverse populations and for analyzing in vitro crosses. Focused Illumina re-sequencing was conducted on seven *P. capsici* isolated from various locations and hosts worldwide. Paired-end sequencing was accomplished around the SgrA1 restriction enzyme cut site and we obtained roughly 1.7 million bases of coverage for each isolate with minimum of 30X coverage. The total number of SNP sites ranged from 14, 637 for an isolate from Tennessee to 22, 431 for an isolate from Argentina with a SNP density of 15.87 SNP/kb and 24.32 SNP/kb respectively. An isolate of *P. tropicalis* was included revealing approximately 3X the number of SNP sites compared to the *P. capsici* isolates. An overview of the SNP marker development and examples of their application will be presented.

An *Arabidopsis thaliana* CC-NBS-LRR resistance gene recognizes a novel conserved effector from *Hyaloperonospora arabidopsidis*

Sandra Goritschnig, Ksenia Krasileva, Douglas Dahlbeck and Brian Staskawicz

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The *Hyaloperonospora arabidopsidis (Hpa)/Arabidopsis thaliana* pathosystem has emerged as an excellent model system to study oomycete pathogenesis and cognate resistance. The recent publication of the *Hpa* genome is now allowing us to more directly address *Hpa* on the genome level. We have used Hidden Markov Model-based searches and expression analysis to predict putative secreted and translocated effectors from *Hpa* and employed a surrogate *Pseudomonas* system to deliver the effectors to the Nordborg collection of *Arabidopsis* ecotypes. While we observed variation in recognition of several alleles of ATR1 and ATR13 in the different ecotypes, only few of the putative effectors were able to trigger a hypersensitive response (HR) in *Arabidopsis* when delivered by *Pseudomonas*. Here we are presenting the recent cloning of a resistance gene recognizing a conserved RXLR effector ATR39 from *Hpa*. The resistance gene (RPP39) in the ecotype Weiningen (Wei-0) is a member of a small cluster of CC-NBS-LRR genes on chromosome 1. The HR triggered by *RPP39* is independent of *NDR1*, which is required for the function of several other CC-NBS-LRR *R* genes. ATR39 is highly conserved among different *Hpa* isolates and expressed as two alleles, which show signatures of heterozygosity at that locus. While both alleles are capable of triggering a hypersensitive response when delivered by *Pseudomonas* at high inoculum, only one of them triggers a resistance response in low inoculum disease assays. Furthermore, despite recognition of ATR39 by its cognate *R* gene, Wei-0 is still susceptible to several *Hpa* isolates containing *ATR39*, indicating an active role of ATR39 or other effectors in suppression of resistance. This novel pair of resistance gene and cognate effector provides new insights into mechanisms of resistance and suppression of immune responses.

A molecular insight into algal-oomycete warfare: cDNA analysis of *Ectocarpus siliculosus* infected with the basal oomycete *Eurychasma dicksonii*

Laura J. Grenville-Briggs[1], Claire M.M. Gachon[2], Martina Strittmatter[2], Frithjof C. Küpper[2], and Pieter van West[1]

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Oomycetes (or water moulds) are non-photosynthetic Stramenopiles, which exhibit either pathogenic or saprophytic lifestyles. Pathogenic oomycetes infect a remarkable range of hosts including, marine algae, crustaceans, plants, nematodes, fungi, insects, fish and mammals. Many of the most devastating agricultural and aquacultural pathogens belong to the oomycetes. *Eurychasma dicksonii* is an abundant, and probably cosmopolitan, obligate biotrophic pathogen of marine brown algae. Molecular evidence indicates that it belongs to the most basal oomycete clade known so far. It occurs in all cold and temperate seas worldwide. As brown algae make up about 70% of the biomass of temperate and polar rocky shores, we infer that *E. dicksonii* like its terrestrial counterparts, probably shapes natural algal populations, profoundly impacting ecosystem functioning. Here we report the first large scale molecular data acquired on the most basal oomycete to date. 9873 unigenes, totalling over 3.4Mb of sequence data, were produced from Sanger sequenced and pyrosequenced EST libraries, of infected *E. siliculosus.* 6787 unigenes (69%) are of algal origin, and 3086 (31%) oomycete origin. Here we discuss the physiology of this host-pathogen interaction, in the light of our recent results.

Ancient and recently-acquired molecular weaponry in animal pathogenic oomycetes

Rays H.Y. Jiang[1], Brian J. Haas[1], Sean Sykes[1], Broad Genome Annotation Group[1], Sarah Young[1] , Irene De Bruijn[2], Pieter van West[2], Chad Nusbaum[1], Brett Tyler[3] and Carsten Russ[1]

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Saprolegnia parasitica is an oomycete pathogen that causes severe diseases in fish, amphibians and crustaceans, resulting in major annual losses to aquaculture and damage to aquatic ecosystems. It represents the first animal oomycete pathogen genome to be sequenced. We annotated the 53 Mb genome using *ab initio* methods and RNA-seq data from multiple life stages. The genome of *Saprolegnia* reflects many aspects in the ancestral oomycete in its gene structure, signaling pathways and metabolism. The canonical host-targeting domains found in plant pathogen oomycetes appear to be absent in *Saprolegnia*. Nevertheless, we found several effector candidates with variant-RXLR motifs, and showed experimentally that they target animal host cells. The largest plant pathogen effector families in *Phytophthora*, such as RXLR, crinkler and Necrosis Inducing Proteins (NIP) are absent in *Saprolegnia*. In contrast, *Saprolegnia* possesses one of largest sets of protease genes among eukaryotes, with about 300 more than typical fungal species. RNA-seq data show a wave-like deployment of proteases at different points during infection. *Saprolegnia* also has a massive kinome of 619 genes, similar in size to mammalian kinomes, 20% of which are induced upon infection. Surprisingly, *Saprolegnia* has ~50 protein domains that otherwise only occur in animals, e.g. disintegrins and Notch-like proteins, which may modulate host cells by molecular mimicry. Many of these genes are likely to be transferred from bacteria and primitive aquatic animals. The comparative analysis enabled us to reconstruct the ancestral oomycete genome and host-driven adaptations in animal and plant pathogens.

