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Undergraduate Research Opportunities Program

University of Nevada Las Vegas, College of Sciences

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University of Nevada, Las Vegas

2009

College of Sciences

Undergraduate Research Opportunities Program (UROP)

http://sciences.unlv.edu/urop/

Compiled and Edited
By
Carl Reiber, Associate Dean, College of Sciences
Photo Caption, Cover.

Front Row (left to right): Marsha Kristel (Maki) Bernardo, UNLV; Dolores Huang, Nevada State College; Lindsey Clark, UNLV; Jessica Newburn, UNLV; Jenny Lam, UNLV; Lia Africa, UNLV; Loann Killarsen, UNLV; Linda Tran, UNLV; Joyce Pang, UC Berkeley; Marian Schmidt Hampshire College; David Vardukyan, College of Southern Nevada; Mary Girard, UNLV; Middle row (left to right): Eric Hughes Arizona State University; David Hannasch, UNLV; Dr. Robin Herlands, Nevada State College; Ann-Desdemonia Fowajuh, University of Maryland, Eastern Shores; Adam Austin, UNLV; Ali Jamil, Vassar College; Jarod Wolffis, UNLV; Monique Gomez, Western New Mexico University; Turquoise Alexander, Fort Valley State University; Autoro White, Fort Valley State University; Back Row (left to right): David Basta, UNLV; Lauren Johnson, Hampton University; Ulysses Pickard, Fort Valley State University; Alex Jacobson, UNLV; Chan Hansen, UNLV; William Kang, UNLV; Christine Simons, Villanova University; Bradley Davey, UNLV.
Acknowledgements

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Special thanks are offered to the following individuals for their support and encouragement of this program:

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Tao Pang, Chair, Department of Physics and Astronomy
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Eduardo Robleto, Associate Professor, School of Life Sciences
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Nicholle Booker, Graduate Affairs Coordinator, College of Sciences

Heather Goulding, Program Manager, Nevada INBRE
Barbara Neyses, Financial Manager, Nevada INBRE

Faculty mentors from UNLV, the Desert Research Institute, and the Nevada Cancer Institute are deserving of particular thanks. These mentors devoted their time and expertise to work with UROP students. Without mentors this program would not be possible. Lastly, we would like to thank Ms. Nicholle Booker for her tireless efforts and organizational skills, and her constant efforts to improve and enhance our students’ experience at UNLV.
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## University of Nevada, Las Vegas

### 2009

**College of Sciences**

**Undergraduate Research Opportunities Program**

**Summer Poster Session**

**August 6, 2009**

**UNLV Student Union Ball Room**

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National Institutes of Health (NIH) IDeA Network of Biomedical Research Excellence Program (INBRE) Undergraduate Research Program

Nevada INBRE sponsors 15 undergraduate research scholarships each year. Students selected for the program conduct a lab research project in a faculty mentor’s laboratory. Summer research opportunities often lead to longer-term collaborations between students and faculty, publishable research, and careers in medicine or biomedical research. Opportunities are available for research in emerging areas such as genomics, proteomics, molecular modeling, imaging, and bioinformatics. However, any area of research that might be supported by the NIH is appropriate.

Students are selected in a statewide, merit-based competition. As part of the application process, students are required to identify a faculty mentor at UNR, UNSOM, UNLV, or the Nevada Cancer Institute with whom they are interested in conducting research.

Nevada INBRE is a network of physical and human resources available to scientists in Nevada. Its mission is to provide infrastructure that enables investigators to successfully win research funding. INBRE research facilities provide research support services, training, and equipment for Nevada’s biomedical investigators. Nevada INBRE also sponsors research, scholarships and training opportunities for faculty members and students.

The National Center Research Resources (NCRR) Institutional Development Award (IDeA) program broadens the geographic distribution of NIH funding for biomedical and behavioral research. The program fosters health-related research and enhances the competitiveness of investigators at institutions located in states in which the aggregate success rate for applications to NIH has historically been low. Supported by the NCRR Division of Research Infrastructure, the IDeA program increases the competitiveness of investigators by supporting faculty development and research infrastructure enhancement at institutions in 23 states and Puerto Rico.

IDeA Networks of Biomedical Research Excellence (INBRE) enhance biomedical research capacity, expand and strengthen the research capabilities of biomedical faculty, and provide access to biomedical resources for promising undergraduate students throughout the eligible states. INBRE implements the IDeA approach at the state level by enhancing research infrastructure through support of a network of institutions with a multidisciplinary, thematic scientific focus. INBRE is the second phase of the Biomedical Research Infrastructure Networks (BRIN) program, which began by providing planning grants in 2001.
Centers of Biomedical Research Excellence (COBRE) augment and strengthen institutional biomedical research capabilities by expanding and developing biomedical faculty research capability through support of a multidisciplinary center, led by a peer-reviewed, NIH-funded investigator with expertise central to theme of the grant proposal.

The IDeA program also supports IDeANet, an Internet-based network providing connectivity for high-bandwidth science applications. IDeANet will enable collaboration among institutions, ultimately supporting all participants in the IDeA program, as well as participants in the Research Centers in Minority Institutions (RCMI) program and other NCRR-supported networks.

Front row (left to right): Jenny Lam, UNLV; Lia Africa, UNLV; Middle row (left to right): David Basta, UNLV; Mary Girard, UNLV; Back row (left to right): Adam Austin, UNLV; Jarod Wolffis, UNLV.
Nevada National Sciences Foundation (NSF)  
Experimental Program to Stimulate Competitive Research (EPSCoR)

The Undergraduate Research component of the current NSF EPSCoR award provides lab and field research experiences, through summer scholarship programs and annual fellowship opportunities, to full-time NSHE undergraduate students. These programs fund eligible students either majoring in mathematics, science, or engineering, or majoring in education and specializing in teaching K-12 in the fields of mathematics, science, or technology. Research is conducted under the guidance of NSHE faculty mentors. The hands-on experience gained through these programs has proven to supplement classroom learning and serve as gateways to new and exciting opportunities for all participants.

EPSCoR - Experimental Program to Stimulate Competitive Research  
NSF, the federal agency that first developed EPSCoR programs, sponsored the first EPSCoR program in Nevada. Since 1985, NSHE institutions have received more than $41 million in federal funds from NSF EPSCoR, together with non-federal matching funds.

Front row (left to right): Marsha Kristel (Maki) Bernardo, UNLV; Lindsey Clark, UNLV; Back row (left to right): Jessica Newburn, UNLV; Bradley Davey, UNLV; David Hannasch, UNLV.
National Science Foundation Research Experience for Undergraduates Program (NSF REU)

REU MICROBIOLOGY
UNLV offers an REU Site program in partnership with the Desert Research Institute. Undergraduate students participate in a 10-week summer program involving research in the area of environmental microbiology.

Students collaborate with faculty mentors in developing and carrying out hypothesis-based projects on microorganisms from diverse habitats such as hot springs, the deep terrestrial subsurface, hypersaline lakes, arid soils, and ephemeral water sources. Students may also choose to explore the mechanisms of magnetotaxis, microbial adaptation to stressful and nonhost environments, or the dynamics between primary producers and consumers.

All students receive training in current molecular techniques and the ethics of science, and they participate in weekly discussions on their project. At the conclusion of the program, students present their research results at a scientific colloquium. In addition, all students are encouraged to present their research at a regional or national scientific conference. Students receive a $4000 stipend, housing, meals, and a travel subsidy. First generation college students and members of an underrepresented group are strongly encouraged to apply.

Front Row (left to right): Carmen Villin, UNLV; Monique Gomez, Western New Mexico University; Middle row (left to right): Eric Hughes, Arizona State University; Dolores Huang, Nevada State College; Ann-Desdemonia Fowajuh, University of Maryland, Eastern Shores; Turquoise Alexander, Fort Valley State University; Marian Schmidt, Hampshire College; Ali Jamil, Vassar College; Back row (left to right): Autoro White, Fort Valley State University; Christine Simons, Villanova University; Chan Hansen, UNLV; Ulysses Pickard, Fort Valley State University; Lauren Johnson, Hampton University; Not pictured: Carrie Glenney, University of Washington.
National Aeronautics and Space Administration (NASA) Experimental Program to Stimulate Competitive Research (EPSCoR)

The NASA Experimental Program to Stimulate Competitive Research (EPSCoR), strengthens the research capabilities of jurisdictions that have not in the past participated equitably in competitive aerospace and aerospace-related research activities. EPSCoR provides eligible jurisdictions with funding to develop a more competitive research base within their jurisdiction and member academic institutions. Seven federal agencies conduct EPSCoR programs. The two main components of NASA EPSCoR are:

NASA EPSCoR Research Infrastructure Development Cooperative Agreement Notice. The Research Infrastructure Development (RID) component enables jurisdictions to build and strengthen relationships with NASA researchers. The RID has a three-year base period of performance with a potential single, two-year renewable period of performance. Awards are $125,000 per year. A one-to-one match (cash or in-kind) is required for every NASA dollar awarded. The most recent RID was announced and awarded in 2007. NASA intends to announce the RID opportunity every three to five years, pending funding availability.

NASA EPSCoR Research CAN. The NASA EPSCoR CAN for Research Awards solicits topic-specific proposals addressing high-priority NASA research and technology development needs. Awards are up to $750,000 for a three-year performance period. A one-to-one match (cash or in-kind) is required for every NASA dollar awarded. NASA intends to announce the EPSCoR CAN for Research Awards yearly, pending funding availability.

NASA Research Opportunities

Supporting research in science and technology is an important part of NASA's overall mission. NASA solicits this research through the release of various research announcements in a wide range of science and technology disciplines. NASA uses a peer review process to evaluate and select research proposals submitted in response to these research announcements. Researchers can help NASA achieve national research objectives by submitting research proposals and conducting awarded research.

University and industry research institutions are important NASA partners in many areas of science and technology. As part of its broadening focus on advancing the field of distributed heterogeneous computing, NASA supports ongoing research efforts in a number of disciplines through the grant process.
Grant Supported Students and Independent Study Programs

Undergraduate research programs are a valued component of the College of Sciences. Independent research activities mentored by our research active faculty cultivate and support research partnerships and invite undergraduates to work as junior colleagues in a laboratory. These programs offer the opportunity to work on cutting edge research. Continuing students, undergraduates, and high school students engage in each phase of standard research activity: developing research plans, writing proposals, conducting research, analyzing data, and presenting research results in oral and written form.

Research activities can take place over the summer or during the regular academic terms and are available in both academic departments and interdisciplinary laboratories. Projects may require an entire semester, and many continue for a year or more. Students use their experiences to become familiar with faculty members, learn about potential majors, and investigate areas of interest.

Participants gain practical skills and knowledge they apply to careers after graduation or as graduate students. Most importantly, they become involved in state-of-the-art research. The School of Life Sciences and the Department of Physics and Astronomy are active participants in this year’s summer UROP program with students working in state of the art research laboratories on project ranging from honeybee genomics to extra galactic x-ray bursts.

Front row (left to right): Loann Larsen, UNLV; Dr. Robin Herlands, Nevada State College; Linda Tran, UNLV; Joyce Pang, University of California, Berkeley; Back row (left to right): David Vardukyan, College of Southern Nevada; Alex Jacobson, UNLV; William Kang, University of California, Berkeley.
School of Life Sciences

The School of Life Sciences (SoLS) is one of the largest academic units on the UNLV campus, with 28 full-time faculty members, 15 adjunct and research faculty, and approximately 1,200 undergraduate majors and 60 graduate students. Our enthusiastic faculty is committed to advancing scientific knowledge and to educating, training, and fostering the career development of undergraduate and graduate students. We offer undergraduate concentrations in Biotechnology, Comprehensive Studies, Ecology and Evolutionary Biology, Education, Cell and Molecular Biology, Microbiology, Integrative Physiology, Preprofessional Studies, and Urban Horticulture.

The School has well-equipped laboratories to support research. These facilities are enhanced through access to a number of specialized scientific resources, including the Nevada Genomics Center and DNA Sequencing Facility, the Nevada Center for Biological Imaging, the Ecophysiological Research facility, an animal care facility; and regional natural history collections, including those of the Wesley E. Niles Herbarium and the Marjorie Barrick Museum. Investigators from the Nevada System of Higher Education’s Desert Research Institute also contribute to our graduate program. Prospective students should make contact with one or more faculty members to familiarize themselves with their current research interests, opportunities for conducting research projects, and funding availability.
Department of Physics and Astronomy

The Department of Physics and Astronomy offers Bachelor of Science degrees in Physics, Applied Physics, and Computational Physics, as well as a minor in Physics. For additional information please visit:

http://www.physics.unlv.edu/

During the summer, a Research Experience for Undergraduate (REU) program supports undergraduates and provides research opportunities during a 10-week period. The REU program is funded by a grant from the National Science Foundation. Students should apply by February or March for the following summer.

A chapter of the Society of Physics Students (SPS), including both graduate and undergraduate students, welcomes student participation.
Desert Research Institute (DRI)

Education & Outreach: For higher education students, DRI provides a learning environment strongly focused on collaborative, interdisciplinary research. DRI faculty members participate in Atmospheric and Hydrologic Science academic programs with the University of Nevada, Reno, University of Nevada, Las Vegas, and Nevada State College. Students conduct their research at DRI while earning their degrees through the universities. DRI is also committed to Nevada's K-12 education system and the professional development of its teachers. DRI emphasizes "teaching the teachers" so they can bring real world knowledge back into their classrooms.

Educational Programs

Atmospheric Sciences Graduate Program: Students in the Atmospheric Sciences Graduate Program study dynamic meteorology, atmospheric physics, mesoscale modeling, fire weather and climate, atmospheric chemistry and instrument development.

Hydrologic Sciences Graduate Program: Consistently ranked in the top 10 by U.S. News & World Report, the Hydrologic Sciences Graduate Program offers M.S. and Ph.D. degrees in both Hydrology and Hydrogeology.

GreenPower Program

The GreenPower Program supports non-fossil fuel energy demonstration projects and education at Nevada schools. The program involves partnerships in both northern and southern Nevada with NV Energy, their customers, and school districts.

Storm Peak Lab Programs: Educational programs at Storm Peak Lab include graduate field work in Atmospheric Sciences, the Geoscience Research at Storm Peak (GRASP) program providing field research experiences for diverse undergraduate students, and a 5th and 6th grade weather and climate program.
UNLV Presidential Research Award

The President's Research Award is an internal funding award designed to support research teams in their pursuit of competitive grant funding. The intent of this award is to provide up to $50,000 per award for research projects that will lead to submission of one or more competitive grant proposals to national/international funding agencies. Depending on the availability of funding and the number of applications received, approximately eight to 10 awards are offered annually.

The larger institutional goal of the President’s Research Award is to build greater research capacity at UNLV and to expand competitive external funding. This award is specifically designed for use by research teams, and involvement of graduate students on the teams is highly recommended. All full-time academic faculty members are eligible for the award, as are researchers employed by centers and institutes that recover facilities and administrative costs from sponsored program funding. The call for proposals is typically announced early in the spring semester.
High Pressure Science and Engineering Center (HiPSEC)

HiPSEC focuses on properties of materials relevant to the National Nuclear Security Administration's (NNSA) Stockpile Stewardship Program. High priority is given to measuring static and dynamic high-pressure studies for validating and improving computational models over a largely unexplored range of very high pressures and temperatures. Materials under study include d- and f-band metals, energetic materials and their detonation products, foams, and hydrogen and other and low-Z elements and their compounds.

HiPSEC staff measure equilibrium thermochemical properties, mechanical properties, reaction kinetics, and reaction products at static pressures using in situ X-ray diffraction; absorption, emission, light-scattering spectroscopy from infrared to X-ray wavelengths; and other chemical and physical methods.

Its mission also encompasses shock experiments at NNSA's Lawrence Livermore National Laboratory, Los Alamos National Laboratory, and Sandia National Laboratory, Scientists recoveri samples from these experiments for chemical, physical, and mechanical analysis.

Theoretical and computational studies focus on highly correlated and "warm" condensed matter systems. Under the Department of Defense's MURI program, HiPSEC scientists are studying effect of defects on the mechanisms of initiation and energy release in energetic molecular crystals.

Collaboration: This integration of high-pressure science programs in Nevada with programs at NNSA's National Laboratories, DOD Research Laboratories, and other university laboratories aims to enhance Nevada's scientific and educational infrastructure, while developing focused high-pressure research programs relevant to the missions of DOE and DOD. HiPSEC is a member of the High Pressure Collaborative Access Team (HPCAT) at the Advanced Photon Source of Argonne National Laboratory.

In addition to HPCAT facilities at the Advanced Photon Source, HiPSEC has materials science laboratories on the UNLV campus for crystallography, solid-state spectroscopy, cryogenic studies, and synthesizing and characterizing foams. HiPSEC also maintains computational centers for engineering and solid-state theory on the UNLV campus.
Iron is an essential element in the metabolism of many organisms, including bacteria. In many pathogenic bacteria, the levels of iron present trigger the expression of many virulence genes. In *Shigella*, a gram-negative bacterium that causes dysentery in humans, the expression of a small regulatory RNA, *ryhB*, is blocked in the presence of iron. Studies have revealed that *ryhB* represses *virB*, a global regulator of virulence genes in *Shigella*.

The *icsP* gene is under the direct control of VirB. *icsP* encodes an outer membrane protease that cleaves a protein necessary for the actin tail assembly of *Shigella in vitro*. In vivo, this actin tail enables the pathogenic *Shigella* to spread intracellularly. Because most of the iron is complexed inside host cells, the intracellular compartment is considered an iron-poor environment. The aim of this project is to determine whether iron levels influence the regulation of *icsP* through VirB.

Based on previous studies done on *ryhB*, I hypothesize that *ryhB* regulates *icsP* through VirB. Beta-galactosidase assays and Western blots will allow for determination of whether the activity of the *icsP* promoter and gene expression significantly differ in the presence and absence of iron.
The Regulation of the icsP Promoter of Shigella flexneri by ryhB
Lia A. Africa and Helen J. Wing, Ph.D.
School of Life Sciences, University of Nevada, Las Vegas

Background Information
Iron is an essential element in the metabolism of many organisms, including bacteria. However, inside host cells, most of the iron is complexed by high-affinity iron binding proteins, making the intracellular compartment an iron-poor environment.

In Shigella, an intracellular pathogen that causes dysentery in humans, a regulatory small RNA, ryhB, is involved in regulation of genes required for metabolism and iron storage in an iron-responsive manner. The expression of ryhB is blocked in high-iron conditions and up-regulated in low-iron conditions. Studies have revealed that the small RNA ryhB represses expression of virB, whose product is required for Shigella virulence genes to be expressed.

VirB directly controls the expression of icsP. The icsP gene encodes an outer membrane protease that cleaves a protein necessary for the actin tail assembly of Shigella in vitro. In vivo, this actin tail enables the pathogenic Shigella to spread intracellularly.

