

Second Annual CREATE-IGERT Symposium



November 20 - 21, 2009 UC Davis Giedt and Kemper Halls

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2009 CREATE-IGERT SYMPOSIUM PARTICIPANTS

CREATE-IGERT UC Davis Participants

- **Arzola, Lucas, Chemical Engineering Graduate Program, Dept of Chemical Engineering & Materials Science, PhD Student
- **Bjornson, Marta, Agronomy & Horticulture Grad Program, Dept of Plant Sciences, PhD Student
- *Blumwald, Eduardo, Dept of Plant Sciences, Professor

Boczar, Barbara A., Innovation Access Technology Transfer Services, Assoc. Director

- **Butterfield, Timothy, Plant Biology Grad Group, Dept of Molecular & Cellular Biology, PhD Student
- **Castillo, Elenor, Plant Biology Grad Group, Dept of Plant Sciences, PhD Student
- **Chiniquy, Dawn, Plant Biology Grad Group, Dept of Plant Pathology, PhD Student
- *Coaker, Gitta, Dept of Plant Pathology, Assistant Professor

Cuevas, Hector, Graduate Studies Outreach, Recruitment & Retention, Director

- *Dandekar, Abhaya, Dept of Plant Sciences, Co-PI CREATE-IGERT
- *Dandekar, Satya, Dept of Medical Microbiology & Immunology, Chair & Professor
- *Dehesh, Katayoon, Dept of Plant Biology, Professor
- **Elmore, J. Mitch, Plant Biology Grad Group, Dept of Plant Pathology, PhD Student
- *Falk, Bryce, Dept of Plant Pathology, Professor
- *Fan, Zhilian (Julia), Dept of Biological & Agricultural Engineering, Assistant Professor
- *German, Bruce, Dept of Food Science & Technology, Professor

Gibeling, Jeffery, Graduate Studies, Dean

**Glavan, Tiffany, Microbiology Grad Group, Dept of Medical Microbiology & Immunology, PhD Student

Gouran, Hossein F., Dept of Plant Sciences, Specialist

Hamann, Bernd, Dept of Computer Science, Professor and Office of Research, Associate Vice-Chancellor

Hu, Wei, Dept of Molecular & Cellular Biology, Post-Doc

Ibanez, Ana M., Dept of Plant Sciences, Project Scientist

Jamison-McClung, Denneal, Biotechnology Program, Associate Director & Program Coordinator CREATE-IGERT

*Jenkins, Bryan, Dept of Biological & Agricultural Engineering, Professor

Joh, Larry, Chemical Engineering & Materials Science, CREATE-IGERT Program Engineer

Jung, Sang-Kyu, Chemical Engineering Graduate Program, Dept of Chemical Engineering & Materials Science, PhD Student

Kingsbury, Nathaniel, Chemical Engineering Graduate Program, Dept of Chemical Engineering & Materials Science, PhD Student

Kjelstrom, Judy, Biotechnology Program, Director & Senior Personnel CREATE-IGERT Klein, Barry M., Office of Research, Vice-Chancellor

*Kliebenstein, Daniel, Dept of Plant Sciences, Assistant Professor

2009 CREATE-IGERT SYMPOSIUM PARTICIPANTS (Cont.)

 st Lagarias, J. Clark, Dept of Molecular and Cellular Biology, Professor

Liu, Bo, Dept of Plant Biology, Associate Professor

*Lebrilla, Carlito, Dept of Chemistry, Professor

Mahan, Kristina, Microbiology, PhD Student

Matvienko, Marta, Genome Center & Bioinformatics Program, Assoc. Project Scientist

*McDonald, Karen, Chemical Engineering & Materials Science, Associate Dean & Director CREATE-IGERT

McCaffrey, Zach, Institute of Transportation Studies, PhD Student

McGee, David, Innovation Access, Executive Director

*Negre-Zakharov, Florence, Dept of Plant Sciences, Assistant Professor

Newell, McGloughlin, Martina, UC BREP, Director & Co-PI CREATE-IGERT

Nitin, Nitin, Dept of Food Science & Technology, Assistant Professor

Overacker, Karina, Dept of Biomedical Engineering, Undergraduate Student

*Parales, Becky, Dept of Microbiology, Professor

Rattanaporn, Kittipong, Chemical Engineering Grad Program, Chemical Engineering & Materials Science, PhD Student

*Ronald, Pamela, Dept of Plant Pathology, Professor, Co-PI CREATE-IGERT

*Savageau, Michael, Dept of Biomedical Engineering, Professor

Shoemaker, Sharon, California Institute Food & Agricultural Research (CIFAR), Executive Director

Theg, Steven, Dept of Plant Biology, Professor

*VanderGheynst, Jean, Biological & Agricultural Engineering, Associate Dean & Co-PI CREATE-IGERT

*Yilma, Tilahun, Dept of Pathology, Microbiology & Immunology, School of Veterinary Medicine, Distinguished Professor

*Yoder, John, Dept of Plant Sciences, Professor

CREATE-IGERT Tuskegee University Participants

**Gayles, Dominique, IBS PhD Trainee

**Lateef, Dayla, IBS PhD Trainee

Miller, Sonni, IBS PhD Affiliated Student

^{**}Lindenmuth, Ben, Chemical Engineering Grad Program, Dept of Chemical Engineering & Materials Science, PhD Student

^{**}Simmons, Chris, Biological Systems Engineering Grad Program, Dept of Biological & Agricultural Engineering, PhD Student

^{*}CREATE-IGERT Faculty Trainer

^{**}CREATE-IGERT Trainee

2009 CREATE-IGERT SYMPOSIUM PARTICIPANTS (Cont.)

- **Odom, LaKisha, IBS PhD Trainee
- **Samuels, Steven, MS Trainee
- **Shange, Raymon, IBS PhD Trainee

CREATE-IGERT Industry Participants

Castle, Linda, Pioneer Hi-Bred Verdia Research Campus, Research Director Huang, Ning, Ventria Bioscience, Director Tricoli, David, Nunhems USA, Scientist Yaver, Debbie, Novozymes, Director

CREATE-IGERT Community Participants

O'Neal, Jeffery, California Community College Biological Technologies Initiative, Director

Savage, Thomas, Sacramento State University Center for Interdisciplinary Molecular Education Research & Advancement, Director and Department of Chemistry, Professor

Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE) IGERT Symposium and Distinguished Lecture

November 20 – 21st, 2009 Giedt Hall. UC Davis

Welcome to the second CREATE-IGERT Distinguished Lecture and Symposium!

The Integrative Graduate Education and Research Traineeship (IGERT) is a National Science Foundation program that encourages new approaches to interdisciplinary graduate education to prepare students to tackle complex, multifaceted real-world problems. The Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE) IGERT, is a multi-institutional, international educational partnership between UC Davis, Tuskegee University, the National University of Ireland at Maynooth, and the Teagasc Oak Park Research Centre, in Carlow, Ireland. CREATE integrates training in the plant sciences, molecular biology and engineering, to advance research and catalyze breakthroughs in the sustainable use of plants for production of non-food products ranging from biofuels to vaccines. In addition to the underlying scientific and engineering principles, trainees develop an understanding of the complex interconnected issues (environmental, ecological, sustainability, public/societal concerns, global impact, regulatory, intellectual property), preparing them as the research, educational, business, and policy leaders of the future.

Thank you for joining us as we honor our 2009 trainees, as well as our Tuskegee partners, faculty trainers, industry affiliates, and Distinguished Lecturer, Dr. Maurice Moloney, Founder and Chief Scientific Officer of SemBioSys.

I'd especially like to thank Dr. Denneal Jamison-McClung, CREATE-IGERT Program Coordinator and Associate Director of the Biotechnology Program, and Dr. Judith Kjelstrom, Director of the Biotechnology Program, as well as the entire Biotechnology Program Staff, particularly Marianne Hunter, for all of their hard work in organizing this symposium.

The CREATE program is made possible through funding by the <u>National Science Foundation (DGE-0653984)</u>, and support from the UC Davis Office of Research, Office of Graduate Studies, Biotechnology Program and Department of Chemical Engineering & Materials Science.

With warmest regards,

Karen McDonald
Director, CREATE-IGERT Program

CREATE-IGERT Distinguished Lecture and Symposium Schedule November 20 – 21st, 2009 Giedt and Kemper Halls, UC Davis

8:00-8:30am	Registration & Coffee	
8:30-8:40am	Welcome (Dean Jeff Gibeling, Office of Graduate Studies)	
8:40-8:50am	Overview of the CREATE-IGERT Training Program (Prof. Karen McDonald, CREATE-IGERT Director)	
8:50-9:00am	CREATE-IGERT Research Experiences for Undergraduates (Prof. Jean VanderGheynst)	
Session1: Plant-Made Products		
9:00-9:25pm	Prof. Karen McDonald introducing Lucas Arzola "High-Level Transient Expression of Anthrax Receptor Decoy Protein in Nicotiana Benthamiana Plants by Agroinfiltration"	
9:25-9:50pm	Prof. Karen McDonald introducing Tiffany Glavan "Utilizing a TRBO-Based Plant Expression System to Accelerate Epithelial Regeneration"	
9:50-10:15am	Prof. Jean VanderGheynst introducing Chris Simmons "A Kinetic Model of the T-Strand Secretion Pathway of Agrobacterium tumefaciens"	
10:15-10:30am	Coffee Break	
Session 2: Biofuels & Biorefineries		
10:30-10:55am	Dr. Denneal Jamison-McClung introducing Dawn Chiniquy "Characterizing Rice Genes Involved in Glucuronoarabinoxylan (GAX) Synthesis	
10:55-11:20am	Prof. Karen McDonald introducing Ben Lindenmuth "Production of a Recombinant Endoglucanase in N Bethamiana and P Pastoris"	
11:20-11:45pm	Prof. Rebecca Parales introducing Mark Wolf "Thermostable Enzymes From Acidothermus Cellulolyticus"	

