Aug 9th, 9:00 AM - 12:45 PM

Undergraduate Research Opportunities Program 2011

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University of Nevada, Las Vegas

University of Nevada, Las Vegas

UNLV
UNIVERSITY OF NEVADA LAS VEGAS

2011

Undergraduate Research Opportunities Program

& Creative Activities Symposium (UROP/URCAS)

August 9, 2011

Science and Engineering Building

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Compiled and Edited
By
Nicholle Booker, Graduate Affairs Coordinator, College of Sciences

Photo Caption, Cover:

Front row (left to right): Pashtana Usufzy, University of Nevada Las Vegas; Rosa Ojeda, California State Northridge; Maryknoll Palisoc, University of Nevada Las Vegas; Diana Ha, University of Nevada Las Vegas; Alexa Khan, University of Nevada Las Vegas; Carmen Vallin, University of Nevada Las Vegas; Daniel Sneed, University of Nevada, Las Vegas; Kyle Childs, South Carolina State;

Second Row (left to right): Mathew Heuton, UNLV; Phil Lotshaw, Willamette University; Anthony Harrington, Institution; Christopher Yip, Juniata College; April Jeffries, State University of New York at Albany; Quinton Guerrero, Monmouth College; Justine Carryer, Colorado College; Jennifer Meoni, North Carolina State; Kimberley Gray, Department of Energy Employee;

Third Row (left to right): Guillermo Esparza, Harvey Mudd College; Trea LaCroix, University of Nevada Reno; Lucy Rivera, Western Arizona; Anna Smith, Davidson College; Mary Evert, Otterbein College; Charles Loelius, Rutgers University; Robert Gex, University of Nevada Las Vegas; Jeanette Perry, University of Nevada Las Vegas;

Back row (left to right): Quinlan Smith, California Lutheran University; Gregory King, University of Nevada Las Vegas; Nick Macholl, Loyola Chicago; Tyler Mosher, Clarkson University; Jack Brangham, Otterbein College; Brandon Stewart, College of Southern Nevada; Stuart Allen, Georgia Institute of Technology; Hassan Rizvi, University of Nevada Las Vegas; Andy Brooks, University of Nevada Las Vegas; Max Olsen, Utah State

Acknowledgements

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Ron Smith, Vice President for Research

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Javier Rodriguez, College of Sciences, Associate Dean

John Farley, Professor, Department of Physics and Astronomy

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Kurt Regner, REU Microbiology

Eduardo Robleto, REU Microbiology

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Ken Czerwinski, Program Director, Radiochemistry Program

Wendee Johns, Radiochemistry, Program Manager

Bradley Childs, Radiochemistry, Doctoral Student

Janelle Droessler, Radiochemistry Doctoral Student

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Mike Wells, Chair, Department of Geosciences

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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.

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University of Nevada, Las Vegas

2011

Undergraduate Research Opportunities Program

& Creative Activities Symposium (UROP/URCAS)

August 9, 2011

UNLV, Sciences and Engineering Building (SEB) Lobby

9:00 a.m. - 10:00 a.m.	Poster Installation
10:00 a.m. - 10:15 a.m.	Opening Remarks,
	College of Sciences Dean, Dr. Timothy Porter
10:15 a.m. - 12:00 p.m.	Public Viewing
12:00 p.m. - 12:45 p.m.	Luncheon, SEB Room #1243
12:45 p.m. - 1:00 p.m.	Acknowledgements, Program Directors

Mission Statement

The University of Nevada, Las Vegas is a research institution committed to rigorous educational programs and the highest standards of a liberal education. We produce accomplished graduates who are well prepared to enter the work force or to continue their education in graduate and professional programs. Our faculty, students, and staff enthusiastically confront the challenges of economic and cultural diversification, urban growth, social justice, and sustainability.

Our commitment to our dynamic region and State centrally influences our research and educational programs, which improves our local communities. Our commitment to the national and international communities ensures that our research and educational programs engage both traditional and innovative areas of study and global concerns. UNLV's distinctive identity and values permeate a unique institution that brings the best of the world to our region and, in turn, produces knowledge to improve the region and world around us.

UNLV is committed to and driven by these shared values that will guide our decision making:

-High expectations for student learning and success;

-Discovery through research, scholarship, and creative activity;

-Nurturing equity, diversity, and inclusiveness that promotes respect, support, and empowerment;

-Social, environmental, and economic sustainability;

-Strong, reciprocal, and interdependent relationships between UNLV and the region around us;

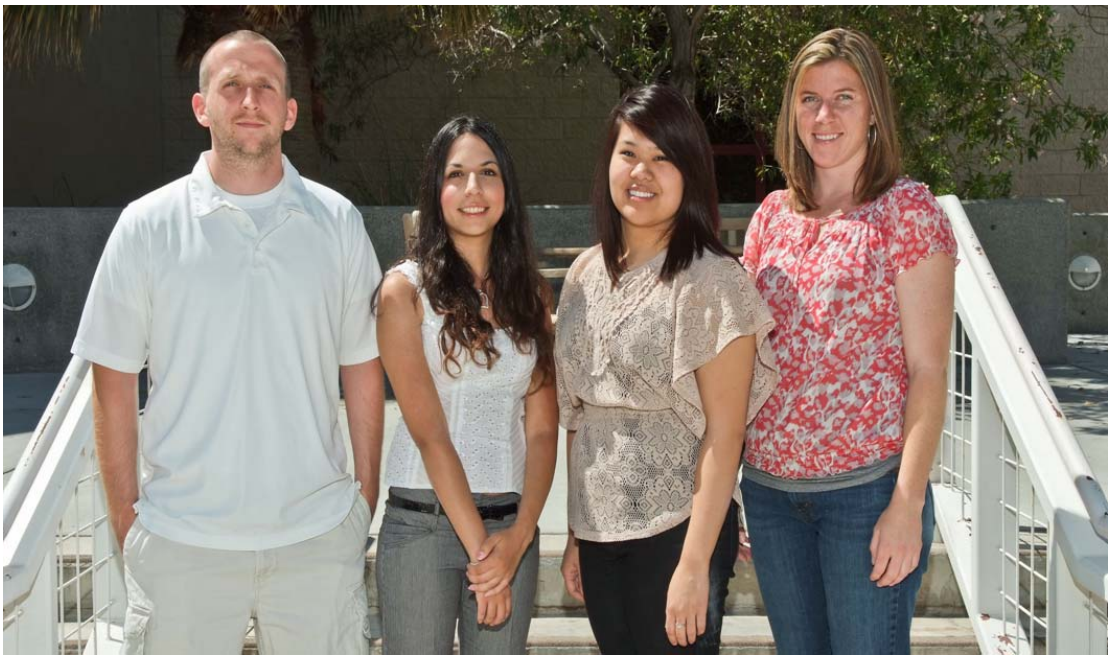
-An entrepreneurial, innovative, and unconventional spirit.

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.



Left to right: Mathew Heuton, UNLV; Alexa Khan, UNLV; Diana Ha, UNLF; Jeanette Perry, UNLV.

Nevada INBRE

IDeA Network of Biomedical Research Excellence

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.

Left to Right: Gregory King, UNLV; Pashtana Usufzy, UNLV; Carmen Vallin, UNLV; Hassan Rizvi, UNLV.

NSF UNLV PHYSICS

RESEARCH EXPERIENCE FOR UNDERGRADUATES PROGRAM

National Science Foundation Research Experience for Undergraduates Program (NSF REU)

REU PHYSICS AND ASTRONOMY

The Research Experience for Undergraduates (REU) program is a program of the National Science Foundation to give undergraduate students an experience in performing research.

Most of a student's career consists of classroom lectures. The REU program is intended to benefit students by offering experiences that go beyond the classroom. The UNLV Physics Department has had a successful REU program since 1987. Initially the program was limited to UNLV students. Beginning in 1992, the program was open to non-UNLV students as well. Students participate in research projects in the summer with follow-up activity during the academic year.

Front row (left to right): Quinton Guerrero, Monmouth College; Justine Carryer, Colorado College; April Jeffries, State University of New York at Albany; Daniel Sneed, University of Nevada Las Vegas; **Second row (left to right):** Phil Lotshaw, Willamette University; Robert Gex, University of Nevada Las Vegas; Guillermo Esparza, Harvey Mudd College; Anna Smith, Davidson College; **Back row (left to right):** Quinlan Smith, California Lutheran University; Jack Brangham, Otterbein College; Nick Macholl, Loyola Chicago; Brandon Stewart, College of Southern Nevada; Tyler Mosher, Clarkson University; **Not Pictured:** Julius Monello.

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 non-host 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.

Back row (left to right): Andy Brooks, University of Nevada Las Vegas; Anthony Harrington, University of Nevada Las Vegas; Max Olsen, Utah State; **Middle row (left to right):** Mary Evert, Otterbein College; Trea LaCroix, University of Nevada Reno; Lucy Rivera; Western Arizona; **Front row (left to right):** Christopher Yip, Juniata College; Rosa Ojeda, California State Northridge; Jennifer Meoni, North Carolina State; Maryknoll Palisoc, University of Nevada Las Vegas

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 rom honeybee genomics to extra galactic x-ray bursts.

Radiochemistry Fuel Cycle Summer School

Description

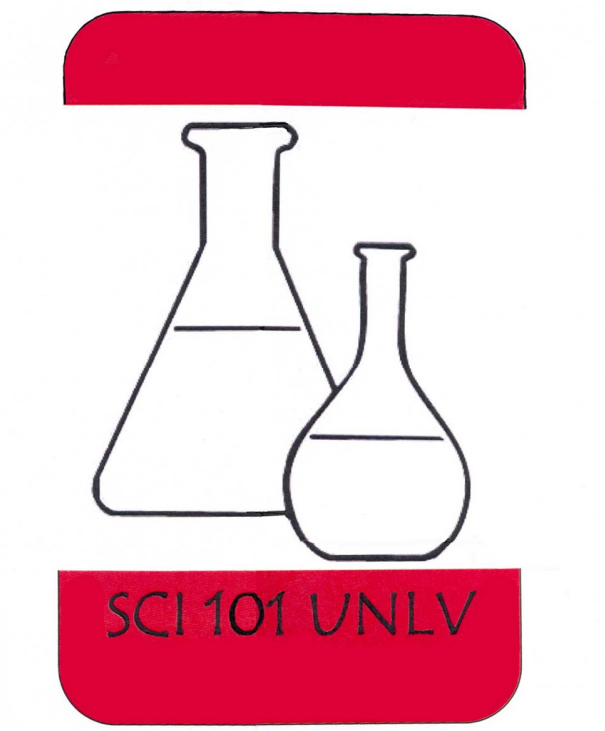
The six week summer school is an intensive course in radiochemistry with a focus on the nuclear fuel cycle. Radiochemistry is introduced through the physics of radioactive decay and chemistry of radioelements, providing an intellectual intersection of the periodic table and chart of the nuclides. The course begins with a description of the chart of the nuclides, detailing the information, concepts, and data used as a foundation for exploring isotopes. Details on alpha decay, beta decay, gamma decay, and fission are presented. The methods and data from the investigation of nuclear properties, nuclear forces and nuclear structure are described. The fundamental chemical properties in radiation and radiochemistry, including the relationship between speciation, kinetics and thermodynamics, the influence of radiolysis on chemistry, radioisotope production and separations will be explored. The use of radiochemistry in research and technology is explored with attention given to the nuclear fuel cycle. The modern nuclear fuel cycle is presented from fundamental chemistry to

Course Outcomes

It is expected students will accomplish the following during the course.

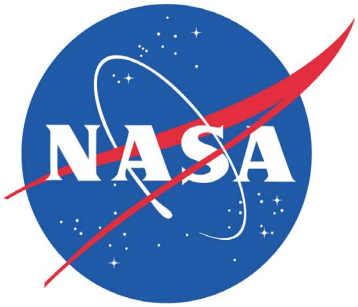
- Understand, utilize, and apply the chart of the nuclides to radiochemistry and nuclear technology
- Understand the fundamentals of nuclear structure
- Understand chemical properties of radioelements
- Comprehend and evaluate nuclear reactions and the production of isotopes
- Comprehend types and descriptions of radioactive decay
- Utilization of radiochemistry in research

From left to right: Kyle Childs, South Carolina State; Kimberley Gray, DOE Employee; Charles Loelius, Rutgers University.



SCI 101 - Introductions to the University for Science Majors

SCI 101 is a First Year Experience (FYE) program designed to help students make the transition to college life by providing opportunities to explore, discover, and connect with the university and its resources. The course syllabus is focused on three themes whose learning outcomes enable the students to navigate their college careers successfully at UNLV. The first third of the course provides students with a tool kit of necessary college skills including note taking, test taking preparation, organization, and stress management. The middle third of the course focuses on research skills and critical thinking. Students participate in projects designed to advance their library literacy, research methods, public speaking, and technical writing. The final portion of the class focuses on university resources including academic and career advising, university academic standards, and



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 equably 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.

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 recover 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, solidstate spectroscopy, cryogenic studies, and synthesizing and characterizing foams. HiPSEC also maintains computational centers for engineering and solidstate theory on the UNLV campus.

Desert Research Institute (DRI)

Education and 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.

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 Leak 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.

Nevada Cancer Institute (NVCI)

Nevada Cancer Institute (NVCI) is the official cancer institute for the State of Nevada. A nonprofit organization, NVCI is committed to reducing the burden of cancer by pursuing the development of a comprehensive cancer center, as defined by the National Cancer Institute. Through the knowledge and expertise of the finest scientists, clinicians, educators and caregivers, the Institute provides hope to communities in Nevada, the Southwest and beyond through research, education, early detection, prevention and high quality patient care. NVCI is striving for a future without cancer that is achieved through initiated and collaborative research in basic, clinical and population science Nevada Cancer Institute is a team of dedicated professionals committed to providing world-class, research-linked cancer care to Nevadans and people throughout the Southwest. Our research-linked, comprehensive cancer facility opened late Summer 2005 and is dedicated to state-of-the-art research and implementation of groundbreaking methods of prevention, detection and treatment of cancer.

Solar and Renewable Energy Minor

Through an industry-education partnership with NV Energy, UNLV has established educational programs in sustainable energy. The undergraduate Solar and Renewable Energy Minor was developed to catapult Nevada and UNLV into an alternative energy leadership role and to meet workforce needs of the region. This has attracted new students to UNLV and provides an excellent opportunity for workforce development in the state and region. The minor is available to all undergraduate students at UNLV.

There are two tracks (Engineering and Science Track and Policy Track) that have appropriate curriculum where students can add this onto their existing degree program. In the Fall semester of

Urban Sustainability Initiative

Two Department of Energy Grants are being used to implement the Urban Sustainability Initiative (USI) at the University of Nevada, Las Vegas. USI is supporting various interdisciplinary research teams, graduate students, and interns. These research efforts in environmental, economic, and social sustainability. They address critical areas of (e.g., energy, water, transportation, health, built environment) that were identified by the Brookings Institution Mega Mountains study (2008) as critical for the regions continued prosperity. Undergraduate students are supported through this initiative to move forward campus sustainability efforts in education, research, and operations.

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. 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.

School of Life Sciences

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

Diana Ha

Email: Diana.Hah@gmail.com
Former High School: College of Southern Nevada High School
Location of High School: Las Vegas, NV
Mentor/Advisor: Jeffery Shen
Educational Institute of Project: UNLV
Department: School of Life Sciences
Research Site: UNLV White Building – Shen Lab

Title: Bioinformatics Approach to Identifying Proteins Mediating Plant Response to Drought Stress

Abstract: The current water shortage is a major concern in regard to the global climate change. A constant decrease in availability of water will have direct effects on the development of plants. This fact is a strong stimulus to identify the proteins involved with activation of stress response in plants. My research focuses on the abiotic stress of drought on rice (Oryza sativa). There are two components to my research: a bioinformatics approach that will analyze genes activated during drought stress, and an experimental approach that will utilize the yeast one-hybrid system to identify the proteins involved with this response.