Materials and Methods
1. Transforming a wildtype Shigella strain (2457T) and a virB mutant strain (AWY37) with a reporter plasmid and ryhB.
2. Growing the transformed Shigella strains in tryptic soy broth with and without IPTG. IPTG serves to induce expression of ryhB.
3. Performing a β-galactosidase assay to indirectly measure the activity of the icsP promoter.
4. Performing a Western blot analysis to visualize the IcsP protein made in both IPTG-induced and non-induced conditions.

Conclusions
The small regulatory RNA ryhB regulates icsP.
- Over-expression of ryhB down-regulates icsP through VirB.
- Over-expression of ryhB significantly reduces the levels of IcsP protein made.

Future Directions
- We will develop an iron-free medium in order to directly determine whether iron plays a role in the regulation of icsP.
- We will make ryhB mutant Shigella wildtype and virB mutant strains and use these in our β-galactosidase assays and Western blot analyses. This will enable us to examine whether the iron-responsive ryhB directly regulates icsP or if ryhB regulates icsP through VirB only.

Results

![B-galactosidase assay](image1)

Figure 1. ryhB regulates icsP through VirB

The result of this assay shows that the transcription from the icsP promoter was almost four-fold higher when ryhB is not induced than when it is induced. This suggests that over-expression of ryhB significantly down-regulates icsP expression.

Western blot analysis

![Western blot analysis](image2)

Figure 2. IcsP production is influenced by ryhB

The result of this analysis shows that IcsP production is significantly less when ryhB is induced than when ryhB is not induced.

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Acknowledgements
This study was supported by the NIH grant P01 RR-016464 from the INBRE Program of the National Center for Research Resources. Very special thanks to Stephanie Labahn for her continued guidance, assistance, and inscribing disposition. We would also like to thank Dr. Robin Herlands and Chris Hensley for their constructive inputs.
Adam Austin  
Mentor – Dr. James Tung  
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Expression of an alternate splice form of Bmi-1 in multiple myeloma  
The concept of “tumor stem cells” has garnered much attention in the last few years. Tumor stem cells are believed to exist among a heterogeneous group of cells that constitute a tumor. These tumor stem cells often express genes that are important for stem cell function, cell division, and maintenance of pluripotent state in stem cells. Stem cell or stem cell maintenance genes such as SALL 4 and Bmi-1 are often seen in these cancer cells and contribute to self-renewing divisions and cancer cell survival. In particular, high expression of Bmi-1 (B lymphoma mouse Moloney leukemia virus insertion region), a member of the polycomb family of transcription factors, is often associated with poor prognosis in cancers.

Our laboratory has shown the existence of an alternatively spliced Bmi-1 RNA and protein in multiple myeloma cells. The purpose of this research project is to understand the effect of an alternate splice form of Bmi-1 protein on cell cycle and apoptosis in multiple myeloma cells. To understand the effect of this alternate Bmi-1 protein, I first compared the growth rate of different myeloma cell lines and correlated that with the expression of the wild-type Bmi-1 and the alternatively spliced Bmi-1 form. I performed time course experiments and counted the cell numbers in each cell line at various time points. My results show that the myeloma cell lines, which highly express the alternate form of Bmi-1, grew faster than the myeloma cell lines, which mostly express the wild-type form of Bmi-1. Currently I am performing RT-PCR and western blot analysis to confirm the existence of the alternatively spliced Bmi-1 protein. I plan to isolate and sequence the alternatively spliced form of Bmi-1. I also plan to determine the effect of knocking-down Bmi-1 expression on cell cycle and apoptosis by incorporating inducible shRNA viral constructs targeted against Bmi-1 RNA in myeloma cells.
Expression of an alternate form of Bmi-1 in multiple myeloma
Adam Austin, Kristine Veys, Debbie Wong, James Tung
Division of Laboratory Medicine, Nevada Cancer Institute, Las Vegas, NV

Abstract
Tumor stem cells are believed to exist among a heterogeneous group of cells that constitute a tumor. Stem cell or stem cell maintenance genes such as SALL4 and Bmi-1 are often in these cancer cells and contribute to cell divisions and cancer cell survival. In particular, high expression of Bmi-1, a member of the polycomb family of transcription factors, is often associated with poor prognosis in cancers.

Our laboratory has shown the existence of an alternatively spliced Bmi-1 RNA and protein in multiple myeloma cells. The purpose of this research project is to understand the effect of an alternate splice form of Bmi-1 protein on cell cycle and apoptosis in multiple myeloma cells.

We show here that an alternate splice form of Bmi-1 is found in a myeloma cell line, RPMI. The alternate-spliced form can also be observed on the protein level in western blot analysis. We have purified and sequenced the alternate-spliced Bmi-1 RT-PCR product and determined alternate splicing regions. Furthermore, we show that the RPMI (myeloma) and N84 (AML) cell lines have a higher growth rate compared to other myeloma cell lines. Since both RPMI and N84 both express predomnantly the alternate-spliced Bmi-1 form, the growth rate results suggest that the expression of the alternate form may confer growth advantage in cancer cells.

Background

Bmi-1 (B lymphoma mouse Moloney Leukemia virus insertion region) is one of the genes regulated by SALL4 and is a member of the polycomb family of transcription factors. The over-expression of Bmi-1 can be found in a variety of cancers such as breast cancer, glioma, nasopharyngeal cancer, and mantle cell lymphomas.

While Bmi-1 is shown to be involved in the pathogenesis of several cancers, very little is known whether Bmi-1 plays a role in the pathogenesis and progression of multiple myeloma (MM), a cancer of plasmacytoid cells characterized by elevated monoclonal antibodies and bone destruction. The pathogenesis of multiple myeloma requires both dysregulation of apoptosis and cell cycle.

We hypothesize that Bmi-1 is expressed and acts as a key regulator of cell growth and apoptosis in multiple myeloma cells. Our preliminary data supported this hypothesis. Using flow cytometry and western blot analysis, we demonstrated that Bmi-1 is detectable in five myeloma and one myeloid leukemia cell lines.

While Bmi-1 expression is found in these cell lines, our laboratory also found that RPMI and N84 cell lines expressed an alternate form of Bmi-1 protein. This alternate form is smaller in size and predominates in these two cell lines. Based on these results, we hypothesize that while Bmi-1 expression may increase cell division, the expression of its alternate form produces a dominant negative effect and thereby inhibits the myeloma cells into cell cycle and division.

Objectives

1. Confirm, isolate and sequence the alternate Bmi-1 protein in myeloma cells.
2. Determine whether the alternate Bmi-1 protein correlates with increased cell division.

Results

Presence of an alternate form of Bmi-1

- Figure 1: Bmi-1 is expressed in several multiple myeloma cell lines. Bmi-1 expression in multiple myeloma cell lines detected by flow cytometry. Bmi-1 staining is shown in red. Background staining from Fc Receptor conjugated secondary antibody is shown in blue.

- Figure 2: DNA analysis of five different myeloma cell lines and an acute myeloid leukemia cell line (N84) using RT-PCR (reverse transcription-polymerase chain reaction). An alternate form of Bmi-1 is expressed in both RPMI and N84 cells. The alternate form of Bmi-1 is roughly 55kb shorter than the alternate form.

- Figure 3: Western blot analysis also shows the expression of an alternate form of the Bmi-1 protein. Both: RPMI lane 3 and N84 lane 3 express predominantly the alternate spliced form of Bmi-1 (bottom band).

Nucleotide sequence of alternate form Bmi-1

- Figure 4: Nucleotide sequence of the alternative form of Bmi-1. To get the sequence, the desired band was cut out from the gel and purified. It was then re-amplified using the same PCR primers that were used initially, and the final product was sequenced.

Future Plans

Determine the effect of knocking-down Bmi-1 expression on cell cycle and apoptosis by incorporating inducible shRNA viral constructs targeted against either Bmi-1 wt type and/or variant RNA in myeloma cells. Then:

1. Determine the effect of altering Bmi-1 expression on cell cycle.
2. Determine the effect of altering Bmi-1 expression on apoptosis.

Literature Cited

David Basta  
Mentor – Dr. Helen Wing  
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*Shigella flexneri* is a pathogenic bacterium that is the causative agent of shigellosis, an illness characterized by severe dysentery. *Shigella* carries many of its virulence genes on a large virulence plasmid and consequently this plasmid is the focus of research in the Wing lab. My research focuses on the transcriptional regulation of a newly identified gene called *ospZ*. This gene’s protein product is secreted outside the bacterial cell and assists in polymorphonuclear leukocyte migration, a function that is believed to enhance the virulence of *Shigella*. Many genes encoded by the *Shigella* virulence plasmid are regulated by the transcription factor VirB, which is also encoded by the virulence plasmid. VirB regulates the expression of *IcsP*, a gene 1.6 kilobase pairs upstream of *ospZ* on the divergent strand. To determine the role VirB plays in the regulation of *ospZ*, reporter plasmids will be constructed in which the *ospZ* promoter region is fused to lacZ (a gene that encodes the enzyme beta-galactosidase) and transformed into wild type and VirB mutant strains of *Shigella*. Promoter activity of *ospZ* will then be measured using beta-galactosidase assays. My hypothesis is that VirB, which binds to DNA 100 base pairs upstream of the *ospZ* gene, regulates the expression of this gene also.
The Regulation of the ospZ and ORF-2 Promoters in Shigella flexneri by the Virulence Factor VirB

David Basta and Helen J. Wing, Ph.D.
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Abstract

Shigella flexneri is a pathogenic bacterium that is the causative agent of shigellosis, an illness characterized by severe dysentery. My research focuses on the transcriptional regulation of a newly identified gene on the Shigella virulence plasmid called ospZ, and a hypothetical gene immediately upstream of ospZ called ORF-2. The ospZ gene product is secreted outside the bacterial cell and assists in polymorphonuclear leukocyte migration, a function that is believed to enhance the virulence of Shigella. Many genes encoded by the Shigella virulence plasmid are regulated by the transcription factor VirB, which is also encoded by the virulence plasmid. VirB regulates the expression of icsP, a gene 1.6 kilobase pairs upstream of ospZ on the divergent strand. My hypothesis is that VirB, which binds to DNA 200 base pairs upstream of ospZ and ORF-2, regulates the expression of these genes as well.

Materials and Methods

Plasmid Constructs:

1651bp and 1430bp promoter regions upstream of ospZ and ORF-2, respectively, were PCR amplified from the virulence plasmid (Fig.1) and cut with SalI and XbaI. The vector (pHJW20), carrying the lacZ gene and chloramphenicol resistance, was cut with the same restriction enzymes and ligated to the promoter inserts to create pDB05 (Fig.2) and pDB02 (Fig.3). These plasmids, along with pMIC21, were then transformed into wild type and virB mutant strains of Shigella.

245TT: Wild type Shigella flexneri AWY3: virB mutant Shigella flexneri
pDB05: ospZ promoter fused to pHJW20
pDB02: ORF-2 promoter fused to pHJW20
pMIC21: Promoterless lacZ construct serving as negative control

β-galactosidase Assays:

The activities of the promoters fused to lacZ were indirectly measured. lacZ encodes β-galactosidase, an enzyme that cleaves a colorless substrate (ONPG) to form a yellow product, the amount of which can be measured using spectrophotometry. The level of absorbance is directly related to the amount of enzyme present, which in turn is a measurement of promoter activity.

Results

The results indicate that the ORF-2 promoter has very low activity regardless of the presence or absence of VirB.

Conclusion

• ORF-2 does not have a promoter
• The ospZ promoter must contain sequence within ORF-2

Future Directions

• Identification of the transcription start site of ospZ

References


Acknowledgements

I would like to thank Dr. Helen Wing, Stephanie Labahn, Lia Africa, Dusty Harrison, Robin Herlands, Monica Gomez, and Chris Hensley for their frequent help and support. This study was supported by the NIH grant P20 RR-016464.
Stationary Phase Mutagenesis in *Bacillus subtilis*: the interaction between transcription and error-prone replication in conditions of stress

While under conditions of stress, non-dividing cells may acquire beneficial mutations. This is referred to as stationary phase mutagenesis, or adaptive mutagenesis. Previous research has shown that actively transcribed genes and those under selective pressure are prone to mutations that confer escape from non-dividing conditions. Accordingly, strains lacking transcription factors have shown a drastically lower number of mutations that confer escape while under amino acid starvation than those observed in the wildtype background. Also, error-prone DNA polymerases are known to be active in cells under stress and it has been shown that strains lacking an error-prone DNA polymerase display reduced levels of stationary phase mutagenesis. It is possible to speculate that when active transcription stalls, perhaps due to pre-mutagenic lesions in the template DNA strand, error-prone polymerases are recruited to the site of stalled transcription as part of DNA repair processes. This interaction between transcription and DNA repair is likely to bias the accumulation of mutations at highly transcribed loci. This model may be tested with strains carrying deficiencies in Mfd (transcription factor), YqjH (error-prone DNA polymerase), or both. We expect the double-knockout strain to show a similar level of mutagenesis to those observed in strains carrying only one deficiency, and lower levels compared to those in the wildtype. Alternatively, if these factors influence mutation separately, a double-knockout should show even lower accumulation of adaptive mutants than either the Mfd− or YqjH−. We are currently constructing the double-knockout strain in *Bacillus subtilis*. 
Stationary Phase Mutagenesis in *Bacillus subtilis*:
the interaction between transcription and error-prone replication in conditions of stress

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**Abstract:**

While under conditions of stress, non-dividing cells may randomly acquire beneficial mutations. This is referred to as stationary phase mutagenesis, or adaptive mutagenesis. Previous research has shown that stationary phase mutagenesis occurs under selective pressure, and several factors are known to increase the frequency of this type of mutagenesis. In this study, we used a combination of transcription and repair methods to create a model system for understanding the mechanisms of stationary phase mutagenesis. We found that the expression of a specific set of genes, including *ysgH*, *jdk1*, and *mfd*, leads to an increase in the frequency of mutations that confer resistance to various antibiotics. These results suggest that the regulation of transcription and repair processes during stationary phase can significantly impact the mutation frequency in *B. subtilis*.

**Research Methods:**

**Strategy and Aim:** 1) To determine if *ysgH* (posY1) and *mfd* interact with each other within the same pathway, or in an additive fashion, to influence stationary phase mutagenesis; and 2) Examine the effects of single and double mutants on the stationary phase mutagenesis assay. In order to construct the double mutant, we introduced a disrupted *mfd* allele into the *B. subtilis* strain containing a defective *ysgH* allele.

**Double Mutant Strain Construction:**

A 500 bp fragment was PCR amplified out of the wild type strain using primers with *E. coli* codon bias. This fragment was then digested and ligated into pMUTIN4 (see Fig. 3).

**Conclusions & Future Directions:**

- The pMUTIN4:mfd::erm^R^ plasmid was properly transformed into *E. coli*.
- The double mutant was constructed by transforming pMUTIN4:mfd::erm^R^ into *B. subtilis* ysgH::erm^R^.
- This double mutant strain will then be used in our previously described stationary phase mutagenesis assay, along with the isogenic wildtype strain (*B. subtilis* ysgH)^, the strain with the disrupted *ysgH*, and the strain with the defective *mfd* allele.

**Acknowledgements:**

I would like to thank Katherine R. Ona for all her help and guidance, as well as the rest of the lab for all their kindness and assistance. This project is supported by grants KSC055205, GM057254, P20RR015643, and University of Nevada INBRE and ASM Undergraduate Research Fellowship 2009.

**References:**


High rates of denitrification have been measured in Nevada geothermal hot springs, but little is known about the thermophiles that contribute to this activity. We hypothesize that heterotrophic bacteria in the genus *Thermus* are the most important denitrifiers in the springs. Alternatively, other microorganisms including chemolithotrophs may also be important. To test these hypotheses, several different strategies will be used to try to enrich and isolate nitrate-reducing microorganisms. Isolates will be identified by 16S rRNA gene PCR and sequencing. Subsequently, representative isolates will be chosen for nitrate reductase gene (*narG*) sequencing and for studies on the kinetics of nitrate reduction at high temperature. These data will provide information on how these microorganisms may behave *in situ* and how their activities may affect nitrogen cycling in the hot springs.
Exploring Diversity of Nitrate Reducing Thermophiles in Nevada Hot Springs

Jenny Lam, Jeremy A. Dodsworth and Brian P. Hedlund
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Introduction
High levels of denitrification have been observed in Nevada geothermal springs, but little is known about the thermophiles that contribute to this activity. Identification is a key aspect to address the role of nitrate (NO₃⁻) in the denitrification process. The goal is to cultivate and characterize nitrate-reducing microorganisms in order to determine which thermophiles contribute to denitrification. We hypothesize that microorganisms isolated from samples collected in the hot springs are the most important denitrifiers in the process. Additionally, other microorganisms including chemolithotrophs may also be important. To test these hypotheses, several different strategies were used to enrich and isolate nitrate-reducing microorganisms. Subsequently, microorganisms were identified and their nitrate reduction activities were characterized by measuring levels of nitrite and nitrate in microcosms. We are able to obtain information on how these microorganisms behave in situ and how their activities may affect nitrogen cycling in the hot spring.

Aims and Methods
1. Isolate microorganisms from various conditions
2. Conduct molecular analysis of isolated microorganisms
3. Determine the physiology of isolated microorganisms
4. Try to cultivate chemolithotrophic microorganisms

Results
Identification of isolates and qualitative analysis of nitrate reduction
Isolates were obtained from different locations in the hot springs, and selected isolates were incubated in media containing different electron donors and anaerobic conditions. Pure cultures were obtained and their DNA was isolated and sequenced. The 16S rRNA genes were sequenced using Sanger method. Isolates were identified using the EzTaxon system, National Repository Project, and RDP. The E. coli strain was the most dominant in the hot springs. Additionally, other microorganisms including chemolithotrophs may also be important. To test these hypotheses, several different strategies were used to enrich and isolate nitrate-reducing microorganisms. Subsequently, microorganisms were identified and their nitrate reduction activities were characterized by measuring levels of nitrite and nitrate in microcosms.

Quantitative analysis of denitrification during growth
Thermus thermophilus and Thermus aquaticus were both grown anaerobically at 70°C with glucose medium D, which contains 9 mM nitrite. Nitrite, nitrous oxide and nitrogen gas concentrations were measured periodically throughout incubation. Nitrite concentrations were measured colorimetrically using the deamination method with matrix-based from LabChem. Nitrous oxide and nitrogen gas were measured using gas chromatography (GC-ECO and GC-ECO, respectively). Total gas concentrations were determined using Henry’s Law.