11:45-12:30pm

Lunch

Session 3: Environmental Sustainability

12:30-12:55pm	Prof. Clark Lagarias introducing Timothy Butterfield "Targeted Regulation of Phytochrome Signaling Using Constitutively Active Alleles"
12:55-1:20pm	Prof. Florence Negre-Zakharov introducing Elenor Castillo "Sulfur Volatile Compounds: An alternative to pesticides for the Citrus disease Huanglongbing"
1:20-1:45pm	Prof. Gitta Coaker introducing J. Mitch Elmore "Investigating Host-Mediated Activation of Bacterial Effector Proteins"
1:45-2:10pm	Dr. Denneal Jamison-McClung introducing Rachel Kerwin "Investigating the Importance of Natural Variation in the Glucosinolate Pathway using Arabidopsis thaliana"
2:10-2:35pm	Prof. Karen McDonald introducing LaKisha Odom, Tuskegee University "Effect of Cotton Plant Genetically Modified with An Antimicrobial Synthetic Peptide D4E1 On Soil Microbial Diversity and Enzyme Activity"
2:35-3:00pm	Prof. Karen McDonald introducing Raymon Shange, Tuskegee University "Assessing the Environmental Sustainability of Coupled Human-Natural Systems as Indicated by Microbial Community Structure"
3:00pm	Closing Remarks—Prof. Karen McDonald

IGERT: Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE) (NSF Award DGE0653984)



UC Davis P.I.s & Co P.I.s

Karen McDonald, Principal Investigator – UC Davis Abhaya Dandekar, Co-Principal Investigator – UC Davis Martina Newell-McGloughlin, Co-Principal Investigator – UC Davis Pamela Ronald, Co-Principal Investigator – UC Davis Jean VanderGheynst, Co-Principal Investigator – UC Davis Denneal Jamison-McClung, Program Coordinator – UC Davis Larry Joh, Program Engineer – UC Davis

Tuskegee University P.I.s & Co P.I.s

Luther Williams, Principal Investigator – Tuskegee University Jesse Jaynes, Co-Principal Investigator – Tuskegee University C.S. Prakash, Co-Principal Investigator – Tuskegee University Deloris Alexander, Program Coordinator – Tuskegee University

IGERT: Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE)

NSF Award DGE-0653984 August 15, 2007 – July 31, 2012

UC Davis has been awarded the multi-institutional IGERT: Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE) grant from The National Science Foundation in the amount of \$599, 824. The grant is under the direction of Karen A. McDonald; Department of Chemical Engineering, with co-PIs: Abhaya M. Dandekar, Department of Plant Sciences; Jean S. VanderGheynst, Department of Biological and Agricultural Engineering; Martina Newell-McGloughlin, UC BREP; and Pamela C. Ronald, Department of Plant Pathology. The lead institution is the University of California at Davis, Davis, CA and collaborating institutions are Tuskegee University, Tuskegee, AL (Luther S. Williams, PI); National University of Ireland, Maynooth, Ireland (Dr. Phil Dix, PI); Teagasc Oak Park Research Centre, Carlow, Ireland (Dr. James Burke, PI).

The IGERT program, entitled Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE), will provide a structured and well-integrated graduate research and educational training program focused on a unifying theme of transgenic plants and *in-vitro* plant systems for the production of industrial non-food products and biopharmaceuticals. Research focus areas are 1) Plant-Made Products, 2) Biofuels and Biorefineries, and 3) Environmental Sustainability. Across the three broad focus areas, specific attention will be paid to the scientific, engineering, environmental, regulatory, economic, intellectual property, societal and global issues associated with plant biotechnology.

The **Project Objectives** for CREATE-IGERT are to:

- 1. CREATE a **framework for interdisciplinary graduate training** that will foster an environment for revolutionary breakthroughs at the interface of plant science, biotechnology, and engineering.
- CREATE new scientific knowledge, engineering technologies, tools, methods, processes, and global understanding to advance the fields of plant science, biotechnology, engineering and areas at the interface of these disciplines, particularly those related to the underlying theme.
- 3. CREATE and cultivate the **integrative skill set** in graduate student trainees, faculty trainers, and postdoctoral scholar participants using the underlying theme as the focus.
- 4. CREATE a training program to attract, retain, and graduate doctoral students from diverse backgrounds who are not only top-rated scientists and engineers but also have the variety of skills and understanding to approach problems from integrated perspectives, allowing them to become the academic, industrial, national laboratory, and/or policy leaders in areas related to the unifying theme.
- 5. CREATE a **Masters to PhD Bridge Program** that strengthens research and graduate training linkages between UC Davis and Tuskegee University in areas related to plant biotechnology and provides a guided transition for MS students at Tuskegee into doctoral programs at UC Davis.

To apply for IGERT support, students must be a member of the DEB



Designated Emphasis in Biotechnology Program (DEB)

Goals and Mission of the DEB

The Designated Emphasis in Biotechnology (DEB) is an inter-graduate group program that allows Ph.D. students to receive and be credited for training in the area of biotechnology. The DEB provides a nurturing interactive environment to promote integration of multiple disciplinary approaches to the conduct of research and to promote learning in biotechnology. The mission is to prepare well-educated students to approach problems with creativity and flexibility. The program will provide tools for the students to be leaders, visionaries, entrepreneurs, researchers and teachers in the broad area of biomolecular technology.

DEB Mission:

- To provide well-coordinated, cross-disciplinary training of graduate students in critical areas of biomolecular technology research.
- To promote interdisciplinary research environments that integrate basic biological science, engineering and computational disciplines.
- To allow cross-disciplinary training and trainee experience in a biotechnology company or cross-college laboratory.

Students come from a wide array of disciplines: Participating graduate programs currently include 28 programs: Agricultural and Environmental Chemistry; Animal Biology; Applied Science; Biochemistry and Molecular Biology; Biological Systems Engineering; Biomedical Engineering; Biophysics; Cell & Developmental Biology; Chemical Engineering; Chemistry; Civil and Environmental Engineering; Comparative Pathology; Electrical & Computer Engineering, Entomology; Food Science Technology; Genetics; Immunology; Materials Science & Engineering; Mechanical and Aeronautical Integrative Engineering; Microbiology: Molecular. Cellular & Physiology: Neurosciences; Nutritional Biology; Pharmacology & Toxicology; Plant Biology; Plant Pathology; Soils & Biogeochemistry and Statistics. The DEB program supplements a student's Ph.D. curriculum and those completing the program will obtain an official designation on their diploma & transcript indicating a qualification in biotechnology. Example: Doctoral Degree in Microbiology with a Designated Emphasis in Biotechnology

*CREATE-IGERT Trainees must be enrolled in the DEB



Session 1: Plant-Made Products

HIGH-LEVEL TRANSIENT EXPRESSION OF ANTHRAX RECEPTOR DECOY PROTEIN IN NICOTIANA BENTHAMIANA PLANTS BY AGROINFILTRATION

Trainee: Lucas Arzola

Faculty Trainer: Karen McDonald

Campus: UC Davis

Lucas Arzola*, Nathaniel Kingsbury, Michael Plesha, and Karen A. McDonald Department of Chemical Engineering & Materials Science, University of California Davis

The 2001 anthrax mailings raised awareness of the devastating effect a massive bioterrorist attack could have. As a result, anthrax was designated as the number one biological warfare threat to the United States. Rapid, large scale and cost-effective production of therapeutic agents is needed to respond in the event of large scale exposure. Recent advances in the field of plant biotechnology have made possible the use of plants as cost-effective factories of therapeutic proteins. *Agrobacterium tumefaciens* mediated transformation, utilizing agroinfiltration, can be used to rapidly induce the transient expression of the protein of interest. Our research focuses on developing an effective production platform for anthrax receptor decoy protein, PBI-220, by agroinfiltration of leaves on *Nicotiana benthamiana* plants. PBI-220 mitigates the effects of anthrax by preventing the production of the deadly anthrax toxins. Transient expression has reached a maximum at 7 days post-infiltration and resulted in a maximum production of (1.3 ± 0.10) mg of PBI-220 per g fresh weight of leaf. We are currently investigating which agroinfiltration conditions lead to increased protein expression. We have also started to study the subcellular localization of the protein by imaging with confocal microscopy.

UTILIZING A TRBO-BASED PLANT EXPRESSION SYSTEM TO ACCELERATE EPITHELIAL REGENERATION

Trainee: Tiffany Glavan

Faculty Trainer: Satya Dandekar

Campus: UC Davis

Tiffany W Glavan*1, Abhaya Dandekar2, Satya Dandekar1, Bryce Falk3, Larry Joh4, Sang-Kyu Jung4, Karen McDonald4

¹Department of Medical Microbiology and Immunology, University of California, Davis, CA

²Department of Plant Sciences, University of California, Davis, CA

³Department of Plant Pathology, University of California, Davis, CA

⁴Department of Chemical Engineering and Materials Science, University of California, Davis, CA

Cells that comprise the intestinal epithelium are continually differentiating and migrating upward into the villi. Regeneration and renewal of this single cell layer is an ongoing process that is integral to gastrointestinal health and immune function. We are interested in developing a plant-based therapeutic aimed at accelerating mucosal regeneration and repair mechanisms, thus increasing barrier function in the gastrointestinal tract. The mitogenic protein of interest is R-spondin1, a growth factor known to positively regulate cell signaling in the Wnt pathway through an interaction with DKK1. As a therapeutic, this protein has the potential to provide a restoration of function in the context of multiple disease states that disrupt the intestinal epithelium, including radiation and chemotherapy-induced mucositis and inflammatory bowel disease.

