

Project No. and Title: NCCC307 Biochemistry and Genetics of Plant-Fungal Interactions

Period Covered: 10/28/2018 to 3/13/2019

Date of Report: 5/10/2019

Annual Meeting Date: 3/13/2019. Held as workshop concurrent with Fungal Genetics Meetings in Asilomar, CA

Participants:

Lorang, Jennifer (lorangj@science.oregonstate.edu) - Oregon State University; Brown, Daren (daren.brown@ars.usda.gov) - USDA-ARS Peoria, Illinois; Friesen, Tim (Timothy.Friesen@ars.uada.gov) - USDA-ARS Fargo, North Dakota; Liu, Zhaohui (zhliu@ndsu.edu) – North Dakota State University; Slot, Jason (slot.1@osu.edu) - Ohio State University; Redman, Regina (rsredman@adsymtech.com) - Adaptive Symbiotic Technologies Seattle, WA; Rodriguez, Rusty (rjrodriguez@adsymtech.com) - Adaptive Symbiotic Technologies Seattle, WA; Rollins, Jeff (rollinsj@ufl.edu) - University of Florida ; Todd, Richard (rbtodd@ksu.edu) - Kansas State University; Keller, Nancy (njkeller@wisc.edu) - University of Wisconsin; Trail, Frances (trail@msu.edu) - Michigan State University; Wolpert, Thomas (wolpertt@science.oregonstate.edu) - Oregon State University; Ciuffetti, Lynda (ciuffetl@science.oregonstate.edu) - Oregon State University; Venkatesh, Nandhitha (thiruvannama@wisc.edu) - University of Wisconsin; Yarden, Oded (Oded.Yarden@huji.ac.il) - The Hebrew University of Jerusalem; Nadal, Marina (marina@bunney.com) - Mycotechnology Inc.; Turgeon, Gillian (bgt1@cornell.edu) - Cornell University; Hunter, Cameron (chunter2@ksu.edu) - Kansas State University; Draper, Martin (maddr@ksu.edu) - Kansas State University; Kubo, Yasuyuki (y_kubo@kpu.ac.jp) - Kyoto University; Valent, Barbara (bvalent@ksu.edu) - Kansas State University; Chumley, Forest (fchumley@ksu.edu) - Kansas State University; Liang, Xiaofei - Northwest University; Xu, Jin-Rong (jinrong@purdue.edu) - Purdue University; Kistler, H. Corby (corby.kistler@ars.usda.gov) - USDA-ARS St. Paul, MN; Steyer, Joel (jsteyer@ksu.edu) - Kansas State University; Bansal, Kamaldeep - University of Florida

Brief Summary of Minutes of Annual Meeting:

NCCC-307 Meeting Minutes - March 13, 2019, Asilomar CA

8:00 pm– 8:30 pm – Remembering Marty Dickman

8:30 pm– 8:45 pm – Welcome by Co-chairs, Jenny Lorang and Daren Brown
Tribute to Dr. Marty Dickman

8:45 pm– 9:15 pm – Nandhitha Venkatesh, student of Nancy Keller
”Plant disease and microbial ecology in polymicrobial settings.”

9:15 pm– 9:35 pm – Joel Steyer, student of Richard Todd

“*LeuR*, a novel transcriptional factor, regulates both leucine biosynthesis and nitrogen assimilation genes in *Aspergillus nidulans*.”

Mrs. Venkatesh and Mr. Steyer both gave excellent presentations describing their work in their respective labs. Questions were many and were fielded with confidence. On multiple occasions, the answers given sparked more questions leading to lively discussions.

9:35 pm – 9:45 pm – Discussion of next NCCC307 Meeting

9:45 pm - 11 pm – Open discussion and sharing memories of Marty Dickman

Meeting Abstracts

Plant Disease and microbial ecology in polymicrobial settings

Nandhitha Venkatesh and Nancy P. Keller

Department of Plant Pathology, University of Wisconsin-Madison. Department of Medical Microbiology and Immunology and Department of Bacteriology, University of Wisconsin-Madison.