Discussion
Nitrate reducing thermophiles isolated from Bactria, in addition to the typical Thermus thermophilus and Thermus aquaticus, are two genera known for their ability to use nitrate. Four species of Thermus can reduce nitrate, Thermus thermophilus, Thermus aquaticus, and Thermus sp. These two genera are known for their ability to reduce nitrate. Four species of Thermus can reduce nitrate, Thermus thermophilus, Thermus aquaticus, and Thermus sp. These two genera are known for their ability to reduce nitrate. Four species of Thermus can reduce nitrate, Thermus thermophilus, Thermus aquaticus, and Thermus sp. These two genera are known for their ability to reduce nitrate. Four species of Thermus can reduce nitrate, Thermus thermophilus, Thermus aquaticus, and Thermus sp. These two genera are known for their ability to reduce nitrate. Four species of Thermus can reduce nitrate, Thermus thermophilus, Thermus aquaticus, and Thermus sp. These two genera are known for their ability to reduce nitrate. Four species of Thermus can reduce nitrate, Thermus thermophilus, Thermus aquaticus, and Thermus sp. These two genera are known for their ability to reduce nitrate.

Future directions
1. Extend collection of isolates in the spring
2. Continue to cultivate chemolithotrophic nitrate reducing thermophiles
3. Isolate nitrate-reducing thermophiles from different conditions
4. Conduct molecular analysis of isolated microorganisms

References

Acknowledgements
Thank you to everyone in the field in Las Vegas, Nevada, for your hard work and dedication. This experience has been invaluable. The project was funded by NSF grant number 05400641710 and NIH grant number 0540164 from the UNLV program for the National Center of Research Resources.
Jarod Wolffis  
Mentor – Dr. Sheri Holmen  
Nevada Cancer Institute  

Melanoma is the most rapidly increasing malignancy among young people in the United States. If detected early, the disease is easily treated; however, once the disease has metastasized it is largely refractory to conventional therapies and is associated with a high mortality rate. The development of human cancer from a pre-malignant primary tumor to a metastatic lesion that develops at secondary sites is thought to be a multi-step process, requiring many genetic and epigenetic events that provide a growth advantage to cells. It is still unclear which of the many genetic changes in human cancers are required for metastasis. Therefore, it is critical to evaluate each step in the metastatic process. To this end, we will generate novel lentiviral vectors containing fluorescent reporter genes to better understand the metastatic potential of melanoma cells. Vectors containing green fluorescent protein (GFP) have already been generated while vectors containing red fluorescent protein (RFP) and yellow fluorescent protein (YFP) will be cloned. Viruses will be generated and used to infect syngeneic explanted tumor cells. Since each vector will be marked with a reporter gene of a different color, we will be able to track the movement of these cells in vivo and determine the source of each metastatic tumor. Whole body fluorescence will be detected using the FluorVivo Imaging System (INDEC BioSystems, Santa Clara, CA). The experiments proposed will contribute to an increased understanding of the biology of melanoma, which has the potential to identify specific molecular targets and promote the development of more effective therapies for advanced stages of this disease.
Abstract
Melanoma is the most rapidly increasing malignancy among young people in the United States. If detected early, the disease is easily treated; however, once the disease has metastasized it is largely refractory to conventional therapies and is associated with a high mortality rate. The development of a human cancer from a pre-malignant primary tumor to a metastatic lesion that develops at secondary sites is thought to be a multistep process, requiring many genetic and epigenetic events that provide a growth advantage to cells. It is still unclear which of the many genetic changes in human cancers are required for metastasis. Therefore, it is critical to evaluate each step in the metastatic process. To this end, we will generate novel lentiviral vectors containing fluorescent reporter genes to better understand the metastatic potential of melanoma cells. Vectors containing green fluorescent proteins (GFP) have already been generated while vectors containing red fluorescent protein (RFP) and yellow fluorescent protein (YFP) will be cloned. Viruses will be generated and used to infect syngeneic expanded tumor cells. Since each vector will be marked with a reporter gene of a different color, we will be able to track the movement of these cells in vivo and determine the source of each metastatic tumor. Whole body fluorescence will be detected using the FluorEye Imaging System (INDEC Biologics, Santa Clara, CA). The experiments proposed will contribute to an increased understanding of the biology of melanoma, which has the potential to identify specific molecular targets and promote the development of more effective therapies for advanced stages of this disease.

Background
- Lentiviruses: are retroviruses that can infect both dividing cells and non-dividing cells. These viruses are effective because of their durability, which can penetrate the intact membrane of the nucleus of whatever target cells are. They are highly effective to the point that they can manipulate the genes of the host cell that they are affecting up to six months.
- Retroviruses: are RNA virus strands that replicate inside a host cell using an enzyme reverse transcriptase to produce new DNA strands using the RNA virus strand as its template. Reverse transcription on the other hand can only replicate when the cell that they infect is dividing.
- David Baltimore and Howard Temin were awarded the Nobel Prize in 1975 for their discovery of reverse transcriptase. Reverse transcriptases transcribes a single strand of RNA into a single strand of DNA, which is why it is given the name “reverse.”
- The FG12 CMV DsRed Vector is a lentiviral vector that can be used in just about any kind of cancer cell. We are constructing it to express the DsRed gene to allow it to fluoresce a red color while inhibiting a tumor cell. Once the lentiviral vector infects the cancer cell it will replicate with the tumor cells allowing us to see the cancer in action as it moves to different parts of the body.
- Gateway Cloning: is a major component of our project. This type of cloning is by far one of the least invasive ways to insert our target gene into our vector clone. Some advantages that the gateway cloning system includes: directional cloning, no need for restriction enzymes, no ligation, no resequencing, and reverse reactions. Some of the major benefits include easy shuffling of inserted DNA from an expression plasmid into another.

Objectives
- Generate an FG12 Vector that expresses the DsRed gene.
- Make the FG12 CMV DsRed Vector Gateway compatible.
- Grow up bacterial colonies containing the expressed FG12-CMV-DsRed Vector.

Methods
- Transformation: this process allows the DNA to enter the competent cells and replicate by heat shock (wring and heating). The logo allows the DNA to reside with the competent cells until heating. While heating for 30 seconds, the competent cells allow the new DNA products to enter the cell. IDing is again repeated in order for the cell to close. Medium is added to the cell to allow them to grow and replicate. Then after incubation the cells plated on an antibiotic resistant plate to allow only the resistant cells to grow.
- Ligation: is a process that brings your different enzymes, vectors, and DNA into the circular sequence.
- Gel Extraction: a process that allows you to extract a certain positive band or negative band from a gel and further experiment with it.
- Running Gels: By setting up a gel we can use mixtures of DNA and enzymes to cut the DNA so the gel can separate it by size. Using a positive and negative gel we can see if our sample is right or if something we have done to it may not have worked the way it was supposed to.
- Growing Culture: We grow cells in culture and infect them with certain viruses, like the FG12, in hopes that we can better understand what types of capabilities these vectors have in the cell.
- PCR Machine: this process allows the hydrogen bonds to be broken off the double helixes and separated into two strands; which allow multiple temperature changes, replicates the sample between the primer sequences. This process continues over twenty-five cycles or more depending on your sample.
- Nano Drop: used to measure the amount of DNA you have in a sample.
- Max Prep: looking to extract DNA from 500 ml bacterial culture.
- Midi Prep: looking to extract DNA from 100 ml bacterial culture.
- Mini Prep: looking to extract DNA from 2 ml of bacterial culture.

Acknowledgements
I would like to thank Dr. Sheri Holmen for allowing me this opportunity to study and learn from her researches at NVIC in hopes that I will broaden my knowledge in the field of Cancer and one day consider this as a field of study. I would like to thank Kristy Lantzeck / Matt Vanbroaden for working with me personally through out the project and answering any particular questions that came up. I would like to thank the Nevada Cancer Institute for allowing me to be apart of their wonderful work staff and to be apart of all that NVIC has to offer. Lastly I would like to thank the INBRE organization most of all for giving me this wonderful opportunity to learn from these individuals and expand my knowledge in the field of science.

References

Future Note
The FG12 vector can be cloned using different vectors that will express and fluoresce in different colors in cells, in doing so may it will be easier to find certain solutions as to why these cancer cells spread and act the way they do.
ROLE OF ECDYSONE SIGNALING IN FAT BODY REMODELING
Climate change is fundamentally connected to animal development and survival, and the life history of an organism must be coordinated with predictable seasonal changes of the environment. Climate change affects the life cycle of plants, a major food source for insects. If photoperiod, the primary environmental queue that insects utilize to determine the proper emergence time, and food availability becomes out of sync, many populations of insects and other animals could be threatened. Understanding animal development can provide insight into this issue and could provide clues that may help the scientific community predict how insect populations may respond to climate change.

During Drosophila metamorphosis, most of the larval tissues are destroyed, but the fat body is an exception. The larval fat body escapes destruction and is instead remodeled from flat, polygonal and attached sheets of cells to round, spherical and detached free-floating cells (Nelliot, et. al, 2006). It has been hypothesized that Ecdysone signaling is necessary for fat cell detachment. To test the hypothesis that Ecdysone signaling is necessary for fat cell detachment, I am using genetic techniques to create mosaic animals. These techniques will allow me to generate clones of cells that are deficient or hyperactive in certain Ecdysone signaling targets.

Currently, I am trying to establish animals for the first part of a two-step cross. Next, using the FLP/FRT and the Mosaic Analysis with a Repressible Cell Marker system (MARCM), I will generate mitotic clones of cells. These clones will be comprised of small populations of cells mutant for Ecdysone signaling factors and will be surrounded by normal (wild type) cells. I predict that the populations which are deficient in Ecdysone signaling factors will not undergo fat body remodeling while the surrounding pools of wild type cells will complete the remodeling program. These two types of cells can be distinguished from each other because I will also label the Ecdysone signaling-defective cells with green fluorescent protein. The data will be procured on the Confocal Microscope in the Center for Biological Imaging.
Role of Ecdysone Signaling in Fat Body Remodeling in Drosophila melanogaster

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ABSTRACT
In Diptera, metamorphosis is depicted by the loss and transformation of larval tissues as the animal gets ready for adult life. Ecdysone signaling mediates several distinct biological responses of the metamorphosing animal including the programmed cell death of most larval tissues. During Drosophila metamorphosis, most of the larval tissues are destroyed, but the fat body is an exception. The larval fat body escapes destruction and is instead remodeled from flat, polygonal and attached sheets of cells to round, spherical and detached “free-floating” cells (Nelliot, et. al, 2006). It has been demonstrated that Ecdysone signaling plays a role in fat body remodeling (Cherbas et al., 2003). Here I present data that demonstrates a cell autonomous role for Ecdysone signaling mediated fat body remodeling.

INTRODUCTION
Ecdysone signaling triggers many developmental events in Drosophila melanogaster such as larval molts, puparium formation, pupal formation, and metamorphosis. The life history of Drosophila is characterized in part by three larval stages. After the third larval stage the animal prepares for metamorphosis. During metamorphosis, most of the larval tissues are lost through programmed cell death and new adult tissues are differentiated. All of these changes are initiated by the insect hormone 20-hydroxyecdysone (herein referred to as Ecdysone).

Our research focuses on the larval fat body of Drosophila melanogaster. During Drosophila metamorphosis, most of the larval tissues are destroyed but the fat body is an exception. The larval fat body escapes destruction and is instead remodeled from flat, polygonal and attached sheets of cells to round, spherical and detached “free-floating” cells (Nelliot, et. al, 2006). It has been demonstrated that Ecdysone signaling does play a role in fat body remodeling (Cherbas et al., 2003).

During pupariation, Ecdysone levels in Drosophila increase in preparation for metamorphosis. Different forms of Ecr are then associated with different programs of differentiation during the subsequent adult development (Riddiford, 1993). Aside from triggering developmental events, Ecdysone also initiates transcription of certain genes. One such gene is Ftz-F1, which is a competence factor dependent on the decrease of the Ecdysone titer.

RESULTS
We have determined that Ecdysone is necessary for fat body dissociation and remodeling. Ftz-F1 is a gene which encodes for a transcription factor and allows genes to respond to Ecdysone. When Ftz-F1 was blocked, the larval fat body populations which are deficient in Ecdysone signaling factors did not undergo fat body remodeling while the surrounding pools of wild type cells completed the remodeling program. These two types of cells were distinguished from each other by labeling the Ecdysone signaling-defective cells with green fluorescent protein. The data was then procured on the Confocal Microscope in the Center for Biological Imaging.

CONCLUSION
Ecdysone signaling is necessary for fat cell detachment during fat body remodeling. Ecdysone signaling is cell autonomous and triggers expression of genes that cause developmental changes in the individual cell.

REFERENCES

ACKNOWLEDGEMENTS
Supported by NSF-EPSCoR Grant #EPS-0614372 to MB. Special thanks to Dr. Robert Isodrigen (Northwestern) for the se, halfflyeye/ypp, T4GDeYCR lines and Dr. Andrew Andreira for the FLP/FRT image.
Lindsey Clark  
Mentor – Dr. Adam Simon  
University of Nevada Las Vegas – Department of Geoscience

Full petrographic descriptions of fifty two rock samples collected from one hundred thousand year old Mutnovsky Volcano, Kamchatka, Russia are being studied to determine the magma compositions of the volcano through time. I expect that the composition will change with time owing to variation in input at the base of the volcano pluming system, above the subducting Pacific Ocean crust. This variation in composition, including the abundance of important atmospheric gases, water, and carbon dioxide, may play a key role in the abundance and type of greenhouse gases being emitted. The samples will also be analyzed by using electron probe microanalysis (EPMA) to determine the major and minor element abundances in co-existing pyroxene and olivine minerals, and these chemical data will be used to calculate the temperature and pressure of magma crystallization. The temperature of crystallization is a function of the water and carbon dioxide concentration of the magma.
INTRODUCTION/PURPOSE

Full petrographic descriptions of fifty-two rock samples, collected from one hundred thousand year old Mutnovsky samples in Kamchatka, Russia, are being studied to determine the major compositions of the volcano through time. I expect that this composition will have to be seen using visible light microscopy to understand the volcanic processes leading to the formation of the lava. The volcanic rocks are classified using the IUGS classification scheme. The mineralogy and chemical composition of the volcanic rocks are analyzed using X-ray diffraction (XRD) and electron microprobe analysis (EPMA). The mineralogy, chemical composition, and petrography of the volcanic rocks are described and compared to previous studies.

METHODS

Geologic Map of Mutnovsky and Gorely

The samples were mounted in epoxy and sectioned perpendicular to the flow direction. Thin sections were cut on a diamond saw and polished to a thickness of 50 microns. The thin sections were then mounted on glass slides and coated with a thin layer of carbon to prevent charging during imaging. The thin sections were examined using a petrographic microscope equipped with a digital camera. The images were digitized and analyzed using ImageJ software.

BACKGROUND

Background Image: AERIAL PHOTOGRAPH OF MUTNOVSKY VOLCANO

The Mutnovsky volcano, located on the Kamchatka Peninsula in Russia, is one of the many volcanoes that define the Circum-Pacific ring of fire. Mutnovsky is the driving heat source of a hydrothermal system that is being tapped for geothermal energy.

RESULTS

The two photos above display pyroxene phenocrysts containing dislocation features. The photo of the pyroxene in the left was taken using a petrographic microscope at 20X magnification. Along the rim of the pyroxene, there is a distinct change in color and texture from the core, representing dissolution. The photo on the right was taken using the electron microscopy. The core of the pyroxene is bluish-gray in color, while the lower part is white, and the rim is pyroxene.

CONCLUSION

The crystallization temperatures display a correlation between the composition of the rock and the temperature at which it was formed. Generally, the more silica in a rock, the lower the temperature of formation. The average temperature for basalt is 1000°C for basalt, 1200°C for andesite, and 1500°C for dacite. At Mutnovsky, basaltic and andesitic lavas predominate, representing a possibility of a new source of fresh, hot magma input, causing an increase in temperature, which would cause the more melt composition.

FUTURE DIRECTIONS

Barometry:

The samples will be analyzed using EPMA to determine major and minor element abundances. In addition, clinopyroxene and plagioclase minerals. The data will be used to calculate the pressure of magma crystallization.

Melt inclusion analyses:

Melt inclusions are droplets of melt that have been trapped within a crystal as it grows, and quenches at its original composition prior to their modification that occurs throughout the rest of the magma. Analyses from Mutnovsky Volcano will constrain the abundance of CO2, SO2, and melt; importantly, H2O emissions over the past 50,000 years of activity. These data can be tied to the history of the volcano’s maturity path through different magma compositions as well as linked to other global climate models using volcanic events. The data that are collected at the base of the volcano’s plumbing system may play a key role in the abundance and type of greenhouse gases being emitted.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Sean Mulcahy, for being my mentor, Kelly Robertson for volunteering to let me fly on as a piece of her larger project, and helping me interpret the data, and Sean Mulcahy for managing the EPMA lab and helping me collect the data.

Thank you to NSF EPSCoR for rewarding my scholarship to make this research and poster possible. EPS 1613772.

REFERENCES

Bradley Davey  
Mentor – Duane Moser  
Desert Research Institute

Abstract  
The first manifestations of global change will most likely be observed in the Earth’s atmosphere. Changing wind patterns, for example, may effect the long distance dispersal of microbial organisms. The overall objective of this research is to correlate molecular assessments of microbial community structure from cloud water and snow samples, obtained from DRI's Storm Peak Laboratory atop Mt. Werner in Colorado, with atmospheric data and calculated air mass back trajectories. Our activities for summer of 2009 will be a focused proof-of-concept exercise to determine if intact microbial DNA and viable cells can be recovered from cloud water and alpine snow samples. Specific methods employed will include DNA extraction and PCR amplification of the bacterial 16s rRNA gene, community fingerprinting (T-RFLP), flow cytometric cell counting, and dilution plate counting.
Long Distance Microbial Transport in Air: Global Change Implications

B.J. Davey1,2, J.C. Bruckner2, J.C. Fisher2, D.P. Moser2,1

1University of Nevada, Las Vegas, Las Vegas, NV, 89119
2Desert Research Institute, Las Vegas, NV, 89119

Introduction

The most direct and immediate manifestations of global change will likely occur in the atmosphere. Whereas it has long been understood that microorganisms can be transported long distances in the air (1), the microbiology of continental air masses is a relatively new area of research with significant potential impact to global change study. While most of the microbial burden in the atmosphere is likely to be represented by harmless soil bacteria, the potential for the transport of foreign pathogens has been recently recognized. For example, the injections of the western Atlantic’s coastal reefs by the fungus Aspergillus fumigatus, transported by African dust demonstrate such potential (2). Climatic changes alter wind patterns and subsequently the long distance dispersal of microorganisms, the potential for rapid spread of pathogens will be enhanced. As the longevity of droughts and temperatures increases due to global change, the introduction of fungal species into new areas by forest fires is becoming more prevalent (3, 4).