Why are you doing this project? I am interested in the growing global climate change crisis that has led us to look into an alternative way to harvesting agricultural crop. My project aims at goal to have the ability to produce the same quality plant with less water.
What problem are you trying to solve? Harvesting rice as an agricultural crop requires an immense amount of water. It is beneficial to our environment if we can find a way to allow our plants to uptake less water yet still produce abundant crop.
What tools or equipment are you using? I will be using bioinformatics tools such as MEME, BioProspector, Gibbs and many others to analyze genes of interest. I will also utilize the yeast-one hybrid system to identify proteins that interact with these genes.
Why is your project worth researching? It is vital for research on drought stress, especially in plants because of the shortage of water our climate is experiencing. The problem we are trying to solve is to find a way to grow rice plants with less water while maintaining an appropriate yield because of its high demand as a world-wide food crop.
What relevance will it have on the community, society, and in your research field? Identifying the proteins that are involved with drought stress responses in rice plants can potentially lead way to genetically modifying these plants to combat drought more efficiently. This means that we can eventually harvest more rice with less water. The identification of these proteins can also pave way to further research in other agricultural crops.
What did you find? There are many genes that are unregulated during exposure to abiotic stress in rice plants. Application of software such as BioProspector allowed for identification of different motif sequences in the promoter regions of the activated genes.
What is the future of your research project? With the identification of proteins mediating drought stress in rice, we can look into genetically modifying rice to grow copiously with less water in the future. Further research on different types of abiotic stresses such as high salinity or cold stress can also be done on the rice plant.

The Role of a Transcription Factor in Regulating Rice Response to Drought Stress

Diana Ha, Liyuan A. Zhang and Jeffery Q. Shen
School of Life Sciences, University of Nevada at Las Vegas

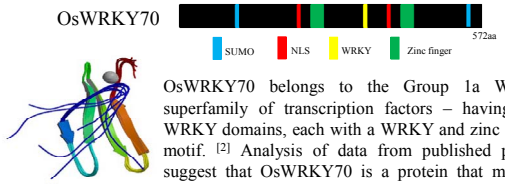
Abstract: The current water shortage is a major concern in regard to our global climate change crisis. A decrease in the availability of water will have direct effects on the development of plants. [1] Some crops, such as *Oryza sativa*, or commonly known as rice, requires an abundant amount of water for adequate growth. With the water shortage crisis, it will become extremely difficult to harvest such crops to meet the world's food demand. However, many plants have evolved mechanisms for overcoming and tolerating stresses such as drought. My research focuses on studying the proteins involved with these mechanisms. The WRKY superfamily is a family of transcription factors that up or down-regulate pathways in response to biotic and abiotic stresses in plants. [2] We propose and hypothesize that *OsWRKY70* plays a role in the abiotic stress of drought in rice. To identify the physiological role of this gene, we studied the phenotype of *OsWRKY70* knockout mutants using an insertional transposon in comparison to its wildtype counterparts. This project aims to study the proteins involved with drought resistance in rice, which will pave the way for the production of genetically engineered crops that will be better at conserving water.

Introduction:

WRKY transcription factors are proteins that bind to genes to turn them on or off [3] (see Figure 1). They are the master switches in regulating plant responses to biotic (pathogenic attack) and abiotic (cold, heat, drought) stress.

Figure 1: The WRKY Protein Binds to the W-box to Express or Repress Target Genes.

Figure 2: OsWRKY70 Belongs to WRKY Group 1a.

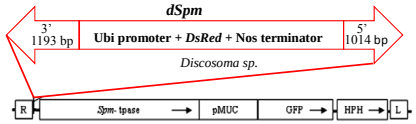


OsWRKY70 belongs to the Group 1a WRKY superfamily of transcription factors – having two WRKY domains, each with a WRKY and zinc finger motif. [2] Analysis of data from published papers suggest that OsWRKY70 is a protein that may be responsible in drought tolerance.

Methods:

- To test the physiological role of *OsWRKY70*, the expression of this gene was knocked out using transposon mutagenesis.
- PCR allowed for selection of mutants that are homozygous for the knockout trait. Quantitative real-time PCR (qRT-PCR) showed the expression levels of *OsWRKY70* in the mutants.
- Seedling germination with growth on MS-media with 3% sucrose provided comparative analysis between mutant and wildtype phenotypes.

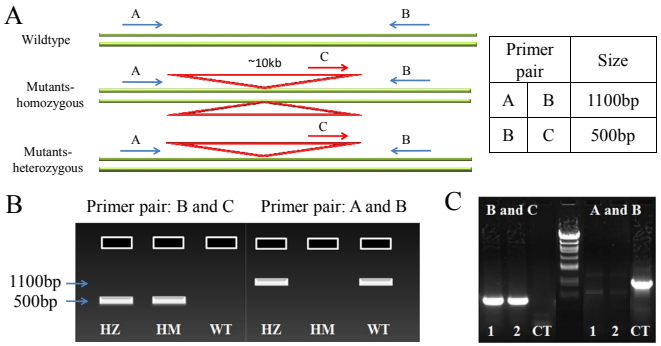
Figure 3: *OsWRKY70* Knockout Mutants Were Created by the *dSpm* Transposon.



Insertion of this transposon causes a disruption in the transcription of the *OsWRKY70* gene. Transposon mutants were obtained from University of California, Davis.

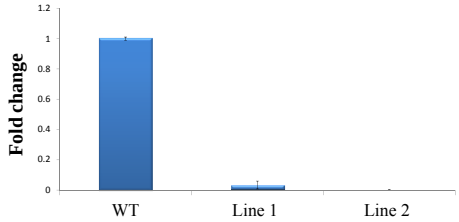
Results:

Figure 4: Homozygous Knockout Lines Were Confirmed by PCR.



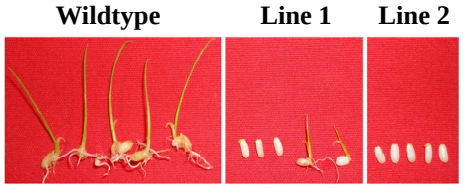
Polymerase Chain Reaction (PCR) was utilized to select for homozygous knockout mutants. Panel A shows the different primers used for wildtype, homozygous, and heterozygous mutant lines. Panel B shows the possible band sizes for each primer pair. A band at 500 bp, using primer pair B and C, indicates both homozygous and heterozygous mutants. Additionally, homozygous mutants will not show a band with primer pair A and B. Panel C shows the actual results of the PCR after running on gel electrophoresis, indicating that both sample 1 and sample 2 are homozygous mutants. CT is the control sample from the wildtype line.

Figure 5: The Expression of *OsWRKY70* in the Two Mutant Lines is Barely Detectable.



The expression of *OsWRKY70* was analyzed by qRT-PCR for each mutant line and wildtype line. Results show that expression was barely detectable in both *OsWRKY70* knockout mutants as compared to that of wildtype. Both knockout mutants were derived from the same line.

Figure 6: Knockout Mutants Germinated One Week Later Than Wildtype Seedlings.



Germination of seedlings on MS-media with 3% sucrose. *OsWRKY70* knockout mutant seedlings did not germinate until one week after its wildtype counterpart.

Conclusions:

- PCR confirmed that the *OsWRKY70* mutant lines we obtained were homozygous for the knockout of *OsWRKY70* (see Figure 4).
- qRT-PCR analysis showed that mutant plants had a barely detectable amount of expression (see Figure 5). This indicates that the transposon was able to knockout the expression of *OsWRKY70*.
- Seedling germination showed that *OsWRKY70* knockout mutants germinated one week after wildtype seedlings (see Figure 6), leading us to speculate that *OsWRKY70* plays a role in germination in rice plants.
- Further analysis is ongoing to determine if *OsWRKY70* plays a role in regulating responses to drought stress.

References:

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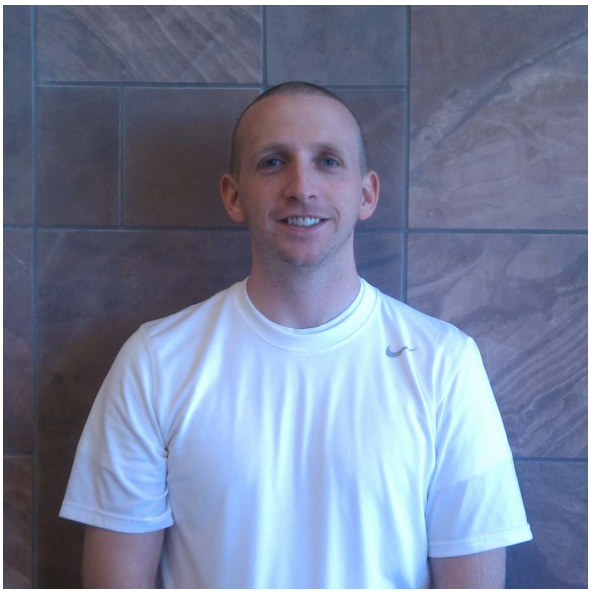
[2] Ross, C. A., Liu, Y. and Shen, Q. J. (2007), *The WRKY Gene Family in Rice (Oryza sativa). Journal of Integrative Plant Biology*, 49: 827–842.
[3] Seki, M., et al., *Molecular Techniques in Crop Improvement.* 2010. 333.

Acknowledgements: I would like to thank my mentor, Dr. Jeffery Shen, for this rewarding opportunity as well as members of Shen Lab for all their endless help and support. I appreciate your guidance through this project; I could not have done it without your help. This project was made possible by the Nevada Infrastructure for Climate Change Science, Education and Outreach. NSF EPSCoR Grant # EPS0814372.



Mathew Heuton

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Former High School: West High School
Location of High School: Las Vegas, NV
Mentor/Advisor: Frank Van Breukelen
Educational Institute of Project: UNLV
Department: School of Life Sciences
Research Site: SEB 3168

Title: Effects of Climate Change on the Viability of the Devils Hole Pupfish

Abstract:
Devils hole is an unusual, cavernous, aquifer-fed spring in Death Valley National Park. It is the only home to a critically endangered species, *Cyprinidon diabolis*, a.k.a. The Devils Hole Pupfish. *C. diabolis* allows us a unique look at the impact of climate change on a species that has no gene flow and no possibility of escape from its current habitat. Climate change affects ectotherms (cold-blooded animals) tremendously since their body temperature is subject to changes in the temperature of their environment. In the case of *C. diabolis*, they are subject to high temperatures year round, around 33°C. The high temperatures cause an increase in oxygen needs for essential metabolic activity as well as an increase in energy needs. The limited energy and oxygen availability of Devils Hole means *C. diabolis* has to work harder to merely stay alive and has less energy to dedicate to reproduction. Most biologists will tell you that animals are adapted to thrive in their environment. But what happens when the environment changes quickly, the genes necessary for adaptation are unavailable in the population, and there is no chance for finding a new habitat? The answer is simple; the population declines rapidly until the stress or stresses they face are removed. In the case of *C. diabolis*, we are investigating just how much stress is being placed on this population by an increase in temperature. Recent climate change models predict a 5 – 6°C increase in average air temperatures across the southwest portion of the United States in the next 80 years (Meehl, et al., 2007). In biological systems, a 1° C change in body temperature may result in a >10% higher energy demand. Although we do not know what the effects of higher air temperatures will be on Devils Hole water temperature, it is likely that even a modest change in temperature will result in greater energetic demands.

Why are you doing this project? We can also hypothesize what effects global climate change will likely have on this population and even use this species as a model for climate change's effects on fish population around the world.
What problem are you trying to solve? What tools or equipment are you using? Our investigation includes measuring resting aerobic metabolic activity based on oxygen consumption at various temperatures. Due to the endangered nature of C. diabolis, we use a possible hybrid fish of C. diabolis and its closest relative, C. nevadensis mionectes for testing purposes. The basis of our experiments is flow-through respirometry.
Why is your project worth researching? Cyprinidon diabolis is a critically endangered pupfish species that is only found in Devils hole. The population contained just 38 individuals in 2006. The current population is estimated at 120 individuals. Most breeding takes place on the shelf at the bottom center of the photograph.
What relevance will it have on the community, society, and in your research field? Devils Hole is a unique pupfish habitat in the Mojave.. We can also hypothesize what effects global climate change will likely have on this population and even use this species as a model for climate change's effects on fish population around the world.
What did you find? Preliminary data show unusual oxygen consumption patterns for 33°C acclimated fish.
What is the future of your research project? As a model, C. diabolis may tell us what will happen to fish populations around the world if human-caused climate change is not controlled.

Effects of Climate Change on the Viability of the Devils Hole Pupfish

Matthew Heuton, Stanley Hillyard, and Frank van Breukelen
School of Life Sciences and School of Dental Medicine, University of Nevada Las Vegas

Abstract

Devils hole is an unusual, cavernous, aquifer-fed spring in Death Valley National Park. It is the only home to a critically endangered species, *Cyprinidon diabolis*, a.k.a. The Devils Hole Pupfish. *C. diabolis* allows us a unique look at the impact of climate change on a species that has no gene flow and no possibility of escape from its current habitat. Climate change affects ectotherms (cold-blooded animals) tremendously since their body temperature is subject to changes in the temperature of their environment. In the case of *C. diabolis*, they are subject to high temperatures year round, around 33˚ C. The high temperatures cause an increase in oxygen needs for essential metabolic activity as well as an increase in energy needs. The limited energy and oxygen availability of Devils Hole means *C. diabolis* has to work harder to merely stay alive and has less energy to dedicate to reproduction. Most biologists will tell you that animals are adapted to thrive in their environment. But what happens when the environment changes quickly, the genes necessary for adaptation are unavailable in the population, and there is no chance for finding a new habitat? The answer is simple; the population declines rapidly until the stress or stresses they face are removed. In the case of *C. diabolis*, we are investigating just how much stress is being placed on this population by an increase in temperature. Recent climate change models predict a 5 – 6˚ C increase in average air temperatures across the southwest portion of the United States in the next 80 years (Meehl, et al., 2007). In biological systems, a 1˚ C change in body temperature may result in a >10% higher energy demand. Although we do not know what the effects of higher air temperatures will be on Devils Hole water temperature, it is likely that even a modest change in temperature will result in greater energetic demands.

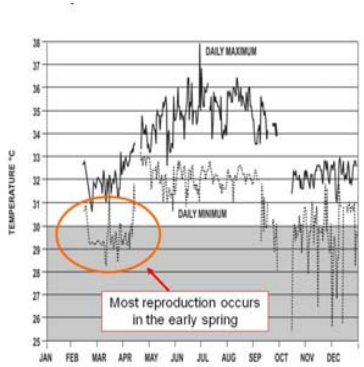
Methods

Our investigation includes measuring resting aerobic metabolic activity based on oxygen consumption at various temperatures. Due to the endangered nature of *C. diabolis*, we use a possible hybrid fish of *C. diabolis* and its closest relative, *C. nevadensis mionectes* for testing purposes. The basis of our experiments is flow-through respirometry. We place the fish in a clear plastic chamber that has water pulled through it by a peristalsis device and a series of tubes. An electrode that senses changes in oxygen pressure takes measurements before the fish enters the chamber to establish a baseline and then continually measures the oxygen pressure over a course of 2-5 hours to determine how much oxygen the fish requires at rest. After determining how much oxygen the fish consumed at rest at various temperatures, we chart those data to determine which temperatures are stressful for the fish in an acute setting. By using fish that are acclimated to different temperatures, 28 and 33˚ C, we can see what is happening to the fish metabolically under chronic conditions. We can also hypothesize what effects global climate change will likely have on this population and even use this species as a model for climate change's effects on fish population around the world.

