Annotation of Three Novel Soil Mycobacteriophages: Sibs6, Roots515, and CBorch11

Poster (Friday, April 20, 2018)

Sara Sybesma Tolsma, Christopher Borchers, Nathan Eide, Emily Geraets Colton Hage, Jacob Jenness Northwestern College Hannah Jorgensen Northwestern College Shay Kamstra Northwestern College Megan Kingsriter Northwestern College Sidney Martin Northwestern College Courtney Mithelman Northwestern College Bethany Muyskens Northwestern College Lily Peschau Northwestern College Peace Preston Northwestern College David Rowley Northwestern College Sabrina Tarchione Northwestern College Shelby Van Den Berg Northwestern College Michaela Van Riesen Northwestern College Byron Noordewier Northwestern College
Northwestern College

We isolated, purified, characterized, and sequenced three novel Mycobacteriophages from soil bacteria. Sibs6 is a member of the A1 cluster, Roots515 is a C1 cluster phage, and CBorch11 is a member of the rare H1 cluster. Sibs6 and CBorch11 are siphoviridae phages with double-stranded DNA genomes and long, flexible, non-contractile tails. Roots515 is a podoviridae phage with a double-stranded DNA genome and a short, non-contractile tail. The 50,210 base-pair genome of Sibs6 has a 63.8 % GC content and includes genes that suggest this phage is lysogenic, consistent with its plaque morphology and other A1 phages. Its left arm contains 38 forward genes and its right arm contains 58 reverse genes. Using bioinformatics, we assigned functions to 41 of its 96 genes. The genome of Roots515 is 156,288 base pairs in size and has a 64.7% GC content. Its 271 genes are mostly forward and include 33 tRNA genes. We assigned functions to 50 of the protein-coding genes in Roots515. CBorch11's genome is 68,508 base pairs in size and has a 57.6% GC content. CBorch11's 93 genes are all forward genes and we assigned functions to 22 of them.

Targeting Leucine and its Metabolism for the Treatment of Bone Sarcomas

Poster (Friday, April 20, 2018)

Elitsa Ananieva, Shailer Martin, William Reiche, Alexander Schultz Nickolas Fifelski, Michael Boyer
Des Moines University

Osteosarcoma and chondrosarcoma are devastating bone sarcomas with low survival rates. The prognosis for individuals with bone sarcomas depends upon the patient's response to chemotherapy and surgical intervention. A novel approach to treat bone sarcomas is to target leucine, an essential amino acid that supports tumor growth. Our objective was to investigate how a pharmacological inhibition of leucine uptake and metabolism by N-acetyl-leucine amide (NALA) and gabapentin, respectively, would affect the ability of osteosarcoma and chondrosarcoma cells to use leucine for energy and biosynthetic needs. To achieve this objective, we treated osteosarcoma and chondrosarcoma cells with NALA (0-25mM) and gabapentin (0-10mM) for 24-48 hours and measured: cell growth, leucine transamination, lactate secretion, the energy sensor AMPK-regulated protein kinase (AMPK) and the S6 ribosomal protein. Results showed that NALA stimulated AMPK but inhibited leucine transamination, lactate secretion, the activity of S6, and the growth of both cell lines. Gabapentin affected the osteosarcoma cells only. Activated AMPK indicated low energy status, while inhibition of lactate secretion and S6 suggested low biosynthesis potential of the cells upon inhibition of leucine uptake by NALA or leucine metabolism by gabapentin. Targeting leucine is thus a promising new solution to combat bone sarcomas.

Production of Anti-Mycobacteriophage Protein Antibodies in Balb/c Mice

Poster (Friday, April 20, 2018)

Sara Tolsma, Jeanna Becker, Chris Borchers, Kelsey Dorhout Hans Epp, Emily Geraets, Northwestern College Courtney Mithelman, Northwestern College Hannah Powell, Northwestern College Peace Preston, Northwestern College Renju Pun, Northwestern College Sabrina Tarchione, Northwestern College Michaela Van Riesen, Northwestern College Byron Noordewier, Northwestern College Northwestern College

We annotated three novel mycobacteriophages: Sibs6 (Cluster A1), Roots515 (Cluster C1), and CBorch11 (Cluster H1). We used 12% SDS-PAGE to visualize high titer lysates from the three phages. Our gels revealed a major Sibs6 protein at 65 kDa, two major Roots515 proteins at 64 kDa and 30 kDa, and four CBorch11 bands at 65 kDa, 42 kDa, 30 kDa, and 23 kDa. We eluted the 64/65 kDa bands from each phage sample, concentrated, and used these eluted proteins to immunize Balb/c mice. We are characterizing these polyclonal anti-phage protein antibodies and plan to determine if they cross react with proteins produced by mycobacteriophages from other clusters. We also hope to use the antibodies to understand the production of these proteins in the context of phage life cycles. In addition, we immunized Balb/c mice with whole CBorch11 mycobacteriophage and are developing monoclonal antibodies to proteins produced by this rare cluster H1 mycobacteriophage.

