Chelsea Agar and Vince J. LiCata, Biological Sciences

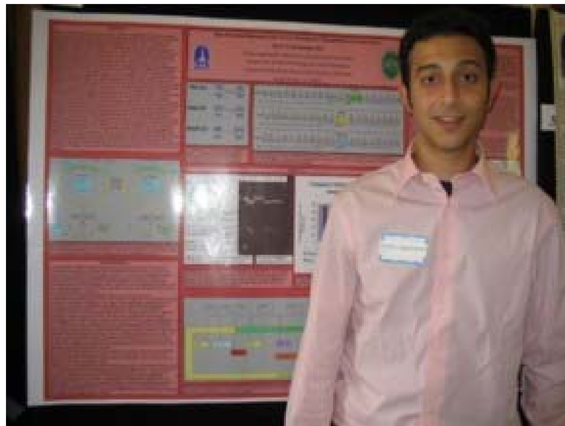
A Comparison of the effects of bound DNA on the thermostability of Type I DNA polymerases from Thermus aquaticus and Escherichia coli

Klenow and Klentaq, the large fragment domains of DNA polymerase I from *Escherichia coli* and *Thermus aquaticus* respectively, were examined when bound to a 13/20mer DNA segment using circular dichroism (CD) spectroscopy and differential scanning calorimetry (DSC). Thermal denaturations of both species, alone and as a DNA-protein complex, were preformed to directly measure the effects of bound DNA on melting temperature (Tm), constant pressure heat capacity (DCp), and enthalpy (DH), and to help elucidate the basis for the more thermally stable nature of Klentaq versus its mesophillic homolog, Klenow. Experimental data from both DSC and CD show essentially no difference in Tm for Klentaq upon DNA binding, whereas both techniques show a significant change in Tm between Klenow and the Klenow-DNA complex. It was determined that bound DNA has a stabilizing effect on Klenow but no stabilizing effect on the already extremely thermally stable protein Klentaq.

Monty Aghazadeh and Norimoto Murai, Plant Pathology and Crop Physiology, LSU Agricultural Center

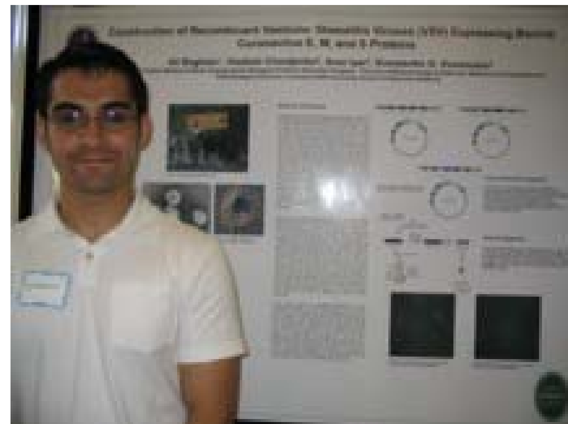
Site-Directed Mutagenesis of the Neomycin Phosphotransferase Gene from Transposon 903

When constructing binary vectors for use in plants, many different antibiotic resistance genes can be used as selectable markers for plant and bacterial expression. For bacterial expression, one of the more widely used selectable markers is the 1.6 kb Neomycin phosphotransferase (NPTII) gene from Transposon 5, which codes for kanamycin resistance. There is a smaller, different 1.2 kb NPT gene that also confers kanamycin resistance, which is found in Transposon 903. Despite its smaller size, this NPT gene from Tn903 is rarely used as a selectable marker because of the fact that 3 commonly used restriction enzyme sites are present within the gene: XhoI, SmaI, & HindIII. These three restriction enzyme sites can thus interfere with processes such as cloning, where restriction enzymes are heavily depended upon. The purpose of this study is therefore to eliminate these 3 restriction enzyme sites from the NPT gene of Tn903 and in so doing, prepare it for use as a selectable marker in a binary vector. In order to eliminate these restriction sites, we designed mutagenic primers for PCR in such a way that only one of six base recognition sequences was changed without altering the codon usage of the affected amino acid (silent mutation). The first step of this process was to clone the Tn903 NPT gene into pBluescript KS-, a vector that is relatively easy to work with. After this, the basic procedure was to perform PCR with the 1st set



of mutagenic primers thereby mutating the XhoI site, ligate the blunt end PCR product, and then perform transformation. This procedure will be repeated 2 more times with the 2nd and 3rd set of mutagenic primers that create the SmaI and HindIII mutations, respectively. At the end of the 7 week period, the Xho I restriction enzyme site was effectively removed from the Tn903 NPT gene, while the kanamycin-resitance activity of the gene was kept intact. This indicates that the method of inverse PCR using mutagenic primers effectively achieved the intended goal. The final 2 mutations of the SmaI and HindIII sites will be accomplished in the same manner, thus generating a Tn903 NPT gene minus the aforementioned restriction enzyme sites, which can be used as a selectable marker in binary vectors. And because the Tn903 NPT gene is smaller than the Tn5 NPT gene, use of the Tn903 NPT gene as a selectable marker will yield a smaller, more compact binary vector, which can be advantageous. Additionally, with these mutations, a binary vector can be constructed using the Tn903 NPT gene as the bacterial selectable marker and the Tn5 NPTII the plant selectable marker without any potential for homologous recombinations between the two genes. This is because of the fact that while both genes have the same function, they share no sequence similarities. Therefore, the results of this study hold great potential for the world of binary vectors.