Aspergillus flavus and *Fusarium oxysporum* are ascomycete plant pathogens infamous for mycotoxin production. The ecological relationships that these fungi share with soil microbes have remained elusive. Our prior in vitro studies identified a bacterial lipopeptide, ralsolamycin secreted by the phytopathogen *Ralstonia solanacearum* that induces chlamydospore formation in fungi, following which *Ralstonia* invades and colonizes the chlamydospore. Further, we identified a secondary metabolite (SM) mediated cross-kingdom interaction between both *Ralstonia* and *Fusarium* spp. and *Ralstonia* and *Aspergillus*. Here, we explore the consequences of microbial interactions between *R. solanacearum* and *F. oxysporum* on wilt disease and the ecological fitness of the microbes. Further, we investigate the role of ralsolamycin-mediated interactions in polymicrobial settings. Co-infections with *R. solanacearum* and *F. oxysporum* show a significant reduction in bacterial wilt severity that appears to be mediated in part by *Fusarium* anti-bacterial SMs. Co-cultures in soil microcosms show a significant increase in retention of bacterial viability upon exposure to cold stress, presumably due to overwintering in chlamydospores. In addition, we find that other bacteria ‘hitchhike’ in chlamydospores of *A. flavus* in the presence of ralsolamycin. Future experiments using microscopy, flow cytometry and relevant genetic mutants of bacteria and fungi will identify the mechanism of interaction in planta and in soil. Understanding the ecological roles of such intimate bacterial-fungal interactions will refine our understanding of inoculum turnover between seasons. Insights into the mechanistic aspects of these interactions will inform novel management strategies for disease control.

LeuR*, a novel transcriptional factor, regulates both leucine biosynthesis and nitrogen assimilation genes in *Aspergillus nidulans

Joel T. Steyer, Damien J. Downes, Cameron C. Hunter, and Richard B. Todd

¹ Department of Plant Pathology, Kansas State University, Manhattan KS 66506, USA

Branched chain amino acids (BCAAs) leucine, isoleucine, and valine are essential amino acids, unable to be synthesized by animals and must be obtained through their diet. Many fungal species, including *Aspergillus*, *Candida*, and *Saccharomyces*, synthesize BCAAs. Proper regulation of BCAA metabolism is important for protein synthesis, growth, and virulence. In *Aspergillus nidulans*, the Zn(II)₂Cys₆ transcription factor LeuB regulates leucine biosynthesis in response to leucine levels. LeuB also regulates expression of *gdhA*, which encodes the key nitrogen assimilation enzyme NADP-glutamate dehydrogenase, NADP-GDH. We have identified a paralog of LeuB, LeuR, and examined its role in leucine biosynthesis and *gdhA* regulation. Phylogenetic analysis shows that while LeuB is conserved in Ascomycetes, LeuR is conserved only within Eurotiomycetes. The *leuB*Δ mutant is a leaky leucine auxotroph. We deleted *leuR* and found the *leuR*Δ mutant to be a prototroph. However, the *leuB*Δ *leuR*Δ double mutant is a tight leucine auxotroph, indicating a role for LeuR in regulating leucine biosynthesis. Using a *gdhA-lacZ* translational fusion reporter gene and exogenous leucine, we show that LeuR regulates *gdhA* expression. Previously, we used a series of deletions in the *gdhA* promoter to show that LeuB acts through two sites of action. We now are using these promoter deletions to compare the wild type strain to *leuB*Δ, *leuR*Δ, and *leuB*Δ *leuR*Δ mutants to identify where LeuR acts in the *gdhA* promoter. Our experiments show that the transcription factors LeuB and LeuR link regulation of nitrogen assimilation and leucine biosynthesis.

Accomplishments:

NCCC307 committee members collectively increased our understanding of biochemistry and genetics of plant-fungal interactions these past six months by: 1) increasing our understanding of the links between plant and fungal viruses, 2) better understanding the function of secondary metabolites, their synthesis, and their evolutionary history, 3) understanding meiotic drive and RNA editing, 4) understanding where inside a cell secondary metabolites are synthesized, and 5) appreciating how convergent assembly of metabolic modules contributes to fungal adaptation to plant defenses. These efforts have produced more than 41 peer-reviewed journal publications from October 2018 through March 2019 reporting period.

Impact Statement:

The publications by NCCC307 members describing their research findings has increased understanding of how plant-fungi interactions impact economically important diseases of crops and food production and threats to humans and animals through the consumption of mycotoxin contaminated foods. The exchange of ideas and biochemical and genetic data among members has contributed to these publications and

fostered new ideas and research directions. This research will be of use to plant pathologists, plant breeders, and other scientists involved in the development of control strategies aimed at reducing crop diseases and mycotoxin contamination problems caused by fungi. The research will also benefit academic, government, and private-sector organizations that assess the risks that fungi pose to human and animal health.

Publications

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