Using PCR to Confirm and Revise Mycobacteriophage Genome Annotations

Poster (Friday, April 20, 2018)

Sara Tolsma, Jeanna Becker, Chris Borchers, Kelsey Dorhout Hans Epp, Emily Geraets, Northwestern College Courtney Mithelman, Northwestern College Hannah Powell, Northwestern College Peace Preston, Northwestern College Renju Pun, Northwestern College Sabrina Tarchione, Northwestern College Michaela Van Riesen, Northwestern College Byron Noordewier, Northwestern College Northwestern College

We annotated three novel mycobacteriophages: Sibs6 (Cluster A1), Roots515 (Cluster C1), and CBorch11 (Cluster H1). We used 12% SDS-PAGE to visualize high titer lysates from the three phages. Our gels revealed a major Sibs6 protein at 65 kDa, two major Roots515 proteins at 64 kDa and 30 kDa, and four CBorch11 bands at 65 kDa, 42 kDa, 30 kDa, and 23 kDa. We eluted the 64/65 kDa bands from each phage sample, concentrated, and used these eluted proteins to immunize Balb/c mice. We are characterizing these polyclonal anti-phage protein antibodies and plan to determine if they cross react with proteins produced by mycobacteriophages from other clusters. We also hope to use the antibodies to understand the production of these proteins in the context of phage life cycles. In addition, we immunized Balb/c mice with whole CBorch11 mycobacteriophage and are developing monoclonal antibodies to proteins produced by this rare cluster H1 mycobacteriophage.

Progesterone is a potent inducer of indoleamine dioxygenase (IDO) in human macrophages

Poster (Friday, April 20, 2018)

Kristin Twedt, Sydney Beals, Daniel Jung
Briar Cliff University

Successful human pregnancy depends on the initiation and maintenance of local immunological tolerance at the fetal-maternal interface. This immunosuppressive microenvironment also regulates the fetal immune system and may be partially responsible for deficiencies in the neonatal immune system. Fetal immune system deficiencies result in a greater susceptibility to microbial infection, a major cause of mortality early in life. Previous researchers have shown that human cord blood-derived macrophages express a higher level of IDO compared to adult macrophages. It is known improper functioning and abnormal expression of IDO can cause atypical pregnancy conditions. Furthermore, IL-27, a regulatory cytokine, is known to regulate IDO expression in human neonatal macrophages.

Since progesterone is produced by the placenta during pregnancy and induces many transcription factors, progesterone may be an inducer of IDO.

Current research showed that IDO is expressed by the treatment of progesterone in a dose-dependent manner. This gene upregulation may be due to the production of IL-27. The elevated expression of IL-27 in human neonatal macrophages positively regulates IDO expression. IDO has been shown to negatively regulate T-cell proliferation and activity. Thus, the ability to mount protective immune responses in newborns and infants may be improved by blocking IL-27.

Molecular mechanism of Indoleamine dioxygenase gene expression in prairie turnip (Psoralea esculenta) extract treated human macrophages

Poster (Friday, April 20, 2018)

Jessica Welter, Jacob Hindman, Paul Weber, Daniel Jung
Briar Cliff University

The enzyme indoleamine 2, 3-dioxygenase (IDO) is present in many cells, including macrophages and is important in the modulation of immune response. The catabolites of tryptophan are involved in immune tolerance, and IDO is a rate-limiting enzyme that catabolizes tryptophan.

IDO is normally upregulated by cytokines, with interferon gamma (IFN-) being the primary source of upregulation. Tumor necrosis factor alpha (TNF-α) synergistically enhances IDO when IFN-γ is present. Psoralea esculenta, found in the prairies of Iowa, contains the flavonoids genistein and daidzein. The flavonoids are known to be an inducer of IDO.

In this research, prairie turnip rind extract was found to upregulate IDO mRNA to an extent similar to IFN- and TNF-α. The mechanism of upregulation was further investigated.