Devils Hole is a unique pupfish habitat in the Mojave.

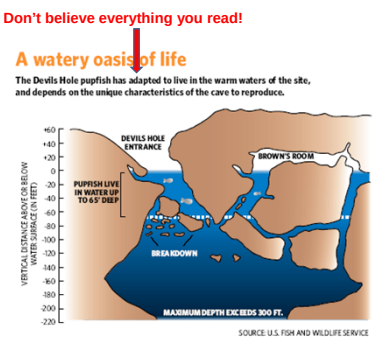
Cyprinidon diabolis is a critically endangered pupfish species that is only found in Devils hole. The population contained just 38 individuals in 2006. The current population is estimated at 120 individuals. Most breeding takes place on the shelf at the bottom center of the photograph.

Seasonal temperature variation on the breeding shelf.



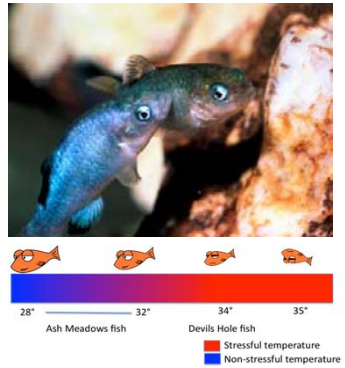
The breeding shelf is the only portion of Devils Hole that is exposed to the surrounding environment and the water temp. changes as the season changes.

Devils Hole's structure helps maintain a stable temperature of 33 – 34˚ C in the main pool.



The stable temperature in the main pool ensure that the fish are acclimatized to approximately 33˚ C. What if the fish are unable to thrive at this temperature? Evolutionary dogma says they should be adapted, but maybe they're not.

C. diabolis likely lives above its ideal temperature range.

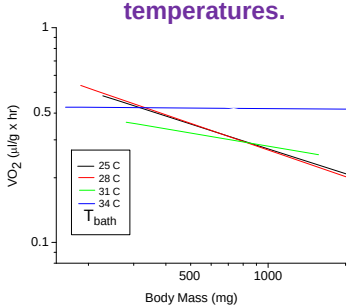


As water temperature increases, the oxygen demand of fish increases and the oxygen capacity of water decreases. This means the fish need more oxygen but there's less of it available.

Using flow-through respirometry, we determine oxygen consumption as a function of temperature and mass.

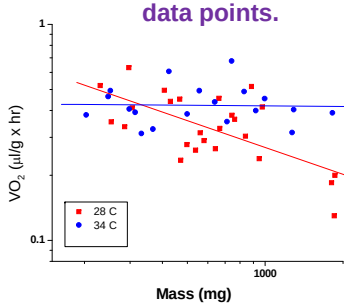
By controlling the temperature of the water in an aquarium, we can determine just how much oxygen *C. diabolis* uses at various temperatures both above and below the temperatures found in Devils Hole.

Pupfish acclimated to 28˚ C were tested at various bath temperatures.



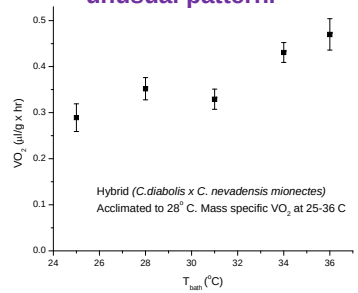
The above graph demonstrates that at temps. between 25 and 31˚ C, the mass-specific metabolic rate decreases, as expected; however, at 34˚ C, the rate is essentially flat, indicating that as mass increases, the fish struggle to meet oxygen needs.

A clear trend is seen when looking at the individual data points.



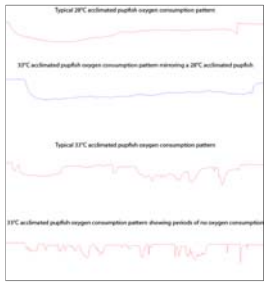
These data demonstrate that small fish appear to be unaffected by the 6˚ C difference. The medium and large fish show a significant increase in oxygen consumption as temperature increases.

Mass-specific oxygen consumption shows an unusual pattern.



In most biological systems, metabolic activity increases 2-3 fold per 10˚ C increase in temperature. We see almost no change between 25 and 31 ˚ C and a modest increase between 25 and 34˚ C.

Preliminary data show unusual oxygen consumption patterns for 33˚ C acclimated fish.



While most of the 28˚ acclimated fish showed consistent oxygen consumption patterns when tested at 28˚ C bath, early testing on 33˚ C acclimated fish shows erratic patterns and in some cases, a complete halt to all oxygen consumption for long periods of time.

Summary and Conclusions

- Increased metabolic activity in medium and large fish tested at 34˚ C suggests temperature may be limiting size, fecundity, and the ability to thrive in Devils Hole.
- A minimal change in mass-specific metabolic rate on the population level over a wide range of temperatures may indicate a redirection of metabolic resources in order to simply survive, precluding the ability to thrive.
- New data are showing that fish acclimated to 33˚ C are very unstable in their metabolic activity and a further increase in habitat temperature may lead to the extinction of *C. diabolis*.
- As a model, *C. diabolis* may tell us what will happen to fish populations around the world if human-caused climate change is not controlled.

Supported by cooperative agreement EPS-08144372 from the National Science Foundation

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Mentor/Advisor: Frank Van Breukelen
Educational Institute of Project: UNLV
Department: School of Life Sciences
Research Site: SEB 3168

Title: Ubiquitylation of Proteins in the Frozen Wood Frog

Abstract:
Wood Frogs (*Rana sylvatica*) are able to withstand freezing temperatures during the winter season. During that time heart rate and breathing approaches a halt. One might ask how are they able to spontaneously come back to life when temperatures come back to ideal. In collaboration with Dr. Ken Storey (Carleton University, Canada), we are going to investigate ubiquitin-dependent proteolysis in the freeze-thaw cycle & how that might give clues to their survival.

Why are you doing this project? I am doing this project to find out how Wood Frogs are able to withstand freezing temperatures that would kill most of other organisms.
What problem are you trying to solve? I am trying to find out if ubiquitin conjugate levels decrease when Wood Frogs enter freezing temperatures.
What tools or equipment are you using? A Lowry Assay of my homogenate samples to find protein concentrations during the freeze-thaw cycle of the wood frog. Western and Dot Blots to find to out ubiquitin conjugates levels in the freeze-thaw cycle of the wood frog.
Why is your project worth researching? My project is worth researching because it is amazing how these vertebrates (Wood Frogs) are able to freeze and have their heart rate, blood flow, and breathing stop and spontaneously come back when they are thawed out. This research may help out in the future with many other organisms that don't have this ability.
What relevance will it have on the community, society, and in your research field? To give people around me an insight of the reasons how these organisms are able to withstand freezing temperature would be an amazing accomplishment.
What did you find? I currently am still working on my research project.
What is the future of your research project? I am hoping when I finish this project I am able to get it published in a scientific journal.

Ubiquitylation of Proteins in the Frozen Wood Frog

Alexa Khan[1], Michael Ulrich[1], Kenneth Storey[2], Frank van Breukelen[1]
[1]School of Life Sciences, University of Nevada, Las Vegas; [2]Carleton University, Ottawa Canada

Abstract

Wood frogs *(Rana sylvatica)* are able to withstand freezing. Respiratory and cardiac activity ceases when frozen. Homeostatic functions like protein synthesis and degradation presumably must also be compromised. We investigated the fate of ubiquitin-dependent proteolysis in the freeze-thaw cycle and how that might gives clues to wood frog survival. We performed western blots for ubiquitin conjugates.

Introduction

Rana sylvatica has the ability to remain frozen for as many as 11 days at -4ºC with 100% survivorship. Few data are available as to the fate of major homeostatic processes like protein synthesis and degradation. Ubiquitin dependent proteolysis is responsible for as much as 80-90% of cytosolic protein degradation. Essentially, a small peptide modifier, ubiquitin, is conjugated to target proteins and marks those proteins for degradation. Ubiquitin dependent proteolysis is essential for the turnover of protein pools but is also critical in how a cell responds to cellular stress and protein damage. While there have been numerous predictions of how global climate change will affect temperatures, few studies have addressed the biological implications

Hypothesis

I hypothesize that when wood frogs *(Rana sylvatica)* recover from freezing, ubiquitin-conjugate concentrations will decrease.

Materials and Methods

Rana sylvatica were collected from the woods in Ottawa, Canada. Hind leg thigh muscle and liver tissue samples were collected from different points of the freeze-thaw cycle. Five individuals of each state were used (n = 5).

Control (C) wood frogs were acclimated 1-2 weeks at 5° C.

Frozen (F) wood frogs were exposed to -3° for 24 h.

Long thaw (LT) wood frogs were frozen 24 h @ -3° C and then thawed for 24 h.

Short thaw (ST) wood frogs were exposed to -3° C and then thawed for 8 h.

Sample Preparation-

Samples from the muscle and liver tissues were homogenized in 50 mM Tris•HCl, pH 8.3, 20% glycerol, 2% SDS, and 0.4 M 2-mercaptoethanol. Samples were centrifuged for 20 min @10,000 • g for 30 min, 4° C. Supernatants were disrupted using a 30 gauge needle and frozen until use.

A modified Lowry protein assay was performed to determine protein concentrations.

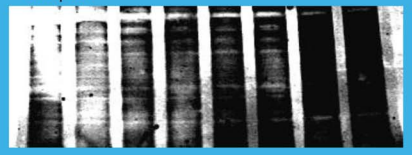
Western Blot

We are asking two questions in regards to the ubiquitylation of proteins. We asked if there were qualitative changes to which proteins were ubiquitylated as a function of state e.g. are there a few proteins that are preferentially ubiquitylated upon freezing? Question 2 was if there were quantitative changes to those proteins tagged for ubiquitylation upon freezing e.g. are more proteins tagged for degradation upon freezing?

We performed western blot analyses. Briefly ,40 µg proteins were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 9% gel. The proteins were electrotransferred to PVDF membrane. The membrane was treated with 0.5% glutaraldehyde for 20 min, then blocked with 5% nonfat dry milk. The blot was incubated with a monoclonal antibody to ubiquitin conjugates, washed, incubated with a HRP-linked secondary antibody, and visualized using ECL+ on a Typhoon imager.

Results

This is a western blot that was done for the liver homogenate tissue samples. See M&M, for key to samples.

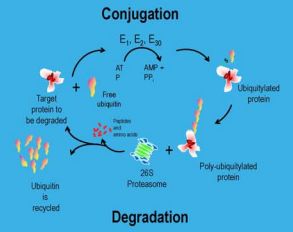


C C F F LT LT ST ST

Conclusion

The data demonstrate no qualitative differences in which liver proteins get ubiquitylated. The data do indicate quantitative differences where freezing and thawing is associated with increased ubiquitin conjugate concentrations. The muscle blot was similar in demonstrating no qualitative differences in ubiquitylated proteins (data not shown).

I will perform more quantitiative dot blot analyses to determine precisely how much ubiqutiylation occurs.



Wood frog *(Rana sylvatica)* thawed

Acknowledgements

We thank NSF EPSCoR for funding this project and the other members of the laboratory for their guidance and assistance. In particular, Michael Treat was instrumental in this study.

Wood frog *(Rana sylvatica)* frozen

Jeanette Perry

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Former School: College of Southern Nevada
Location: Las Vegas, NV
Mentor/Advisor: Brett Riddle
Educational Institute of Project: UNLV
Department: School of Life Sciences
Research Site: FMA 102

Title: Evaluating species responses to climate change using genetic data

Abstract: I examine the impact of historical climate change on the distribution of the Great Basin Pocket Mouse, Perognathus parvus, a sagebrush species from the Great Basin and Columbia Plateau. I evaluate whether the species distribution shifted predictably between the last glacial maximum and present time and whether we can correctly predict the change in species distribution between the two time periods. I use ecological niche modeling to generate a null hypothesis of a range shift assuming that the species has identical climatic requirements between the two time periods. I then use genetic data to evaluate whether the genetic signals of populations correspond to geographic processes (stability or expansion) inferred from the models. Alternatively, if the species climatic requirements do not remain identical through time, the genetic data will not support the models and indicate a different response to climate change, such as range stability.

Why are you doing this project? The current and projected future warming trends together with degradation of habitats throughout much of the Great Basin and Columbian Plateau represent real threats to many species occupying these regions.
What problem are you trying to solve? If we can determine the impacts climatic changes have had on the distribution of species, we can obtain a better understanding of the future impacts that projected climatic trends will have on many species in similar regions. Our results with the Great Basin Pocket Mouse may be relevant to other species, some of which are Endangered Species Act candidates. To determine whether the species distribution shifted predictably (accordingly with the climate), the first approach used was ecological niche modeling.
What tools or equipment are you using? This is a computer model that uses information on species environmental requirements and creates a map of the species distribution according to habitat suitability.
Why is your project worth researching? This approach assumes the species environmental requirements have not changed between the last glacial maximum (when ice sheets were at their maximum between 26,500-20,000 years ago) and present time. The second approach used was to contrast these models with genetic patterns. By utilizing DNA sequences, we can determine if the population has been expanding, shifting, or stable.
What relevance will it have on the community, society, and in your research field?
What did you find? I cannot include my findings yet, as I have not completed my research for the modeling or genetic markers.
What is the future of your research project? To complete the modeling and genetic markers to conclude my findings.

Evaluating Species Responses to Climate Change Using Ecological Niche Modeling and Genetic Data

Jeanette Perry, School of Life Sciences, University of Nevada, Las Vegas

1 Introduction

The current and projected future warming trends together with degradation of habitats throughout much of the Great Basin and Columbian Plateau represent real threats to many species occupying these regions. If we can determine the impacts of past climatic changes on the distribution of species, we can obtain a better understanding of the future impacts of projected climatic trends on many species in these regions. My results with the Great Basin Pocket Mouse (*Perognathus parvus*) may be relevant to conservation ecologists and resource managers attempting to protect several Endangered Species Act candidates, such as the pygmy cottontail (*Brachylagus idahoensis*). I used ecological niche modeling and molecular genetics to determine if *P. parvus* distribution shifted according to predictions of climate-driven habitat changes between the Last Glacial Maximum (LGM; when ice sheets were at their maximum between 26,500-20,000 years ago) and present time.

2 Predictions

I tested the null hypothesis that the species habitat requirements remain identical through time as climates change, and therefore *P. parvus* responded to the warming climate after the LGM by shifting its range (Fig. 1a; Hewitt 1996). If that's the case, this range shift will be congruently supported by the ecological niche models and the genetic data. Alternatively, if the species habitat requirements do not remain identical through time, the genetic data will not support the niche models and show a different response to climate change, such as range stability despite the climatic changes (Fig. 1b).

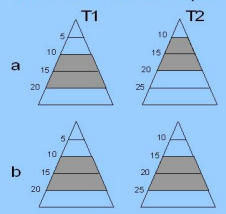

Figure 1. Two types of species response to climate change. In **a)** species habitat requirements remain identical between two time periods in which climate has shifted and a species responds to with a range shift. In **b)** the species habitat requirements shift through time and the species persists in place despite climatically-driven habitat shift.

3 Methods

To determine whether the species distribution shifted as predicted under the niche conservation model, I first used the methodology of ecological niche modeling to reconstruct the species current and LGM distributions. I employed the software Maxent (version 3.3) which evaluates environmental (e.g. climatic) data taken from the species occurrence records. This information on species environmental requirements was used to identify suitable habitat across the landscape and project species expected distribution on a map. Fourteen climatic variables were used to build the models, including precipitation, temperature, and diurnal range. The program was masked (limited to) the ecoregions where the species actually occurs.