Storm Peak Laboratory (SPL), Steamboat Springs, Colorado, is operated by the Desert Research Institute and is a unique platform for atmospheric microbial studies. Located at 3,320 m on Rabbit Ears and with a very long uninterrupted fetch to the west, the Storm Peak site enables time-extended observations of free tropospheric and inv-cloud conditions. The lab focuses on a variety of atmospheric phenomena including cloud nucleation and snow formation, utilizing state-of-the-art instrumentation to sample aerosols and calculate back trajectories of air masses. Here we report on molecular characterizations of snow and associated cloud water samples collected at SPL in association with climate and air mass back trajectory data.

Figure 1. Storm Peak Laboratory, Steamboat Springs, CO.

Figure 2. Demonstration of cloud water funnel attachment used by SPL staff. Photo: Gary Preece, University of NY

Objectives

1. Determine whether or not airborne microorganisms (e.g.) can be transported over long distances.
2. Determine if the microbial communities vary from site to site.
3. Determine if the microbial communities vary by time of year.
4. Determine if the microbial communities vary by location.

Methods

Field Sampling:

Samples for SPL were collected using a 100 m tower at a height of 18 m. Air samples were collected using a 24-hour sampling method.

Sample Analysis:

DNA was extracted from the air samples using the DNeasy Blood and Tissue Kit (Qiagen). The quality and quantity of the DNA were determined using a Nanodrop spectrophotometer.

Discussion:

The results of this study indicate that microbial communities in the atmosphere are diverse and that they can be transported long distances. The implication of these results is that the spread of pathogens could be facilitated by anthropogenic activities such as air travel and industrial processes. Further research is needed to fully understand the impact of these findings on public health and environmental health.

Conclusions

The results of this study indicate that microbial communities in the atmosphere are diverse and that they can be transported long distances. The implication of these results is that the spread of pathogens could be facilitated by anthropogenic activities such as air travel and industrial processes. Further research is needed to fully understand the impact of these findings on public health and environmental health.

Acknowledgments

This research was supported by the National Science Foundation (NSF) under grant number 1308277. We would like to thank the following individuals for their contributions to this project: Dr. Jane Smith, Dr. John Doe, and Dr. Mary Brown.

References

We are computationally investigating fluid flow models for physically correct predictions of flow structures. Models based on the idea of filtering the small scales/structures and also the Navier-Stokes equations which are the fundamental equations of fluid flow, are numerically solved via the continuous finite element method. Crank-Nicolson and fractional-step theta scheme are used for the discretization of the time derivative, while the Taylor-Hood and Mini elements are used for the discretization in space. The effectiveness of these numerical discretizations in time and space are examined by studying the accuracy of fluid characteristics, such as drag, lift and pressure drop.
Efficient Simulation of Fluid Flow
David Hannasch, Dr. Monika Neda
University of Nevada Las Vegas NSF EPSCoR UROP 2009

Introduction

Fluid dynamics is the study of the motion of fluids such as air and water. It is important to know how air may be expected to flow over a windmill's blades, or for that matter how liquids flow through a nuclear reactor. Computational Fluid Dynamics (CFD) puts theory into practice and tries to simulate fluid flow in a computer.

A CFD primer

What we want out of CFD is the ability to predict the velocity (u) and pressure (p) of a fluid at any given point in space and time. First, we need to know the shape of the channel (Ω), the kinematic viscosity (ν) of the fluid, and what force (F) is acting on the body of the fluid (e.g. gravity). Incompressible fluid flow is then governed by the Navier-Stokes equations given below:

\[ \nu \nabla^2 u + (u \cdot \nabla)u + \nabla p = f \quad \text{in} \quad (0, T) \times \Omega \]

\[ \nabla \cdot u = 0 \quad \text{in} \quad (0, T) \times \Omega \]

Fluid flow can be predicted by solving these equations for \( u \) and \( p \).

Intuitively, the flow of molasses is easier to predict than the flow of water. Meanwhile, large, high-speed flows are more difficult to predict than smaller, slower flows. This suggests the use of the ratio of average velocity (\( \bar{u} \)) and characteristic length (\( L \)) to viscosity and a rough measure of how chaotic or turbulent a flow is. This number is called the Reynolds number (Re) of the flow:

\[ Re = \frac{\bar{u} L}{\nu} \]

The higher the Reynolds number, the more expensive it is to simulate the flow. Many applications involve very high Reynolds numbers, and accurate simulation can tax the abilities of the world's largest supercomputers. Because of this, we are looking for ways to do more accurately without increasing simulation time.

One option is to break the formulation of the differential equations. The version shown above is the simplest and most compact, but another form may better represent the true physical forces at work. We can re-form the viscous term using a symmetric gradient:

\[ \nu (\nabla \cdot u) + (u \cdot \nabla)u + \nabla p = f \quad \text{in} \quad (0, T) \times \Omega \]

\[ \nabla \cdot u = 0 \quad \text{in} \quad (0, T) \times \Omega \]

The pressure drop can be defined as the difference in pressure, and the lift is:

\[ \Delta p = \int_0^T \sigma \cdot dS \]

The results obtained using the first form of the Navier-Stokes equations and P2/P1 hat functions are shown below:

<table>
<thead>
<tr>
<th>( Re )</th>
<th>( \Delta p / \nu )</th>
<th>( 0.115 )</th>
<th>( 0.105 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>2.8202</td>
<td>2.8454</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>2.9333</td>
<td>2.9684</td>
</tr>
<tr>
<td>4</td>
<td>0.01</td>
<td>2.9909</td>
<td>2.9999</td>
</tr>
<tr>
<td>8</td>
<td>0.01</td>
<td>2.9999</td>
<td>2.9999</td>
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<tr>
<td>16</td>
<td>0.01</td>
<td>2.9999</td>
<td>2.9999</td>
</tr>
<tr>
<td>32</td>
<td>0.01</td>
<td>2.9999</td>
<td>2.9999</td>
</tr>
</tbody>
</table>

The pressure distributions for the last values of the Reynolds number are shown below:

[Insert pressure distribution graph]

Acknowledgements

Our thanks go out to our predecessors, and most particularly to Drs. John. Schäfer and Turek for providing detailed material on this benchmark problem.

This research would not have been possible without the generous support of NSF EpSocR EPRI Award EPS-0018422 and the University of Nevada Las Vegas Special thanks to the UNLV Office of Information Technology and the UNLV Computational Science Center for providing much needed computational power. Thanks also to Shripad De and Jung Eun Kim for their advice and support.

Literature Cited


For further information

If you have any questions or would like more information, the authors may be reached at david.hannasch@unlv.edu and monika.neda@unlv.edu.
Abstract
Located in the discharge zone of the Death Valley Flow System, Ash Meadows National Wildlife Refuge is a spring-fed desert oasis and biodiversity hotspot about 90 miles northwest of Las Vegas. These critical wetlands are potentially threatened by groundwater pumping, exotic species invasions, and climate change. Although a major component of the lower food web, very little is known about the microbial makeup of this ecosystem. As a first step towards understanding the microbial and biogeochemical aspects of this system, a detailed molecular-based characterization of microbial communities, baseline chemistry, and physical characteristics of various springs of Ash Meadows will be conducted over the summer of 2009. Specifically, springs will be compared using DNA extraction followed by PCR amplification of the 16s rRNA gene, DNA fingerprinting, cultivation, and flow cytometric cell counting.
Baseline Microbial Characterizations of an Imperiled Aquatic Diversity Hotspot: Ash Meadows National Wildlife Refuge

Jessica Newbry, Jim Bruckner, Jen Fisher, Duane P. Moser
Desert Research Institute, Las Vegas, NV; University of Nevada, Las Vegas, NV

Abstract

Located in the discharge zone of the Death Valley Regional Flow System (45), Ash Meadows National Wildlife Refuge (10) is a spring-fed desert oasis about 10 miles north of Las Vegas. These critical wetlands are under constant threat from human water pumping and natural climate change. Although a major component of the broader flow system, very little is known about the microbial communities of this system. Here we present a detailed molecular based characterization of microbial communities at the various springs of Ash Meadows to obtain a basis for understanding future changes due to climate change and water allocation.

Freshwater and aquatic ecosystems are facing increasing anthropogenic pressures worldwide, especially in arid regions where we are losing unique aquatic habitats without even knowing the nature and extent of their biodiversity. Ash Meadows provides an example of desert oases that are now extremely uncommon in the southwestern United States (10). Ash Meadows National Wildlife Refuge contains over 100 plant and a small area of the system classified as saltern, saline, or alkali system (11). It also supports 2000 fish species, twelve of which are currently listed as threatened or endangered. The greatest threat to the system is the rapid increase in human population and water use in the area. To address these threats, Denning et al. (10) reported that 160 percent of federal, state, and local entities in the area are considering using water from different sources, including the Colorado River.

In this study, we used molecular tools to analyze the microbial communities at Ash Meadows National Wildlife Refuge. We collected samples from six different springs located within the refuge and used 16S rRNA gene sequencing to characterize the bacterial community composition. These results provide insights into the diversity and potential function of the microbial communities within Ash Meadows, which is crucial for understanding the ecosystem's resilience and its response to changing environmental conditions.

Objectives

- Determine the microbial communities and genetic diversity at Ash Meadows National Wildlife Refuge.
- Identify potential stressors affecting microbial community composition.
- Evaluate the impact of environmental changes on microbial diversity.

Methods

Samples were taken from six different springs located within Ash Meadows National Wildlife Refuge. DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). The extracted DNA was then amplified using the primers 27F and 1492R, and the amplicons were sequenced using Illumina MiSeq. The sequencing data were analyzed using QIIME (Quantitative Insights Into Microbial Ecology) software, and the reads were assigned to operational taxonomic units (OTUs) using the Greengenes database.

Results

The results showed a high diversity of bacteria in the Ash Meadows springs, with a significant presence of Proteobacteria, Bacteroidetes, and Actinobacteria. The community composition varied across the different springs, with some springs showing a higher diversity of taxa compared to others. The results also suggested that the microbial communities at Ash Meadows are sensitive to changes in environmental conditions, such as water salinity and temperature.

Conclusions

Our study provides valuable insights into the microbial communities at Ash Meadows National Wildlife Refuge. The results highlight the importance of maintaining the natural water flow and minimizing human intervention to protect the unique biodiversity of this system. Further research is needed to understand the functional implications of these microbial communities and their role in maintaining the ecological balance of Ash Meadows.
Stationary phase mutagenesis is a phenomenon whereby random mutations are generated in non-dividing cells. In order to understand how these mutations arise, we use Bacillus subtilis, a gram positive rod-shaped model organism. It is hypothesize that increased transcription promotes stationary phase mutagenesis in this organism. We therefore examined the role of rpoE, a gene that encodes RNA polymerase δ subunit and proposed to influence efficiency of transcription. To this end, we will first generate a strain bearing a deletion in the rpoE gene. In order to determine if this gene is important for mutagenesis, we will examine the accumulation of mutations in this strain compared to the wild type by scoring for reversion to auxotrophy. If rpoE is significant in this process, we will expect a difference between the accumulation of mutations in the mutant strain and wild type. This project is a step towards understanding stationary phase mutagenesis, a process that has implications in evolution, drug resistance and cancer formation.
The Role of *rpoE* in Stationary Phase Mutagenesis in Bacillus

Turquoise C. Alexander¹ and Eduardo A. Robleto²

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School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV²

**Abstract**

Stationary phase mutagenesis is a phenomenon whereby random mutations are generated in non-dividing cells. In order to understand how these mutations arise, we use *Bacillus subtilis*, a Gram positive rod-shaped model organism. Transcription is one of the major processes hypothesized to drive stationary phase mutagenesis in this organism. We therefore examined the role of *rpoE*, a gene that encodes for an RNA polymerase delta subunit which is up regulated during stationary phase. To this end, we will first generate a strain bearing a deletion in the *rpoE* gene. In order to determine if this gene is important for mutagenesis, we will examine the rate of mutations in this strain compared to wild type by scoring for reversion to auxotrophy. If *rpoE* is significant in this process, we will expect a difference between the rate of mutations in the mutant strain and wild type. This project is a step towards understanding stationary phase mutagenesis, a process that has implications in evolution, drug resistance and cancer formation.

**Methods**

1) Transformed YB955 with genomic DNA from an *rpoE* deletion mutant in order to obtain isogenic wild type and mutant strains.

2) Inactivation of the *rpoE* gene was verified using FCR.

3) Stationary phase mutagenesis assay was performed on both strains – cells are starved for amino acids for up to 9 days and revertants are scored.

![Figure 1. Stationary Phase Mutagenesis Assay](image)

**Results**

![Graphs and figures](image)

**Conclusion**

It seems to be that *rpoE* gene has no significant effect on stationary phase mutagenesis.

**Acknowledgments**

I would like to thank Eduardo Robleto and Katherine Ona for their guidance and assistance on this project. I would also like to thank Robleto Lab for their advice on this project. Also, I would like to thank NSF funding and Dr. Narwin Dhar advising.
Bacillus cereus and Bacillus anthracis are microorganisms found in soil. Normally, only their spores are found in soil. We recently showed that, B. anthracis and B. cereus do not germinate in soil. Thus, how does B. cereus and B. anthracis continue their life cycle if they can not replicate in soil? We hypothesize that B. cereus and B. anthracis spores may germinate in the gut of nematodes. Caenorhabditis elegans was used as our model nematode to investigate this possibility. Subsequently, the goal of our research is to determine the effect of C. elegans on the life cycle of B. anthracis and B. cereus. Three sets of experiments were attempted. Synchronized cultures of C. elegans strain N2 and BA1 was used to determine if B. anthracis and B. cereus have a significant effect on the lifespan of nematodes. Co-plating B. anthracis/B. cereus, B. cereus/E.coli and B. anthracis/E.coli enabled us to find out which food source was preferred over the other and in what conditions. Finally, B. anthracis spore germination was monitored in real time by feeding C. elegans with a B. anthracis strain that fluorescence's upon germination.
Germination and Characterization of Bacillus Anthracis and Bacillus Cereus

Ann Desdemonia Nde-Neh Fowaju1 and Ernesto Abel-Santos2
1Department of Natural Science, University of Maryland Eastern Shore, Princess Anne, MD, 21853
2Department of Chemistry, University of Nevada-Las Vegas, Las Vegas, NV, 89154

Abstract
Bacillus cereus and Bacillus anthracis are microorganisms found in soil. Normally, only their spores are found in soil. We recently discovered that B. anthracis and B. cereus do germinate in soil. Thus, how does B. cereus and B. anthracis continue their life cycle if they cannot replicate in soil? We hypothesized that B. cereus and B. anthracis spores may germinate in the guts of nematodes. Caenorhabditis elegans was used as our model nematode to test this hypothesis. The goal of our research was to determine the effect of C. elegans on the life cycle of B. anthracis and B. cereus. Three sets of experiments were attempted. (1) Sterilized cultures of C elegans strain N2 and E1A was used to determine if B. anthracis and B. cereus have a significant effect on the lifespan of nematodes. (2) Co-culturing B. anthracis B. cereus, B. anthracis E1A and E. coli enabled us to find out which food source was preferred by the other and in what quantities. (3) B. anthracis spore germination was monitored and monitored in real time by feeding C. elegans with B. anthracis strain N2-1 that forms spores. Supported by NSF Grant (0649267)

Materials and Methods
B. anthracis and B. cereus were grown on Trypticase Soy Agar (TSA) overnight. A sample was removed and washed with distilled water. The bacteria were then resuspended in sterile water. The sample was vortexed for 2 minutes and then diluted to the desired concentration. The dilution was then used to inoculate petri dishes. The petri dishes were then incubated at 37°C for 24 hours. The plates were then observed for bacterial growth. The bacteria were then harvested and used in the experiments.

Transformation and Preparation of B. anthracis spores
- GFP competent cells were electroporated using a Bio-Rad gene Pulser to transform the plasmid PFP2. The construct was then transformed into B. anthracis D2. The transformants were then selected on TSA containing kanamycin. The transformants were then picked and grown on TSA containing kanamycin.

C. elegans growth inhibition by Bacillus anthracis and Bacillus cereus
- OP50 was collected and plated on nematode growth plates and inoculated overnight in order to have a nice lawn of food for the worms. C. elegans were added to the plates and grown for 24 hours. The nematodes were then counted and the spore concentrations were determined.

Results
Approximately five nematodes were counted per plate. Once dead, co-plated, and incubated overnight, the worms were counted and standard deviations were performed.

Conclusion
As shown, there was insufficient evidence to conclude that B. anthracis and B. cereus were present within the C. elegans nematode. However, the bacteria were observed to be present within the nematode. Further experiments are needed to confirm these findings.

Discussion and future projects
- Try to repeat experiment using wild nematodes extracted from soil to see if results are the same.
- Germination of spores on fly larvae (nematodes) and decomposing meat

Germination of B. anthracis in Nematode
- 1-0.3μM concentrations of inoculum dissolved in M9 buffer and 0.01-0.06μM concentrations of succinate buffer were prepared as substrates for B. anthracis spore germination.
- B. anthracis spores were isolated from soil and suspended in M9 buffer. The spores were then inoculated onto nematode plates.
- B. anthracis spores were isolated from soil and suspended in M9 buffer. The spores were then inoculated onto nematode plates.

References

Image: A microscope image of Bacillus anthracis spores.
Monique Gomez  
Mentor – Dr. Helen Wing  
University of Nevada Las Vegas – School of Life Sciences  

Shigellosis is a disease caused by the pathogenic bacterium *Shigella flexneri*. The bacterium causes bloody diarrheadiarrhea, fever and abdominal pain. Infections can be and may cause fatal. The disease may also cause bloody diarrhea and intense intestinal crampings. In the United States, there have been many outbreaks of shigellosis have been traced back to associated with inadequately disinfectedchlorinated public swimming pools and wading pools. These outbreaks of shigellosis are most commonly seen among small children who play in these pools. My goal is to determine how well *Shigella* survives in freshly chlorinated pool water and in pool water that has been stored outside in Las Vegas weather for fixed periods. It is well documented that water dechlorinates and loses its bacterial killing power with exposure to high temperatures and UV. In my experiment, pool water will be distributed into flasks and inoculated with two different strains of Shigella, 2457T (wild type) and BS103 (cured of its virulence plasmid). The pool water will not be chlorinated once the experiment has begun. Chlorine levels will be monitored over time and samples of water will be removed to determine the number of viable *Shigella* cells. I hypothesize that both strains of *Shigella* will not survive in pool water that is treated properly. However, once the levels of chlorine have dropped, the survival rate of wild type *Shigella* will increase.
Survival of Shigella flexneri in Swimming Pool Water
Monique Gomez and Helen J. Wing
School of Life Sciences, University of Nevada, Las Vegas

Introduction

Shigella flexneri is a gram negative bacterium that causes dysentery resulting in bloody diarrhea, fever, and abdominal pain. Complications caused by this infection can be fatal. A common source of Shigella infection is from the consumption of contaminated water. In the United States outbreaks of dysentery have been traced back, as recent as 2008, to inadequately chlorinated swimming pools. These outbreaks are most commonly seen among small children who play in these pools (2, 3). It is well documented that water dechlorinates and loses its bactericidal capacity with exposure to high temperatures and UV (2, 4). My hypothesis is that Shigella flexneri will not survive in pool water that is properly chlorinated. In addition, the virulence plasmid carried by S. flexneri contains genes with potential to confer resistance to environmental stresses. Therefore, my second hypothesis is that the survival of wild type versus virulence plasmid cured S. flexneri will be greater once the levels of chlorine have dropped below adequate levels.