Antioxidant compound M11 isolated from Psoralea esculenta inhibits the growth of two pathogenic bacteria; Streptococcus pyogenes and Staphylococcus aureus

Poster (Friday, April 20, 2018)

Olivia Matz, Abagail Gerhardt, Jacob Hindman, Paul Weber Daniel Jung
Briar Cliff University

Psoralea esculenta, found in the prairies of Iowa, contains the flavonoids genistein and daidzein. These compounds are known to exhibits high antioxidant activity. Previous research by colleagues at this institution have shown that a previously unreported component, M-11, isolated from this plant also exhibited potent antioxidant activity.
Previous research in this lab revealed that the extracts from Psoralea esculenta inhibits the growth of Staphylococcus aureus significantly. Current research revealed the same pattern of inhibition on the growth of Streptococcus pyogenes and these inhibition were partially due to the potent antioxidant M-11.

Using PCR to Confirm and Revise Mycobacteriophage Genome Annotations

Poster (Friday, April 20, 2018)

Sara Tolsma, Bethany Muyskens, Chris Borchers, Byron Noordewier
Northwestern College

We discovered CBorch11, a novel cluster H1 mycobacteriophage, and annotated its 68,508 base pair genome. CBorch11 is the sixth H1 phage to be discovered, which makes comparative genomics difficult. Autoannoation software suggested gene 90 began at nucleotide 64331 and ended at 64456 and gene 91 began at nucleotide 64411 and ended at 64803. This created an 81 base pair overlap, which is uncommon in mycobaceriophage genomes. We compared these genes to similar H1 phages and found that some annotations deleted gene 90, but others did not. When gene 90 was deleted, some annotations extended the start site of gene 91, but others did not. We isolated RNA from an M. smegmatis culture actively infected with CBorch11 and prepared cDNA from the RNA transcripts. We designed primers to distinguish gene 90 from gene 91 and an extended version of gene 91. We are using PCR to determine which products are present in our cDNA preparation. We will use these results to definitively confirm or revise our genome annotation.

Investigating Listeria p60 Protein Enzymatic Activity on Various Biological Substrates

Poster (Friday, April 20, 2018)

Brett Cornforth, Rebecca Schmidt
Upper Iowa University

Listeria monocytogenes (Lm) is an intracellular bacterial pathogen which causes the food-borne illness listeriosis in humans. Listeriosis is the cause of 400-600 deaths each year. During a Listeria infection, the most highly secreted protein is p60, which is currently categorized as an Lm endolysin. Based on the knowledge that p60 is highly expressed in Listeria infections, along with the research showing that p60 is very ineffective at hydrolyzing Lm peptidoglycan, the purpose of this study is to investigate the biological substrates upon which p60 is active, including the mammalian phospholipid lecithin as a possible substrate. This hypothesis is based upon LRAT (lecithin retinol acyltransferase), a p60 homolog found in mammals, which acts as a phospholipase. Previous preliminary results suggested possible p60 lecithinase activity on egg agar plates. This study aims to replicate those results using freshly purified p60 protein that was collected from transfected E. coli. In the future, peptidoglycan from other bacterial sources may be examined as p60 substrates in order to determine if p60 aids in Listeria microbial antagonism. A better understanding of p60 enzyme activity and its various roles, such as during infection, could be used to minimize health risks associated with Listeria-related food-borne illness.

Properties of Antibacterial Extracts and Isolated Components from the Prairie Turnip (Psoralea esculenta)

Poster (Friday, April 20, 2018)

Jacob Hindman, Emily Joines, Carlye Polacek, Cristhian Trujillo Paul Weber, Daniel Jung Briar
Briar Cliff University

Native Americans of the prairie regions have employed the prairie turnip (Psoralea esculenta) as both a dietary staple and a medical remedy for generations. Previous work at this institution has demonstrated that extracts from the rind skin of this plant and a fraction isolated by preparative chromatography possess selective and potent antibacterial activity. Reported herein are the results of studies on the effect of light, heat and exposure to oxygen on extract stability. Also determined are features of chemical structure important for antibacterial activity of extracts.

GENERATION OF TAU OVER-EXPRESSING CELL LINES TO STUDY ABNORMAL MAPK SIGNALING IN NEURODEGENERATIVE TAUOPATHIES

Poster (Friday, April 20, 2018)

Elvis Castro, Hayley Lange, Jessy Huff, Ellie Freebern Destiny Boettger
Morningside College

