Abstract booklet

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Presented by:

GOBS

UWM's Graduate Organization of Biological Sciences

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https://uwm.edu/biology/spring-2022-biological-sciences-research-symposium/ or scan the QR code.

# Talk Abstracts

## Undergraduate talk

### Ecology

1. **Metabolic Fingerprints of Pitcher Plant Microbiomes Show Dynamic Community Succession**

*By David Deshpande* & Erica Young*

The carnivorous pitcher plant Sarracenia purpurea supplements its nutrient needs by trapping and digesting insect prey. Pitchers are initially sterile, and microbes are recruited which help digestion by producing hydrolytic enzymes to break down prey. However, the development of microbiome metabolic functional capacities has not been characterized. This project aimed to use metabolic fingerprinting of communities over early succession by applying Biolog EcoPlates™, which have 31 diverse carbon substrates for screening microbial metabolic capacity. Pitcher plants in the Cedarburg Bog were identified and water sampled from 6 newly-opened pitchers over 27 days after opening. EcoPlate™ screening and hydrolytic enzyme activity assays (chitinase, lipase) were conducted. Microbiome metabolic functions changed drastically during early succession; there was no metabolic substrate use capacity in newly opened pitcher communities (indicating sterility), but by day 6, most substrates could be metabolized. Substrate use patterns over day 3 - 27 showed marked differences between replicate pitcher communities. Principal component analysis (PCA) demonstrated some functional convergence between replicate communities over day 3-27. PCA also showed more similar responses across communities for amino acids than across all substrate types. Chitinase and lipase activities varied by pitcher, but chitinase activity peaked on day 13 while lipase increased through day 27. Application of Ecoplate™ and biochemical enzyme assays to pitcher microbial communities demonstrates rapid acquisition of community metabolic functions during microbiome recruitment and succession in S.

purpurea. These findings emphasize the importance of microbiome diversity in nutrient breakdown that supports the host plant.

## Graduate Talk

### Cell and Molecular Biology

1. **Searching for Jun Dimerization Partners that Promote Regeneration-Associated Gene Expression after CNS Injury**

*By Heather L. Leskinen*, Shama P. Mirza, Ava J. Udvadia*

In humans and other mammals, injury to the central nervous system (CNS) can cause a permanent loss of neuronal function, leading to cognitive defects, limb paralysis, and other neurological disabilities. In contrast, some non-mammalian vertebrates, like zebrafish, have the remarkable ability to functionally regenerate axons after CNS injury by reactivating and sustaining the expression of regeneration-associated genes. Our recently published work identified the jun gene as a putative target of regeneration-specific enhancers (Dhara et al., 2019). Our combined RNA- and ATAC-seq analyses revealed the Jun protein as a potential master regulator of stage-specific regeneration after optic nerve injury in zebrafish. Jun functions as an obligate dimer and is known to both homo- and hetero-dimerize to regulate gene expression in response to injury of the peripheral nervous system. However, it is not known if these same binding partners interact with Jun during CNS regeneration. To investigate this, we will use the zebrafish optic nerve crush as a CNS injury model to identify Jun-interacting proteins over the course of regeneration using proximity labeling assays, followed by mass spectrometry. Once candidate binding partners are identified, the functional significance of specific Jun interactions in vivo with forced expression of heterodimers by examining axon growth in larval zebrafish optic nerve transection models. Elucidating the zebrafish gene regulatory program will aid in developing new therapeutic approaches in human CNS regeneration.

