CURE Symposium

undergraduate discovery science

December 3, 2016 • University of Colorado • Boulder, CO

ABSTRACTS

Participating Labs

1161 – From Dirt to DNA: Phage Genomics Laboratory
1171 - The Discovery Lab
3140 Cell Biology Laboratory
4100 - CRISPR Mutagenesis in *Xenopus*
4202 – The Python Project

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Schedule of Events:

9:00 am – 10:00 am Student speakers

9:00 am Welcome
9:05 am Phage Minime, a new Mycobacteriophage, suspected to be in Cluster C1
Matthew Bertelson & Chad Brokaw
9:15 am Structure-based prediction of novel antibiotic targets
Rachel Anderson
9:25 am Mutated oral groove may enhance phagocytic rate in NP1 Tetrahymena
Kimberly Lugo, Jessica Miller, Phil Rubin
9:35 am CRISPR+CAS9 mutagenesis of tumor protein p63 regulated 1-like
Julia Mo
9:45 am HSD3B7 shown to potentially mediate cholesterol homeostasis in the Burmese python
Stuart Sommers

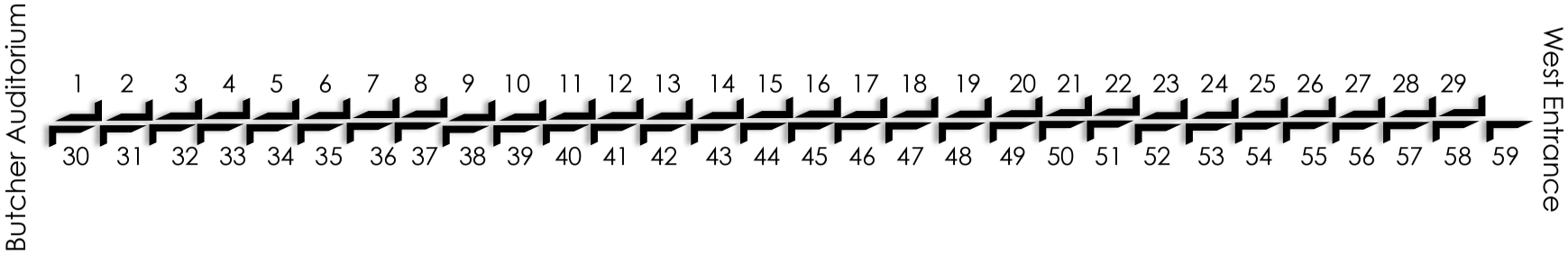
10:00 am – 10:45 am Poster Session I

10:45 am – 11:30 am Poster Session II

Table of Contents:

Poster Map

Posters can be viewed in the Butcher Atrium and Gallery of the Jennie Smoly Caruthers Building from 9:00 am to 11:30 am. Abstract numbers are indicated on the posters.



Poster Session I

1 P1	16 D8	31 D23	46 C15
2 P2	17 D9	32 C1	47 C16
3 P3	18 D10	33 C2	48 C17
4 P4	19 D11	34 C3	49 C18
5 P5	20 D12	35 C4	50 M1
6 P6	21 D13	36 C5	51 M2
7 P7	22 D14	37 C6	52 PY1
8 P8	23 D15	38 C7	53 PY2
9 D1	24 D16	39 C8	54 PY3
10 D2	25 D17	40 C9	55 PY4
11 D3	26 D18	41 C10	56 PY5
12 D4	27 D19	42 C11	57 PY6
13 D5	28 D20	43 C12	58 PY7
14 D6	29 D21	44 C13	59 PY8
15 D7	30 D22	45 C14	

Poster Session II

1 P9	16 D31	31 C19	46 C34
2 P10	17 D32	32 C20	47 C35
3 P11	18 D33	33 C21	48 C36
4 P12	19 D34	34 C22	49 M3
5 P13	20 D35	35 C23	50 M4
6 P14	21 D36	36 C24	51 M5
7 P15	22 D37	37 C25	52 PY9
8 P16	23 D38	38 C26	53 PY10
9 D24	24 D39	39 C27	54 PY11
10 D25	25 D40	40 C28	55 PY12
11 D26	26 D41	41 C29	56 PY13
12 D27	27 D42	42 C30	57 PY14
13 D28	28 D43	43 C31	58 PY15
14 D29	29 D44	44 C32	59 PY16
15 D30	30 D45	45 C33	

P1

EXPERIMENTAL RESULTS SUGGEST BACTERIOPHAGES WITH SIPHOVIRIDAE MORPHOLOGIES, BUT TWO DIFFERENT LIFECYCLES

Austin Hammermeister Suger, Avery Langley, Dylan Gessner, Ian McAdams

Bacteriophages are categorized into either lytic or temperate lifestyles. There are key characteristics which tend to suggest which how a specific phage should be categorized. Our groups performed a series of experiments which gave us information about our two unique phages. There are still some uncertainties about all the factors that impact the which lifecycle a temperate phage will enter. By connecting the information we have about our phages lifecycles to information from other experiments, we may be able to gain a better understanding of factors that impact bacteriophage behavior. Our two groups isolated two Mycobacteriophages with similar EM morphologies and very different plaque morphologies. Better understanding of bacteriophage behavior could be crucial to advancing the medical applications of bacteriophages to combat bacterial infections. Bacterial infections are becoming ever more problematic as the rate of antibiotic resistance in bacteria increases, but bacteriophages have the potential to change this.

P2

BACTERIOPHAGES CAPTAINHOOK AND HANNIBAL WERE FOUND TO BE LYTIC AND IN THE SIPHOVIRIDAE FAMILY

Nadine Salvador, Hanna Lavassani, Jessica Granados, Maile Anthony

Both phages, Hannibal and CaptainHook, have similarities by being lytic phages due to clear plaques performed in the Titer Assay experiment, as well as both phages being a part of the siphoviridae family when shown under an electron microscope. Hannibal still has a way to go in terms of determining its Cluster phage, and CaptainHook confirming it being a part of the Q Cluster family. Our hypothesis of both of these phages is that they both will have similar characteristics to determine and authenticate a PCR cluster, as well as further validate their lytic and siphoviridae counterparts. In order to do this, our methodology must consist of repetition of certain experiments in order to confirm our hypothesis, and follow specific guidelines within the Phage Genomics Lab Manual (Fillman, 92). Our results will signify the understanding of further developing research into antibiotics and antibiotic resistance.

P3

ISOLATIONS OF, TINYHIPPO AND BUBBLEWRAP, SIPHOVIRIDAE TEMPERATE BACTERIOPHAGES

Kayla Albo, Jillian Blumberg, Abraham Alcazar, Shrihari Kote, Arthur Bremner

Bacteriophages can either undergo the lysogenic or lytic lifecycle. Lysogenic bacteriophages incorporate their DNA into the host chromosome. Lysogenic phages produce plates that have cloudy or turbid plaques, this means that those plaques represent lysogens or bacteria containing prophages. Meanwhile, phages that have entered the lytic cycle produce clear plaques because all of the bacteria has been lysed. Bacteriophages are important for the field of medicine because of their ability to kill antibiotic resistant bacteria.

P4

NEWLY ISOLATED LYTIC AND SIPHOVIRIDAE MAKAYLAFOND AND LYTIC DREVIL

Alexa Esler, Will Gibbons, Brittney Lafond, Makaylah Waddle

The study of bacteriophage has become widespread as the idea of phage therapy has grown in popularity. Phage therapy utilizes bacteriophage to infect and kill antibiotic resistant bacteria. Bacteriophage are viruses that coevolve with their host bacteria to infect and eventually lyse the cell or integrate into the host bacteria (lysogenic). The bacteriophages, MakayLafond and DrEvil, were uncharacterized phages that were isolated from a soil sample. Through a variety of experiments, enrichment, plaque streak for purification, and other experiments vital information was able to be discovered about both phages. MakayLafond was isolated and was found to have lytic qualities. The restriction digest suggests that MakayLafond could be a Cluster B phage. In addition, an electron microscope photo of MakayLafond indicates that it most likely belongs in the Siphoviridae bacteriophage family due to the long, flexible tail. Experimentation with DrEvil indicated that this phage exhibits lytic qualities. The experimentation performed helps add to the growing knowledge of phage research,

and identify potential phages that can be used for therapies to fight bacterial infections. This is due to the phages being able to infect *Mycobacterium smegmatis* which is similar in nature to *Mycobacterium tuberculosis*.

P5

NOVEL MYCOBACTERIOPHAGES INFECTING *M. SMEGMATIS* ISOLATED FROM SOIL SAMPLES

Moses Bravo, Christopher Sharkey, Travis Arnold, Aidan Barker, Valentina Pena, Colton Paterson

The population of bacteriophages is large and highly genetically diverse. Limited genomic data exists. Isolation and characterization of novel phage species can contribute to expansion of data for understanding patterns in phage evolution and genetic architecture. Here, we aimed to characterize three novel mycobacteriophages isolated from Boulder area soil samples. The phages were grown in cultures of *Mycobacterium smegmatis* and purified through plaque assays. Plaque morphologies indicated lytic and lysogenic lifecycles. Electron micrographs (EM) showed a common *Siphoviridae* morphotype. In restriction digest analysis of the three phages, one had visible DNA fragmentations.

P6

ISOLATION AND CHARACTERIZATION OF TWO NOVEL SIPHOVIRIDAE MYCOBACTERIOPHAGES: MICKJU & SPIKEWAVE

Nick Kolesky, Megan Strimbu, Hanju Kim, Jeremy Taylor, Candace Alai

A large portion of the microbial genomic ecosystem is comprised of bacteriophage genomes, of which mycobacteriophage constitute a portion of. Due to evolutionary mechanisms, a wide array of mycobacteriophage genomic sequences have yet to be discovered. Therefore, discovering these genomic sequences could lead to a greater understanding of the symbiotic relationship between bacteria and phage. Additionally, new biotechnologies and medical treatments could be developed (especially in the case of combating anti-biotic resistant strains of bacteria). We found that wet soil samples collected from the creek bank of Boulder Creek, Boulder, Colorado contained new Siphoviridae lysogenic & lytic mycobacteriophages, Spikewave & Mickju.

P7

TEMPERATE PHAGES TINYTEMPER AND RABBLEROUSER ISOLATED ON THE UNIVERSITY OF COLORADO BOULDER CAMPUS

Alia Alsaif, Madison Pinkard, Siena Rigatuso, Taylor Stinson

Many different types of phages have been discovered and archived from the area around the University of Colorado Boulder campus, showing the diversity of the viruses. In our experiment, our most significant findings were of the unique, temperate bacteriophages, TinyTemper and RabbleRouser that infect *M. smegmatis* in our isolation of phage from a soil sample around the campus. The plaque morphologies consist of circular shaped plaques approximately 2 mm-3 mm in diameter that are completely transparent in the center with cloudy rings. Due to their turbidity, the phages are likely temperate phages that entered the lysogenic life cycle, thus, capable of lysing and killing bacteria cells after genome replication and specific conditions are met. The finding of these types of bacteriophages is important because it can be studied and tested for the infection and lysis of *M. tuberculosis* cells. It can also play a role in fighting disease, replacing antibiotics with bacteriophage. Not many types of phages have been identified as compared to the whole population. As new and unique phages are discovered, they can be studied and tested in their ability to counteract disease either through lytic or lysogenic means. Since we were unable to characterize our bacteriophages, the identities are unknown and therefore, we cannot identify their cluster. However, there are clusters our bacteriophages can likely belong to due to their morphology and its ability to infect *M. smegmatis*. The possible clusters that our temperate phages would identify with would be cluster A and cluster K.

P8

DISCOVERY OF SIPHOVIRIDAE LYTIC AND LYSOGENIC PHAGES MADVIN AND VOTEX COLTANASA

Maddy Batiz, Kevin Ziznewski, Manasa Ponnapalli, Colton Slaughter

Bacteriophages (phages) are a type of virus that infect bacteria and common applications include treating antibiotic resistant diseases with them. Phage

therapy is possible due to the fact that lytic phage disrupt bacterial metabolism and force bacterium to lyse. Phage are coevolving with bacteria, which means that they can never develop a resistance to phage. It also means that they are very specific to the bacteria which they infect, to ensure that they only kill harmful bacteria. While phages therapy may become a possible future alternative to antibiotics, we still currently know too little about bacteriophages. Phage are typically thought to be harmless to human cells, but it is still unknown how phages interact with human cells, leaving scientists to wonder how they affect external factors such as the immune system.

Phages can also be characterized through their morphology, allowing scientists to help predict the cluster of the phage. The aim of this experiment was to add to the bacteriophage database by finding and characterizing different types of phage. Specifically, we found, isolated, and characterized a phage from soil using *Mycobacterium smegmatis* as a host bacteria. We discovered two phages, Votex Coltanasa and Madvin, temperate and lytic respectively, both of which possess the siphoviridae morphology.

P9

A COMPARATIVE ANALYSIS OF LYTIC AND TEMPERATE PHAGE EXHIBITING SIPHOVIRIDAE MORPHOLOGY UPON ELECTRON MICROSCOPY

Jordan Affinati, Shaan Sharma, Johna Thaut, Suchita Lulla, Sophia Reyes

Bacteriophages are viruses that can infect bacteria. They are known to inhabit a myriad of environments, and are specific to the species of bacteria they infect. In this study, we sought to detect, isolate, and characterize two separate species of phage exhibiting unique plaque morphologies using the host *Mycobacterium smegmatis (M. smeg)*. This particular strain of bacteria was the ideal host since, in addition to being nonpathogenic, many viruses known to infect *M. smegmatis* have been known to infect pathogenic bacteria such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* (Gordon and Smith, 1953). Two separate soil samples were collected and enriched before subsequent purification. The phage lysate of both samples was streaked for further purification before a titer assay was performed to determine the phage's concentration. Interestingly, each sample yielded distinct plaque morphology. The first sample exhibited turbid plaques indicative of temperate phage while the plaque's of the second sample were clear and representative of lytic phage. Despite distinct plaque morphologies and size, an electron micrograph revealed Siphoviridae morphology in both samples. Further characterization of these unique phages will be pursued in the future to adequately place both species in their appropriate clusters. Through the isolation and comparison of two new species of phage, we are contributing to the known database of bacteriophages; this contribution could be used to aid future research in phage therapy.

P10

DISCOVERY AND CLASSIFICATION OF BACTERIOPHAGE B0WS3R AND WANDERER

Kayla Boyd, Erin Burgmaier, Deborah Tesema, Kerrie MacMillan

There are currently 129 classified and documented clusters and subclusters of mycobacteriophages. These series of experiments isolate mycobacteriophages from soil samples in order to add phages to this database, and assign them to a cluster. The following discusses two phages that were isolated: Wanderer and B0WS3R. Wanderer was classified as a C cluster phage, and B0WS3R remains an unclassified, possibly singleton.

P11

PHAGES POTUS AND MADMAXIMUS EXHIBIT SIMILAR CHARACTERISTICS IN TERMS OF CLUSTER, MORPHOLOGY, AND RESTRICTION DIGEST

Erin Char, Kathryn Hay, Jessica Rea, and Angela Shah

Bacteriophages have the potential to change the way antibiotic resistant bacterial diseases are treated. Phages MadMaximus and POTUS were isolated, and their appearance, plaque morphology, and DNA were characterized. Data were obtained using electron microscopy, titer assay, and restriction digestion. We hypothesize MadMaximus is an A2 or A6 cluster phage based on its lytic plaque morphology, siphoviridae characteristics, and restriction digestion pattern. While POTUS had similar characteristics, restriction digest results were inconclusive. Bacteriophage research furthers our understanding of the diversity of viral genes and how they can be used to infect multiple hosts. Due to similarities between *Mycobacterium smegmatis (M. smeg)* and *M. tuberculosis,* classification can assist in the medical treatment of diseases and advance phage therapy.

P12

ISOLATING PHAGE: PHANGEL, A B CLUSTER PHAGE, AND CHARACTERIZING HARSHBUTFAIR

Katelyn Skeen, Logan Schwanz, Greyson Contag

We isolated and characterized bacteriophages from soil samples and determined their concentration and the characteristics of their genome. We isolated two mycobacteriophages, Phangel and HarshButFair. Phangel is a small, lysogenic phage with a long tails and turbid plaques. Restriction digest gave us the strong evidence that Phangel is in the B cluster. In the isolation of HarshButFair we discovered that there were two phage in the lysate. We then attempted to isolate a singular phage from the lysate. As phage usage increases and the field expands, new frontiers in phage treatment of diseases as a replacement for antibiotics and food purification more possible. By isolating phage, scientists are developing a better understanding of phage biology.

P13

CLASSIFICATION OF TEMPERATE A-CLUSTER PHAGE WITH A HIGH RATE OF INFECTION USING *M. SMEGMATIS*

Nathan Do, Gavin Chiem, Jack Johnson, Valentine Miller

The number of known mycobacteriophages has exponentially grown since 2009, and there are still thousands of unknown phages waiting to be classified. A phage was isolated from a soil sample, and plated on a petri dish. The plaques produced were hazy and halo like, indicating the phage was temperate. Its long tail and proportional head size indicated a siphoviridae morphology. The phage DNA digestion revealed a pattern which classified it as an A-Cluster phage.

P14

CLAETUS IS A LYTIC MYCOBACTERIOPHAGE IN THE SIPHOVIRIDAE FAMILY

Samuel Ahoor, Olivia Amster, Jinny Jin, Andrew Folta

The goal of this experiment is to identify characteristics of bacteriophages that are capable of infecting *Mycobacterium smegmatis*. Claetus, the bacteriophage that we isolated, is predicted be an A cluster phage because it is the most common phage cluster and because Claetus has a similar morphology to other A cluster phages. Claetus was isolated through enrichment. The phage was then purified to ensure that it was not contaminated with a different phage. A titer assay was performed to create and determine the strength of Claetus's high titer lysate. Its DNA was isolated then digested by various restriction enzymes, and a gel electrophoresis was performed to visualize the resulting cut genome.

