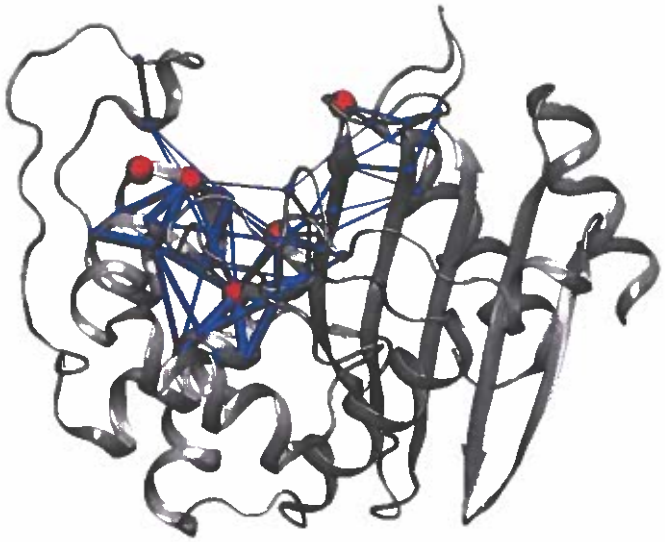
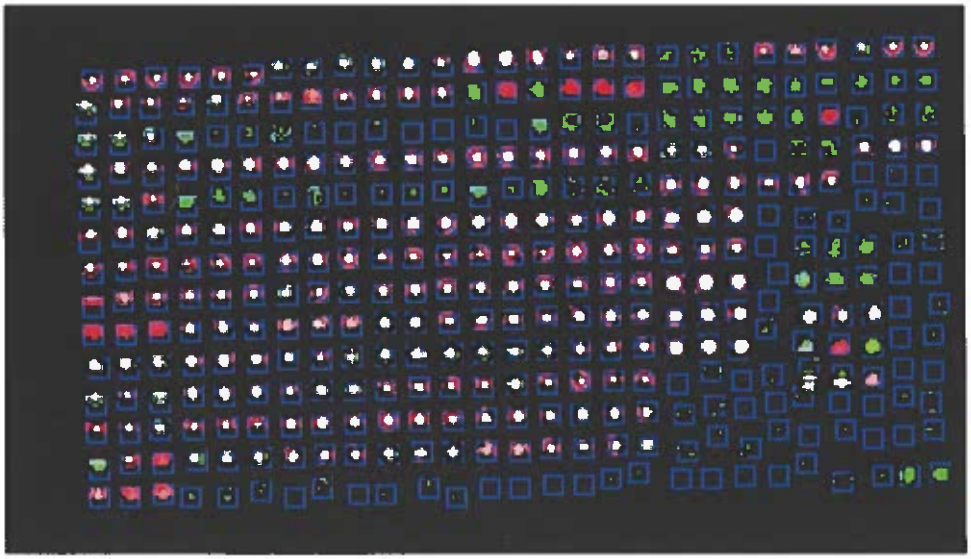


III Annual Cell & Molecular Biology Research Symposium

Friday, March 23rd and Saturday, March 24th, 2018



GRAND VALLEY STATE UNIVERSITY

III Annual CMB Research Symposium

Friday

Session 1: Cancer biology
Chair: Dominic Petriella

1-1:10	Opening
1:10-1:30	Luke Pardy
1:30-1:50	Alexander Ward
1:50-2:10	Madison Spratt
2:10-2:20	Coffee break
2:20-2:40	Madison Schmidtmann
2:40-3	Madeline Fugate
3-3:05	Chair's summary
3:05-3:25	Refreshment break

Session 2: Plant biology
Chair: Daniel Caylor

3:25-3:45	Michael Michalski
3:45-4:05	Samuel Henson
4:05-4:25	Steven McKenzie
4:25-4:45	Christopher Avey
4:45-4:50	Chair's summary
4:50-5pm	Best talks of Sessions 1-2 - award ceremony

Saturday

Session 3: Biomarkers
Chair: John Hall

9:-9:20	Sarah Robertson
9:20-9:40	Macie Weiland
9:40-10	Emma Hahs
10-10:20	Ashleigh Harrah
10:20-10:40	Paul Gingerich
10:40-10:45	Chair's summary
10:45-11	Coffee break

Session 4A: Human disease
Chair: Emily David

11:0-11:20	Bradley Howlett
11:20-11:40	Melanie Edwards
11:40-12	Johnathan Hall
12:00 - 1pm	Lunch break

Session 4B: Animal models
Chair: Emily David

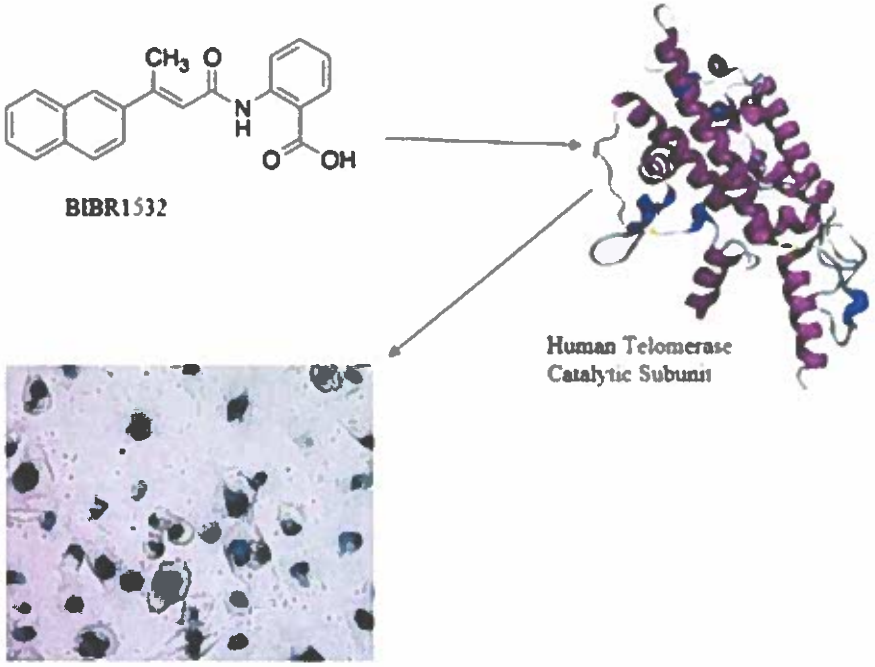
1-1:20	Liz Croff
1:20-1:40	Taylor Paas
1:40-2	Dominic Petriella
2-2:05	Chair's summary
2:05-2:20	Coffee break

Session 5: Microorganisms and antibiotic resistance
Chair: Emma Hahs

2:20-2:40	Alyssa Benn
2:40-3	Emily David
3-3:20	Daniel Caylor
3:20-3:40	Andrew Carlson
3:40-4	Nathan Baker
4-4:05	Chair's summary
4:15	Best talks of Sessions 3-5 - award ceremony

Adjuvant/Neoadjuvant Antitelomerase Treatment in Chemotherapy of Triple Negative Breast Cancer

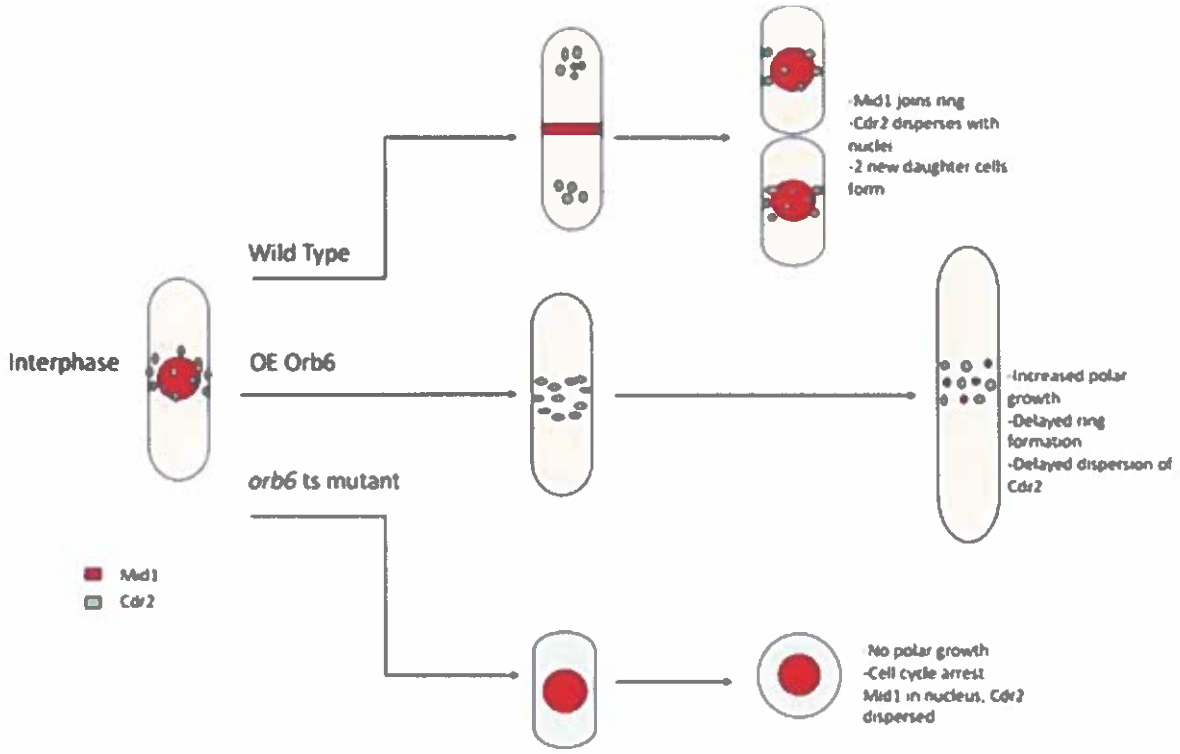
Luke Pardy[1], Chelsea Reiber[1], Robert Smart[2], William Schroeder[2]
Mentor: Dr. Osman V. Patel[1]
[1]Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI 49401
[2]Department of Chemistry, Grand Valley State University, Allendale, MI 49401



Antitelomerase based chemotherapeutics have been extensively studied since the effects of the enzyme telomerase on cancer cell immortality have been elucidated. This work sought to determine the effects of adjuvant/neoadjuvant antitelomerase treatment with low-dose chemotherapeutics on Triple Negative Breast Cancer (TNBC) growth, senescence, and gene expression. Serious research identified telomerase inhibitors and their effects on cancer cell growth. However, these studies have either been focused on the single compound of interest, or have only done short term treatments with the compound and other chemotherapeutics. In this study we used long-term low-dose adjuvant or neoadjuvant antitelomerase treatment with low-dose applications of either a taxane or anthracycline, to determine if this treatment potentiated the effects of the chemotherapies on TNBC growth. To determine these effects, we grew TNBC cells for 6 weeks, and alternated their exposure to the anti-telomerase compounds and chemotherapeutics in order to determine the effect on cell growth rate. We then carried out senescence assays at weeks 3 and 6 of the trials to determine senescent cell percentage, as well as took RNA samples at the same times for RT-qPCR analysis of gene expression in these cells. Cell densities decreased by 25% ($p<0.05$) and the number of senescent cells increased ($p<0.05$) in the neoadjuvant setting. hTERT, hTR, and Bax gene expression all increased ($p<0.05$) relative to the control, whereas Ki-67 decreased ($p<0.05$), all in the neoadjuvant setting. This work provides a novel means for TNBC treatment that circumvents the toxic effects of typical chemotherapy regiments but maintains their therapeutic effects.