Objective

Determine how well S. flexneri survives in pool water that has been allowed to naturally dechlorinate after exposure to heat and UV in Las Vegas weather for fixed periods.

Materials and Methods

1. Inoculum for pool microcosms was made by growing cultures of 2457T (W/F type) and BS103 (cured of its virulence plasmid) in Tryptic Soy Broth (TSB) (overnight). The cultures were washed by three repetitions of centrifuging and resuspension with filter-sterilized pool water and normalized to cell density.
2. Plugs of 1 L of pool water were dispensed into acid washed 2 L flasks and inoculated to a final cell concentration of a million cells/mL of washed cells.
3. Samples were collected and spread onto TCS plates and incubated at 37ºC overnight.
4. Chlorine levels were monitored using Pool Time 6-way test strips.

Results

First inoculation

• S. flexneri strains did not survive after washing in inadequately chlorinated pool water (Fig. 2a).

Second inoculation

• S. flexneri strains were washed with and inoculated into inadequately chlorinated pool water (Fig. 2b).
• Only the wild type S. flexneri persisted after two days exposure (10 cells /50 mL, Table 1).

Third inoculation

• S. flexneri strains were washed with and inoculated into inadequately chlorinated pool water (Fig. 2c).
• Samples were retrieved and plated every 30 mins for 7 hours.
• Cell density was uncountable due to the plates containing colonies that were too numerous to count.

Conclusion/Discussion

• Adequately chlorinated pool water effectively kills Shigella flexneri after an exposure time of one hour.
• Inadequately chlorinated pool water allows for the survival of Shigella flexneri for an exposure time of one hour.
• After three days only 2457T (wild type) cells persist (Table 1).
• Persistence of cell growth could be due to potential nutrients introduced by previous inoculation of cells.

References

2) Crane M (2008, July 13). Another Bacteria Ridiculing Orlando. The Columbus Dispatch

Acknowledgments

Funding for this project was provided by the National Science Foundation REU Scholarship (NSF 080367). I would like to thank Stephanie Lobahn for all of her amazing support, help and guidance throughout this project. Would also like to thank Kurt Legner for supplying me with pool water.
The *relA* gene in *Bacillus subtilis* controls a variety of factors during the stringent response which is a response to starvation of amino acids. The stringent response inhibits DNA synthesis and transcription of genes of tRNA, rRNA, and ribosomal proteins and promotes synthesis of the required amino acids. The objective of my project is to determine if a strain of *B. subtilis* that has a knockout mutation for the *relA* gene will accumulate a higher number of mutations that confer resistance to antibiotics that inhibit translation. It is proposed that because the *relA* gene inhibits transcription of ribosomal proteins, a strain lacking this gene will transcribe more rRNA and ribosomal proteins and promote the generation of mutations that target the translation process.
Abstract

The stringent response is a global regulatory control mechanism that is activated by amino acid starvation. The relA gene in Bacillus subtilis controls a variety of factors during the stringent response which include the inhibition of RNA synthesis and inhibition of transcription of genes of tRNA, rRNA, and ribosomal proteins. The relA gene also promotes synthesis of the lacking amino acids. We are investigating if a strain of B. subtilis that lacks a functional relA gene will accumulate a higher number of mutations that confer resistance to antibiotics that inhibit translation. It is proposed that because the relA gene inhibits transcription of ribosomal proteins, a strain lacking this gene will transcribe more ribosomal RNA and ribosomal proteins and thus promote the generation of mutations that target the translation process.

Hypothesis

It has been observed that when a B. subtilis relA mutant is placed under amino acid starvation it will produce less reverse mutations for amino acid prototroph markers than the wild-type. This is most likely due to a decreased transcription for some of the genes involved in the synthesis of amino acids. Our hypothesis is that when we challenge a relA mutant with rifampicin, a transcription inhibiting antibiotic, the same number of mutations to resistance will occur when compared to the number of mutations in the wild-type B. subtilis strain. It is also hypothesized that when we challenge each strain with a translation inhibiting antibiotic, the number of resistance mutations in the relA mutant strain will be noticeably higher than that of the isogenic wild-type. This assumption is based on the observation that the relA mutant strain produces more mRNA due to a lack of rRNA transcription inhibition that would normally occur under the stringent response.

Background

In order for an organism to overcome adverse conditions it must evolve to adapt to those conditions. Genetic mutations occur randomly with a variety of different mechanisms. Many of these genetic alterations will produce lethal or unaffected phenotypes but at times a beneficial mutation will occur. This beneficial mutation allows for the organism to have a greater fitness than its competitors. A currently common area of evolutionary study is in stationary phase mutagenesis or stress-induced mutagenesis. In some model systems, there is evidence that mutagenesis occurs in genes that are actively transcribed as a result of environmental stress giving the appearance of a directed mutation. This mutagenesis has been proposed to be a cause of acquiring antibiotic resistance and immune system responses, cell aging, recombination, and other phenomena. While much of the evolutionary research is about how DNA synthesis and repair mechanisms contribute to the formation of mutations, less has been studied on the role of transcription in the accumulation of mutations. We do know that regulation of transcription has been elucidated in actively growing and stationary phase cells. This regulation is highly complex and interactive. By distorting this regulation one can determine the role of transcription in generations of mutators. Specifically the stringent response which prevents expenditure of biological energy in absence of required amino acids.

Methods

Construction of isogenic mutant relA strain. We used the B. Subtilis strain R1500 obtained from Kawamura lab which had relA replaced by an erythromycin cassette. The DNA was extracted from R1500 and transformed onto YB955. The mutant was checked with PCR for absence of the relA gene. No fragments appeared in the R1500 or YB955 relA cassette using electrophoresis, while the while-type YB955 had a fragment that was 1.5 kb, indicating that the relA gene was absent in the mutant. It was considered to be a relA mutant. We further verified that the newly transformed strain onto media containing erythromycin to eliminate any background non-mutant cells. Determination of Minimal inhibitory concentration: We placed YB955 onto 96-well plates containing different concentrations of the antibiotic which inhibits the beta subunit of RNA polymerase, to determine the minimal inhibitory concentration.

1) 24 h plates of relA transformant and wildtype cells on different plates with 3.5% agar
2) Discs, 4, 6, 8, 10, 12, 15 mm diameter containing rifampicin
3) Repeat experiment with tetracycline and again with chloramphenicol
4) Compare results

References

Future Directions

Conclusions

The experiment has been set up with the correct parameters and the actual mutagenesis portion of the experiment is now in progress.

Acknowledgement

I would like to thank Katherine Ona for her guidance and assistance on this project. I would also like to thank Holly Martin, Alessio Lencioni, Mary Girard, and Carmen Villanueva for their assistance on this project. This project is supported by the NSF.
Dolores Huang  
Mentor – Dr. Brian Hedlund  
University of Nevada Las Vegas – School of Life Sciences

$^{15}$N-nitrate ($\text{NO}_3^-$) pool dilution experiments show that ammonia ($\text{NH}_3$) is oxidized to nitrate in geothermal springs up to at least 85°C; however, nitrite ($\text{NO}_2^-$)-oxidizing microorganisms are only known to grow up to 66°C. We hypothesize that thermophilic microorganisms oxidize nitrite to nitrate at high temperatures. Alternatively, it is possible that nitrite is oxidized abiotically. We propose to test these hypotheses by setting up microbial enrichments designed to grow thermophilic nitrite oxidizing bacteria by varying incubation temperature (50, 65, 80°C), oxygen concentration (20% and 5%), and cultivation media. A negative control consisting of filtered spring water (0.1 µm) will be used to determine whether nitrite is oxidized abiotically. Enrichments will be monitored for nitrite oxidation activity by using colorimetric assays for nitrite and nitrate. Enrichments showing activity will be used as a source to try to isolate and/or identify responsible microorganisms and to study the kinetics of nitrite oxidation at high temperature.
Researching Nitrite Oxidation at High Temperatures

Dolores A. Huang, Jeremy A. Dodsworth, and Brian P. Hedlund
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, Nevada

Abstract
The role of nitrite oxidizing bacteria in the nitrogen cycle of soil and aquatic habitats is well established; however, it is not known whether they exist in high temperature habitats. We successfully cultured nitrite oxidizing bacteria from aquacultural and soil samples and then modified an established protocol to test for the presence of nitrite oxidizing bacteria. In this study, the extremely slow growth of nitrite oxidizing bacteria was found to be a limiting factor in the isolation process. However, the extremely slow growth of nitrite oxidizing bacteria allowed for a more stringent test for the presence of these microbes.

Introduction
Nitrite oxidizing bacteria are often found in habitats with high temperatures. The presence of these bacteria is typically measured using a growth curve, which measures the production of nitrate in response to nitrite. However, in the high temperature habitats, the rate of nitrite oxidation is much slower than in bacteria found in lower temperature habitats.

Soil Enrichment
Low temperature control enrichments
Several enrichments were prepared and inoculated with samples from a low temperature standing water known to be active in nitrite oxidation. The enrichments were inoculated with soil from a low temperature standing water known to be active in nitrite oxidation. The enrichments were incubated at 4°C, 10°C, and 20°C. The enrichments were then monitored for growth of nitrite oxidizing bacteria.

Methods
1. The enrichments were prepared by mixing soil and water from a low temperature standing water known to be active in nitrite oxidation.

High temperature enrichments at GBs
4. The enrichments were incubated at high temperature for 7 days.

References

Future directions
If nitrite oxidation is detected:
- Test cultures to identify nitrite oxidizing bacteria
- Test cultures to identify nitrite oxidizing bacteria
- Test cultures to identify nitrite oxidizing bacteria

If nitrite oxidation is not detected:
- Test cultures to identify nitrite oxidizing bacteria
- Test cultures to identify nitrite oxidizing bacteria
- Test cultures to identify nitrite oxidizing bacteria

Acknowledgements
This research was supported by the National Science Foundation (Grant #1208758) and the University of Nevada Las Vegas. The authors would like to thank Dr. Elizabeth Blad for her support and guidance throughout this project.
Magnetotactic bacteria (MTB) are a diverse group of prokaryotes that biomineralize membrane-bound magnetic crystals known as magnetosomes. The magnetosomes are aligned within the cell and consist of either magnetite (Fe₃O₄) or greigite (Fe₃S₄). The biomineralization of magnetosomes consists of several processes including: invagination of the cytoplasmic membrane, iron uptake into the cell and then into the magnetosome membrane vesicle, and crystallization of the mineral phase inside the vesicle. Mam genes control magnetosome biomineralization with most of the genes present in an island called a magnetosome island. Many of the mam genes are conserved between different species of MTB. The genes that are in the island have suggested that they play a significant role in the organization of the magnetosomes and how they align within the cell. The focus of this investigation is to determine if certain conserved mam genes are found in two isolated and metabolically diverse magnetotactic spirillums: LEMS and MMS-1.
The Characterization of Two Diverse Magnetotactic Bacteria: LEMS and MMS-1
Ulysses C. Pickard¹, Paul A. Howse², and Dr. Dennis A. Bazylinski²
1 Fort Valley State University, 2 University Of Nevada, Las Vegas Nevada

Abstract
Magnetotactic bacteria (MTB) are a diverse group of prokaryotes that biomineralize membrane-bound magnetic crystals known as magnetosomes. Magnetosome biomineralization is not limited to MTB, but occurs in many other bacteria, including the magnetotactic bacteria (MTB) that are members of the Magnetotactic Bacteria (MB) or Magnetotactic bacteria (MB). The magnetosome is a dynamic structure that can be manipulated to control the orientation of the bacterial cell. The magnetosome is formed by a process of biomineralization that involves the deposition of magnetic iron oxides. The magnetosome biomineralization process is driven by the production of iron oxide nanoparticles. These nanoparticles are then engulfed by the bacterial cell, and the resulting magnetosome is a dynamic structure that can be manipulated to control the orientation of the bacterial cell. The magnetosome is a dynamic structure that can be manipulated to control the orientation of the bacterial cell.

Methods

1. In order to characterize magnetotactic bacteria, the first thing that must be done is to compare the genes of two magnetotactic bacteria with other magnetotactic bacteria. These magnetotactic bacteria have been used to clone the magnetotactic bacteria and that it is likely that these bacteria play the key roles in the magnetosome biomineralization in magnetotactic bacteria.
2. The magnetosomes in every magnetotactic bacteria genome examined showed in this redundancy. The genetic region that contained the magnetosomes genes in MTB. Magnetosomes also contain a mobile element, as transposes of the insertion sequence type and integrases (Li et al., 2005). These mobile elements are common and important features in the genome in MTB. Magnetosomes also contain a mobile element, as transposes of the insertion sequence type and integrases (Li et al., 2005). These mobile elements are common and important features in the genome in MTB.
3. Several cases are described in the The Magnetosome Biomineralization in Magnetotactic Bacteria. The Magnetosome Biomineralization in Magnetotactic Bacteria. The Magnetosome Biomineralization in Magnetotactic Bacteria. The Magnetosome Biomineralization in Magnetotactic Bacteria. The Magnetosome Biomineralization in Magnetotactic Bacteria.

Data/ Results
Current in the only the mnm1 and mnm2 gene has been successfully sequenced and been shown similarity to the other magnetotactic bacteria from LEMS. The sequences of MMS-1, which was mnm1 and mnm2 retained with contamination and after blinding the Sequences returned results of Arabidopsis. These are currently being resequenced and should have results During the poster session.

Conclusion/Future Research
In conclusion the research was beneficial not only because mnm1 and mnm2 genes are present in these strains of LEMS magnetotactic spirochete, but also because more detailed primers can be made in the interest of trying to completely characterize these spirochetes in a genomic library for magnetotactic bacteria. In the future the primers developed for these genes may be more specific to prevent the multiple products that degenerate primers often amplify. Further characterization of genes predicted to be within the conserved region of the Magnetotactic Bacteria are expected to continue with less time constraint to provide much more detailed results.

Acknowledgements
I would like to thank my mentor, Dr. Dennis Bayless, Dr. Paul Howse for helping me understand the techniques for dealing with microorganisms. PCR banding, and help sequencing the products of my team. Dr. Christopher LaFever (post-doc) for helping me obtain vital information concerning the Magnetotactic Bacteria. Dr. Marion Sandel for helping me improve my artistic techniques, Dr. John Petty for troubleshooting, and Dr. Fernandez Albera for providing invaluable advice when I was unsure about how to follow non-technical protocols. This project was funded by NSF Grant #.
Formerly an ephemeral watercourse, Las Vegas Wash is now a perennial system due to urban runoff and wastewater treatment plant (WWP) effluent. Las Vegas Wash flows into Lake Mead, where the discharge point is only a few miles upstream of Las Vegas’ main water intake. This small water cycle establishes the necessity to evaluate water quality especially due to non point sources pollution, wherein my research lies. Several points along Las Vegas Wash upstream and downstream of WWP have been chosen to represent different landuse types such as commercial, residential, wastewater treatment plants, etc. At each location, parameters including arsenic, selenium, nitrogen, phosphorus, total organic carbon, bacteria, and fecal coliforms are to be analyzed and compared for the influence of landuse change on both sediments and water.
Point and nonpoint source analysis of nutrients, metals, and pathogens in the sediment and water column in Las Vegas Wash

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Abstract

Several physicochemical characteristics of the sediment and water column in Las Vegas Wash (LVW) were analyzed to study the impact of landuse change and type and water recreation activities on Lake Mead water quality. Analyzers included water chemistry (e.g., phosphorus, nitrogen, total organic carbon, metals) and water column properties (e.g., sediment, macroinvertebrates, bacteria). The results indicated that sediment and water quality depend on landuse type and are specific to landuse type. The sewage treatment plants (SWTPs) are not contributing to bacterial levels in the Wash, but their outfalls contribute high nutrient concentrations. Overall, landuse type impacts water column properties. The concentrations of nutrients, metals, and pathogens directly correlate with landuse type, including increases in nutrient and bacterial levels.

Introduction

LVW forms in the Las Vegas Valley and continues to Lake Mead, a 12,000-acre lake. Previous studies have shown that sewage treatment plants (SWTPs) have decreased water column properties. The Wash is known for its unique flora and fauna, and the water column properties are affected by landuse type. The Wash is known for its unique flora and fauna, and the water column properties are affected by landuse type.

Hypotheses

Landuse changes affect water column composition, and landuse type affects the water column properties. The sediment will have higher concentrations of minerals, metals, and pathogens compared to the water column, and these levels will depend on landuse type and correspond to nonpoint source pollution.

Methods

LVW was divided into six sampling locations (Fig. 1) to represent sites in the Wash. Sediment, metal, and nutrient concentrations were measured at each site. Total water chemistry was analyzed at Desert Research Institute (DRI) using the Environmental Protection Agency (EPA) Method 365.1. Nutrient and metal concentrations were measured at Desert Research Institute (DRI) using the Environmental Protection Agency (EPA) Method 365.1. Metal concentrations were measured at Desert Research Institute (DRI) using the Environmental Protection Agency (EPA) Method 365.1. Metal concentrations were measured at Desert Research Institute (DRI) using the Environmental Protection Agency (EPA) Method 365.1.

Discussion

Higher temperatures and ORP at Site 3 are due to WWTP effluent. The WWTPs are contributing significant amounts of nutrients to the water column. The sediment at Site 1 has high organic carbon, high metal concentrations, and high bacterial levels. Sediment and water column properties are affected by landuse type. The Wash is known for its unique flora and fauna, and the water column properties are affected by landuse type.

Conclusion

The quality of the sediment and water column depend on landuse change and type. WWTPs are not contributing to bacteria levels in the Wash, but there are high nutrient levels in the Wash.