## Ecology

### 1. Among-female variation in preference function structure for two acoustic traits (Hyla versicolor)

*By Kane D Stratman* and Gerlinde Hoebel*

Female choice is a widely researched topic of sexual selection. Biologists have characterized both the variation in male mating displays and shape of female preferences for such behaviors across a variety of taxa. A female's reference functions represents both the most preferred male trait value and her willingness to accept non-ideal values. In the wild, females are expected to choose based on preferences for multiple aspects of a male display, however the previous research has only measured female-by-female preferences for single traits. In this study we built two preference functions per individual (Eastern gray treefrogs); one exploring responses to calling duration, and another to calling rate. There was considerable variation in general preference shapes, implying that population-level measures in fact hide strongly divergent behaviors. In particular, we found that females are nearly evenly split in their assessment of very rapid calls; many prefer the most extreme rates, while the rest strongly prefer an intermediate value. Knowledge of a female's preference for one trait did not predict her preference for the second. Comparing these bivariate functions to the acoustic distributions of actual recorded males, we found support for directional selection, however the wide range of trait preferences predicts the maintenance of male signal variation.

## Microbiology

### 1. AraC Family Transcription Factor VfmE Differentially Regulates Pectate Lyase Production by Binding to c-di-GMP in Plant Pathogen Dickeya Dadantii

*By Biswarup Banerjee*, Manda Yu, Ching-Hong Yang*

*Dickeya dadantii* is a phytopathogenic bacterium that infects a wide range of host plants. The bacterium secretes pectate lyases (Pel) through the type II secretion system (T2SS) that degrades the cell wall in host plants. The virulence of D. dadantii is regulated by the second messenger cyclic diguanylate monophosphate (c-di-GMP), and the equanimity of c-di-GMP is maintained by a number of diguanylate cyclases and phosphodiesterases. Deletion of a phosphodiesterase ecpC repressed pelD transcription, and this repression was found to be relieved by an additional deletion in vfmE. VfmE is an AraC type of transcriptional regulator belonging to the Vfm quorum-sensing system. Our results suggest that VfmE is a c-di-GMP binding protein that functions as an activator of pel at low c-di-GMP concentrations and a repressor of pel at high c-di-GMP concentrations through regulation of the transcriptional activator SlyA. Multiple sequence alignment with known c-di-GMP binding proteins revealed a “RWIWR” motif in VfmE that is essential for c-di-GMP binding. Lastly, since VfmE binds c-di-GMP via the RxxxR motif, mutation in R93D of VfmE eliminated the c-di-GMP-related phenotypes in Pel activity. Our results demonstrate that VfmE not only regulates the Vfm quorum-sensing but also is a c-di-GMP effector, suggesting that D. dadantii integrates the c-di-GMP signaling network with the Vfm quorum-sensing pathway during environmental adaptation thereby regulating its virulence.

# Poster Abstracts

## Undergraduate

### Cell and Molecular Biology

1. **The "bloody red" of Hemimysis anomala: A spectral analysis of the color pigments in the chromatophores of the invasive opossum shrimp in Lake Michigan.**

*By Lavinia Bauer* and Dr.John A. Berges*

Hemimysis anomala is a relatively new but fast-spreading invasive species in the Great Lakes and little is known about its success or its place in the food web. One of the crustaceans most obvious characteristics is its "bloody red" color and its ability to change the intensity of its coloration by contracting and extending chromatophores across its body in changing environments. The pigments responsible for the color as well as the specific environmental conditions that cause color changes are poorly understood. To identify the color pigments causing the red color we sonicated individuals in ethanol (100%) and DMSO (10%) and measured absorbance (200-800nm) of the full body, eggs, embryos, and single body parts using spectrophotometry. Absorbance peaks at wavelengths around 480nm suggest carotenoid pigments while peaks at about 668nm suggest the presence of chlorophyll, most likely ingested through prey items. We have observed enhanced red color when organisms are kept at very cold temperatures (4°C) or in darkness. As a next step, we will test combinations of light and temperature to understand how they affect coloration.