Polarity Kinase Orb6 Mediates Localization of Cytokinesis Nodes Mid1 and Cdr2 in *S. pombe*

Madison Spratt, Jessica Thoe,
Mentor: Dr. Dawn Clifford Hart
Grand Valley State University, Cell and Molecular Biology Department

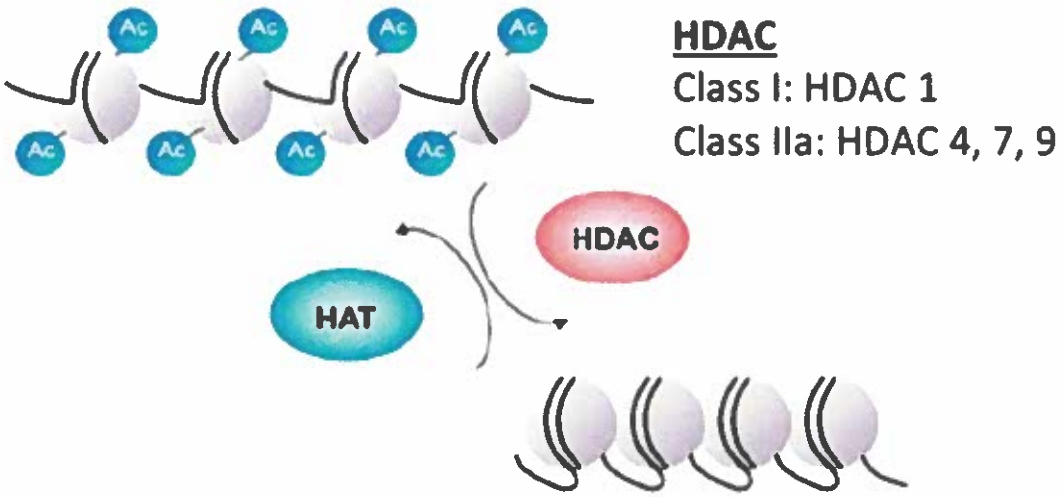


Atypical cell division in human cells is the underlying cause of numerous diseases including cancer. Fission yeast *Schizosaccharomyces pombe* is a model organism that is ideal for defining eukaryotic mechanisms of division. The cell cycle of *S. pombe* is characterized by polar elongation of the cell during interphase and formation and contraction of the actin-myosin ring during division. While interactions between these two processes have been identified, the mechanisms that regulate both are not fully understood. To investigate this, the effects of the polarity promoting kinase Orb6 were evaluated on the contractile ring scaffold protein Mid1 and the node protein that stabilizes its association to the membrane, Cdr2. Orb6 overexpression and temperature sensitive mutations were performed to visualize variances in Mid1 and Cdr2 localization. Results demonstrate that the overexpression of Orb6 prevents dispersion of Cdr2 from the medial cortex and the two potentially bind. In addition, Mid1 is exclusively localized to the nucleus in *orb6* mutations and increasingly localized to the nodes when Orb6 is overexpressed. Combined, these results suggest a direct interaction between Cdr2 and Orb6 that mediates the localization of Mid1. Further investigation into these interactions may help define a novel connection between cell polarity and ring formation, allowing for a more complete understanding of the process of cytokinesis.

Research funded by National Science Foundation RUI Award #1157997.

Survey of Expression of Histone Deacetylases in Patient Samples and Lung Cancer Cell Lines

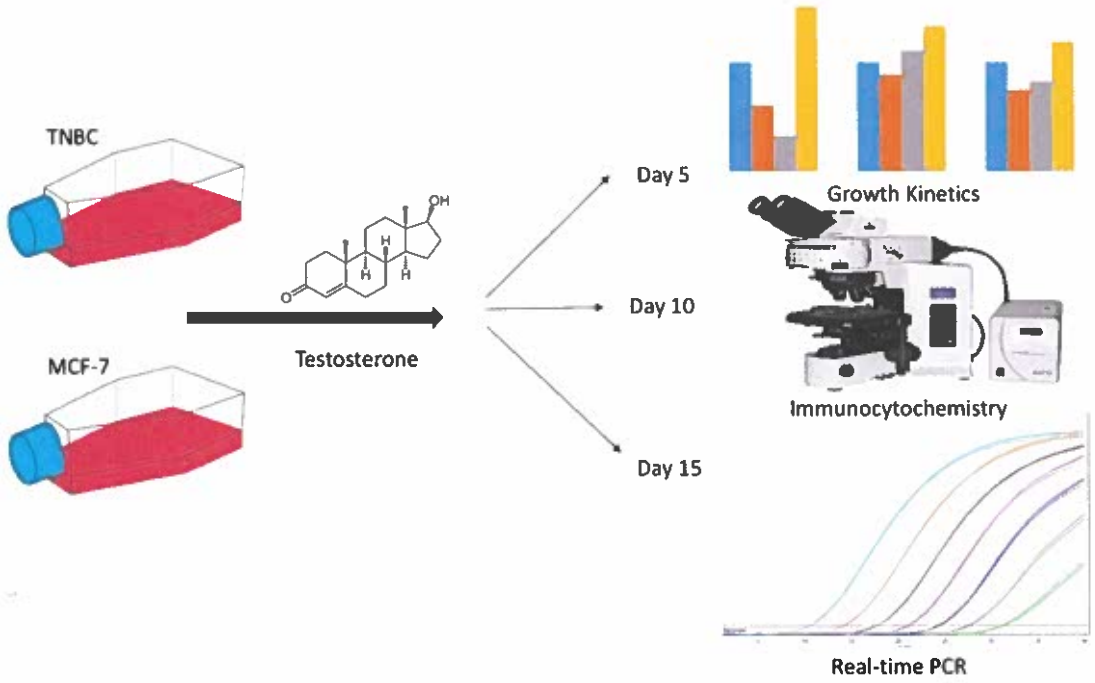
Madison Schmidtmann[1], Jheng-Yu Wu, Ph.D.[2]
Mentor: Dr. Xiaohong Zhang[3]
[1]Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI
[2]Department of Pathology and Cell Biology, University of South Florida, Tampa, FL
[3]Department of Oncology, Wayne State University, Detroit, MI



Histone deacetylases (HDACs) play an important epigenetic role in chromatin structure, gene expression, and other cell regulation mechanisms. Generally, high expression levels of HDACs are associated with a lower rate of survival in cancer patients. However little is reported on the expression of HDACs specifically in lung cancers. In addition, only pan-HDAC inhibitors are currently used to treat lung cancer and only a few HDAC-specific inhibitors are being developed further. In this project, we examined the mRNA and protein expression levels of HDACs 1, 4, 7, and 9 in various lung cancers. Using Western blot analysis we determined that protein expression levels of HDACs 1, 4, 7, and 9 are higher in comparison to healthy control cell lines. Additionally, HDAC 7 demonstrated much higher protein expression in mucoidepidermal carcinomas and squamous cell carcinoma. Through Kaplan Meier plots and Oncomine™ data we determined there is a significant correlation in survival rate of lung cancer patients in relation to mRNA expression of HDACs 1, 4, 7, and 9. We expect this analysis to facilitate the development of of HDACs 1, 4, 7, and 9 specific inhibitors to be used for lung cancer treatment. HDAC 7 could provide an ideal target for mucoidepidermal carcinomas and squamous cell carcinomas.

Effects of Testosterone Supplementation on Breast Cancer Cells: A Preliminary Investigation

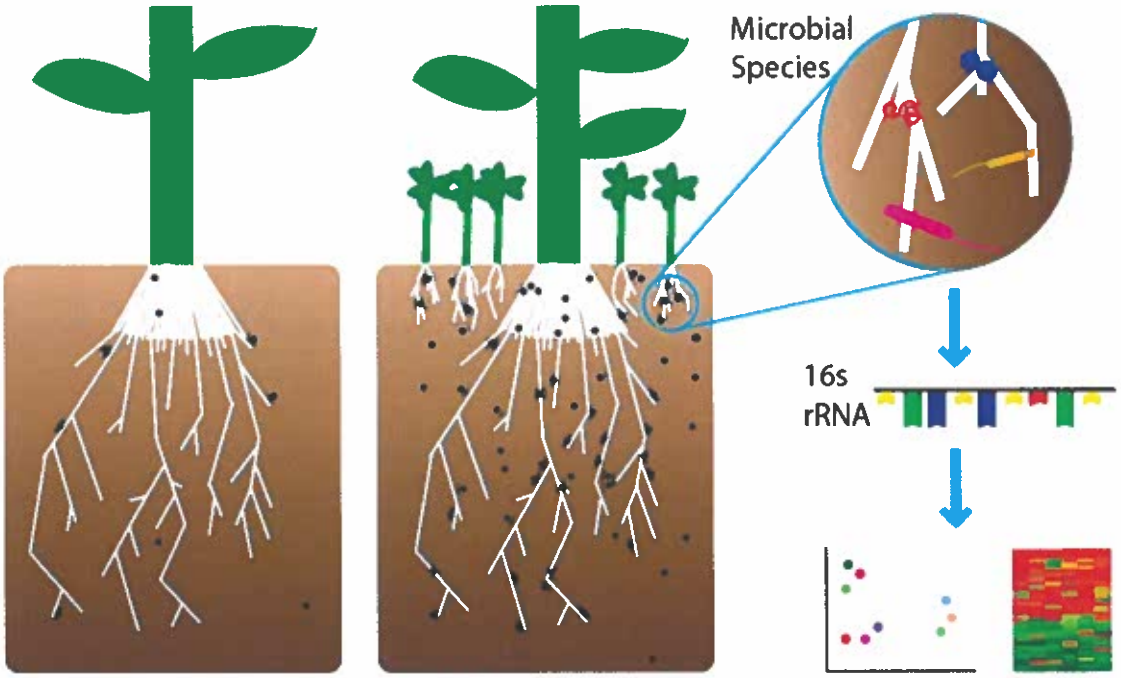
Madeline Fugate
Mentor: Dr. Osman Patel
Department of Cell and Molecular Biology, Grand Valley State University, Allendale MI



Triple-Negative Breast Cancer (TNBC) accounts for about 15% of all breast cancer cases, but has a worse prognosis and a higher mortality rate than other subtypes. This particular subgroup is referred to as 'triple-negative' as it lacks the expression of receptors commonly targeted [estrogen, progesterone, human epidermal growth factor 2] for treatment. Recent studies indicate that the androgen receptor (AR) may be a target for treatment and better prognosis, but its role in TNBC remains unclear. Therefore, the objective of this study was to compare the effects of testosterone supplementation on the growth kinetics of TNBC and ER receptor-positive breast cancer (MCF-7) cells. Our study measured the effects of varying testosterone concentrations on growth response of TNBC and MCF-7 cells using immunocytochemistry, Western blotting and real-time PCR techniques. We visualized and quantified the subcellular androgen receptor localization and the abundance of AR transcripts in TNBC and MCF-7 cells. Preliminary results show a dose-dependent increase in AR abundance in both TNBC and MCF-7 cells. Further work will provide a better understanding of the role of AR in TNBC and MCF-7. This information will aid in understanding the role of AR in breast cancer development, progression, and metastasis.