The composition and development of flagellated oomycete spores revealed though cell biology and comparative genomics approaches

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Most but not all oomycetes produce zoospores, which have basal body-anchored flagella as their prominent feature. Prior workers identified potential components of eukaryotic basal bodies and flagella through comparative genomics and proteomics studies across kingdoms. The dichotomy between zoospore-forming and zoospore-lacking oomycetes (e.g. *Phytophthora infestans* and *Hyaloperonospora arabidopsidis,* respectively) provides an opportunity to refine our definition of the proteins required for flagella development and function, and understand the processes that caused some species to lose the flagellated stage. Of about 200 proteins previously identified as being within eukaryotic flagella or basal body proteomes, genes encoding most were detected within the genome of *P. infestans* but nearly 160 were absent from *H. arabidopsidis*. A bioinformatics strategy identified relics of about 20% of the lost genes in *H. arabidopsidis*, in which the accumulation of deletions and frame-shift mutations evidenced the degradation of the flagella pathway in that species. In *P. infestans,* orthologs of most genes lost or present only as fossils in *H. arabidopsidis* had expression patterns consistent with roles in the flagellated stage. Localization of the proteins to flagella, basal bodies, or other cellular compartments is a logical next step towards illuminating their roles. To facilitate such studies, vectors were constructed to allow proteins of interest to be expressed as N- or C-terminal fusions with GFP, CFP, YFP, or mCherry, in backbones allowing selection with G418 or hygromycin. Fusions between the fluorescent tags and proteins from diverse organelles validated the function of the plasmids and provided insight into hyphal growth and spore development. The plasmids should be a useful resource for studies of oomycete biology.

Characterization of Oomycete Effector Entry in Plant and Animal Cells

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Oomycetes cause significant damage to crops and forest each year. Analysis of several oomycete genomes shows that these genomes contain a reservoir of putative RXLR effectors. These effectors are utilized during the biotrophic phase of infection to suppress defense responses. Several oomycete and fungal effectors have been shown to enter host cells via a conserved N-terminus RxLR motif without any pathogen-encoded machinery. RxLR mediated effector entry relies on binding to a newly identified external phospholipid, PI(3)P. Binding to PI(3)P can be blocked either by sequestering PI(3)P on the outer leaflet of the plasma membrane or by competitively inhibiting the PI(3)P binding site of the effector. We present our latest findings on the mechanism of entry of oomycete and fungal RxLR effectors along with further characterization of the PI(3)P binding mechanism.

Host induced epigenetic alteration in *Phytophthora ramorum*

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An oomycete plant pathogen *Phytophthora ramorum* is responsible for two distinctive diseases; (1) Sudden Oak Death, which is characterized by lethal bole cankers on oaks, and (2) Ramorum blight, which causes necrotic lesions on leaves of diverse shrub species such as bay laurel and Rhododendrons (foliar hosts). It has been noticed that although a single clonal lineage dominates in Californian forests, isolates originating from oaks tend to be less virulent on both oak and foliar hosts than those from foliar hosts, and colonies of oak isolates look irregular and are somatically unstable. We hypothesized that because *P. ramorum* in California is exclusively clonal, most of the aforementioned phenotypic variations should be due to difference in gene regulation rather than genetic polymorphism. We have conducted microarray mRNA profiling and found that hundreds of genes encoding for transposable elements were highly active in some isolates derived from oak trees, which we termed transposon derepressed phenotype (TDP). RT-qPCR was then employed to measure the expression level of transposons in one hundred *P. ramorum* isolates derived from diverse host species. It was found that 64% of isolates derived from oak hosts showed TDP, whereas only 9% of isolates from foliar hosts showed TDP. We hypothesize that *P. ramorum* incurs epigenetic alterations within and beneath oak bark, which resulted in derepression of transposons.

Functional characterization of cytolytic and non-cytolytic members within the NLP effector superfamily

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NEP1-like proteins (NLPs) represent a superfamily of effector proteins which are distributed amongst various oomycetes, fungi and bacteria. Among the best characterized NLPs are those that are expressed and secreted by the pathogens during the late stage of infection and that trigger necrosis in dicotyledonous plants. We could demonstrate that this necrotic activity makes them virulence factors in the host organisms by supporting a necrotrophic life style. The three-dimensional structure of an NLP from *Pythium aphanidermatum* (NLP_{Pya}) is similar to the fold of actinoporins, pore-forming toxins from marine invertebrates. Like actinoporins, NLPs affect the integrity of plasma membranes from dicot plants, thereby leading to necrosis. However, the detailed molecular mechanism and the target molecule of NLPs at the plasma membrane are currently unknown. Several lines of evidence suggest a lipid-like interaction partner at the plasma membrane. A surface-exposed cavity was determined in the NLP_{Pya} crystal structure that would be suitable as the binding site for such a molecule. NLP versions mutated in the vicinity of the cavity are used in a photo-affinity labeling approach for capturing the target molecule. Recent progress in characterizing the mechanism of pore formation and the host docking site of NLPs will be presented. Besides cytolytic NLPs, another group of NLPs exists which is expressed in the early infection stage. As expected due to the lack of conserved residues required for cytolysis, these NLPs are incapable of triggering necrosis. They are most abundant in oomycetes and fungi, and are found in biotrophic organisms or pathogens with monocot hosts. Considering the structural relation of NLP_{Pya} not only to actinoporins but also to fungal lectins, non-cytolytic NLPs could contribute to host cell adhesion. We will provide recent progress in the functional analysis of non-cytolytic NLPs and discuss their putative role in the early infection process.

Natural variations in the perception of the oomycetal effector CBEL in *Arabidopsis*

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The plant cell wall, one of the first barrier against pathogen intrusion, is targeted by cell-wall degrading enzymes secreted during infection. Wall degradation results in the release of cell wall fragments that can be detected as DAMPs (Danger Associated Molecular Patterns) inducing plant immunity (Boller and Felix, 2009). Structural modification of plant cell wall could also occur during the interaction of cellulose with non-enzymatic microbial proteins harboring CBM1 (Carbohydrate-Binding Module family 1). CBM1s were originally characterized in fungal cellulases where they enhance substrate accessibility, and in non-enzymatic CBEL proteins of *Phytophthora* where they play an essential role in adhesion and recognition of cellulosic substrates (Gaulin et al., 2002). CBEL-like proteins were detected in all oomycete genomes analysed so far, including the legume pathogen *Aphanomyces euteiches* (Gaulin et al., 2008). CBEL from *Phytophthora parasitica* induces defense responses on several plant families including the model plant *Arabidopsis thaliana* where it causes a hypersensitive-like cell death. Strikingly, CBM1 of CBEL acts as a PAMP (Pathogen Associated Molecular Pattern) by inducing plant immune responses in several plant species including *Arabidopsis* and *tobacco*. This activity is correlated with the ability of CBM1 to bind cellulose (Gaulin et al., 2006). Based on these data a model was made linking CBM1-induced structural modification of cellulose to defense induction (Dumas et al., 2009). To validate this model, a reverse-genetic approach was developed in *A. thaliana*. Several mutant lines and ecotypes were tested for the CBEL-induced cell death. This screening assay pointed out natural variations in CBEL perception among *Arabidopsis* accessions. Defense genes expression profile was compared between two *Arabidopsis* wild-type lines, Col0 and Ws4, which showed a strong polymorphism in the CBEL-induced cell death. Natural variations of CBEL-induced responses in *Arabidopsis* will be presented.