## 2. Investigation of gene expression in the kidney of an MYH9-Related Disease zebrafish model

*By Matthew R. Wagner*, Laura A. Rolfs, Marissa E. Davies, and Jennifer H. Gutzman*

MYH9-Related Disease encompasses five different diseases: May-Hegglin anomaly, Sebastian, Fechtner, and Epstein syndromes; and non-syndromic deafness DFNA17. These diseases present with a variety of conditions including thrombocytopenia, leukocyte inclusions, renal disease, cataracts, and hearing loss. Each disorder is due to mutations in the MYH9 gene, which codes for non-muscle myosin IIA (NMIIA). NMIIA is an intracellular motor protein important for cell division, cell migration, and cell shape changes during embryogenesis. Although we know mutations in MYH9 cause these diseases, we do not know the mechanisms underlying the development of disease. Using CRISPR we generated a zebrafish myh9b mutant, myh9b is the zebrafish MYH9 homolog. Studying these mutants has enabled us to examine early developmental defects due to the loss of NMIIA protein. Using these mutants, we discovered an embryonic phenotype consistent with kidney failure that appears between 48 and 72 hours post fertilization (hpf). However, this phenotype quickly reverses between 96 hpf and 5 days post fertilization. As a first step towards understanding the role of myh9b in kidney development, we examined gene expression patterning in the developing pronephros. We hypothesized, based on the reversal, that the expression patterns for the genes cdh17 and wt1b would be normal in our myh9b mutants. cdh17 encodes for cadherin 17 and is expressed in the pronephric ducts and duct progenitors. wt1b encodes for a zinc-finger transcription factor and is expressed in the pronephric glomeruli. Using in situ hybridization, we found that there were no obvious differences in gene expression

between wild-type and the myh9b mutant zebrafish at the time points investigated. These data suggest that kidney pattering is not compromised in myh9b mutants leading to the hypothesis that the kidney defect is due to abnormal glomerular filtration. Future experiments will examine glomerular filtration rate and ultra-structure of the glomeruli during development.

### 3. Reactive oxygen species (ROS) activated prodrugs: Mechanisms and Combination Therapies

*By Dhivyashree Senthil Murugan*, Taufeeque Ali, Heli Fan, Muhammad Asad Uz Zaman, Wenbing Chen & Xiaohua Peng*

Triple Negative Breast Cancer (TNBC) tests negative for the presence of hormonal receptors such as progesterone and estrogen receptors and excess human epidermal growth factor (HER2 protein). TNBC is unaffected by hormonal treatments that target these three growth factors. After diagnosis, there is less time than other cancers to treat TNBC. These characteristics of TNBC result in having poorer prognosis among all types of breast cancer. Therefore, there is dire need for a better understanding of the cancer and of potential drugs. Based on previous research, we have concluded that two Phenylboronic acid nitrogen mustard prodrugs, CWB-20I45 (1) and FAN-NM-CH3 (2) are effective in reducing tumor sizes due to prodrugs' enhanced activity in the presence of hydrogen peroxide (H2O2). Prodrugs are initially inactive that are then turned into active compounds upon metabolism. Inside cells, these prodrugs cause DNA cross-linking that ceases DNA replication and leads to cell death, making them superior to common chemotherapy drugs. Cancer cells have higher levels of reactive oxygen species (ROS) such as H2O2. The prodrugs are thus more selective to cancer cells and less toxic to normal cells. A series of in-vivo experiments determined that the prodrugs are safe in mice. To understand the drug's mechanism inside cells, we are investigating biological pathways by looking at protein expression levels, which vary in drug-treated cells. These proteins include tumor suppressor p53. This was done using RT-qPCR technique to amplify RNA upon extracting mRNA between cancerous cells that are untreated versus drug-treated.

## Ecology

### 1. Panic in their Voices

*By Alexander Sweet*, Olivia Feagles, Kane Stratman, Gerlinda Hobel*

How a species reacts to a change in social environment can be critical in understanding it's overall behavior, particularly during courtship. Especially for lekking species in which social environment is determinant of their entire mating cycle. Flexible reactions to social context during male mating displays, for instance, have the potential to improve the likelihood of mating successfully. Many species of anurans exhibit flexible mating behaviors, but here we use male Eastern Gray Treefrogs (*Hyla versicolor*) to explore plasticity in calling strategies in response to social cues. H. versicolor are a local species of anuran in which males attract females using advertisement calls consisting of a series of short pulses (ranging from 5-30). The call traits females find most attractive include longer call duration and/or faster call rate, but a single male has limited energy and can only invest effort in one calling strategy. We recorded male calls in two settings and social environments: in a natural context pre-amplexus and an artificial context after interrupting amplexus (breeding position). The results show that male frogs with interrupted amplexus had shorter calls than pre-amplexus state, but faster call rates. Interestingly, males in both groups invested the same amount of energy (measured as 'duty cycle'), suggesting a shift in call strategy and flexible responses to social cues.