The Effect of Intercropping White Clover on Aspects of the Microbial Soil Ecosystem in Small Scale Market Gardens

Michael Michalski-[1]
Mentors: Dr. Jennifer Winther[2], Dr. Sheila Blackman[1]
[1]Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI, 49401, USA.
[2]Department of Biology, Grand Valley State University, Allendale, MI, 49401, USA.

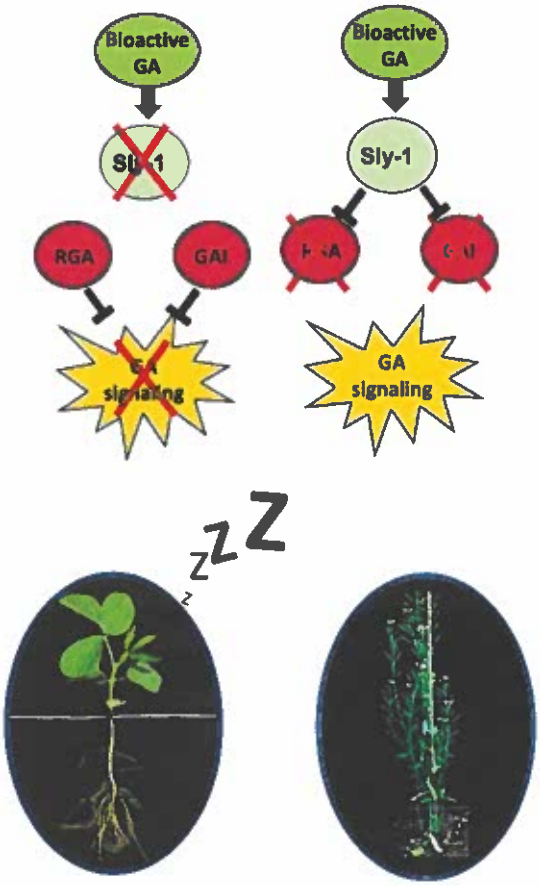


Current industrial agriculture systems rely on fossil fuels to produce nitrogen and phosphorus, key nutrients for plant growth. Two factors that affect the nutrient content are soil bacteria, and the application of cover crops. To improve the natural recycling, legume cover crops can be added to absorb nutrients unused by high yield crops then the legume can decompose after the growing season. Thanks to advances in sequencing techniques, the diverse makeup of this microbial ecosystem can now be quantified. However, there is limited data showing how soil microbial niches are impacted by the addition of a clover cover crop (*Trifolium repens*) to the field. In this study we used 16s rRNA sequencing to measure how the soil ecology changes in three different sets of market gardens maintained by Grand Valley State Universities' 2016 summer Global Agriculture Sustainability class at the Sustainable Agriculture Project (SAP). Our results show the addition of *T. repens* increases microbial diversity at the species level. Additionally, the number of microbial species increases over time as mature niches are able to develop. We expect this data to help strengthen the argument for introducing clover crops as part of a developing sustainable agriculture system that increases recycling of organic nutrients and reduces the dependence on fossil fuels.

Funded by the Office of Undergraduate Research and Scholarship 2016 Student Summer Scholars grant.

Sorting Out Multiple Soybean Orthologs Of SLEEPY-1

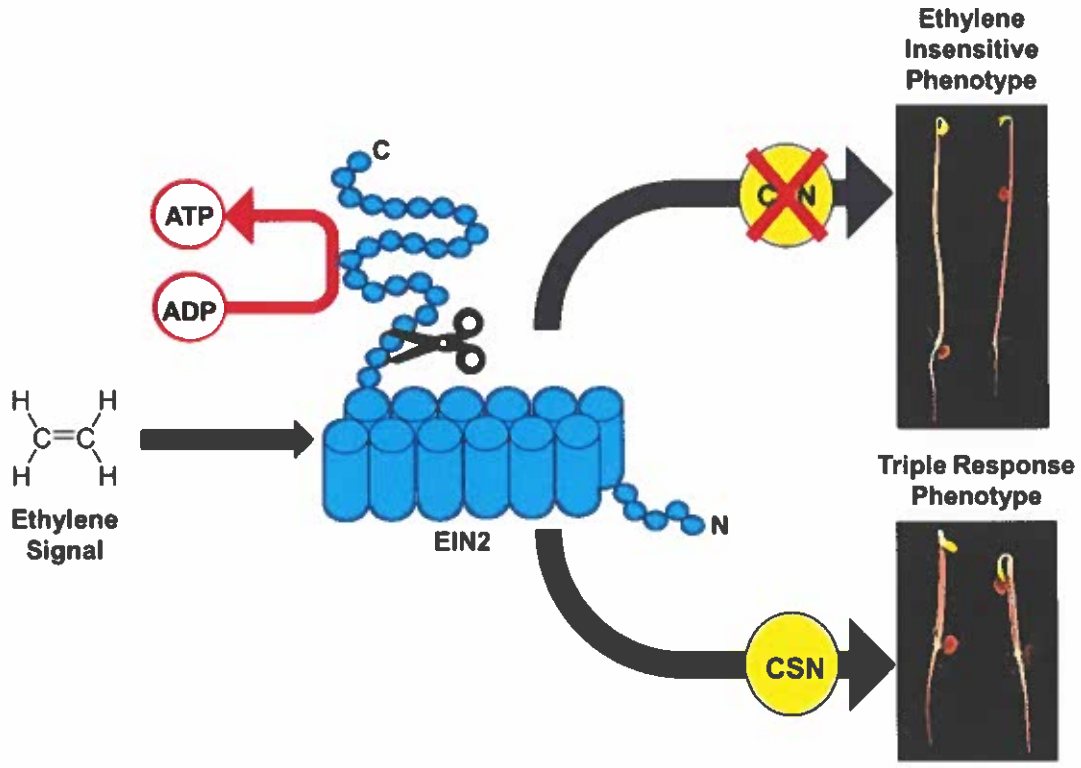
Sam Henson[1], Derek Janssens[2]
Mentor: Dr. Pei-Lan Tsou[1]
[1] Grand Valley State University, Department of Cell and Molecular Biology,[2] Department of Biomedical Science



Giberellic acid is an important plant hormone that regulates growth, cell division, and seed germination. Sleepy 1 (SLY1) functions as a positive regulator of gibberellic acid (GA) signaling in *Arabidopsis*. Components in the GA response pathway in *Arabidopsis* have been identified and studied. SLY1 is an F-box protein that is up regulated in response to increased bioactive GA and functions by degrading DELLA proteins. These DELLA proteins, RGA and GAI, inhibit GA signaling. We have identified GmSLY1a and GmSLY1b, two orthologs of *SLY1* in soybean. Both of these putative orthologs contain an F-Box motif as well as several other domains found in the *Arabidopsis* SLY1 protein. However, it has not yet been determined whether these orthologs maintain the same function as SLY1. We measured the relative expression of GmSLY1a and GmSLY1b in different soybean tissues and compare them to the expression of SLY1 in *Arabidopsis* in order to gain insight to the functionality of the two genes. The *sly1* mutants in *Arabidopsis* result in a dwarf phenotype and show variable seed dormancy. We also transformed both genes into *Arabidopsis sly1* mutants to further analyze the soybean ortholog and access their ability to rescue the *sly1* mutant phenotype in *Arabidopsis*.

Ethylene Induced Modification of EIN2 C-Terminus in *Arabidopsis Thaliana*

Steven D. McKenzie
Mentor: Dr. Matthew J. Christians
Grand Valley State University, Department of Cell and Molecular Biology, Allendale MI 49401, USA

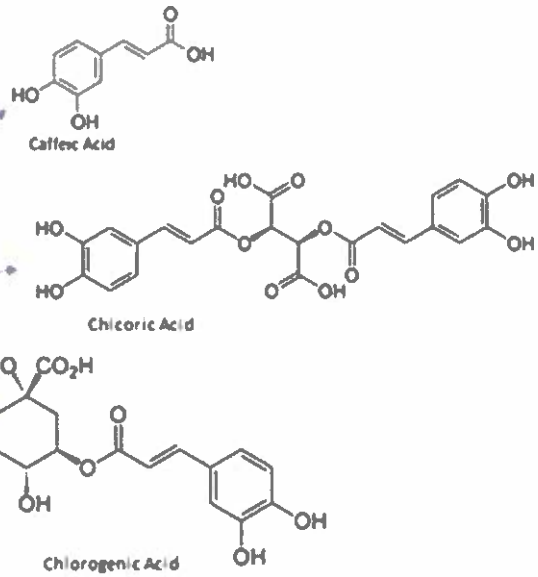


Ethylene is used by plants for a variety of hormonal functions, including fruit ripening, leaf senescence and abscission, and seedling development. The endoplasmic reticulum (ER) membrane bound protein Ethylene Insensitive 2 (EIN2) plays a vital role in ethylene signal transduction. In the presence of ethylene, the C-terminus of the protein is cleaved and translocated into the nucleus. The cleavage ultimately contributes to a unique triple response phenotype characterized by short hypocotyls, pronounced apical hooks, and shorter roots. However, the protein component involved in the cleavage event has yet to be determined. There is evidence that various subunits of the COP9 Signalosome (CSN), a metalloprotease, interact with EIN2, possibly resulting in its cleavage. In this study we obtained several CSN subunit *Arabidopsis thaliana* mutants (*csn 5a-1, csn 5b-1, csn 6b-1*) and found that *csn 5b-1* dark grown seedlings are ethylene insensitive, showing greater hypocotyl growth when grown on ACC, an ethylene precursor. Wildtype seedlings treated with the metalloprotease inhibitor 1,10-phenanthroline (OPT) have also displayed a seemingly ethylene insensitive phenotype. This data shows that the catalytic subunits of the CSN contribute to a downregulation of ethylene responses in seedlings. Further research will provide insight into the role of the CSN subunits in EIN2 cleavage and the ethylene signaling pathway, and could lead to the development of efficient agricultural practices.