A multidisciplinary collaborative project has been initiated to develop an *Agrobacterium*-based plant expression system to produce this protein in tobacco plants. The human gene for R-spondin1 has been re-designed for expression in *N.benthamiana* through codon optimization and the addition of Kozak's context sequence, a six histidine tag, and mRNA secondary structure aimed at decreasing degradation. This synthetic gene construct was inserted into a tobacco mosaic virus RNA-based overexpression (TRBO) vector, which was then electroporated into *Agrobacteria*. *N.benthamiana* leaves were co-infected with this construct along with a vector coding for the p19 gene silencing suppressor. Initial dotblot, SDS-PAGE, and western blot analyses of the crude protein extract reveal positive expression of R-spondin1 with a molecular weight of approximately 32.8kDa. We plan to purify the protein using nickel-based affinity chromatography and analyze its bioactivity using a BrdU assay on treated Caco-2 cells. Transcriptional kinetics will be investigated using pRT-PCR and post transcriptional modifications will be determined through mass spectrometry.

A KINETIC MODEL OF THE T-STRAND SECRETION PATHWAY OF AGROBACTERIUM TUMEFACIENS

Trainee: Chris Simmons

Faculty Trainer: Jean VanderGheynst

Campus: UC Davis

Chris Simmons*, Jean VanderGheynst

Department of Biological and Agricultural Engineering, UC Davis

Agrobacterium-mediated gene transfer (agroinfiltration) is a common plant transformation technique. However, the efficacy of agroinfiltration varies widely between plant species. We are interested in studying the kinetics of gene transfer from A. tumefaciens to plant cells. In the process, we plan to create a methodology for identifying the rate limiting steps in the gene secretion pathway that act to reduce transformation efficiency in various plants. During agroinfiltration, genetic material from Agrobacterium, the T-strand, is exported from the bacteria into plant cells. Ultimately, genes housed on the T-strand may be expressed by the infected plant cells via transient expression. A variety of virulence (Vir) proteins encoded by Agrobacterium facilitate this DNA secretion process. Unique Vir proteins form complexes with the T-strand at distinct steps in the pathway. Immunofluorescence microscopy and fluorescence in situ hybridization (FISH) will be used to quantify T-strands at key pathway steps from extracts of agroinfiltrated plant tissue based on their associations with certain Vir proteins. We will collect T-strand concentration data over time for each pathway step. This data will be used to fit parameters in a power-law mass balance describing the flux of T-strands through each step of the agroinfiltration pathway. The resultant model will be used to identify rate-limiting steps of the pathway. This model will be a powerful tool for optimizing in planta transient expression of agroinfiltrated genes and will have implications for the biopharmaceutical and biofuel industries, where plant-based transient expression may be desirable.



Session 2: Biofuels & Biorefineries

CHARACTERIZING RICE GENES INVOLVED IN GLUCURONOARABINOXYLAN (GAX) SYNTHESIS

Trainee: Dawn Chiniquy

Faculty Trainer: Pamela Ronald

Campus: UC Davis

Dawn Chiniquy* 1, 2, Miguel Vega-Sanchez 1, 2, Laura Bartley 1, 2, Peijian Cao 1, 2, Henrik Scheller 2, and Pamela Ronald 1, 2

¹ Joint BioEnergy Institute, 5885 Hollis St, Emeryville, CA 94608

² Department of Plant Pathology, University of California, Davis, CA 95616

Biofuels derived from cellulosic biomass have the potential to reduce US dependence on foreign oil, decrease net carbon emissions, and provide underdeveloped countries with a means to gain greater energy resources. However, the enzymatic and chemical conversion process of cellulosic biomass is inefficient and expensive. It is thought that certain modifications to xylan could lead to more efficient break down of cellulosic biomass for fermentation into fuels. Xylan is the second most abundant polysaccharide on the planet, after cellulose, yet the genes involved in its synthesis are largely unknown. To gain a greater understanding of xylan synthesis and provide a basis for future xylan modifications, we will be conducting a reverse genetics screen—generating and screening mutant rice plants in each of 35 genes from two glycosyltransferase families. These genes have been implicated in xylan synthesis through bioinformatic analysis and molecular characterization of six xylan synthesis mutants in *Arabidopsis*. We will screen plants by looking for alterations in hemicellulose and cellulose composition, and also changes in saccharification efficiency to determine if the cellulosic material from the mutant plants is easier to break down into component sugars. Those plants with altered cell wall composition or saccharification efficiency will be further characterized to gain a greater understanding of xylan synthesis in rice.

PRODUCTION OF A RECOMBINANT ENDOGLUCANASE IN N. BENTHAMIANA AND P. PASTORIS

Trainee: Ben Lindenmuth

Faculty Trainer: Karen McDonald

Campus: UC Davis

Ben Lindenmuth* and Karen McDonald

Department of Chemical Engineering & Materials Science, University of California, Davis, CA

Biofuels such as ethanol are fermented from glucose, and the cellulose in biomass is a potential source of this sugar. Large quantities of low-cost enzymes are needed to degrade the cellulose into glucose. In this project, leaves harvested from *Nicotiana benthamiana* plants are infiltrated with recombinant *Agrobacteria tumefaciens* to produce these enzymes. These bacteria carry the gene encoding endoglucanase from the thermophilic bacteria *Acidothermus cellulolyticus*, which is transferred to the host plant and expressed transiently. The endoglucanase can then be recovered and combined with other synergistic enzymes for cellulose degradation.

This presentation focuses on the quantification of endoglucanase recovered from plant tissues. A fluorescence-based activity assay is used to measure enzyme activity in leaf extracts. However, this assay cannot report deactivated enzyme, and cannot correctly quantify enzymes with suboptimal activity. As the first step toward developing an ELISA assay to overcome these issues, an endoglucanase standard has been produced in the yeast *Pichia pastoris*. The active enzyme is secreted into the culture media, facilitating recovery and purification by ultrafiltration and immobilized metal affinity chromatography. Without optimization, the protein is expressed in flask cultures at a concentration of up to 50 mg/L. This endoglucanase will be characterized and used as an ELISA standard to evaluate the endoglucanase produced by agroinfiltration of *N. benthamiana* plant tissues.

THERMOSTABLE ENZYMES FROM ACIDOTHERMUS CELLULOLYTICUS

Trainee: Mark Wolf

Faculty Trainer: Rebecca Parales

Campus: UC Davis

Mark S. Wolf*1, Juan V. Parales1, Ravi D. Barabote2, Alison M. Berry2, and Rebecca E. Parales1

¹Department of Microbiology, University of California, Davis, CA ²Department of Plant Sciences, University of California, Davis, CA

Acidothermus cellulolyticus 11B is a Gram positive actinomycete that was isolated from hot springs in Yellowstone National Park using cellulose as the carbon source. A. cellulolyticus is an acid tolerant (pH 4-6; optimum pH 5.5) thermophile (37-70°C; optimum growth temperature 55°C). Due to these characteristics, it has potential as a source for thermostable enzymes with the ability to depolymerize components of the plant cell wall. Development of advanced fuels and other industrial products derived from lignocellulosic biomass depends upon the ability to access and depolymerize the components of plant cell walls. Plant cell walls, however, are organized in a complex crosslinked matrix of crystalline cellulose microfibrils, hemicellulose, pectin, and lignin. The lignin and hemicellulose provide structural and chemical protection from depolymerization by enzymes. The cellulolytic capabilites of A. cellulolyticus have been intensively investigated, and one cellulase (E1) is currently used commercially. In contrast, hemicelluloses degradation by A. cellulolyticus remains unexplored. Hemicelluloses are heterogenous branched polysaccharides that account for 25-35% of lignocellulosic biomass. Xylan is the primary component of hemicellulose and is a polymer of pentoses such as xylose and arabinose linked by β-1,4-glycosidic bonds. Microbes employ xylanases to cleave these bonds and depolymerize the polysaccharide. A. cellulolyticus is capable of robust growth on xylan and the complete genome sequence revealed 35 predicted glycosyl hydrolases and eight carbohydrate esterases. Presented here is the purification and characterization of a family 10 endo-[-] 1,4-xylanase (Xyl-1). Purified Xyl-1 showed enzymatic activity on xylans from oat spelts and birchwood and was active between 30°C and 100°C, and from pH 3-9. Optimal activity was at 90°C and the optimal pH (4.5-6.0) varied with the reaction temperature. Xyl-1 retained activity for extended periods of time at high temperatures when in the presence of xylan substrates. Work is currently being done to purify and characterize a second xylanase as well as an esterase. Synergistic activity of these enzymes on xylan substrates will be investigated.



Session 3: Environmental
Sustainability

TARGETED REGULATION OF PHYTOCHROME SIGNALING USING CONSTITUTIVELY ACTIVE ALLELES

Trainee: Timothy Butterfield Faculty Trainer: J. Clark Lagarias

Campus: UC Davis

Timothy Butterfield*, Wei Hu and J. Clark Lagarias

Section of Molecular and Cellular Biology, College of Biological Sciences, University of California, Davis, CA

Plants must optimize their growth habit to maximize the capture of limiting resources, including photosynthetically active radiation found in the Red (R) and Blue (B) wavelengths. Given the importance of R and B wavelengths for fitness, it is not surprising that photoreceptors that perceive R and B have evolved in cyanobacteria, lower, and higher plants. Phytochromes (Phys) are a family of photoreceptors that covalently bind a bilin chromophore and perceive R, Far Red (FR), and B radiation. Upon absorption of R, the chromophore rearranges, inducing a structural change in the holoprotein, thereby activating the photoreceptor. Photoreceptor activation promotes large-scale developmental changes including seed germination, photomorphogenesis, growth habit, and flowering. Our laboratory has identified a class of dominant, constitutively active mutant alleles of phyA (YHA) and phyB (YHB) 1; more recently, we have reported that YHB faithfully recapitulates phyBregulated gene expression networks in a light-independent manner². By co-transforming a collection of Arabidopsis thaliana mutants with affinity-tagged YHA or YHB alleles, and the cyanophage bilin biosynthetic enzyme PebS³ we are building a system to manipulate and probe the biochemistry of YHA- or YHB-regulated signaling. PebS produces a chromophore that fails to support photochemistry in WT phys, but is sufficient to activate YHA and YHB; thus our system will permit activation of the YH alleles under dark or light conditions – without activating any WT phytochromes, but permitting B signaling regulated by the Phototropin and Cryptochrome photoreceptors. Our query is three-fold: we are seeking to 1.) identify unique and shared target genes of PhyA and PhyB throughout development, 2.) characterize YHA and YHB functional interactions at discrete developmental time points, and 3.) isolate novel mutations in the Phy molecule that suppress the constitutive activation of YHA or YHB. We expect that an increased understanding of phy biochemistry and developmentally specific interacting partners may be leveraged to regulate photomorphogenic traits of agronomically important crop species to produce robust, environmentally sustainable crops of value to biofuel and plant-made products applications.