## 2. Little Effect of Climate Change on Insect Abundance at the UWM Field Station

*By Ryan Nicol*, Peter Dunn*

Changing weather patterns have been gaining more attention in recent years. For example, temperatures have increased, and wind speeds have decreased continent-wide over the last century. Recent studies have also shown that insect populations are declining worldwide. This study examined the hypothesis that insect abundance is affected by climate change. We estimated insect abundance over the last 22 years at the UWM field station in Saukville, Wisconsin. Insect biomass was estimated from a suction trap operated daily during May and June from 2000 to 2021. Weather data were also collected daily from an automated weather station 100 m from the trap. We found that wind speeds are decreasing, and temperatures are increasing in southeastern Wisconsin over the last 22 years. Declining wind speed was correlated with an increase in insect abundance, probably as a consequence of increased trapping efficiency. Increasing temperature was also correlated with an increase in insect abundance. Despite the positive correlation between insect abundance and temperature, there was no long-term change in total biomass of insects over the 22-year study. Overall, weather affects insect abundance, but there is little evidence of long-term effects at the UWM Field station. Nonetheless, insect abundance is influenced by temperature and wind speed, and thus, longer-term climate change will likely affect insect abundance, even in protected areas such as the UWM Field Station.

## Microbiology

### 1. Comparing Influenza A and Sars-CoV-2 Structure, products, and Vaccines

*By Filip Saitis*, Madhusudan Dey*

The Influenza (flu) and SARS-CoV-2 (Covid-19) viruses are global threats that target the human respiratory system. The effective preventative measure for these viral infections is to develop a vaccine, which requires a thorough understanding of the viral pathogenic behavior. Although the genome sequences of both flu and COVID-19 viruses are known, and their mutants have been achieved, the pathogenic mechanisms are still not fully understood. In this Bioinformatics-based project, published works of literature were examined to understand the mechanisms by which RNA viruses invade and utilize the translational machinery in human cells. The focus is on two viruses that cause respiratory diseases. These viruses are Influenza and SARS-CoV-2. Analysis of their RNA sequences, the encoded proteins, their macromolecular structures, and the viral replication methods was performed. Comparisons are made between three-dimensional models of spike proteins of different strains of RNA viruses in order to understand their varying ability to infect cells. These proteins' primary structure is examined to discover where viral strains differ in their protein products. Following this analysis, the development and mechanism of the mRNA vaccine are explored. Its functionality, risks, and benefits are also analyzed. Finally, the mRNA vaccine is compared with the traditional protein/peptide vaccine.

## Graduate

### Cell and Molecular Biology

1. **A quantitative ERK biosensor as a tool to understand FGFR signalling in C. elegans**

*By Claudia Sofia Rodriguez*, Melissa Garcia Montes de Oca, Te-Wen Lo , Cindy Voisine, Michael Stern, Claire de la Cova*

The hypodermis of the roundworm Caenorhabditis elegans is critical for fluid homeostasis. EGL-15 is the sole Fibroblast Growth Factor Receptor (FGFR) of C. elegans and is expressed in the hypodermis. Upon ligand binding, EGL-15 activates signal transduction by the RAS-RAF-MEK-ERK pathway that has been implicated in many human diseases. EGL-15 is negatively regulated by CLR-1, a receptor tyrosine phosphatase. In the hypodermis, loss of clr-1 activity causes a fluid balance defect termed the “clear” (Clr) phenotype. EGL-15 dysregulation also results in defects that include the hyperactive Clr phenotype, as well as a “suppressor of clear” (Soc) phenotype, caused by mutations that compromise EGL-15 activity. We also describe a novel adaptor protein denoted SOC-3 that may offer an indirect coupling pathway towards ERK activation. We hypothesize that the activation state of the downstream kinase ERK is increased in Clr mutants and decreased in Soc mutants.