Mitochondrial haplotype analysis for differentiation of isolates of *Phytophthora cinnamomi*

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While *Phytophthora cinnamomi* is heterothallic, analysis of field populations indicate a clonally reproducing population. In the absence of sexual recombination the ability to monitor mitochondrial haplotypes may provide an additional tool for identification of clonal isolates and analysis of population structures. To determine haplotypes for this species seven mitochondrial loci spanning 6,961 bp were sequenced for 62 isolates representing a geographically diverse collection of isolates with A1 and A2 mating type. 45 haplotypes were identified with difference due to SNPs (totaling 152 bp) and length mutations (17 indels greater than 2 bp representing 910 bp). SNPs were the predominate mutation in the four coding regions and their flanking intergenic regions while SNPs and length mutations were observed in the other three primarily intergenic regions. Some of the length mutations in these regions were due to indels of unique sequences while others were due to variable numbers of subrepeats (3-12 copies of a 24 bp subrepeat). Network analysis of the haplotypes identified 8 primary clades with the most divergent clade representing A1 isolates from Papua New Guinea. With three exceptions isolate grouping in the network corresponded to mating type and previously published isozyme classifications; a haplotype representing an A1 mating type (H29) was placed well within the haplotype grouping having A2 mating type, one haplotype (H26) had isolates with two isozyme classifications and one isozyme group was on separate network clades. Among the 62 isolates examined several examples were identified of isolates recovered from different geographic regions from the same or similar host having the same mitochondrial haplotype, indicating movement of isolates via plant material.

Suppression and elicitation of plant immunity by two *Pseudomonas* type III effectors

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Pseudomonas syringae pv. *tomato* (*Pst*) causes bacterial speck disease of tomato (*Solanum lycopersicum*) by utilizing its type III secretion system to deliver ~30 effectors into the plant cell. Two of these effectors, AvrPto and AvrPtoB, act early in the infection process by interacting with the kinase domains of PAMP receptor complexes. This interference in PAMP-mediated signaling enables the virulence activity of later-acting effectors. Some tomato varieties are immune to speck disease because they express the Pto ser/thr kinase that detects the presence of either AvrPto or AvrPtoB and acts in concert with the NB-LRR protein Prf to activate effector-triggered immunity. Thus, Pto may have evolved as a molecular mimic (a decoy) of the true virulence targets of these effectors. The crystal structures of the complex between Pto and AvrPto or AvrPtoB have been solved and revealed both common and unique interfaces between Pto and the effectors. This suggests that tomato has evolved independently to recognize these effectors. We have identified wild species of tomato that recognize only AvrPto, only AvrPtoB, both effectors, or neither one. Our current work is focused on integrating knowledge of the Pto and AvrPto/AvrPtoB crystal structures with what we are learning about natural variation in *Pst* recognition in the *Solanaceae* from characterization of wild tomato species. Supported by NIH R01GM078021, NSF-IOS 0841807 and USDA-AFRI-2010-65108-20503.

The Aspartic Proteinase Family of Three *Phytophthora* Species

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Pepsin-like aspartic proteinases (APs) are produced in a wide variety of species and contain conserved motifs and landmark residues. APs fulfil critical roles in infectious organisms and their host cells. Phytophthora species are oomycete plant pathogens with major social and economic impact. Several of which have been sequenced. The genomes of Phytophthora infestans, P. sojae and P. ramorum contain 11-12 genes encoding APs, resolved into 5 clades by phylogenetic analysis. Several subfamilies contain an unconventional architecture, as they either lack a signal peptide or a propart region. One of the Phytophthora APs is an unprecedented fusion protein with a putative G-protein coupled receptor as the C-terminal partner. The others appear to be related to well-documented enzymes from other species including a vacuolar enzyme that is encoded in every fungal genome sequenced to date. The oomycetes also have enzymes similar to plasmepsin V, a membrane-bound AP in the malaria parasite *Plasmodium falciparum*, that cleaves effector proteins during their translocation into the host red blood cell. The translocation of Phytophthora effectors to host cells is topic of intense research in which APs might be involved.

Understanding oomycete gene evolution using evolutionary gene networks

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Parasitism is the most common consumer strategy among organisms and is a life history strategy that has arisen independently innumerable times across the tree of life. Nevertheless, the mechanisms underlying the transition from a free-living lifestyle to a parasitic mode of existence are largely a mystery. It is widely believed that parasites are under selective pressure to streamline their genomes based on the fact that most have nuclear genomes that are smaller in size and coding capacity of canonical housekeeping genes, relative to free-living species. Parasites are not, however, simply genetic subsets of free-living organisms. For example, as a consequence of having to import nucleotides, amino acids, proteins and other molecules, most inter- and intracellular parasites encode more transporters than their free-living relatives. Parasites must continue to adapt to different selective pressures than their free-living ancestors and employ novel strategies to counteract host defenses. This adaptation has a genetic component that involves gene duplication and sub-functionalization in multigene families. Parasitic oomycetes, especially plant pathogenic species, are widely studied and have a wealth of genomic information available. However, facultative and free-living oomycetes are much less understood and lack sufficient data for wide scale comparative analysis. We have sequenced the genome of *Achlya hypogyna* (facultative parasite) and the transcriptome of *Thraustotheca clavata* (free living), two Saprolegnian oomycetes, in order to fill this gap. Using these data, we present a method to elucidate the evolution of gene families related to a parasitic lifestyle, using evolutionary gene networks (EGNs). EGN's are mathematically based graphs that can be used to visualize gene relationships that traditional phylogenetic would fail to resolve. Using this method we have identified unique gene family expansions, contractions, and combinations that are potentially key to the evolution of parasitism within this diverse group of organisms.