SULFUR VOLATILE COMPOUNDS: AN ALTERNATIVE TO PESTICIDES FOR THE CITRUS DISEASE HUANGLONGBING

Trainee: Elenor Castillo

Faculty Trainer: Florence Negre-Zakharov

Campus: UC Davis

Elenor Castillo*, Abhaya Dandekar and Florence Negre-Zakharov Department of Plant Sciences, University of California, Davis, CA

The goal of this project is to develop prototype plants that will be components of a 'transgenic push-pull system' a novel transformative strategy to manage Huanglongbing (HLB) also known as citrus greening disease. Currently there is no cure for HLB and once infected death occurs within two years. The destructive insect vectored bacterial disease of citrus is caused by *Candidatus* Liberibacter asiaticus (CaLas) in Asia and *Candidatus* Liberibacter africanus (CaLaf) in Africa. Once developed and deployed, our system will be compatible with biocontrol and other integrated pest management (IPM) practices, and will allow small growers to successfully grow citrus in areas where the disease is endemic. Furthermore, it would reduce the input of chemical pesticides, lower production costs and improve the yield and quality of the fruit. Our system has a behavior modifying, transgenic 'push component' where plants are engineered to express a volatile insect repellent and proteins that disrupt feeding behavior. Push plants will be inter-planted in existing citrus orchards or used as a rootstock in new plantings, grafted to conventional scion varieties to ward off (push) the disease carrying insect vector. The transgenic push system is designed to manipulate the behavior of the insect vector such that the presence of repellents make the protected citrus plants unattractive and clear the pathogen.

INVESTIGATING HOST-MEDIATED ACTIVATION OF BACTERIAL EFFECTOR PROTEINS

Trainee: J. Mitch Elmore Faculty Trainer: Gitta Coaker

Campus: UC Davis

J. Mitch Elmore*, Gitta Coaker

Department of Plant Pathology, University of California, Davis, CA

We are interested in understanding how the plant innate immune system functions to provide effective resistance against a broad spectrum of pathogens. To this end, we are currently using bacterial effector proteins as tools to probe different aspects of plant innate immunity. Effector proteins are essential virulence determinants for many Gram-negative bacterial plant pathogens and several have been demonstrated to suppress various aspects of plant immunity. These proteins are delivered into the host cell cytoplasm via the Type III Secretion System (T3SS) during infection and collectively contribute to pathogen fitness on host plants. Due to size constraints of the T3SS, effectors are delivered as either partially or completely unfolded proteins and it is hypothesized that many exploit plant folding catalysts for activation. One such folding catalyst, cyclophilin, has been identified previously. Cyclophilins are molecular chaperones that catalyze the *cis/trans* isomerization of peptidyl-prolyl bonds, a rate-limiting step during protein folding.

We have designed and implemented a targeted protein interaction screen to identify effectors that interact with the *Arabidopsis* cyclophilin ROC1. To date, eight effectors from different species of phytopathogenic bacteria have been identified in this screen. Preliminary data indicates that several of these effectors are indeed activated through interaction with host cyclophilins. Identification of the host activators of bacterial effectors will enable the production of enzymatically active effector proteins *in vitro* and facilitate investigations into their functions during the infection process. Innovative strategies for sustainable disease control in agriculture can be developed by understanding the molecular mechanisms underlying plant-pathogen interactions.

INVESTIGATING THE IMPORTANCE OF NATURAL VARIATION IN THE GLUCOSINOLATE PATHWAY USING ARABIDOPSIS THALIANA

Trainee: Rachel Kerwin

Faculty Trainer: Dan Kliebenstein

Campus: UC Davis

Rachel Kerwin* Dan Kliebenstein

Department of Plant Sciences, University of California, Davis, CA

Glucosinolates (GLS) are a class of secondary metabolites found in leaf tissue and seeds of plants from the order Brassicales, of which *Arabidopsis thaliana* is a member. These metabolites are natural pesticides that are activated upon herbivore attack (e.g. a caterpillar chewing on the leaves of the plant). In nature, there are hundreds of accessions of Arabidopsis thaliana that live in different environments and all produce this class of compounds. Due to natural variation in genes encoding transcription factors and biosynthetic enzymes, wild accessions of Arabidopsis thaliana show significant intraspecific variation in GLS phenotypes. However, analysis of 300 wild accessions suggests that natural populations contain only a subset of the potential GLS genotypes that can be generated in the laboratory by crossing these plants (Kliebenstein et al., 2001; Lambrix et al., 2001; and unpublished data). This suggests that there is selection for and against specific phenotypes that restricts which genotypes survive and persist in wild populations. In addition, variation in GLS profiles is also likely being selected for due to diversity in herbivore communities. In order to test these hypotheses, I will create the majority of potential aliphatic GLS variation using mutants in Arabidopsis thaliana accession Columbia-0 (Col-0). By using mutants that mimic natural polymorphisms (mutations) present within 9 genes representing 8 loci involved in glucosinolate diversity, a population expressing the different combinations of alleles in this metabolic pathway will be generated. This population will then be used to directly test the proposed hypotheses.

EFFECT OF COTTON PLANT GENETICALLY MODIFIED WITH AN ANTIMICROBIAL SYNTHETIC PEPTIDE D4E1 ON SOIL MICROBIAL DIVERSITY AND ENZYME ACTIVITY

Trainee: Lakisha Odom

Faculty Trainers: Jesse Jaynes and Ramble Ankumah

Campus: Tuskegee University

Lakisha Odom*, Ramble O. Ankumah, Conrad Bonsi, Jeff Cary, Marceline Egnin, Jesse Jaynes, Desmond Mortley, Lanell Ogden, and Kanniah Rajasekaran

Department of Agriculture and Environmental Science, Tuskegee University, AL

Cotton seedling disease is a fungal disease complex comprised of several fungal pathogens. In Alabama, those pathogens are Rhizoctonia Solani and Pythium. Cotton Seedling Disease, which results in loss of cotton production revenues totaling over 10 million dollars per year, in Alabama alone, has no known disease resistant cultivars. Synthetic antimicrobial peptides have been used in previous research in order to confer phytopathogen control. In an effort to confer resistance through genetic modification, a synthetic antimicrobial peptide D4E1, which has been shown in vitro and in-plants to have broad spectrum antimicrobial action against many fungal orders, has been transformed into cotton seeds to examine the efficacy of this peptide on the control of Cotton Seedling Disease Complex in transformed cotton plants in a field setting. Three 150 x 150 ft test plots, over two field seasons, were arranged in a completely randomized design and were assigned either one of 3 lines of cotton seed transformed with D4E1 (designated 357, 358, and 373) or a control line containing a GUS marker gene. In test plot 1, there is a significant difference (P < 0.05) between lines and the control. When comparing mean cotton seedling germination scores, in field plot 1, there was a significant difference (P≤ 0.05) between each of the lines and test field 2 there was no difference between the lines and the control, while test field 3 showed enhanced resistance in the isogenic lines. Soil samples were also subjected to Phosphatase enzyme assays and there were no differences between the control and the three isogenic lines, although there were differences that were associated with time. Soil samples were also subjected to pyrosequencing to ascertain microbial diversity. Preliminary results how *D4E1* was not shown to have an effect on plant productivity, enzymatic activity or microbial diversity, but was shown to be effective in reducing disease symptoms associated with cotton seedling disease.

ASSESSING THE ENVIRONMENTAL SUSTAINABILITY OF COUPLED HUMAN-NATURAL SYSTEMS AS INDICATED BY MICROBIAL COMMUNITY STRUCTURE

Trainee: Raymon Shange

Faculty Trainer: Ramble Ankumah and Robert Zabawa

Campus: Tuskegee University

Raymon S. Shange*, Ramble Ankumah, and Robert Zabawa

Department of Agriculture and Environmental Science, Tuskegee University, AL

The spatial effects of human ecological involvement via land use and management on biochemical and microbial activity in soils remains an area of interest to microbial ecologists. The aim of this study is to characterize microbial community structure across landscapes through biochemical, physiochemical, and metagenomic measures. Using a modified FLX pyrosequencing approach we evaluated bacterial diversity of the soils of two agricultural systems, both characterized by heterogeneously managed landscapes. In addition, phosphatase enzymes and selected edaphic factors were assayed to give an integrative representation of the microhabitat. Site 1 is characterized by three distinct land use types: poultry houses (B), grazing pasture (L), and poultry litter storage (X). Site 2 also is characterized by three land use types: grazing pasture (C), pine forest (F), and cultivated area (V). Differences among land use types were observed in phosphatase enzyme activity, pH, and soil particle size. Enzyme activities, sometimes examined as an indicator of microbial community status, were found to be the highest in areas of animal grazing and poultry litter storage, and lowest in pine forest and poultry house areas. Predominant phyla in the soil across both agroecosystems were Proteobacteria, Actinobacteria, Acidobacteria, and Bacteriodetes. The relative abundances did not reflect the differences found amongst the land use areas in enzyme activity with the exception of Actinobacteria at Site 2. The sequencing technique in conjunction with selected enzyme activity and soil properties proved to be a powerful method to characterize the bacterial ecosystem of soil under different land use not only in terms of presence or absence, but also in terms of distribution.