To quantify ERK activity in egl-15 and clr-1 mutants, we utilized a fluorescent biosensor termed ERK-Kinase Translocation Reporter (ERK-KTR). The ERK-KTR protein is an ERK substrate and its phosphorylation state is monitored through nuclear/cytoplasmic localization within the cell. In the presence of ERK activity, the ERK-KTR is phosphorylated and its localization becomes cytoplasmic. In the absence of ERK activity, the ERK-KTR remains nuclear-enriched. We quantified ERK-KTR localization in the hypodermis as a ratio of

cytoplasm/nucleus signal, where a higher Cyto/Nuc ratio indicates higher ERK activity. We demonstrate that the ERK-KTR is a faithful reporter of ERK activation in the hypodermis. In addition, we show that clr-1 mutants have significantly elevated ERK activity compared to wild type, indicative of increased EGL-15 activity. We aim to use the ERK-KTR to better understand FGFR regulation and the quantitative impact of egl-15 and soc-3 mutants.

## 2. The UNC-116 kinesin functions with the NEKL-3 kinase to promote axon targeting

*By Cody J. Drozd* & Christopher C. Quinn*

Formation of a healthy and stable nervous system requires precise regulation of neuronal development. Failures in axon targeting could lead to a neurodevelopmental disorder, such as intellectual disability or autism. De novo variants of kinesin KIF5C have been identified in patients with neurodevelopmental disorders. However, the role of KIF5C in neuronal axon development is poorly understood. Here, we analyze kinesin KIF5C by using genetic analysis to observe C. elegans KIF5C ortholog unc-116 in axon termination. Specifically, we find that a loss-of-function of UNC-116 causes axon termination defects in the Posterior Lateral Microtubule (PLM) axon of C. elegans. These termination defects are also observed in two conserved missense mutations of UNC-116: E239K and R288W. Furthermore, a gain-of-function of UNC-116 causes the PLM axon to terminate prematurely. In addition to UNC-116, we observe the effects of NEK kinase (NEKL-3) in PLM axon termination. By observing fluorescently-tagged NEKL-3, we find that NEKL-3 moves bidirectionally along the PLM axon and becomes more stable towards the axon tip. When crossed with UNC-116 loss-of-function mutants, the amount of NEKL-3 significantly decreases in both the proximal and distal ends of the PLM axon. Additionally, a loss-of-function of NEKL-3 causes PLM axon termination defects. By using genetic analysis, we find that NEKL-3 interacts with a major axon termination pathway, the RPM-1 pathway. We aspire for our research to aide in understanding the roles of UNC-116 and NEKL-3 in axon termination and to help explain axon formation in neurodevelopmental disorders.

### 3. Laminin-111 mutant study reveals a hierarchy within laminin-111 genes and their requirement for MHB tissue folding