Tau, a neuronal microtubule-associated protein, has been shown to enhance MAPK signaling through the ERK pathway. Over-activation of ERK and other MAPK pathways such as JNK and p38 in response to chronic inflammation can lead to significant neuronal atrophy and cell death in neurodegenerative diseases such as Alzheimer's disease. Since the role of tau in JNK and p38 activation due to chronic inflammation remains unclear, we aimed to further investigate tau's impact on these pathways downstream of inflammatory stressors such as Tumor Necrosis Factor Alpha (TNFα). We have previously shown that over-expression of the 3R isoform of human tau enhances TNFα -induced cell death, but the effects of 4R tau isoforms on MAPK signaling remains unclear. To better understand the impact of 4R tau isoforms, we have generated several 4R tau over-expressing neuronal cell lines to be used in future studies. Three clonal cell lines and one pooled polyclonal cell line were established and screened to verify the over-expression of 4R tau. TNFα -induced MAPK signaling will be compared in the 4R and 3R tau over-expressing cell lines. The findings from these experiments will increase our understanding of tau's interactions with the abnormal MAPK signaling that occurs in neurodegenerative diseases.

Domestication effects of tb1 gene (teosinte branched 1) on teosinte and maize

Poster (Friday, April 20, 2018)

Kimberly Hults, Destiny Einerwold, Noah Schmitt, James Hampton
Buena Vista University

Modern corn is derived from a Mesoamerican wild grass called teosinte and the result of selection for a surprisingly small number of mutant alleles. One of the most important genes in this domestication process was in alterations of the tb1 gene, present in both teosinte and maize. Previous research has found that the tb1 gene has effects on inflorescence sex and number and length of internodes in the lateral branches and inflorescences. Tb1 has major phenotypic effects in teosinte, but these are not seen in maize. This sequence comparison may help elucidate the unique role that the tb1 gene played in the domestication of the Heartland's most valuable crop. We will report our research on tb1 in teosinte and maize.

The Role of FEA3 in Cob Development in Teosinte

Poster (Friday, April 20, 2018)

Devin Wagenman, Rebecca Peters, Ryan Exline, James Hampton
Buena Vista University

Our research group investigates the arrangement of cells in the production of the ear of corn. as it is produce by organizing center in the apical meristem. In particular, we are looking at the role of the FEA 3 gene, which is involved in the proliferation of stem cells in the ear. We have compared the DNA sequence of this gene between teosinte, the earliest progenitor of corn, and modern maize. We will report the results of our investigation.

MICROTUBULE-ASSOCIATED PROTEIN TAU IS ABNORMALLY UPREGULATED IN EWING'S SARCOMA

Poster (Friday, April 20, 2018)

Christian Burford, Chad Leugers
Morningside College

Ewing's sarcoma is characterized by a chromosomal translocation involving the EWS gene and a member of the ETS family of transcription factors. This translocation causes a series of genes to be upregulated and downregulated as a result of abnormal transcription factor activity. Recent studies have shown one particular gene known as Microtubule-Associated Protein Tau (MAPT) has elevated mRNA expression levels in Ewing's sarcoma. However, no one has verified whether this increased mRNA expression corresponds with increased tau protein production. Tau has been reported to enhance abnormal cell signaling that can occur in cancer, and tau expression has also been correlated with resistance to certain chemotherapeutic drugs, such as taxols, in some breast cancers. Ewing's sarcoma treatment typically consists of non-taxol drugs, but the survival rate for metastatic cases is less than 30%. This poor prognosis highlights the need for new therapeutic drug targets, which may include tau. In order to confirm the upregulation of tau in Ewing's sarcoma, we have performed Western blots that demonstrate tau protein is present in these cancer cells. We have also found evidence of a possible cleaved tau isoform, which may increase the effects of tau on signaling pathways responsible for abnormal cell division.

Prairie turnip (Psoralea esculenta) extracts inhibit the growth of pathogenic bacteria; Streptococcus pneumonia and Enterobacter aerogenes.

Poster (Friday, April 20, 2018)

Abigail Furlich, Elizabeth Havenridge, Mishell Garcia, Tracietaylor Turner Ikram Omar, Grace Whitlaw, Nicole Wiles, Jacob Hindman, Paul Weber, Daniel Jung Briar Cliff University
Briar Cliff University

Research in this lab revealed anti-bacterial activity of the extracts from Psoralea esculenta. Bacterial survival test (percent survival of bacteria) showed the inhibition of bacterial growth on following pathogenic and non-pathogenic bacteria. Percent survival levels of tested bacteria were dose dependent. Furthermore, of the organisms studied, Mycobacterium and Staphylococcus are known invasive human pathogens while Pseudomonas and Klebsiella are opportunistic pathogens. The results showed that the survival rates of invasive pathogens are quite low (~10%), whereas those of opportunistic pathogen are considerably higher (35%).

To further investigate if this inhibition pattern is a general trend, we tested the effects of extracts on Streptococcus pneumoniae (invasive pathogen) and Enterobacter aerogenes (opportunistic pathogen). The results showed the unique pattern of growth inhibition.