P15

MOSTLYHARMLESS AND MUFFIN CONCLUDED TO BE CLUSTER K AND CLUSTER A PHAGES

Michaela Deck, Harrison Puscher, Valerie Olsen, Bernadine Hammond

The application of phage therapy to fight antibiotic resistant bacteria is a potentially life-saving technique. By discovering and categorizing the new phages MostlyHarmless and Muffin, we have the possibility to contribute to this work. In this series of experiments, we isolated phage DNA, ran gel electrophoresis with enzymes, and observed high titer assay plates. The phages have been concluded to be in cluster K and cluster A respectively. They are also both lytic and Siphoviridae**.** A property of their clusters is the ability to infect *Mycobacterium tuberculosis*, and therefore could contribute to ongoing research concerning the cure of this increasingly antibiotic resistant disease (Pope, 2011).

P16

ISOLATION OF TEMPERATE SIPHOVERDE BACTERIOPHAGE; WALTER WHITE

Nate Hammel, Lauren Shepard, Samuel Haman, Kenzie Atkin

Mycobacteriophage bind to very specific strains of bacteria and undergo lytic and lysogenic life cycles. Bacterial viruses play a very important part in a wide variety of diseases. In this experiment, mycobacteriophage were isolated and purified using *Mycobacterium smegmatis as a model organism*. The bacteriophage were examined under an electron microscope. DNA was extracted from the phages to provide further information for its classification. During isolation, there was an extraordinary amount of phage with long, straight tails ~200 nm in length and the heads were ~60 nm in diameter. The phage demonstrated characteristics similar to that of

the Siphoviridae family because of its nonenveloped head and a long noncontractile tail.

D1

STRUCTURALLY SIMILAR COMPOUNDS INHIBIT GROWTH OF *SALMONELLA* IN A REVERSE DOSE-DEPENDENT MANNER

Emma E. Burt

Typhoid fever, caused by a systemic salmonella infection, is becoming increasingly dangerous. Because of the rise of antibiotic resistance, deaths due to typhoid fever are predicted to outnumber deaths due to cancer by the year 2050 if new antibiotics are not discovered. Research is being conducted using the strain *Salmonella Typhimirium*, a typhoid fever causing strain in rats, allowing us to study the effects of novel antibiotics in a systemic typhoid fever model.

In this study, we observed the effects of the Diversity Set V. This is a set of compounds that have yet to be tested on *Salmonella Typhimirium*. 17 out of 1,590 compounds were discovered to be lethal in more than one trial. Drug 132-6 was lethal in two of three trials and was determined to be a candidate for antibiotic research. The chemical structure of this drug was compared to compounds in the mechanistic set. Two compounds from the set were determined to be structurally comparable to 132-6. These were tested for antibiotic properties and after 20 hours of incubation were shown to promote growth in doses greater than 12.5 μM concentration and inhibit growth in a bacteriostatic manner for doses smaller than and including 12.5 μM.

Mechanistic set drugs 78365 and 166464 showed antibiotic properties and will be sent to the Detweiler lab for further testing. The compounds may prove promising for use in antibiotics. They will be altered and studied *in vivo* to study toxicity and determine the possible viability of use in human trials.

D2

USE OF *SALMONELLA* FOR DISCOVERY OF NEW ANTIBIOTICS

Jordan Shapiro

Salmonella bacteria are the cause of the illness that is of question in this experiment. We tested salmonella's response to certain drugs that were determined as 'hits' in order to try to lessen the gap of the rising rate of antibiotic resistance. Typhoid fever is a potentially life-threatening illness caused by infection by *Salmonella* bacteria. This disease is easily treated with antibiotic medicines, but as people continue to misuse and overprescribe antibiotics, these bacteria are becoming resistant to these medications.

During this experiment the mechanisms of antibiotics were observed as we searched for possible new drugs. Throughout this experiment we tested drugs in a diversity set and determined the compounds that showed to inhibit the growth of bacteria as hits.

After doing a verification set and observing contradictory data, we searched for different drugs from the diversity set to choose to test. The three drugs that were chosen had been hits in the diversity set, yet as I had observed the data from the plates made twice, the dosing series demonstrated that overall these drugs did not inhibit the growth of the salmonella. Based on these findings the data will be forwarded to the Detweiler Lab, who may choose not to pursue this compound in future studies due to its ineffectiveness in our lab or continue in an attempt to improve.

D3

INHIBITING GROWTH OF *SALMONELLA* IN A DOSING SERIES FOR DISCOVERY OF NOVEL ANTIBIOTICS

Micah Salazar

Multi drug resistant bacteria are a severe threat to public health. There are 18 drug resistant strains of bacteria recognized by the Centers for Disease Control and Prevention in the United States alone. Due to rapid and less regulated DNA replication, bacteria will develop drug resistance by random chance. Due to lack of antibiotic research, most bacteria will soon develop resistance to all antibiotics and their mechanisms and infection could mean a death sentence.

In this study, we tested the growth of *Salmonella typhimurium* in response to a variety of compounds in the Drug Diversity Set V made available by the National Cancer Institute Developmental Therapeutics. Compounds were tested in M9 media to mimic a nutrient poor environment similar to mammalian physiological conditions. Of all the compounds examined, 37 were found to have inhibited bacterial growth while 20 were found to have enhanced bacterial growth. One compound examined, 144-7, was found to have inhibited bacterial growth at 25μM and 12.5μM concentrations dissolved in DMSO solution. Another compound examined, 59-6, was found to have enhanced bacterial growth. 59-6 is under currently

pharmaceutical investigation to be used as a hypertension medication.

The data gathered from this study suggests that 144-7 is a candidate for further examination in lab mice and further preclinical investigation. Compound 144-7 will be sent to the D. lab who will investigate its medicinal chemistry, in vivo applications, and needed alterations to the compound to reduce toxicity, improve molecular function after metabolism, and lengthen or shorten its biological half life.

D4

HYDROXYUREA AND IFOSAMIDE PROMOTE THE GROWTH OF *SALMONELLA TYPHIMURIUM*

Avery Bell, Emily Vogt

Salmonella can cause a sickness called typhoid fever, which could be life threatening. Since *Salmonella* is becoming antibiotic-resistant there is a huge need for new antibiotics. To approach this problem, we used the novel methods of the Detweiler Lab to test a library of oncology drugs from the National Institute of Cancer to see whether those Food and Drug Administration (FDA)-approved drugs would also work as an antibiotic to treat *Salmonella*. The library was tested next to a control of Ampicillin to determine which drugs had an effect on the growth. Once the library was tested, we identified two drugs that increased the growth. We identified Hydroxyurea and Ifosfamide, which increased growth. With Hydroxyurea, we performed multiple experiments such as dosing series to see that at higher doses it promoted the growth of *Salmonella* and a timing series to see how time changed the efficacy of the drug. Then with Ifosfamide we did a dosing series to see that at high concentration growth was increased but then took it further by looking at the mechanistic set to pick out five other compounds that had a similar structure to see that those had the same effect as Ifosfamide. This work is significant because cancer patients already are more susceptible to getting sick due to how Chemotherapy compromises the immune system and are unable to fight it therefore if they caught *salmonella* and the drug they were taking increased growth that could have a life-threatening effect on the patient.

D5

THE EFFECT OF METHYL 5-FLUORO-4-METHOXY-2,6-DIOXO-1,3-DIAZINANE-5-CARBOXLATE AND 5-HYDROXYIMINO-2,3,5-ITRPHENYLPENTANENTRILE ON *SALMONELLA* GROWTH

Anthony Partrick

Salmonella is a typhoid-causing bacterium that thrives within the intestines, macrophages, and blood of organisms. It is a systemic infection that expresses severe and deadly symptoms throughout the human body, such as extreme headaches and diarrhea that can lead to intestinal rupture. If not treated appropriately, the *Salmonella* infection can potentially result in the death of its host. Antibiotics, like ampicillin, can be used to successfully treat *Salmonella* infections, but the typhoid-causing bacteria have become more and more resistant to certain antibiotics over time.

During this experiment, we used *Salmonella typhimurium* to observe how the 1,700 compounds from the Diversity Set V affected the growth of the bacteria. The *Salmonella* were tested in M9 media because its chemical properties are similar to the conditions present within a macrophage. Methyl 5-fluoro-4-methoxy-2,6-dioxo-1,3-diazinane-5-carboxylate and 5-hydroxyimino-2,3,5-triphenylpentanenitrile, abbreviated as methyl 5 and 5-hydroxy, imitated the behavior of ampicillin because they seemed to inhibit the preliminary growth of *Salmonella*. Dosing series confirmed that both of the compounds remained effective at most administered concentrations, while the timing series demonstrated that both compounds keep the bacteria stable after 48 hours of incubation.

These findings suggest that methyl 5 and 5-hydroxy may be novel compounds that can be used to treat *Salmonella* infection. This information will be sent to the Detweiler Lab, which may choose to experiment with the compounds by using mice to further determine the effectiveness, side effects, toxicity, and metabolism rate of the compounds during *in vivo* trials.

D6

INVESTIGATION OF ANTI-CANCER DRUGS TO INHIBIT *SALMONELLA* GROWTH

Mason Valdez

Typhoid fever is endemic in tropical countries, but specifically in south-central Asia and south-east Asia. Antimicrobial resistant strains of *Salmonella* first appeared towards the end of the 20th century and still have yet to be resolved. With 81% of Typhoid fever cases in the United States attributed to foreign travel, resolving the issue of antimicrobial resistant strains on

a worldwide scale will help resolve the issue back at home.

Over the course of the semester we worked on testing many compounds to see if they inhibited the growth of *Salmonella typhimurium* in an M9 media. The M9 media was representative of accurate cell-like conditions. It was decided to study the growth of the *Salmonella* when exposed to two individual compounds used to treat cancer, Floxuridine and Azacitidine. In the experiment the compounds' effectiveness was tested in multiple dilutions. This experiment will hopefully reveal (1) if the compounds effectively inhibit the growth of the *Salmonella* and (2) which dilutions are the most effective.

D7

(5Z)-5-HYDROYIMINO-2,3,5-TRIPHENYLPENTANENITRILE INHIBITS GROWTH OF *SALMONELLA* IN A DOSE-INDEPENDENT MANNER

Kyle Iezzi

Typhoid fever is a life-threatening acute illness caused by infection by *Salmonella* bacteria. Typhoid fever, an infection affecting the entire body, causes dangerously high fever, gastrointestinal distress including severe diarrhea, and headache that progresses to systemic toxicity, intestinal rupture, and death in approximately 20% of patients. Typhoid fever can be treated with prompt antibiotic treatment, however, increasingly, typhoid-causing *Salmonella* are developing resistance to these treatments.

In this study, we observed the responses of *Salmonella typhimurium* to 1,590 compounds in Diversity Set V available from the National Cancer Institute Developmental Therapeutic Program. Thirty-seven compounds were found to inhibit growth and twenty compounds were found to promote growth of the bacteria in M9 media, which mimics conditions in which *Salmonella typhimurium* replicate *in vivo*. One compound, *(5Z)-5-hydroxyimino-2,3,5-triphenylpentanenitrile*, was chosen as a promising antibacterial candidate due to its preliminary inhibition of growth in a manner similar to ampicillin, an effective treatment for typhoid fever in humans. A dosing series demonstrated that (5Z)-5-hydroxyimino-2,3,5-triphenylpentanenitrile was effective in killing or preventing growth of *Salmonella typhimurium* at all doses further confirming its effectiveness.

These data support the preliminary conclusion that *(5Z)-5-hydroxyimino-2,3,5-triphenylpentanenitrile* is a promising candidate compound requiring further study. This data will be forwarded to the Detweiler Lab, who may pursue this compound in future studies including measuring effectiveness *in vivo* and initiation of medicinal chemistry experiments to alter the structure of the compound in an attempt

D8

FLUOROURACIL INHIBITS GROWTH OF *SALMONELLA* IN A DOSE-INDEPENDENT MANNER

Pranathi Durgempudi, Lauren Lamb, Emma Russell

Salmonella typhi is bacteria found in food and water that causes infections when digested. In some cases, the infection can lead to Typhoid fever, which is fatal. Antibiotic resistance is on the rise so our goal for this research was to find new drugs that could possibly be used as antibiotics for *Salmonella*. We plated 90 microliters of bacteria with 10 microliters of the drug we were testing in a well and see if after 24 hours there would be any growth. Fluorouracil is a chemotherapy drug that inhibits the growth of cells. Using the method above it was a "hit" and caused no growth of the bacteria. However, fluorouracil is toxic, though it has been approved by the Food and Drug Administration (FDA), so we also tested a dosing series to see if low amounts of fluorouracil would still have antibiotic properties with the salmonella. We tested concentrations of the drug mixed with water at 25, 12.5, 6.25, 3.125, 1.625, and 0.812 uM. Even at low doses, fluorouracil still kills *Salmonella typhi*. The significance of our work is that we found a possible new antibiotic that has already been approved by the FDA. This can be used when antibiotic resistance gets extremely high and results in death in those infected. It's also rewarding to see the drug kill the bacteria at even low doses so we have a chance of getting fluorouracil to a dose where is won't cause significant side effects in its patients.

D9

THE EFFECT OF 4-(2-THIAZOLYLAZO) RESORCINOL (TLR) ON *SALMONELLA* TYPHIMURIUM GROWTH

Kristi Kuehneman

Typhoid fever is a bacterial infection caused by *Salmonella Typhi* bacteria. Typhoid fever is a serious public health concern in developing countries because access to clean water is limited and sanitation is poor. There are several antibiotics that are effective for curing typhoid fever by inhibiting the growth of

Salmonella. However, there is an increase in antibiotic resistant bacteria. Therefore, certain strains of *Salmonella Typhi* do not have a cure.

We tested a total of 1,590 compounds. There was a 2.1% success rate giving us a total of 33 compounds that were considered "hits." We saw that 21 compound's absorbency was minus two standard deviations from the mean of DMSO. We also saw 12 drugs that were "hits," however, they were plus two standard deviations from the mean, which leads us to believe that those compounds promoted growth of *Salmonella*. Testing a dosing series on *Salmonella* exposed to 4-(2-Thiazolylazo) resorcinol (TLR) was able to show us the minimum concentration of TLR needed to inhibit the growth of *Salmonella*. After the fourth dilution (3.125uM) *Salmonella* was not affected and grew normally. This data comes from a plate that was read 48 hours after the TLR was added to the *Salmonella*.

TLR has antibiotic qualities that could potentially stop the growth of Typhoid fever bacteria. We were able to stop the growth of *Salmonella Typhi* in media that resembled a macrophage. Our dosing series demonstrated that TLR could inhibit the growth of *Salmonella* bacterium effectively up until 3.125uM.

D10

CAPECITABINE INHIBITS THE GROWTH OF *SALMONELLA* IN A DOSE-DEPENDENT MANNER

Isabella Shelby

Salmonella is a gram-negative bacterium present in contaminated food or water. Typhoid fever is a systemic infection caused by the serotype *Salmonella typhi* and can be fatal without proper intervention. Instances of resistant typhoid are high in less developed countries. Resistance occurs when genetic mutations prevent antibiotic mechanisms of action, making current antibiotics less effective. The purpose of this drug screen was to target oncological drugs and diversity library compounds to identify novel antibiotic substances.

To perform the initial drug screen, we exposed *Salmonella typhimurium* to various compounds and chemotherapeutic drugs. The positive and negative controls were ampicillin and Dimethyl sulfoxide (DMSO), respectively. We used spectrophotometry to measure the growth of *salmonella* in the plate. Hits were identified as compounds with an absorbance below 2 standard deviations of the mean DMSO value, meaning that hits would have an absorbance below 0.065. We identified that the FDA-approved drug capecitabine had an average absorbance of 0.035. To perform a dosing series, capecitabine was diluted in a 1:2 ratio, making 13 dilutions total. The dosing series revealed that capecitabine is most effective at doses lower than 6.25 μM.

Through this process, we determined that the oncological drug Capecitabine (Xeloda) has dose-dependent antibiotic properties. Capecitabine is a prodrug to U5 fluorouracil, used to treat colorectal, gastrointestinal, esophago-gastric, and metastatic breast cancer. It is an antimetabolite that inhibits the enzymatic activity of thymidylate synthase, preventing DNA synthesis. Determining the antibiotic properties of capecitabine opens research to the exploration of this and other fluoropyrimidines as antibiotics.

D11

EFFECTIVENESS OF COMPOUNDS CYCLOHEXEN-1-YLOXY(PHENYL)PHOSPHORYL)BENZENE AND 4-AMINO-6,7-DIHYDRO-5H-CYCLOPENTA[B]PRIDIN-2YL 4-METHYLBENZENESULFONATE IN KILLING SALMONELLA

Naomi Eps, Katelynn Fegan

The bacterium *Salmonella typhi*, causes typhoid fever when ingested, which is a serious infection. The symptoms of the infection are fever and diarrhea, but without proper treatment, can progress to ruptured intestines and death. To prevent these serious side effects, immediate treatment with antibiotics is necessary. *Salmonella typhi* is becoming resistant to antibiotics, so more antibiotics are needed to combat these dangerous bacteria.