Secondly, genetic patterns were used to evaluate the niche conservation model. I generated mitochondrial DNA sequences for 71 individuals of *P. parvus*. I calculated genetic variation among sampling localities which was then interpolated across the landscape in ArcGIS. The patterns of genetic variation can be used to infer which populations have recently expanded and which have remained stable over time according to available population genetic models (Fig. 2) (Hewitt 1996).

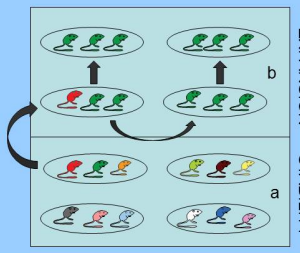

Figure 2. A simplistic diagram of the predicted decrease in genetic variation in the direction of range expansion. In **a)** an area of stable populations shows high genetic variation (represented by different colors). In **b)** the arrows represent the direction of the range expansion with only a subset of haplotypes expanding to a new area, creating areas of low genetic variation.

4 Results and Discussion

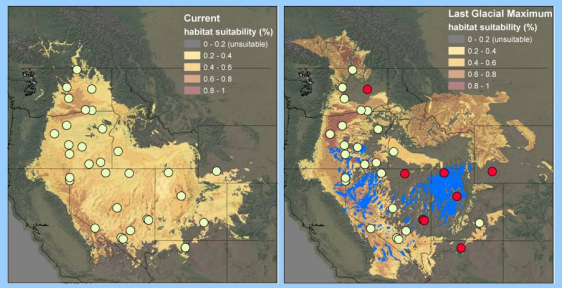

Figure 3. Ecological niche models for *P. parvus* for the current climatic conditions (left) and those of the LGM (right). The shading gradation progresses from yellow (the least suitable habitat), orange to brown (the most suitable habitat). Pluvial lakes (blue) are shown on the LGM model. Shaded circles indicate sampling localities used in analysis, with populations predicted to occur in areas of recent expansion shaded red on the LGM model.

The LGM model generally projected a broader suitable habitat range than the current model, stretching southwest into the Mojave Desert and northward into Washington, Idaho, Wyoming and Montana. The LGM model indicated unsuitable habitat within the eastern parts of the Great Basin, as opposed to more suitable habitat throughout the western Great Basin. Based on these models, I predicted a genetic signal of stable populations (Fig. 2a) in areas of overlap between the LGM and current models (Fig. 3; yellow shaded circles). I also predicted a genetic signal of populations in areas of recent expansion (Fig. 2b) as suitable habitat increased into the eastern Great Basin after the LGM (Fig. 3; red shaded circles).

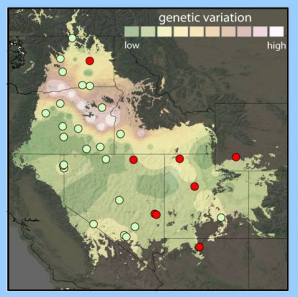

Figure 4. Interpolated genetic variation across the landscape of the current climatic niche model for *P. parvus*. The shading gradation progresses from green (lowest genetic variation), yellow, orange, brown to white (highest genetic variation).

Interpolated genetic variation across landscape (Fig. 4) did not match our predictions based on the models. The areas where we predicted a genetic signal of recent expansion did not exhibit lower genetic variation than areas where we predicted population stability. The highest genetic variation was detected within the northern parts of the species range, within the Columbian Plateau. The genetic variation within the Great Basin was overall lower, with the lowest values found within the western Great Basin.

In conclusion, my prediction of species response to the past climatic event under the assumption of niche conservation was not supported by contrasting the ecological niche model with the actual pattern of genetic variation. The disagreement between the models and genetic data imply that *P. parvus* did not shift its range predictably under this assumption, which could mean that the species habitat requirements did not remain identical between the two climatically very different time periods. Alternatively, environmental factors (e.g., soils) other than those associated with the climatic variables used to build the models might be important in shaping the species distributions through time.

5 References

1. Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.
2. Painting by Elizabeth McClelland from Kays and Wilson's *Mammals of North America*, © Princeton University Press (2002).

6 Acknowledgements

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Title: Defining the role of NRAS in melanoma maintenance

Abstract: The incidence of melanoma has increased 600 percent over the last four decades; it is the most rapidly increasing malignancy among young people in the United States and is currently the leading cause of cancer death in women aged 25-29. 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 cancer from a pre-malignant primary tumor to a metastatic cancer that develops at secondary sites is a multi-step process, thought to require many genetic and epigenetic events that provide a growth advantage to cells. It is still unclear, however, which of the many genetic changes are required late in tumor progression. The increased incidence of melanoma, combined with the poor prognosis of patients with advanced disease, make it imperative that we increase our understanding of the underlying genetic causes of melanoma such that better targeted therapeutic strategies can be developed.

Why are you doing this project? I'm working on this project because it gives me not only exposure to biology and chemistry of cancer but also helps me learn and experience a variety of procedures and techniques using different equipment. The project sounded interesting to me because it involves research in the field of oncogenic gene expression and the suppression of it.

What problem are you trying to solve? The fact that resistance plays a major role in treating cancer is known. However, the degree to which this resistance can be understood and dealt with is currently still not impressive. The problem I'm trying to solve is a similar one. Combination therapy to treat cancer is the ultimate goal, and blocking multiple targets that affect cancerous growth is the means we want to do it by.

What tools or equipment are you using? In the lab, there are various exciting and very interesting tools/equipment that I get to work with. Beginning with a pipette to a spectrophotometer, we use a wide array of biotechnology. I get to work with the bio-hood, the PCR machine, Gel Electrophoresis machines, centrifuges of different sizes and etcetera. While specific machines/kits are available for the respective tasks, it is interesting and availed through experience to use personalized tricks or protocols to perform certain procedures.

Why is your project worth researching? This project focuses on treating resistant tumors. When people have cancer and the cells respond positively to the treatment, it is always possible that the cancer will come back and more powerfully the next time. This time, there will be a lot of tumor/growth that is resistant to previous treatment. However, if combination therapy is used to knock out such tumors, there is a possibility that the recurrence of such growth can be prevented. Considering the incidence of melanoma and the frequency of the recurrence of growth, this project might open a good gateway toward treating people with recurring tumors along with the original anomalies.

What relevance will it have on the community, society, and in your research field? This project will create a mouse model that can positively influence future research that will be carried in the field of such resistant tumors. Specific selection for such resistant tumors will allow us to better learn the tumors and this will help the community in the long run. It will definitely make understanding the recurrence of cancer and tumor growth easier and treating it more palpable.

What did you find? We found that tumors are inducible and that extension of tumor growth to other organs, such as the brain and the kidney and a true metastasis to the lung has also occurred on one occasion. The growth of resistance tumors in the mouse model is rapid. Short term cultures from the primary tumors from such growth were established and these cells were syngeneic, forming tumors in all recipient mice. Tumors regressed in all mice treated with Doxycyclin and suppression of NRAS expression significantly increased survival compared with untreated tumor bearing mice.

What is the future of your research project? To take an unbiased approach using gene expression analysis to identify novel mechanisms that may be responsible for resistance.

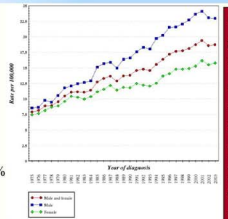
Defining the Role of NRAS in Melanoma Maintenance

Sravya T. Challa **PI: Sheri L. Holmen, PhD.**

Nevada Cancer Institute, Las Vegas, NV

Melanoma is the most rapidly increasing malignancy among young people in the U.S.

- The incidence of melanoma has increased by more than 600 percent over the last forty years (ACS statistics www.cancer.org)
- Melanoma is the leading cause of cancer death in women aged 25-29
- 5-yr survival for advanced stages of the disease is <20%



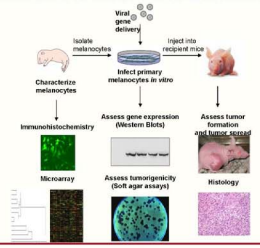
- **Goal**: to generate an in vivo model of melanoma that is resistant to inhibition of MAPK signaling in the context of mutant NRAS.
- **Hypothesis**: resistant tumors will develop that are no longer dependent on NRAS for continued growth.
- **Method**: to use a novel mouse model of melanoma, generated through the somatic introduction of NRAS-encoding avian retroviruses into transgenic mice expressing the avian retroviral receptor, TVA, specifically in melanocytes to induce melanoma in vivo. The MAPK pathway will be inhibited genetically by doxycycline mediated suppression of NRAS expression to select for resistant tumors.
- **Long-term goal**: to identify additional targets for rational combination therapy for advanced melanoma.

Molecular Analysis of Human Melanoma*

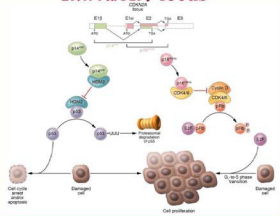
Gene/Locus	Familial(F)/Sporadic(S)	Alterations
9p21:p16^{INK4a}p19^{ARF}	F, S	Point mutation,deletion, promoter^{M}
p53	S	Point mutation
p27	S	Decreased expression
10q23: PTEN	S	LOH and Point mutation
11q22-23	S	LOH
NRAS	S	Point mutation
C-myc	S	Overexpression
B-RAF	S	Point Mutation
MC1R (melanocortin receptor)	S	Point mutation

*Adapted from Castellano and Parmiani Melanoma Research 1999:9;421-432

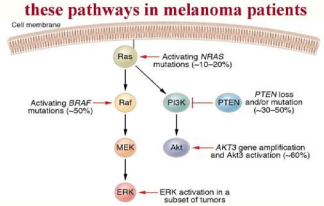
Initial Validation of Melanoma Associated Genes



Ink4a/Arf locus



Schematic of the canonical RAS effector pathways RAF-MEK-ERK and PI3K-Akt and the mutations that most often activate these pathways in melanoma patients



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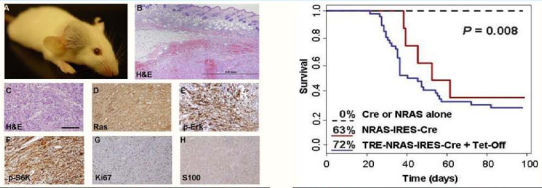


Induction of melanoma in DCT-TVA/*Ink4a/Arf lox/lox* mice. Mice were injected with *NRASQ61R* and *Cre* viruses at birth. (A) A representative mouse (age 42 days) with a tumor at the site of injection. (B-C) H&E stained tumor sections. (D) IHC for RAS. (E) IHC for phosphorylated Erk (p-Erk) (F) IHC for phosphorylated p70 S6 kinase (p-S6K). (G) IHC for Ki67. (H) IHC for S100. All sections were counterstained with hematoxylin. Scale bars represent 1.0 mm for (B) and 200 μm for (C-H).

Kaplan-Meier percent survival curves. DCT-TVA/*Ink4a/Arf lox/lox* mice were injected at birth with the NRAS^{Q61R} and Cre viruses indicated. Dashed line: Cre alone $n = 20$ and NRAS alone $n = 15$. Black line: NRAS^{Q61R} and Cre viruses in combination $n = 22$. Blue line: TRE-NRAS-IRES-Cre and Tet-off $n = 46$. Red line: NRAS–IRES-Cre ($n = 8$). The comparison between the solid lines and the dashed line (controls) was highly significant ($P = 0.008$) demonstrating that both Cre and NRAS are required for tumor formation in this context.

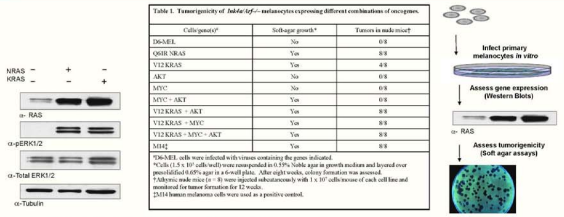


Table 1. Tumorigenicity of *Ink4a/Arf*−/− melanocytes expressing different combinations of oncogenes.

Cells (gene(s))*	Soft-agar growth*	Tumors in nude mice†
D6-MEL	No	0/8
Q61R NRAS	Yes	8/8
V12 KRAS	Yes	4/8
AKT	No	0/8
MYC	No	0/8
MYC + AKT	Yes	0/8
V12 KRAS + AKT	Yes	8/8
V12 KRAS + MYC	Yes	8/8
V12 KRAS + MYC + AKT	Yes	8/8
M14‡	Yes	8/8

*D6-MEL cells were infected with viruses containing the genes indicated.
†Cells (1.5 x 10^6 cells/well) were resuspended in 0.35% Noble agar in growth medium and layered over preestablished 0.65% agar in a 6-well plate. After eight weeks, colony formation was assessed.
‡Athymic nude mice (n = 8) were injected subcutaneously with 1 x 10^7 cells/mouse of each cell line and monitored for tumor formation for 12 weeks.
‡M14 human melanoma cells were used as a positive control.

Expression of RAS in D6-MEL cells and growth in soft agar. Cell lysates from uninfected D6-MEL melanocytes (–) or cells infected with retroviruses containing either Q61R NRAS or V12 KRAS (+) were collected in SDS lysis buffer, separated on a 4-20% gradient gel and immunoblotted for total RAS expression (α-RAS), activated phosphorylated ERK p44/42 (α-pERK1/2), total ERK p44/42 expression (α-Total ERK1/2), and α-tubulin.

RCAS/TVA Melanoma Mouse Model System

TVA: receptor for subgroup A Avian Leukosis Virus (ALV-A)
- Confers susceptibility to ALV-A infection when introduced into cells normally resistant to infection, can be expressed in a tissue-specific manner allowing efficient gene targeting

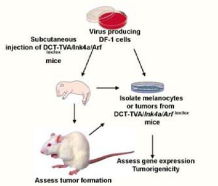
RCAS: replication competent retroviral vector (in avian cells), derived from ALV

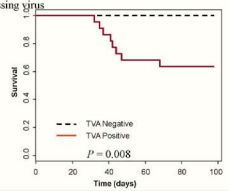
In replicating mammalian cells expressing TVA, RCAS can stably integrate into the DNA and express the inserted gene at high levels
- Replication defective in mammalian cells; does not spread in target animals. Multiple oncogenic alterations can be introduced into the same cell or animal without the cost of generating multiple strains of transgenic mice

No endogenous viral sequences with which these vectors could recombine

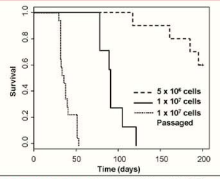
Gene expression can be reduced by RNAi using a miRNA expressing virus

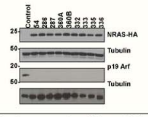
Delivery of RCASBP(A) viruses to murine Cells *in vitro* and *in vivo*



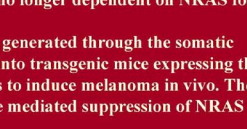


Kaplan-Meier percent survival curve. All mice were injected with *NRASQ61R* and *Cre* viruses at birth. For DCT-TVA negative mice $n = 16$ (dashed line) and for DCT-TVA-positive mice $n = 22$ (solid line).