Transcriptional gene regulation in the oomycetes

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Genome annotation have identified more than 1000 members of transcription factors in each genome of six oomycete species; *Phytophthora sojae, P. ramorum, P. infestans, Pythium ultimum, Hyaloperonospora arabidopsidis* and *Saprolegnia parasitica*. Apetala2 (AP2) protein is a family of transcription factors that are believed to be unique for plants. *Arabidopsis*, rice and soybean each contains 122, 139 and 98 putative genes, respectively. Comparative analysis identified a single gene model in all of the sequenced oomycete genomes except *P. ultimum*. In plants, they play various roles in growth, development, response to biotic and abiotic stresses, hormone metabolism and signaling. AP2 proteins appear to be highly conserved (59-65% identity) across the oomycetes. In *P. sojae*, it is expressed in zoospores and during infection. Thus, it is likely involved in the regulation of a protein network unique to oomycetes, and could represent a potential target for controlling oomycete pathogens.

Deployment of Rapid Diagnostic Tools for *Phytophthora* on horticultural crops in Central America

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Plant disease is a limiting factor in agricultural production in Latin America. Diseases are difficult to control due to high rainfall conditions and the presence of a diversity of plant pathogenic microorganisms. Plant pathogens produce a large number of diseases on tropical crops and cause losses estimated to be as high as $30 billion per year. The accurate identification of plant pathogens has important implications for growers in Latin America and the US and can improve our knowledge of pathogen biology and ultimately treatment and control of tropical plant diseases. The risk of accidental introduction of *Phytophthora* species with trade requires continued monitoring and improved diagnostic capabilities. Our overall objective is to produce a platform of tools needed to detect, identify, and ultimately prevent entry of novel species of *Phytophthora* into the US with a major focus on development of surveillance tools for common and high threat species of *Phytophthora* on horticultural crops including potato, cacao and floriculture crops from Central America. We deployed a series of "shovel ready" technologies including: a Phytophthora diagnostics workshop held in San Jose Costa Rica in 2010, a Lucid key for species identification, molecular and digital diagnostic identification systems to identify *Phytophthora* species and improve the diagnostic capabilities for important plant disease clinics in the region. We are working with collaborators including, FHIA in Honduras, Universidad de Costa Rica, CATIE, The World Cacao Foundation, DOLE Foods, and the Organization of Tropical Studies to conduct surveys of *Phytophthora* species on horticultural crops in the region.

Genome reconstruction of oomycete pathogens

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Oomycetes are the causal agents of devastating diseases on plants, animals and insects. Recently several genomes of these pathogens became available. These fungi-like organisms have large and flexible genomes with expanded gene families that are implied to play an important role in the host-pathogen arms race. Hence, we want to systematically investigate when gene families in these pathogens duplicated, e.g. in the common ancestor or continuously along the tree, and conversely, whether the adaptation of oomycetes to their host and life style is in part due to the loss of certain families. Therefore, we analyzed the predicted proteomes of six pathogenic oomycetes and four non-pathogenic sister taxa (diatoms, brown and golden-brown algae). We constructed ~12,000 multi-species gene (protein) families as well as their gene (protein) trees and reconciled these with a reliable species phylogeny. The inferred evolutionary events were projected onto the species tree. We observed a high number of duplications and losses, especially within the oomycetes, that shape the genome content of the extant organisms. The evolutionary signature of duplications and losses along the branches of the species tree differed significantly between functional classes. Our results corroborate and generalize recent observation in the study of individual gene families. Moreover, this will aid in the understanding of the evolutionary processes that shape the genome content of extant oomycetes.

Elucidating the role of *Hyaloperonospora arabidopsidis* RxLR effector proteins on the suppression of the plant immune system

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A partially open question is how microbial pathogens manipulate the plant immune system to establish disease. Oomycete effector proteins have been reported to be delivered to the host cell to alter host immunity and therefore allowing successful pathogen colonization. The *Hyaloperonospora arabidopsidis* genome potentially encodes approximately 140 candidate effector proteins likely involved in the alteration of plant defense processes to allow successful growth and reproduction. Using a high throughput yeast two hybrid technique a plant immune interactome was generated using 99 RxLR-motif containing effectors proteins against a collection of more than 8000 Arabidopsis proteins. The results made it possible to generate an interaction network and interesting candidates have been investigated further using fluorescence microscopy, Arabidopsis T-DNA knock-out lines and RXLR over expression lines to deduce the effect of these proteins on plant susceptibility.

Computational and Experimental Identification of Elite Effectors in the Genome of *Phytophthora sojae*

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Oomycete pathogens, including *Phytophthora* and *Pythium* species and many downy mildews, cause billions of dollars of damage to crops, forestry and ornamental plantings each year, and genome sequences have now been generated for a number of these pathogens. The genome of the soybean pathogen *Phytophthora sojae* contains nearly 400 genes encoding candidate effector proteins carrying the motif RXLR that mediates host cell entry via binding to cell surface phosphatidylinositol-3-phosphate. We have carried out a broad survey of the transcription, variation and functions of a large sample of the *P. sojae* candidate effectors. Of 169 effectors tested, most could suppress programmed cell death triggered by BAX, effectors and/or the PAMP INF1, while several triggered cell death themselves. Among the most strongly expressed effectors, two general patterns of expression were observed. Based on the genome sequences of three more major genotypes of *P. sojae* obtained by 454 pyrosequencing, 45 (12%) RXLR effector genes showed high levels of polymorphism among *P. sojae* isolates and significant evidence for positive selection. We also used transcriptional profiling, including both Affymetrix GeneChips and ABI Solid™ sequence tags, to identify *P. sojae* effector genes that may play an active role in promoting infection. Combining this information with mathematical modeling revealed an elite subset of effectors comprising around 10% of the repertoire that appears to be responsible for most of the contribution of this family to virulence. Several family members are individually indispensible for virulence. Moreover, coordinated interplay among key effectors appears to maximize defense suppression; effectors expressed earliest in infection could suppress the cell death triggered by several effectors expressed later in infection that could suppress INF1-triggered cell death. In support of this hypothesis, mis-expression of key effectors severely reduced the virulence of *P. sojae* transformants.