Investigation of Lactococcus lactis chain length under varying growth conditions

Poster (Friday, April 20, 2018)

Leah Davenport, Rebecca Schmidt
Upper Iowa University

Lactococcus lactis is a well-studied bacterium used historically in the food industry as in cheese, yogurt, and fermented vegetables. Now Lactococcus lactis is an emerging model organism in health for expression, secretion of proteins, and metabolites in live vaccine delivery. Lactococcus lactis typically grows in streptococcal form, in chains of spherical cells. Depending on the application, variations in morphology could influence efficiency of bacterial behaviors such as attachment to surfaces in vitro and in vivo, or secretion and production of desired compounds. This study investigated two hypotheses regarding whether cell size and cell chain length varies depending on growth conditions. The first hypothesis predicts that Lactococcus lactis chain length is influenced by the environment and could therefore be manipulated based on the needs of the investigator. Alternatively, the chain length of Lactococcus lactis is genetically determined and therefore resistant to the influence of growth conditions. Preliminary findings indicate that there is a difference in the chain length between cultures grown under shaking vs still conditions. This suggests that researchers could alter growth conditions to influence morphology in Lactococcus sp. and possibly other organisms in future experiments.

Staphylococcus aureus Biofilm Formation

Poster (Friday, April 20, 2018)

Jordyn Ostrowski, Rebecca Schmidt
Upper Iowa University

Many bacteria can exist as surface-attached accumulations known as biofilms. Biofilms assemble from surface-associated microbial cells that are enclosed in an extracellular polymeric substance matrix secreted by the microbial community. Biofilms can lead to many infectious diseases and can play a role in medical device-related infections. This study examines the early stages of biofilm creation using static biofilm assays, measuring matrix production after initial bacterial adherence to the surface and microcolony formation. Biofilm production was assayed by crystal violet staining of the matrix, with readouts based on analysis of optical density and microscopy. Staphylococcus aureus, a significant biofilm-forming microbe in medical device-related infections, was the main bacterial strain of bacteria investigated and compared to Staphylococcus epidermidis and other species. Preliminary observations indicate that S. aureus produces more robust biofilm than S. epidermidis when both strains are inoculated using cultures at equivalent optical densities. These studies

Inhibition of Malic Enzyme Enhances Survival in a Fruit Fly model of Emery Dreifuss Muscular Dystrophy

Poster (Friday, April 20, 2018)

Hannah Apolinar, Maria Valdes, Lori Walrath, Gary Coombs
Waldorf University

Previous studies have shown that mutations in Drosophila Melanogaster Lamin C that are homologous to LMNA mutations found in human Emery Dreifuss muscular dystrophy (EDMD) patients impair larval locomotion and survival of pupae to adulthood. These mutant Lamin C variants also activate the CncC transcription factor homologous to human Nrf2. Activation of CncC leads to reductive stress in the body wall muscle cells of larvae. RNAi mediated knockdown of the enzymes glucose 6 phosphate dehydrogenase, 6 phosphogluconate dehydrogenase, and malic enzyme, which increase reducing equivalents in the cell, increases survival to adulthood, suggesting that the redox stress caused by expression of mutated Lamin C underlies organism level symptoms of muscular dystrophy. To further test this conclusion, we searched the literature for small molecule inhibitors of malic enzyme and found a report of inhibition of human maleate dehydrogenase 2 by pamoic acid with an IC50 of 1.4 ± 0.4 □M. We show here that low millimolar concentrations of pamoic acid significantly increase survival to adulthood of flies expressing muscular dystrophy associated mutant Lamin C at 22°C and 25°C. We also evaluated the effect of pamoic acid on larval locomotion, but the results are inconclusive.

Single Nucleotide Polymorphisms and Microsatellites in the Canine Glutathione S-transferase Pi 1 (GSTP1) Gene Promoter

Poster (Friday, April 20, 2018)