Ali Baghian and Knostantin Kousoulas, Pathobiological Sciences, LSUSVM
Construciton of recombinant vesicular stomatitis viruses (VSV) expressing Bovine Coronavirus E, M and S Proteins

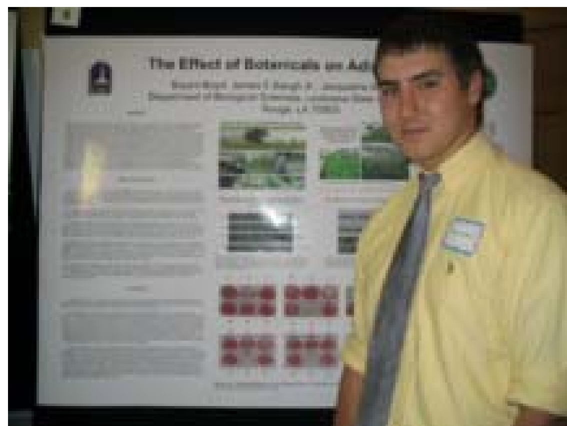


Genes encoding BCoV M, E, and S proteins were cloned into intermediate plasmids containing specific protein epitopes used for detection purposes. The 3X FLAG epitope was designed to be located at the 5' end of S gene while the same 3X FLAG epitope was placed at the 3' end of the M gene and V5 epitope was located at the 3' end of E gene. E, M, and S genes with their respective epitopes were independently inserted between the G and L genes of plasmid pVSV-XN2 containing the entire VSV genome, thus making three different large plasmids named pVSV-E, pVSV-M, pVSV-S. VSV gene containing plasmids pBS-N, pBS-P and pBS-L were all under a T7 promoter which is essential for efficient expression of viral RNA. Cells were first infected for an hour with a Vaccinia virus possessing T7 polymerase and subsequently transfected with the mix of plasmids. Vaccinia virus was removed by filtration through 0.2 μ m filter, while the much smaller recombinant VSV was recovered and purified after serial passages. Infected cells were examined and proteins expressed with their antibody epitopes were visualized using immunofluorescence microscopy.

Bryant Boyd and Jacqueline Stephens, Biological Sciences
The Effects of Botanicals on Adipogenesis

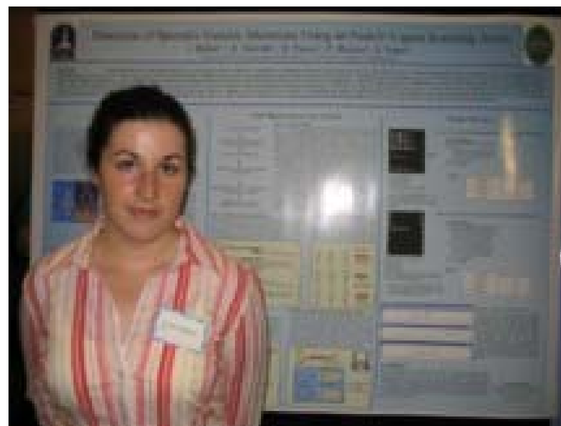
Obesity is the primary disease of fat cells and a major risk factor for the development of non-insulin dependent diabetes mellitus (NIDDM), cardiovascular disease, metabolic syndrome, and hypertension. Obesity and its related disorders result in dysregulation of the mechanisms that control the differentiation and expression of genes in adipocytes. Currently, botanicals are

used to treat a variety of pathological conditions. However, most botanicals are not regulated by the FDA and their ?over-the-counter? use has not been extensively studied by scientists. Therefore, the NIH has awarded funds for five centers of excellence in the country to study the effects of botanicals for the treatment of various diseases. Through funds obtained via the Pennington Biomedical Research Center, we are screening a large number of botanical extracts. Our goal is to determine if these compounds affect adipogenesis. We have used the 3T3-L1 cells to examine the effects of over 50 botanical extracts on fat cell differentiation. We have examined lipid accumulation by performing Oil Red O staining and examined adipocyte marker gene expression by performing Western blot analysis. The eventual goal of our screening efforts is to identify compounds that will be used to test in vivo and used for the development of a drug for the treatment of metabolic diseases. Our studies have identified at least one botanical that enhances adipogenesis and several that attenuate fat cell differentiation. Hence, our results demonstrate that some botanical extracts can modulate fat cell differentiation. Further characterization of these candidate botanicals will provide a strong foundation for in vivo studies that may lead to the development of a drug for use in the treatment of metabolic diseases.



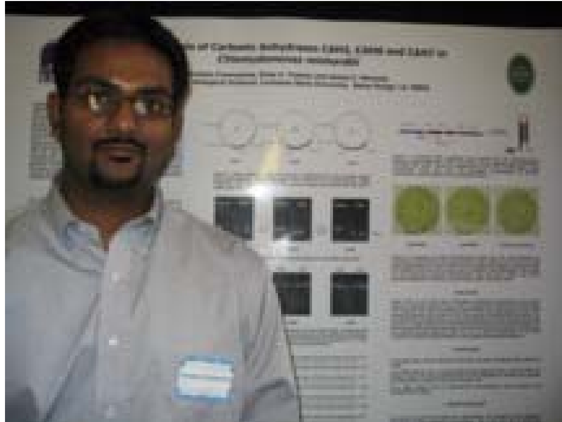
Jillian Bybee (Carroll College) and Steven Soper, Chemistry
Detection of Sporadic Genetic Mutations Using an EndoV/Ligase Scanning Assay

The identification of sporadic point mutations in tumor-suppressor genes, which are responsible for regulating cell growth, has been linked to the early detection of cancer, as well as with identifying those individuals with a genetic predisposition to developing cancer. Our research investigates the use of a single-step endonuclease V/ligase scanning assay in the detection of unknown point mutations in genomic DNA. Specifically, the research focuses on the K-Ras gene, which has been linked to several different types of cancer, including colorectal cancer. A universal PCR strategy using fluorescently labeled universal primers and unlabeled gene specific primers allowed for amplification of K-Ras oncogene sequences. Amplification was followed by heteroduplex generation from the universal PCR products. This procedure resulted in products with one of two mismatches (A/C or G/T) that could be used for endonuclease treatment. Endonuclease V recognizes and cleaves mismatches, as well as some correctly paired bases, within the gene sequence. A highly specific ligase was used to reseal miscleaves, which eliminates artifacts, thus increasing signal intensity. Capillary gel electrophoresis was employed to distinguish the single-stranded products of the EndoV/ligase assay based on fragment-size differences. The early detection and identification of mutations could lead to increased survival



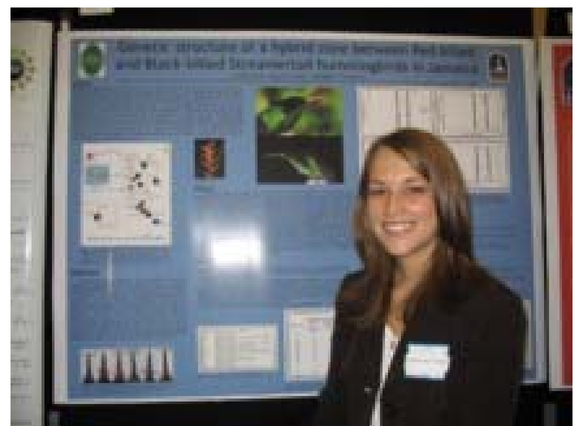
rates for individuals testing positive for those mutations. Further research should include the potential to transfer the assay to a microelectrophoretic device with possible clinical applications.