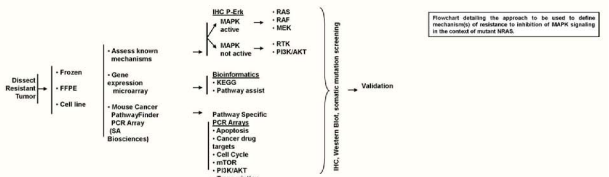
Kaplan-Meier percent survival curve. DCT-TVA/*Ink4a/Arf lox/lox* mice were injected subcutaneously with a cell line derived from an explanted melanoma: 5 × 106 cells $n = 10$ (large dashed line) and 1× 10^7 cells $n = 7$ (solid line). 1× 10^7 cells that had been passaged *in vivo* were re-injected $n = 17$ (small dashed line). **Protein expression in explanted melanoma cells.** Expression of NRAS^{Q61R}-HA from eight different mice (54, 286, 287, 360A, 360B, 332, 333, 335 and 336). The A and B designation indicates two separate tumors from the same mouse. Expression was detected with an antibody to the HA epitope tag on NRAS. D6-MEL immortal melanocytes were used as a negative control for HA expression and normal mouse astrocytes were used as a positive control for p19 Arf expression. The blots were re-probed with a-tubulin as a loading control.



Protein expression in explanted melanoma cells. Expression of NRAS^{Q61R}-HA from an explanted tumor sample derived from a DCT-TVA/*Ink4a/Arf lox/lox* mouse injected with TRE-NRAS-IRES-Cre and Tet-off viruses. The cells were grown in the absence (-) or presence (+) of Dox for 1 week. NRAS expression was detected with a pan-RAS antibody. The virally delivered NRAS is larger than endogenous RAS due to the HA epitope tag on NRAS. The blots were re-probed with an antibody that recognizes a-tubulin as a loading control. **Kaplan-Meier percent survival curves.** DCT-TVA/*Ink4a/Arf lox/lox* mice were injected at birth with TRE-NRAS-IRES-Cre and Tet-off. Dashed line: Tumor-bearing mice were treated with Dox when tumors reached 1,000 mm3 $n = 12$ Black line: untreated $n = 33$. The comparison between the two groups was highly significant ($P = 1.29$ x 10-7) demonstrating that inhibition of NRAS expression in this context significantly increases survival. **Comparison of primary and resistant tumors.** Tumors were induced in DCT-TVA/*Ink4a/Arf lox/lox* mice by delivery of viruses containing TRE-NRAS-IRES-Cre and Tet-off. Top Panel: Representative H&E, NRAS-HA IHC, and P-Erk IHC of a primary tumor. Bottom Panel: Representative H&E, NRAS-HA IHC, and P-Erk IHC of a tumor that became resistant while on Dox treatment. As expected, virally delivered NRAS-HA expression was detected in the primary tumor but not in the resistant Dox treated tumor.

Results and Conclusions

- Both *INK4a* and *ARF*, DCT-TVA mice were crossed to *Ink4a/Arf lox/lox* mice to generate DCT-TVA;*Ink4a/Arf lox/lox* mice.
- No tumors were detected in TVA-negative mice but in 12 weeks, more than one-third of DCT-TVA;*Ink4a/Arf lox/lox* mice developed melanoma that was histologically similar to the human disease.
- Delivery of a virus in which *NRASQ61R* and *Cre* expression was linked by an internal ribosomal entry site (IRES) resulted in tumor formation in nearly two-thirds of TVA positive mice. Short term cultures from the primary tumors were established and were syngeneic with the DCT-TVA/*Ink4a/Arf lox/lox* strain, forming tumors in all recipient mice.
- In the context of Tet-on, the Tet-responsive gene is only expressed in the presence of doxycycline (Dox); in the context of Tet-off, the Tet-responsive gene is repressed in the presence of Dox. Using this approach in the context of Tet-off and the TRE-NRAS-IRES-Cre vector we observed tumor formation in 72% of DCT-TVA/*Ink4a/Arf lox/lox* mice.
- In contrast to tumors from mice not exposed to Dox, resistant tumors from Dox treated mice lacked exogenous NRAS expression indicating that tight Dox-mediated NRAS regulation remained intact. Because reactivation of the MAPK pathway is one possible mechanism of resistance, we used IHC to detect active phosphorylated Erk (P-Erk) in the resistant tumors. In contrast to the primary tumors, very little P-Erk expression was detected in the resistant tumors.
- These results suggest that reactivation of the MAPK pathway upstream of Erk is not the mechanism of resistance. Several other mechanisms of resistance are possible, including activation of alternative signaling pathways.



Future Directions

- Preservation and evaluation of samples to identify the mechanism(s) of resistance to either genetic or pharmacological inhibition of the MAPK pathway.
- Evaluation of resistant tumor samples to ensure suppression of virally delivered NRAS expression by both IHC of FFPE tissue and by Western blot analysis from established cell lines.
- Gene expression analysis to identify novel mechanisms that may be responsible for resistance in this context using GeneChip 12X135k microarrays.

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Mentor/Advisor: Andrew Andres
Educational Institute of Project: UNLV
Department: School of Life Sciences
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Title: The Role of an ABC Transporter as a Steroid Antagonist in Drosophila

Abstract: Drosophila melanogaster are holometabolous insects that have several distinct life stages including larvae and a winged adult. The larval stage is mainly a time of feeding and growth, while the adult stage is optimized for sexual reproduction and dissemination. The larval stage can itself be divided into three time periods, or instars: 1st (L1), 2nd (L2), and 3rd (L3) (Figure 1). Larval growth – both between instars and beyond – depends on specific signaling pathways controlled by a cholesterol derived steroid, 20-hydroxyecdysone (20E). Although 20E is a systemic developmental signal, little is known about the molecular details of how different tissues respond to the hormone. We have been studying one gene induced by 20E in some target tissues [1]. This gene, E23, encodes an ATP binding cassette (ABC) transporter protein that may function to limit hormone exposure in tissues where it is expressed.

Why are you involved in research as an undergraduate/graduate student? I originally joined a research lab to gain hands-on experience involving the things I had been learning through my coursework. I was considering graduate school at the time, and I knew that the reality of working in a lab would be different from my visualization of it; joining a lab would put me in the correct mindset for seeing if I would actually enjoy research as a career.
What is your background? I am originally from Phoenix, Arizona and went to high school in Arizona, Texas, and finished in Nevada. I have been at UNLV for four years, although I spent two of those years going to business school for a degree in Finance. I eventually converted to Biology when I realized how bored I was with school!
How did you get involved/interested in science? All aspects of science have captivated me for as long as I remember. Being curious is a part of being human, and science attempts to answer the burning questions that we all have about ourselves, the world around us, and how we fit into that world.
How did you go about applying for / getting into the lab? I had been in Tribeta, an Honors Biology group, which had weekly talks from professors and graduate students about their research. I saw one of the upcoming talks was involving Alzheimer's research, which interested me. The professor giving the talk was one I had also been talking a course from that semester and I approached him to see if he had space in his lab. Luckily, he had just formed a group for a summer research project, and I was able to work with the group.
What is your project and how would you describe a typical day in the laboratory? I am currently studying some aspects of molting in Drosophila melanogaster, fruit flies. The main focus is on larval growth and the timing and molecular characteristics of their distinct growth stages. My normal day includes tending to the flies – making sure they have good food and lots of friends. For the project, I also have to create crosses between specific stocks and then collect the eggs and larvae that arise and observe their growth and physiology.
What are your plans in the future? I am currently applying to schools for medical research programs. I hope to return to Texas, and I would love to work in the fields of Neuroscience and clinical research and trials.
How do you feel this experience will prepare you for those plans? Any real experience in the lab is a learning opportunity. I have many years ahead of me in labs working on projects and I feel more prepared for some of the work that entails. I have also been able to write grant proposals, a necessary evil that I will face many more times.

The Role of an ABC Transporter as a Steroid Antagonist in *Drosophila*

Gregory King and Andrew J. Andres
University of Nevada, Las Vegas School of Life Sciences

Introduction

Drosophila melanogaster are holometabolous insects that have several distinct life stages including larvae and a winged adult. The larval stage is mainly a time of feeding and growth, while the adult stage is optimized for sexual reproduction and dissemination. The larval stage can itself be divided into three time periods, or instars: 1st (L1), 2nd (L2), and 3rd (L3) (Figure 1). Larval growth – both between instars and beyond – depends on specific signaling pathways controlled by a cholesterol derived steroid, 20-hydroxyecdysone (20E). Although 20E is a systemic developmental signal, little is known about the molecular details of how different tissues respond to the hormone. We have been studying one gene induced by 20E in some target tissues [1]. This gene, *E23*, encodes an ATP binding cassette (ABC) transporter protein that may function to limit hormone exposure in tissues where it is expressed.

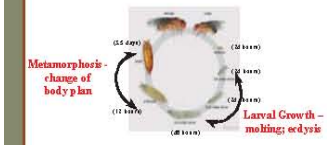

Figure 1: Drosophila Life Cycle

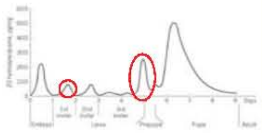

Figure 2: Predicted 20E titers during *Drosophila* development

We have used transgenic *Drosophila* containing DNA constructs in which *E23* is ectopically overproduced. We have shown that several target tissues do not respond to 20E at metamorphosis, consistent with the hypothesis that E23 acts as a 20E antagonist to pump hormone out of cells before a genomic response can occur (Figure 3) [Paladino and Andres, manuscript in prep].

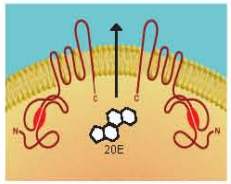

Figure 3: *E23* is predicted to encode an ABC transporter

To test this hypothesis further, I analyzed transgenic flies overexpressing *E23* in the Inka cells, which are responsible for larval ecdysis. My data support our model in which E23 works as a 20E antagonist, pumping hormone during all developmental stages.

Why *Drosophila* and E23?

Drosophila, the common fruit fly, has been used as a model organism to study genetics and biology for a century. Numerous mutant and transgenic fly lines are available, and can be used to study virtually any conserved cellular function. The powerful genetic tools allow the overexpression or silencing of any *Drosophila* gene in any specific tissue. As a result, *Drosophila* is an important model in particular for the general study of conserved signaling pathways in animals, including humans.

A more thorough understanding of cell biology is essential to medicine. E23 itself is specific to *Drosophila* but it is closely related to ABC pumps found in many species, including humans. ABC transporters are highly involved in several diseases, including in the resistance of cancers to chemotherapy [2]. Often, a cancer patient will respond well to chemotherapeutic agents, even for weeks or months. However, the cancer can quickly return, and with a resistance to the drugs. This is often due to the amplification of ABC transporters in these cancer cells. Study of the basic science involved in ABC transporters could give indications of potential silencing effects and future medication.

Hypotheses

E23 functions as a 20E antagonist, meaning when it is overproduced in any target cell the cell will not respond to hormone and produce the normal developmental response.

Since the Inka cells respond to 20E by stimulating larval ecdysis, E23 overproduction in these specific cells should result in the larvae displaying ecdysis defects that prevent normal growth.

Molting vs. Ecdysis

During larval growth in Drosophila, animals must shed their rigid cuticular skins as they progress from one instar to another. This includes the separation and absorption of parts of the old cuticle, creation of new cuticular structures, and final removal of old structures [3].

Ecdysis describes the final step of the molting process, specifically focusing on the wiggling behavior that allows the larva to escape from the smaller cuticle. This process is thought to be controlled by special gland cells, known as Inka cells, that line specific sites on the trachea [4]. Ecdysis triggering hormone (ETH) is a small peptide which seems to promote ecdysis and is produced within these gland cells in response to 20E. Once produced, it stimulates the brain to initiate the ecdysis behavior [5]. Therefore, if E23 is overproduced in the Inka cells the larvae should be able to produce a new cuticle, but show defects in the ability to escape the old cuticle.

Figure 4: Arrows mark the nuclei of Inka cells, which can be genetically modified to overproduce E23.

Materials and Methods

To control the production of E23 within specific tissues, a powerful genetic system can be used: the *UAS-Gal4* system (Figure 5). This system, which has been adopted from yeast, consists of two parts: the *Upstream Activator Sequence* (*UAS*), and the GAL4 "driver" protein which binds to *UAS*. This allows for transcription and production of the *UAS* controlled *E23* only in cells that express the *Inka-GAL4* construct. Figure 6 is a diagram of the procedure to assay for defects.

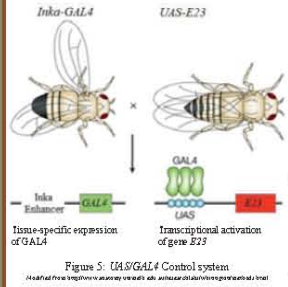

Figure 5: *UAS/GAL4* Control system

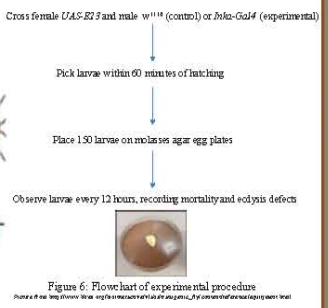

Figure 6: Flowchart of experimental procedure

The larvae were observed on molasses agar egg plates every 12 hours out to 84 hours from hatching. Their mortality was recorded by assay of remaining living larvae, and ecdysis defects were noted by presence of supernumerary mouth hooks and pharyngeal plates. As a control, the *UAS-E23* stock was also crossed to a w^{1118} stock, a commonly used unmodified control stock and the same analysis was performed.

Results and Conclusion

Shown below is a graph comparing the control (*UAS-E23/*w^{1118}) and experimental (*UAS-E23/Inka-GAL4*) larvae (Figure 7). The graph, from data following 150 larvae each, compares mortality by measuring living animals. On the right is a comparison of the third instar larvae (Figure 8). Ecdysis defects are indicated by a second set of mouth hooks, cuticular structures that are created during molting. If the larva cannot undergo ecdysis, the mouth hooks remain attached rather than falling off with the old cuticle.

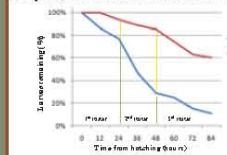

Figure 7: Mortality comparison of *UAS-E23/*w^{1118} vs. *UAS-E23/Inka-GAL4*

Figure 8: *Wildtype/UAS-E23* vs. *UAS-E23/Inka-GAL4*

There is clearly a difference in both ecdysis defect and resulting growth. The molting defects in the experimental are clear, and indicate that a new cuticle was formed, but was not removed between instar stages. Data are consistent with E23 production antagonizing 20E in Inka cells, perturbing ecdysis. At the stages of ecdysis, 24 hours to 48 hours (1st to 2nd and 2nd to 3rd instar), the mortality gap increases to only 29% remaining for the experimental versus 85% remaining for the control.

Future Work

Along with observing the effects of Inka cells on the molting and ecdysis process, I will also be studying the effect of 20E suppression throughout the larval epidermis. The epidermal cells, which secrete the cuticular structures, can be controlled through another recently acquired another stock, *A58-GAL4*. We hypothesize an overproduction of E23 in these cells would halt molting; preliminary data with this driver results in an extension of the larval L1 stage and lethality.

To approach the question of which 20E receptor is used for molting, I have access to transgenic fly stocks that have silencing abilities for the EcR/USP receptors – well characterized 20E receptors – and can cross these to the *Inka-GAL4* stock [6]. If this action prevents molting it will provide good evidence that the molecule tested is functioning as a receptor for 20E, and show again the importance of 20E hormone in *Drosophila* development.