In this study, we tested the effectiveness of 1,590 compounds in Diversity Set V from the National Cancer Institute Developmental Therapeutic Program in prohibiting or accelerating the growth of *Salmonella typhimurium*. Out of the twelve total hits, we examined two compounds, 4-amino-6,7-dihydro-5H-cyclopenta[b]pyridin-2-yl-4-methylbenzenesulfonate (ADCM) and cyclohexen-1-yloxy(phenyl) phosphoryl]benzene (CYPB) that were found to be effective at inhibiting bacteria growth. A dosing series revealed that 121-4 was effective in preventing growth of *Salmonella typhimurium* at doses of 6.25 μM and above. A dosing series for compound ADCM revealed that it was not effective at inhibiting growth of *Salmonella typhimurium* at different doses. The results for compound CYPB are comparable to those of ampicillin dosing series, which revealed that ampicillin

inhibits growth of *Salmonella typhimurium* at doses of 6.25 uM and above.

These results will be forwarded to the Detweiler Lab, where they may choose to further investigate the compound for important characteristics such as its absorbances at different times after ingestion and its chemical structure.

D12

[2-[2-(DIMETHYLAMINO)-2-OXOETHYL]SULFANYLPHENYL] N-METHYLCARBAMATE INHIBITS GROWTH OF *SALMONELLA TYPHIMURIUM* IN M9 MEDIA

Eleni Patera

Several types of *Salmonella* infections have an immense impact worldwide despite the improvements in hygiene and availability of antibiotic drugs and vaccines. In our study, we focused on *Salmonella Typhimurium,* which is the main cause of Typhoid fever. The National Cancer Institute Developmental Therapeutic Program provided us with two chemical libraries: Oncology Set and the Diversity Set V, which consists of 1,590 compounds. In this study we pursued the follow compounds from the Diversity Set: Antor (compound 5) , [2-[2-(dimethylamino)-2-oxoethyl] sulfanylphenyl] N-methylcarbamate, also known as BRN 2291159, and Isopropylamino Ethanol Hydrochloride. We predominantly focused on the compound [2-[2-(dimethylamino)-2-oxoethyl] sulfanylphenyl] N-methylcarbamate, which was validated to observe how it affects *Salmonella Typhimurium* and determine if it promotes or inhibits *Salmonella Typhimurium* growth. We made a dosing series for the three compounds mentioned above, and the dosing series demonstrated that BRN 2291159 was able to inhibit growth of *Salmonella* growth only at a dose of 25 μM. Doses lower than 25 μM proved that the compound was not effective for inhibiting growth. However, dose of this compound at 25 μM remains effective. These data supports that BRN 2291159 possibly inhibits the growth of *Salmonella Typhimurium* at a dose of 25 uM. Further studies can include a timing series and testing similar compounds to determine if there is an

D13

MITOXANTRONE AFFECTS GROWTH OF *SALMONELLA* IN A DOSE-DEPENDENT MANNER

Erin Moriarty, Kathy Nguyen

Salmonella-induced typhoid fever can be a potentially life-threatening disease. Its ability to thrive in hostile environments affords it the ability to infect systemically, manifest a widespread array of symptoms, and induce numerous consequences on the host. Due to the imminent danger of antimicrobial resistance, demand for novel antibiotics and screening methods are on the rise.

In this study, *Salmonella typhimurium* was exposed to 127 compounds from the National Cancer Institution Oncology library. The experiment took place in conditions mimicking that of a macrophage, using M9 media. Of these, 13 compounds appeared to inhibit growth of *Salmonella typhimurium* and 9 appeared to promote growth. One compound, Mitoxantrone, was chosen as a promising compound due to its apparent promotion of growth of *Salmonella typhimurium* and was used as a subject for further testing. A dosing series was performed and demonstrated that Mitoxantrone significantly promotes growth at the highest dose, 25 μM, and began to inhibit growth at doses of 1.563 μM and below. One caveat is that, during the dosing series, *Salmonella typhimurium* did not grow as expected in the water control.

These data support the preliminary conclusion that while Mitoxantrone promotes growth at high concentrations, lower doses of the drug is promising as a potential antibiotic. Furthur investigation into the dose- and time-dependant properties of the drug will take place, and then the data will be forwarded to the Detweiler lab for further testing.

D14

FLOXURIDINE, AZACITIDINE, AND CAPECITABINE INHIBIT *SALMONELLA* GROWTH IN DOSING SERIES

Kirby Peterman, Ben Marshall, Brenden Erikson

Salmonella typhimurium is a strain of *Salmonella* that commonly lives in macrophages of infected humans and can be found in soil and water. This strain of *Salmonella* causes typhoid-like symptoms in rats and food poisoning symptoms in humans such as diarrhea, fever, and headache.

In this study, *Salmonella typhimurium* was exposed to 128 drugs used for chemotherapy treatment in cancer patients from the National Cancer Institute. 7 potential hits were identified by using phenotypic screening to determine if *Salmonella* growth was inhibited by the drug using ampicillin, a known

antibiotic, as a positive control and DMSO as a negative control.

Floxuridine, Azacitidine, and Capecitabine were chosen to be used in further research in a dosing series because they substantially inhibited growth at 25 uM concentration in the first round of tests. A dosing series was used to see inhibition results for doses as low as 0.195 uM.

Although these drugs were initially developed as chemotherapies, using extremely low doses on *Salmonella typhimurium* could prove effective as an antibiotic-type treatment without producing adverse effects seen in high dosage chemotherapy. This would require further research on effective dosing.

D15

COULD TRADITIONAL MEDICINES HOLD THE KEY TO NOVEL ANTIBIOTICS?

Zeena Nisar

This laboratory conducted a semester long research project related to the research of Dr. Corrie Detweiler. We investigated the Diversity set from the library of Food and Drug Administration-approved drugs using a screening method with Salmonella. The screening method consisted of placing the drugs in duplicates on a plate with a set amount of Salmonella as part in vitro testing. The antibiotic, Ampicillin, was also placed on the plate as a positive control and dimethyl sulfoxide was placed as a negative control. A spectroscopy method was used to analyze the absorbance of the wells, which indicates the amount of bacteria remaining in the wells. An absorbance value higher or lower than two standard deviations of the mean is considered a "hit" and the drug is investigated further. After finishing the screening, I decided to independently investigate various botanical treatments in order to gauge their effectiveness as potential antibiotic treatments. I used a similar screening method in which I conducted a dosing series. Various doses of the natural extracts were placed parallel to various doses of Ampicillin (the positive control) in order the analyze the effectiveness of the extracts. Dill weed, turmeric, and green tea were tested in a dosing series. Turmeric and dill weed showed the most promising results in terms of antibacterial properties and could be investigated further.

D16

SALMONELLA TYPHIMURIUM DRUG INHIBITION AND DOSING SERIES TRIALS

Kyler Bartlett, Marlee Lederer, Shelbi Davenport

Salmonella typhimurium is defined as, pathogenic Gram-negative bacteria predominately found in the intestinal lumen. This study is based on our hypothesis which states, lower doses of a compound will have less an effect on the growth of *Salmonella,* meaning the bacteria will grow better in lower doses. The goal is to identify compounds that kills or stops the growth of *Salmonella typhimurium*

Everything was tested in *Salmonella typhimurium* and M9 media in hopes of replicating growth in a human macrophage. Dimethylsulfoxide (DMSO) was used for our negative control because it is the compound in which the diversity set compounds are dissolved. The growth of bacteria should not be inhibited when bacteria are exposed to DMSO. Ampicillin was used as a positive control because it is a known bacterial antibiotic. The growth of bacteria was expected to be inhibited in the wells containing ampicillin. After testing the compounds in the diversity set as a class, the study focused on compounds that were almost statistically significant to retest these compounds in order to determine whether they inhibited growth of *Salmonella* more than DMSO alone. A dosing series was used to test 6 compounds to determine if they inhibited growth of *Salmonella*, and to test the dose required for the compound to be effective.

Based on our results, our hypothesis was not proven correct because the lower the concentration in our experiments, the lower the absorbance, however these results may have been atypical, with ampicillin being the only drug that behaved as expected.

D17

FLUOROURACIL, AN ONCOLOGY DRUG, IS EFFECTIVE IN INHIBITING GROWTH OF *SALMONELLA TYPHIMURIUM* AND HAS CERTAIN DOSAGE CHARACTERISTICS

Abby Blake

Salmonella typhimurium is a pathogenic Gram-negative bacteria that is mainly found to affect the intestinal tract. This bacteria is found to cause typhoid fever in humans and animals. Tyhhoid fever is a systemic infection that is characterized by headaches, diarrhea, lethargy, and overall aches. Typhoid fever can be treated with the use of several antibiotics, however antibiotic resistance is increasing.

In this study, we tested 1,590 compounds from Diversity Set V (provided by the National Cancer Institute Developmental Therapeutic Program) for their

effectiveness in killing or significantly reducing growth of the salmonella bacteria. We used an M9 media to grow the bacteria in order to replicate the conditions in which salmonella replicate *in vivo*. Another lab section also tested compounds from an Oncology Set and around 30 compounds were found to inhibit growth. Of these compounds I chose Fluorouracil, a topical medication used to treat cancerous skin growths. Fluorouracil is in a class of drugs known as anti-metabolites, and it works by blocking the growth of abnormal cells that cause the skin growth. With this drug, I performed a dosing series which demonstrated that the drug was most effective at mid range dosages (12.5 uM and 6.25 uM).

This study was helpful in determining Flurouracil as a promising potential drug to be used for medical purposes and requires further testing. This data can be used in other laboratories to further test the drug for effectiveness in both *in vivo* and *in vitro* studies.

D18

CAPECITABINE INHIBITS THE GROWTH OF *SALMONELLA TYPHIMURIUM* EFFECTIVELY WHEN COMPARED TO AMPICILLIN

Jack McLeod

Typhoid fever is an illness that has been present throughout much of recorded history. The prevalence of Typhoid fever is much reduced in countries with well-supported infrastructures such as the United States, though still present and estimated to be about 6,000 cases per year. However, in 2013 11 million cases were reported worldwide and caused around 160,000 deaths. Typhoid fever is still a deadly and infectious disease that is prevalent in the world.

In this study the research team examined the response of over 1,590 compounds in the "Diversity Set V" from the national cancer institute along with an Oncology set. 12 drugs were found from the Diversity Set to inhibit or significantly slow growth of *Salmonella typhimurium* and 11 from the Oncology set. One drug was found to inhibit growth of *Salmonella typhimurium* more effectively the Ampicillin used as a control. The ability to inhibit the synthesis of thymidine was promising in its results during the experiment. This also led to an other drug, Fluorouracil being noticed due to the body metabolizing Capecitabine into Fluorouracil or (5-FU)

This led to study being directed towards the potential usefulness of Capecitabine as an antibiotic due to its low toxicity and use currently to treat many types of GI cancers and breast cancer. Since the dosing currently for Cancer treatment can be as high as 6 grams per day for months it could hopefully be scaled back to a more conservative approach and still be effective in reducing the growth of *Salmonella typhimurium*.

D19

ESSENTIAL OILS INHIBIT GROWTH OF *SALMONELLA TYPHIMURIUM*

Rachel Horstmeyer

Found inside of raw chicken and eggs as well as on the scales of snakes, *Salmonella typhi* infections can cause severe food sickness. Due to antibiotic resistance, fewer antibiotics are available to treat such infections and alternative treatments of infections need to be researched. A common alternative, essential oils (EOs), have been used for their antimicrobial properties for many decades, **D21**

THE EFFECT OF ESSENTIAL OILS ON *SALMONELLA TYPHIMURIUM* GROWTH

Kelsi Zueger

The salmonella bacteria is responsible for over 50% of all human infections in the United States every year as well as responsible for killing 500 of the 1.4 million infected every year. This bacterium may cause the infected to develop diarrhea, fevers, as well as abdominal cramps and headaches. In some more developing countries the bacterial infection could result in typhoid fever, which leads to death in 10%-30% of cases.

With the boom of essential oils and their uses for both home and health uses, an essential oil mixture was tested for its antibacterial abilities. After researching different oils from the book *Modern Essentials,* oregano was found to be antibacterial, antifungal, and antiphrastic. Along with this information I came across a recipe for the mixture, which I tested that also contains other oils such as ginger, peppermint, grapefruit, cinnamon, and thyme. A dosing series showed that in smaller concentrations of the solution was effective in killing or preventing growth of the salmonella tested. Oregano tested singularly also showed the same results that oregano does have some ability at preventing or killing salmonella.

These data shows that essential oils could be used as new modern medicine, but it also requires much more testing, the results did not show to be better than the already known treatment of salmonella, but

with different mixtures and concentrations, we could find a better and more plant based treatment.

However, lab research supporting their effectiveness is limited. In this experiment, fourteen different EOs were tested against *Salmonella typhimurium*: tea tree, clove bud, cinnamon, orange, Breathe, melaleuca, eucalyptus, On Guard, lemon, peppermint, Digest Zen, and lavender. *Salmonella typhimurium* was used because it replicates *Salmonella typhi* in mice. These were plated alongside a negative control, sterile water, as well as a positive control, ampicillin. Sterile water was used as a negative control because the added water allowed the concentration of bacteria in the well to be equal to those in the oil wells. Ampicillin was used as a positive control because it is a known inhibitor of *Salmonella*. In this study, each of the EOs weas tested in triplicate in a dosing series with *Salmonella typhimurium*. There were four different doses of oil/water/amp: 10%, 5%, 2.5%, and 1.25%. All EOs produced hits throughout the dosing series, equal to our better than ampicillin.

D20

BENZYL DIONE, A POSSIBLE NEW ANTIBIOTIC

Madi Sanchez

Antibiotic resistance is a growing problem across the globe. People are getting infections from bacteria but the antibiotics being used to treat them are resistant and therefore ineffective. These leave 2 thousands dying every year from a simple infection.

In this study, *Salmonella Typhi* is the bacterium being studied. We examined the Diversity Set V, a set of 1,590 compounds, so see how each compound interacted with the bacterium. Our goal was to find compounds that have antimicrobial properties. 37 of the compounds were shown have lower bacteria absorbencies and 20 were shown to enhance the growth of bacteria.

From there one compound, Benzyl Dione, was shown to have antimicrobial properties was further tested. A dosing series was done in order to understand the relationship between concentration of the compound and its efficiency in preventing or killing bacteria. This resulted in an indirect relationship between the concentration and the number of bacteria, meaning the more concentrated the compound the less bacteria there was. Because of these results Benzyl Dione is a good candidate for further study to see if it could potentially be used as an antibiotic in mammals.

D21

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These data shows that essential oils could be used as new modern medicine, but it also requires much more testing, the results did not show to be better than the already known treatment of salmonella, but with different mixtures and concentrations, we could find a better and more plant based treatment.

D22

AXITINIB SHOWS PROMISING ANTIBIOTIC PROPERTIES AGAINST *SALMONELLA TYPHIMURIUM*

Dylan Price

Since the fortuitous discovery of Penicillin in the late 1920's, antibiotics have been implemented extensively to greatly reduce the human health threat created by infectious bacteria. Since then, numerous other antibiotics have been created, but the threat of infectious diseases remains and threatens to become more severe due to the development of multidrug resistance in bacteria. As a result, there is a pressing need to develop novel antibiotics to keep pace with resistance. In response, Dr. Corrie Detweiler at the

University of Colorado Boulder has pioneered a rapid screening process designed to identify previously undiscovered antibiotics. Through this process, axitinib (C22H18N4OS), an FDA-approved tyrosine kinase inhibitor, revealed promising results. Four different undergraduate researchers confirmed that it effectively terminated the growth of *Salmonella Typhimurium*. These data prompted further exploration of axitinib from both the patient care perspective and the microbiological perspective. From a patient care perspective, axitinib shows promise in that it can be administered orally, and the side effects of are predictable and manageable. From a microbiological perspective, it has some key distinctions from the other tyrosine kinase inhibitors, and it has a number of structural properties reminiscent of methicillin. Moving forward, more tests should be done to observe the efficacy of axitinib in treating *Salmonella Typhimurium* with variable doses and under various conditions.

D23

GROWTH RESPONSE OF *SALMONELLA TYPHIMURIUM* TO DIFFERENT ANTIMICROBIAL ESSENTIAL OILS

Katie Franks

As the production of new antibiotics has slowed, the antibiotic resistant "bug" is growing and continuously growing through the abuse of antibiotics. In this research, we took to finding the new antibiotic to help slow the growth of resistant bacteria. Likewise, essential oils were also tested to determine whether or not the claims that homeopathic antibacterial remedies were sound. The effects salmonella and DMSO had on salmonella were tested as controls, to compare to effects four different essential oils, Oregano, Eucalyptus, Melaleuca and On-Guard, had on the bacteria as well. Along with the initial tests of the oils against Salmonella, we also put the oils through a dosing series to determine which had the greatest antimicrobial effect at the lowest dose.

D24

STRUCTURE-BASED PREDICTION OF NOVEL ANTIBIOTIC TARGETS

Rachel Anderson

Typhoid fever is a highly infectious disease endemic to South America, Africa, and South Asia. Prior to the advent of antibiotics, patients would be bedridden for up to a month and faced a 20% mortality rate. Although currently treatable with antibiotics, resistance to all current treatments is growing at an alarming rate. Without intervention, typhoid fever may be difficult or impossible to treat in the coming decades.

Research from the Detweiler lab shed light on how a *S. typhi* bacterium outwits the immune system, reproducing within the macrophage attempting to kill it. This study attempted to build on that work by growing *Salmonella* in the hostile conditions (low pH, limited nutrient access) found inside macrophage phagosomes.

Diversity Set V, a 1,593 compound library, was obtained from the National Cancer Institute Developmental Therapeutics Program (NCI-DTP). *Salmonella* was exposed to individual compounds from the library, then incubated 24 hours at body temperature (37 °C). 22 compounds were discovered to prevent bacterial growth as effectively as Ampicillin, an antibiotic. 6 compounds were found to increase bacterial growth.