Krishen Cunnusamy and James Moroney, Biological Sciences
RNAi Analysis of Carbonic Anhydrases Cah3, Cah6 and Cah7 in *Chlamydomonas reinhardtii*



Carbonic anhydrases (CAs) are a widely expressed family of zinc-containing metalloenzymes that catalyze the reversible hydration of CO_2. First identified in 1933, in red blood cells, CAs have since been found to be abundant in bacteria and all mammalian tissues where they play a key role in the regulation of pH and fluid balance. In plants and algae, CAs are involved in photosynthesis and in the Carbon Concentrating Mechanism. In *Chlamydomonas reinhardtii*, the eight CAs that have been identified. They facilitate CO_2 and HCO_3^- uptake, thus enhancing photosynthesis by overcoming the slow diffusion of CO_2 in water. Our project is aimed at targeting some of those CAs namely CAH3, CAH6 and CAH7 by RNA interference and determining the relative effects of their respective silencing. To accomplish this, we amplified particular fragments of each gene from the cDNA library and genomic DNA and fused them into the pSL72 vector. The resulting constructs were linearized and transformed into *Chlamydomonas* by electroporation. Successful transformants, grown on paromomycin plates, were then screened on low CO_2 to determine whether the reduction of these CA messages affect photosynthesis. Preliminary evidence suggests that the expression of CAH3 and CAH6 has been reduced using these constructs. Additionally, some of the strains with potentially reduced CA expression grow slowly under low CO_2 conditions.

Katherine Faust and Robb Brumfield, Biological Sciences and Museum of Natural Sciences
Genetic structure of a hybrid zone between two species of Jamaican Streamertail Hummingbirds



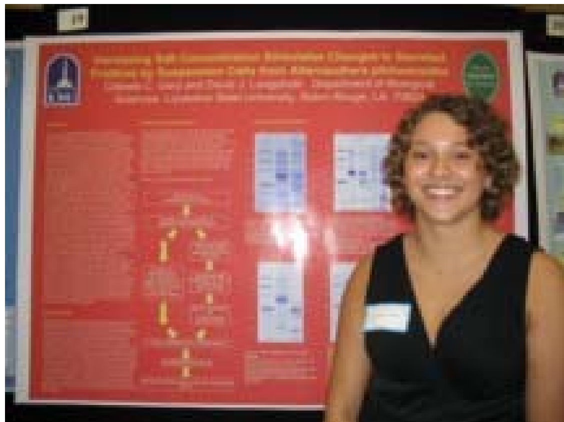
When the geographical distributions of two closely related species come into contact, one potential outcome is the formation of a stable hybrid zone, a narrow geographic region in which the two species interbreed. Such hybrid zones offer a window on the speciation process, because the evolutionary dynamics involved in the intermingling of divergent genomes can be observed directly. Hybrid zones also offer the opportunity for adaptive genes or traits to move from one species to the other. Here, we performed the first genetic characterization of a hybrid zone between Red-billed and Black-billed Streamertail (Trochilus) hummingbirds in Jamaica. We sequenced three nuclear genes in a series of 100 hummingbirds collected along a transect spanning the transition from red-billed to black-billed birds. We found that bill color changes from red to black over only 15 kilometers (~9 miles), but over this same region nuclear

gene frequencies did not change significantly. These results suggest that the forces maintaining the narrow transition in bill color (and the genes controlling bill color) are not acting on other regions of the genome. Future work will focus on increasing both the geographic sampling and the number of genes to help clarify what forces are maintaining the differences in bill color.

Celeste Gary and David Longstreth, Biological Sciences

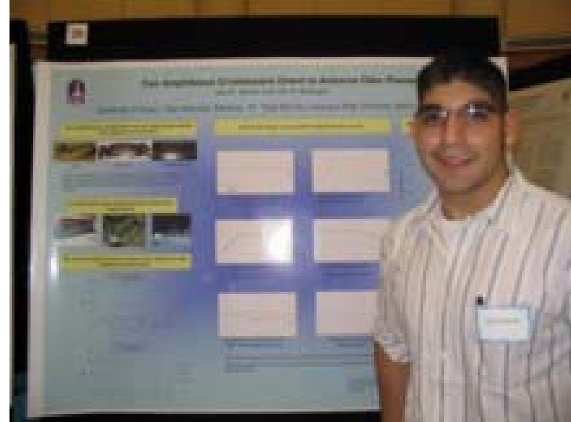
Increasing Salt Concentration Stimulates Changes in Secreted Proteins by Suspension Cells from *Alternanthera philoxeroides*