Distinguished Lecture

NSF CREATE-IGERT 2009 Distinguished Lecturer

Co-Sponsored by the Storer Endowment in Life Sciences

Dr. Maurice Moloney
Founder & Chief Scientific Officer, SemBioSys



From construct to clinic: Plant-made pharmaceuticals and drug development using green technology

Friday, November 20, 2009 1002 Giedt Hall, UC Davis 4:10 – 5:00 pm

The UC Davis NSF CREATE-IGERT program is honored to host Dr. Maurice Moloney, the Founder and CSO of SemBioSys. With over 20 years of experience in plant biotechnology, Dr. Moloney is recognized as an international expert in transgenic oilseed crops, developing the first transgenic canola while head of the Cell Biology Group at Calgene, Inc.

Dr. Moloney has published over 70 research papers and is listed as an inventor on more than 20 issued or pending patents, including the landmark patents that laid the foundation for Monsanto's Roundup Ready® and Bayer's Liberty Link® canola products.

In 1987, Dr. Moloney joined the faculty in the Department of Biological Sciences at the University of Calgary, where he held the Natural Sciences and Engineering Research Council of Canada (NSERC) Industrial Research Chair in Plant Biotechnology from 1995 to 2004.

He is currently a member of the NSREC Council and serves as the Chairperson of NSERC's Committee on Research Partnerships, in addition to a number of federal and corporate advisory boards. Dr. Moloney has been recognized with a number of prestigious awards, including the Alberta Science and Technology (ASTECH) Award for leadership in Alberta Technology.

Dr. Moloney earned a B.Sc. in Organic Chemistry from Imperial College at the University of London and a doctorate in Plant Biochemistry from Leicester Polytechnic in the United Kingdom. The University of Lethbridge recently honored Dr. Moloney with a D.Sc. *honoris causa*.





Poster Session

Focus Area: Biofuels & Biorefineries

THERMOSTABLE ENZYMES FROM ACIDOTHERMUS CELLULOLYTICUS

Mark S. Wolf*1, Juan V. Parales1, Ravi D. Barabote2, Alison M. Berry2, and Rebecca E. Parales1
1Department of Microbiology, University of California, Davis, CA
2Department of Plant Sciences, University of California, Davis, CA

Acidothermus cellulolyticus 11B is a Gram positive actinomycete that was isolated from hot springs in Yellowstone National Park using cellulose as the carbon source. A. cellulolyticus is an acid tolerant (pH 4-6; optimum pH 5.5) thermophile (37-70°C; optimum growth temperature 55°C). Due to these characteristics, it has potential as a source for thermostable enzymes with the ability to depolymerize components of the plant cell wall. Development of advanced fuels and other industrial products derived from lignocellulosic biomass depends upon the ability to access and depolymerize the components of plant cell walls. Plant cell walls, however, are organized in a complex crosslinked matrix of crystalline cellulose microfibrils, hemicellulose, pectin, and lignin. hemicellulose provide structural and chemical protection from depolymerization by enzymes. The cellulolytic capabilities of A. cellulolyticus have been intensively investigated, and one cellulase (E1) is currently used commercially. In contrast, hemicelluloses degradation by A. cellulolyticus remains unexplored. Hemicelluloses are heterogenous branched polysaccharides that account for 25-35% of lignocellulosic biomass. Xylan is the primary component of hemicellulose and is a polymer of pentoses such as xylose and arabinose linked by β-1,4-glycosidic bonds. Microbes employ xylanases to cleave these bonds and depolymerize the polysaccharide. A. cellulolyticus is capable of robust growth on xylan and the complete genome sequence revealed 35 predicted glycosyl hydrolases and eight carbohydrate esterases. Presented here is the purification and characterization of a family 10 endo-[-] 1,4-xylanase (Xyl-1). Purified Xyl-1 showed enzymatic activity on xylans from oat spelts and birchwood and was active between 30°C and 100°C, and from pH 3-9. Optimal activity was at 90°C and the optimal pH (4.5-6.0) varied with the reaction temperature. Xyl-1 retained activity for extended periods of time at high temperatures when in the presence of xylan substrates. Work is currently being done to purify and characterize a second xylanase as well as an esterase. Synergistic activity of these enzymes on xylan substrates will be investigated.

INDUCING PHYTOCHROME B SIGNALING WITHOUT ACTIVATION OF OTHER PHYTOCHROMES

Timothy Butterfield*, Wei Hu, and J. Clark LagariasDepartment of Molecular and Cellular Biology, University of California, Davis

Phytochrome B (phyB) plays a dominant role in red light sensing in plants, with its regulatory roles redundantly shared and modulated by other members of the phytochrome protein family. This redundancy has made it difficult to identify genes and gene products specific to the phyB signaling pathway. Recently, our laboratory identified a class of dominant constitutively active mutant alleles of phyB that faithfully recapitulate phyB-regulated gene expression networks in a light-independent manner (1; 2). By exploiting the chromophore-dependent activity of the Y²⁷⁶H allele (YHB) of Arabidopsis phyB, we are developing a bilin-inducible system to manipulate phyB signaling. This system not only permits investigation of phyB signaling in darkness without activation of other phytochromes, but also offers the potential to study phyB-specific signaling pathways in light-grown plants under conditions where other phytochromes are inactive. Through expression of the cyanophage bilin biosynthetic enzyme PebS to produce the unnatural bilin precursor phycoerythrobilin (3) in transgenic plants, YHB alleles are activated while wild-type alleles are photoinactive. In this way, signaling by phyB can be selectively activated in light-grown plants without activation of other phytochromes.

IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL EFFECTORS THAT INTERACT WITH THE PLANT PROTEIN FOLDING CATALYST CYCLOPHILIN

J. Mitch Elmore* and Gitta Coaker

Department of Plant Pathology, University of California, Davis, CA

Effector proteins are essential virulence determinants for many Gram-negative bacterial plant pathogens. These proteins are delivered into the host cell cytoplasm via the Type III Secretion System (T3SS) during infection and collectively contribute to pathogen fitness on host plants. Due to size constraints of the T3SS pilus, effectors are delivered as either partially or completely unfolded proteins and it is hypothesized that many exploit plant folding catalysts for activation. One such folding catalyst, cyclophilin, has been identified (Coaker, et al. 2005). Cyclophilins are molecular chaperones that catalyze the *cis/trans* isomerization of peptidyl-prolyl bonds, a rate-limiting step during protein folding.

We have designed and implemented a targeted protein interaction screen to identify effectors that interact with the *Arabidopsis* cyclophilin ROC1. We have focused our efforts on effectors that contain putative cyclophilin binding motifs (Piotukh, et al. 2005). To date, eight effectors from different species of phytopathogenic bacteria have been identified in this screen. Functional analysis of effectors that require cyclophilin for activation is underway. Identification of the host activators of bacterial effectors will enable the production of enzymatically active effector proteins *in vitro* and facilitate investigations into their functions during the infection process.

CHIMERIC ANTIMICROBIAL PROTEIN PROVIDES RESISTANCE TO PIERCE'S DISEASE IN GRAPEVINES

Hossein F. Gouran¹, Ana M. Ibáñez¹, Kevin Quach¹, Sandie L. Uratsu¹, George E. Bruening², Cecilia Agüero¹, Gupta Goutam³ and Abhaya M. Dandekar¹

- ¹Deptartment of Plant Sciences, University of California, Davis
- ²Deptartment of Plant Pathology, University of California
- ³Biosciences Division, Los Alamos National Laboratory

Pierce's disease (PD) in grapevines is a vector transmitted disease where the causative agent a Gram-negative bacterium Xylella fastidiosa is deposited into the xylem tissue by the feeding action of the Glassy Winged Sharpshooter (GWSS), an insect vector that efficiently transmits the disease and is of greatest concern to growers in California. The virulence of the bacterium is associated with its ability to colonize xylem and its ability to move through pit pore membranes into adjacent water conducting elements. Because *X. fastidiosa* is xylem-limited, xylem-targeted expression of potential antimicrobial therapeutic proteins needs to occur to prevent and control PD infestations. We have designed a chimeric anti-microbial protein with 2 functional domains, one a surface recognition domain, SRD that specifically targets the bacterium's outer membrane accomplished using human neutrophil elastase (HNE) that recognizes MopB, the major outer membrane protein of X. fastidiosa. The second domain connected to the first by a flexible linker contains Cecropin B (CecB) a lytic protein domain to lyse the outer/inner membrane and to clear X. fastidiosa from xylem elements. Transgenic grapevines expressing this HNE-CecB chimeric anti-microbial gene have been generated. Individual lines have been propagated in the greenhouse and mechanically inoculated with *X. fastidiosa*. Four out of eleven mechanically inoculated transgenic lines expressing HNE-CecB showed significant resistance to Xylella fastidiosa. Furthermore, magnetic resonance imaging (MRI) of stem sections from these resistant transgenic grape lines above the point of inoculation revealed noticeably less number of clogged xylem vessels than controls.

EFFECT OF ANTIMICROBIAL SYNTHETIC PEPTIDE *D4E1* ON INFESTATION OF COTTON SEEDLING DISEASE.

Lakisha Odom*, Conrad Bonsi, Ramble O. Ankumah, Jesse Jaynes, Marceline Egnin, Lanell Ogden, and Desmond Mortley

Department of Agriculture and Environmental Science, Tuskegee University, AL

Cotton seedling disease is a fungal disease complex comprised of several fungal pathogens. In Alabama, the two fungal pathogens associated with Cotton Seedling Disease are Rhizoctonia Solani and Pythium. Cotton Seedling Disease, which results in loss of cotton production revenues totaling over 10 million dollars per year, in Alabama alone, has no known disease resistant cultivars. In an effort to confer resistance through genetic modification, a synthetic peptide *D4E*1, which has been shown in vitro and in planta to have broad spectrum antimicrobial action against many fungal orders, has been transformed into cotton seeds, to examine the efficacy of this peptide on the control of Cotton Seedling Disease Complex in transformed cotton plants in a field setting. Two 150 x150 ft test plots were arranged in a completely randomized design and were assigned either one of 3 lines of cotton seed transformed with D4E1 (designated 357, 358, and 373) or a control line containing GUS marker gene. In test plot 1 there was a significant difference (P<0.05) between line 358 and the control and line 373 indicating that those lines had overall healthier cotton seedlings than the control. In field plot 2 there was no significant difference between lines and the control. When comparing mean cotton seedling germination scores, in field plot 1, there was a significant difference (P<0.05) between each of the lines and the control and in test field 2 there was no difference between the lines and control.