The structures of the 22 compounds showing antibacterial activity were grouped by similarities. It was hypothesized that additional antibacterial compounds could be located by looking for structural similarities. This simple search method did not produce additional results.

The data from this study will be returned to the Detweiler lab for further investigation, such as toxicity assays and potentially *in vivo* testing on mice for especially promising candidate compounds.

D25

EFFECTIVENESS OF ESSENTIAL OILS AS ANTIBIOTICS AGAINST *SALMONELLA* INFECTION

Estrella Guerrero

Salmonella infections are widespread throughout the globe and range in their severity from food poisoning to enteric fever. *Salmonella typhi* is a strain of salmonella that causes typhoid fever in humans. Typhoid fever is a potentially life-threatening illness that can be treated with antibiotics. This illness is not very common in the United States but in countries such as South Africa, Southeast Asia and South America it still presents a problem and has grown to show antibiotic resistance. In this study I used *Salmonella typhimurium*, a strain of salmonella that causes typhoid fever-like symptoms in mice but causes food poisoning in humans. The use of this

specific strain enabled me to see how essential oils could be useful in inhibiting typhoid fever.

In this study four popular essential oils including grapefruit (*Citrus paradisis*), peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), and lavender (*Lavandula angustifolia*) were tested on their effectiveness to inhibit *Salmonella typhimurium*. These essential oils have shown antimicrobial properties in other studies against food-spoiling microbes. The essential oils were obtained from a local store and their effectiveness was analyzed through a dilution series and a spectrophotometer. Out of the four oils I tested, none were found to inhibit the growth of *S. typhimurium*.

D26

DOSE DEPENDENT DRUGS INHIBIT *SALMONELLA* GROWTH

Diana Gasiouk, Emma Russell

Antibiotic drug discovery peaked in the 1980's, but since then there have been zero new classes of antibiotics discovered. This is a problem because overuse of the current antibiotics has led to enormous increases in the frequency of bacterial resistance, rendering current antibiotics ineffective. This research gives the chance for discovery of new antibiotics. Mimicking the environment of Salmonella *Typhimurium* with M9 media, drugs from the National Cancer Institute Oncology Set Library were pipetted with Salmonella to test their effectiveness at killing the bacteria. Ampicillin was used as a positive control and DMSO was used as the negative control. The effectiveness of the drugs was evaluated using a spectrometer, which showed the change in absorbance of Salmonella when the drug was added into the well. This research focuses on Lapatinib and Floxuridine, two oncology drugs from the FDA approved drug library. Chemotherapy drugs are known to be toxic, but in discovering oncology drugs that kill bacteria, we were able to test how low of a dose of the drugs would still be able to kill Salmonella.

D27

2-ARSONBENZOIC ACID, NSC 15571, INHIBITS *SALMONELLA TYPHIMURIUM* GROWTH IN A DOSE-DEPENDENT MANNER

Rathan Kumar

The prevalent problems of Typhoid fever and antibiotic resistance if found all over the world and makes treating bacterial infections such as that of *Salmonella* hard. The current method of developing new antibiotics to reliably treat these problems is progressing too slowly to help. Similarly, there have been numerous compounds not intended or tested for having antibacterial properties. Thus, it is logical that some of these untested compounds could act as a possible antibiotic to *Salmonella Typhimurium*. Therefore, these untested drugs were screened for antibacterial properties and tested along with other drugs of similar structures. The initial screen of the Diversity Set resulted in NSC 15571 being identified as a hit and inhibiting the growth of the bacteria. A dosing series were conducted on compounds NSC 15571. The compound was tested initially with a common concentration found in the blood, 25μM in the solution with the *Salmonella*. The solutions were tested in triplicate with 50μg/ml Ampicillin and DMSO and deionized Water as the controls. They were incubated at 37°C and tested for absorbance at 600nm. and In the primary dosing series, NSC 15571 stopped bacteria growth at 25μM, 12.5μM, 6.25μM, 1.56μM and 0.78μM. However, at 3.125μM, the drug appears to not inhibit growth.

D28

NEW COMPOUNDS FOUND TO INHIBIT *SALMONELLA TYPHIMURIUM* GROWTH

Caeli Best

Currently, *Salmonella typhi* infections affect 21.5 million people a year. The majority of those affected live in developing countries where sanitation is not as advanced. With the increase rise in antibiotic resistance, the number of those affected/year will continue to grow. By the year 2050, the number of deaths caused by antibiotic resistance will surpass cancer and cause roughly 10 million deaths/year. In Discovery-based Laboratory 1, we teamed up with the Detweiler lab to find new compounds that diminish bacterial growth. The bacteria were cultured in M9 media and then exposed to different compounds. The microplates incubated at 37° for 24 hours. They were then read by a spectrophotometer set to 600 nm to measure absorbance. The Salmonella growth and the absorbance have a positive correlation. We were able to determine 'hits' by calculating two standard deviations (st. dev.) on either side of the mean absorbance. If the hit fell beyond -2 st. dev. from the mean, then it inhibited bacterial growth. If the hit fell beyond +2 st. dev. from the mean, then it promoted bacterial growth. After testing over 1,600 compounds and drugs, many hits were determined. From the hits

determined, a dosing series was preformed to determine which concentrations are most effective.

D29

PHENOTYPIC SCREENING FOR NOVEL ANTIBIOTICS IN *SALMONELLA TYPHIMURIUM*

Eric Schaedig

Antibiotic-resistant infections are currently on the rise while antibiotic research is rapidly declining. It is projected that infectious diseases will soon be the leading cause of mortality in the world. Namely, multidrug-resistant *Salmonella* infections are becoming increasingly common across the globe. The dangers of these resistant infections can be mitigated via the discovery of new antibiotics.

In this study, the Diversity Set V from the National Cancer Institute Developmental Therapeutic Program and several plant extracts–Echinacea, oregano, and wheatgrass–were screened in order to identify compounds possessing antibiotic properties. *Salmonella typhimurium* were cultured in M9 minimal media and inoculated with the compounds. Each sample was then incubated for twenty-four hours. Absorbance of each sample at 600 nm was then used to quantify the bacterial growth. Compounds and extracts possessing antibiotic properties were identified using an absorbance threshold two standard deviations below the mean of the negative control trials.

Out of the 1,593 compounds in the Diversity Set V library, twenty-two compounds were identified to have antibiotic properties. The plant extract data were inconclusive. The twenty-two compounds identified in the library screen are excellent candidates for more intensive studies on their efficacy as antibiotics.

D30

THE EFFECT OF TRANS-RESVERATROL ON *SALMONELLA TYPHIMURIUM*

Krista Gould, Almut Herzfeld Mayer, Rebecca Lockyear

Salmonella, while a relatively minor inconvenience in the United States and other developed nations, affects millions of people worldwide and typhoid fever (*S. typhi*) alone claims the lives of over 200,000 people annually. Given that trans-resveratrol has not been extensively tested yet is hailed as an anticarcinogen, antioxidant, and antibiotic, we wished to test the effectiveness of this compound against *Salmonella typhimurium*, a relatively innocuous strain of *Salmonella* for humans, which causes typhoid fever symptoms in mice, similar to *Salmonella typhi*.

We created six distinct dilutions (1mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL, 0.03125mg/mL) of resveratrol in distilled water, added 10 μL of each dilution to 90 μL of *S.* typhimurium six times (in addition to several controls as detailed in the 'Methods' section), and ran a timing series for 38 hours to determine if the resveratrol had inhibited, promoted, or did not affect growth of *S. typhimurium*. This was done for three distinct plates.

Trans-resveratrol was shown to be neither bactericidal nor bacteriostatic in our experiment. Resveratrol did not inhibit the growth of salmonella to a significant extent. However, the highest dose came closest to being a "hit", although it was not at all close. Therefore, higher doses of trans-resveratrol should be tested next. If those results display similar findings, then we will abandon trans-resveratrol and seek out other compounds, which may be more effective against *S. typhimurium*.

D31

EFFECTS OF 2,2 BIS(1,2-DIMETHYLINDOL-3 YL), PROPIONIC ACID & SPIRO[3,3]HEPTANE-2,2,6,6,-TETRAMETHANOL ON *SALMONELLA TYPHIMURIUM* GROWTH

Arjun Saravanan

With the emergence of bacterial drug resistance, current drugs are starting to become ineffective against gram-negative bacteria. *Salmonella typhimurium* was specifically used as a model to test a diverse range of drugs from NCBI. At the drug concentration (100ug/ml), novel efficacious antibiotics were identified if there was statistical reduction in Salmonella T. growth. Statistical significance of growth was measured at a standard deviation x<-2, x>2 (where(x) = SD growth). To have a standard base drug hit threshold, Ampicillin (50ug/ml AMP) is the positive control (DMSO solvent was negative control). With relatively successful hits (comparison with AMP), 2,2 bis(1,2-dimethylindol-3-yl)Propionic Acid (Drug1:D1) & Spiro[3,3]heptane-2,2,6,6-tetramethanol (Drug2-D2) were selected for the dosing series test (100ug/ml drug concentration w/ 1:10 dilution, measured after 24 hour period). Both individualistic and combined drug effects were measured. For D1, y=100ug/ml (y= drug concentration) matches efficacy of AMP, at y=0.1ug/ml:.01ug/ml shows statistically significant decrease in Salmonella. However, at the y=

10ug/ml: 1ug/ml, drug had no change in growth. For D2, y = 100ug/ml:.1ug/ml drug was ineffective; y = 10ug/ml:1ug/ml:.01ug/ml drug showed a statistically significant decrease growth. Combined D1/D2 (1:1 drug volume) y= 100ug/ml:10ug/ml:1ug/ml:.1ug/ml:.01ug/ml all showed significant decrease; only the 100ug/ml was as effective as AMP. Results suggest that the combination drug shows a synergistic effect in killing Salmonella T., as it worked in all concentration. Further testing is required: timing series is necessary to test the Combination drug's effect on growth over a longer period of time.

D32

ONCOLOGY DRUG EFFECTS ON *SALMONELLA*

Oula Kareem, Anne Dickson

Salmonella is a dangerous bacteria is found in uncooked food and if ingested can result in sickness and even death. Currently, salmonella has been treated using antibiotics such as ampicillin, but due to the overuse and misuse of antibiotics resistant strains have developed. The purpose of our research project was to test various chemotherapy drugs and determine their efficacy in inhibiting the growth of Salmonella. DMSO-treated cells were used as a control because they result in proliferation of Salmonella. Ampicillin-treated cells were also used as a control because they are proven to inhibit growth. We treated Salmonella with various chemotherapy agents and compared their growth to the controls to determine whether or not they could be as effective as ampicillin in treating Salmonella. The results of our research showed that drugs Hydroxyurea and Capecitabine were effective in killing Salmonella. These results could potentially shed light into alternative treatments for Salmonella with further research.

D33

THE EFFECT OF ESSENTIAL OILS ON LIMITED THE GROWTH OF *SALMONELLA TYPHIMURIUM*

Brandon Smith

Salmonella poisoning has often been treated throughout the world with natural remedies. The remedies seem to stem from natural defense mechanisms developed by plant cells acting upon Salmonella in a host body. To determine which types of oils are most effective against the salmonella, I exposed cultures of *Salmonella typhimurium* to essential oils diluted in a 1:10 mixture with distilled water. Upon examining the growth of the cultures via a spectrophotometer, it appears that leaf or stem based oils with curing properties, such as peppermint and eucalyptus, have a profound killing impact on the salmonella, while oils derived from fruits have a null effect on the bacteria. Afterwards, a dosing series was conducted on both Eucalyptus and Oregano oils by creating multiple 1:1 dilutions with distilled water and oil. Results show that the eucalyptus oil begins to become effective when they make up 6.25% of solution with water, while oregano oil is effective at a 25% concentration.

D34

SULFONAMIDE GROWTH INHIBITION IN *SALMONELLA TYPHIMURIUM*

Niccolo Degliantoni, Caitlin Tiehen

Antibiotic resistance in today's society is largely due to misunderstandings of how antibiotics work and the resulting overuse of these drugs. Research and development for the discovery of new antibiotics is currently at a standstill due to economic, scientific and regulatory barriers. By testing drugs within the same class as the first known antibiotics, findings lead to the ability to combat antibiotic resistance. The specific antibiotic class our group is studying is the Sulfonamide class, an old antibiotic class discovered in the 1930's.

D35

INHIBITORY EFFECTS OF DOSE-DEPENDENT COMPOUNDS IN *SALMONELLA TYPHIMURIUM*

Charles Satterlee

The fight against bacterial pathogens is one of humanity's enduring struggles. With the discovery of antibiotics everything changed. We have lived in privileged era, mostly protected from the fear our ancestors experienced. Yet with the increase in antibiotic resistance our communities are once again at risk of life threatening illness. Our research focuses on developing new antibiotics for Typhoid fever.

In this study, we incubated *Salmonella Typhimurium* in M9 media, and tested its response to a compound library provided by National Cancer Institute Developmental Therapeutic Program. Compounds, which demonstrated growth inhibitory

effects similar to Ampicillin, were selected for a second round of validation testing. This validation included a dosing series to test the effects of the compound with variable doses. After the first round of screening, the compounds dichlorophenoxy[2] and dimethylamino[3] inhibited growth and qualified for further testing. Once moved to the dosing series, these compounds were found to have no effect on the growth of *Salmonella Typhimurium* between the ranges of 10 microliters and 2 microliters.

Our screening process proved effective at differentiating between compounds, which have potential to become new antibiotics and those that will not. Early examination of these compounds showed promising effects by multiple researchers. Yet within a single round of screening they were disqualified. The value of quickly identifying false leads cannot be overstated, given the scope of potential antibiotics and the size of the libraries in need of testing.

D36

PLANT-BASED EXTRACTS AS ANTI-BACTERIAL AGENTS INHIBITING THE GROWTH OF *SALMONELLA*

Doreen Roberts

For the extent of human history, herbs and spices have been used to treat and prevent illness. Many have shown to possess antibiotic properties. This study tested various herbs and spices to determine if any inhibit the growth of Salmonella in vitro. The results showed that ginger oil and garlic, which has been filtered possesses antibacterial properties at higher concentrations. Clove oil showed antibacterial activity at all concentrations. The data shows that garlic, ginger oil, and clove oil can potentially be used to treat Salmonella contamination.

D37

DISCOVERING NEW ANTIBIOTICS FOR *SALMONELLA TYPHIMURIUM*

Danae Shae

We tested 1,593 compounds against *Salmonella typhimurium*, which has never been done before. This is a bacteria in many parts of the world leads to high mortality rates and is increasingly becoming resistant to the existing classes of antibiotics. Our study used typhoid fever-causing Salmonella in an attempt to identify novel antibiotics to address this problem. In my experiment, I decided to test some compounds that were not in this initial screen, berberine, oregano oil, and activated charcoal, to see if they had an effect on *Salmonella* growth, one of which was actually very successful.

D38

ESSENTIAL OILS WITH BENZENE RING CONSTITUENTS INHIBIT SALMONELLA TYPHIMURIUM GROWTH AND HAVE OTHER POTENTIAL BIOMEDICAL USES

Elisse Headrick

Pathogenic disease mortality rates have decreased the past century due to antibiotics. However, with emerging antibiotic resistance in bacteria, pathogenic disease mortality is predicted to increase if new antibiotic drugs are not discovered.

In this study, we examined the responses of *Salmonella typhimurium* grown in M9 media to Essential Oils using different dosage series at 1.4uM, 5uM, 10uM and 15uM. To determine what constituted a hit and inhibited *Salmonella typhimurium* growth, we used data from previous studies conducted by the CU Boulder MCBD department that included testing 1,590 chemicals from the Diversity Set V and 129 chemicals from the Oncology Drug Set this semester. We later used this data to also compare chemical structures of antibiotics and cancer drugs. For the Essential oils study, ten oil compounds were tested and four were found to inhibit *Salmonella typhimurium* growth. The ten compounds were chosen due to their promising results in oncological studies that have or are being conducted. If the oils have anti-cancer effects, then the oils may also have anti-microbial effects. Chemicals compounds with a multitude of biomedical uses can be highly valuable and potentially lower mortality rates.

The data gathered supports the preliminary conclusion that Clove Oil, Oregano Oil, Eucalyptus Oil, and Rosemary Oil contain promising compounds that require further study. The data will be forwarded to the Detweiler Lab, who may choose to further study the chemical compounds to synthesize medicinal isomers that may become new antibiotics.

D39

COMPOUND MLS002703918 (NSC-106464) INHIBITS GROWTH OF SALMONELLA TYPHIMURIUM

Paul DeHay

Typhoid fever poses a serious threat to the

health of individuals (mostly children) in the developing world. The illness is caused by a bacterium called *Salmonella typhi*, and can be fatal if proper treatment is not administered. Typhoid fever can be treated with antibiotics, but increasing antibiotic resistance has been developing in these typhoid-inducing strands of *Salmonella*.

Salmonella typhimurium causes a typhoid-like disease in mice. We examined this bacteria and its response to 1,590 compounds found in Diversity Set V, which was made available. Of the 1,590 compounds tested, 21 compounds had shown to inhibit the growth of the bacteria, and 12 compounds had shown to encourage the growth of the bacteria in M9 media. This media helped us examine the bacteria in conditions similar to that of the bacteria in vivo. Of the 21 compounds, which inhibited growth, three were chosen and tested in different dosing series. Of the three compounds, compound MLS002703918 (NSC-106464) had shown to kill or prevent the growth of *Salmonella* at doses above 25 μM, which is similar to the effectiveness of Ampicillin (an effective treatment of typhoid fever administered to humans).