Sarah Mann, Anastasia Yablochkin, James Sacco
Drake University

Background: Genetic polymorphisms within the Glutathione S-transferase P1 (GSTP1) gene affect detoxification activity of the GSTP1 enzyme and may be associated with canine cancer. This study aimed to identify polymorphisms in the GSTP1 promoter of 278 purebred dogs and several canids, compare polymorphism prevalence between breed groups and select breeds, and predict their effects on gene expression. Results: Of the 15 single nucleotide polymorphisms (SNPs) and two microsatellites discovered, three were unique to dogs and three were unique to canids. The microsatellite located in the 5’ untranslated region (5’UTR) was a GCC tandem repeat, consisting of alleles ranging from 10 to 22-repeat units, with 16 and 17 repeat-alleles most commonly-seen. The microsatellites may arise from unequal recombination. Twenty-eight haplotypes were constructed in dogs and eight in canids. The most common haplotype was the wild-type *1A(17). In Siberian Huskies and Boxers, there was minimal diversity. The compound 16*2 allele may interfere with transcription factor binding and/or stability of the transcript. Conclusions: Dogs and other canids exhibit variation in the GSTP1 promoter. Distinct haplotypes were prevalent in certain breeds. Unequal crossing-over explains most of the microsatellites observed. Certain variants may affect gene expression and are currently being investigated via promoter characterization studies.

Toxicology studies of aluminum on the model organism, Caenorhabditis elegans, and the molecular analysis of expression changes in genes linked to breast cancer.

Poster (Friday, April 20, 2018)

Samantha Redmond, Kyleigh McLaughlin, Adam Hoffman, Rasika Mudalige-Jayawickrama Kelly Grussendorf,
University of Dubuque

Many deodorants that are commonly used contain aluminum, specifically aluminum chloride. Studies have shown that aluminum chloride could be linked to cases of breast cancer, altering levels of gene expression of various genes, as well as interfering with the function of estrogen receptors. To study the effect of aluminum and its link to breast cancer we used the model organism Caenorhabditis elegans. C. elegans serves as an ideal organism in this type of study due to ease of use, transparency, invariance, and uncomplicated manner of subjecting them to various elements. Lastly, homologous genes that have been linked to breast cancer in humans have been found to carry out similar cellular roles in C. elegans. We subjected C. elegans to various concentrations of aluminum chloride in a wild-type background and strains with the following genetic mutations: cep-1 (homolog p53), brc-1 (homolog BRCA1), and brc-2 (homolog BRCA2). After treatment, worms were analyzed for reproductive rates, behavioral change, as well as expression analysis of the genes of interest. Past and current studies show reproductive rates decrease as the concentration of aluminum chloride is increased. Lastly, current studies are addressing observed behavioral changes as well as initial testing and verification of expression levels of breast cancer linked genes.

Assay Development of the Innate IMD Pathway in Drosophila melanogaster

Poster (Friday, April 20, 2018)

Tara Hicks, William Jones
Upper Iowa University

The purpose of this project was to determine the kinetics of Drosophila melanogaster's IMD pathway. The D. melanogaster mutant 55707 has a modified diptericin gene, an antimicrobial peptide (AMP), containing a β-Galactosidase reporter gene (Dipt2.2-LacZ). Diptericin, and thus the β-Galactosidase protein, is induced by the IMD pathway in response to infection by gram negative bacteria. An assay for the β-Galactosidase activity was developed using the ortho-nitrophenyl-β-galactoside (ONPG) substrate. This ONPG assay was used to measure the expression of β-Galactosidase activity in E. coli infected 55707 D. melanogaster. At various times post infection, the infected flies were frozen then homogenized. The homogenate was cleared by centrifugation and the supernatant assayed for β-Galactosidase activity. An increase in β-Galactosidase activity was first detected at four hours after infection and increased thereafter. This system will allow for future studies testing factors affecting the innate immune system's response to various environmental factors or testing of in vivo activities of antibiotics.

ANALYZING THE EFFECTS OF ENDOCRINE DISRUPTOR EXPOSURE ON CD - 1 MICE

Poster (Friday, April 20, 2018)

Makky Mousa-Makky, Gerald Zuercher, Adam Hoffman, Kelly Grussendorf
University of Dubuque

Hormones are essential signaling molecules that are necessary for many cellular and organismal properties, particularly homeostatic processes. Regulation of these hormones is an intricate and necessary process that, unfortunately can be challenged by many external and environmental molecules. . Our study looked at a chemical compound, formononetin, which is believed to be endocrine disruptor, particularly affecting estrogen. Due to the structural similarities to estrogen, formononetin is believed to mimic estrogens effect and is considered a phytoestrogen (derived by plants). To study the effects of formononetin we subjected CD-1 mice to various concentrations of formononetin over a four week period. During and at the completion of the study, mice were analyzed by many different means. Of phenotypic traits that were observed, there were changes weight as well as changes in levels of food and water consumption. Current work is being carried out to determine the correlation of these phenotypic changes with other known mimicking molecules of estrogen. Also, as this was the first study of this type at the University of Dubuque, many experimental approaches and techniques have now been analyzed and hope to be incorporated into future studies of hormone disruptors at the University of Dubuque