Meet the Dutch; population genetics of *Phytophthora infestans* in the Netherlands in the last decade

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A representative set of over a thousand isolates collected from commercial potato fields provide a comprehensive survey of Dutch late blight populations from 2000 to 2009. The Dutch population diversity of *P. infestans* was assessed at high resolution using twelve highly informative microsatellite markers, the mating-type and the mitochondrial haplotype. The population analysis revealed both the complexity of the Dutch population, with over 322 unique genotypes identified, as well as the increasing importance of clonal lineages. The fluctuations of the relative importance of these clonal lineages during the potato grown season and their stepwise displacement over the years are remarkable. The results demonstrate the importance of the sexual cycle to generate the high genetic diversity of which the best adapted genotypes spread all over the country and beyond by the asexual cycle. We identified regional differences that could be related to late blight management strategies, soil substance or the use of cultivars. In addition we developed a functional marker for virulence on potato lines that carry the *Blb1* resistance gene. Using this molecular marker we demonstrate that in the Netherlands a low frequency of Blb1 breakers is present (<0.5%), which could be found even before the introduction of the *Blb1* resistance gene in the Netherlands. In total 10 independent genotypes of Blb1 breakers were found that seem to be generated during the sexual cycle and did not spread efficiently.

The oomycete RxLR-effectors AVR3a and SpHtp1 show cell type specific import and their RxLR-leaders mediate dimerisation

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The fungus-like oomycetes contain several species that are devastating pathogens of plants and animals. During infection oomycetes translocate effector proteins into host cells where they interfere with host defence responses. Several oomycete effectors have a conserved Arg-Xaa-Leu-Arg (RxLR)-motif that is important for their delivery. We found that, whereas the RxLR-leader sequence of SpHtp1 from the fish pathogen *Saprolegnia parasitica* shows fish cell-specific translocation, the RXLR-leader of AVR3a from the potato-late-blight pathogen *Phytophthora infestans* promotes efficient binding of the C-terminal effector domain to several cell types. Our results demonstrate that the RxLR-leaders of SpHtp1 and AVR3a are dimerisation sites, able to form heteromers. Furthermore, cell surface binding of both RxLR-proteins is mediated by an interaction with modified cell surface molecules. These results reveal a novel effector translocation route based on effector dimerisation and receptor modification, which could be highly relevant for a wide range of host-microbe interactions.

MicroRNA regulation in soybean during *Phytophthora sojae* infection

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Small RNAs, including microRNAs (miRNAs) and small interfering RNAs (siRNAs), have essential regulatory roles in many plant processes including development, metabolism and responses to abiotic and biotic stresses. Prior studies have shown that specific miRNA expression changes are induced in plant hosts upon interaction with pathogens. Some small RNAs are involved in plant innate immunity against pathogen infections. Furthermore, viral and bacterial pathogens evolved effector proteins to manipulate host small RNA pathways in order to suppress defense and facilitate disease development. To date, the functions of small RNA in plant interactions with fungal and oomycete pathogens remains largely unknown. In order to fill this gap in our knowledge, we analyzed small RNA populations in soybean upon infection with *Phytophthora sojae*. Our central hypothesis is that *P. sojae* infection will trigger expression changes in specific small RNA species, which may play a role in plant immunity by regulating their respective target genes. Following high throughput Illumina sequencing and bioinformatic analysis, we have identified ten known microRNAs and approximately 38 potentially novel microRNAs that are differential expressed in the *P. sojae*-infected soybean roots. These differentially expressed miRNAs are likely to have potential roles in soybean defense response. For example, we found miR393 to be up-regulated in soybean root upon *P. sojae* infection. miR393 is induced in *Arabidopsis* treated with flagellin of the bacterial pathogen *Pseudomonas syringae*. It has been shown that miR393 is involved in *Arabidopsis* immunity against bacterial infection by its regulatory role in auxin signaling pathway. Our findings suggest that miR393 may have a conserved function in protecting plants from infection of both bacterial and fungal/oomycete infection. Current progress is underway to elucidate the molecular mechanism underlying the functions of selected miRNAs during soybean interaction with *P. sojae*.

Regulation of primary and secondary sporulation by Myb transcription factors in the oomycete *Phytophthora infestans*

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In our previous studies, a Myb transcription factor binding site was determined to be involved in regulating sporulation-specific gene expression, and genome-wide analysis identified several distinct phylogenetic groups of Myb transcription factors in the genome. In this study, the regulatory roles of these Myb proteins in sporulation are examined by gene overexpression. The phenotypes induced by overexpression distinguish two kinds of sporulation as follows: 1) Primary sporulation. This is the canonical developmental process during which sporangia are produced from vegetative mycelia. The overexpression of *Myb2R4*, a R2R3 Myb gene, increased sporangia production significantly. qRT-PCR shows that a few other Myb genes, especially those of the R2R3 type, are up-regulated by *Myb2R4* overexpression, suggesting some Myb genes form a regulatory cascade. 2) Secondary sporulation. This is a phenotype in which a sporangium germinates and then directly produces a new spore without an obvious vegetative growth phase. The overexpression of *Myb3R6*, an oomycete-specific R1R2R3 Myb gene, induced a high percentage of secondary spore production. The overexpression of *Myb2R5*, a R2R3 Myb gene, increased secondary sporulation significantly under inducing condition, in which sporangia were briefly stimulated to germinate in rich media and then transferred into water. Our assumption is that the overexpression of *Myb3R6* or *Myb2R5* reduces the dormancy of sporangia; however, the spores undergo secondary sporulation when the conditions do not favor direct germination.

POSTER PRESENTATIONS

Relative disease susceptibility of cultivated varieties of potato when infected with different isolates of *Phytophthora infestans*

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Late blight, caused by the Oomycete pathogen *Phytophthora infestans*, continues to be the most devastating and economically important disease affecting potato crops worldwide. Recent geographical migrations of the pathogen have displaced the 'old' US-1 clonal lineage with 'new', genotypically distinct, lineages. These new genotypes have shown increased pathogenic fitness and diverse environmental requirements. Previous field studies, as well as our own laboratory observations, have suggested variable levels of susceptibility among different cultivars of potato when exposed to isolates of these newer genotypes. Interactions between two different isolates of the US-8 race (NC092ba and PSUPotB, each sampled from distinct geographic regions during different years) and potato (*Solanum tuberosum)* were analyzed through *in planta* infection under controlled laboratory conditions. Three potato cultivars (Kennebec, Red Norland, and Russet Burbank) were chosen for these experiments. Disease development over time was rated for each experimental unit by determining incubation period, foliar lesion area and relative number of leaves displaying necrosis. Data suggest that differential pathogenicity between the US-8 isolates exists. Cultivar susceptibility was also variable. Contrary to previous studies, it was found that cv. Kennebec was least susceptible to either pathogen. Along with *in planta* infection, effect of temperature on the disease cycle was also evaluated using a detached leaf assay (DLA). Three biologically relevant temperatures were evaluated (12ºC, 18ºC and 24ºC). Our findings suggest that there is a differential temperature effect on disease development between isolates and between cultivars. Unexpectedly, data suggest that at 24ºC, disease severity was highest and incubation period lowest. Confirmation of pathogen presence in infected leaf tissue was successfully obtained using previously developed *P. infestans* specific primers (PINF and ITS5) in a Polymerase Chain Reaction (PCR) assay. Further results of these investigations will be presented and discussed.