The data from compound MLS002703918 (NSC-106464) has shown it to be a candidate deserving of further investigation as an effective treatment against *Salmonella bacteria*. This data will be forwarded to the Detweiler Lab, in which they may pursue the effectiveness of this compound *in vivo*, and to study and alter its chemical structure to increase effectiveness and minimize toxicity.

D40

DETERMINING IF HYDROXYUREA AND AZACITIDINE IMPACT THE GROWTH OF *SALMONELLA* AT VARIOUS CONCENTRATIONS.

Henry Denny

Throughout the semester, several tests were performed where compounds were pipetted with *Salmonella* in well plates, and bacterial growth was examined. The compounds were analyzed based on how well they controlled the growth of *Salmonella*. From the Oncology Drug Set, two compounds that were confirmed hits were selected, as they showed high absorbance levels. The compounds in the Oncology Set were pre-approved by the FDA for the treatment of cancer. The compounds selected from this set were Hydroxyurea and Azacitidine. The objective was to examine the absorbance levels of these compounds when they were tested at different concentrations. As a result of doing so, the compounds ability to control the *Salmonella* growth could be determined. Thus, a dosing experiment was performed with Azacytidine and Hydroxyurea. It was hypothesized that as the concentration of the compound decreased, so would the absorbance level. From examining the results, the hypothesis proved to be correct. As each drug was diluted to a lesser concentration, the absorbance level also decreased, and the compound was no longer a hit. However when each compound was tested with *Salmonella* at a high concentration, each compound produced a high absorbance level, and was again validated to be a hit. From completing this research, it can be concluded that these compounds that are pre-approved by the FDA for their chemotherapeutic properties do have an impact on the growth of *Salmonella* when tested at various concentrations.

D41

NATURAL ALLIES IN THE WAR AGAINST ANTIBIOTIC RESISTANCE OF *SALMONELLA*

Jess Colmenero

"Salmonellosis is one of the most common and widely distributed foodborne diseases and is caused by the bacteria *Salmonella*. It is estimated that tens of millions of human cases occur worldwide every year and the disease results in more than [a] hundred thousand deaths." (WHO, 2016) Under the recognition of this serious endangerment and the growing degeneration of antibiotic effectiveness, this research is being conducted in a manner that reexamines the structures of natural products and their ability to kill or inhibit growth of *Salmonella*. The products being tested include pure essential oils such as fresh ginger, cinnamon, tea tree, lemongrass, eucalyptus, and fractionated coconut oil. The preliminary data suggests that tea tree oil is the most effective in high doses, although eucalyptus does present other interesting results. The long-term goal of this approach is to produce a library of natural, viable structures to use in creating new antibiotic possibilities, recognizing the potential of compounds currently in use, or producing a means of naturopathic treatment.

C1

CALORIC RESTRICTION IN *T. THERMOPHILA* BY LIMITING GLUCOSE AVAILABILITY

Jack Goldstein, Conor Kelly, Erica Molaro

Carbohydrates are a ubiquitous food source for all organisms - a necessity for the maintenance of organismal health. In humans, on one hand, the lack of glucose in the form of food is attributed to malnourishment and health decline. On the other, however, the overabundance of glucose is known to cause oxidative stress, contributing to senescence. Studies exploring caloric restriction in various model organisms have arisen within the past decade, in which organisms are provided a limited amount of food and the consequent impact on health is observed. Our group's aim was to study the effect of caloric restriction on *T. thermophila* on cell health, measurable through cell counts taken over three days at 24 hour intervals. *T. thermophila* were grown in cultures containing 0%, 20%, 40%, 60%, 80%, and 100% of a baseline amount (0.2% m/v) of glucose, established through experimentation with minimal proteose peptone media. We hypothesized that restricting glucose to 40% of the normal baseline would result in a higher sustained growth of *T. thermophila*. *T. thermophila* grown in cultures containing 0% and 20% of the baseline glucose exhibited higher cell densities than cells grown in cultures containing 80% and 100% glucose at 24, 48, and 72 hours for all counts taken. However, the hypothesis cannot be accepted because cultures of various glucose concentrations, at a maximum of 60% glucose, were suggestive of optimal growth, not just 40%.

C2

THE EFFECTS OF CAFFEINE ON WILD TYPE *TETRAHYMENA THERMOPHILA* CU428

Madison Adamthwaite, Jacob Donahoe, Anna Schneider

Caffeine's effect on the rate of phagocytosis in *Tetrahymena* was observed via the introduction of dissolved caffeine to the aqueous environment in which the *Tetrahymena* existed. Five different concentrations of caffeine 0mM, 1mM, 2.5mM, 5mM and 20mM were used to observe the rate of phagocytosis in *Tetrahymena* by looking at the number of food vacuoles filled with India ink and Red fluorescent beads over a 20 minute time period. In an environment absent of caffeine, the *Tetrahymena* showed the highest number of filled food vacuoles and thus the highest level of phagocytosis. It was found that caffeine concentrations do in fact inhibit the rate of phagocytosis in wild type CU428 *Tetrahymena thermophila*. It was observed that as the concentration of caffeine increased, phagocytosis decreased linearly. In addition, the rate of phagocytosis was found to increase with time in all conditions. The hypothesis that increasing the concentrations of caffeine will result in decreasing rates of phagocytosis is supported. Our findings can further be used to conclude the possible effects caffeine has on human cells.

C3

EFFECT OF ANTIHISTAMINE AND HISTAMINE CONCENTRATIONS ON THE RATE OF PHAGOCYTOSIS IN *TETRAHYMENA THERMOPHILA*

Samantha Daily-Malysa, Bronwyn Duffy, and Amanda Haeberle

Many people experience allergic responses to environmental irritants like pollen and bee stings. Histamine is a molecule that responds to these foreign molecules by inciting an inflammatory reaction. In comparison, antihistamine acts as an antagonist that inhibits histamine's response. In order to study the effects of histamine and antihistamine on eukaryotic phagocytosis, the model organism *Tetrahymena thermophila* was used. *T. thermophila* was exposed to four concentrations ranging from 1 μM to 1000 μM of histamine and antihistamine, respectively. At increments of ten minutes, we counted the number of food vacuoles in multiple cells to observe the rate of phagocytosis. Histamine was found to minimally increase food vacuole formation, while antihistamine was found to significantly reduce food vacuole formation at high concentrations. This data could be used to establish a relationship between eukaryotic responses to histamine and antihistamine. These results suggest that antihistamine reduces the rate of phagocytosis and histamine has no effect on the rate of phagocytosis in *T. thermophila*.

C4

EFFECTS OF ALCIAN BLUE AND CAFFEINE ON REGULATED SECRETION IN *TETRAHYMENA THERMOPHILA*

Madeline Lane, Nishika Virmani, Kristyn Waggoner

Caffeine and Alcian Blue induce regulated secretion in wild type *Tetrahymena Thermophila* (*T. thermophila*). Caffeine sensitizes the ryanodine receptors on the membrane through calcium induced mucocytosis. Alcian blue triggers mucocytosis through an unknown pathway. Mucocyst exocytosis is the most visible form of regulated secretion. The effects of caffeine on Alcian Blue induced regulated secretion were examined. *T. thermophila* were exposed to varying concentrations of Alcian blue and caffeine in an effort to induce a response. After several trials of differing conditions no regulated exocytosis was induced. This suggests that the pathway for regulated secretion is complex and requires specific conditions. To further investigate the effects of caffeine on *T. Thermophila*, wild type cells were also exposed to varying concentrations of only caffeine and changes in behavior and osmoregulation were recorded. The results were inconclusive and did not suggest any correlation between caffeine concentration and behavior.

C5

WILD TYPE VS. POC1Δ BASAL BODY EFFICIENCY: VISCOSITY'S EFFECT

Katie Abboud, Chaerin Lee, Maria Meihaus

In *Tetrahymena thermophila* cells, the Poc1 protein stabilizes basal bodies. When exposed to high temperature and high viscosity, the Poc1Δ strain, cells lacking the Poc1 protein, exhibit disorganized and destabilized basal bodies. Cilia, which extend from the foundational basal bodies, are then less effective in performing cell movement and phagocytosis. To test the effects of viscous solutions upon Poc1 deficient cells, four different groups were observed at differing time periods: wild type cells in SSP media, Poc1Δ cells in SSP media, Poc1Δ in PEO (a highly viscous solution), and wild type cells in PEO. Each group was introduced to red fluorescent, latex beads to induce phagocytosis. Basal body function was then inferred by observing both the amount of food vacuoles present in each sample and the organization of basal bodies within the cell. Immunostaining allowed for the observation of basal body organization underneath a fluorescent microscope, as well as the illumination of the fluorescent red latex beads, which were found within food vacuoles. The hypotheses included expectations of Poc1Δ cells in PEO solution to display the least amount of food vacuoles, while wild type cells in SSP solution were expected to exhibit the most, no matter the time period. The experimental results were inconclusive.

C6

CONJUGATION OF *TETRAHYMENA THERMOPHILA* STRAINS RAD51 AND EPC1 AND THE FREQUENCY OF THE RESULTING PHENOTYPES

Rachel Kastanek, Ethan Bailey, Charlotte Vonstein

This experiment investigated the outcomes of breeding two mutant strains of *Tetrahymena thermophila*, Rad51↑ mutant and Epc1 knockout, to examine which mutant phenotypes would be expressed in the progeny. The *T. thermophila* mutant strains were bred by way of sexual conjugation at 35°C and then examined for the absence of a macronucleus and atypical cell shapes. It was hypothesized that the progeny cells would exhibit both mutations due to the different gene locations of the mutations. The phenotype that was observed with the highest frequency were cells with a macronucleus and atypical cell shape. This indicated that the majority of the mutant progeny had inherited only one of the mutant phenotypes. However the statistical significance of our results was reduced due to large populations of inviable cells that caused a lack of consistent data. Therefore the hypothesis was neither confirmed nor rejected.

C7

INDUCED CBF EFFECTS ON BASAL BODY STABILITY IN POC1 DEFICIENT MUTANTS

Daniel Cox, Ronnie Jensen, Nigel Moore

This experiment aimed to determine whether certain chemical substances could directly alter basal body structure in mutant *Tetrahymena* via ciliary beating frequency. Research involved observations via inversion microscopy, immunostaining, and fluorescence microscopy to visualize chemically induced changes. During the experiment aggregation was observed in both the normal and mutant *Tetrahymena* strains in caffeinated environments. While the results of the initial experiment were uncertain, *Tetrahymena* aggregation under caffeinated conditions may suggest that caffeine can be used to physically manipulate selected groups of *Tetrahymena*, allowing more direct experimental control methods with the organism in future research.

C8

INVESTIGATION OF WILD TYPE PHENOTYPE RECLAMATION FROM A RAD51 OVEREXPRESSING MUTANT

Phillip J. Martinez, Dasha T. Cogswell, Allyson B. Kang

Model organism *Tetrahymena thermophila's* meiotic cycle has been well characterized, however the molecular role of the overexpression of double stranded break (DSB) repair enzyme Rad51p on macronuclear division has not been well understood. An overexpression of Rad51p in mammalian cells has been implicated to contribute to the development of malignancy in particular cancers [1,2] but little is known about how the overexpression of Rad51p in *T. thermophila* effects the behavior of the cell on a molecular basis. Past studies [3,4] have implicated an underexpression of Rad51p to be linked with micronuclear deterioration as well as meiosis I arrest most likely due to Rad51's role in DSB repair. Overexpression of Rad51p however, has been observed to inhibit the organism's ability to replicate the macronucleus during cell cycle at higher temperatures resulting in the absence of a macronucleus in one of the daughter cells after each division. Here, we observe a small fraction of the mutant strain at higher temperatures gradually obtain the wild type phenotype over the course of six weeks while still maintaining a high level of Rad51p expression. This could suggest that, with the negative pressure of high temperatures, some of the population can regain the ability to regulate cell cycle successfully even in the presence of elevated levels of Rad51.

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C9

IMPACT OF ARTIFICIAL SWEETENER ON *T. THERMOPHILA*

Mehdi Adibi, Joshua Mak, Shawn D'Souza

Tetrahymena thermophila are unique eukaryotic organisms which can mitotically divide under optimal conditions or meiotically fuse under stressful conditions. This experiment focused on the mitotic division of Tetrahymena strain B2086. cAMP, an effector molecule for a multitude of processes, has been linked to regulating mitosis of *Tetrahymena*. The introduction of certain chemicals have been found to affect the production of cAMP in *Tetrahymena*. Lower cAMP concentrations are known to increase *Tetrahymena* division rates whereas higher cAMP concentrations have opposite effects. Basic mechanisms of these chemical pathways have been mapped for caffeine and glucose. The question explored was how Splenda, an artificial sweetener, affects mitotic division rates of *Tetrahymena thermophila*. This experiment also explores if a basic mechanism can be mapped out for Splenda cAMP pathway based on the experimental results and literature pathways for glucose and caffeine. Results will shed light on the effects of artificial sweeteners on division of eukaryotic cells and validate if these substances are safe for long term consumption by eukaryotes, specifically humans. Due to inability to metabolize the components of Splenda, its use as an energy source is inhibited. This should lead to the opposite effects of glucose, an energy source of cells, and decrease mitotic rate due to an increase in cAMP. The results validated this hypothesis. Splenda showed a reduced rate of division similar to the control caffeine. Without specific biochemical analysis, determination of how Splenda act on the cAMP pathway remains unknown, however, we can speculate cAMP increased similar to caffeine's effect.

C10

MICROTUBULE INHIBITION SHOWS DIFFERENCE BETWEEN WILD-TYPE STRAINS OF *TETRAHYMENA THERMOPHILA*

Matthew Genelin, Kendal Osborn, Ashleigh Van Deusen

Two wild type strains of *Tetrahymena thermophila*, CU428 and B2086, were treated with Colchicine and the rates of phagocytosis and exocytosis were observed to compare both strains. The role of microtubules, a component of the cytoskeleton, is not well defined. To provide insight of their function, the two strains of *Tetrahymena thermophila* were incubated in Colchicine, a cytoskeletal drug that inhibits further dimer polymerization of microtubules. The two strains of *Tetrahymena* were first incubated in India ink to visualize uptake. Later, they were exposed to Colchicine and incubated in carmine dye. Due to the difference in color of carmine dye and India ink, food vacuoles that were recently phagocytosed and those that were recently exocytosed became distinguishable. It was hypothesized that when colchicine is added to *Tetrahymena thermophila* CU428 and B2086, phagocytosis will be inhibited, showing few to no red food vacuoles in the cells, and exocytosis will also be affected with more black food vacuoles remaining inside the cells. The results show that phagocytosis was significantly inhibited when cells were incubated with colchicine and exocytosis of the black food vacuoles was also hindered. These results suggest that microtubules may play a role in both processes. Between the two wild type strains of *Tetrahymena thermophila*, there are slight differences in the retention of black vacuoles as well as the uptake of red vacuoles after treatment with Colchicine: strain CU428 has lower retention and higher uptake, while B2086 has higher retention and lower uptake.

C11

INDUCING ENDOSYMBIOSIS BETWEEN E. *COLI* AND TETRAHYMENA *THERMOPHILA* USING DIFFERENT ENVIRONMENTAL STRESSORS.

San Ho. Alyssia Lam. Nate Moore

In previous experiments, when studying an endosymbiotic relationship between Tetrahymena *thermophila,* and E. *coli,* it took multiple years of lab time and resources just to allow these two organisms to start an endosymbiotic relationship. The amount of time it takes to make this relationship capable of study makes these types of experiments rare and expensive. The purpose of our experiment was to try to induce the two types of bacteria, into an endosymbiotic relationship by stressing the Tetrahymena with multiple environmental stressors. Throughout six consecutive weeks the Tetrahymena environment was changed by temperature, pH, nutrients and UVC radiation in separate cultures to attempt to force them into an endosymbiotic relationship with the E. *coli*. This experiment showed that in a relatively small sample size of cells, all of the stressors showed no significant increase in the amount of fluorescent E. *coli* in the Tetrahymena cells. Despite having observed a small number of tetrahymena cells with E. Coli harboring inside them, we were unable to determine whether these E. Coli cells are still viable and can still replicate. The number differences were not significant enough to determine that these stressors were able to induce an endosymbiotic relationship between E. *coli* and Tetrahymena *thermophila,* and a longer period of time and more replication cycles would be needed to establish this relationship.

C12

THE IMPACTS OF HEAT ON POC 1 DEFICIENT TETRAHYMENA

Kaylynn Fernando, Mark Fisher, Michelle Reynaud

We took the poc1Δ strain of tetrahymena and exposed them to an array of different temperatures ranging from room temperature all the way to 40° celsius. The poc1 deficient cells have a temperature sensitive lethal phenotype, making them an ideal subject for our experiment. Their temperature sensitivity reduces basal body frequency, organizational defects in basal bodies, and ultimately loss of oral apparatuses. The basis of our experiment was to observe how phagocytosis and the nuclei of the cells were affected by changing the temperature of their environment compared to other wild type strains. We exposed the cells to each temperature for 15 minutes, and then observed how many food vacuoles they made for 30 minutes. Our main finding was that at 36° celsius the poc 1 deficient strain began to have abnormal trends in food vacuole production, but they did not cease to phagocytize until 40° celsius which is the same temperature as all tetrahymena wild type strains die. This work provides insights into how poc1Δ tetrahymena functions at different temperatures and how some of the trends might not be what is expected.