DEVELOPMENT OF TRANSGENIC SWEETPOTATO [Ipomoea batatas (L. lam)] EXPRESSING GENES jc41N AND jc41ND AS A PLANT-BASED VACCINE AGAINST HIV

Steven Samuels*, Marceline Egnin, Jesse Jaynes, Sy Traore, B. Min, Jacquelyn Jackson, Frieda Sanders

Department of Agriculture and Environmental Sciences, Tuskegee University, Tuskegee AL 36088

Recombinant proteins are now being used in the medical field as a method of treatment for many diseases such as cancer and HIV. Plants have been found to be an inexpensive and efficient means of production, and expression of these therapeutic proteins. Based on these facts and the principles of plant transformation technology, synthetic antiviral peptides; jc41n and jc41nd have been developed at Tuskegee University by Dr. Jesse Jaynes that are capable of inhibiting the progression of HIV. These synthetic peptides are of lytic nature, thus toxic to any cell, however jc41nd contains an additional amino acid sequences that render it inactive and non toxic to cells when expressed. In order to express these peptides in planta, to *de novo* synthetic gene constructs were designed with an intron sequence to facilitate cloning in bacterial and the accumulation in plant without any penalty or lethality. The objective of this research is to clone and express these peptides in PI 318846-3 (D-3) as an alternative production and delivery system for HIV treatment. A CAMV 35 promoter sequence was fused to each construct, and the resulting recombinant plasmid will be used to transform sweetpotato. Transformation of D-3will is done via *Agrobacterium tumefaciens*-mediated gene transfer, followed by molecular screening to verify the presence of the *JC* series in sweetpotato. Confirmation of JC genes in DNA of D-3 will be done via PCR and southern blotting, which will confirm the stable integration of genes into plant genome. RNA will be analyzed by RT-RCR to confirm expression at transcriptional level, followed by Western blot to show the presence of expressed proteins. After transformation of sweetpotato the ability of jc41n and jc41nd to inhibit action of HIV infecting normal cells will be tested by clinical trials.

Focus Area: Plant Made Products

UTILIZING A TRBO-BASED PLANT EXPRESSION SYSTEM TO ACCELERATE EPITHELIAL REGENERATION

Tiffany W Glavan*1, Abhaya Dandekar2, Satya Dandekar1, Bryce Falk3, Larry Joh4, Sang-Kyu Jung4, Karen McDonald4

- ¹Department of Medical Microbiology and Immunology, University of California, Davis, CA
- ²Department of Plant Sciences, University of California, Davis, CA
- ³Department of Plant Pathology, University of California, Davis, CA
- ⁴Department of Chemical Engineering and Materials Science, University of California, Davis, CA

Cells that comprise the intestinal epithelium are continually differentiating and migrating upward into the villi. Regeneration and renewal of this single cell layer is an ongoing process that is integral to gastrointestinal health and immune function. We are interested in developing a plant-based therapeutic aimed at accelerating mucosal regeneration and repair mechanisms, thus increasing barrier function in the gastrointestinal tract. The mitogenic protein of interest is R-spondin1, a growth factor known to positively regulate cell signaling in the Wnt pathway through an interaction with DKK1. As a therapeutic, this protein has the potential to provide a restoration of function in the context of multiple disease states that disrupt the intestinal epithelium, including radiation and chemotherapy-induced mucositis and inflammatory bowel disease.

A multidisciplinary collaborative project has been initiated to develop an *Agrobacterium*-based plant expression system to produce this protein in tobacco plants. The human gene for R-spondin1 has been re-designed for expression in *N.benthamiana* through codon optimization and the addition of Kozak's context sequence, a six histidine tag, and mRNA secondary structure aimed at decreasing degradation. This synthetic gene construct was inserted into a tobacco mosaic virus RNA-based overexpression (TRBO) vector, which was then electroporated into *Agrobacteria*. *N.benthamiana* leaves were co-infected with this construct along with a vector coding for the p19 gene silencing suppressor. Initial dotblot, SDS-PAGE, and western blot analyses of the crude protein extract reveal positive expression of R-spondin1 with a molecular weight of approximately 32.8kDa. We plan to purify the protein using nickel-based affinity chromatography and analyze its bioactivity using a BrdU assay on treated Caco-2 cells. Transcriptional kinetics will be investigated using pRT-PCR and post transcriptional modifications will be determined through mass spectrometry.

Focus Area: Plant Made Products

QUANTITATIVE PCR MONITORING OF T-STRAND POPULATIONS IN AGROINFILTRATED LEAF TISSUE

Chris Simmons* and Jean VanderGheynst

Department of Biological and Agricultural Engineering, University of California, Davis

Agrobacterium tumefaciens-mediated gene transfer, or agroinfiltration, is a common plant transfection technique. However, the efficacy of agroinfiltration varies widely between plant species. We are interested in studying whether populations of T-strands, the genetic material transferred from Agrobacteria to plant cells, change over time as plant cells are transfected by the bacteria. The number of T-strand copies within plant cells is related to the ultimate *in planta* expression level of recombinant genes housed on the T-strand. Changes in T-strand levels within plant tissue may yield clues as to why transgene expression varies between plant species following agroinfiltration. Moreover, the behavior of T-strand populations in agroinfiltrated plant tissue may suggest targets for improving agroinfiltration.



CREATE-IGERT

Trainee Biographies

Cohort 1 Trainees Selected Fall 2007, funded Jan 2008-Dec 2009)



Butterfield, Timothy
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Department of Molecular & Cellular Biology, Plant Biology Graduate
Group

Research Focus: Environmental Sustainability

Bridging multiple disciplines, including plant biology, biochemistry and biophysics, Tim is pursuing research that will lead to new strategies for agronomic crop improvement. Specifically, his doctoral work will investigate a gain-of-function allele of plant phytochromes for use in engineering altered light-responsiveness and yield gain in crop plant species.

Presentations

- "Molecular Mechanisms of Phytochrome Signaling." Talk presented at the UC Davis Plant Cell Biology Training Grant Retreat; Sept. 11 2009, Asilomar CA.
- "Manipulation of Phytochrome-Mediated Signaling in Transgenic Plants." Talk presented at the CREATE-IGERT Distinguished Lecture and Symposium; October 16th 2008, Davis CA.
- "Into the Light: Information, Signal Transduction & Response Regulation." Talk presented at the UC Davis Plant Biology Graduate Group Colloquium; September 16th 2008, Davis CA.

Posters

- "Inducing phytochrome B signaling without activation of other phytochromes." Poster presented at the 9th Annual International Conference on Tetrapyrrole Photoreceptors of Photosynthetic Organisms. July 26-31 2009; Asilomar CA.
- "Manipulation of phytochrome-mediated signaling in transgenic plants." Poster presented at the UC Davis Eighteenth Annual Biotechnology Training Retreat; April 4 2009, Napa CA.

Awards & Honors

 October 2009, Nominated for membership in the UC Davis Chapter of Gamma Sigma Delta, the Honor Society of Agriculture

Outreach Activities

 2008 & 2009 Picnic Day Ag Biotech Outreach Display. Spoke with general public about the safety and benefits of agricultural biotechnology

- 2008 & 2009 Teen Biotech Challenge. Volunteered as a webpage contest judge and event host.
- Fall 2008-Spring 2009 Graduate Student Assembly Representative. Served on behalf of the Plant Biology Graduate Group.



Chiniquy, Dawn
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Department of Plant Pathology, Plant Biology Graduate Group

Research Focus: Biofuels & Biorefineries

Engineered disease resistance in bioenergy crops may prove to be a critical factor in maximizing biomass production for cellulosic ethanol. Dawn's research will contribute to the development of rice strains improved for use as bioenergy feedstocks. Specifically, her doctoral work will involve the characterization a family of glycosyltransferase enzymes required for cellulose biosynthesis in rice, as well as a novel gene participating in rice defense responses.

Publications

• The ParA resolvase catalyzes site-specific excision of a transgene from the *Arabidopsis* genome. James G. Thomson, Yuan-Yeu Yau, Robert Blanvillain, Dawn Chiniquy, Roger Thilmony, and David W. Ow. Transgenic Research, *in press*

Presentations

 "Characterizing Glycosyltransferases to Improve Cellulosic Conversion to Biofuels", Talk presented at the CREATE-IGERT Distinguished Lecture and Symposium; October 16th 2008, Davis CA.

Posters

 Analysis of Putative Feruloyltransferase Transcript Levels and Cell Wall Composition during Rice Development. Dawn Chiniquy, Laura Bartley, Jesper Harholt, Brian Conlin, Chithra Manisseri, Peijian Cao, Henrik Vibe Scheller, and Pamela Ronald. Cell Wall Biosynthesis and American Congress on Plants and Bioenergy, 2008



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Department of Medical Microbiology & Immunology, Microbiology
Graduate Group

Research Focus: Plant-made Products

Tiffany's project is focused on the development of plant-derived therapeutic proteins to treat gastrointestinal dysfunction through the regeneration and renewal of the epithelial layer of the gut mucosa. She is collaborating with multiple groups on campus in an effort to express the protein in N.benthamiana and evaluate its activity in epithelial cell culture.

Publications

- George, M., Verhoeven, D., Sankaran, S., Glavan, T*., Reay, E., & Dandekar, S. (2009) Heightened cytotoxic responses and impaired biogenesis contribute to early pathogenesis of the oral mucosa in SIV infected rhesus macaques. Clinical and Vaccine Immunology: 16(2), 277-281.
- Dandekar, S., Sankaran, S., & *Glavan, T. (2008). Chapter 17: HIV and the Mucosa: No Safe Haven. In Vajdy, M. (Ed.), Immunity Against Mucosal Pathogens. New York, NY: Springer Science + Business Media B.V.