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Title: Effects on Cell Migration with the Inhibition of GSK-3 kinase in Glioblastoma and Fibrosarcoma cell lines

Abstract: Cell migration is a vital component of metastasis. In order for a cell to migrate, during metastasis or under other conditions, cell-cell contacts must be broken, and cell-substrate contacts (i.e. focal adhesions) must be formed to give the moving cell traction. An enzyme called GSK-3 kinase regulates the function of a bifunctional protein known as β-catenin. In the cytoplasm, β-catenin serves as a key structural component of adherens junctions, the points of contact between neighboring cells. These cell-cell contacts contain transmembrane cadherins that link to dynamic membrane and cytoskeletal substructures, of which β-catenin is a key constituent. Under normal conditions, GSK-3 kinase phosphorylates β-catenin, which then undergoes ubiquitin-dependent proteolysis. However, using a GSK-3 kinase inhibitor, such as BeSO4, would allow for the stabilization of β-catenin and suggest an increase in its adhesive behavior. Thus, we hypothesized that cell migration is suppressed by GSK-3 inhibitors.
This experiment investigated a glioblastoma tumor cell line called A172 and a fibrosarcoma cell line called HT-1080. To test our cell migration hypothesis, we used an In-vitro FluoroBlok Tumor Cell Migration Assay and the Wound-Healing Assay. For the cell migration experiment to work, we must initially make sure that each cell line is sensitive to BeSO4 (by carefully observing patterns in their growth), as many cell lines are not sensitive and do not respond to BeSO4. After confirming the-sensitivity of A172 and HT-1080 to BeSO4, the use of the In-vitro FluoroBlok Tumor Cell Migration Assay and the Wound-Healing Assay demonstrated that both cell lines showed a dramatic decrease in cell migration when treated with BeSO4. These results provide evidence that tumorigenic cells (A172 and HT-1080) treated with BeSO4, causes stabilization of β-catenin and allows for stronger cell-cell contacts, and therefore decreased cell migration.

Why are you doing this project? I am doing this project because I am fascinated by the intricate systems involved in cancer cell progression, such as the ability of cancerous cells to invade and migrate during metastasis.
What problem are you trying to solve? We are trying to see if treating tumorigenic cells with BeSO4 will decrease cell migration, which may potentially aid in developing better treatments in the future.
What tools or equipment are you using? We are using various tools and equipments for this experiments. Some of these equipments include: Tissue Culture Hood, Pipettes, different types of microscopes, cell-plate readers to count cells, Cell migration assay kits, and Wound healing assays which include items such as petri dishes and pipette tips.
Why is your project worth researching? Cancer is a very deadly disease. One of the big problems of cancer is metastasis; and if we can find a way to prevent invasion of tumorigenic cells from one region to another region, then we can better treat those suffering from this condition.
What relevance will it have on the community, society, and in your research field? It will have a very profound impact on society as a whole. Millions of people around the globe suffer from cancer, and finding better treatments would not only help those suffering from cancer, but it will also aid in future studies with hopes of curing cancer.
What did you find? We found out that treating the glioblastoma cell line and the fibrosarcoma cell line with BeSO4 significantly decreased cell migration, a key part of metastasis.
What is the future of your research project? The future for this research project is very promising. We now have evidence that inhibition of GSK-3kinase, by treatment with BeSO4 ,(which causes stability of β-catenin) leads to a dramatic decrease in cell migration. Therefore, we can begin to explore the possible mechanistic connections between GSK-3 kinase/β-catenin signaling and cell migration.

Cell Migration Dynamics After Alteration of Cell-Cell Contacts in Fibrosarcoma and Glioblastoma Cell Lines

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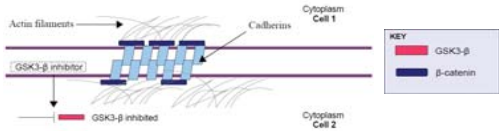
Abstract

Cell migration is a vital component of metastasis. In this study, our intent was to study cell migration by alteration of the Wnt/GSK-3 Pathway. Since $BeSO_4$ is a known GSK-3 kinase inhibitor, we hypothesized that this agent would cause cell migration to decrease as a result of β-catenin stabilization. Two human cell lines, HT-1080 (fibrosarcoma) and A172 (glioblastoma), were used to observe migration levels in the presence and absence of $BeSO_4$. Our results show that cell migration is diminished for cells that were pre-treated with $BeSO_4$, in comparison to the untreated (control) cells.

Background

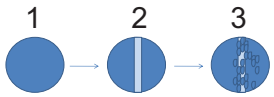
In order for a cell to migrate, during metastasis or under other conditions, cell-cell contacts must be broken, and cell-substrate contacts (i.e. focal adhesions) must be formed to give the moving cell traction. An enzyme known as GSK-3 kinase regulates the function of a bi-functional protein called β-catenin. In the cytoplasm, β-catenin serves as a key structural component of adherens junction, the points of contact between neighboring cells. These cell-cell contacts contain transmembrane cadherins that link to dynamic membrane and cytoskeletal substructures, of which β-catenin is a key constituent. Under normal conditions, GSK-3 kinase phosphorylates β-catenin, which then undergoes ubiquitin-dependent proteolysis. However, using a GSK-3 kinase inhibitor, such as $BeSO_4$, would allow for the stabilization of β-catenin, and thus potentially increase cell-cell adhesion and decrease cell migration.



Methods

- Coulter Counter
  - Used to count cells in order to assess effects on cell division.
  - $BeSO_4$ is known to affect cell division in many cell types, but its effect on HT-1080 cells has never been studied.
- In Vitro FluoroBlok Tumor Cell Migration Assay
  - $BeSO_4$ treated and untreated cells were allowed to migrate from the apical surface to the basal surface through the membrane via 3 µm pores. Cells that had migrated to the basal surface were stained with a fluorescent dye and mean fluorescence was measured to determine cell migration.
- Wound-Healing Assay
  - A pipette tip was used to make an initial wound (cell-free area), which was then monitored over time to observe the amount of cell migration.

1. Grow cells to preferred confluence in +/- $BeSO_4$ media.
2. Use a pipette tip to scrape away cells in a line across the dish.
3. Observe cell migration back into the cleared area.



Results

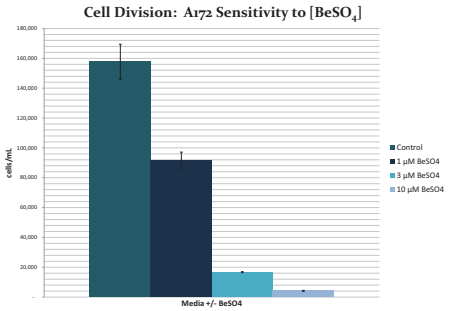


Figure 1a. *Low micromolar $BeSO_4$ inhibits cell proliferation in glioblastoma cells. A172 cells were dosed with $BeSO_4$ on Day 0 and counted after 9 days with 1:4 splits every 3 days.*

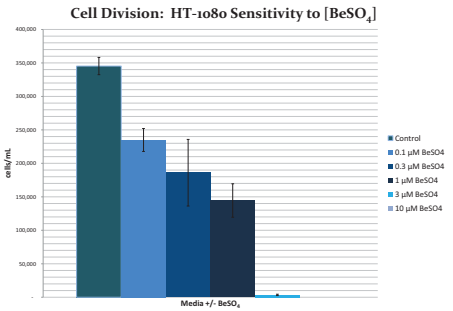


Figure 1b. *Low micromolar $BeSO_4$ inhibits cell proliferation in fibrosarcoma cells. HT-1080 cells were dosed with $BeSO_4$ on Day 0 and counted after 8 days with 1:8 splits every 2 days.*

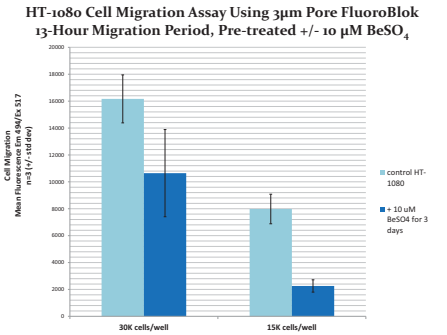


Figure 2a. *Untreated and 10µM $BeSO_4$ (3-days pre-treated) HT-1080 cells were allowed to pass through 3µm pores within the membrane via cell migration. Cells that had migrated to the basal side of the membrane were stained with a fluorescent dye, and the mean fluorescence was measured to quantify cell migration. Cells were seeded into the migration assay chambers at higher density (30,000 cells/well) and lower density (15,000 cells/well) to determine the optimal conditions for the experiment.*

Figure 3a. *HT-1080 cells were pretreated with +/- $BeSO_4$ for 36 hours. Plates were approximately 80-85% confluent. A wound was made at t=0 (using a pipette tip) and cell migration was observed at t=0, t=3, t=6, and t=9 hr.*

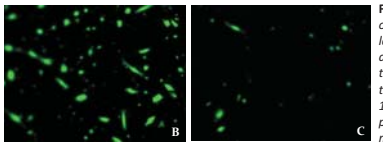


Figure 2 (b & c). *Microscopy of basal side of membrane, showing fluorescently-labeled cells that had migrated from the apical side. B) Untreated HT-1080 cells that had passed through the 3µm pores in the membrane. C) Pre-treated (3 days) 10µM $BeSO_4$ HT-1080 cells that had passed through the 3µm pores in the membrane.*

Figure 3b. *A172 cells were pretreated with +/- $BeSO_4$ for 74 hours. Plates were approximately 95-100% confluent. A wound was made at t=0 (using a pipette tip) and cell migration was observed at t=0, t=13, and t=23 hr.*

Conclusion

After confirming the sensitivity of A172 and HT-1080 to $BeSO_4$ in the Proliferation Assay, the FluoroBlok Tumor Cell Migration Assay and the Wound-Healing Assay demonstrated that both cell lines show a decrease in cell migration when treated with $BeSO_4$. We hypothesize that $BeSO_4$ treatment causes stabilization of β-catenin, which allows for stronger cell-cell contacts, and therefore decreased cell migration. Further studies are planned to test this hypothesis.

Acknowledgements

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Title: Combination of VirB binding site mutations to evaluate collective impact on icsP promoter activity in Shigella flexneri

Abstract: Past research studying Shigella flexneri has examined the regulation of the promoter region of icsP, a virulence gene. Conventionally, the binding of transcription factors occurs within
200 base pairs of the transcription start site of a gene. The Wing Lab has demonstrated that two binding sites of the transcription factor VirB are required for the regulation of the icsP promoter. However, these two sites are located more than 1 kb upstream of the transcription start site of the icsP promoter. Seven additional binding sites are located between the two distal sites and the transcription start site. The impact of the two distal sites on regulation leads us to wonder as to the impact of the closer sites on the regulation of promoter activity. Past research in the Wing Lab has shown no decrease in promoter activity when each of the seven binding sites is replaced with a mutated version of itself. I hypothesize that my experiment will reveal a collective impact upon icsP promoter activity not demonstrated in tests of individual mutations. We can witness this effect by combining all seven mutations in one plasmid construct through an organized experimental plan. To do this, I utilized restriction enzymes to cut previously prepared plasmids carrying each of the VirB binding site mutations. I combined one binding site mutation with another through ligation, and I transformed cells of DH10B E. coli to verify that a new plasmid was created. By repeating this process, I will combine all the mutations into one construct. I will then measure the activity of the new construct and the original plasmid constructs using beta-galactosidase assays. The overall aim of this project is to further characterize the relationship between binding sites of VirB and expression of icsP.

Why are you doing this project? I'd like to learn more about the research being done on campus, and I find the subject matter truly interesting and important. In terms of scientific reasoning, my hope is that, through this project, we will develop a better understanding of the workings of Shigella through exploring how the binding sites of the VirB transcription factor affect promoter activity.

What problem are you trying to solve? I am trying to assess the impact of VirB binding sites on the regulation of promoter activity of icsP, given past data that show two more distal VirB binding sites do have an impact upon this activity.

What tools or equipment are you using? PCR, DNA ligations, transformations and the application of restriction enzymes. I will utilize everything from a balance to a spectrophotometer to achieve my goal of creating a single construct containing the seven VirB binding site mutations.

Why is your project worth researching? It will further the information available on the relationship between binding sites of the transcription factor VirB and expression of the virulence gene icsP.

What relevance will it have on the community, society, and in your research field? Shigella flexneri is an invasive bacterial pathogen that causes shigellosis, an illness triggering severe dysentery. According to the World Health Organization, "More recent estimates fix the Shigella disease burden at 90 million episodes and 108,000 deaths per year." An investigation into the impact of the VirB binding sites is part of a larger effort to examine the promoter activity and workings of icsP. Because icsP can cleave the icsA protein from the surface of Shigella, it can affect the actin-based motility of Shigella. This will be important in helping to limit the intercellular spread of Shigella and, therefore, in reducing the impact on those affected by the pathogen.

What did you find? The results are pending.

What is the future of your research project? This will depend upon whether my results show that the VirB binding sites between the transcription start site and the two distal VirB binding sites have an impact upon the regulation of icsP promoter activity. If this is the case, then more work would have to be done to assess the level of impact.

Combination of VirB binding site mutations to evaluate collective impact on *icsP* promoter activity in *Shigella flexneri*

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INTRODUCTION

Shigella flexneri is a gram-negative, invasive bacterial pathogen that afflicts the human colonic epithelium, causing shigellosis, an illness triggering severe dysentery. The World Health Organization cites the disease burden of shigellosis near 90 million episodes and 108,000 deaths per year [1].

The motility and spread of *Shigella* is modulated by *icsP*, a virulence gene. The transcription factor VirB positively regulates many virulence genes encoded by the *Shigella* virulence plasmid. Two distal binding sites of VirB have been shown to regulate the promoter activity of *icsP*, despite their location of more than 1 kb upstream of the transcription start site [2]. Five VirB binding sites are located between these two sites and the transcription start site, and two are located in close proximity downstream of the transcription start site [2].

Investigation into the impact of the VirB binding sites is part of a larger effort to understand the workings of VirB, which is the major switch that controls virulence gene expression in *Shigella*.

BACKGROUND

-Previous truncation analysis has shown that two distal binding sites of VirB more than 1 kb upstream play a role in regulating *icsP* promoter activity [2].
- Five other sites are located between the transcription start site and the promoter. Two are located downstream, within 30 base pairs of the start site [2].
- Individual mutations have been inserted in each binding site to measure promoter activity. No single mutated binding site showed great impact upon activity (unpublished work, see Fig. 1 & 2).

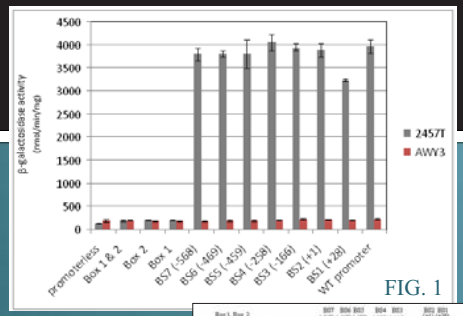

FIG. 1

FIG. 2

HYPOTHESIS

The combination of mutated VirB binding sites into a single plasmid construct will result in decreased activity of the *icsP* promoter.

OVERALL GOAL AND REASONING

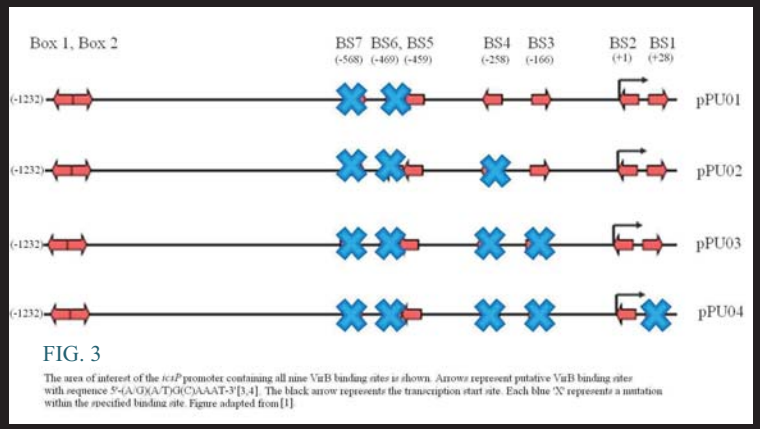

FIG. 3

The area of interest of the *icsP* promoter containing all nine VirB binding sites is shown. Arrows represent putative VirB binding sites with sequence 5'-(A/G)(A/T)G(C)AAAT-3'[3,4]. The black arrow represents the transcription start site. Each blue 'X' represents a mutation within the specified binding site. Figure adapted from [1].