C13

EFFECTS OF RAD51 OVEREXPRESSION ON *TETRAHYMENA THERMOPHILA*

Sam Avoian, Michelle Chiok, Katie Priest

In this study, the effect of Rad51 overexpression on *Tetrahymena thermophila* was

examined, specifically in relation to the cell's ability to carry out phagocytosis. Initially, the size of Rad51 overexpressed cells was compared with that of CU428 wild type cells. Through this, it was determined that Rad51 overexpression, which results in loss of the macronucleus, yields cells of a smaller size than their wild type counterparts. The wild type cells are on average 1.17 times larger, and this difference was found to be statistically significant. Following this, the number of food vacuoles observed after zero, five, and ten minutes in the presence of food were compared in order to determine if Rad51 overexpression results in less efficient phagocytosis. Ultimately, it was found that there is indeed a significant difference between the Rad51 overexpressed cells and the CU428 wild type cells in this aspect, The Rad51 overexpression cells' rate of phagocytosis was 10.9% less than the CU428 wild type cells. This study provides new insight into the effects of Rad51 overexpression in *Tetrahymena*, specifically on the uptake and consumption of food particles, which is a vital part of cellular survival.

C14

DRUGS SUPPRESS APPETITE IN *TETRAHYMENA*: INHIBITION OF PHAGOCYTOSIS BY CYTOSKELETAL DRUGS

Shoshana Brown, Cole Leichty, Madison Paul

Actin and tubulin proteins, present in the cytoskeletons of most eukaryotic cells, are vital for mobility and cell division. In the single-celled model organism *Tetrahymena Thermophila,* actin and tubulin proteins have been detected near the oral groove and cilia, facilitating in the phagocytotic processes of the cells. Inhibiting these proteins through specialized drugs results in a decrease of phagocytosis, as observed through limited food vacuole formation over a duration of time. Previous studies have found that actin levels remain constant throughout the cell cycle, while concentrations of tubulin fluctuate during cell development, growth, and death. Based on this research, and with the knowledge that the cells from the cultures would vary in their developmental stages, this experiment was constructed with the proposal that the actin-targeting inhibitor drug, Cytochalasin B (5 mg/ml), would have more of an effect on phagocytosis of the cells than the tubulin-targeting inhibitor Colchicine (40 mg/ml). To test the hypothesis, cell cultures were exposed to five testing conditions: A (healthy concentration of Cytochalasin B), AC (high concentration of Cytochalasin B), T (healthy concentration of Colchicine), TC (high concentration of Colchicine), and NC (no drug exposure). Food vacuole formation was observed by administering India Ink to the cell cultures and fixing the cells at 1, 5, 10, and 20 minutes using formaldehyde. Averages from four trials revealed the A and AC cell exposures have less food vacuoles than the T and TC cell groups, supporting the hypothesis that Cytochalasin B limits phagocytosis greater than the Colchicine in *Tetrahymena*.

C15

SHEARING DECILIATION PROMOTES FASTER CILIA REGENERATION THAN DIBUCAINE DECILIATION

Tiera Mack, Laura Thomas, Sam Penhale

Our aim was to determine if dibucaine or shearing deciliation is more effective in removing oral and body cilia of *Tetrahymena thermophila* B2086, in our lab, given our time and materials constraints. The dibucaine method involved exposing cells to dibucaine; shearing involved making cilia rigid with ficoll mixture and passing cells through a needle to break cilia off. Following both techniques culture media was added to aid in recovery. We observed phagocytosis and cell/cilia motion for 80 minutes after performing each deciliation. Observations were made using videos and pictures of the cells taken every 10 minutes. For the dibucaine trials, our data indicated cells never fully recovered to the level of motion observed prior to deciliation. Cells in the shearing trials steadily recovered and appeared to regain full motion by 80 minutes. In phagocytosis, cells deciliated with dibucaine formed about one third of the vacuoles that ciliated cells formed. Sheared cells often formed double the number of vacuoles of those in the dibucaine trials. We interpret these results to mean our procedure for dibucaine deciliation affected Tetrahymena more than our procedure for shearing, possibly in a way that could prevent full recovery given any amount of time, and definitely preventing recovery within our expected window of 40-80 minutes based on prior research findings. We're hesitant to conclude which technique is more effective given the range of deciliation purposes in Tetrahymena research, but if cell survival and recovery is important to the experiment, the shearing method appears to be the better option.

C16

"CHAPPED LIPS", INVESTIGATION OF ORAL APPARATUS DEGRADATION IN NP1 *TETRAHYMENA*

Tanner Foster and Leanna Clarkson

The NP1 mutant strain of *Tetrahymena thermophila* is known to have a structured, functional oral apparatus at lower temperatures and a degraded, non-functional oral apparatus at higher temperatures. In contrast, the wild type (CU428) strain of *Tetrahymena thermophila* is known to have a structured, functional oral apparatus within the entire range of temperatures. This was investigated further by both quantitatively and qualitatively assessing oral apparatus degradation in the NP1 strain. Qualitatively, oral apparatus degradation was assessed in both strains by immunostaining the basal bodies of the cells with antiCentrin at each of the four temperatures listed previously in order to view the oral apparatus degradation under the fluorescent microscope. Quantitatively, phagocytosis rates (directly related to oral apparatus structure) were assessed in both strains at four different temperatures within the range of 28-37°C by incubating each strain at each temperature, then incubating each sample in India Ink for 15 minutes and counting the number of food vacuoles stained in 30 cells of each sample and averaging the counts. India Ink phagocytosis rates generally decreased as temperature increased in both strains. In the NP1 strain the phagocytosis rate decreased, increased, and then decreased dramatically at the critical temperature. Therefore, the oral apparatus in the NP1 cells was concluded to not function as well at 30°C, while it fully degrades at 37°C. This is relevant to humans because we also have ciliated cells, and this could help us understand how those cilia function at higher temperatures.

C17

THE NATURE OF POC1Δ *TETRAHYMENA* IN VISCOSITY

Brian List and Rachel Saggau

Motility in Tetrahymena is facilitated by cilia that are located on the outer surface of the cell and within the oral groove. Basal bodies are structures made up of triplet microtubules that anchor cilia with the help of proteins such as the microtubule regulator, poc1, which has been found to stabilize basal bodies through microtubule-binding. Poc1Δ Tetrahymena have been observed degrading in 30 to 38°C due to loss of cilia as result of unstable basal bodies that are affected by loss of the poc1 MAP protein. Through the observation of Poc1Δ mutant Tetrahymena in a charged depression slide to see galvanotaxis, with B2086 wild type Tetrahymena as a control, the direction of movement was controlled due to the depolarizing calcium channels in the organism. Tetrahymena were observed in different levels of viscosity created from serial dilutions made of 15% polyethylene oxide (PEO). Higher percentages of PEO should lead to faster degradation of Poc1Δ Tetrahymena with cells lysing or unable to move as quickly. With limited data due to problems with the galvanotaxis slide and complications that arose with microscope observation, this experiment proposes that higher viscosities put more strain on ciliary beating and lead to break down of the basal bodies in Poc1Δ Tetrahymena.

C18

THE ADMINISTRATION OF CYTOCHALASIN-B AND NOCODAZOLE INHIBITS PHAGOCYTOSIS IN *TETRAHYMENA THERMOPHILA*

Kyle Parmley, Rob Turner, Caitlin Vogt

The inability to form phagosomes through the process of phagocytosis in *Tetrahymena thermophila* has been linked to the addition of both Nocodazole and Cytochalasin-B. Variability in the quantity of phagosomes formed after addition of the two drugs has been observed at different concentrations; however, not all concentrations of both drugs have been tested. Thus, this lab set out to test varying concentrations of Nocodazole and Cytochalasin-B on wild-type *Tetrahymena thermophila*, strain B2086, which have never been tested before on this organism. This experiment provided insight on the ability of *Tetrahymena thermophila* to perform phagocytosis at these drug concentrations. The *Tetrahymena* were incubated in 7.5 ug/ml as well as15 ug/ml of Cytochalasin-B and 10 ug/ml as well as 25 ug/ml of Nocodazole. The dosage concentrations of 7.5 ug/ml and 15 ug/ml of Cytochalasin-B as well as 25 ug/ml of Nocodazole resulted in phagosome formation that fell between the results of the negative and positive controls. The dosage concentration of 10 ug/ml of Nocodazole had inconclusive results. These findings suggest that the addition of these concentrations of Nocodazole and Cytochalasin-B to *Tetrahymena thermophila* decrease in phagosome formation.

C19

THE EFFECTS OF CAFFEINE AS A STIMULANT ON *TETRAHYMENA THERMOPHILA*

Summer Foyle, Ezra Kurzban, Zachary Stensland

This experiment focused on the longitudinal effects of adding caffeine to a media containing Poc 1 tetrahymena thermophila as well as 2086 wild type in order to see differences among different types that could be present in an environment. We tested the caffeine threshold at which tetrahymena could no longer survive and found that at a concentration of caffeine over 0.001M in a concentration of wild type tetrahymena at 19.2x10^4 cells/mL as well as in a concentration of Poc 1 mutated strain at a concentration of 14.4x10^4 cells/mL, died at approximately the same rate as cells not treated with caffeine. We also tested food vacuole formation to see if the addition of caffeine was making the cells more metabolically activity than the control group. Staining the cells with tubulin tracker green helped us to see the physical effects of caffeine on basal body formation. From these experiments we were able to conclude that at a concentration of 0.001M the tetrahymena are less likely to uptake the india ink we presented to them as food as the group that was not treated with caffeine. Staining with tubulin tracker green after treating the cells both with and without caffeine we could not find any significant differences that lead us to believe that caffeine had an effect on the formation or performance of the basal bodies.

C20

THE ROLE OF CALCIUM IN THE REPARATION AND INHIBITION OF ACTIN FILAMENTS BY CYTOCHALASIN B IN *TETRAHYMENA (CU428 WT)*

Rachael Hempy, Levent Ozdemir, and Simone Vigil

Cytochalasin B has been shown to reduce the formation of food vacuoles (i.e., the rate of phagocytosis) in wild-type *Tetrahymena*. Calcium has been shown to upregulate the rate of phagocytosis. If calcium is able to upregulate phagocytosis, it may then be able to compete with cytochalasin B if present in a high enough concentration. This would have great implications for cancer patients undergoing chemotherapeutic treatment with cytochalasin supplementation. Cytochalasins have shown great promise in improving efficacy rates and reducing the prevalence of drug resistance when used to supplement current chemotherapeutic treatments.

This research hoped to show that in the presence of a high concentration of calcium, cytochalasin B is unable to reduce the formation of food vacuoles effectively. The belief being that focusing on the regulation of calcium in cancer patients and supplementing their treatment with cytochalasins may lead to faster recovery times and less drug resistance. Wild-type *Tetrahymena (Cu428WT)* was chosen as the research subject as it grows quickly and exhibits similar processes to human cells. The *Tetrahymena* were tested at various time intervals (0, 5, and 10 minutes) with varying concentrations of $CaCl_2$ to confirm that calcium in fact did play a role in upregulating phagocytosis. The highest concentration of $CaCl_2$ [10^{-3}] was then tested in the presence of cytochalasin B [50 ug/ml]. The results were not conclusive, but did indicate what future research could improve upon.

C21

INHIBITING *ESCHERICHIA COLI* COLONY GROWTH *IN VITRO* WITH *TETRAHYMENA THERMOPHILA*

Emily Oddo, Emily Oldani, and Elena Patera

Bacterial infections are an increasing problem worldwide as antibiotic resistance is increasing in frequency and antibiotic discovery is decreasing. Antibiotic resistance is a problem caused by natural selection, and the rate of its occurrence is increased by the overuse of antibiotics in industrial fields as well as improper prescription use. In this research, *Tetrahymena thermophila* was introduced in three different concentrations to two systems of media that were designed to mimic the conditions of the human small intestine and ileum in temperature and acidity with added *Escherichia coli*. In the experiment, *T. thermophila* health and their phagocytosis of the *E. coli* cells were monitored. The hypothesized results were a decimated or weakened *E. coli* colony, which was characterized by a high rate of phagocytosis and a population of *T. thermophila* that began the experiment appearing to be healthy and then was weakened significantly. It was found that the *T. thermophila* were able to have health comparable to that of the control in the media that mimicked both the small intestine and ileum, and that they were effective in reducing the amount of *E. coli* present in the media. The only variable that demonstrated a positive correlation was the difference in pH, rather than the concentration of *T. thermophila*. The results of this experiment on the response of *T. thermophila* to conditions that mimic the human GI tract can be utilized in furthering research towards finding an alternative to traditional antibiotic use to weaken or eliminate a severe bacterial infection.

C22

MICROTUBULES: A MOLECULAR MISSION WITHIN VACUOLAR MEMBRANE TRANSPORT

Caitlyn Cochran, Jessica Hartley, Kevyn Jackson

Vacuolar membrane transport is an essential process that relies on cytoskeletal dynamics that are conserved among many organisms, both prokaryotes and eukaryotes. The present study evaluates the role of microtubules, an essential cytoskeletal element, in the cellular transport processes of phagocytosis and exocytosis in *Tetrahymena thermophila*. *T. thermophila,* a ciliated unicellular protozoa, was used as the model organism because it grows readily in culture, is accessible to students, and has a wide range of functions that can be modified and observed. Phagocytosis and exocytosis are the mechanisms by which *T. thermophila* ingest food particles from extracellular space and excrete undigested waste. To determine whether microtubules play a vital role in phagocytosis and exocytosis in *T. thermophila,* we monitored the formation of carmine stained vesicles and the loss of India-ink stained vesicles over time in the presence of and absence of two microtubule inhibitors Colchicine and Nocodazole. Colchicine inhibits microtubule polymerization by interacting with the ends of the microtubules, whereas Nocodazole binds to tubulin and quickly depolymerizes microtubules in cells. The use of microtubule inhibitors to alter natural microtubule assembly can help us to determine whether they are a key component in transport processes. This experiment demonstrated that the presence of microtubule inhibitors altered both phagocytosis and exocytosis in *T. thermophila* when compared to cells with no inhibitors. Our results support the idea that both phagocytosis and exocytosis may be microtubule-dependent physiological processes.

C23

GLYCOGEN AND AMYLOSE AS CHEMOTAXIC AGENTS IN *TETRAHYMENA THERMOPHILA*

Katherine Rose and Allison Haun

The use of ciliates as biosensors in the health and food industry for the detection of certain chemicals has become a topic of increasing interest within the scientific community. Chemotaxis, the movement of a cellular organism dictated by a distinct molecular gradient of chemicals in the environment is categorized as either attractive or repulsive and allows for highly specific directional movement of these cells. Tetrahymena *thermophila* are sensitive to chemotaxic agents such as glucose and fructose, which can elicit attractive and repulsive movement in the cells, respectively. In our experiment, two additional saccharides, glycogen and amylose, were tested as possible chemotaxic agents for tetrahymena. We recorded tetrahymena motility over a short period of time in respect to cell count using capillary tubes to observe the addition of diluted saccharide samples. Upon final analysis, only amylose resulted in strong cell movements (total number) toward the samples, leading us to conclude that it elicits a likely chemotactic-attractive effect on Tetrahymena *thermophila*.

C24

SURVIVAL OF FITTEST: *THERMOPHILA* VS. *VORAX* AND THE EFFECTS OF CON. A INTERFERENCE

Charly Mendoza, Lindsay Nikolaeff, Yishi Wang

The macrostome formation of the tetrahymena vorax is an area that hasn't been as studied or manipulated like the thermophila strain. In order to induce the macrostome change, the tetrahymena vorax is exposed to a unique chemical signal called stomatin. Stomatin is a chemical component within the thermophilic strain that acts as an inducer for the vorax strain to undergo a conformational change in order to promote the survival ability of the vorax strain if it lacks an appropriate food source such as E. coli. Analysis of cellular interactions between vorax, thermophila, and p-glo e-coli support the confirmation of the transformation of the vorax from the microstome to the macrostome form. This induced change allows vorax to now be able to consume the thermophila but it can't consume e-coli. Concanavalin A was found to be an active inhibitor to the transformed vorax macrostome. Con A's role in this investigation is that this chemical component basically come sin and causes an interference in the intercellular communication between the vorax itself and its cellular membrane. Once this interference is present, the macrostome form vorax can't consume anything. Based on this, the inhibitor is used to see whether or not the experimental vorax could be starved while in its macrostome formation while in the presence of E. coli and thermophila. The main findings are that the concanavalin A did inhibit the proficiency of the vorax macrostome to be able to properly capture and digest

thermophila cells at a high concentration within its environment and as a result the exposed vorax sample starved.

C25

VIABILITY OF RAD51 OVEREXPRESSION MUTANTS

Monica Pilewskie, Robert Spradlin, Emily Tran

In eukaryotic DNA replication, double-stranded DNA breaks are formed by the RAD51 protein. A mutation that causes an overexpression of RAD51 is correlated with cancer in mammalian cells, but viability of cells with this mutation is unknown. Tetrahymena with a RAD51 overexpression mutation were observed in order to determine the viability of cells without a macronucleus. This research will further the understanding of the role of RAD51 and homologous recombination in eukaryotes. The research was conducted by observing viability and function of wildtype and mutant Tetrahymena overexpressing RAD51. Viability of the cells was measured using a hemocytometer and Trypan Blue, phagocytosis was observed with India ink, and the presence of a macronucleus and micronucleus was observed using DAPI staining. The results of the experiment show that the wildtype strain was generally more viable than the mutant strain after incubation at 35° C. The data shows that the wildtype Tetrahymena were more resistant to stress than the mutants, but that basic functions within the mutants, such as phagocytosis, remained unaffected.