Plants and cell-suspension cultures of *Alternanthera philoxeroides* (alligator weed) tolerate significant increases in salinity. Our goal was to learn more about the production of a secreted, 22 kD polypeptide from suspension cultures of *A. philoxeroides* that is stimulated by exposure to 200 mM NaCl. Heterotrophic suspension cells, not previously exposed to high salinity, were sub-cultured in nutrient media (control) or nutrient media with varying concentrations of NaCl or KCl for various periods of time. After removal of cells, media proteins were precipitated with 60% $(NH_4)_2SO_4$, and then dialyzed in dilute buffer. Cells were ground in buffer to extract soluble proteins for comparison. Media and cell proteins were separated by gel electrophoresis (SDS-PAGE). For seven-day old cultures a polypeptide, approximately 22 kD in size, was found in high-salt (200 mM NaCl) media but not in control media. The 22 kD polypeptide was not present in SDS-PAGE of soluble cell proteins grown in either the control or high-salt treatments. In fact, SDS-PAGE proteins of the control and high-salt soluble cell proteins were the same. The presence of the 22 kD polypeptide was examined at different times after transfer to 200 mM NaCl media. The intensity of the 22 kD polypeptide was weak on days two through four. After day four, the intensity of the 22 kD polypeptide increased through day six but then decreased on day ten. The effect of 200 mM KCl on the SDS-PAGE profile for media proteins was also examined. The 22 kD polypeptide was present in the media containing KCl indicating it is not just stimulated by NaCl. Suspension cells were also cultured in 50 mM and 100 mM NaCl for seven days. Since the 22 kD polypeptide was more intense in the 200 mM NaCl than at lower NaCl media concentrations, stimulation of the 22 kD polypeptide appeared to be affected by NaCl concentration. We examined the possibility that the 22 kD polypeptide was present in the cell wall of control cells and was only released by high NaCl concentration. Control cultures grown for seven days were treated with 200 mM NaCl for five minutes. The 22 kD polypeptide was not present in this media. Also, filtered cells from control cultures grown for seven days were exposed to 100 mM $CaCl_2$ and 90 mM sucrose for 30 minutes. Although this treatment did release proteins from the cells, the 22 kD polypeptide did not appear to be among them. Preliminary data indicates that the 22 kD polypeptide may be related to osmotin, a protein that appears to be part of a plant signal pathway stimulated by stress.



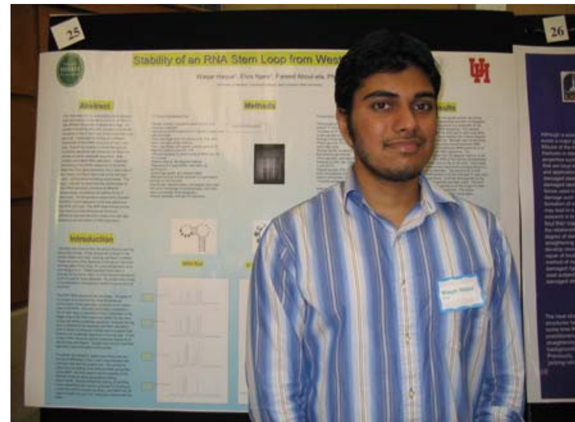
Jose M Garza (UTPA), Jim Belanger, Biological Sciences
Neuroethology – Crustacean Orientation in Airborne Odor Plumes

Numerous studies have been completed to determine crustacean orientation behavior in plumes. These studies are carried out in the attempt to discover insight to crustacean physiological and behavioral characteristics involved in locating food sources. Most studies test these odorants in a flume where test subjects and odorant source is submerged partially or entirely within water. In this study a different approach was taken by using airflow as the medium of odor transport for crabs and crayfish that display aquatic and terrestrial characteristics. Numerous trials were done at three speeds of laminar flow. Three odor sources were also used to have differing chemical stimuli. Results suggest that although the majority of the subjects being tested traveled upwind towards the source there was no evident olfactory mediated guidance. It can be concluded that these invertebrates are not able to display positive rheotactic behavior in response to chemical stimuli with airflow as the transport medium.



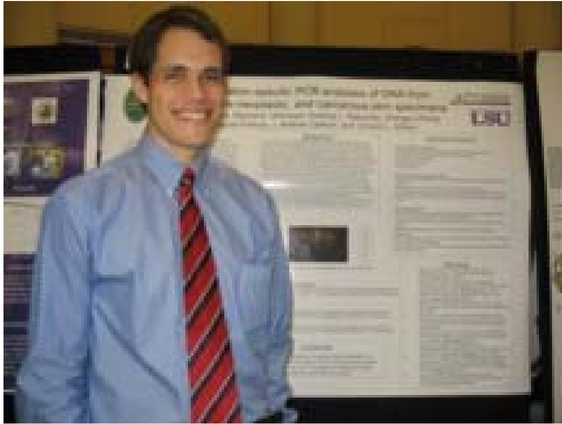
Waqar Haque (University of Houston) and Fareed Aboul-ela, Biological Sciences
Stability of an RNA Stem Loop in the West Nile Virus RNA

The West Nile virus is a potentially lethal disease, and since being introduced to the U.S. in 1999, it has afflicted thousands of people each year. My project involved working with the penta-nucleotide sequence in the 3' stem-loop of the West Nile virus genome. I examined the folding in 2 different sequences of the mRNA transcript of the 3' stem loop. One of the mutants involved flipping a six nucleotide sequence that contained the flavivirus-conserved penta-nucleotide sequence. This mutation eliminated RNA replication. I observed the folding of the mRNA sequence of the entire West Nile Virus gene sequence, the 3' stem loop of the mutant, and the 3' stem loop of the wild type. Later, I did temperature folding experiments. This way, I was able to determine the conformation of the mRNA secondary structure at different temperatures, elucidating the stability of the 3' stem-loop. The temperature experiments showed the WNV mutant sequence to be less stable than the WNV wild type. The NMR experiments proved that there is a three dimensional structural difference between the WNV mutant and wild type, leading to the elimination of RNA replication.



Eric Harris (Troy) and Vince Wilson, Biological Sciences
Methylation-specific PCR Analyses of DNA from Norma, Neoplastic, and Cancerous Skin Specimens

The addition of methyl-groups to cytosines residues in CpG islands is involved in the transcription inactivation of two tumor suppressor genes (*p16* and *DAPK*). The *p16* gene protein product is an inhibitor of cell division. The *DAPK* gene (death-associated protein kinase) codes for a protein involved in apoptosis (programmed cell death). The absence of either or both of these genes can lead to the uncontrolled cell division characteristic of neoplasia. Analyzing, cataloging, and understanding the patterns of the CpG islands of these genes is important for understanding tumor suppressor gene silencing in human cancer. MSP (methylation-specific PCR) can be used to determine the methylation status of these CpG sites. MSP requires that the template DNA be first modified with a sodium bisulfite treatment, which converts all unmethylated, but not methylated, cytosines to uracil. Methylated- and unmethylated-specific primers are then used to amplify the DNA. In this study, we demonstrate the use of MSP to identify hypermethylation changes in the promoter regions of *p16* and *DAPK* in DNA extracted from normal, pre-neoplastic, and squamous cell carcinoma skin specimens of female vulvar tissue.