Presentations

• "Restoring Epithelial Integrity with Plant-Based Therapeutics", Talk presented at the CREATE-IGERT Distinguished Lecture and Symposium; October 16th 2008, Davis CA.

Posters

- "Restoring Epithelial Integrity with Plant-Based Therapeutics", Poster presented at the UC Davis Eighteenth Annual Biotechnology Training Retreat; April 4 2009, Napa CA.
- "IL-17 Depletion in HIV/SIV Infection: A Breakdown in Mucosal Defense", Poster presented at the CREATE-IGERT Distinguished Lecture and Symposium; October 16th 2008, Davis CA.

Outreach Activities

- 2008 & 2009 Picnic Day Ag Biotech Outreach Display. Spoke with general public about the safety and benefits of agricultural biotechnology
- 2008 & 2009 Teen Biotech Challenge. Volunteered as a webpage contest judge and event host.
- February 2009 Graduate Group Recruiting. Spoke on behalf of the CREATE-IGERT training program to ~25 potential new students in the Microbiology & Immunology Graduate Group.



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Engineering Graduate Program

Research Focus: Biofuels & Biorefineries

Ben's research is focused on the development of a plant-based expression system for the production of cellulose degrading enzymes. He is investigating the expression of these enzymes *in planta*, as well as their potential use in an exogenously applied biomass pre-treatments.

Patent Applications

• McDonald, K. A., Dandekar, A., Falk, B. & Lindenmuth, B*. (2008). U.S. Patent Application Serial No. 61/090,221 Production of Cellulase Enzymes in Plant Hosts Using Transient Agroinfiltration.

Presentations

• "Transient In Planta Expression of Cellulose-Degrading Enzymes: Plant Tissues as Bioreactors." Talk presented at the CREATE-IGERT Distinguished Lecture and Symposium; October 16th 2008, Davis CA.

Posters

- "In Planta Production of Cellulose-Degrading Enzymes" Poster presented at the Chevron-UC Davis Joint Research Symposium; July 30, 2009, University Club, UC Davis, Davis, CA.
- "Transient In Planta Expression of Cellulose-Degrading Enzymes: Plant Tissues as Bioreactors." Poster presented at the UC Davis Eighteenth Annual Biotechnology Training Retreat; April 4, 2009, Napa CA.
- "Transient In Planta Expression of Cellulose-Degrading Enzymes: Plant Tissues as Bioreactors." Poster presented at the Agricultural Biotechnology International Conference; August 24, 2008, Cork, Ireland.
- Transient In Planta Expression of Cellulose-Degrading Enzymes: Plant Tissues as Bioreactors." Poster presented at the ACS National Meeting; August 20, 2008, Philadelphia, PA.
- "In Planta Production of Cellulose-Degrading Enzymes" Poster presented at the Chevron-UC Davis Joint Research Symposium; July 17, 2008, Kemper Hall, UC Davis, Davis, CA.
- "A Plant-Based Expression System for In Planta Production and Localization of a Cellulose-Degrading Enzyme" Poster presented at the Seventeenth Annual Biotechnology Training Retreat, Christian Brothers Retreat & Conference Center; March 29, 2008, Napa, CA.

Awards & Honors

• October 2009, Nominated for membership in the UC Davis Chapter of Gamma Sigma Delta, the Honor Society of Agriculture

Outreach Activities

- 2008 & 2009 Teen Biotech Challenge. Volunteered as a webpage contest judge.
- Mentored an undergraduate student participating in the UC Davis-Zhejiang University Global Research in Advanced Technology Program



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Department of Biological & Agricultural Engineering, Biological
Systems Engineering Graduate Program

Research Focus: Plant-made Products

Through kinetic modeling of the T-DNA strand transfer pathway from *Agrobacterium tumifaciens* to host plant cells during agroinfiltration, Chris will focus on identifying key factors and rate-limiting steps in plant transformation. Ultimately, this model will be used in designing more efficient, rapid and cost-effective plant expression systems for plant-made products.

Publications

- Simmons, C. W*., VanderGheynst, J. S. & Upadhyaya, S. K. (2008). Physical factors that affect the vacuum infiltration of A. tumefaciens into harvested leaf tissue and subsequent in planta transgene transient expression. Biotechnology & Bioengineering: 102(3), 965-970.
- Simmons, C. W. & VanderGheynst, J. S. (2007). Transient co-expression of post-transcriptional gene silencing suppressors and beta-glucuronidase in harvested lettuce leaf tissue does not improve recombinant protein accumulation in planta. Biotechnology Letters, 29:641-645.
- VanderGheynst, J.S., Gou, H.-Y. & *Simmons, C. W. (2008). Response surface studies that elucidate the role of infiltration conditions on Agrobacterium tumefaciens-mediated transient transgene expression in harvested switchgrass (Panicum virgatum). Biomass and Bioenergy, 32(4), 372-379.

Presentations

 "A Kinetic Model for T-Strand Transfer During Agroinfiltration." Talk presented at the CREATE-IGERT Distinguished Lecture and Symposium; October 16th 2008, Davis CA.

Posters

- "Identification and Characterization of Bacterial Effectors that Interact with the Plant Protein Folding Catalyst Cyclophilin." Poster presented at the UC Davis Eighteenth Annual Biotechnology Training Retreat; April 4 2009, Napa CA.
- "A Kinetic Model for T-Strand Transfer During Agroinfiltration." Poster presented at the CREATE-IGERT Distinguished Lecture and Symposium; October 16th 2008, Davis CA.
- "A Kinetic Model of the T-DNA Secretion Pathway for *Agrobacterium tumefaciens*." Poster presented at the Annual Biotechnology Retreat, Napa, CA.

Awards & Honors

• October 2009, Nominated for membership in the UC Davis Chapter of Gamma Sigma Delta, the Honor Society of Agriculture

Outreach Activities

• Jan-March 2009 and October-December 2009 Sheldon HS Biotech Academy Ementor. Participates in a program for one-on-one e-mentoring with biotech high school students. Topics include academic preparation for STEM careers, resume writing, time management, tips for success in high school and college, etc... The recurrent projects last for 8-weeks during the academic year.

Cohort 2 Trainees Selected in Spring and Fall 2008, funded Jan 2009-Dec 2010

Arzola, Lucas
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McDonald Laboratory, 3069 Bainer Hall
Department of Chemical Engineering & Materials Science, Chemical
Engineering Graduate Program

Research Focus: Plant-made Products

The constant threat of bioterrorism and the current H1N1 flu pandemic highlight the importance of developing the rapid, scalable, and cost-effective production of therapeutic agents. Recent advances in the field of plant biotechnology have made possible the use of plants as cost-effective factories of therapeutic proteins. My research focuses on the development of a plant based expression system for the production of an anthrax receptor decoy protein that can mitigate the effects of anthrax.

Posters

• "AntaRx: A New Plant-Produced Treatment for Anthrax." Poster presented at the 2009 ISPE Annual Meeting; Nov 9, 2009, San Diego, CA.

- "AntaRx: A New Plant-Produced Treatment for Anthrax." Poster presented at the IGERT PI Meeting; May 18, 2009, Alexandria, VA.
- "AntaRx: A New Plant-Produced Treatment for Anthrax." Poster presented at the ISPE SF/Bay Area Poster Competition; May 9, 2009, Foster City, CA.
- "Cryopreservation and Viability Analysis of Transgenic Rice (Oryza sativa) Cells."
 Poster presented at the CREATE-IGERT Distinguished Lecture and Symposium;
 October 16th 2008, Davis CA.
- "Cryopreservation and Viability Analysis of Transgenic Rice (Oryza sativa) Cells."
 Poster presented at the Agricultural Biotechnology International Conference; August 24, 2008, Cork, Ireland.

Awards & Honors

- November 8-11, 2009, Will compete in the International ISPE Poster Competition in San Diego, CA.
- October 2009, Nominated for membership in the UC Davis Chapter of Gamma Sigma Delta, the Honor Society of Agriculture
- May 9, 2009, 1st Place in the International Society of Pharmaceutical Engineers (ISPE) SF/Bay Area Poster Competition

Outreach Activities

- October 22-25, 2009, Attended conference "Institute on Teaching and Mentoring" in Washington, DC. This conference offers workshops for minority students on graduate school survival, mentoring, and career development for becoming a faculty member.
- 2009-2010 (academic year), ISPE Student Chapter President, UC Davis. Organizes the ISPE student chapter activities, such as tours of local biotech companies, and represents the group at local symposia and outreach events.
- May 2009, Teen Biotech Challenge. Volunteered as a webpage contest event host and staffed the College of Engineering information booth at the symposium, answering questions on university training and STEM careers for high school students, parents and teachers.

Castillo, Elenor
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Department of Plant Sciences, Plant Biology Graduate Group

Research Focus: Environmental Sustainability

Elenor's project focuses on elucidating the metabolic pathways that underlie production of aromatic volatiles in fruits, which has direct commercial application in extending fruit shelf-life. On a broader scale, understanding the role of volatile chemical signals within and between plants in field populations may also play a part in increasing

crop yields/biomass, engineering insect and pathogen resistance, and fine-tuning other agronomic and quality-related crop traits.

Publications

Rockwell, N. C., Njuguna, S. L., Roberts, L., Castillo, E.*, Parson, V. L., Dwojak, S., Lagarias, J. C., & Spiller, S. C. (2008). A second conserved GAF domain cysteine is required for the blue/green photoreversibility of cyanobacteriochrome Tlr0924 from Thermosynechococcus elongatus. Biochemistry: 47(27), 7304-16.

Presentations

- Center for Biophotonics Science and Technology, 2008 Annual Retreat, Abstract and Oral Presentation
- "HPL-Derived Metabolites as a Vehicle for Production of Superior Stress-Tolerant Plants" Poster presented at the UC Davis Eighteenth Annual Biotechnology Training Retreat; October 16, 2008, Davis, CA.