Improving phenolic yield from *Echinacea* via hormonal manipulation and control of in field growth conditions

Christopher Avey[1], Megan Goy[1], Carrie Monroe[2]
Mentor: Dr. Sheila Blackman[1]
[1]Grand Valley State University, Department of Cell and Molecular Biology, Allendale, MI
[2]Grand Valley State University, Department of Natural Resource Management, Allendale, MI

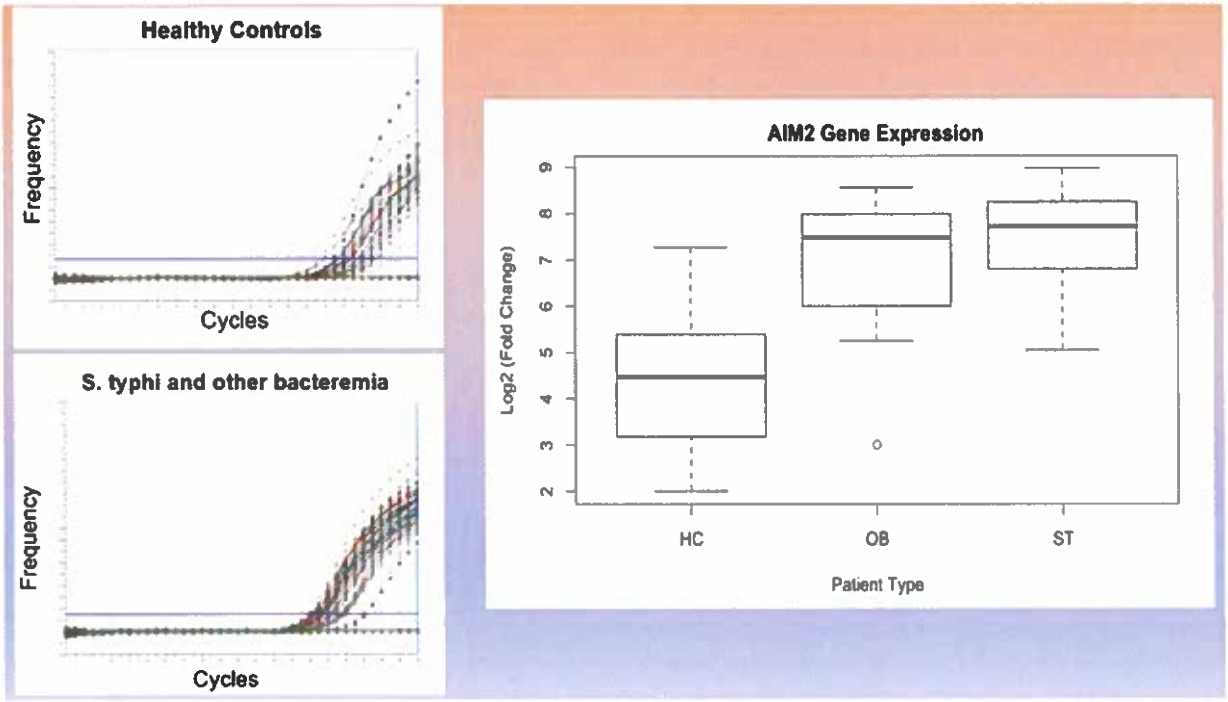
Echinacea used as Bioreactor



Phenolic acids are useful compounds in the synthesis of various pharmaceutical agents, and it would be beneficial to increase production of them from natural sources such as Echinacea. In this project we aim to develop in vitro culture techniques which produce tissue with elevated levels of useful phenolics in a manner which can be efficiently scaled up. It is known that use of callous culture allows for abnormally high production of certain chemicals, while also improving ease of growth and ability to scale up production, allowing more general access to this yield enhancement technique. Callous generation is achieved with auxin and cytokinin additives, with liquid culture of phenolic rich callouses allowing a simple method of increased production. In this project we have tested various hormone concentrations to optimize phenolic acid production. The most effective hormone concentrations were used to sustain the callous tissue in liquid media, from which phenolics could be extracted. A defined ratio of hormones for use in callous culturing was determined, as well as a method of extracting and reliably assaying the phenolic compounds present in a given sample without significant loss of products. Further research and testing of samples obtained in this method is necessary, but growth procedures have been put forward that allow efficient production of samples and extraction of said samples from plant tissue.

AIM2 (Absent in Melanoma 2) gene as diagnostic biomarker for typhoid fever

Sarah Robertson[1], Brooke Armistead[1], Sango Otieno[2],
Mentor: Dr. Sok Kean Khoo[1]
[1]Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI
[2]Department of Statistics, Grand Valley State University, Allendale, MI

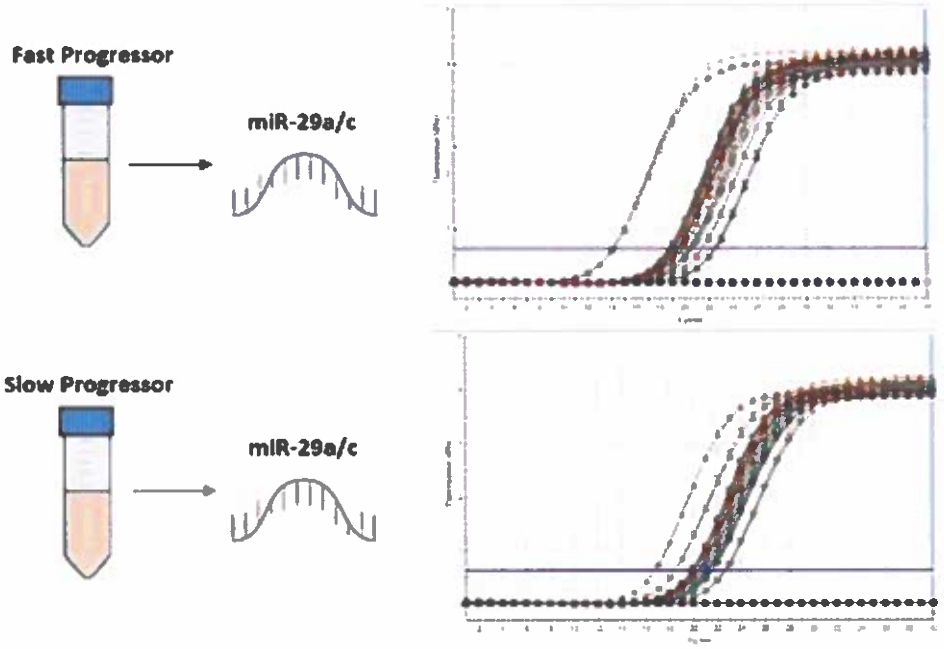


Typhoid fever (TF) is an infectious disease caused by *Salmonella typhi* (*S. typhi*), transmitted via contaminated water. Early treatment of TF involves a broad-spectrum antibiotic due to the similarity of symptoms between TF and other bacterial diseases. Antibiotic resistant strains of *S. typhi* are now on the rise due to inappropriate treatment. Therefore, it is necessary to identify accurate diagnostic biomarkers of TF to ensure the most effective antibiotic is used. Quantitative real time PCR was used to investigate gene expression of Absent in Melanoma 2 (*AIM2*), an inflammatory gene, which triggers an innate immune response to infectious microbes. *AIM2* expression in healthy controls (HC) was compared to TF patients, and other bacteremia patients. *AIM2* was significantly up-regulated in TF patients compared to HC (p-value=0.0002) while there was no significant difference between TF and other bacteremia patients. In summary, *AIM2* can serve as a potential diagnostic biomarker to differentiate TF from HC. Further work is warranted to identify biomarker candidates to differentiate TF and other bacteremia.

Partially funded by the National Institute of Allergy and Infectious Disease of National Institutes of Health and Grand Valley State University Office of Undergraduate Research and Scholarships Modified Student Summer Scholars Program.

Leucine-rich repeat kinase 2 (*LRRK2*)-targeted microRNAs as disease progression biomarkers for Parkinson's disease.

Macie Weiland and Brooke Armistead
Mentor: Dr. Sok Kean Khoo
Grand Valley State University, Department of Cell and Molecular Biology, Allendale MI

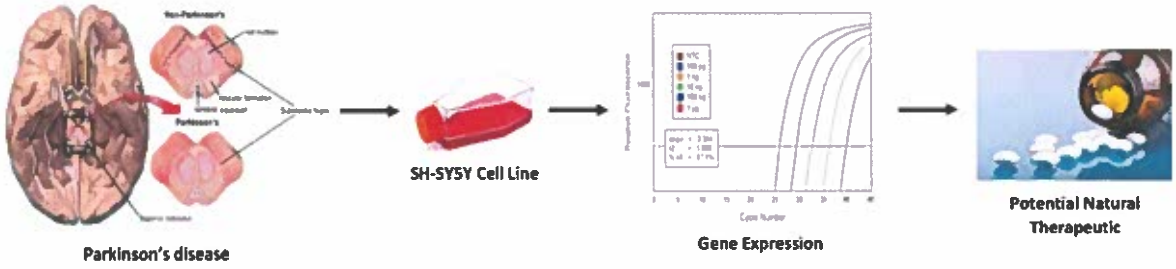


Parkinson's disease (PD) is a neurodegenerative disorder that causes motor, cognitive, and gastrointestinal dysfunctions, and affects over 6 million people worldwide. The progression of PD varies among patients in which slow progressors will develop mild to moderate symptoms 10-20 years after diagnosis, and fast progressors will develop severe symptoms in less than 10 years of diagnosis. Currently, there is no laboratory test to reliably determine the progression of PD upon diagnosis. Therefore, it is essential to identify non-invasive and low-cost blood-based biomarkers to accurately differentiate fast progressors from slow progressors for better disease management. Two microRNAs (miRs)—miR-29a and miR-29c— regulate the leucine-rich repeat kinase 2 (LRRK2) gene, which is known to be involved in the pathogenesis of PD. We hypothesized that miR-29a/c are more highly expressed in fast progressing PD than slow progressing PD. Quantitative real-time PCR was used and analyzed with Markov Chain Monte Carlo algorithm (MCMC) and logistic regression to evaluate gene expression of miR-29a/c in blood serum samples from fast and slow progressing PD patients collected at their time of diagnosis. While there was no significant statistical difference in miR29a/c expression between fast and slow progressors, fast progressors had overall higher expression.