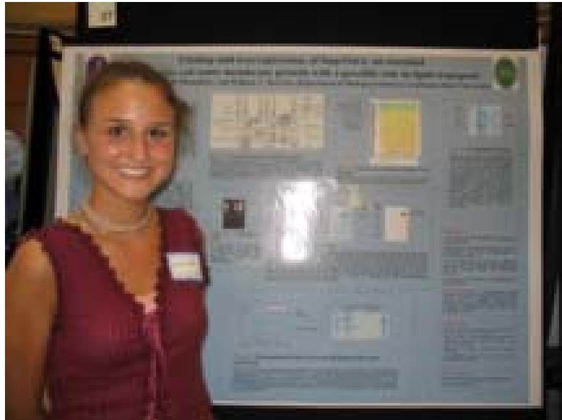


Jared Landry and M.C. Rush, Plant Pathology and Crop Physiology, LSU Agricultural Center
In Vitro Selection for Mutants Resistant to Toxic Compounds in Rice

Blast disease, caused by *Pyricularia oryzae*, is one of the most widespread and serious diseases in rice. α-picolinic acid (C6H5NO2) is a toxin produced by *P. oryzae* in support of its penetration and infection activities. Another toxic compound, Liberty herbicide, used for weed control is a toxic compound of microbial origin, which will be used on rice plants transformed for resistance to Liberty. This system is being developed commercially and will be sold under the name "Liberty-Link". We are trying to develop an alternative non-transformed Liberty resistance based on *in vitro* mutations to avoid the complications of using GMO rice crops. In this project, cells were screened *in vitro* for mutants resistant to these toxins. Calli were obtained on solid callus induction medium using seed of Taipei 309 rice. After three months, these calli were transferred to liquid medium in flasks to begin *in vitro* screening. Multiple concentrations of Liberty herbicide and α-picolinic acid were each used to screen calli for mutants resistant to these toxins. The calli showed resistance at 10 ppm of Liberty herbicide and 20 ppm of α-picolinic acid. Mutants at these concentrations were transferred to solid regeneration medium to obtain plants. Plants resistant to α-picolinic acid were regenerated and are currently being tested for their resistance to the toxin.

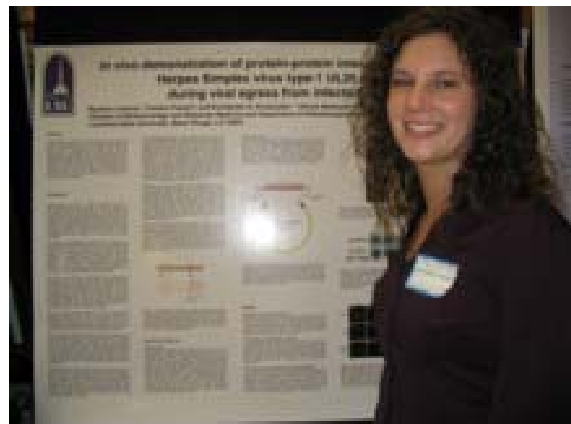
Plants resistant to α-picolinic acid will be tested for resistance to infection by *P. oryzae*. Genes for resistance to rice blast can be transferred to commercial varieties by conventional breeding procedures. When developed, Liberty resistant plants can be crossed with commercial varieties to replace Liberty resistance based on transgenes with the resistance based on a mutant gene.

Leslie Lekatz (Bemidji State) and William T. Doerrler, Biological Sciences
Cloning and overexpression of Imp/OstA, an essential *Escherichia coli* outer membrane protein with a possible role in lipid transport



Lipopolysaccharide (LPS), an essential component of the *Escherichia coli* (*E. coli*) outer membrane (OM) is synthesized inside the cell and must cross the inner membrane (IM), periplasm, and OM in order to reach their location in the cell, the outer surface of the OM. MsbA, an IM protein of the ABC transporter superfamily, is believed to play a role in the transport of newly synthesized LPS across the IM. The mechanism of transport of LPS across the outer membrane is not understood, but recent evidence suggests that Imp/OstA (increased membrane permeability/organic solvent tolerance), an essential and highly conserved *E. coli* OM protein, plays a role in lipid transport across the OM. This study was conducted to look more closely at Imp and its role in membrane biogenesis. The *imp* gene was amplified from *E. coli* genomic DNA by polymerase chain reaction and cloned into an expression vector behind a T7 promoter, which was then used to transform *E. coli* cells. Imp was overexpressed and localized correctly to the OM. The protein was resistant to solubilization by several detergents, even though other OM proteins were solubilized. This work begins our efforts to purify, crystallize and structurally characterize this novel OM protein.

Rachael Liesman (Illinois Wesleyan University) and Konstantin G. Kousoulas, LSUSVM Pathobiological Sciences
In Vivo demonstration of Protein-Protein Interactions between Herpes Simplex Virus Type-1 UL20 and gK during viral egress from infected cells

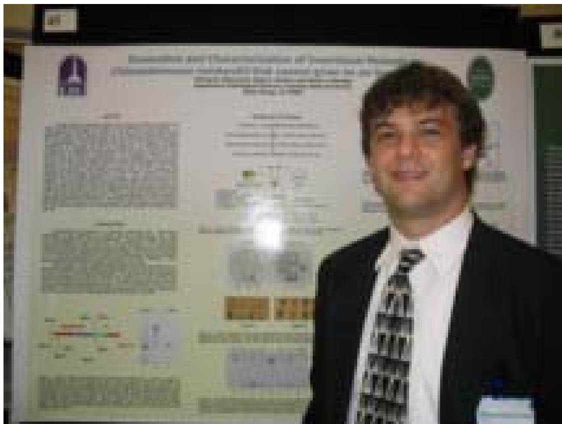


Recent evidence suggests a functional relationship between Herpes Simplex virus type-1 UL20 and gK. We sought to demonstrate that UL20 and gK specifically interact using a split-ubiquitin yeast two-hybrid system. Contrasting with traditional yeast two-hybrid experiments, the split-ubiquitin yeast two-hybrid system allows us to investigate interactions between integral membrane proteins such as gK and UL20. Confocal microscopy was also used to determine the intracellular localization of UL20 and gK. UL20 has been successfully fused into the bait vector and shown, through interactions with a control prey vector, to be properly

inserted into the membrane. UL20 and gK have been shown to co-localize to the *trans*-golgi network (TGN) by confocal microscopy.