*By Elizabeth Falat, Gabriella Voit*, Jennifer Gutzman*

During development, organ formation is reliant on the correct formation of tissue shape. Tissue morphogenesis is a process carefully regulated by both cell signaling, and external forces. Using the highly conserved zebrafish midbrain-hindbrain boundary (MHB) as an epithelial tissue model we have also identified the basement membrane protein laminin-111 as a key factor in basal tissue folding. Laminin-111 is a highly conserved, heterotrimeric protein that lines the basal surface of the neuroepithelium. Laminin-111 is comprised of the alpha, beta and gamma chains encoded by lama1, lamb1a, and lamc1 genes respectively. Based on mutations in individual genes resulting in disparate human disease phenotypes, we hypothesized that result each laminin gene would have a unique role in tissue morphogenesis. Using zebrafish mutants for each laminin-111 gene, we compared tissue and cell shape during MHB morphogenesis. We found that all three mutants have similar MHB tissue folding defects at 24 somites. However, when we quantified cell shape changes and localization of myosin, we discovered that lamc1 is the most critical gene in MHB morphogenesis. lama1 follows closely with moderate defects compared to lamc1 while lamb1a appears to have the least severe defects. Our results examining the MHB tissue fold later in development were consistent with the hierarchy identified by cell shape and signaling analysis. These findings are critical for novel techniques in tissue engineering and will help to elucidate differences in human diseases due to specific chain mutations.

## 4. Zebrafish as a Model System for MYH9-Related Disease

*By Laura Rolfs*, Elizabeth Falat, Jennifer Gutzman*

There are five clinical disorders resulting from different mutations in the MYH9 gene that are classified as MYH9-related disease (MYH9-RD): May-Hegglin anomaly, Sebastian, Fetchner, and Epstein syndrome; and non-syndromic deafness DFNA17. These diseases are characterized by symptoms including abnormal blood composition, kidney dysfunction, visual defects and hearing loss. MYH9 encodes the highly conserved non-muscle myosin IIA protein (NMIIA), which has essential roles in cell division, cell migration, and cell shape changes. Therefore, our lab has obtained and generated null mutants for the myh9a and myh9b genes respectively. Through our studies we have identified myh9b, not myh9a, as the critical myh9 gene required for normal zebrafish development and morphogenesis. Consistent with this finding, myh9a homozygous mutants are viable through adulthood and do not develop any visible phenotypes. However, myh9b homozygous mutants are semi lethal and develop pericardial edema, which is a phenotype consistent with kidney dysfunction. This phenotype develops between 48-96 hours post fertilization and reverses shortly after onset. Due to this reversal, we hypothesize that there is compensation by other non-muscle myosin proteins occurring in these mutants. Current experiments involve investigating NMIIA and NMIIB protein levels in myh zebrafish mutants during the time of pericardial edema presentation. Future experiments will examine kidney patterning and glomerular structure in our mutant models.

## Ecology

### 1. Are treefrog personality traits reflected in sexually selected behaviors?

*By Olivia Feagles* and Gerlinde Hoebel*

Both natural and sexual selection give rise to a variety of unique traits and behaviors, most of which vary widely between individuals of the same species. Here, we explore the link between sexually selected traits (male advertisement displays) and animal personality - in Eastern Gray Treefrogs (Hyla versicolor). In H. versicolor, calls are the primary focus of female mate choice, with specific preferences for longer call duration, faster call rate, and more energy investment (duty cycle). To explore animal personality, we employ novel methods to investigate behaviors related to predator evasion, foraging, hiding affinity, and natural activity levels, and score those along a shy-bold continuum. We find substantial individual variation in boldness across tasks, suggestive of animal personality. We also find some behavioral linkage between naturally and sexually selected behaviors: Frogs that scored bolder in an exploration task also called more (higher call rate and duty cycle), and those that scored bolder in the foraging task called less (lower call rate and duty cycle).