Partially funded by the Michael J. Fox Foundation and GVSU's Office of Undergraduate Research and Scholarships P. Douglas Kindschi Undergraduate Research Fellowship in the Sciences.

microRNA 34b/c and alpha synuclein gene expression in SH-SY5Y cells for Parkinson's disease study

Emma Hahs
Mentor: Dr. Sok Kean Khoo
Grand Valley State University, Department of Cell and Molecular Biology, Grand Rapids, MI 49503, USA

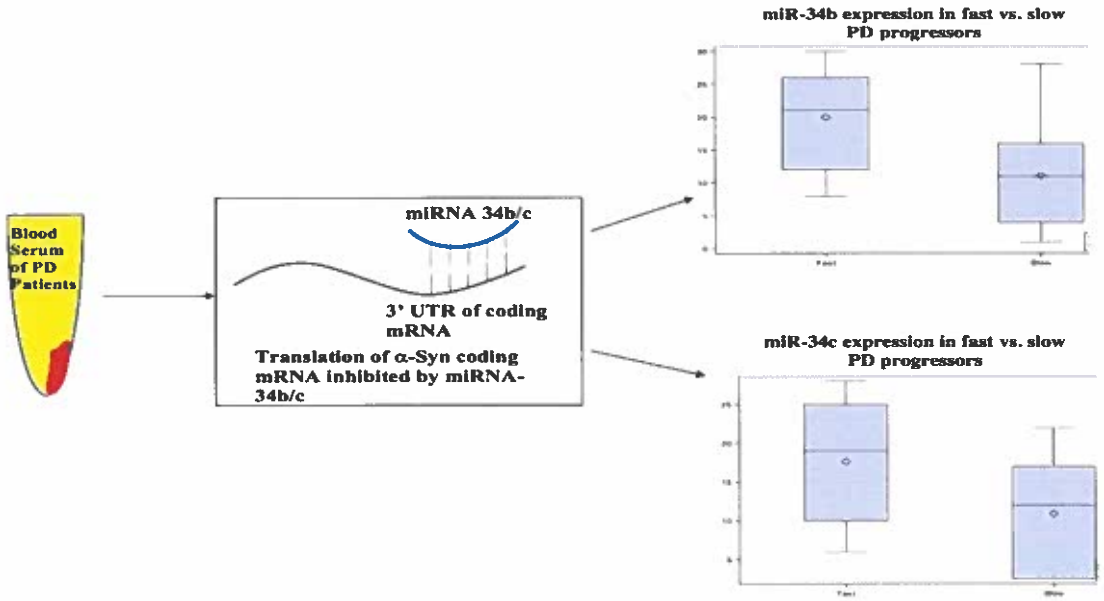


Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting 1-2% of adults over the age of 60. The pathological hallmark of PD is alpha synuclein (aSyn) protein inclusions in the brain. Accumulation of this misfolded protein, especially in the dopaminergic neurons, is thought to cause PD. Thus, developing new drug therapies that block aSyn aggregation could potentially slow or stop the disease progression. MicroRNAs (miRNAs) are small RNAs that bind to messenger RNA (mRNA) and regulate their gene expression. miRNA-34b and 34c are predicted to target aSyn and are shown to be down-regulated in PD brains. Here, we aim to establish a cell model to study the effects of miRNAs on aSyn aggregation. Our hypothesis is that miRNA-34b/c gene expression will be down-regulated, whereas the aSyn gene expression will be up-regulated. Using quantitative real-time PCR to measure gene expression, we found miR-34b/c expression down-regulated in rotenone treated SH-SY5Y cells that mimic a PD phenotype when compared with the control. However, aSyn gene expression was not up-regulated in rotenone treated cells, which is unexpected. Our next step is to measure aSyn protein expression because gene expression was not reflective of the PD phenotype. After establishing this PD model, miRNA mimics or inhibitors will be added to investigate their effects on aSyn aggregation to provide valuable information on future miRNA-based therapies for PD.

Partially funded by the Michael J. Fox Foundation for Parkinson's Research and Grand Valley State University's (GVSU) Office of Undergraduate Research and Scholarships Student Summer Scholars Program. GVSU facilities were also used to complete the research.

MicroRNA-34b and 34c as disease progression biomarkers for Parkinson's Disease

Ashleigh Harrah, Brooke Armistead,
Mentor: Dr. Sok Kean Khoo
Grand Valley State University, Department of Cell and Molecular Biology, Grand Rapids, MI 49503, USA

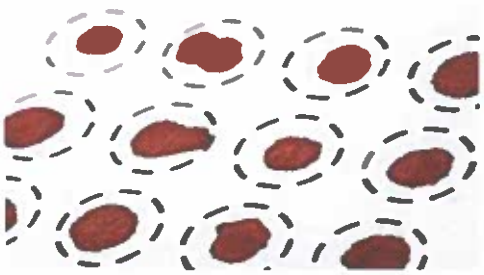


Parkinson's Disease (PD) is a neurodegenerative disorder with heterogeneous symptoms including motor and cognitive impairments. While the cause of PD is unknown, it has been found that PD patients have decreased levels of dopamine due to aggregation of alpha-synuclein (α-Syn) protein in their dopaminergic neurons. MicroRNAs (miRNAs) are small molecules that bind to complementary messenger RNAs to regulate protein expression. Down-regulation of miRNA-34b/c is known to increase α-Syn expression in cell cultures and PD brain tissues. To date, there is no laboratory test to monitor disease progression upon diagnosis. miRNA-34b/c have been found to serve as biomarkers to distinguish PD from healthy controls, but a biomarker to monitor disease progression has not been identified. Using quantitative real-time PCR to evaluate miRNA34b/c expression in sera of fast and slow PD progressors, we aimed to develop a blood-based progression biomarker. It was found that fast PD progressors have statistically significant higher expression of miRNA-34b (p-value= 0.0025) and miRNA-34c (p-value=0.0156), when compared with slow PD progressors. Thus, miRNA-34b/c may be used as potential biomarkers to differentiate fast from slow progressing PD to enable more effective disease management and treatment.

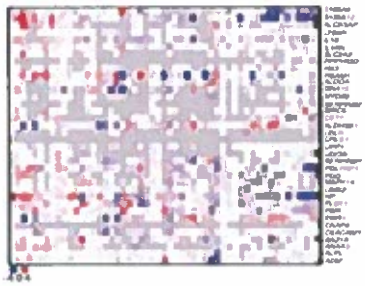
Partially funded by the Michael J. Fox Foundation for Parkinson's Research and GVSU Office of Undergraduate Research and Scholarships Student Summer Scholars Program.

Determining S100A9 as an Indicator for the Development of Cerebral Palsy in Infants through Quantitative Analysis of Gene Expression.

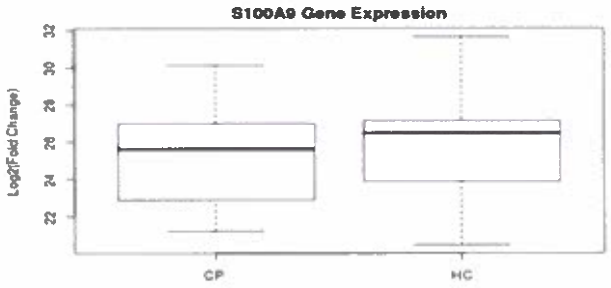
Paul Gingerich, and Brooke Armistead.
Mentor: Dr. Sok Kean Khoo
Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI
Partially funded by the National Institute of Neurological Disorders and Stroke of National Institutes of Health

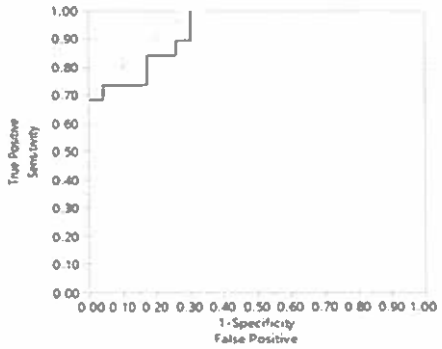


Infant Blood Spots



Heatmap of gene expression





Cerebral palsy (CP) is a type of chronic brain damage developed before, during, or soon after birth. Though nonprogressive in nature, CP has a wide range of physical and mental severity, which can be mild, or it can result in more debilitating conditions like quadriplegia and severe mental retardation. Currently, it is unknown what exactly causes an infant to develop CP, and there is no conventional test to determine if an infant will acquire CP. Risk factors, including gestation, delivery, gender, race, and APGAR scores are thought to influence the prevalence of developing CP, however with no prenatal test for the likely development of CP, steps cannot be taken to prevent infants from developing the condition, or alleviate the severity. In this project, I will be measuring the expression of the gene S100A9, a calcium binding protein, which showed promise as an indicator in the likely development of CP. Through logistic regression, the model produced had a specificity of 0.6957, sensitivity of 1.000, and p-value of 0.0011. The defined model could permit a definitive diagnosis of CP to allow treatment earlier in an infant's development to reduce severity of the disease.

Protein Kinase B (AKT) Isoform-Specific DNA Hypermethylation with Maintenance Atypical Antipsychotic Treatment

Bradley Howlett[1], Berhane Seyoum[2], Sabrina Dass[3], Elani Sanders[3], Abdullah Mallisho[2], Zhengping Yi[3]
Mentor: Dr. Kyle Burghardt[3]
[1]Grand Valley State University, Department of Cell and Molecular Biology, Allendale, MI
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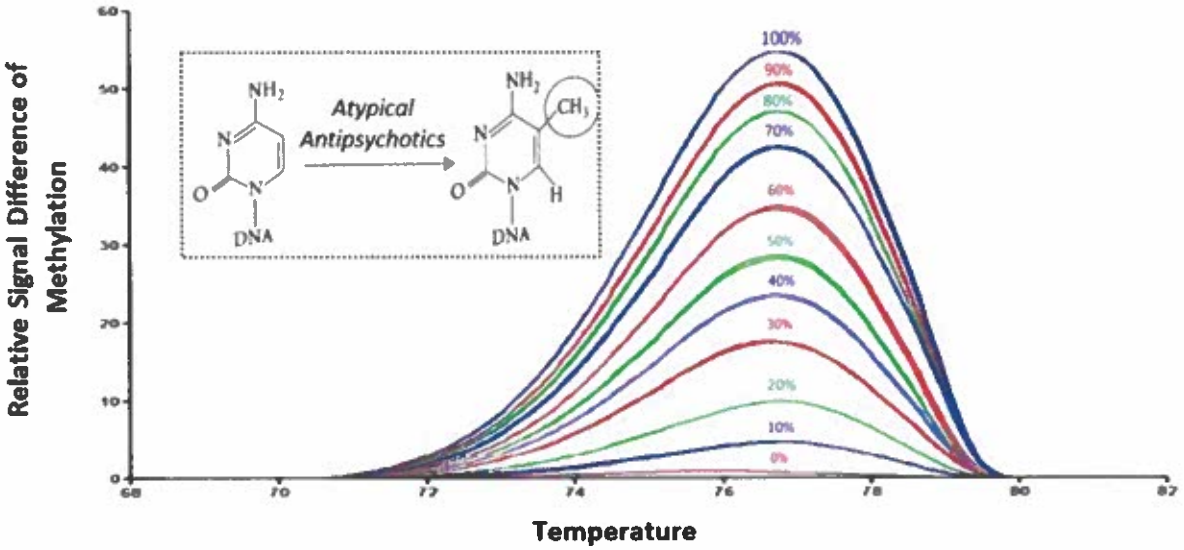

Atypical antipsychotics can cause insulin resistance that leads to an increased risk of diabetes and cardiovascular disease, however the mechanism by which antipsychotic-induced insulin resistance occurs is not completely known. Protein kinase b (AKT) is an important serine/threonine kinase that facilitates many vital functions in the tissue cells of the body, and contributes to the overall muscle health and glucose uptake into the skeletal muscle. The objective of this study was to measure *AKT* isoform-specific gene methylation in the skeletal muscle of bipolar patients on atypical antipsychotic treatment compared to patience on mood stabilizer maintenance therapy. Skeletal muscle *AKT* gene methylation near the promoter for *AKT1, AKT2* and *AKT3* was measured by methylation-sensitive high-resolution melting. In participants treated with atypical antipsychotics, *AKT1* and *AKT2* methylation was increased compared to participants on mood stabilizers. Overall our findings suggest that AKT is differentially methylated in the skeletal muscle of participants on atypical antipsychotics or mood stabilizer maintenance therapy. These results may direct future strategies to avoid damaging side effects of atypical antipsychotic treatment.