Michael McCormick and James Moroney, Biological Sciences
Generation and Characterization of Insertional Mutants in *Chlamydomonas reinhardtii* that Cannot Grow on Air Levels of CO_2

A basic approach in genomic studies is to identify a mutant strain defective in some process and compare that strain to the wild-type organism. In *Chlamydomonas reinhardtii,* the process of interest is the Carbon Concentrating Mechanism (CCM). The function of the CCM is to capture and concentrate various forms of inorganic carbon around Rubisco, the main CO_2 fixing enzyme, in preparation for photosynthesis. It has been found that under low CO_2 conditions, the genes responsible for activating the CCM are up-regulated. By utilizing insertional mutagenesis, it is possible to interrupt genes that are involved in the CCM and create mutants that are defective in this process. The phenotypic evidence for such transformants can be seen in colonies that grow well on elevated CO_2 but minimally on low CO_2. The insert used in these screenings was the *APHVIII* gene, which encodes resistance to the antibiotic paromomycin. In the screen employed in this study, mutants that showed a weak growth phenotype under low CO_2 conditions and resistance to paromomycin were selected. In some of these mutants, the inserted *APHVIII* gene has possibly disrupted a CCM gene. Once a promising mutant is obtained, the site of DNA insertion can be identified using *i*PCR and Adaptor PCR methods. Both methods operate by exploiting insert-specific primers to amplify the region flanking the insert. About 70 transformants were generated that showed continuous poor growth under low CO_2 conditions. Finally, the sequences that have been obtained will be matched up to the *Chlamydomonas* database in order to determine where the insert landed in the genome. The location of the insert in two mutants has been determined. One insert is in a gene called *cia7*, which appears to be associated with the CCM and another is in the *RbcS2* gene which encodes the Rubisco small subunit.



Daren Molina (UTPA) and F.A. Rainey, Biological Sciences
Survival of Soil Bacterial Communities Following Desiccation

The Atacama Desert is located in northern Chile and Southern Peru along the Pacific coast. This desert has been called the driest place in the earth. According to previous studies, there is almost no detectable trace of bacteria in the soil of the hyper-arid core region of the desert around 24º and 25º S. The soil bacterial counts increase to the south along the precipitation gradient. It was hypothesized that there would be a reduction in the colony forming units (cfu’s) of the soil bacteria if samples from the

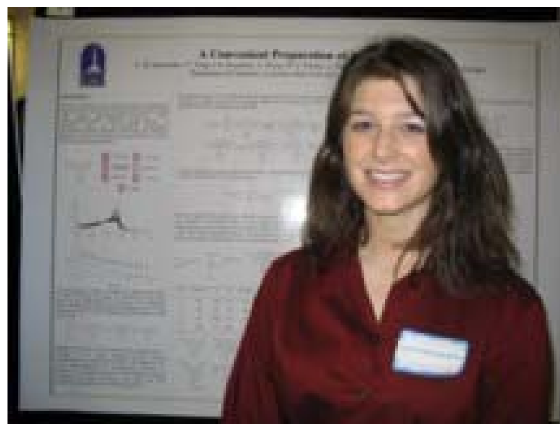
moister region of the desert were desiccated. Soil samples from three regions in the desert (AT05 176 – Altamira, AT05 177 – Brad's Site, AT05 22 – Vineyard) and a Baton Rouge cornfield (CF05) were tested. Soil from each site was placed in separate Petri dishes and place inside a Secador Desiccator along with an amount of silica gel and allowed to dry. There were four replications of each sample placed in four desiccators. One desiccator was opened per week for the next four weeks and plated out on marine agar and 1/10 plate count agar (PCA). The impregnated plates were incubated at 28° for approximately 20 days and the counts were then analyzed for any trends and compared to a control. Humidity reading were taken of the four desiccators as well as the desert itself and then compared graphically. Desiccation appeared to have somewhat of an initial effect on the sites such as 1/10 PCA AT05 22. Less of a drop in CFU's was determined in 1/10 PCA AT05 177 and MA CF05. Desiccation did not appear to affect the cornfield site more than the desert sites. The data gathered will have to be further analyzed via PCR, DNA sequencing and organism identification to determine the types and quantities of organisms in the soil for comparison purposes.

Dayle Parnell-Lampen (Providence) and Kirsten Prufer, Biological Sciences
Developing an immunoprecipitation method to identify nuclear transport protein interactions of LXRα and LXRβ

LXRα and LXRβ are nuclear receptor proteins involved in cholesterol metabolism. The precise mechanism of the nuclear transport of these proteins is not yet understood. In this experiment, the objective was to better understand this transport by developing a method to identify which nuclear transport proteins bind to LXRα and LXRβ in the cytoplasm and in the nucleus. 293 cells used in this experiment contained plasmids bearing a gene for YFP spliced next to a gene for either LXRα or LXRβ so that the genes would be translated into a single YFP-LXR protein. Once enough protein was synthesized, two different extraction methods were used. A cellular fractionation using a Dounce homogenizer was done to isolate nuclear and cytoplasmic extracts and RIPA whole cell extracts were also made. These extracts were immunoprecipitated with a GFP antibody and a rabbit IgG antibody as a control. Western blot analysis was performed on the isolated proteins and staining was done for RXR. RXR was chosen because it is a heterodimer of LXR and is expected to interact very strongly with LXR. The gels consistently showed bands at the molecular weight of RXR but the protein did not appear to be specifically pulled down by the GFP antibody. Extracts that had not been subjected to immunoprecipitation were also stained for RXR as an additional control. While the fractionation method using the Dounce homogenizer showed RXR staining, the RIPA extracts did not. This indicates that the RIPA extraction method is unsuitable for this procedure as it probably causes degradation of the proteins. Overall, these results indicate that this method was unsuccessful. An optimized immunoprecipitation method, using the cellular fractionation by Dounce homogenizer technique, will need to be developed in order to identify the nuclear transport protein interactions of LXRα and LXRβ