- This project involves combining the individual mutations of VirB binding sites into a single construct (see Fig. 3 for plan).
- Decreased promoter activity would suggest that BS1 and the four VirB binding sites between the transcription start site and the two distal binding sites play a role in *icsP* promoter activity regulation.

MATERIALS AND METHODS

- Beginning with previously created plasmids carrying mutations, I used restriction digests, as seen in Fig. 4, to cut specific segments of the promoter carrying the desired VirB binding sites.
- I used blunt-end ligation techniques by setting my ligation at 16 degrees Celsius overnight. After dialyzing a ligation, I transferred it into viable cells of DH10B *E. coli* through bacterial transformation.
- After a diagnostic digest to confirm that my construct has been created, I cloned the segment carrying the desired binding sites into a pHJW20 reporter construct, which allows usage of a β-galactosidase assay to measure promoter activity through color produced.

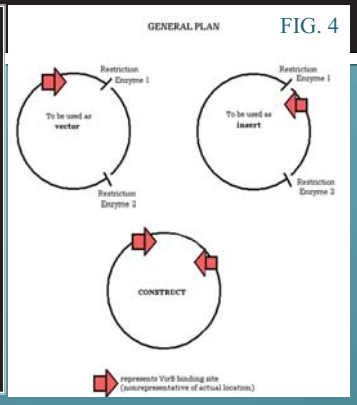

FIG. 4

RESULTS

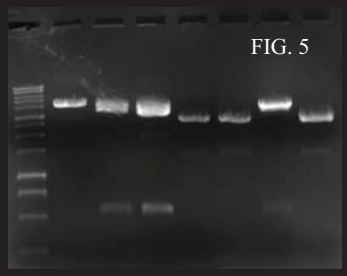

FIG. 5

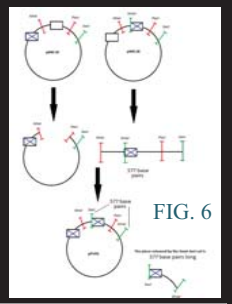

FIG. 6

Fig. 5. The agarose gel image to the left shows a diagnostic digest of pPU01 candidates 1-5 cut with *Sma*I and *Sac*I. Left to right: ladder, vector, insert, candidates 1-5. Candidates 1 and 4 display the expected pattern.
Fig. 6. This scheme shows the construction of pPU01, illustrating why cutting with *Sma*I and *Sac*I would release a 579 base pair piece.

CONCLUSIONS AND CURRENT WORK

The diagnostic digest done on my first construct shows that the desired construct is present. Cutting with *Sma*I and *Sac*I reveals that pPU01 releases a piece approximately 579 base pairs long, the same size piece released by pMIC28, the desired insert. This construct is now being moved into pHJW20, a reporter plasmid that will allow me to test its effect upon promoter activity. Construction of my second construct, using the same process as seen in Fig. 4, is under way.

FUTURE DIRECTIONS

- Combination of VirB mutations will continue. My vector, pPU01, and insert, pJCD03, will form the basis for a construct including sites 4, 6 and 7.
- Sites 3 and 1 will be added individually to form pPU03-pPU04.
- Each combination of binding sites will be utilized as an insert to the reporter plasmid pHJW20 to measure promoter activity.

ACKNOWLEDGEMENTS

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Educational Institute of Project: UNLV
Department: School of Life Sciences
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Title: Transcription Associated Mutagenesis in Stressed B. subtilis cells: The Role of Transcription Associated Stem-loop Structures

Abstract: 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 as stationary phase mutagenesis. Much of what is known come from studies in eukaryotic and bacterial models. It has been proposed that in non-growing cells, the process of transcription plays an important role in mutagenesis. We test the hypothesis that DNA secondary structures, formed during transcription, promote mutagenesis. The transcription-generated structures are speculated to be prone to mutations by exposing regions of single stranded DNA to lesions. We 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 prototrophy in cells under conditions of starvation. Ultimately, these experiments will increase our understanding of how mutations occur in cells of all domains of life.

Why are you doing this project? The mechanism behind the generation of mutations is an important process to understand because not only could it provide insight for eukaryotic systems but also help us understand how antibiotic resistance and evolution occur in prokaryotes.

What problem are you trying to solve? I am trying to find out what specific aspect of transcription could be influencing those mutations. Transcription is part of the process of making proteins and stationary phase refers to cells that are in a non-growing phase.

What tools or equipment are you using? I am trying to find out what specific aspect of transcription could be influencing those mutations. Transcription is part of the process of making proteins and stationary phase refers to cells that are in a non-growing phase.

Why is your project worth researching? My project is worth researching because at this point my lab is unable to answer exactly how transcription generates mutations. My results should bring us closer to understanding the mechanism behind transcription associated mutagenesis.

What relevance will it have on the community, society, and in your research field? Mutations affect everyone in society since many eukaryotic diseases are due to mutations, and since every day antibiotics are becoming less and less effective. Any research that aims to understand the mechanisms that lead to mutations can provide results for others to build upon and potentially use to begin to solve the many problems mutations lead to.

What did you find? The results are still pending

What is the future of your research project? The future for my project includes doing a stationary phase assay with the strains I constructed to test whether stem loop structures have a role. Depending on the results of the stationary phase assay the project could continue in a number of different directions.

DNA secondary structures and their contribution to mutagenesis in *B. subtilis* stationary phase cells.

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Abstract

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 as stationary phase mutagenesis. Much of what is known come from studies in eukaryotic and bacterial models. It has been proposed that in non-growing cells, the process of transcription plays an important role in mutagenesis. We test the hypothesis that DNA secondary structures, formed during transcription, promote mutagenesis. The transcription-generated structures are speculated to be prone to mutations by exposing regions of single stranded DNA to lesions. We 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 prototrophy in cells under conditions of starvation. Ultimately, these experiments will increase our understanding of how mutations occur in cells of all domains of life.

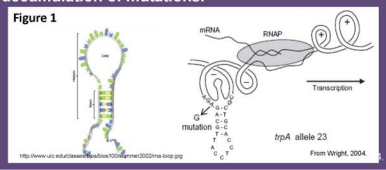
Background

- Stationary phase mutagenesis was first evidenced in the 1950s by Francis J. Ryan in a paper in Genetics.
- Later on, Cairns and coworkers revisited the concept of mutagenesis in conditions of carbon starvation and showed that cells under stress accumulated Lac$^+$ mutations (1990).
- Two pathways in the *E. coli* FC40 *lac* system has been proposed. One that generates point mutations and another that generates amplifications.
- Recent evidence in *Bacillus subtilis* suggests that aspects of transcription mediate the formation stationary phase mutations.
- Transcription and its transient changes in DNA topology have been linked to formation and stabilization of secondary structures such as hairpins and Z-DNA G4 structures in both the transcribed and non-transcribed strand. These structures are stabilized with increased transcription and increased negative supercoiling.
- Recent work has shown how G4 DNA, when located in the non-transcribed strand, can blockT7 RNAP and Mammalian RNAPII. This arrest has the potential to initiate a "gratuitous" TCR response that can lead to mutagenesis.
- Work in *E.coli* has also evidenced a role for stem loop structures or hairpins in mutagenesis in association with transcription and replication.
- The likelihood of forming SLS is sequence-dependent and may be estimated by calculating Gibbs free energy value, which suggests that transcription-associated mutations occur at hotspots in the genome.
- Here, we report the construction of *thiF* alleles that differ in their ability to form SLS. These alleles will be use to test whether stationary phase mutations are dependent on transcription and take place at hot spots.

Hypothesis

Transcription associated mutations in stationary phase are dependent on the formation of SLS. SLS stability, as measured by free energy of formation, influences the accumulation of mutations.

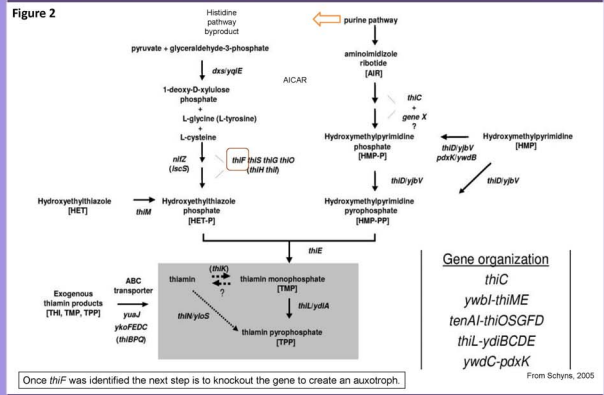
Figure 1



Methods

Find a marker gene that forms SLS in *Bacillus subtilis*

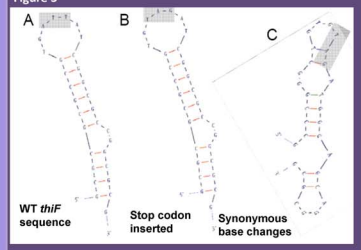
Figure 2



Once *thiF* was identified the next step is to knockout the gene to create an auxotroph.

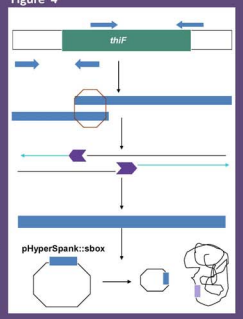
Construct alleles that differ in ability to form SLS using PCR

Figure 3



WT: Contains 30bp sequence that forms stable stem loop.
GCG-CGC-GCC-GTG-ATT ATC-GGC-GCC-GGC-GCG
Strain 1: STOP-
GCG-CGC-GCC-GTG-TGA ATC-GGC-GCC-GGC-GCG
Strain 2: STOP+UNSTABLE-
GC T CG A GC T GTG- TGA AT C GG A GC A GGC-GCG

Figure 4



Results

- Defective *thiF* alleles differing in their ability to form SLS have been constructed and transformed into WT strain (see figure 3).

Future Plans

- Knockout *thiF* gene eliminating stem loop sequence to prevent recombination.
- Conduct a stationary phase assay and score mutant reversion to thiamine prototrophy.
- Conduct stationary phase assay in the presence of subinhibitory concentrations of gyrase inhibitors.
- Conduct stationary phase assay without transcription strand specific repair pathways such as knocking out *mfd* gene.
- Sequence analysis of Thi$^+$ reversions.

Acknowledgements

I would like to thank the Nevada INBRE program for funding this work. A special thanks to everyone in the Robleto lab for sharing their knowledge with me. This project was supported by the following grants MCB0843606, and 2 P20 RR016463 Nevada INBRE. I would also like to acknowledge Katherine Ona for her involvement in the research project.

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Title:

Abstract: A high pressure study is being conducted on 1,1-diamino-2,2-dinitroethene (DADNE or Fox-7) using micro Raman spectroscopy. A Merrill-Basset diamond anvil cell is being used to produce the high pressure for the experiment. Fox-7 will be studied under both non-hydrostatic and quisihydrostatic conditions to study if there is any differences in the phase changes seen. Liquid nitrogen is used as the quisihydrostatic medium for its ability to maintain hydrostatic conditions up to 10 GPa. The micro Raman spectrum is collected every few GPa from 0 to 28 GPa for both experiments. During the non-hydrostatic study a phase change is suspect to happen around 18 GPa. This phase change is believed to be due to a change from a chair conformation of the molecule to a planar conformation and due to a change in the hydrogen bonding between the molecules in the crystal. The quisihydrostatic experiment is suspected to show the same results as non-hydrostatic experiment with the usual phase change shift to higher pressures. The information gained from these experiments will lead to a better understanding of these energetic materials. This will hopefully allow other people to create new materials that will not only be safer to use, but also new effective. It could also lead to current materials being able to be used in a more effective and safer way as well.

Why are you doing this project? The goal of this experiment is to better understand the reasons for Fox-7's insensitivity and high performance. It is very similar to other explosives in composition but different in structure. This different structure is believed to be the reason for Fox-7's unique characteristics.
What problem are you trying to solve? Using Raman spectroscopy along with high pressure techniques we hope to better understand this molecule and how it handles extreme conditions.
What tools or equipment are you using? A diamond anvil cell (DAC) was loaded with Fox-7 and no medium in a stainless steel gasket. The DAC used was a Merril-Basset design and had a pair of 400 micron diamonds. The gasket was preindented to about 60 microns in thickness and then a 145 micron hole was drilled for the sample. The sample was loaded with ruby sphere, which would be used to measure pressure. Once the DAC was loaded and a spectrum of the ruby was taken to find the initial pressure. The Raman spectrum was then collected on the Fox-7 at 537, 571 and 615 nm, 0-1400, 1400-2500 and 2700-3700 cm-1 Raman Shifts respectively. The pressure was then increased and the ruby and sample were scanned again and this process continued until a pressure of 20.34 GPa was reached with approximately 1 GPa steps. The DAC was then decompressed to ambient pressure and a spectrum was taken of the sample again. A spectrum of a new sample of Fox-7 was then taken to measure the ambient spectrum of the sample prior to compression. The data was then analyzed and the peaks were mapped against pressure and the spectrums were stacked according to pressure.
Why is your project worth researching? 1,1-diamino-2,2-dinitroethene (Fox-7) is an insensitive secondary explosive that was developed recently by Sweden. Thus there is very little known about it.
What relevance will it have on the community, society, and in your research field? This explosive, or energetic material, has a higher insensitivity and performance than RDX, making it a more ideal explosive
What did you find? The phase transitions are believe to be in largely due to a change in hydrogen bonding and possibly a change from the zig zag crystal pattern to a planar crystal pattern.
What is the future of your research project? To further study these phenomena an analysis of each peak should be done to determine what vibrational mode it corresponds to.

High Pressure Study of 1,1-diamino-2,2-dinitroethene with Raman Spectroscopy

Jack Brangham, Michael Pravica, Martin Galley

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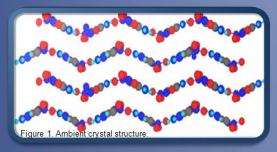


Figure 1. Ambient crystal structure.

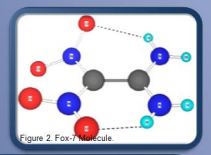
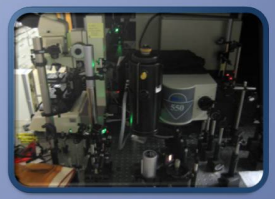
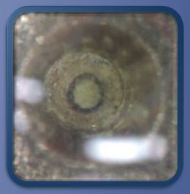


Figure 2. Fox-7 Molecule.





Abstract

The goal of this experiment is to better understand the reasons for Fox-7's insensitivity and high performance. It is very similar to other explosives in composition but different in structure. This different structure is believed to be the reason for Fox-7's unique characteristics. Using Raman spectroscopy along with high pressure techniques we hope to better understand this molecule and how it handles extreme conditions.

Introduction

1,1-diamino-2,2-dinitroethene (Fox-7) is an insensitive secondary explosive that was developed recently by Sweden. Thus there is very little known about it. The material is known to form a zig zag crystalline pattern as seen in Figure 1. The molecule itself consists of two carbon atoms connected by a double bond, one carbon has two amino groups and the other has two nitro groups. The structure of the molecule is shown in Figure 2. The molecule is also known to have hydrogen bonding[1].