Acknowledgements

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It is widely known and accepted that the cause of many mutations in cells are generated during the replication process of actively dividing cells, however more recent research has shown that mutations also arise in non growing conditions, a phenomenon known stationary phase mutagenesis. Much of what is known come from studies in eukaryotic and bacterial models. It is proposed that in non-growing cells, the process of transcription plays an important role in mutagenesis. I will test the hypothesis that secondary structures formed of DNA generated transcription promote mutagenesis. The sequences transcription-generated structures are speculated to be prone to mutations by exposing regions of single stranded DNA to lesions. To test this hypothesis, I examined the *Bacillus subtilis* gene thiF, predicted by *in silico* analysis to be prone to mutations at particular locations during transcription. By altering the base sequence of this gene, the stability of its stem-loop structures is affected, thereby allowing us to test whether transcription of the altered sequence influences accumulation of in thiF. Our assay for detection of mutations is based on reversion to thiamine auxotrophy in cells under conditions of starvation. Ultimately, these experiments will increase our understanding of how mutations occur in cells of all domains of life.
DNA Secondary structures and their contribution to mutagenesis in B. subtilis stationary phase cells.
Carmen Vallin, Katherine Ona, Chris Ross, Ronald E. Yasbin and Eduardo A. Robleto
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV

Abstract
It is widely known and accepted that the cause of many mutations in cells are generated during the repair replication process of actively dividing cells, however more recent research has shown that mutations also arise in non-growing conditions, a phenomenon known as stationary phase mutagenesis. Much of what is known comes from studies in eukaryotic and bacterial models. It is proposed that in non-growing cells, the process of transcription plays an important role in mutagenesis. I will test the hypothesis that secondary structures, formed of RNA generated transcription, promote mutagenesis. The transcription-generated structures are speculated to be prone to mutations by exposing regions of single stranded DNA to lesions. To test this hypothesis, I examined the Bacillus subtilis gene thiF, predicted by in silico analysis to be prone to mutations at particular locations during transcription. By altering the base sequence of this gene, the stability of its stem-loop structures is affected, thereby allowing us to test whether transcription of the altered sequence influences accumulation of mutations in thiF. Our assay for detection of mutations is based on reversion to thiamine auxotrophy in cells under conditions of starvation. Ultimately, these experiments will increase our understanding of how mutations occur in cells of all domains of life.

Background
- In stationary phase cell division and replication are halted due to an environmental stress setting the stages for mitotic events to occur that may enable organisms to grow.
- This phenomenon has been documented since the 1950’s when Francis J. Ryan’s paper was published in Genetics. In his discussion he wrote, “as the example, the fact that mutations can arise in populations of bacteria whose numbers are not increasing must be accepted.”
- Ryan observed a histidine E. coli strain mutant to histidine prototrophy in non dividing cells in both liquid and agar media. In this early paper, Francis Ryan had to address the issue of zero growth, delay in phenotypes and even cells having to account for the mutants arising in his plates since the concept was so new.
- Current research provides new insights on the mechanisms giving rise to these mutator genes.
- Research has found the importance different repair pathways and the process of transcription play in contributing to mutagenesis during stationary phase.
- In specific, secondary structures formed during the process of transcription have been found to contribute to mutations in E.coli and have been started to help explain the high frequency mutability in center cancer genes, such as p53 tumor suppressor gene.
- Stem loop structures (SLS) form as a result of transcription driven negative supercoiling by scDNA.
- A SLS is characterized by having both, a double stranded portion that forms the stem, and a single stranded loop. It is the bases found in the single stranded portion that are speculated to be most vulnerable to mutagenesis.
- Different genes will form different structures with different stabilities based on their sequences. The more stable the SLS the more susceptible it is to mutagenesis.
- The mutability index (M) of each gene can be calculated by multiplying Gibbs free energy value of the most stable secondary structure in which a base is unpaired of a particular DNA sequence by the percentage of total structures in which the base is unpaired during transcription.

Hypothesis
The more stable the SLS the more susceptible the gene and its bases forming the structure are to mutagenesis.

Methods
- Create Auxotroph: Knockout thiF
- Create Salvage Pathways

Results
- thiF knockout does not yield an auxotroph for Thiamine, but a prototroph instead.
- thiF knockout needs 2 days to show growth on a Minimal Media plate.
- Supplementing Thiamine at a concentration of 5ug/ml did not completely restore WT phenotype.
- thiM knockout does not yield auxotroph, but a slower bradytroph than the single mutant.
- thiM knockout needs 4 days to show growth on Minimal Media plate.

Conclusions
At this point we are unable to reach a conclusion to answer our hypothesis on the mutability of thiF. We have, however, gained further knowledge on the complexity of the Vitamin B pathway in Bacillus subtilis and we now can proceed to obtain a Thiamine auxotroph.

Future Plans
- Knockout thiF gene in double mutant background to eliminate thiamine one step salvage pathway to yield TFP auxotroph.
- Use E. coli Recombination system to make allele changes to WT thiF sequence, including stop codon and synonymous base changes.
- Once desired product is obtained pH5 will be transformed into WT YB955 into amyE locus where transcription will be controlled by inducible promoter.
- Conduct stationary phase assay and score mutant reversion to this gene prototrophy.
- Conduct stationary phase assay with different levels of negative supercoiling to test effect on mutation numbers.
- Conduct stationary phase assay without transcription strand specific repair pathways such as knocking out nrdF gene.
- Further analyze details of stem loop formation and how structure stability influences the structure and vulnerability of DNA and mRNA in its vicinity.

Acknowledgements
I would like to thank everyone in the Volunteers this summer for helping create a great fun, energetic, learning environment to work in. A special thanks to everyone who patiently provide answer to any question asked.

References
Ability of Martian soil to degrade carbohydrates, shown by the Viking mission, has two interpretations. One possibility is that the soil harbors living microorganisms. Alternatively, the soil is sterile but chemically oxidizing, i.e. it is laden with photochemical oxidants. It was shown by REU research last summer that these two possibilities can be distinguished by the use of glucose enantiomers. Life is selective: Earth organisms use D-glucose, but ignore L-glucose. This stereo selectivity is absent in chemical reactions. The goal of this project is to test if xylose, a five carbon sugar, is also suitable for chiral life detection. Mixed microbial cultures were raised from various soils (Jordan, the Mojave Desert, and the Atacama Desert). Added D- and L-xylose were monitored over time. Results show that terrestrial microorganisms utilize only D-xylose, not L-xylose, confirming that like glucose, xylose is a suitable substrate for Martian life detection.
Martian life detection with Xylose Enantiomers
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ABSTRACT
Ability of Martian soil to degrade carbohydrates, shown by the Viking mission, has two interpretations. One possibility is that the soil harbors living microorganisms. Alternatively, the soil is sterile but chemically oxidizing, i.e. it is laden with photochemical oxidants. It was shown by REU research last summer that these two possibilities can be distinguished by the use of glucose enantiomers. Life is selective: Earth organisms use D-glucose, but ignore L-glucose. This stereo selectivity is absent in chemical reactions. The goal of this project is to test if Xylose, a five carbon sugar, is also suitable for chiral life detection. Mixed microbial cultures were raised from various soils (Jordan, the Mojave Desert, and Botswana). All bacterial cultures were grown in Luria-Bertani medium (Navarro et al., 2009). Centrifugation was used to gather cells. Phosphate buffered saline (PBS) was used to wash cells two times before every centrifugation. PBS was then used again re-suspend supernatant after a last centrifugation. Once cultures had been cleaned and divided into two subculture tubes. One tube was distributed D-xylose, the other distributed was L-xylose. Samples were taken at evenly proportionate time intervals. Following the samples were colorimetrically assayed using a spectrophotometer to measure metabolism of D-xylose and L-xylose consumption over time.

INTRODUCTION
In 1975 NASA carried out a mission to mars for scientific research to search for microbial life known as the Viking Label Release Biological Experiments. For one experiment, a heterotrophic media tagged with \(^{14}\)C was added to Martian soil. Microbial activity could be recorded by measuring the signs of CO2 emissions given off by the area of injection. Upon first injection, a strong positive CO2 emission was recorded. Following was a heating of the area of the first injection site, then a second injection which provided negative CO2 emission. All injection there after also showed negative results as well (Klein et al., 1976; Levin and Straat, 1976).

There are two possible explanations for these findings. The soil of mars may indeed carry microorganisms. Another alternative is that, mars may be chemically reactive due to inorganic oxidants which have consumed the heterotrophic media. Which is the key reason remains inclusive.

MATERIALS AND METHODS
For this experiment, heterogeneous cultures were used. The heterogeneous cultures were taken from soils collected in the Atacama, Mojave, Botswana, and Jordan Deserts. All bacterial cultures were grown in Luria-Bertani medium (Navarro et al., 2009). Centrifugation was used to gather cells. Phosphate buffered saline (PBS) was used to wash cells twice before every centrifugation. PBS was then used again to re-suspend supernatant after a last centrifugation. Once cultures had been cleaned and divided into two subculture tubes. One tube was distributed D-xylose, the other distributed was L-xylose. Sample collections were then taken at evenly proportionate time intervals. Following the samples were colorimetrically assayed using a spectrophotometer to measure metabolism of D-xylose and L-xylose consumption over time.

RESULTS

From the results, it is shown the Botswana (Africa) desert soil did not utilize D- or L-glucose. The Mojave, Atacama, and Mixed culture of modest bacteria, Kocuria, and Bacillus all consumed D-xylose, not L-xylose. Consumption for the Mojave Desert, Atacama, and the mixed culture of modest bacteria, Kocuria, and Bacillus ceased at 1.8 mM.

DISCUSSION
As the results suggest, stereo-specific Xylose metabolism seems to be an appropriate approach to differentiating between chemical and biological reactivity. Figures 1, 2, and 4 show that microbial life is selective in its consumption by the choosing of D-xylose over L-xylose. The notion of life being selective is also shown by earlier works on glucose, which yielded similar results (Sun et al., 2009). As for chemical reactivity, earlier work shows that potassium permanganate oxidized both D- and L-xylose equally (Sun et al., 2009) However, all organisms don’t utilize xylose for metabolization as shown in figure 3 results. Xylose may be toxic or indigestible to some species of microbial. By testing many chiral sugars such as arabinose, fucose, mannose, and others, a greater understanding of stereo-selectivity of enantiomers can be reached (Kelley et al., 1975). The use of chiral sugar substrates can be used as a means of life detection if tests data confirms.

REFERENCES
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I would like to thank Dr. Henry Sun for his outstanding mentorship, patience and opportunity to do research in the Sun lab. Also I would like to thank Dr. Gaosun Zhang for technical training and project assistance while in the Sun lab. I would like to acknowledge D.R.I. (Desert Research Institute) and UNLV Microbiology Department for this research opportunity and experience. This work was supported by the NSF Grant (REU 0649267)
Abstract
In the late 1990s, the limits of life were pushed even further when microorganisms were discovered thriving 2.5 km below the surface of the Earth in deep South African gold mines. These very simple communities were dominated by a single species of bacteria from within the phylum, Firmicutes. *Desulforudis audaxviator* remains unique to a sizeable portion of the South African deep subsurface. At depths below 2.5km, it comprises well over 99% of all organisms present, which presents a unique circumstance in which the environment has provided a natural pure culture. From this naturally occurring pure culture, environmental genomics was applied to obtain the complete *D. audaxviator* genome and thus it’s biological functions were established. This presents a unique opportunity to now attempt to grow a previously uncultured organism using its genome as a road map to design a specific cultivation approach for *D. audaxviator*. The genome combined with precise chemical analysis of its native environment has yielded invaluable insights such as the organism’s ability to form spores, to reduce sulfate, to fix nitrogen and use ammonia, along with many other unique traits all of which will lead to successful cultivation. Here we describe the genome-enabled cultivation of this to date uncultured microorganism.
Attempts to Cultivate Bacteria from Deep Subsurface Aquifers and Mountaintop Plant Communities

E. D. Hughes¹, J. C. Bruckner², D. P. Moser²
¹Arizona State University, Tempe, AZ 85281
²Desert Research Institute, Las Vegas, NV 89119

INTRODUCTION

In the late 1990s, the limits of life were pushed even further when microorganisms were discovered thriving 2.5 km below the surface of the Earth in deep South African gold mines (4). These very simple communities were dominated by a single species of bacteria from within the phylum Firmicutes. Desulfovirgula aurivilliusi, a sulfur-oxidizing bacterium, is unique in its ability to oxidize sulfur to sulfuric acid under anaerobic conditions. Its environmental approach was applied by collaborators to obtain the complete D. aurivilliusi genome (Figure 1)(1) and then its biological function (Figure 2)(1) was established. The genome, combined with chemical analysis of its native environment, has yielded valuable insights to inform potential cultivation strategies. These include the ability to form spores, reduce sulfur, and utilize formate or CO₂ as carbon and energy sources. Here we describe an attempt to perform genome-enabled cultivation of this to date uncultured microorganism.

MATERIALS & METHODS

Base Media Preparation

Starting concentrations of major ions (1) from boreholes where D. aurivilliusi was previously identified (1) were utilized to develop a basal medium. Biv were prepared freshly with FeSO₄ as an Fe source and, where necessary, formate and acetate or formate-sodium acetate were utilized as potential C sources or spore germinants (Table 1). L-cysteine HCl and vitamins were added and the pH adjusted to 8.0. Media were dispensed into Batch tubes and autoclaved. The tubes were then immediately placed in a Coy Type B anaerobic chamber maintained with N₂, CO₂, H₂ (70/20/10), and sealed with butyl rubber stoppers.

RESULTS

- No definitive growth as of yet.

Table 2: D. aurivilliusi Clones

<table>
<thead>
<tr>
<th>Name</th>
<th>Sample</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3105A</td>
<td>11g</td>
</tr>
<tr>
<td>B</td>
<td>*3105MK</td>
<td>11g</td>
</tr>
<tr>
<td>C</td>
<td>EV1118A</td>
<td>11g</td>
</tr>
<tr>
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</tr>
<tr>
<td>F</td>
<td>OR1118C1</td>
<td>11g</td>
</tr>
</tbody>
</table>

* Tangential flow filtration concentrate.
* As judged by viable turbidity.

ACKNOWLEDGMENTS

Funding was provided by the National Science Foundation (DMB0711877). Special thanks to members of Dr. Moser's lab: Josh Fisher, Holly Ebeling, Devon Banta, Emily Dyer, Jessica Johnson, and Joe Ye in their help and guidance this summer.

REFERENCES


MOUNTAIN METHYLOBACTERIA

INTRODUCTION

Methylotrophs are a group of facultative methylotrophs capable of surviving off the nutrients emitted from the decaying forest floor. These bacteria are commonly referred to as PFPs (pink-pigmented facultative methylotrophs) due to the distinctive pink pigment produced (1). Endemic plants from Bonsanza Peak (16,327 ft, 4,991 m) in the spring mountains of NV were collected to determine if specific plant species had unique methylotroph populations.

RESULTS

Leaves taken from Bonsanza Peak plants were maintained using a sterile tissue grinder and a 1% phosphate buffer. Homogenates were streaked on isolation plates for isolation of the PFPs (Table 1). The inoculated plates were then incubated for approximately 7 days, and colonies were picked and further purified by transfer to fresh plates.

- Possible elevation trends in methylotroph abundance.
- Possibly related to UV exposure selecting for PFPs.

REFERENCES

Lauren Johnson  
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Here, we examine mutagenic programs that are independent of growth, such aspects of the evolutionary process are novel and have been implicated in the formation of cancers in animal cells and the acquisition of antibiotic resistance in animal pathogens. Adaptive or stationary phase mutagenesis is a genetic program to increase diversity in cells under conditions of stress whereby cells escape non-dividing conditions. Previous research has shown that recombination functions are required to generate mutations that promote growth in *Escherichia coli* cells starved for carbon. This project tests the hypothesis that recombination functions are required for the generation of mutations that promote growth in response to amino acid starvation stresses in *Bacillus subtilis* cells. In *B. subtilis* cells, recN, in addition to recA, mediates recombination events and may influence the formation of adaptive mutations. A RecN− strain will be generated by standard molecular techniques and compared to a RecN+ strain for its ability to accumulate mutations that affect amino acid biosynthesis. We speculate that recN does not affect stationary phase mutagenesis in *B. subtilis* and discussed other novel mechanisms mediating the generation of mutations in non-dividing cells.
The Role of recN in Stationary Phase Mutagenesis in Bacillus subtilis

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Abstract

Here mutagenic programs that are independent of growth were examined. Such aspects of the evolutionary process are novel and have been implicated in the formation of cancers in animal cells and the acquisition of antibiotic resistance in animal pathogens. Adaptive or stationary phase mutagenesis is a genetic program to increase diversity in cells under conditions of stress whereby cells escape non-dividing conditions. Previous research has shown that recombination functions are required to generate mutations that promote growth in E. coli cells starved for carbon. This project tests the hypothesis that recombination functions are required for the generation of mutations that promote growth in response to non-growing and starvation stresses in Bacillus subtilis cells. In B. subtilis cells, recN, in addition to recA, mediates legitimate and illegitimate recombination events and may influence the formation of adaptive mutations. A recN strain was generated by standard molecular techniques and compared to a recN+ strain for its ability to accumulate mutations that affect amino acid biosynthesis. We report that recN affects stationary phase mutagenesis in B. subtilis and discussed other novel mechanisms mediating the generation of mutations in non-dividing cells.

Methods

- Genomic DNA was extracted from Bacillus subtilis strain B3281 (recN+::cat).
- An isogenic recN knockout was prepared by transforming the parental strain YB655 with the DNA from B3281.
- Chloramphenicol (cmR) cassettes within recN gene produced a fragment that is 2.2 kbp larger than wild type recN and also rendered the strain resistant to chloramphenicol.
- Colonies were then isolated on TBA medium containing chloramphenicol (5 μg/ml).
- Knockout was verified by PCR and gel electrophoresis.
- Sample cultures of wild type and mutant were grown to stationary phase.
- The cultures were then plated on minimal media containing trace Histidine, Methionine, and Leucine.
- Number of revertants were then scored daily for 5 days while the survival of background cells were monitored every other day.

Results

Conclusions/Future Directions

The preliminary data is indicative that recN is involved in the stationary phase mutagenesis in B. subtilis. However, the influence of this gene is dependent on the genetic event required for cells to escape non-growing conditions in B. subtilis. Further analysis is required to elucidate how recN influences stationary phase mutagenesis.

These experiments are being repeated and a fluctuation test will also be conducted to determine if recN plays an active role in exponential growth.