Preliminary Study of DNA Preservation in Water Samples from Rural Haiti Using PCR

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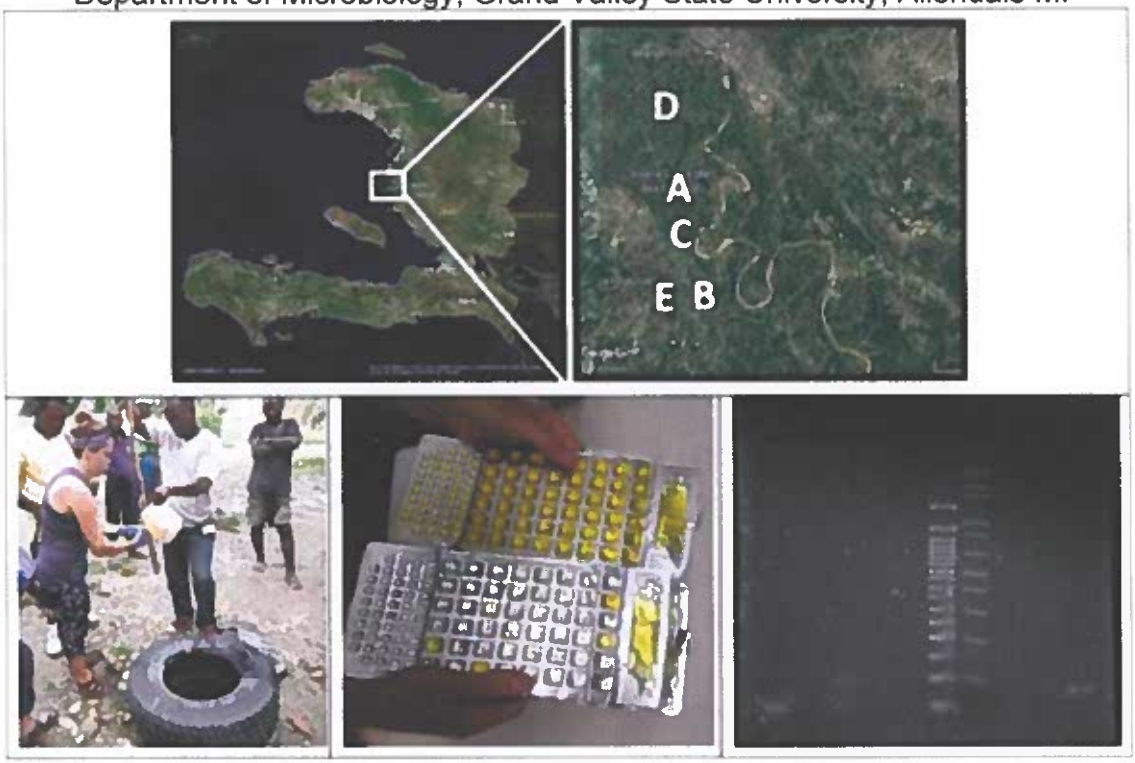

Over 1.8 billion people worldwide are expected to drink bacterially contaminated water annually. Nearly 3.6 million people worldwide, half of them being children, die from diseases contracted from this contaminated water. Contamination is a widespread problem in third world countries such as Haiti, yet the source of *E. coli* contamination is unclear. Before DNA analyses can be used to determine the source, samples require successful preservation to be transported for further testing. Freezing is a successful method of DNA preservation; however, it may not always be available in under-developed countries. Four additional preservation methods using desiccation, glutaraldehyde, formalin, and a local alcohol, clairen, were performed upon water samples from five different sites in Haiti. We are working towards developing a PCR assay using primers from the Annis Water Research Institute (AWRI). AWRI had developed specific primers for statewide use to develop qPCR assay of beach water samples as an alternative to bacterial culturing. DNA extraction, PCR, and agarose gel electrophoresis were performed on these samples in hopes to define a minimum and maximum DNA template volume resulting in an *E. coli*-specific PCR product. A method was designed in order to one day use PCR to determine the best preservation method. Troubleshooting and refinement are still required in this study, along with an answer to if the present *E. coli* is from human or animal sources.

Improving a Computational Analysis Method for Blood and Serum Microarrays

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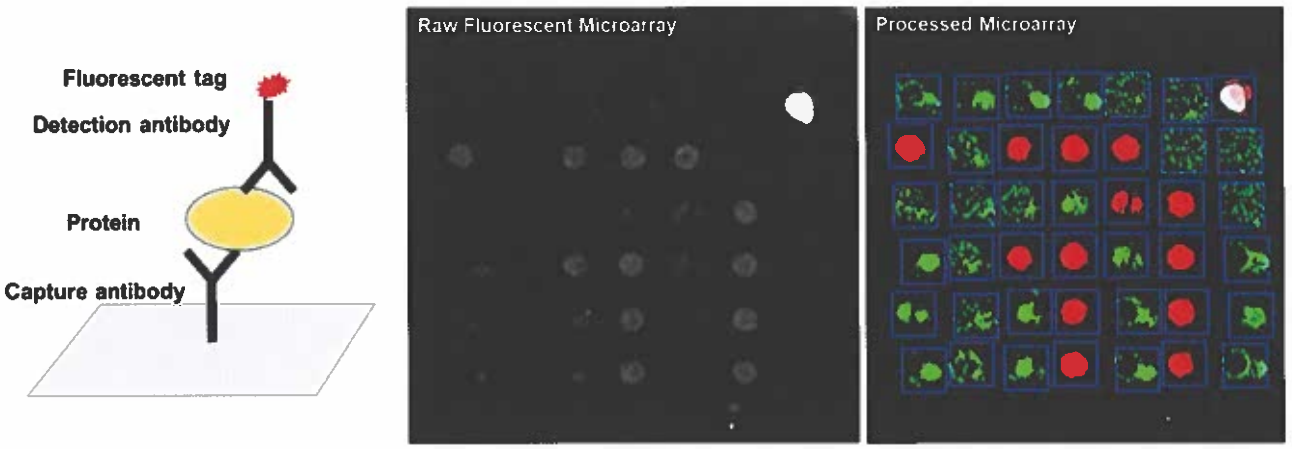

Antibody arrays are an efficient way to measure protein content from low volume biological samples. Despite this, relatively large volumes of blood or serum samples are still required to produce signals that can be detected visually. Advances in image capturing techniques enable taking high-resolution, high contrast images of samples, but these images still need to be analyzed manually. This project was to improve upon an existing software developed by the Haab lab at the Van Andel Research Institute which separates tissue signal from background signal of very low signal antibody arrays. The software was developed and tested in MATLAB. The program was improved by creating functions to analyze up to 4 times more blocks of arrays at a time than the previous version, or as few as 1 array, for a range of 1 to 192 blocks per analysis. The program was also improved by increasing processing speed to be 4 times faster in most cases using parallel processing, refining spot box positions using a multiple-pass overlap correction, creating a minimum signal threshold for arrays that present no signal, and creating a program in Java which produces files of spot statistics. The statistics provided to users for each spot are background subtracted signal intensity, outlier removed signal intensity, and many others. This software will allow researchers to use significantly smaller volumes of their biological samples for a rapid, objective analysis.

Generation of pHdc-containing transgenic flies to study Hdc promoter function

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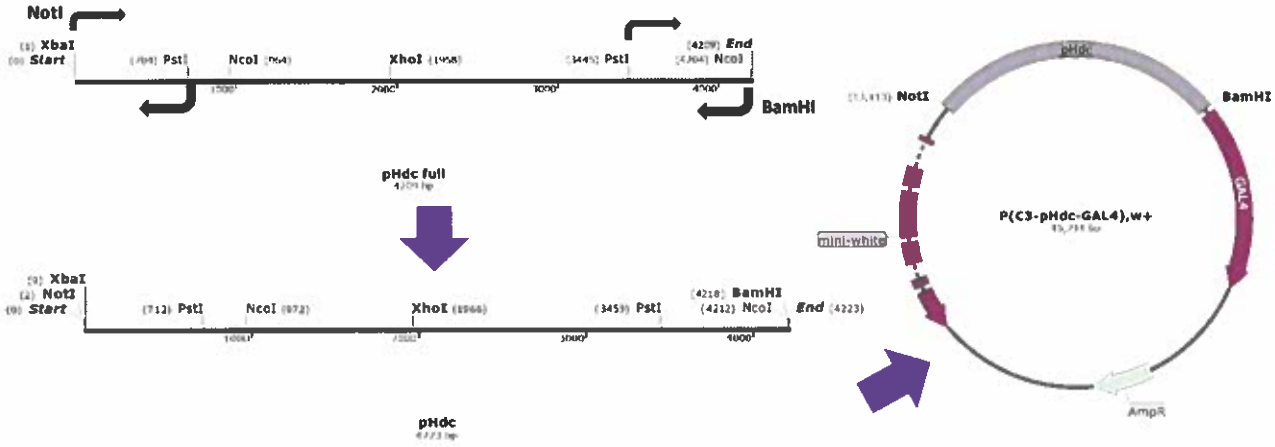

The spatial and temporal expression of a gene is typically controlled by its promoter. This study's goal is to generate tools to examine the functional limits of the histidine decarboxylase gene's (*Hdc*) promoter in *Drosophila melanogaster*. To carry this out, a full version ($pHdc_{LONG}$) and a short version ($pHdc_{SHORT}$) are cloned into transformation plasmids containing the eGFP reporter and then grown in *E .coli*, and injected into embryos, with transgenic flies being currently selected. Additionally, a $pHdc_{LONG}$-GAL4 plasmid is being synthesized that will induce expression of GAL4 in histaminergic cells. Oligonucleotide primers were designed to add unique restriction enzyme sites at the ends of $pHdc_{LONG}$ using a polymerase chain reaction (PCR) approach. These new end fragments of $pHdc_{LONG}$ are cloned and sequenced to select the proper clone to insert these end fragments back into $pHdc_{LONG}$ to enable cloning of this promoter into various transformation plasmids for injection into *D. melanogaster* embryos. Characterization of the pHdc-GFP chimeric plasmids will allow the determination of the extent of the promoter needed for functional gene expression, which can then be used to create tools to manipulate gene expression of *Hdc*.