C26

MUTATED ORAL GROOVE MAY ENHANCE PHAGOCYTIC RATE IN NP1 *TETRAHYMENA*

Kimberly Lugo, Jessica Miller, Phil Rubin

The goal of our experimentation was to characterize NP1 mutant *Tetrahymena thermophila*, which experience temperature dependent malformation of the oral groove at 37°C. We were interested in assessing how malformation of the oral groove affects phagocytosis, and whether this mutation occurs gradually with increasing temperature, or if there is a sharp cutoff at 37°C. To analyze structural differences in basal body alignment in NP1 mutants and wild type *Tetrahymena*, we fluorescently labeled centrin protein via immunocytochemistry. We stained NP1 and wild type cells with anti-centrin antibody, tagged by red fluorescence. This antibody labeled basal bodies, a fundamental component of the oral groove. To assess how the NP1 mutation affects phagocytosis, we observed the uptake of India Ink in both cell types and quantified phagocytosis through the rate of food vacuole formation over time. We performed this phagocytosis protocol at multiple temperature points, via heating in an incubator as well as a heat block. Through multiple trials of phagocytosis in NP1 mutants and wild type *Tetrahymena* at varying temperatures, we consistently observed higher rates of food vacuole formation in NP1 mutants compared to wild type. This was supported through observation of fundamental differences between the structure of the oral groove in mutants as opposed to wild type cells after fluorescent labeling. Based on these results, we can tentatively conclude that the phenotypic difference in structure of the oral groove in NP1 mutants is advantageous, and enhances the uptake of food particles.

C27

CYTOSKELETAL INHIBITOR EFFECT ON PHAGOCYTIC ACTIVITY IN *TETRAHYMENA*

Rachel Walker, Matthew Weber, Maria Nino

Cytoskeletal inhibitors affect specific cellular processes in different ways. Phagocytosis, a specialized form of endocytosis, is one method of ingesting extracellular material and exocytosis is a process in which intracellular vesicles fuse with the plasma membrane and release their contents into the extracellular space. Inhibiting either actin or microtubules is known to decrease phagocytic activity in *Tetrahymena thermophila*. In this experiment, we tested the effects of inhibitors in different concentrations on each cytoskeletal element, Colchicine (microtubules) and Cytochalasin (actin).

To determine how a range of Colchicine and Cytochalasin concentrations affected phagocytosis and exocytosis, we conducted a pulse-chase experiment in which the number of vacuoles previously stained with India ink were counted over time as a measure of exocytosis while the red vacuoles were simultaneously being taken into the cell from Carmine dye added to the cell solution were counted to measure phagocytosis. A decrease in the number of red vacuoles formed over time suggests an inability to undergo phagocytosis and a constant number of black vacuoles shows impaired exocytosis.

For the Colchicine trials, the number of red vacuoles decreased as we added more inhibitor, while the number of black vacuoles did not change much. For the Cytochalasin trials, the number of red vacuoles

decreased as we added more of the inhibitor while the number of black vacuoles did not change much. We noticed that generally as the concentration increased, the number of red and black vacuoles decreased, although the number of black vacuoles remained more constant in the Colchicine trials.

C28

FOOD VACUOLE FORMATION IN *T. THERMOPHILA* IN HIGH NaCl ENVIRONMENTS

Luke Lemons, Sydney E. Sontag, Courtney Sowa

Tetrahymena thermophila are single-celled freshwater Eukaryotes living in ponds and rivers. In this experiment, we explore the effects of increased extracellular amounts of NaCl on *T. thermophila*. Our motivation for this experiment was to extrapolate our data to predict future effects that NaCl based pollutants bring to *T. thermophila* and microbial ecosystems. We incubated *T. thermophila* in 0.05 M NaCl, 0.1 M NaCl, 0.15 M NaCl for 15 minutes at room temperature to induce osmotic stress in the *T. thermophila*. To more easily view the food vacuoles, we incubated our *T. thermophila* in india ink for 5, 10, 15, and 20 minutes. At each time, we observed the number of food vacuoles present, and where the vacuoles were relative to the membrane and oral groove.
We found that as external osmolarity increased, the rate of food vacuole formation decreased. Across the concentrations tested, food vacuole formation slowed over time. The location of food vacuoles did not vary based on the concentration of NaCl that they were exposed to. Based off our experimental results, it appears that while NaCl concentration influences vacuole formation, it does not impact the vacuole traffic patterns. It would be good to test the response to high extracellular concentrations of other salts to determine whether the NaCl drew out the water of the cytoplasm or interfered with ion-dependent complexes in the cell. Also, the impact of freshwater environments being salinated could potentially be measured using the above method.

C29

MANIPULATION OF ENVIRONMENTAL FACTORS OF *TETRAHYMENA THERMOPHILA* PROVIDES FURTHER INSIGHT INTO THE ROLE OF RAD51 IN CELLULAR DIVISION AND DNA REPAIR

Kyla Foster, Rachel Mahoney, and Anne Waddle

Tetrahymena thermophila are single-celled, eukaryotic, protozoan that have been widely researched because they are an ideal eukaryotic model in both their phylogenetic relations to other organisms as well as their logistical advantages in the laboratory setting. Rad51 is a well characterized, double-stranded DNA break repair protein found in eukaryotes. *Tetrahymena* were used as a model organism to examine how the manipulation of environmental factors affects Rad51's role in *Tetrahymena*. Wild type and Rad51-overexpressing *Tetrahymena* cells were incubated over a range of heightened temperatures to examine Rad51's impact on macronuclear formation. At increased temperatures, the Rad51-overexpressing *Tetrahymena* had inconsistent formation of both nuclei as compared to wild type. These results suggest that when Rad51-overexpressing *Tetrahymena* are subject to increased temperatures, it severely hinders a portion of the population from undergoing successful cellular division. Rad51's role was also explored through *Tetrahymena's* response to the induction of double-stranded DNA breaks with a chemical mutagen. Fluorescence imaging was utilized to qualitatively hypothesize that upon induction of DNA damage, the expression of Rad51 increased in the nucleus. These experiments furthered understanding of Rad51's role and response to stressed conditions, in this case, increased temperature and exposure to mutagens. In a broader sense, these experiments suggest the idea that due to *Tetrahymena's* native habitat of freshwater ponds, the expression of Rad51 is likely highly regulated and that the protein is a critical component of the DNA repair pathway of *Tetrahymena thermophila*.

C30

HOW MOLECULE SIZE INFLUENCES THE RATE OF PHAGOCYTOSIS IN *TETRAHYMENA*

Sydney Foster, Michael Phoutrides, Olivia Squalls

One of the most essential functions of *Tetrahymena* is its ability to take in food using phagocytosis. Previous research suggests that particle size does not affect the rate of phagocytosis. We have decided to explore this idea further in hopes of verifying the validity of this statement. We have designed an experiment that explores the effect of Cytochalasin,(an actin inhibitor) and particle size on tetrahymena in order to test whether particle size affects phagocytosis. To address this question, we have analyzed how particle size influenced the rate of phagocytosis in *Tetrahymena*. We used different sized species including biotic/abiotic factors which included:

GFP labeled *E. Coli*, Red Fluorescent Beads, India Ink, and Carmine Ink. We quantified our data by counting the number of food vacuoles formed in each *tetrahymena* and recorded the amount of vacuoles formed at 1, 5, 10, and 20 minutes for each variable. We hypothesized correctly that the rate of phagocytosis in *tetrahymena* would decrease as the size of the particle increases. When comparing India Ink and Carmine, India Ink formed a relatively large amount of uniformly spherical vacuoles, whereas Carmine formed a smaller amount of less uniform more mosaic looking vacuoles. When comparing the red fluorescent beads and GFP labeled E. Coli, many more E. Coli vacuoles were formed then beads, however, the vacuoles formed with the beads were larger than the E. Coli vacuoles. We incorporated a second component into our experiment where we introduced Cytochalasin (actin inhibitor) along with one variable to the tetrahymena cell. We also hypothesized correctly that when the cytochalasin was introduced, the amount of food vacuoles formed would decrease dramatically. When a variable was coupled with cytochalasin, an average of less than one vacuole was formed for all of the times (1,5,10,20).

C31

POC1 Δ MUTANT HAS A HIGH CELL MOTILITY AT LOW VISCOSITIES

Laura Dunn, Claire Gerland and Meaghan Mannel.

The model organism *Tetrahymena thermophila* is a unicellular eukaryote used to inhabiting fresh water where it uses cilia for cell movement. Basal bodies help to stabilize the cilia of *tetrahymena* and are very important in the locomotion of the cell. The Poc1 gene stabilizes the basal bodies, providing the mechanism for movement and allowing the cell to move through viscous solutions of polyethylene oxide. There is an unknown point where the cell can begin to move between 0% and 3%. Various polyethylene oxide solutions were created by diluting 15% polyethylene oxide and combining it independently with B2086 and the Poc1 Δ mutant. These solutions were then compared under a DME microscope and analyzed using Fiji Software. It was found that the Poc1 Δ mutant was able to move in a 2.5% polyethylene oxide solution and also moved more efficiently in a 2% and 1% solution than the B2086 wildtype strain of *Tetrahymena thermophila*. These results provide a asis for further research, investigating how the lac Poc1 gene in tetrahymena has allowed the Poc1 Δ mutant to move more efficiently in different viscosities compared to B2086.

C32

TETRAHYMENA THERMOPHILA AS AN EFFECTIVE CONTROL FOR *ESCHERICHIA COLI* POPULATIONS

Molly Slack and Jared Darling-Munson

Escherichia coli (*E. coli*) are a Gram-negative bacteria, commonly found in the soil and many aqueous environments around the world. They have also been found to residing in the lower gastrointestinal tract of many mammalian species in a symbiotic manner. However, if *E. coli* are introduced to the upper GI tract, they tend to have a pathogenic effect on humans. Due to their widespread presence in water reservoirs and many environments where food is mass produced, it would be beneficial to find a method which can effectively control the population of *E. coli* in a closed ecosystem. This article examines the possibility of controlling such populations by introducing a specific ratio of the protist organism *Tetrahymena thermophila* (*T. thermophila*) to the environment. To determine the mean rate at which *T. thermophila* consumed *E.coli*, multiple solutions containing variable ratios of the two organisms were incubated for 120 minutes. The final concentration of *E. coli* was compared to the initial concentration in order to determine the remaining percentage of the *E. coli* population. *T. thermophila* was observed to consistently predate upon *E. coli* in a controlled environment, to the point of nearly eradicating the the bacteria from the media. Use of a non-pathogenic organism to control populations of pathogenic organisms is prefered over the use of non-specific antibiotics, since it reduces the likelihood of developing a “superbug” bacteria through overuse of antibiotics.

C33

THE EFFECTS OF MIRACLE-GRO® ON OSMOREGULATION RATES IN *TETRAHYMENA THERMOPHILA*

Eric Klostermann, Kelsey Arbogast, McKenna Russen

Agricultural fertilizer runoff leaches excess nitrate into the environment. This disrupts the nitrogen cycle, allowing nitrate to accumulate within aquatic ecosystems and groundwater causing organismal death in humans and aquatic life. *Tetrahymena thermophila* is a model organism and many cellular systems are similar to those of human cells. If *T. thermophila* are

subjected to nitrogen-based fertilizer then slower vacuole contraction rates will be observed due to a decreased pH and excess nitrate ions in solution. Nitrate molecules accumulate within *T. thermophila* and block channels that secrete toxins leading to toxic accumulation and death. We observed *T. thermophila* and the contraction rates of their contractile vacuoles when exposed to varying concentrations of Miracle-Gro® to measure the health of the organism. Two factors within the *T. thermophila* contributed to cell death: low pH and excess nitrate ions. Our results suggest a connection between minute amounts of fertilizer and deleterious effects on eukaryotic cells. *T. thermophila's* inability to survive in the presence of excess nitrate ions suggests a link between environmental contamination and negative health side effects in humans and aquatic life.

C34

EFFECTS OF CONJUGATION OF *TETRAHYMENA THERMOPHILA* ON GROWTH INHIBITION BY THE ANTIMICROBIAL TOXIN TRICLOSAN

Luke Camp, Stefan Grampp, Karim Mahmoud

Triclosan (TCS) is a xenobiotic chemical frequently used as an antimicrobial agent. Triclosan has been widely used in personal care products and has been found in soil, surface waters, and human tissues. In recent years, concerns about triclosan toxicity have arisen, and it has been confirmed toxic to a number of organisms including various tetrahymena, small invertebrates, fish, and amphibians. Due to its relatively high genetic similarity to humans, the unicellular eukaryote *T. thermophila* is often used in toxicity assays for water contaminants. Another unique property of *T. thermophila* is its ability to reproduce clonally while accumulating mutations or other alleles in the micronucleus, resulting in hidden genetic diversity. We investigated whether the hidden genetic diversity of *T. thermophila* populations can confer increased resistance to TCS toxicity. Two strains of *T. thermophila* (CU428, B2086) were mixed and conjugation was induced, allowing the previously hidden genotypes to be expressed. The mixed cultures were placed in growth media containing low concentrations of triclosan. It was expected that clonal tetrahymena populations (unconjugated) would be more sensitive to triclosan because of reduced genetic diversity. The results were inconclusive, because the *T. thermophila* concentrations were not affected to a significant extent by the triclosan concentrations used. The B2086 clonal population was more affected by triclosan addition than the conjugated culture, but the CU428 clonal strain and the conjugated culture showed very similar results.

C35

FLUORESCENCE MICROSCOPY STUDIES OF COLCHICINE AND MICROTUBULES DURING *TETRAHYMENA THERMOPHILA* CONJUGATION

Ambria Benavides, KimNgan (Lina) Tat, Ty Miller

Tetrahymena thermophila reproduce through binary fission, but under starved conditions, they engage in conjugation to exchange the genetic materials in their micronucleus which increases their chance of survival. During conjugation, microtubules maintain the stability of the cell structure through the formation of a pronuclear transfer basket around the pronucleus. Other studies have examined different proteins and nucleic acids involvement in conjugation of *Tetrahymena thermophila,* but the role of microtubules have not been thoroughly investigated. Colchicine is a drug that enhances microtubule depolymerization by binding to the beta subunit which will inhibit further addition of tubulin. Without the microtubules envelopment of the pronucleus, the pronucleus would be unable to move and fuse with the pairing cell's pronucleus to form a new micronucleus which would induce apoptosis. Because colchicine is a microtubules depolymerization enhancer, we hypothesized that in the presence of high concentration of colchicine, *Tetrahymena thermophila* would fail to conjugation or apoptosis. We analyzed the effect of microtubules depolymerization on conjugation by exposing the conjugating wild-type CU428 and B2086 cells to different colchicine concentrations and observed these depolymerization effects using Fluorescence Microscopy. In this study, our result was inconclusive but we greatly enhanced our protocol for future experiment.

C36

DOES THE ADDITION OF EPINEPHRINE TO *TETRAHYMENA THERMOPHILA* CELL ENVIRONMENT CHANGE THE RATE OF PHAGOCYTOSIS?

Shivani Dixit, Paul Charles, Oscar Whitney

Tetrahymena thermophila (Tetrahymena) endogenously produce hormones, such as epinephrine, also found in higher eukaryotes, but the function of those hormones in *Tetrahymena* is still largely

unknown. Other catecholamines, have been found to lower the rate of phagocytosis in *Tetrahymena*, and therefore it follows that epinephrine might function similarly. Epinephrine has been found to "imprint" extracellular membrane receptors, which change the hormone concentration within the cell. This change may affect more complex cellular processes such as phagocytosis. The number of food vacuoles present in 10-20 cells at various time intervals were counted and then averaged in order to quantify the change in rate of phagocytosis. Using *Tetrahymena* B2086, a negative control (no addition of hormone) was run along with two positive controls: addition of 100 mM epinephrine and addition of 100 mM diphenhydramine. The two experimental trials added 200 uM and 400 uM epinephrine to *Tetrahymena* B2086. The student T-test was used to inferentially analyze differences between the observed trends in the data. These analysis show there was no significant difference in the rate of phagocytosis. Therefore, it cannot be concluded that the addition of epinephrine decreases the rate of phagocytosis in *Tetrahymena*. This could suggest that epinephrine is not actively involved in regulating phagocytosis or the cells did not uptake any epinephrine. Further research can be conducted to propose a different mechanism by which hormones are introduced into a *Tetrahymena* cell.

M1

KNOCKOUT OF *XENOPUS LAEVIS AFF4* USING CRISPR-CAS9 MUTAGENESIS: A POTENTIAL LEUKEMIA DRUG TARGET

Joe Englbrecht

Human AFF4 protein is crucial for successful transcription by poised RNA Polymerase II upon stress, as well as for the stability of the Super Elongation Complex. Recent research on infant acute lymphoblastic and mixed lineage leukemia suggests the *AFF4* gene is a key regulator of its pathogenesis, thus making it a potential target for drug therapy in patients. The homologous *Aff4* gene in the frog, *Xenopus laevis,* may be used to investigate the effects of different levels of *Aff4* expression using loss and gain of function studies and gene expression analysis. *X. laevis* embryos allow for a much quicker and cheaper process compared to using human or mouse embryos. Currently, there are no publications on *Xenopus laevis Aff4*. To determine the function of *Aff4* in *Xenopus laevis*, the CRISPR-Cas9 gene editing technique was employed with the goal of knocking out the *Aff4* in a single cell embryo, and observing the effects on development. A guide RNA construct to specifically target both variants of *Xenopus laevis Aff4* gene was designed and in vitro transcribed guide RNA complexed with Cas9 enzyme was injected into one-cell stage *Xenopus* embryos. In order to further characterize *Aff4* expression, an *Aff4* specific probe was generated for in situ hybridization analysis. Characterization of *Aff4* function in early embryogenesis may allow for further understanding of pathways linked to the pathogenesis of leukemia and drug screens for treatments using *Xenopus laevis* as a model organism.