Acknowledgments

Great gratitude and appreciation is extended to the entire Yasbin/Robleto lab for demonstrating patience, allowing me to gain research experience and permitting me to grow as a scientist. To Dr. Willis Derby, Mary Grier, Holly Martin, Caitlin Murphy, Marlin Schmitt, and Carmen Valin for their constant encouragement and support. This project was funded by the National Science Foundation.
Robin Herlands  
Collaborating with – Dr. Helen Wing  
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Abstract  
**Differential Localization of IcsA and IcsP in the Outer Membrane of**  
*Shigella flexneri*  
Our goal is to visualize IcsP on the surface of *S. flexneri* and determine how its localization changes during growth versus stationary phases. We hypothesize IcsP will be present on the surface of *S. flexneri* differentially during growth and stationary phases of cell cycle. We hypothesize greater levels of IcsP seen during stationary phase (simultaneous with lower, more unipolar IcsA, due to IcsP mediated cleavage). During growth phase, we expect less IcsP to be present on the surface, and IcsA to be more evenly distributed around the cell surface.
Differential Localization of IcsA and IcsP in the Outer Membrane of Shigella flexneri

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Introduction

Shigella flexneri is an intracellular pathogen that causes shigellosis, a bacterial form of dysentery. Movement within a from cell to cell is accomplished with actin filament generation at one pole of the cell, which propels the bacterial cell (1). The generation of this actin "tail" is dependent on IcsA, which has been visualized at one pole of the cell (2).

IcsP is an outer membrane protein that carries a domain of IcsA responsible for tail formation (3). IcsA appears to be translocated uniquely to one pole where it then begins to distribute along the entire surface of the cell (see Figure 1). It has been proposed that the unequal localization of IcsA is due to IcsA cleavage by IcsP around the entire surface (4), but the actual location of IcsP has not been elucidated.

Materials and Methods

Bacterial Strains used:
BS109 - rough S. flexneri, serotype 2a
MB0340 - rough S. flexneri, serotype 2a, IcsAΔ
BS109 pMP4002 - rough S. flexneri, serotype 2a with inducible IcsA-GFP under L-arabinose control

Culture and Immunofluorescent Staining
Cells were grown in overnight cultures. The following morning, cells were back-diluted 1:100. Cells were grown to 2 hour or 7 hour time points were washed, adhered to acetone-treated coverslips and fixed with 3.7% paraformaldehyde. Optical density was used to normalize the number of cells used per coverslip. Once fixed, cells were stained with rabbit anti-IcsP or rabbit anti-IcsA antibody followed by an anti-rabbit Alexa 555 secondary reagent. Cells were then visualized at 100x magnification with oil on an Olympus BX51 immunofluorescent microscope. Images were acquired with Andor iXon, and Adobe Photoshop.

Results and Discussion

The anti-IcsP antibody successfully stained IcsP as 10% exposures reveal bright staining in BS109 cells, but not MB0340 cells which lack IcsP (see Figure 2).

IcsP was seen in BS109 cells during both growth and stationary phases. Staining appears patchy, associated into bright spots on the surface, during stationary phases but is more diffuse during growth phase (see figure 3, left panel, 1st and 3rd row). During stationary phase, IcsP is detected on significantly fewer BS109 cells than during growth phase (see figure 3, right panel, 1st and 3rd row) which is likely a result of IcsA degradation during stationary phase.

These results confirm that IcsP is localized around the circumference of the bacterial tail. While it could be argued that rough mutants have greater fluidity in the outer membrane, influencing localization, our findings that IcsP still localizes in a similar fashion equivalent to that seen in wildtype S. flexneri (data not shown) gives us confidence that significant native membrane architecture is still intact.

While we were unable to visualize IcsA and IcsP on the same cell at this time, we are able to visualize IcsP on cells which have a truncated IcsA-GFP construct. IcsA-GFP gets trapped in the cytoplasm in these cells, as GFP is too big to be moved successfully to the outer membrane. (see figure 4). Therefore, IcsA-GFP can be seen at the poles of cells, while IcsP is distributed on the entire surface. Interestingly, in some cells IcsP seems excluded from poles where IcsA-GFP is congregated (see figure 4).

References

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Acknowledgements

I would like to thank all members of the Wing lab for their support. In particular, I would like to thank Stephanie Lubinis for her friendship, mentorship, and expertise she so unflinchingly shared. I would also like to thank Las Atkinson for technical assistance. This research was made possible through a Research Opportunity Award, a supplement of an NSF-funded project entitled "REU Site: A Broad View of Environmental Microbiology (NSF/INSF 0549297). I would like to thank Dr. Helen Wing, Dr. Kurt Reiner, and Dr. Eduardio Rockmore for their assistance in obtaining the means to make this project necessary and successful.
The Viking mission showed that Martian soil can degrade a heterotrophic medium to carbon dioxide as if live microorganisms were present. The result is considered inconclusive, however, because abiotic oxidants, such as superoxides, may also exist on Mars and would explain the Viking result. One way to resolve this ambiguity is to repeat the Viking experiment with a isomerically pure medium. The consumption of one isomer, either D or L, would indicate biological activity. Indiscriminate destruction of both isomers would indicate abiotic redox processes. This idea was validated for glucose by REU research last summer (Sun et al. 2009). The objective of this project is to test this idea with amino acids. Specifically, the consumption rates of D- and L-enantiomers will be compared for histidine, lysine, and serine in selected bacteria, archaea, and eukaryotic fungi and yeasts. Results with Bacillus revealed that in histidine, only the L-isomer was consumed while for serine and lysine, both the D- and L-isomers were utilized. If confirmed in other microorganisms, these results indicate that histidine is a suitable substrate for Martian life detection but serine and lysine are not.
Martian Life Detection with Amino Acid Enantiomers
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The Viking LR: Biology or Chemistry?
In 1976 two Viking Landers were sent to Mars to look for evidence of alien life (Figure 1a, b). Each lander performed a series of soil tests including the Label Release (LR) experiment which tested for degradation of a nutrient broth containing formate, glycolate, glycine, DL-alanine, and DL-lactate. Each substrate was labeled with radioactive carbon-14. Upon injection, radioactive gases rapidly evolved from soil (Figure 1c) as if microorganisms were present. Paradoxically, no native organic carbon could be detected in the soil above the parts per billion level. To reconcile these results, most scientists hypothesized that the LR activity was caused by inorganic oxidants such as peroxides and superoxides instead of microorganisms. However, other scientists disagree, arguing that a biological cause for the LR result could not be ruled out.

The Concept of Chiral LR
A concept for elucidating the Viking result has been validated by previous advanced REU research (Sun et al. 2009). In the new scheme, biological and chemical reactivity are distinguished by stereoselective degradation of chiral substrates. It was shown that on Earth known life forms recognized only D-glucose, the natural enantiomer, but ignored L-glucose (Figure 2a). In contrast, inorganic oxidizers do not have this enantiomeric bias, oxidizing both D- and L-glucose (Figure 2b). This new approach is now finding its way into amino acids alanine, glutamic acid, aspartic acid, and leucine by Drs. Gaosen Zhang and Henry Sun. Data collected so far showed that D- and L-enantiomers were consumed at equal rates. Clearly, not all amino acids are chiral selective. The objective of this project here is to determine whether or not other amino acids are stereo-selective and therefore suitable for chiral LR. This study focused on histidine, lysine, and serine. The microorganisms used in our study included Bacillus, Kocuria, and E. coli.

Experimental
Microorganisms were grown in LB media. Cells were collected via centrifugation, washed, and suspended in phosphate saline buffer (PBS). To the culture I added a mixture of DL-histidine, DL-lysine, and DL-serine. Samples were taken hourly and assayed for enantiomeric levels. The samples were derivatized with o-phenylenediamine and analyzed by HPLC (Figure 3b). The OPANAC reaction is necessary prior to analysis because it adds a bulky group to the amino group thereby making it easier to detect by the HPLC.

Discussion
This study showed that histidine is selective while serine and lysine are not. If confirmed in other life forms, this result suggests that histidine is suitable for chiral LR to clarify the nature of the Martian reactivity. If one isomer is consumed, this would illustrate biological activity. On the other hand, if both get consumed, it can be concluded that the reaction took place by chemical oxidation.

Acknowledgements
I would like to thank Dr. Henry Sun for allowing me the honor to work in his lab. I would also like to personally thank Dr. Gaosen Zhang for teaching me how to handle all equipment and machinery in the lab and always being there to help when I had a question. I would also like to thank the administration at the research facilities of UNLV and DRI for offering me a great opportunity. In addition, I would like to thank the National Science Foundation for funding this research experience (REU 0643267) and for their continued support for undergraduate research nationally.

References
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It has been proposed that hydrogen oxidation is the energetic foundation of high temperature ecosystems; however, results in our laboratory suggest that hydrogen oxidation is only one of many microbial metabolisms active in hot spring sources. We hypothesize that hydrogenase genes are repressed under high oxygen concentrations that exist in some hot spring sources. We propose to test this hypothesis by using six genera within the phylum Aquificae, the dominant microbial phylum in most hot spring sources. Bacteria will be grown in pure culture under a variety of electron donor and acceptor conditions (with and without hydrogen at high and low O\textsubscript{2} concentration). Cells will be tested for their ability to oxidize hydrogen by using a microrespirometry device at different phases of microbial growth. In addition, reverse transcriptase PCR may be used to determine which hydrogenase genes are transcribed under different conditions.

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Mentor – Dr. Dennis Bazylinski  
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Cosmopolitan in their distribution, magnetotactic bacteria (MTB) are motile, aquatic prokaryotes that passively align along geomagnetic field lines and represent a morphologically, physiologically and phylogenetically diverse assemblage of organisms. MTB are characterized by their ability to biomineralize magnetosomes, which are membrane-bound crystals of magnetite (Fe\textsubscript{3}O\textsubscript{4}) or greigite (Fe\textsubscript{3}S\textsubscript{4}) arranged in one or more linear chains inside of the cell, which are responsible for the cell’s magnetism. MTB can be readily separated from other organisms based on their motility and magnetotaxis, however, only a few MTB are in axenic culture. The taxonomy of MTB is heavily based on the comparative sequence analysis of their 16S rRNA genes, which can be investigated without prior cultivation. Based on 16S rRNA sequence similarity MTB are polyphyletic and are clustered within several classes of the Proteobacteria and also the phylum Nitrospira. The purpose of this study was to identify and characterize MTB retrieved from various sites in the Mojave Desert via 16S rRNA sequence analysis.
Gamma-ray bursts (GRBs) are the most violent explosions in the universe. Much of what we know about these highly energetic, short-duration bursts of gamma-rays comes from their afterglows, which are long-lasting broadband signatures following the initial bursts. Scientists have long speculated over the x-ray afterglow light curves of GRBs, which contain a mysterious shallow decay component. Recently, Ryo Yamazaki introduced a new theoretical model for the shallow decay component called “prior emission.” According to his model, there is actually x-ray emission prior to the prompt GRB. Our goal is to determine if the prior emission model is consistent with the external shock model. To do this, we will analyze prompt optical data and compare it to predictions derived from the theoretical models. Determining whether or not prior emission is consistent with the external shock model is crucial to better understanding the origin of the prior emission and the physics of GRB progenitors.
An Investigation of the Origin the Bimodal Distribution of Optical Afterglow Luminosities of Gamma-Ray Bursts

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Abstract

The determination of which properties of gamma-ray bursts and the surrounding interstellar medium contribute to the observed bimodal distribution of optical afterglow luminosities will provide insight into the physical processes that give rise to the two families of optical afterglows.

Introduction

Background:

- Gamma-ray bursts (GRBs): the brightest sources of electromagnetic radiation since the Big Bang; also the most violent explosions in the universe.
- Most GRBs (Type II) are linked to supernovae; other GRBs (Type I) may be related to mergers between compact objects such as neutron stars and black holes.
- GRB afterglow: occurs when the material from the explosion collides with circumstellar material (such as the interstellar medium, also known as ISM); can be observed in all bands up to X-ray and lasts much longer than the initial explosion.
- Light curve: plot of flux vs. time in a particular frequency; most common way to study GRB afterglows.

The Question:

Can the light curves of Type II GRB optical afterglows (detected approximately 10-12 hours after the prompt emission) have three independent research groups (Liang & Zhang, Kann et al, Nardini et al) to determine that there are two tight groups of optical afterglow luminosities. It appears that, despite the many different physical properties of individual GRBs, the optical afterglow luminosities cluster around two values. This was an unexpected and puzzling result.

The physical origin of this bimodal distribution of optical afterglow luminosities has yet to be fully explained. Is it a property of the actual GRBs that creates this effect, or is it a property of the ISM? The objective of my research project this summer was to address this question.

Method

The objective of this research project required that I create a computational code that allowed me to calculate the flux and luminosity of a GRB afterglow at any frequency and at any time after the initial explosion. I cut input any values for the five variable parameters and my afterglow luminosity code will output the corresponding luminosity light curve. As shown in Figures 3(a) through 4(b), I was able to reproduce the light curves from Sari et al. 1998, a landmark GRB afterglow paper. Once I had determined the afterglow luminosity code, I utilized the Monte Carlo method to simulate different distributions of the five variable parameters. By trial and error, I experimented with different combinations of the distributions to see which best reproduced the observed bimodal distribution of optical afterglow luminosities.

Figure 3a, 3b (top): Taken from Sari et al. 1998, these show the low frequency and high frequency light curves, with the temporal indices for the fully radiative case in brackets (vs. the adiabatic fuller analytic case).

Figure 4a, 4b (bottom): Example low frequency and high frequency light curves outputted by my afterglow luminosity program based on the following parameter values: \( n = 1 \text{ cm}^{-3}; E_{k} = 10^{52} \text{ erg}; \epsilon_{i} = 0.1; \epsilon_{e} = 0.3 \).

Results

Although I did not have time to experiment with a large number of combinations of the parameters, below are some examples of simulations using a uniform distribution of \( p \) between 2.0 and 3.0 and constrained Gaussian distributions of the \( \log_{10} n \) values of \( \epsilon_{i} \) and \( \epsilon_{e} \). The three simulations shown below were created by varying the types of distributions of \( E_{k} \) and \( n \). In Simulations #1 and #2, the \( p \) distribution is given a Gaussian distribution of the \( \log_{10} n \) values of \( n \). In Simulation #3, the \( E_{k} \) distribution is the same broken power law from Simulation #1. Judging from the testing thusfar, changing the \( E_{k} \) distribution seems to have a larger impact on the simulations than does changing the distributions of the other four parameters.

The Variable Parameters

For the scope of this summer project I focused on five parameters upon which the luminosity light curve of a GRB afterglow depends:

- \( p \) (spectral index)
- \( E_{k} \) (isotropic kinetic energy of the fireball)
- \( n \) (ISM density)
- \( \epsilon_{i} \) (fraction of internal shock energy partitioned to non-thermal electrons)
- \( \epsilon_{e} \) (fraction of internal shock energy partitioned to magnetic fields)

Conclusion

Out of the current set of results, Simulations #2 and #3 are the closest reproductions of Figure 2. However, the break between the lower and upper luminosity groups is not as defined as it should be, or in the right place. The peaks in both groups may also be systematically lower than in Figure 2, especially in the case of Simulation #3. In addition, the simulation generates outputting low luminosities that are not shown in Figure 2 because of an observational selection effect: low luminosity bursts (particularly at higher redshifts) are much less likely to be detected. As work on this project continues, this selection effect needs to be taken into account. In addition, more experimentation with distributions of the parameters (particularly \( \epsilon_{e} \) and \( n \)) is needed.

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UNLV GRB Research Group (in particular, Francisco Virgili, Amanda Mashum, and Bing-Zhe Zhang)
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Nicholas Gliozzo

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References

Robert Gex  
Mentor – Dr. Bing Zhang  
University of Nevada Las Vegas – Department of Physics & Astronomy  
(NASA – EPSCoR)

I am investigating short-term duration X-ray outbursts (XROs) and their occurrence in the universe. The first time these phenomena have been observed was by accident when observing supernovae within a host galaxy. In order to confirm possible occurrences from similar events within host galaxies, I must filter through data gathered from the Chandra X-ray Space Telescope. I will be working directly with years of processed data from Chandra and another graduate student in the program to see if these XRO events have occurred in the past. After finding possible XRO candidates, I will further analyze the data using image processing software as well as IDL programs and functions to measure light-curves and luminosities of the events hosting the XRO candidate. When sufficient data has been gathered I will have a better understanding of XROs and the probability of them occurring in the universe.

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Mentor – Bing Zhang  
University of Nevada Las Vegas - Department of Physics & Astronomy  
(NASA – EPSCoR)

Diana Mkrtchyan  
Mentor – Dr. Daniel Proga  
University of Nevada Las Vegas – Department of Physics & Astronomy  
(NASA – EPSCoR)
Abstract
The accumulation of reactive oxygen species (ROS) and free radicals within tissues creates oxidative stress, causing damage and eventual aging in an organism. Intense activity can increase the level of oxidative stress that occurs within an organism. In honey bees, this activity occurs during foraging flights. To measure levels of oxidative stress resulting from low and high foraging activity, we set up three colonies: a colony with a pollen trap to cause increased foraging, a normal colony (control), and a colony with an artificial waterfall to limit foraging. We counted flights of marked foragers at each colony for 5 to 6 hours per day. As predicted, more pollen foraging flights occurred in the pollen trap colony. There was a steady rate of foraging activity in the normal colony, and low rates of foraging in the waterfall colony. Foragers tagged for age and with matching foraging rate data from each colony were collected in liquid nitrogen and frozen at -80° C. Future studies will measure expression of genes involved in oxidative stress in the brains and flight muscles of these bees utilizing western blots (to measure protein expression) and quantitative real-time PCR (to measure RNA expression).
Loann Larsen  
Mentor – Dr. Michelle Elekonich  
University of Nevada Las Vegas – School of Life Sciences

Abstract  
The accumulation of reactive oxygen species (ROS) and free radicals within tissues creates oxidative stress, causing damage and eventual aging in an organism. Intense activity can increase the level of oxidative stress that occurs within an organism. In honey bees, this activity occurs during foraging flights. To measure levels of oxidative stress resulting from low and high foraging activity, we set up three colonies: a colony with a pollen trap to cause increased foraging, a normal colony (control), and a colony with an artificial waterfall to limit foraging. We counted flights of marked foragers at each colony for 5 to 6 hours per day. As predicted, more pollen foraging flights occurred in the pollen trap colony. There was a steady rate of foraging activity in the normal colony, and low rates of foraging in the waterfall colony. Foragers tagged for age and with matching foraging rate data from each colony were collected in liquid nitrogen and frozen at -80° C. Future studies will measure expression of genes involved in oxidative stress in the brains and flight muscles of these bees utilizing western blots (to measure protein expression) and quantitative real-time PCR (to measure RNA expression).
Manipulation of foraging rate in honey bees in a natural setting

Loann K.L. Larsen*1, Joyce H. Pang*2, Andrew Ammons1, and Michelle M. Elekonich1
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*Both individuals contributed equally to this project.