Funded by the Office of Undergraduate Research and Scholars at Grand Valley State University for the Student Summer Scholars (S3) Program

Characterization of deletions in the *Hdc* gene of *Drosophila melanogaster* produced by transposon-excision mutagenesis

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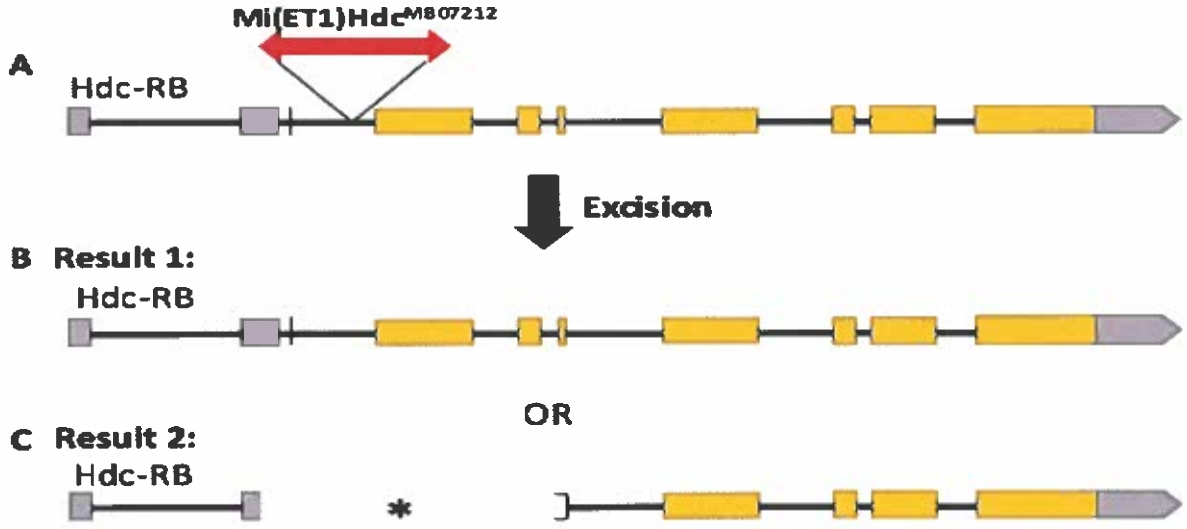

Histamine, a small molecule that is produced from histidine by histidine decarboxylase (HDC), functions as a neurotransmitter in the central and peripheral nervous systems. In *Drosophila melanogaster*, mutations of the *Hdc* gene have shown disruptions in histamine synthesis and in behavior. Previous work on this topic has failed to produce a mutant with total knockdown of the *Hdc* gene resulting in all histamine studies to be done thus far with fruit flies that express some amount of histamine. Using *Minos* transposon-excision mutagenesis part of the *Hdc* gene can be removed and the results of the deletion can be tested using polymerase chain reaction (PCR) to determine how much of the *Hdc* gene was deleted. If enough of the Hdc gene is removed, the gene will no longer be able to produce histamine. In the lab, 12 mutant flies were characterized with imprecise deletion and were histamine negative. Using mutagenesis, these 12 mutants acquired a deletion in the *Hdc* gene large enough to total disrupt the ability to produce histamine. Being able to study the the effects and behaviors of an organism in the absence of a molecule that has functions in the central and peripheral nervous systems can pave the way into discovering mechanisms for disordered that can be caused by lack of histamine and help develop treatments.

Cold-tolerance and Survival Impact of Invasive Alien Species *P. clarkii* in Freshwater Michigan Waterways

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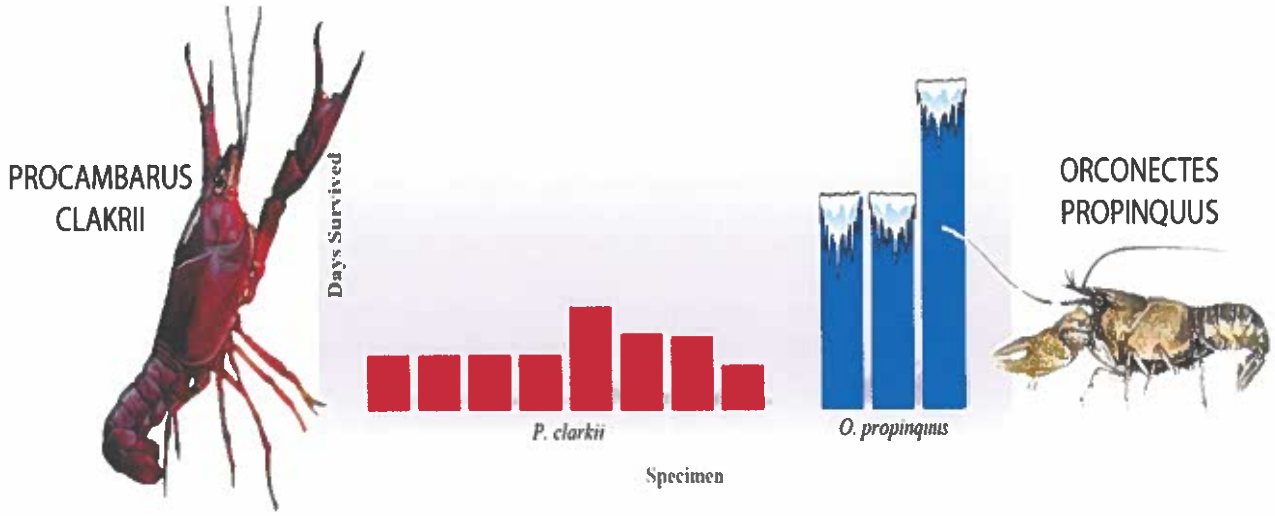

In the advent of a changing climate, sensitive freshwater ecosystems are at a risk of invasive alien species. Particularly hardy alien organisms such as cambarid freshwater crayfish *Procambarus clarkii* severely interrupt delicate aquatic ecosystems, threatening biodiversity of local predators and prey alike. While *P. clarkii* is known for rapid reproduction and resistance to hypoxia, desiccation, and freshwater salinity, *P. clarkii* does not inhabit northern temperature zones, possibly due to its poor tolerance at low temperatures. Often used in the commercial food industry, *P. clarkii* growing conditions suggest subtropical temperatures for *P. clarkii* growth and it is unknown how long *P. clarkii* would survive when exposed to frigid water temperatures similar to a Michigan freshwater ecosystem. By simulating a Michigan winter through use of aquatic refrigeration and food scarcity, the survival of *P. clarkii* in local waters can be compared to native crayfish species *O. propinquus*. Results of *P. clarkii* cold tolerance trials indicate a significant decline in longevity and activity when compared to the native *O. propinquus*. Cold tolerance measurements will provide insight to predict *P. clarkii's* effect on aquatic biodiversity, commercial fishing and agriculture and the possibility of freshwater eutrophication.

Comparison of Residue Network Patterns in Clinical Mutants of β-Lactamase OXA-66

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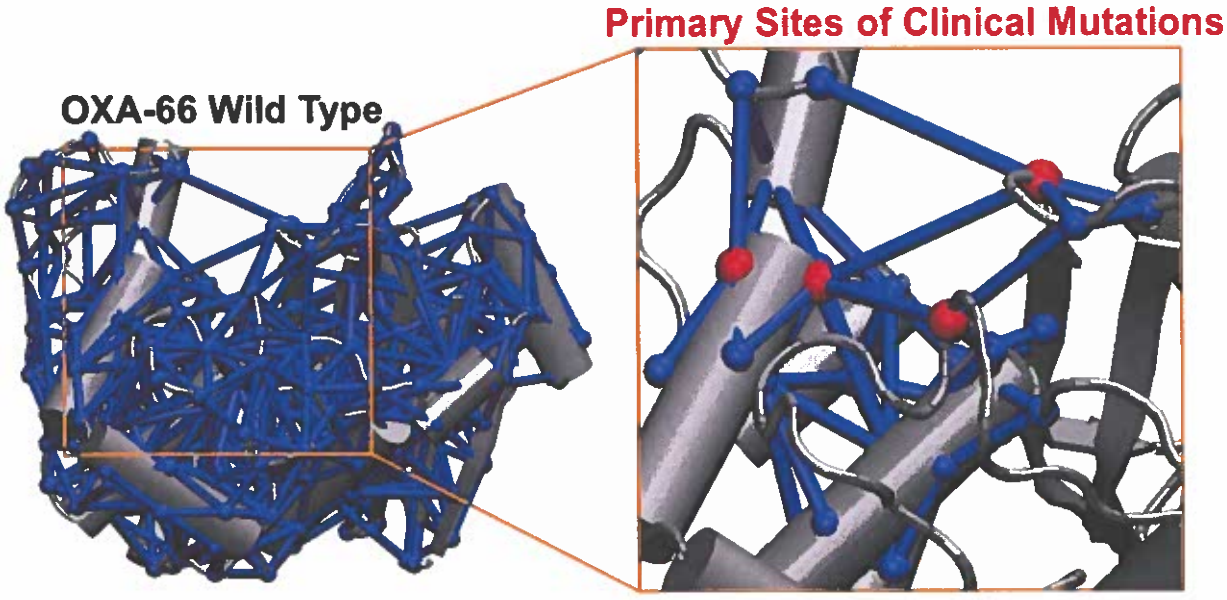

OXA β-lactamases are a major mechanism of antibiotic resistance in *Acinetobacter baumannii*, a common nosocomial pathogen. OXAs have been evolving rapidly toward extended spectrum and/or carbapenemase activity, for example OXA-66 has clinical variants with improved carbapenem binding, such as P130Q, P130A, I129L, L167V, and W222L. Some aspects of the mechanism of their gain of function have been explained, but others remain unclear; e.g. the altered loop dynamics in each variant. To determine changes in protein dynamics, we analyzed 250ns molecular dynamics simulation trajectories of fully hydrated structures of OXA-66 WT and each mutant. We have compared hydrogen bonds and dynamical network models to identify changes in the tertiary contacts between the P loop and the mutation site (helix α5). Our data indicate that mutations near the opening to the binding pocket, in α5, β5/β6 loop, or ω loop, impact the network connections locally and globally, with changes not only in the neighboring P loop but also in the catalytic center of the protein. P loop is more flexible in all mutants and moves away from the main pocket contributing to disruption of the hydrophobic bridge at the entrance to the pocket. The networks of mutants also have markedly different community structures. Our results are consistent with the enhanced activity of the clinical mutants, and help determine how mutations at α5, β5/β6 loop, or ω loop impact the structure and dynamics of the OXA-66 binding pocket.

Simulations were completed at the GVSU Phoenix high performance cluster, supported by the National Science Foundation (NSF) Grant No. CNS-1228291.