Suppression of defense responses in distantly related plants by homologous RXLR effectors

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We are using the interaction between the model plant *Arabidopsis* and its downy mildew pathogen *Hyaloperonospora arabidopsidis (Hpa*) to investigate how plant cells are manipulated by oomycete effectors. The *Ha* genome has been sequenced and bioinformatic analyses have revealed at least 130 candidate effector genes. We are focusing on a pair of homologous effectors: *Ha98* and *Ps163* from *H. arabidopsidis* and *Phytophthora sojae,* respectively. Both effectors are expressed during the host-pathogen interaction. Furthermore, Ha98 and Ps163 carry a functional host targeting (HT) sequence that is required for translocation into the host cell. Transient assays indicate that they suppress diverse elicitors of programmed cell death in soybean including mammalian Bax and the *P. sojae* elicitor Avr4/6. In addition, transgenic *Arabidopsis* plants expressing either *Ha98* or *Ps163* partially suppress *RPP4*-mediated resistance to *Ha* EMOY2 and are more susceptible to *Ha* EMCO5. *Ha98* and *Ps163* expressing plants suppress the callose response to avirulent bacteria suggesting that both effectors are capable of suppressing PAMP-triggered immunity. Transient expression of *Ps163* alleles in *Nicotiana benthamiana* triggers cell death response that is allele-specific and requires RAR1 and Hsp90-1. These experiments suggest that these homologous effectors from distantly related oömycetes can suppress and induce defense mechanisms in diverse plant species.

Secreted protein from human fungal pathogens use an RxLR-meditated strategy for entering the host cell

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Humans are colonized by different fungal species and most of the time colonization is harmless under normal circumstances. However, during immunosuppression, opportunistic pathogenic fungi have the capability to proliferate and eventually cause a disease. Effectors molecules are a key weapon used by pathogens to evade or circumvent host defense mechanisms in order to succeed in the pathological event. Using a bioinformatic approach, we have identified candidate RxLR-like motifs in three fungal predicted effectors from the human pathogens *Aspergillus flavus*, *Cryptococcus neoformans* and *Coccidioides immitis*. In these effectors we identified double RxLR-like motifs, and both the RxLR sequences are involved in the phospholipid, phosphatidylinositol-3-phosphate (PI-3-P) binding. Using a targeted mutagenesis approach we demonstrated that just one of the two RxLR is critical for the traffic across the membrane of mammalian cells. These observations lead us to speculate that one of the RxLRs is strictly required for the human cell uptake while the other one is mainly involved in membrane anchor process. Theoretically we hypothesize the presence of a primary and of a secondary RxLR with the latter mainly involved in the initial adsorption of the effector proteins around PI-3-P rich membrane domains to enhance the effective concentration of the protein at the specific membrane sites facilitating its penetration.

Comparative genomics suggests the presence of RNA interference in Oomycetes

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RNA interference is a natural process eukaryotes use to regulate gene expression. Here we used comparative genomic approaches to identify the genes involved in RNA interference within available Oomycete genomes. Amino acid sequences of proteins known to be involved in RNAi biogenesis from human, *Drosophila*, and *Arabidopsis* were collected and used as references to search Oomycete genomes for the presence of homologs. Dicer, drosha, argonaute, and pasha protein sequences were used as queries as they are known to be crucial for RNAi biogenesis and are heavily conserved between different organisms. Searches yielded that *Phytophthora ramorum, P.capsici, P.infestans, P.sojae*, and *Saprolgenia parasitica*, as well as outgroups *Thalassiosira pseudonana* (diatom) and *Ectocarpus siliculosus* (brown alga), all contain proteins that are homologous to the reference sequences. Pfam was used to verify that each homolog contained the appropriate protein domains known to be involved in RNAi biogenesis. Phylogenetic analysis of both protein and nucleotide data suggest that these genes have also experienced multiple rounds of duplication within Oomycetes. These results suggest that Oomycete genomes contain the appropriate genes necessary for RNA interference. Currently, nucleotide alignments are being used to design primers for both genomic PCR and RT-PCR for test for the presence and expression of these genes in locally collected isolates of *Phytophthora* and *Pythium*.

Characterization of Conserved Effector Proteins from *Hyaloperonospora arabidopsidis* and *Phytophthora sojae*

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Numerous plant pathogens are known to secrete effector proteins that are exported to the interior of host cells [1]. Effectors have been well characterized in bacteria [2] but the roles of effectors in oomycete and fungal pathogenicity are poorly understood [3]. Bioinformatic analysis of recently obtained genome sequences from oomycete pathogens *Phytophthora sojae*, *P. ramorum* and *Hyaloperonospora arabidopsidis (Ha)* have led to the identification of a large number of candidate effector genes [4]. These effector genes are defined by characteristic motifs (Signal peptide, RxLR and dEER) that target the effectors into plant cells [5, 6]. Although these effector genes are very diverse, certain genes are conserved between *P. sojae* and *H. arabidopsidis*, suggesting that they fulfill key roles in pathogenicity. The goal of my project is to characterize a selected set of conserved effector candidates in regard to pathogenicity in *H. arabidopsidis* and *P. sojae*. We hypothesize that these effectors have important conserved roles in infection. The primary objectives of my research center on identifying effector functions and *in planta* targets using both transient assays and stably transformed plants. From experiments conducted to date, Ha23 seems to be a promising candidate effector. Firstly, Ha23 is expressed during the course of *Ha* infection of *Arabidopsis*. Secondly, Ha23 triggers an ecotype-specific defense response in *Arabidopsis*, suggesting that it is recognized by the host. Thirdly, Ha23 can suppress both, immunity triggered by pathogen associated molecular patterns and by effectors *in planta.* The *P.sojae* homolog, Ps73 can also suppress effector triggered immunity *in planta*. Results also show that Ha23 positively contributes to bacterial virulence in *Arabidopsis.* Finally, experiments with transgenic *Arabidopsis* expressing Ha23 also suggest suppression of immunity triggered by pathogen associated molecular patterns and enhancement of bacterial virulence.