Corin Schowalter and Robert Strongin, Chemistry
A Convenient Preparation of Xanthene Dyes

A facile synthetic route utilizing readily available reagents affords a series of regioisomerically pure xanthene dye derivatives. Advantages include relatively mild conditions and good to excellent yields. Non-polar, highly crystalline intermediates are isolable by standard chromatographic techniques. The intermediates are in the requisite xanthene oxidation state, thus avoiding the need for relatively inefficient oxidation chemistry and/or harsh conditions. The methods described herein are now being utilized in our lab towards the synthesis of new naphthofluorescein dye architectures currently unattainable via other methodology. During the course of this work, a new boron–mediated 1,2-aryl migration reaction was discovered

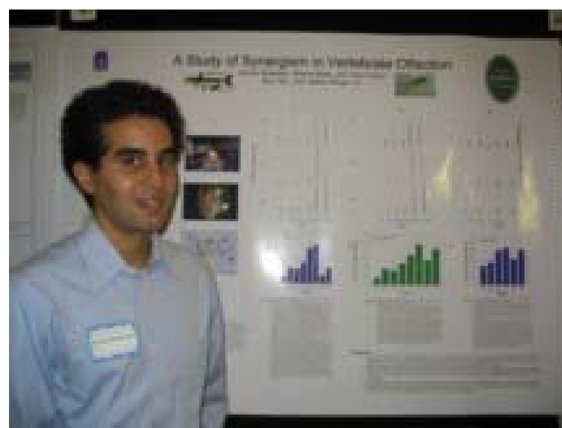


Daniel Serna (UTPA) and Dr. Randall Gayda, Biological Sciences
Analysis of *Salmonella* Isolates from Turtles by Repetitive DNA PCR Primers

The selling of freshwater turtles in Louisiana is a multimillion dollar industry. There has been an ongoing struggle to eliminate *Salmonella* bacteria from the red- ear slider turtles. Turtles examined from the Ponchatoula area, Pierre Part/Napoleonville area, and Jonesville area (all in Louisiana) have tested positive for containing *Salmonella.* The objective of this study is to separate species of *Salmonella* by using PCR to ultimately develop a bacteriophage cocktail that will eliminate *Salmonella* from the turtles. PCR analysis with primers ERIC2 and BOXA1R allowed us to separate strains of *Salmonella* into three different genera; *S. enterididis, S. arizonae,* and *S. diarizonae*.

Arman Sheybani and John Caprio, Biological Sciences
A Study of Synergism in Vertebrate Olfaction

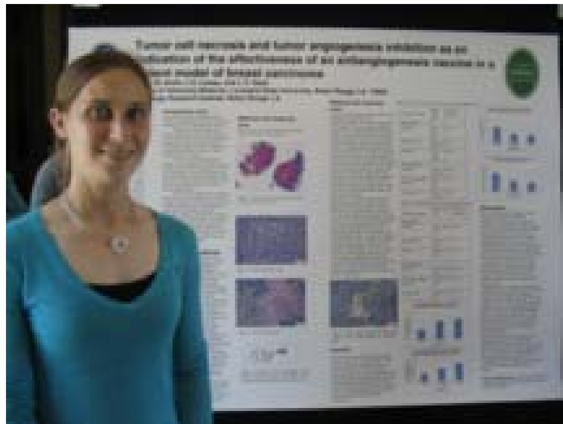
Electrophysiological experiments indicated that channel catfish (*Ictalurus punctatus)* and goldfish (*Carassius auratus*) detect a variety of odorants, such as bile salts, amino acids, polyamines, and nucleotides. While previous experiments investigated olfactory receptor responses to specific compounds representing each of these odorant classes, few studies have combined odorants from different odorant classes to



determine possible synergistic effects. In the present investigation, the fish were adapted to mixtures of polyamines and nucleotides, respectively, while mixtures representing the different classes of odorants were tested to determine if an enhancement of responses occurred. The underwater electro-olfactogram (EOG), a slow negative potential change in response to odorant stimulation that is comprised of olfactory generator current, was recorded *in vivo* with calomel electrodes from the water that continuously bathed the olfactory organ (4 mls/min). Test stimuli were presented at varying concentrations that provided similar EOG response magnitudes. The fish were immobilized with an injection of Flaxedil (0.1mg/100g body weight), and the gills of the fish were irrigated using water which contained the anesthetic MS-222 (0.005%). Synergism was determined to occur if the peak EOG response to a stimulus under a particular adapting regime was greater than its response under control water. The EOG responses were recorded on both tape and chart recorders. The results indicated that synergistic odorant responses occurred in catfish between ATP (adapting regime) and polyamines (test stimulus). For goldfish, synergistic responses occurred between ATP (adapting regime) and amino acids and polyamines as test stimuli.

Corey Smith and Larry Lomax, LSUSVM Pathobiological Sciences
Tumor Cell Necrosis and Tumor Angiogenesis Inhibition as an Indication of the Effectiveness of an Antiangiogenesis Vaccination in a Rodent Model of Breast Carcinoma