M2

CRISPR+CAS9 MUTAGENESIS OF TUMOR PROTEIN P63 REGULATED 1-LIKE

Julia Mo

The course objective was to mutate a gene of interest in *Xenopus laevis* to elucidate its function and characterize its expression pattern. I chose Tumor protein p63-regulated protein 1-like (TPRG1l) because there were no prior studies done on it in frogs. The papers published on the human and mouse TPRG1l homologs have not provided a definitive function, but have proposed a broad range of functions. It's speculated that TPRG1l is a presynaptic protein, a marker for familial idiopathic scoliosis, and is a novel microRNA-21 target in liver fluke-associated Cholangiocarcinoma. Taking advantage of *Xenopus laevis* as a model, we may gain insight into TPRG1l function. The CRISPR + CAS9 endonuclease system was used to target the gene in frog embryos to mutate the gene to become nonfunctional. Any phenotype that may arise may provide evidence of the gene's function. I picked a guideRNA target sequence specific to *Xenopus* TPRG1l and cloned it into DR274 plasmid. I synthesized a single stranded guideRNA by in vitro transcription and injected this guideRNA together with Cas9 enzyme into 1-cell stage frog embryos and observed development until tailbud stages. Along with mutagenesis, our goal was to characterize the expression pattern of the gene via in-situ hybridization. To this end, I amplified the coding sequence of TPRG1l by PCR and cloned it into another expression vector to synthesize an anti-sense in situ probe. The results will corroborate the gene's function in conjunction with any mutant phenotype observed.

M3

FUNCTIONALLY LINKING *REELIN* TO OTOSCLEROSIS USING CRISPR/CAS9 MUTAGENESIS

Andrew W. Hansen

Reelin (*RELN*), which encodes a soluble extracellular glycoprotein similar to those involved in cell adhesion (D'Arcangelo et al., 1995), was originally named for the severe motor impairment of knockout mice, and the most well characterized functions of RELN are those involved with brain development and neurological disorders. Expression data in mice show the gene is expressed in pioneer neurons during cortical migration and functional data in mice has demonstrated a link between *reln* mutation and a failure to properly stratify the cerebral cortex and cerebellum. While cortical stratification is the most well studied function of *reln*, genome wide association studies have recently drawn links between *reln* mutation and otosclerosis, the partial resorption and ossification of the otic capsule. Otosclerosis has been found to be correlated to a *reln* mutation is several populations (Schrauwen et al, 2010; Hong et al, 2001; Khalfallah et al, 2010), yet Sommen et al, 2014, reported no association between *reln* and histologically confirmed cases of otosclerosis. To establish a causative link between the condition and *reln* mutation functional tests are necessary. *Xenopus laevis* is a malleable study system that possess a single copy of *reln*, the expression of which has been characterized only in the context of brain development (Costagli et al, 2002). Using CRISPR/Cas9 mutagenesis, histological analysis, and *in situ* hybridization, we sought to observe the developmental role of *reln* in *Xenopus* head skeleton formation, specifically structures homologous to those forming the middle ear in humans and draw a causational link between *reln* mutation and otosclerosis.

M4

SEMAPHORIN4C TARGETING THROUGH CRISPR-CAS9 MUTAGENESIS

Brian Lee

Semaphorin4C (SEMA4C) codes for a transmembrane protein that guides axons in development, sorts out the pools of motor neurons and modulates the pathfinding of afferent and efferent axons from these pools. I chose to study SEMA4C because it belongs to class 4 of Semaphorins that hasn't been as explored as the class 3 of Semaphorins, and it is highly expressed maternally as well as zygotically in *Xenopus laevis* embryos and tadpoles, and is predicted to regulate formation and function of the neural and immune system, as well as skeletal structure. *Xenopus laevis* was selected as a model organism because it is easily bred and maintained, produces many eggs at once all year round, and its eggs can be observed outside the body once they are fertilized, allowing us to easily observe phenotypic effects of SEMA4C loss of function.

To study the function of SEMA4C in early embryos, CRISPR-Cas9 mutagenesis system was utilized to specifically target the SEMA4C gene. A SEMA4C specific guideRNA was injected together with Cas9 enzyme into one-cell stage embryos that were grown to tailbud stages for phenotype and genotype analysis. My work is unique because Semaphorins are the largest family of proteins responsible for the development of vertebrate neural and immune systems, yet very little information is known about Semaphorins outside of class 3. Using loss and gain of function studies in *Xenopus laevis* as a model organism; we can characterize SEMA4C function in detail and provide further insight into its role in normal development.

M5

DELETION OF THE MUSCLE INHIBITOR GENE MYOSTATIN IN *XENOPUS LAEVIS*

Kevin Jang

Myostatin, also known as growth/differentiation factor 8, is a peptide hormone belonging to the transforming growth factor beta-family. It inhibits both hyperplasia and hypertrophy of skeletal muscle cells in animals. As a result, myostatin-deficient animals can have more than twice the muscle mass of wild-type animals with less adipose tissue. One case study by Schuelke et. al. shows a human most likely myostatin-deficient reaching the age of 4.5 with more muscle and strength than normal children without any observed health problems. Myostatin appears to show a promising role in the treatment of muscle wasting disorders, which currently have no treatments that can reverse muscular atrophy. *Xenopus laevis*, also known as the African clawed frog, will be used as a model organism to profile *myostatin* in developing embryos and to study the effects of *myostatin* mutagenesis. *X. laevis* has quick and well-documented early developmental stages, females can lay many eggs year round and eggs are easily injectable. The collaborative effort Xenbase that documents genetic and phenotypic data for *X. laevis* does not have any information on developmental myostatin expression. There are also no publications showing the phenotypic profiles of myostatin-deleted *X. laevis*. Single guide RNA and Cas9 enzyme was

injected into *X. laevis* eggs, where the Cas9 complex localizes to its target sequence and creates dsDNA breaks. These breaks are subject to non-homologous end joining which can generate insertion/deletion errors. In situ hybridization will also be used to visualize the expression of *myostatin* mRNA in developing tadpoles.

PY1

REGULATION OF SOLUTE CARRIER FAMILY 10 MEMBER 2 IN THE BURMESE PYTHON'S LIVER

Alexandra Krinsky

Liver diseases is a growing problem in the world and with the help of the Burmese Python, this research can help to combat these awful diseases.

Since the Burmese Python is so efficient at digesting its meals with no adverse health effects they make for the perfect model organism to use in this research. Looking at the python's liver can aid in the understanding of how they are able to digest so efficiently, so that maybe one day humans livers can also do the same.

This research focuses on the expression of Solute Carrier Family 10 Member 2 (SLC10A2),which is a sodium-dependent bile acid transporter. This protein spans the membranes of cells and helps in recycling bile acids from different parts of the body. It is found in the ileum, the liver, and the kidneys of humans.

The use of the techniques, traditional PCR and Real-Time-PCR (polymerase chain reaction) was used to see when it is regulated and if SLC10A2 is expressed in the liver during different feeding points. If so, then this gene could be a key to understanding the python's efficient liver. This research showed that SLC10A2 is not heavily expressed in the liver of the Burmese Python. It is still unclear whether three-days post- fed is when SLC10A2 is upregulated, although, it can be concluded that SLC10A2 is not the main bile-acid transporter in the liver of the Burmese Python.

PY2

FOXO1 AS A POTENTIAL REGULATOR OF CHOLESTEROL HOMEOSTASIS IN BURMESE PYTHON

David Ramirez

The extreme physiology of the postprandial Burmese Python has made it a model organism for studying. Upon digestion of a meal, pythons show an increased level of serum fatty acids and cholesterol; levels which would be toxic in other species (Figure 1). The fatty acids act as a growth factor for multiple organs, which eventually regress back to normal size within 15 days. To understand the mechanisms that prevent lipotoxicity and regulate organ growth, our lab studies fluctuations in liver gene expression throughout digestion. Forkhead Box O1 (FOXO1) is a transcription factor whose roles in metabolism and adipogenesis make it a promising gene of study. FOXO1 acts on G6Pase, ATG1, and PPARG, which regulate blood sugar, autophagy, and fatty acid uptake respectively. Upregulation or downregulation of these genes by FOXO1 may play a key role in regulating the aberrant physiology seen after feeding. Understanding this role may provide us with promising drug or gene therapy targets to treat disease such as fatty acid liver disease, diabetes, and more.

PY3

THE ROLE OF BETA-KLOTHO CO-RECEPTOR IN PYTHON LIVER AND BILE ACID HOMEOSTASIS

Alex Kaaua

Beta klotho (KLB) is an integral membrane protein that binds to the C-terminus of fibroblast growth factor 19 (FGF19), allowing FGF19 to bind its N-terminus to fibroblast growth factor receptor 4 (FGFR4). The binding of FGF19 to the receptor complex triggers a downstream signal, which ultimately causes a decrease in bile acid synthesis in the liver. KLB was chosen for study because of its essential role in the FGF19 signaling pathway. KLB is not only necessary for signaling, but also contributes to FGF19 specificity for liver tissue. Several qPCR experiments were conducted to measure the changes in mRNA expression in python liver tissue after feeding. In all qPCR experiments, KLB mRNA expression decreased at 3 days post fed (3dpf) and increased drastically at 10dpf. It is possible that the decrease in mRNA expression of KLB at 3dpf is evidence of the inhibition of the FGF19 signaling pathway. This is consistent with the current understanding of FGF19 signaling as an inhibitor of bile acid synthesis. Therefore, it follows that the FGF19 pathway would need to be inhibited to allow for increased bile acid production. KLB and FGF19 are important factors in bile acid homeostasis and their further study in an extreme model such as the python could prove beneficial.

PY4

MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) REGULATION IN BILIARY TRANSPORT

Esther Choi

The Burmese python has extraordinary adaptive physiology when it consumes a meal up to one-quarter of its body weight. Previous studies have shown that after a meal is consumed, the python's heart, pancreas, liver, and kidneys increase of about 40%, 94%, 106% and 72% in mass, respectively. The levels of fatty acids in the serum during digestion also increase significantly; reaching levels that would be toxic to humans. Human diseases can accumulate high levels of fatty acids in the serum; however, if the same levels seen in the python were present in the human serum, it would cause organ failure and death. The liver is a key organ in digestion and bile acid transport. Biliary transport occurs on the canalicular membrane of the hepatocytes, where transport proteins reside to mediate the secretion of bile into the intestines. This process is required for the emulsification of fats from circulation to maintain a homeostatic level. Multidrug Resistance-associated Protein 2 (MRP2) is a key biliary transport protein known to have a variety of responses such as cholestasis and drug resistance when gene expression is altered. Studying any changes in MRP2 gene expression levels in the Burmese python during digestion may further elucidate the mechanism underlying this extraordinary physiology and the importance of bile transport protein regulation in human diseases.

PY5

EXPRESSION OF TGR5 ECREASES IN THE POSTPRANDIAL PYTHON BIVITTATUS LIVER

Jeremy Herder

After a large meal, the fatty acid content in the Burmese python's blood serum increases dramatically. Bile acids are the molecules responsible for removing cholesterol from blood. TGR5 is the cell surface G protein-coupled bile acid receptor. Using real-time PCR, expression levels of TGR5 mRNA in homogenized python liver tissue were quantified in fasted, 1 day post-fed, 3 days post-fed, and 10 days post-fed Burmese pythons. In comparison to its expression in the fasted python, expression of TGR5 decreased at one and three days, and increased at 10 days

PY6

HEPATOCYTE NUCLEAR FACTOR 4ALPHA AND BILE ACID TRANSPORT

Anne Cathryn Cox

Over 1 in 5 adults in the US has metabolic syndrome (MetS), which is a collection of symptoms including some or all of the following: obesity, high fasting plasmaglucose, high blood pressure, high triglyceride levels, and low HDL cholesterol levels (Beltrán-Sánchez, Harhay, Harhay, & McElligott, 2013). Although this syndrome is well studied, there is still much to learn about how humans process fat in the liver. The Burmese python has several key physiological processes that make it such a pertinent animal for research on human disease involving the metabolism of cholesterol and other fats, including MetS, pathological cardiac hypertrophy, and hepatic steatosis (fatty liver disease). One of these processes is the rapid metabolism of serum fatty acids post-meal. To explore this process, hepatocyte nuclear factor 4 alpha (HNF4A) expression in python liver tissue was tested using real-time PCR. HNF4A has two separate functions important to bile acid homeostasis in hepatocytes in humans: (1) to upregulate the transcription of transport proteins (largely NCTP) moving recycled bile acid into hepatic cells while downregulating transport proteins which move bile acids from hepatic cells into the canaliculus, and (2) to increase binding of HNF1A to its promoter thereby increasing the transcription of CYP7A1 and the downstream synthesis of bile acids. This research found that expression of HNF4A mRNA doubles from the fasted tissue at 1 day-post-fed (DPF), and then returns to normal by 3 DPF, suggesting that the python uses similar pathways of bile-acid sequestration in hepatocytes as humans.

PY7

EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTOR 4 INCREASES IN BURMESE PYTHON LIVER AFTER FEEDING

Natalie Graham

Burmese pythons demonstrate extreme physiological changes after eating, including an increase in size of all organs involved in digestion and an increase in fatty acids in the serum. While the python routinely manages such a severe increase in fatty acids, humans could not endure this change

without taking on damage. The liver, which plays an integral role in the digestion of fats, must help the snake handle the influx of fat.

Fibroblast Growth Factor Receptor 4 (FGFR4), is the most prevalently expressed fibroblast growth factor receptor in liver cells. FGFR4 helps to downregulate bile production as well as stimulate the proliferation of cells. For these reasons, I examined the expression of FGFR4 in python liver after feeding.

In order to see changes in expression over time, RNA was isolated from Burmese python livers at four different time points: fasted, 1 day post fed, 3 day post fed, and 10 day post fed. I designed primers using the Burmese python WGS, generated cDNA from the RNA, and measured expression using qPCR. The PCR product was then run on a gel to confirm its identity.

Using these techniques, I verified that I amplified product from my gene of interest and found that expression of the FGFR4 gene increases in expression after feeding, then gradually declines in expression as time progresses.

PY8

HSD3B7 SHOWN TO POTENTIALLY MEDIATE CHOLESTEROL HOMEOSTASIS IN THE BURMESE PYTHON

Stuart Sommers

Non-alcoholic fatty liver disease (NAFLD) affects up to 25% of people in the United States. NAFLD is caused by the accumulation of fat in the liver, which in turn is caused by: being overweight or obese, insulin resistance, hyperglycemia, and high levels of fats in the blood. In this study, we examined the Burmese python and its incredible ability to digest an enormous amount of lipids and handle the resulting high concentration of fatty acids in its blood. HSD3B7 is known in humans to be involved in bile acid synthesis, a crucial pathway involved in lipid metabolism, and so was chosen as a gene of interest. To evaluate its role in the Burmese python, tissue samples were taken from the liver at several time points after feeding, and relative gene expression was then measured using quantitative PCR. A clear increase in HSD3B7 gene expression was observed shortly after feeding, indicating that HSD3B7 may indeed be involved in Burmese python lipid metabolism and more specifically in cholesterol homeostasis and the production of bile acids. With more and more fat in our diets and therefore our arteries, it is crucial that we investigate ways to optimize lipid metabolism and protect our livers from the resulting diseases, such as fatty liver disease. The python continues to prove a promising model organism for finding these potential optimization methods.

PY9

THE ROLE OF PROTEIN TYROSINE PHOSPHATASE NON-RECEPTOR TYPE 11 IN LIPOGENESIS AND BILE ACID SYNTHESIS

Jesslyn Connors

Heart disease is one of the worldwide leading causes of death and is associated with high levels of cholesterol and hypertrophy. The Burmese python was used as a model organism to study genes associated with metabolism due to their ability to ingest meals high in fat while maintaining a healthy heart and liver. Protein Tyrosine Phosphatase Non-Receptor Type 11, also known as SHP2, was identified as a gene of interest for this study. Gene specific primers were designed and qPCR was conducted to quantify expression at various stages of digestion. Knowing the abundance of gene expression for SHP2 could give insight into what factors allow the python to handle physiological stress better than humans and what treatments could be developed for human heart disease.

PY10

MULTIDRUG RESISTANCE TRANSPORTER 3: A WINDOW INTO THE PYTHON LIVER

Diana Damian-Mosqueda

Although studies of the liver in human beings are nothing new, there are few studies, which seek to unveil the drastic changes that the python undergoes after being feed. A significant increase in fatty acids in the blood of the python after feeding results in a large increase in bile acids as well. Managing flux of bile acids in the python's altered state requires different levels of transport proteins throughout the digestive process.

The Burmese Python liver, in this study, was tested for levels of protein MRP3 (Multidrug Resistance Transporter 3) at different time points both when fasting and after feeding. MRP3 functions in the liver to transport both anionic drugs and to regulate efflux of bile acids. However, different organisms use the protein differently. To test how much MRP3 is used in the python, MRP3 was first isolated in the python genome and primers were specifically made for it. The liver was then homogenized, RNA was isolated, cDNA was made with the specific primers, and finally qPCR was used to analyze the differing levels of

MRP3 protein during these time points. Preliminary results indicate that MRP3 levels remain the same comparatively to the fasted levels at 1 days post feed (DPF) and increase at 3DPF and remain at that level at 10DPF.

Although these results are preliminary and need to be re-tested with new liver samples, they indicate that the python does indeed use and upregulate MRP3 during its digestive process.

PY11

QPCR REVEALS CHANGES IN ALPHA-METHYLACYL-COA RACEMASE GENE EXPRESSION

Whitney Stanton

The Burmese python, native to Southeast Asia, is a model organism used to study its physiological changes upon fasting or feeding in the Python Project. During feeding, the liver doubles in size due to an increase of fatty acids in the serum. The liver then mediates the removal of cholesterol and fats through the production of bile acids. Discovery of how the python manages the hypertrophy and regression of its liver may be applied to the 30 million Americans who currently suffer from liver disease.