Functional Characterization of Anthurium andraeanum DFR Gene in Petunia W80 mutant line

Poster (Friday, April 20, 2018)

Janaan Fink, Stephanie Pfab, Makky Mousa-Makky, Rasika Mudalige-Jayawickrama Teresita Amore
University of Dubuque

Anthocyanins are colored flavonoid glycosides which accumulate in vacuoles giving characteristic colors such as red, purple and blue to flowers, fruits and other specialized plant organs. The main objective of this study is to express dihydroflavonol 4-reductase (DFR) gene of Anthurium andraeanum in a petunia mutant line that lacks DFR enzyme. The DFR enzyme is one of the key enzymes that determine the color and the type of anthocyanin produced in different plants due to its substrate specificity. The full open reading frame of the Anthurium DFR gene was cloned into an expression vector, pORE-E2, under the CaMV 35S promoter and nopalene synthase terminator. The petunia W80 mutant was transformed with the pORE-E2-DFR plasmid via Agrobacterium-mediated method. We have selected 8 individual transformed lines and tested the expression of the transgene via reverse transcription-PCR (RT-PCR). Our results indicate 6/8 lines express DFR in leaves. Once the transformants produce flowers, we will determine the type of anthocyanin produced via thin layer chromatography. These results will help us to understand the substrate specificity of the Anthurium DFR enzyme and its potential value as a genetic tool in manipulating flower color in other floricultural commodities such as Dendrobium orchids, which lack red flowers.

Effects of ethinylestradiol and ethanol on corticotrophin-releasing factor-levels of Zebrafish

Poster (Friday, April 20, 2018)

Hailey Dollen, Devin Stane
Buena Vista University

The purpose of this experiment is to test the effects of 17-□ -ethinylestradiol (EE2) and ethanol on the amount of corticotrophin-releasing factor (CFR) levels present in Zebrafish brains and kidneys. The CRF system functions to maintain homeostasis during stress by regulating cortisol production via the hypothalamus-pituitary-inter-renal axis. To conduct this experiment, six fish were placed in seven tanks with one control tank, three tanks with different concentrations of EE2, and three tanks with different concentrations of ethanol. At five separate time periods, we sacrificed the fish to remove the brains and kidney, which allowed us to carry out a semi quantitative reverse transcription-polymerase chain reaction assay to assess gene expression and view the differences in CRF levels. We expected to see an increase in CRF levels from the controlled level the longer the fish were in their solution as well as an increase in CRF levels from the controlled level as the levels of EE2 and ethanol increased. This information would tell us that the effects of EE2 and ethanol would elicit the increased levels of corticotrophin- releasing factor in the Zebrafish brain and kidneys. This would be due to increased anxiety and stress caused by the EE2 and ethanol.

Understanding the function of SH3PXD2b by identifying interacting proteins using DEEPN: Dynamic Enrichment for Evaluation of Protein Networks.

Poster (Friday, April 20, 2018)

Zachariah Steffen, Whitney Christiansen, Tabitha Peterson, Robert Piper Alesia Hruska
Mount Mercy University

The SH3PXD2B protein is an important component of podosomes, actin-based membrane protrusions that help with adhesion to and degradation of the extracellular matrix. This protein is made of several domains: a PX domain, four SH3 domains, PXXP motifs and tyrosine phosphorylation sites. These domains can act as binding sites for protein-protein interactions. A one base pair deletion of the SH3PXD2b gene found in patients with Frank Ter Haar syndrome and inbreed nee mice leads to truncation of the protein in the third SH3 domain. Glaucoma amongst other symptoms develops due to this mutation. The focus of this research was to produce bait constructs to be used in a modified Yeast two-hybrid screen: DEEPN, Dynamic Enrichment for Evaluation of Protein Networks, to discover novel proteins that interact with SH3PXD2B. Various bait constructs were constructed including full length and individual wild type and mutant SH3 domains, using the pTEF-GBD plasmid by Gibson Assembly. Once baits are validated (testing for protein production, auto-activation, growth defects, and stringency requirements) multiple baits will be used in DEEPN allowing the evolution of interacting proteins in batch. Analysis of next-generation sequencing from these matings will ultimately identify novel proteins that interact with SH3PXD2B.