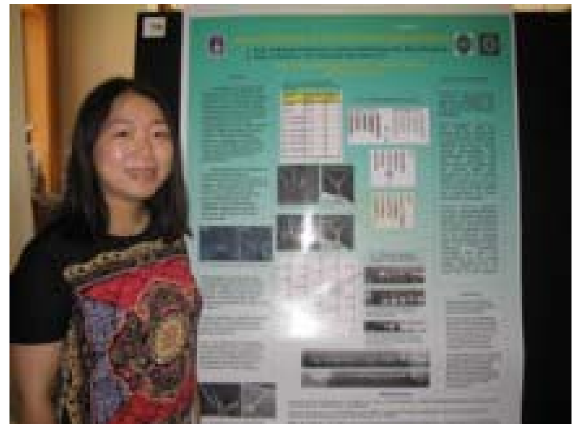
Angiogenesis is required for tumors to grow beyond the size of 1 mm. Tumors secrete vascular endothelial growth factor (VEGF) to induce angiogenesis. Antiangiogenic therapy is a promising area of cancer treatment. A vaccine made by the Mastology Research Institute aims to have the body produce antibodies against VEGF. The efficacy of this vaccine was examined in two experiments (I and II) in a Fisher 344 rat mammary cancer model. After the rats had been vaccinated they were injected with 1 X 106 13762 Mat B III rat mammary adenocarcinoma cells (ATCC). After being sacrificed the tumors were taken for histological analysis. Using the percent of necrotic area versus total tumor area and the number of vessels per “hot spot”, the effectiveness of the vaccine against angiogenesis was determined. The results showed that for experiment I the percent of tumor cell necrosis was: control had 20.2% necrosis, vaccine plus Incomplete Freund’s (IF) had 38.4% necrosis, and vaccine without IF had 42.1% necrosis. The percents of tumor cell necrosis for experiment II were: the control had 18.6% necrosis, vaccine with antigen attached to Bovine Serum Albumin (BSA) and IF had 29% necrosis, and the vaccine with IF had 39.4% necrosis. For experiment I the results for the vessel count per hot spot were: control 14.7, vaccine plus IF 8.6, and vaccine without IF 7.7. The results for experiment II were: control 13.6, vaccine with antigen attached to BSA and IF 7.7, and vaccine with IF 6.3 mean number of vessels per hot spot. For both experiments there was significant difference between the control and the two treatment groups for both percent necrosis and mean number of vessels per hot spot. Animals that were vaccinated had an increase in percent of tumor cell necrosis and a decrease in the number of blood vessels in the mammary tumor. These studies demonstrate the effectiveness of the vaccine in retarding angiogenesis in the rodent mammary tumor and thereby causing more



tumor cell necrosis as compared to controls. There were no significant differences between the different vaccine groups.

Jing Zhou and John Larkin, Biological Sciences
Genetic Mapping of Siamese Phenotypic Modifiers

Arabidopsis trichomes (leaf hairs) are cells that extend from the epidermis of leaves and sepals. They are large and easily visible, making it easy to identify mutant phenotypes. During development, wild type (WT) trichomes exit the mitotic cycle and enter an endoreplication cycle. After such a switch, DNA replication occurs without nuclear and cellular division, resulting in cells with more than 2C DNA content. This characteristic makes trichomes well-studied models for cell differentiation.



The s*iamese (sim)* mutation is recessive and produces multicelluar trichomes. It seems to trigger extra rounds of cell division that are not observed in WT trichomes, suggesting that the gene encodes a cell cycle regulator. It is likely that the SIAMESE (SIM) gene product interacts with other cell cycle components.

Homozygous *sim* seeds have been mutagenized with ethyl methane sulfonate to identify genes functioning together in the same pathway. In particular, several mutations that result in strongly enhanced multicellularity have been recovered, and they are thus called enhancer of *sim* (*ens*). We present here phenotypic characterization of the mutations. We also determined their chromosomal location using bulk segregant analysis. Because the *Arabidopsis* genome is sequenced, we can isolate these *sim* phenotypic modifiers based on their chromosomal location. This may allow us to identify other cell cycle components that interact with the SIM gene product and help to explain the complexity of plant cell cycle regulation.

Other Students

EXROP

Utibe Bickham and Paul Ahlquist, Howard Hughes Medical Institute and Institute for Molecular Virology, University of *Wisconsin-Madison*
Characterization of Newly Identified Kaposi Sarcoma-Associated Herpesvirus (KSHV) Transcripts

The frnk repeat region in the KSHV genome has not previously been predicted to have protein coding potential because there are no start codons for the initiation of translation nor are there stop codons to signal the end of translation in any of its three open reading frames (ORF). Nevertheless, transcriptional activity in this region can be readily detected.

L to r: Alquist, Bickham and Johan den Boon

Therefore, we hypothesized that proteins might be synthesized from the frnk transcripts using an alternate translation initiation codon. PCR-based single and triple FLAG-epitope tagging and DNA transfection was used to express the frnk RNAs in 293T cells under the direction of the CMV promoter, but no protein production from any of the three open reading frames of the frnk region was detected. The positive control, FLAG-tagged P53, was clearly detected on both the single FLAG western blot and showed increased detection sensitivity on the triple FLAG western blot. Northern blotting used for RNA analysis proved that the frnk transcripts were produced from the CMV promoter and available. Our inability to detect protein synthesis from the frnk RNAs suggests a non-mRNA type function for these novel KSHV transcripts.

Individual REUs

Brittany Marchant (University of Science, Philadelphia) and Gudrun Schmidt, Chemistry

Flow Induced Structures in Polymer-Clay Hydrogels

The purpose of this study is to determine how the structures of clay-polymer gels made be manipulated by observing and measuring the rheological properties of the gels. Strain sweep, frequency sweep, and creep tests were performed. The goal is to produce a substance with good barrier properties that may be used to protect tissue of people such as burn victims.

Amanda Steffens (U Wisconsin – River Falls) and Paul Russo, Chemistry

Temperature Response of Poly- £`-carbobenzyloxy-L-lysine

Polarized light microscopy and polarimetry were used to characterize the helix-coil transition in poly (ε-carbobenzyloxy-L-lysine), PCBL, at a molecular weight of 480,000. The polarimeter data for a solution at 1% concentration by weight of PCBL in *m*-cresol showed that the change from random coil to α-helix conformation happened at 26.8 ± 0.2 °C, in agreement with Matsuoka et al.[1] Three solutions of PCBL, dissolved in *m*-cresol, each at a different concentration, were observed through a range of temperatures using polarized light microscopy. A solution at 25.07% by weight formed liquid crystals immediately at room temperature—i.e., at a temperature where dilute PCBL exhibits a random coil conformation. A solution at 15.04% required three days at 35°C to form liquid crystals around the edge of the sample. A 20.00% solution took less than 24 hours to form liquid crystals along the edges of the sample at 35°C. All samples exhibited the cholesteric "texture" that resembles a person's fingerprint. After being warmed, the liquid crystal solutions were allowed to cool to room temperature, where they did not revert to their previous states. This suggests that liquid crystal formation in PCBL/m-cresol

solutions exhibits a strong history dependence, at least for the very high molecular weight of 480,000.