Posters

• "HPL-Derived Metabolites as a Vehicle for Production of Superior Stress-Tolerant Plants" Poster presented at the UC Davis Eighteenth Annual Biotechnology Training Retreat; April 4 2009, Napa CA.

Awards & Honors

- 2008 "Above and Beyond" Award for Baccalaureate Research and Outstanding Achievement in Education, The Center for Biophotonics Science and Technology (NSF center)
- 2008 Jill Barrett Research Scholar

Outreach Activities

- May 2009 Teen Biotech Challenge webpage contest. Served as an event volunteer at the awards banquet & symposium.
- April 2009 Picnic Day Ag Biotech Outreach Display. Spoke with general public about the safety and benefits of agricultural biotechnology
- Society for the Advancement of Native Americans and Chicanos in Science (SACNAS)
 Member
- Co-chair, Latino Graduate Student Association, University of California, Davis
- Sisters Inspiring Sisters, Vice President Mentoring high school girls in the East Oakland Community



Elmore, James "Mitch"
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Coaker Laboratory, 210 Hutchison Hall
Department of Plant Pathology, Plant Biology Graduate Group

Research Focus: Environmental Sustainability

Innovative strategies for sustainable disease control in agriculture can be developed by understanding the molecular mechanisms underlying plant-pathogen interactions. Mitch's research seeks to identify the plant targets and virulence mechanisms of proteins essential to the lifestyle of phytopathogenic bacteria.

Publications

- Govindarajulu, M., Elmore, J.M., Fester, T., and Taylor, C.G. (2008) Evaluation of constitutive viral promoters in transgenic soybean roots and nodules. Molecular Plant-Microbe Interactions. 21(8):1027-1035 [front cover article].
- Lui, J., Elmore, J. M., Fuglsang, A. J., Palmgren, M. G., Staskawicz, B., & Coaker, G. (2009). RIN4 functions with plasma membrane H+-ATPases to regulate stomatal apertures during pathogen attack. PLoS Biology 7(6): e1000139.
- Wilton, M., Subramaniam, R., Elmore, J.M., Felsensteiner, C., Coaker, G., and Desveaux, D. (2009) The Type III Effector HopF2*Pto* DC3000 Targets Arabidopsis RIN4 Protein to Promote *Pseudomonas syringae* Virulence. *Submitted*. Proceedings of the National Academy of Sciences.
- Elmore, J.M. and Coaker, G. (2009) Multiple effector proteins from phytopathogenic bacteria interact with the plant cyclophilin ROC1. *In Preparation*. Target journal: PLOS Pathogens.
- Elmore J.M. and G. Coaker. (2009) Biochemical Purification of Protein Complexes. *In Press*. Methods in Molecular Biology: Plant Immunity. Humana Press, Inc.
- Liu, J.*, Elmore, J.M.*, and Coaker, G. (2009) Investigating the functions of the RIN4 protein complex during plant innate immune responses. *In Press.* Plant Signaling & Behavior 4(12). * These authors contributed equally to this work

Presentations

• Elmore, J.M. Investigating Host-Mediated Activation of Bacterial Effector Proteins. Oral presentation. UC Davis Plant Pathology Retreat. September 2009.

Posters

- Elmore, J. M., and Coaker, G. Identification and characterization of bacterial effectors that interact with the plant protein folding catalyst cyclophilin. Poster presentation. Meeting of the International Society of Molecular Plant-Microbe Interactions. Quebec, Canada. July 2009.
- Elmore, J. M., and Coaker, G. Cyclophilin-Mediated Effector Activation: A Tool to Investigate Effector Function. Poster presentation. Annual UC Davis Biotechnology Program Retreat. Napa, CA. April 2009.
- Elmore, J.M. and Coaker, G. Characterization of the *Pseudomonas syringae* effector HopG1. Poster presentation. UC Davis Plant Pathology Retreat. September 2008.

Outreach Activities

• Fall 2009-Spring 2010 Graduate Student Assembly Representative. Served on behalf of the Plant Biology Graduate Group.



Kerwin, Rachel
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Department of Plant Sciences, Plant Biology Graduate Group

Research Focus: Environmental Sustainability

Rachel seeks to understand the metabolic pathways underlying glucosinolate production in plants. She will characterize nine genes involved in glucosinolate metabolism and investigate glucosinolate's potential as plant-made product to deter herbivory in the field.

Publications

 Hansen, B. G., Kerwin, R. E.*, Ober, J. A., Lambrix, V. M., Mitchell-Olds, T., Gershenzon, J., Halkier, B. A., Kliebenstein, D. J. (2008). A novel 2-oxoacid-dependent dioxygenase involved in the formation of the goiterogenic 2-hydroxybut-3-enyl glucosinolate and generalist insect resistance in Arabidopsis. Plant Physiology: 148(4), 2096-108.

Outreach Activities

 April 2009 FFA GMO Debate Advisor. Participated on a scientific advisory panel to critique and guide Davis HS students in preparing them for a debate on the technical, regulatory and policy issues related to agricultural biotechnology.



Wolf, Mark
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Parales Laboratory, 225 Briggs Hall
Department of Microbiology, Biochemistry & Molecular Biology
Graduate Group

Research Focus: Biofuels & Biorefineries

Mark will characterize a number of lignocellulose-degrading enzymes isolated from *Acidothermus cellulolyticus*, a hot springs microorganism isolated in Yellowstone National Park. His goal is to identify thermostable enzymes appropriate for use in the production of biofuels and industrial chemicals.

Outreach Activities

 April 2009 Picnic Day Ag Biotech Outreach Display. Conducted experiments with school-age children and spoke with general public about the safety and benefits of agricultural biotechnology



Lateef, Dalya
Department of Agriculture and Environmental Sciences, Tuskegee
University

Research Focus: Environmental Sustainability

Dalya Lateef is working at the interface of nutritional medicine and agricultural sciences. The overall goal of her project is to investigate the role of the enteric nervous system of the gastrointestinal tract in the short

term control of food intake by cholecystokinin (CCK). Cholecystokinin (CCK), a gut-brain peptide that is released from the endocrine I cells of the gastrointestinal tract upon the stimulation food, has been implicated as a hormone in the regulation of food intake. Dalya will be investigating plant nutritional components involved in stimulating CCK release and alterations to these components that may be fine-tuned via transgenic crop technologies.



Miller, Sonni-Ali
Department of Agriculture and Environmental Sciences

Research Focus: Environmental Sustainability

Sonni received a M.S. from Tuskegee University in Food and Nutritional Sciences and has worked as a serological technician with Antech Diagnostics Inc., in Lake Success, NY. Having worked for over four years as a laboratory technician, he brings a lot of experience with him in his PhD endeavor.



Odom, LaKisha

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Ankumah Soil and Water Quality Lab, 301 Milbank Hall
College of Agricultural, Environmental, and Natural Sciences and College of
Veterinary, Nursing, and Allied Health. Tuskegee University.

Research Focus: Environmental Sustainability

Evaluating through field trials, the efficacy of a transgenic cotton plant which has been transformed with a synthetic antimicrobial peptide, *D4E1*, on the progression of cotton seedling disease, soil microbial diversity, and enzymatic activity.

Presentations

- "Effect of Cotton Plant Genetically Modified with An Antimicrobial Synthetic Peptide D4E1
 On Soil Microbial Diversity and Enzyme Activity." Talk presented at the CREATE-IGERT Distinguished Lecture and Symposium; October 16th 2008, Davis CA
- "Effect of Transgenic Cotton Plants Transformed with Antimicrobial Synthetic peptide D4E1 on Cotton Seedling Disease and Soil Microbial Diversity." Presented at the 66th Professional Agricultural Workers Conference; December 7-9, 2008, Tuskegee, AL.
- "Effect of Transgenic Cotton Plants Transformed with Antimicrobial Synthetic peptide D4E1 on Cotton Seedling Germination Rate." Presented at the Association of Research Directors, Inc., 15th Biennial Research Symposium, March 28, 2008- April, 1, 2008, Atlanta, GA.

Posters

- "EFFECT OF ANTIMICROBIAL SYNTHETIC PEPTIDE *D4E1* ON INFESTATION OF COTTON SEEDLING DISEASE AND ON SOIL MICROBIAL DIVERSITY." Presented at In Vitro Biology Meeting, June 6-10, 2009, Charleston, SC.
- "Effect of Cotton Plant Genetically Modified with An Antimicrobial Synthetic Peptide D4E1 On Enzyme Activity". Presented at the Agronomy Society of America, November 1-4, 2009. Pittsburgh, PA

Awards & Honors

- April 2008, Nominated for membership in the Tuskegee University Chapter of Gamma Sigma Delta, the Honor Society of Agriculture
- April 2009, Nominated for membership in the Tuskegee University Chapter of Sigma Xi, the Scientific Research Society

Samuels, Steven

Department of Agriculture and Environmental Sciences, Tuskegee University

Research Focus: Plant-made Products

Steven is working on the development of transgenic sweetpotato [*Ipomoea batatas (L. lam)*] for expression of proteins to be used as a plant-based vaccine against HIV. Similar recombinant proteins are currently being used in the medical field as a method of treatment for many diseases such as cancer and HIV. Synthetic antiviral peptides capable of inhibiting the progression of HIV, jc41n and jc41nd, have been developed at Tuskegee University by Dr. Jesse Jaynes. The objective of Steven's research is to clone and express these peptides in transgenic sweetpotato lines. The peptides will be recovered and purified from the transgenic sweetpotato, and ultimately, vaccine safety & efficacy will need to be evaluated in animal models and in clinical trials.

Shange, Raymon

Department of Agriculture and Environmental Sciences, Tuskegee University

Research Focus: Environmental Sustainability

Raymon's research interests include environmental sustainability, soil microbial ecology, and and the use of geographic information systems to enhance natural resource conservation.