## Microbiology

### 1. Role of Adaptor Proteins in the Composition of Pseudomonas aeruginosa Chp Chemosensory Arrays

*By Alyssa Kline*, Zachary Hying, Sonia Bardy*

The opportunistic pathogen, Pseudomonas aeruginosa, relies on the expression of type IV pili (T4P) for aspects of its virulence. T4P are long, flexible appendages used by bacteria in surface adhesion, biofilm maturation, and twitching motility. Expression of T4P-related genes and modulation of T4P activity in P. aeruginosa is dependent on the Chp chemosensory system. The core signaling complex of the Chp system consists of a single methyl-accepting chemotaxis protein (MCP; PilJ), two adaptor proteins (PilI, ChpC), and a histidine kinase (ChpA). These proteins organize themselves into large, highly ordered chemosensory arrays that are localized towards the poles. Classical models of these chemosensory systems are based on the E. coli Che system, which contains only one adaptor protein. We investigated the roles of the two adaptors in Chp array composition and signal transduction. Our initial findings indicated that PilI functions as the primary adaptor in the Chp system and is necessary for signal transduction and array formation to occur, while ChpC is likely an auxiliary protein needed for signal amplification and array stability. Based on the results from bacterial two-hybrid (BACTH) protein-protein interaction studies, we constructed a model for Chp array formation. Using fluorescence microscopy, our newest data on protein localization and array formation further supports this model.

## 2. Understanding the role of MADS-box transcription factor Rlm1 in ER protein homeostasis

*By Kimberly Mayer*, Jagadeesh K Uppala, Madhusudan Dey*

Protein homeostasis, or the balance of protein synthesis, folding, and transport, is essential for normal cellular function. Any disturbances in the endoplasmic reticulum (ER) leads to accumulation of unfolded or misfolded protein in the lumen, causing a situation called ER stress. To counter ER stress, cells evoke adaptive signaling networks collectively known as the unfolded protein response (UPR). The MADS-box transcription factor plays a key role in regulating protein expression and folding in eukaryotes. In budding yeast Saccharomyces cerevisiae, the MADS-box transcription factor Rlm1 has a paralog Smp1. To understand the role of Rlm1 in ER stress response, we deleted both genes RLM1 and SMP1 from the yeast chromosome and made rlm1Δsmp1Δ double deletion strain. The rlm1Δsmp1Δ strain was exposed to tunicamycin, an ER stress inducing agent, to simulate accumulation of unfolded proteins in the ER lumen. We observed that the double deletion strain rlm1Δsmp1Δ showed a sensitive phenotype to tunicamycin, suggesting that Rlm1 could play a role in UPR. To further investigate the role of Rlm1 in UPR, we treated both wildtype (WT) and rlm1Δsmp1Δ strains with tunicamycin; RNA was isolated and subjected to RNA sequencing to understand the differential gene expression. RNA-sequencing results showed several upregulated genes, including SSA1 and HSP26, as well as a wide range of downregulated genes, including BCK2, YPK2, and CHS7. Ongoing research is in progress to characterize these genes and determine their role in protein homeostasis.

### 3. The effect of mineral nitrogen on the symbiosis between Mimosa pudica and Paraburkholderia phymatum.

*By Shashini U. Welmillage*, Prasad Gyaneshwar*

The influence of mineral N (MN) on legume-rhizobial symbiosis has been widely studied focusing more on crop legumes that belong to the subfamily Papilionoidea. It has shown that the available MN in soil inhibits or delays nodulation and symbiotic nitrogen fixation. However, no studies have determined if nodulation in primitive legumes is also responsive to MN. For the determination of the effect of MN on nodulation, M. pudica seedlings were grown in small cups (12 oz) containing sterile vermiculite and were supplied with plant growth media containing 0 mM nitrogen, 5 mM, and 10 mM $NO_3^-$ and $NH_4^+$, then were inoculated with P. phymatum MP20GUS. Uninoculated plants were negative controls. At 28 DAI, plants were carefully removed, and the total number of nodules on each plant was counted. In contrast to the known inhibition of nodulation by MN, no differences were observed in the number of nodules formed with or without added nitrogen. However, at 10 mM concentration $NH_4^+$ seemed to become toxic to the plants. Furthermore, the shoot dry weights (SDW) of plants grown with nitrogen were significantly higher than the no-N growth condition. The higher SDW of plants with N shows that M. pudica assimilated $NO_3^-$ and $NH_4^+$ but still formed nodules. These results considered together, indicate that nodulation of M. pudica by P. phymatum is likely independent of N availability.