Role of bZIP transcription factors in the asexual development of the plant pathogen *Phytophthora infestans*

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Phytophthora infestans is the causal agent of late blight in potato and tomato crops. Plant infection by this pathogen can involve two modes of asexual development. One involves entry of hyphae from germinated sporangia through wounds or natural openings. In addition, sporangia can produce zoospores which encyst and germinate to form infection structures called appressoria that helps to penetrate the host. Gene expression in all developmental stages must be tightly regulated to allow a successful asexual cycle and infection. bZIP transcription factor family have essential roles in development in many eukaryotes. Identification and functional characterization of bZIP transcription factors in *P. infestans* will contribute to our understanding of their role in the asexual development and pathogenicity of this oomycete. Bioinformatic studies showed that *P. infestans* contains around 20 bZIP TFs. qRT-PCR showed that most were differentially expressed at different developmental stages. Overexpression and RNAi-based gene silencing methods, using both constitutive promoters and a chemically-inducible gene expression system based on the ecdysone receptor, are currently being used to study gene function. So far, a monopartite and two-hybrid ecdysone inducible system were tested. The monopartite version showed high expression levels of the reporter but high background without the inducer. The two-hybrid system showed lower expression levels but displayed a very low background in the absence of inducer.

Salisapiliaceae – A new family of oomycetes from marsh grass litter of southeastern North America

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From marine environments, several filamentous oomycete species of the genus *Halophytophthora* have recently been described, mostly from subtropical and tropical ecosystems. During a survey of oomycetes from marsh grass leaf litter, six isolates were recovered that bore similarity to some members of *Halophytophthora* but were highly divergent from isolates of *Halophytophthora* sensu stricto based on a multilocus analysis of three nuclear loci and one mitochondrial locus. In phylogenetic analyses, these isolates were placed basal to a monophyletic group comprised of *Pythium* of the Pythiaceae and the Peronosporaceae. Sequence and morphology of these taxa diverged from the type species *Halophytophthora vesicula*, which was placed within the Peronosporaceae with maximum support. As a consequence a new family, the Salisapiliaceae, and a new genus, *Salisapilia*, are described to accommodate the newly discovered species, along with some species previously classified within *Halophytophthora*. Morphological features that separate these taxa from *Halophytophthora* are a smaller hyphal diameter, the mode of oospore production, lack of vesicle formation during sporulation, and a plug of hyaline material at the sporangial apex that is displaced during zoospore release. Our findings offer a first glance at the presumably much higher diversity of oomycetes in estuarine environments, of which ecological significance requires further exploration.

Inferring evolutionary relationships of *Phytophthora* species in the Ic clade using nuclear and mitochondrial genes.

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Phytophthora infestans, the causative agent of potato and tomato late blight is an important pathogen worldwide and caused the Irish potato famine of the 1840's. Two sisters species of *P. infestans* in the Ic clade, *P. andina* and *P. mirabilis* have been described in Ecuador and Mexico, respectively. The *Phytophthora* Ic clade, consisting of *P. infestans*, *P. phaseoli*, *P. ipomoeae*, *P. mirabilis*, and *P. andina*, contains some of the world's most destructive plant pathogens. We sequenced the mitochondrial genomes of the sister species of *P. infestans* in the Ic clade including *P. phaseoli, P andina, P. mirabilis* and *P. ipomoeae* and used the whole genome sequences and coalescent analysis to resolve the evolutionary histories of members of the Ic clade. Both gene order and content were conserved among the Ic clade *Phytophthora* species, with the only difference in coding genes occurring in ORF79 of *P. andina,* where there is a 12 amino acid deletion. The rooted tree inferred documents the earliest split separating *P. phaseoli* from the rest of the Ic clade species. Next, the lineage ending at *P. andina* diverged from the lineage leading to *P. infestans. P. mirabilis* and *P. ipomoeae* diverged most recently from a *P. andina* ancestor. The inferred tree has *P. mirabilis* and *P. ipomoeae* being more closely related to each other than either is to *P. infestans*. Our data are consistent with the hypothesis that the Andean region is the center of evolutionary origin for all the species in the clade since *P. andina* and *P. infestans* coexist there and bridging hosts occur there. Further surveys are needed to test this hypothesis and the occurrence of *P. mirabilis* and *P. ipomoeae* in the Andean region.

Exploring *P.infestans* RD46 effector function using yeast functional genomics analysis

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During infection *Phytophthora infestans* secretes an armada of effector proteins that compromise host systems for the benefit of the pathogen. Many of the secreted effectors are directed into the host cell by the N-terminal amino acid motif RXLR in combination with downstream acidic residues. To elucidate the function of RXLR effectors, we are using yeast functional genomic screens, which have been effective in discerning the function of bacterial effectors. Previously, we identified numerous RXLR effectors whose expression inhibited growth in yeast, presumably by targeting conserved eukaryotic pathways. Currently, we are exploring the potential targets of one effector, RD46, using yeast microarrays. The results of this analysis will be presented.

Comparative Genomics of ABC Transporters

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The ABC superfamily of transporters are found in bacteria and all eukaryotes and constitute one of the largest family of membrane transporters. In eukaryotes, there are seven classes of membrane transporters and two or more families of proteins that have conserved ATP Binding Cassettes (ABC). Each of the families of membrane transporter has a unique conserved type of ABC domain and a specific orientation and number of ABC cassettes that facilitate rapid classification to a particular sub-family. Free-living oomycetes have slightly more ABC membrane localized transporters than that of the simplest plant genome. However, since sequence homology cannot be used to predict the substrates that are transported other approaches are needed. For example ABC transporters that can transport xenobiotics may be associated with five different families. While we see comparable numbers of ABC transporters in different oomycete genomes, in each of the families there are only a small number of predicted orthologs that have been conserved in all genomes. In fact much of the expansion in the ABCC family of S. parasitica has been derived from paralogous expansion of a single progenitor. Again in the PDR family of S. parasitica, most of the models are derived from paralogous expansion of a few progenitors. Thus comparative genomics may be useful in predicting the function of only a subset of these membrane transporters.