Heavy metal resistance and metal concentrations among glacial and riverine wetlands bacteria across Upper Midwest

Poster (Friday, April 20, 2018)

Matthew Nieland, Brittany Gill, Miyu Okada, Taylor Hixson Anni Moore,
Morningside College

The purpose of this study was to determine levels of heavy metal resistance of microbial communities in glacial and riverine wetlands in Iowa, South Dakota, and Minnesota. Soil/sediment samples were collected from permanent, temporary, and former wetlands (now agricultural land) from each site. Bacteria were extracted from the soil/sediment and grown in the presence of increasing concentrations of arsenic, cobalt, copper, mercury, zinc, determining the minimal inhibitory concentration (MIC) for each metal. The preliminary results show mercury and zinc resistance more variable in riverine wetlands than glacial wetlands. Agricultural soils (former wetlands) had higher mercury resistance (up to 250 µg/ml $HgCl_2$) than permanent wetland soil (up to 50 µg/ml $HgCl_2$) among both glacial and riverine wetlands. Arsenic, copper, and zinc resistance was relatively even across the permanent, temporary, and former wetland soils in all wetlands (600 µg/ml Na_3AsO_4, 500 µg/ml $ZnSO_4$, 500 µg/ml $CuSO_4$). Cobalt resistance was low (2 mM $CoCl_2$), and there appears to be no significant difference between the sites.

Using PCR to Improve the Chances of Identifying a Novel SEA-PHAGE

Poster (Friday, April 20, 2018)

Elizabeth Heeg, Perkins Aiyegbeni, Madison Beaver, Rebecca Gritters Peter Hollinger, Inge, Katie Marie Nwaelugo, Nnenna Stephanie Powell, Hannah Elizabeth Preston, Peace Antonia Pun, Renju Sevcik, Kristina Marie Sitzmann, Haley Lynn Van Den Berg, Shelby Elizabeth Van Der Werff, Brenden Scott Van Riesen, Michaela Rae
Northwestern College

We will present a diagnostic protocol to utilize in order to increase the likelihood of choosing a novel SEA-PHAGE to sequence and characterize. The HHMI SEA-PHAGES project at Northwestern College involves culturing bacteriophages and deciding which isolated phage a student should continue working with. Ideally, a student will culture, purify and analyze an undocumented phage in order to contribute novel data to the growing SEA-PHAGES database. By designing primers to annotated SEA-PHAGES we were able to explore the feasibility of a PCR diagnostic step to help students identify which viruses they should select to further characterize.

Role of Efg1 in management of glycogen storage and starvation in Candida albicans

Oral (Saturday, April 21, 2018)

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Candida albicans is a dimorphic fungus that is common to the human digestive tract. In response to host factors like temperature, CO2 or serum, the fungus transitions from the commensal yeast form to a virulent hyphal morphology. In its virulent form, C. albicans can establish painful infections of the mouth and the vagina that can progress to life-threatening systemic infections in immunocompromised patients. The present study examines the effects of a key C. albicans virulence factor, Efg1, on the fungus' ability to store carbohydrates and to survive periods of starvation. Efg1 is a transcription factor that effects broad morphological and metabolic adaptations in response to protein kinase A signaling. We found that mutants lacking Efg1 have low carbohydrate (glycogen) stores and do not maintain viability on solid media well. These findings implicate Efg1 in control of glycogen synthesis and suggest that the reduced virulence of an efg1 mutant might be due to poor management of carbohydrate storage. The results suggest that glycogen synthesis is a virulence factor for C. albicans as it allows the pathogen to persist through periods of starvation within the host.

EFFICACY OF DIFFERENT PLANT TRANSFORMATION METHODS IN DELIVERING TRANSGENES INTO DENDROBIUM ORCHIDS

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The ability to introduce new genes that precisely target a single biosynthetic pathway for modification of nutritional quality, flower color, vase life or productivity is an important breakthrough in understanding and manipulating gene function. Genetic transformation by adding a single gene has already facilitated functional genomics, discovery of new gene functions through complementation of mutants, production of knockout mutants to study gene functions and opened the explosive new field of functional RNA and gene regulation. We have isolated many commercially important anthocyanin biosynthetic genes that can potentially be used to create novel colors for the orchid cut-flower industry. Our objective is to find an efficient, inexpensive method to introduce these genes into orchid protocorms (undifferentiated seedlings). We have tested biolistic bombardment (gene gun), Agrobacterium-mediated plant transformation, and silicon carbide (SiC) whisker mediated transformation in delivering β-glucuronidase (GUS) reporter gene and antibiotic selectable marker gene into Dendrobium orchids. We successfully produced transformed seedlings through biolistic bombardment and Agrobacterium-mediated transformation. However, SiC whisker method alone has not been successful in delivering the transgene into orchid protocorms. We are currently testing the combination of SiC whiskers and Agrobacterium-transformation in delivering the transgene into orchids. Comparison of all the methods used and their success rate will be presented.