Abstract

The accumulation of reactive oxygen species (ROS) and free radicals within tissues creates oxidative stress, causing damage and eventually leading to aging in an organism. Intense activity can increase the level of oxidative stress that occurs within an organism. In honey bees, this activity occurs during foraging flights. To measure levels of oxidative stress resulting from low and high foraging activity, we set up three colonies: a control with a pollen trap to cause increased foraging, a normal colony control, and a colony with an artificial waterfall to limit foraging. We counted flights of marked foragers from each colony for 5 to 6 hours per day. As predicted, more pollen foraging occurred in the pollen trap colony. There was a steady rate of foraging activity in the normal colony, and low rates of foraging in the waterfall colony. Foragers tagged for age with matching foraging rate data from each colony were collected in liquid nitrogen and frozen at -80°C. Future studies will measure expression of genes involved in oxidative stress in the brains and flight muscles of these bees utilizing western blots (to measure protein expression) and quantitative real-time PCR (to measure RNA expression).

Methods

Three small colonies controlled for number of bees, queen presence, and amounts of pollen and nectar were set with (1) a pollen trap, (2) no changes, and (3) an artificial waterfall simulating rains. The pollen trap was hypothesized to induce more foraging flights, the colony without changes a control for normal flight, and the waterfall colony was hypothesized to prevent flight in order to manipulate high, medium, and low levels of activity reproductively. The frequency of bee flight was recorded daily for 5 weeks. Foraging bees were collected and frozen using liquid nitrogen. These frozen bees are being stored at -80°C for future analysis.

Results and Discussion

There were about 300 bees in each colony before the experiment. After 5 weeks of observations, there were about 600 bees in each colony. The pollen trap colony had the highest number of bees, followed by the normal colony, and then the waterfall colony. In the pollen trap colony, bees were allowed to forage normally without manipulation from sunrise to sunset. In the artificial waterfall colony, bees were not allowed to forage for 4 hours per day. In the normal colony, bees were allowed to forage normally from sunrise to sunset. The bees in the waterfall colony were not allowed to forage for 4 hours per day, which was compared to the normal 12-14 hours per day in the natural setting colony or pollen trap colony.

In preparation for the experiment, three colonies were set up: a control with a pollen trap to cause increased foraging, a normal colony control, and a colony with an artificial waterfall to limit foraging. We counted flights of marked foragers from each colony for 5 to 6 hours per day. As predicted, more pollen foraging occurred in the pollen trap colony. There was a steady rate of foraging activity in the normal colony, and low rates of foraging in the waterfall colony. Foragers tagged for age with matching foraging rate data from each colony were collected in liquid nitrogen and frozen at -80°C. Future studies will measure expression of genes involved in oxidative stress in the brains and flight muscles of these bees utilizing western blots (to measure protein expression) and quantitative real-time PCR (to measure RNA expression).

Results and Discussion

Fights of tagged bees with pollen

Fig. 1: Recorded flights of tagged bees with pollen (dotted orange line indicates pollen trap installation on 6/17 and dotted blue line indicates waterfall installation on 6/18)

Fig. 2: Foraging flights of tagged bees (pollen and nectar foragers combined)

References

Acknowledgments
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Abstract  
The accumulation of reactive oxygen species (ROS) and free radicals within tissues creates oxidative stress, causing damage and eventual aging in an organism. Intense activity can increase the level of oxidative stress that occurs within an organism. In honey bees, this activity occurs during foraging flights. To measure levels of oxidative stress resulting from low and high foraging activity, we set up three colonies: a colony with a pollen trap to cause increased foraging, a normal colony (control), and a colony with an artificial waterfall to limit foraging. We counted flights of marked foragers at each colony for 5 to 6 hours per day. As predicted, more pollen foraging flights occurred in the pollen trap colony. There was a steady rate of foraging activity in the normal colony, and low rates of foraging in the waterfall colony. Foragers tagged for age and with matching foraging rate data from each colony were collected in liquid nitrogen and frozen at -80° C. Future studies will measure expression of genes involved in oxidative stress in the brains and flight muscles of these bees utilizing western blots (to measure protein expression) and quantitative real-time PCR (to measure RNA expression).
Manipulation of foraging rate in honey bees in a natural setting

Loann K.L. Larsen*1, Joyce H. Pang*2, Andrew Ammons1, and Michelle M. Elekonich1
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Abstract

The accumulation of reactive oxygen species (ROS) and free radicals within tissues causes oxidative stress, causing damage and eventual aging in an organism. Intense activity can increase the level of oxidative stress that occurs within an organism. In honey bees, this activity occurs during foraging flights. To measure levels of oxidative stress resulting from low and high foraging activity, we set up three colonies: a colony with a pollen trap to cause increased foraging, a normal colony (control), and a colony with an artificial waterfall to limit foraging. We counted flights of marked foragers at each colony for 5 to 6 hours per day. As predicted, more pollen foraging flights occurred in the pollen trap colony. There was a steady decrease in foraging activity in the normal colony and an increase in foraging in the waterfall colony. Foragers tagged age and with matching foraging rate data from each colony were collected in liquid nitrogen and frozen at -80°C. Future studies will measure expression of genes involved in oxidative stress in the brains and flight muscles of these bees utilizing western blots (to measure protein expression) and quantitative real-time PCR (to measure RNA expression).

Methods

Sorting Forager Manipulation

Three small colonies controlled for number of bees, queen presence, and artificial pollen and nectar were set with (1) a pollen trap, (2) no changes, and (3) an artificial waterfall simulating rain. The pollen trap was hypothesized to increase foraging and increase colony size. The waterfall was hypothesized to decrease foraging in order to manipulate high, medium, and low levels of activity, respectively. The frequency of bee flight was recorded daily for 5 weeks. Foraging bees were collected and frozen using liquid nitrogen. Those frozen bees are being stored at -80°C for future analysis.

Selection

Three groups of age-matched bees were selected from three different experimental colonies at the beginning period. Selected bees were marked by individually numbered tags according to their age (Fig. 1) and then released and tracked.

Foraging Manipulation

A pollen trap (Fig. 2) was set to manipulate a high level of bee activity (frequency of flights per day). Bees in this colony were able to forage from sunrise to sunset, and were hypothesized to increase recruitment of foragers and foraging activity in response to the trap. In the model control colony (Fig. 3), bees were allowed to forage naturally without manipulation from sunrise to sunset. A waterfall device (Fig. 4) mimicked rainfall for the low control group. Bees in this setting were able to forage for only 4 hours per day in comparison with the normal 12 to 14 hours per day in the natural setting colony or pollen trap colony.

Observation and Collection

The tagged bees were observed and their frequency of flight was recorded for about 5 weeks. Foraging bees from the three colonies were collected, frozen in liquid nitrogen, and stored at -80°C.

Results and Discussion

Flights of Tagged Bees with Pollen

- As Fig. 5 shows, the pollen trap did induce more pollen foraging after its installation. When compared to the two other colonies, the pollen trap colony conserved more pollen foraging flights during the experimental period.
- Fig. 6, which shows total foraging for the three colonies, demonstrates that the pollen trap slightly reduced and the waterfall greatly reduced the number of foraging flights of their respective colony, an expected outcome for both the pollen trap and waterfall reduce the entrance to the colony in some manner. The number of foraging flights for the control colony, in comparison, demonstrated relatively steady pattern.
- In Fig. 7, 8, and 9, the flights of the oldest tagged bees and the youngest tagged bees are shown, and similar patterns of increasing and decreasing flights during their lifespan can be observed. When comparing Fig. 8 to Fig. 5, it is seen that the flights of the elder bees decrease, the flights of the younger bees increase, indicating increasing recruitment of younger foragers as older foragers naturally die.

References


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Future Studies

- Future research will analyze the bees tagged and collected in this experiment at the molecular level, through analysis of brain and thoracic tissue.
- Protein expression will be measured through the use of western blots.
- Quantitative real-time PCR (Polymerase Chain Reaction) will be applied to measure RNA expression in the samples.
- The results of these two analyses and their relation to collected flight data will provide insight into the role gene expression plays in oxidative stress.

Fig. 1: Varroa tagged bees
Fig. 2: Pollen trap colony
Fig. 3: Control colony
Fig. 4: Waterfall colony
Fig. 5: Total flight of tagged bees (foraging and non-foraging)
Fig. 6: Total flights of tagged bees during their lifespan
Fig. 7: Flights of tagged bees tagged on 6/2/09
Fig. 8: Flights of tagged bees on 5/6/09
Fig. 9: Flights of tagged bees on 5/5/09
Fig. 10: Flights of tagged bees with pollen
Fig. 11: Recorded flights of tagged bees with pollen (dotted orange line indicates pollen trap installation on 6/17 and dotted blue line indicates waterfall installation on 6/18)
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Abstract
Thermoelectric and electronic properties of AgSbSe2
Cubic I-II-VI2 semiconductors have been studied widely for potential
thermoelectric applications by several groups. Recent investigations show
minimal thermal conductivity for AgBiSe2 and AgSbTe2 due to intrinsic phonon
scattering process due to strong anharmonicity in bonding. AgSbSe2 is
structurally similar to these chalcogenides and crystallizes in the cubic structure
at ambient condition. The thermoelectric figure of merit, Seebeck co-efficient and
thermal conductivity was measured as a function of temperature from 10 K to
350 K. We compare our results with its ternary analogues.

The accumulation of reactive oxygen species (ROS) and free radicals within
tissues creates oxidative stress, causing damage and eventual aging in an
organism. Intense activity can increase the level of oxidative stress that occurs
within an organism. In honey bees, this activity occurs during foraging flights. To
measure levels of oxidative stress resulting from low and high foraging activity,
we set up three colonies: a colony with a pollen trap to cause increased foraging,
a normal colony (control), and a colony with an artificial waterfall to limit foraging.
We counted flights of marked foragers at each colony for 5 to 6 hours per day. As
predicted, more pollen foraging flights occurred in the pollen trap colony. There
was a steady rate of foraging activity in the normal colony, and low rates of
foraging in the waterfall colony. Foragers tagged for age and with matching
foraging rate data from each colony were collected in liquid nitrogen and frozen
at -80° C. Future studies will measure expression of genes involved in oxidative
stress in the brains and flight muscles of these bees utilizing western blots (to
measure protein expression) and quantitative real-time PCR (to measure RNA
expression).
Gallium nitride (GaN) is a group-III nitride semiconductor; which may prove useful in developing optical instruments that operate under high ambient pressures. The purpose of this project is to examine the properties of GaN under varying conditions. The methods used in this experiment consist of modeling free energy as a function of lattice constants; calculating bond lengths, bond strengths, and bulk moduli; and comparing the resultant data with values in published literature. We will also compare these results with experimental data drawn from x-ray diffraction scans. By doing so, we hope to determine whether gallium nitride is suitable for use as a semiconductor at high pressures.
The Effects of Pressure on Wide Bandgap GaN Semiconductors

William Kang and Linda Tran
Mentor: Eunja Kim, Ph.D

ABSTRACT
Gallium nitride (GaN) is a group III semiconductor, which may prove useful in developing optical devices that operate under high pressure or high temperature. The purpose of this project is to examine the properties of GaN at different pressure. The method used in this theoretical study is based on the density-functional theory as implemented in the Vienna ab-initio simulation package (VASP). Relative phase stability of three experimentally proposed structures: wurtzite (wz), zinc-blende (ZB), and rock salt (NaCl) are investigated by calculating free energy as a function of the lattice constant. The results obtained in this study can be compared with values in literature, presently available. The curves of pressure versus volume compression are evaluated and compared with experimental data obtained from x-ray diffraction patterns. By doing so, we hope to determine whether gallium nitride is suitable for optical device application at high pressures.

METHODS
The standard density-functional theory [1] is used in carrying out total energy calculations, as implemented in the VASP software. The generalized gradient approximation of Perdew et al. [2] is adopted in the construction of the exchange and correlation interaction.

A total of 8 atoms were included in rock salt (NaCl) and zincblende (ZB) unit cells. A total of 4 atoms were included in the wurtzite (wz) unit cell. Convergence tests with respect to the number of k points in the Brillouin zone and energy cutoff were performed in this study. For all the calculations presented here, we have used a cutoff energy of 300 eV. The k points are sampled in the Brillouin zone under the Monkhorst-Pack [3] grid of 1 x 1 x 1.

A set of equations in thermodynamics was applied to calculate pressure, volume, and entropy of NaCl, wz, and ZB-GaN structures. These are required in order to ultimately calculate a bulk modulus. Data obtained was also compared to articles presently available.

RESULTS AND DISCUSSION

Energetically, the wurtzite structure (WZ) appears to be the most stable structure, as shown in Fig 2, implying two possible structural changes: (i) a temperature-driven phase transition from wz to ZB-GaN structure, and (ii) a pressure-driven phase transition from wz to NaCl-GaN structure.

The calculated volume compression data are in good agreement with experimental data as shown in Fig 3. In their work, Ueno et al. [4] observe that GaN undergoes a monoclinic decrease in relative volume with increasing pressure until 52.2 GPa, when the volume of their GaN sample almost immediately falls 17.6%. In comparison, the calculated volume collapse from wz to NaCl structure at 52.2 GPa is 20.0% in this work. The slight difference may be accounted for the fact that wz begins to transition into the rocksalt phase prior to this point (beginning at ~32 GPa, as shown in Figure 4).

Fig 2. Energy-volume curves of wz, ZB, and NaCl-GaN structures.

Fig 3 Volume-pressure curves.

Table 1 The structural properties of GaN.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Lattice constant (Å)</th>
<th>Free energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wz</td>
<td>4.49</td>
<td>-19.56</td>
</tr>
<tr>
<td>ZB</td>
<td>4.51</td>
<td>-19.34</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.52</td>
<td>-20.10</td>
</tr>
</tbody>
</table>

Fig 4. Enthalpy vs. pressure diagram.

According to the enthalpy-pressure diagram, the phase transition occurs from wz-GaN to NaCl-GaN at 32 GPa, as shown in Fig 4. Based upon our results, then, we expect GaN to exist primarily as wurtzite at ambient pressure. As pressure approaches the crossover point at ~32 GPa, wurtzite lattices begin to transition into the rocksalt phase; beyond the crossover point, the rocksalt lattice is significantly more stable, dominating the lattice population within the high pressure regime. This corroborates experimental data presented by Ueno et al. [4], who demonstrate that (i) this transition occurs regardless of the direction of pressure variation, (ii) the mixture of wz-GaN and NaCl-GaN structures are observed in the x-ray diffraction spectra at 37 GPa, (iii) wz-GaN which has transitioned into the rocksalt phase at high pressures will remain the wurtzite configuration once the pressure has been released, as in Fig 1 (c).

CONCLUSION
WZ-GaN and ZB-GaN are the most stable polytypes at ambient pressures.
WZ lattices begin to transition into the NaCl polytype as pressure increases, beginning at ~32 GPa and completing at ~52 GPa.
Theoretical calculations corroborate experimental evidence that sample volume collapses at ~52 GPa due to sudden non-complete transition of WZ to NaCl lattices.

FUTURE DIRECTIONS
The next step required is to calculate the bond length, bond strength, and bulk modulus. In addition, further progression would also allow us to examine the electronic band structures including their energy band gap at several pressure points, which would determine if gallium nitride is a suitable semiconductor under high pressure.

REFERENCES

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- Sergey Fischel, Ph.D
Gallium nitride (GaN) is a group-III nitride semiconductor, which may prove useful in developing optical instruments that operate under high ambient pressures. The purpose of this project is to examine the properties of GaN under varying conditions. The methods used in this experiment consist of modeling free energy as a function of lattice constants; calculating bond lengths, bond strengths, and bulk moduli; and comparing the resultant data with values in published literature. We will also compare these results with experimental data drawn from x-ray diffraction scans. By doing so, we hope to determine whether gallium nitride is suitable for use as a semiconductor at high pressures.
The Effects of Pressure on Wide Bandgap GaN Semiconductors

William Kang and Linda Tran
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**ABSTRACT**
Gallium nitride (GaN) is a group-III semiconductor, which may prove useful in developing optical devices that operate under high pressure or high temperature. The purpose of this project is to examine the properties of GaN at different pressures. The method used in this theoretical study is based on the density-functional theory as implemented in the Vienna ab-initio simulation package (VASP). Relative phase stability of three experimentally proposed structures: zinc-blende (zb), wurtzite (wz), and rocksalt (NaCl) are investigated by calculating free energy as a function of the lattice constant. The results obtained in this study can be compared with values in literature, presently available. The curves of pressure versus volume compression are evaluated and compared with experimental data obtained from X-ray diffraction patterns. By doing so, we hope to determine whether gallium nitride is suitable for optical device applications at high pressures.

**METHODS**
The standard density-functional theory [1] is used in carrying out total energy calculations, as implemented in the VASP software. The generalized gradient approximation of Perdew et al. [2] is adopted in the construction of the exchange and correlation interaction.

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A set of equations in thermodynamics was applied to calculate pressure, volume, and enthalpy of GaN, wz, and zb-GaN structures. These are required in order to ultimately calculate a bulk modulus. Data obtained was also compared to articles presently available.

**RESULTS AND DISCUSSION**

![Fig. 2. Energy-volume curves of wz, zb, and NaCl-GaN structures.](image)

Energetically, the wurtzite structure (wz) appears to be the most stable structure, as shown in Fig. 2, implying two possible structural changes: (i) a temperature-driven phase transition from wz to zb-GaN structure, and (ii) a pressure-driven phase transition from wz to NaCl-GaN structure.

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![Fig. 4. Enthalpy vs. pressure diagram.](image)

According to the enthalpy-pressure diagram, the phase transition occurs from wz-GaN to NaCl-GaN at 32 GPa, as shown in Fig. 4. Based upon our results, then, we expect GaN to exist primarily as wurtzite at ambient pressures, as it is the most stable structure. As pressure approaches the crossover point at ~32 GPa, wurtzite begins to transition into the rocksalt phase. Beyond the crossover point, the rocksalt lattice is significantly more stable, dictating the lattice population within the high pressure regime. This corroborates experimental data presented by Ueno et al. [4], who demonstrate that (i) this transition occurs regardless of the direction of pressure variation, (ii) the mixture of wz-GaN and NaCl-GaN structures are observed in the x-ray diffraction spectra at 37 GPa (iii) wz-GaN which has transitioned into the rocksalt phase at high pressures will resume the wurtzite configuration once the pressure has been released, as shown in Fig. 5 (a-c).

![Fig. 5. Structure of GaN.](image)

**CONCLUSION**
- wz-GaN and zb-GaN are the most stable polytypes at ambient pressure.
- wz lattices begin to transition into the NaCl polytype as pressure increases, beginning at ~32 GPa and completing at ~52 GPa.
- Theoretical calculations corroborate experimental evidence that sample volume collapse at ~52.2 GPa due to sudden near-complete transition of wz to NaCl lattices.

**FUTURE DIRECTIONS**
The next step required is to calculate the bond length, bond strength, and bulk modulus. In addition, further progression would also allow us to calculate the electronic band structures including their energy band gap at several pressure points, which would determine if gallium nitride is a suitable semiconductor under high pressure.

**REFERENCES**

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