Crystallographic & Fluorescence-Based Ligand Binding Analysis of BshC: The Final Enzyme in the Bacillithiol Biosynthesis Pathway

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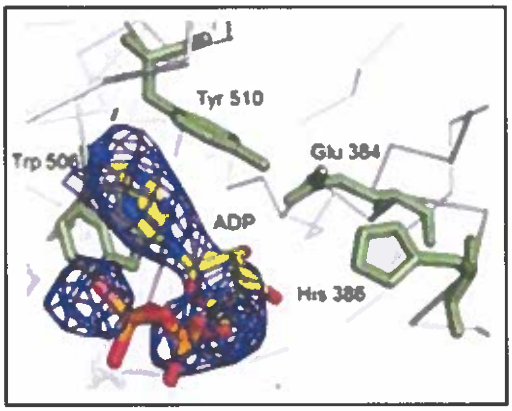

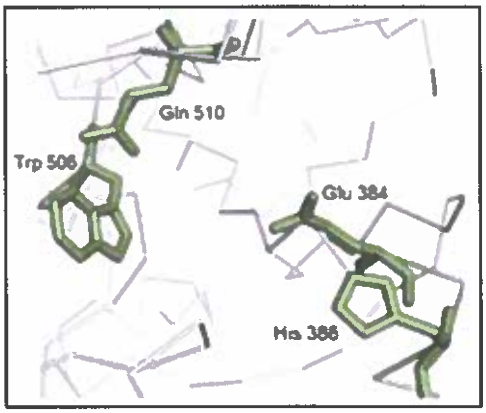

BshC is the final enzyme in a three-step pathway for the biosynthesis of bacillithiol, a compound that enables resistance to the antibiotic fosfomycin. BshC is unique among other enzymes in its family due to its inactivity *in vitro* and an additional ligand binding pocket. Crystallographic data reveals ADP bounding within this pocket. To explore BshC function, several site-directed mutants have been produced in the second ligand binding pocket including: Y510Q, Y510L, H386A, and W506L to understand what is essential to ADP binding. Fluorescence assays have been utilized on the wild-type BshC and four mutants and revealed that all mutants do not bind ATP as effectively as wild-type BshC. X-ray crystallography was used to analyze Y510Q mutation, which revealed that there is indeed no density in the area of the second ligand binding pocket. Together these results confirm that ATP is binding within the second ligand binding site and not in the active site of BshC. Gaining more understanding of the structure of these mutants and how they bind ATP will yield a better understanding of what is necessary for BshC to bind its substrate *in vitro*, which will facilitate development of inhibitors to combat fosfomycin resistance.

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X-Ray Crystallography for the Structural Analysis of BSH Antibiotic-Resistance Enzyme BshB and its Paralog Bca

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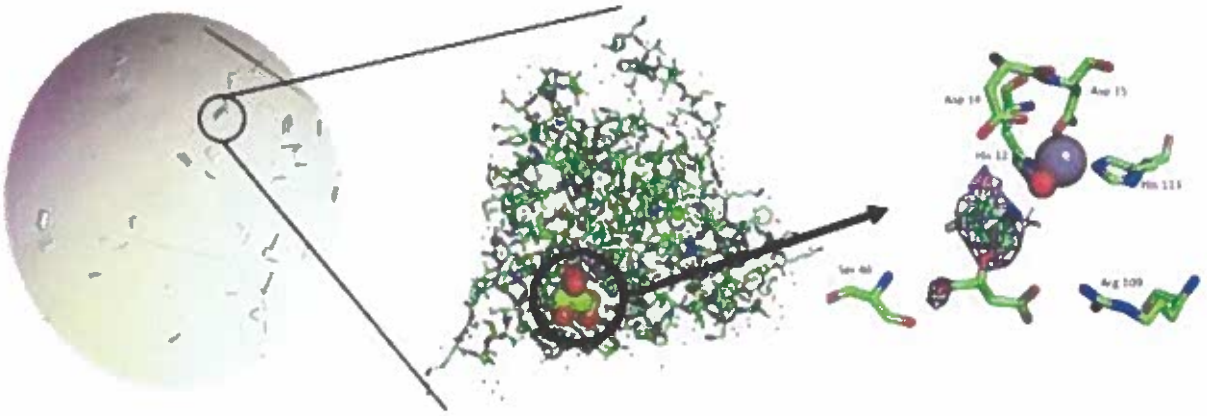

BshB crystals | **Putative BshB crystal structure** | **Putative BshB active site structure**

Bacillithiol (BSH) is a low-molecular-weight thiol that detoxifies reactive oxygen species in certain gram-positive bacteria. In addition to this role, it is also mediates resistance to the antibiotic Fosfomycin by serving as a cofactor to the antibiotic-resistance enzyme FosB. BSH is produced from UDP-*N*-acetylglucosamine (UDP-GlcNAc) by a three-enzyme pathway that includes the enzymes BshA, BshB, and BshC. BshB is a deacetylase, responsible for converting *N*-acetylglucosaminyl-malate (GlcNAc-mal) to glucosaminyl-malate (GlcN-mal). Bca is a paralog deacetylase that can perform the same function as BshB in addition to removing CysS-conjugated toxins from bacterial cells. As the issue of antibiotic resistance is of high clinical relevance, it is essential to understand how each enzyme performs its function. Our objective is to elucidate the structural and functional properties of BshB and its paralog Bca. Crystal structures for BshB and Bca have not been published due to low resolution, and valid kinetic parameters for the enzymes need to be determined. We used X-ray crystallography to attempt to determine the structures of these enzymes and developed a novel discontinuous HPLC fluorescence assay to test the kinetics of BshB. While the crystal structures of BshB and Bca remain elusive due to poor crystal diffraction, our novel functional assay has proven that BshB follows Michaelis-Menten kinetics. Further use of this assay in conjunction with BshB active site mutations will help determine the functional roles of key active site residues in BshB. Ultimately, a complete characterization of these enzymes' role in the BSH pathway will benefit the overall effort to decrease the detrimental effect that antibiotic-resistant bacteria have on human health.

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Localization of the Filamentation-Associated Protein Encoded by *Orf 19.2302* in *C. albicans*

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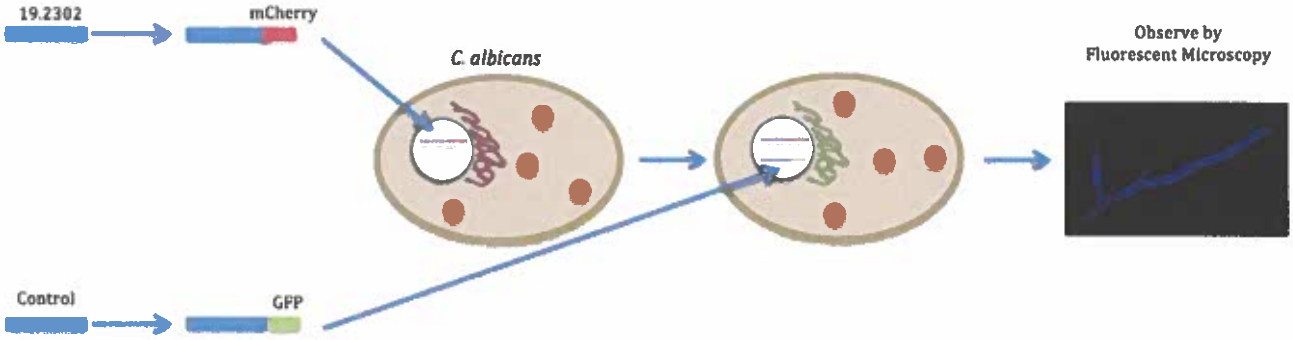

Cell morphology plays a vital role in *Candida albicans* pathogenesis. Nrg1 is a key repressor of *C. albicans* morphology. However, little is known about some of its targets. One such gene, *orf19.2302*, encodes a protein expressed in hyphae, and is predicted to be an ER membrane protein with no known homologues outside of *C. dubliniensis*. To better understand this protein, we transformed mCherry tagged *orf19.2302* and GFP tagged *ERG11* genes into the wild type strain of *C. albicans* to determine the localization of the protein within the cell. We used PCR to construct a version of *orf19.2302* labeled with the mCherry fluorescent tag. This DNA was then transformed into the *C. albicans* genome. Subsequently a GFP tagged version of *ERG11*, known to localize to the ER, was transformed into the strain to examine colocalization of the tagged proteins. These proteins were studied by fluorescent microscopy to determine their localization. Preliminary microscopy results may indicate that this protein performs a larger role than previously anticipated. Also, a deletion strain constructed in the lab does not appear to be sensitive to calcium depletion, as predicted by similarities to a *Saccharomyces* protein in the same family. Further microscopy will be necessary to determine a more precise localization of the protein. From further investigation we hope to gain a better understanding of *orf19.2302* and the role that the encoded protein plays in the morphological changes observed in *C. albicans*.

Collection, Isolation and Identification of Wild Yeasts for Brewing Purposes

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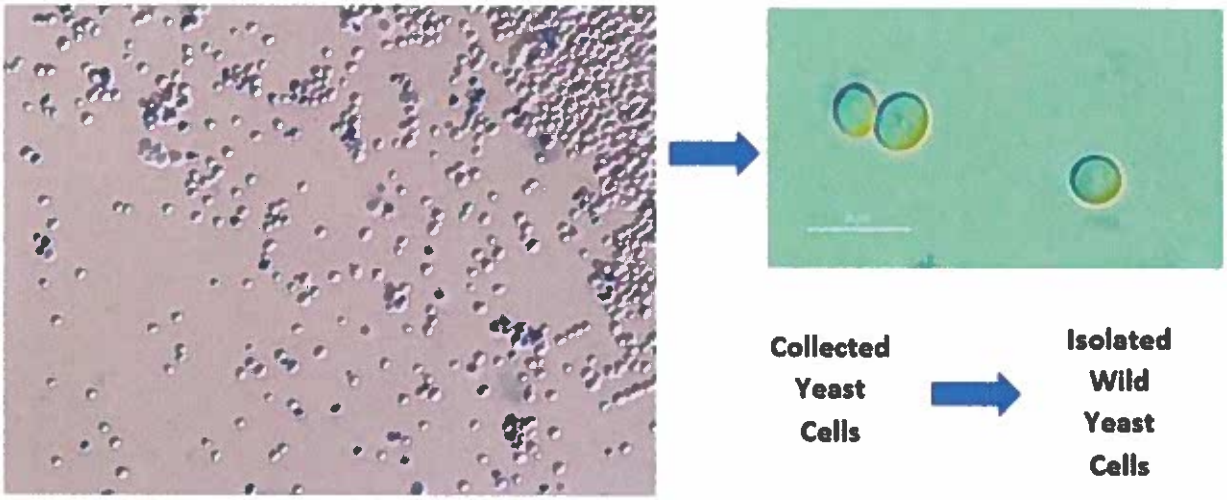

Wild yeasts can be found almost everywhere and have been used solely or in conjunction with brewer's yeasts to brew beer. However, being able to effectively isolate and identify wild yeasts, and determine if they could be potentially used to brew beer can be difficult. Isolating and identifying wild yeasts from other yeasts has been accomplished by plating yeasts using certain plating techniques on yeast peptone dextrose (YPD) plates and then on Lin's Cupric Sulphate plates to select for wild yeasts, but the plating techniques used do not always lead to efficient isolation and identification. Here we present changes in plating techniques and provide additional observations that can be used to substantially increase the effectiveness of isolating and identifying single wild yeasts using YPD and Lin's Cupric Sulphate plates. We describe different streaking techniques that drag yeast cells versus making one long streak on YPD and Lin's Cupric Sulphate plates. We have also measured growth rates and performed PCR on single wild yeast colonies to identify them. We expect these improved techniques to increase the number of wild yeasts that can be isolated and identified in a quicker and more efficient manner from yeast samples collected and incubated in malt extract.