4. **Inhibitory effect of novel bacterial compound RejuAgro A against fish pathogens: A step towards sustainable aquaculture**

*By Shreyashi Mitra*, Dr. Ching-Hong Yang, Dr. Jian Huang.*

Fish infections cause by bacterial pathogens widely affect aquaculture. The most common management controls include the use of human antibiotics. The increasing indiscriminate usage of antibiotics leads to the selection of resistant bacterial pathogens. This forms a reservoir for the antimicrobial-resistant gene and ultimately affects the human population. RejuagroA is a novel bacterial compound produced from pseudomonas sp. which has shown promising results in the inhibition of common fish pathogens. Using this compound against fish pathogens not only will stop the horizontal gene transfer of the antibiotic-resistant gene but also prevent the high amount of antibiotics from entering the marine ecosystem and adversely affecting the environment.

## 5. A Novel Aspect of Eukaryotic AMPK Regulation: Lessons from Yeast

*By Vidhya Basak*, Marianna Orlova, Sergei Kuchin*

Eukaryotic AMP-activated protein kinase (AMPK) is the master regulator of energy homeostasis from yeast to humans. Mammalian AMPK has been implicated in diseases from diabetes to cancer. AMPK of baker's yeast (Saccharomyces cerevisiae) serves as an important model of eukaryotic AMPK signaling. Yeast AMPK responds to stresses caused by glucose/energy limitation and promotes utilization of alternative carbon sources. Like mammalian AMPK, yeast AMPK is a heterotrimeric complex. Yeast AMPK is composed of the catalytic α subunit (Snf1), one of three alternative targeting/scaffolding β subunits (Sip1, Sip2, or Gal83), and a regulatory γ subunit (Snf4). When energy levels drop, yeast AMPK is upregulated by two main mechanisms: 1) catalytic activation by phosphorylation of Thr210 of Snf1; 2) Gal83-dependent nuclear enrichment of the activated yeast AMPK complex. However, many aspects of yeast AMPK regulation still remain unknown. Thus far, the AMPK complex has been assumed to assemble "by itself". Our study in yeast suggests that the interaction of catalytic α subunit Snf1 and the nuclear-targeting beta subunit Gal83 is disrupted in the absence of two paralogous WD40 repeat proteins provisionally dubbed Snf13 and Snf14. WD repeats are conserved in evolution and play various roles in protein-protein interactions. Proteomic studies indicate that Snf13/14 physically interact with Snf1. Here, we show that in yeast cells lacking Snf13/14, Snf1 and Gal83 become uncoupled from each other suggesting that Snf13/14 promote the integrity of the Snf1-Gal83 complex. These results may inform research in the health-giving benefits of mammalian AMPK (diabetes, obesity, cancer) and the role of Snf1/AMPK in fungal pathogenesis.

### 6. From nature to drug: Novel secondary metabolites RejuAgroA from Pseudomonas T307 as a promising biocontrol agent against phytopathogen Erwinia amylovora

*By Vy Huyen*, Ching-Hong Yang*

Fire blights caused by Erwinia amylovora have damaged many economically important crops such as apples, and pears throughout North America. Despite over many years of intense research and attempt to control fire blights, this disease continues to cause devastating losses, estimated to be 100 million dollars annually in North America, and have spread worldwide. The current management tool to control fire blight is to eradicate infected plants or by using human antibiotics, streptomycin and tetracycline. However, California, Michigan, New York, and Georgia have reported streptomycin resistance in E. amylovora. With the need to combat antimicrobial resistance to human antibiotics and to control fire blights, mining for novel antimicrobial compounds from nature has been the main target. Here, we identified a potent novel metabolite RejuArgoA (RAA) from Pseudomonas T307 (T307) that can inhibit E. amylovora at a much higher efficacy than streptomycin and as demonstrated in our greenhouse and field assays. Production of RAA can be boosted by understanding the important genes and intermediate metabolites involved in RAA synthesis. The metabolic pathway in T307 has been encrypted through bioinformatics tools and in gene mutagenesis. In summary, T307 can be used as a new promising biocontrol agent, not only to sustainably manage fire blights but also to reduce the use of current human antibiotics.