Marker discovery and application for the Taro (*Colocasia esculenta)* pathogen *Phytophthora colocasiae*

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Taro, a non-graminaceous monocot, is a major staple crop in the Pacific, and is grown widely in the Caribbean, Africa, and Asia. *Phytophthora colocasiae*, causal agent of Taro Leaf Blight (TLB), seriously threatens the sustainability of this important crop. Resources for studying TLB are limited and a next generation sequencing approach was implemented to develop single nucleotide polymorphism (SNP) markers useful for studying natural populations and tracking virulence/pathogenicity in crosses. Sequencing was focused on the 200-400 bases surrounding the SgrA1 restriction enzyme cut site and a total of approximately 6M 55-75bp Illumina reads were generated for two isolates (an A1 and an A2 mating type) recovered from Taro at different locations in Vietnam. Reads were aligned to the *Phytophthora capsici* reference genome and variable sites identified. We will present an overview of the sequence coverage, heterozygosity, total number of putative SNPs and application of a subset of the identified SNPs to natural populations of *P. colocasiae* in Vietnam.

Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals plant pathogen-like effectors

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The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae and therefore has been seen as a potential biological control agent against disease vector mosquitoes. However, little is known about the pathological process of *L. giganteum* in its mosquito host. In order to detail the molecular basis of entomopathogenicity, Expressed Sequence Tags (EST) were generated using 454 pyrosequencing. To date, a total of 58,931 'Titanium chemistry' reads have been produced, and homology searches have led to the annotation of ca. 20,000 transcripts based on significant similarity to known proteins. Among these, plant pathogenic oomycete effector orthologs have been identified, and include a full complement of crinkler- and elicitin-like transcripts. In plant pathogenic oomycetes, these effectors have been associated with host cell cytotoxicity. Based on the phylogenetic closeness between *Lagenidium giganteum*, *Phytophthora* spp. and *Pythium* spp., the identification of these transcripts is not surprising. However, it provides a basis to evaluate the role and biological activity of crinkler and elicitin effectors in the context of entomopathogenicity.

Analysis of RNAseq data reveals most of the transcriptionally active unreported exons are unique to *Phytophthora sojae*

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We have analyzed RNAseq data from P.sojae mycelia with 4 experimental replicates each having an average of approximately 18 million reads. We used Bowtie short read aligner for initial alignment of reads to the genome sequences. Our attempts of including Tophat junction mapper for predicting junctions failed because of the read length; Tophat is optimized for paired end reads of length >=75 bp. As a work-around to determine how many reads were lost in the junction regions, we mapped the reads to the predicted transcripts and the unigenes derived from *P.sojae* EST libraries. We finally assembled the mapped reads to the genome sequence using Cufflink. Approximately 55% of the reads mapped to the genome sequence, whereas only 45% could be aligned to the predicted transcripts. The 10% reads that did not have a match with the transcripts were assembled to form crude exons. We analyzed these exons and studied their expression pattern. The most highly expressed exons that are not present in the existing gene models are found to be unique to *Phytophthora sojae* with no known similarities within NCBI's nr database. We have also curated the non-coding RNAs from the RNAseq data. Our transcriptomics database will temporarily store the assembled transcripts and raw aligned reads. Our browser is being upgraded to accommodate RNASeq viewing as well as text alignment viewing. Our transcriptomic database is available at http://est.vbi.vt.edu/

Uracil Phosphoribosyltransferase inhibition as a complementary target for *Phytophthora infestans* control

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Phytophthora infestans, the causal agent of tomato and potato late blight, produces high economic and environmental losses worldwide and it is also one of the worst diseases of potato crops in Colombia. Because of the emergence of new more aggressive strains and their resistance to traditionally used fungicides, it is important to generate new strategies for pathogen control. Our research group has followed a strategy, which has been already used with parasites of the class Apicomplexa that affects humans. It is based on the rational design of inhibitors against the enzymes involved in the *de novo* pyrimidine biosynthesis pathway, specifically dihydroorotate dehydrogenase and uridine monophosphate synthase. The enzymes in this pathway are promising targets because their blockage could affect replication and virulence of *P. infestans*.To complement this strategy we propose blocking at least one enzyme in the pyrimidine salvage pathway, which allows the pathogen to get pyrimidines by using the available intra and extracellular pools. After a genome search of the putative enzymes involved in pyrimidine salvage in *P. infestans*, we chose uracil phosphoribosyltransferase (UPRTase E.C. 2.4.2.9) because it is a central enzyme in this pathway. It catalyzes the formation of uridine monophosphate from uracil and phosphoribosyl pyrophosphate. It has 227 amino acids, a predicted molecular mass of 24.90kDa and a predicted isolectric point of 5.33. Its identity with UPRTase from *Solanum tuberosum*, *Homo sapiens, P. capsici, P. ramorum* and *P. sojae* is 32%, 36%, 91%, 93% and 95%, respectively. PiUPRTase was cloned in *E. coli* and it is in the process of being expressed and purified in its recombinant form. Once we achieve the biochemical characterization of the enzyme, we will be able of test uracil analogues for their inhibitory ability over PiUPRTase.

Towards engineering novel resistance against oomycete and fungal pathogens by interfering with phosphoinositide mediated entry of effectors

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Recent evidence suggests that oomycete and fungal effectors enter the plant cell via phosphoinositide binding. RXLR and RXLR-like motifs in these effectors were shown to be required for entry in soybean leaves and root cells. *E. coli* expressed proteins that specifically bind to the phosphoinositide PI3P were able to block effector entry. Based on these previous results from our lab, here we aim to design and test different strategies to protect plants against oomycete and fungal pathogens by targeting the phosphoinositides of the surface of the host cell. Using Agrobacterium-mediated transient expression we established a system for secretion of PI3P-binding domains and other proteins to the apoplast. The addition of the PR1a secretory leader directed most of the proteins to the apoplast of *N. benthamiana*. Furthermore, the addition of a fluorescent tag allowed us to monitor the expression and localization of the secreted proteins in planta. Using this system we are testing the stability of diverse secreted PI3P-binding domains as well as their ability to block pathogen growth in *N. benthamiana*. Stable transformants of *A. thaliana* are also being analyzed for resistance to different pathogens. Additionally, we are expressing non-secreted PI3P- and PI4P-binding domains in planta to analyze phosphoinositide distribution in the cell and its re-organization upon pathogen attack.

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