This explosive, or energetic material, has a higher insensitivity and performance than RDX, making it a more ideal explosive. A higher insensitivity means that it takes a higher energy to start a reaction and thus it is less likely to be detonated unintentionally, while it maintains the desired performance when detonated[1].

Procedure

A diamond anvil cell (DAC) was loaded with Fox-7 and no medium in a stainless steel gasket. The DAC used was a Merril-Basset design and had a pair of 400 micron diamonds. The gasket was preindented to about 60 microns in thickness and then a 145 micron hole was drilled for the sample. The sample was loaded with ruby sphere, which would be used to measure pressure. Once the DAC was loaded and a spectrum of the ruby was taken to find the initial pressure. The Raman spectrum was then collected on the Fox-7 at 537, 571 and 615 nm, 0-1400, 1400-2500 and 2700-3700 cm^{-1} Raman Shifts respectively. The pressure was then increased and the ruby and sample were scanned again and this process continued until a pressure of 20.34 GPa was reached with approximately 1 GPa steps. The DAC was then decompressed to ambient pressure and a spectrum was taken of the sample again. A spectrum of a new sample of Fox-7 was then taken to measure the ambient spectrum of the sample prior to compression. The data was then analyzed and the peaks were mapped against pressure and the spectrums were stacked according to pressure.

Conclusion

Figures 4, 5 and 6 show strong evidence of a phase transition around 3 GPa by the appearance and disappearance of peaks. Upon further inspection Figures 3 and 7 also support this with a change in the shift of peaks. Figures 3, 4 and 8 show evidence of a phase transition around 5 GPa with the strongest evidence being the appearance of a peak in Figure 4. Figures 6 and 7 show evidence of another phase transitions around 9-10 GPA. The change in the shift of the lowest peak in Figure 6 is the strongest evidence of this. The phase transitions are believe to be in largely due to a change in hydrogen bonding and possibly a change from the zig zag crystal pattern to a planar crystal pattern.

To further study these phenomena an analysis of each peak should be done to determine what vibrational mode it corresponds to. From there you could see which vibrational modes are involved in each phase change to help understand what is happening on the molecular level during the transitions.

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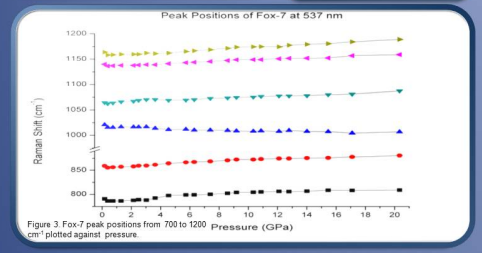

Figure 3. Fox-7 peak positions from 700 to 1200 cm^{-1} plotted against pressure.

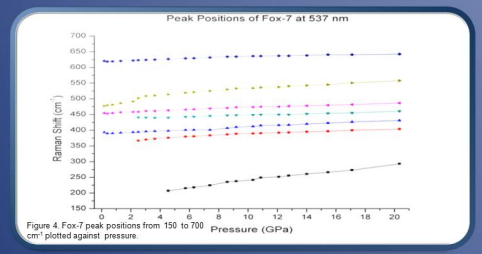

Figure 4. Fox-7 peak positions from 150 to 700 cm^{-1} plotted against pressure.

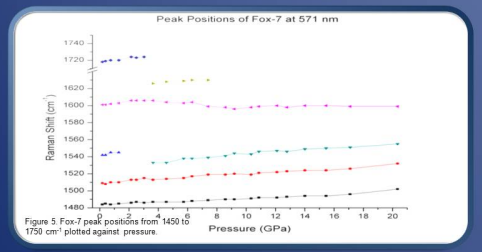

Figure 5. Fox-7 peak positions from 1450 to 1750 cm^{-1} plotted against pressure.

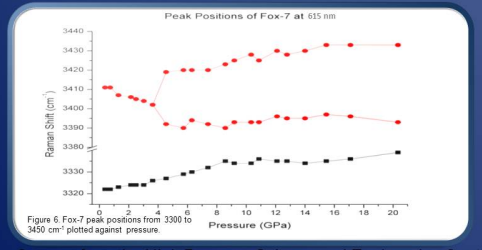

Figure 6. Fox-7 peak positions from 3300 to 3450 cm^{-1} plotted against pressure.

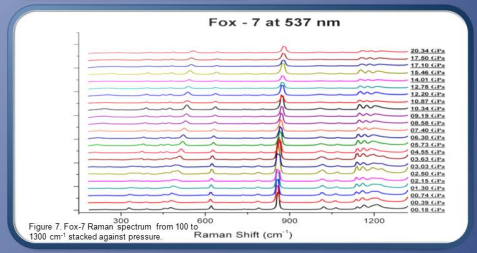

Figure 7. Fox-7 Raman spectrum from 100 to 1300 cm^{-1} stacked against pressure.

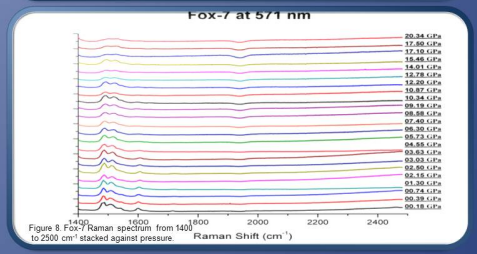

Figure 8. Fox-7 Raman spectrum from 1400 to 2500 cm^{-1} stacked against pressure.

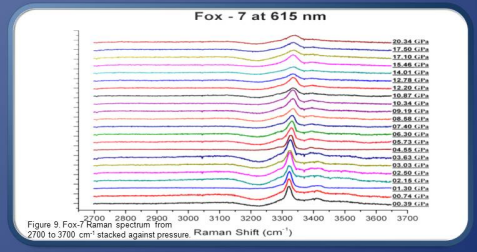

Figure 9. Fox-7 Raman spectrum from 2700 to 3700 cm^{-1} stacked against pressure.

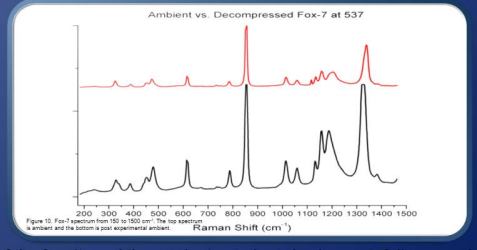

Figure 10. Fox-7 spectrum from 150 to 1500 cm^{-1}. The top spectrum is ambient and the bottom is post experimental ambient.

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Title: Pressure Induced Structural Transitions in BaCo3

Abstract: The crystal structure of witherite BaCo3 was studied using powder X-Ray diffraction through a Merrill-Bassett diamond anvil cell to high pressures up to 20 GPa using synchrotron x-rays at Argonne National Laboratory. BaCo3 crystallizes in the orthorhombic structure with space group Pmcn and the lattice parameters are found to be a = 5.3017, b = 8.90274, and c = 6.42761 Å. Between 4 and 8 GPa a pressure induced first-order phase transition is observed to a hexagonal structure with space group P-31c. After the phase transition, an axial anisotropic compressibility (as the b axis intersects with the a axis) is observed in our experiments. This experiment was conducted with an aim of understanding the phase transition behavior of BaCO3 under pressure and providing more detailed analysis of the high pressure phase by extending the pressure range previously investigated. Our experimental results show that the hexagonal phase is stable up to 20 GPa.

Why are you doing this project? I am doing this research to develop an understanding of how physics research is inspired, planned, conducted, and analyzed. In particular, I am doing research on BaCO3 to learn how pressure affects crystalline properties
What problem are you trying to solve? I am investigating a phase change that occurs at around 8GPa (Giga-pascals) in Barium Carbonate (BaCO3) in order to find an equation of state and a bulk modulus
What tools or equipment are you using? Through this research I am gaining knowledge of the advanced technologies of X-Ray diffraction using X-Rays from the synchrotron at Argonne National Laboratories, high pressure diamond anvil cells, and the analysis programs Fit 2D, Jade, Igor, Origin, and EOS Fit.
Why is your project worth researching? Barium Carbonate is the chief source of barium salts. It is used in the preparation of rat poison and in the production of glass, porcelain, and ceramics. Understanding how this material reacts under pressure can help further deepen our awareness of the application of this compound.
What relevance will it have on the community, society, and in your research field? The potential commercial and artistic applications of Barium Carbonate could have economical impact as well as developmental impact on companies and individuals who produce and use the materials listed above. For the scientific community it will deepen an understanding of Barium Carbonate in particular as well as how high pressure can affect crystalline properties.
What did you find? I found that BaCO3 changes from an orthorhombic structure to a hexagonal structure starting at as little as 4.31 GPa. I also found the equation of state for BaCO3.
What is the future of your research project? I plan to write a paper summarizing the research conducted and results found. Additionally, further scientific analysis of my data collected as well as additions to the collected data may occur.

High Pressure Structural Studies on $BaCO_3$ up to 20 GPa

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BACKGROUND

Carbonate compounds are thought to make up a minor portion of the Earth's upper mantel. Shock heating of the surfaces of carbonate rocks, as in the instance of a meteor impact, has the potential to affect CO_2 concentrations in the Earth's atmosphere. The bulk-modulus of carbonate materials is directly proportional to the rate of devolatilization under these conditions [1]. The interest in Barium Carbonate ($BaCO_3$) specifically is motivated by its structural proximity to aragonite carbonates. Crystalline phase transitions occur in aragonite under extreme conditions that are difficult to maintain in a laboratory; $BaCO_3$ is isostructural with aragonite and therefore is expected to have similar phase transitions at conditions easier to simulate in a laboratory. Studies of Barium carbonate will not only lead to conclusions about witherite carbonates, but aragonite carbonates as well [1]. Another, use of Barium carbonate is in ceramic glazes [4]. Under extreme conditions (firing in a kiln) the structural transition of $BaCO_3$ provides changes necessary for interesting glazes. This experiment was conducted with an aim of understanding the phase transition behavior of $BaCO_3$ under pressure and providing more detailed analysis of the high pressure phase by extending the pressure range previously investigated [1,2,3].

EXPERIMENTAL

The crystal structure of witherite $BaCO_3$ was studied using powder X-Ray diffraction through a Merrill-Bassett diamond anvil cell to high pressures up to 20 GPa. The powder form was loaded with ruby in a Re gasket with a 150 μm hole and 4:1 methanol-ethanol pressure medium. X-rays used for the experiment were of incident wavelength 0.36793 Å and were generated by the synchrotron at Argonne National Laboratory at the ID-B station in sector 16. The pressure was measured at room temperature using an offline ruby system and was increased in intervals of approximately 2 GPa. XRD patterns were taken for an exposure time of 15-30 seconds. The patterns were integrated using Fit2D. The structural analysis was performed using JADE and EOS Fit.

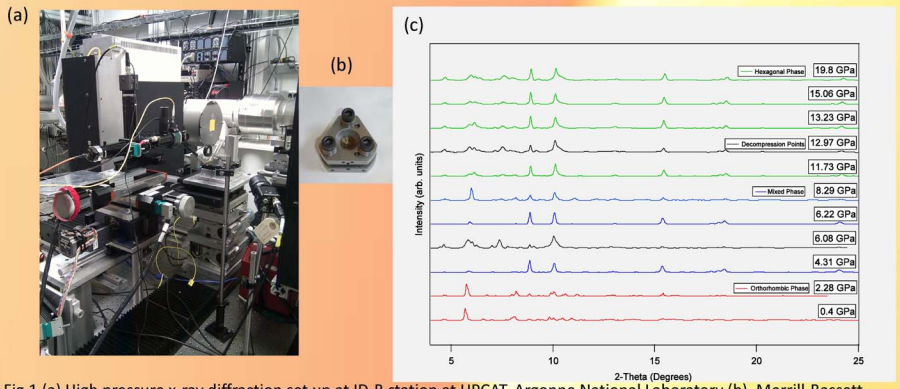

Fig.1 (a) High pressure x-ray diffraction set up at ID-B station at HPCAT, Argonne National Laboratory (b). Merrill-Bassett type high pressure diamond anvil cell. (c). Representative x-ray diffraction patterns at various pressures up to 19.8 GPa for $BaCO_3$.

RESULTS

$BaCo_3$ crystallizes in the orthorhombic structure with space group *Pmcn* and the lattice parameters are found to be a = 5.3017 Å, b = 8.90274 Å, and c = 6.42761 Å. These parameters compare well with literature [1,2]. Between 4 and 8 GPa a pressure induced first-order phase transition is observed to a hexagonal structure with space group *P-31c*. During this interval, a mixed phase is observed. After the phase transition, an axial anisotropic compressibility (as the *b* axis intersects with the *a* axis) is observed in our experiments. The variations of d-spacings as a function of pressure are shown in figure 2(a). From the cell parameter values found in JADE, the volume has been obtained for each pressure and is shown in figure 2(b) where the orthorhombic, mixed, and hexagonal phases are plotted. A third-order Birch-Murnaghan equation was used to fit the high-pressure phase of our P-V data.

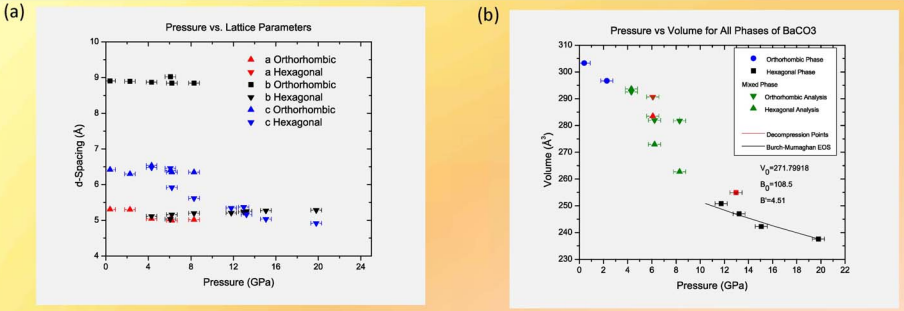

Fig.2 (a). Variation of lattice parameters of $BaCO_3$ phase as a function of pressure (b). P-V plot of all phases

CONCLUSIONS AND SUMMARY

The orthorhombic structure is stable up to 4.3 GPa. At 4.3 GPa diffraction peaks representing the hexagonal phase begin to appear compared to the previously found exposure range of approximately 8 GPa [1,2,3]. In our experiment, diffraction peaks from both the orthorhombic structure and the hexagonal structure co-exist until after 8 GPa. After 8 GPa, a complete phase transformation to the hexagonal phase with space group P-*31c* was observed. The phase change represented in the P-V data does not reflect a steep change in volume as pressure increases as found previously [1].

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Title: Neutron Diffraction of NaBD4: Phase Transition, Rietveld Structure Refinements, and Equation of State

Abstract: NaBH4 is a hydride with possible applications as a hydrogen storage material for future renewable energy technologies. It's dehydrogenation properties are enhanced with the mixture of particular catalysts through ball-milling techniques during which local pressures may exceed several GPa's. It is for this reason that understanding the behavior of pressure induced phase changes of its crystalline unit cell is an area of interest.

Why are you doing this project? NaBH4 is a hydride with possible applications as a hydrogen storage material for future renewable energy technologies.
What problem are you trying to solve? It's dehydrogenation properties are enhanced with the mixture of particular catalysts through ball-milling techniques during which local pressures may exceed several GPa's. It is for this reason that understanding the behavior of pressure induced phase changes of its crystalline unit cell is an area of interest.
What tools or equipment are you using?
This study makes use of neutron diffraction data collected from NaBD4 up to about 12 GPa. The sample was held in a Paris-Edinburgh cell in non-hydrostatic pressure conditions. The program Topaz was used to perform Rietveld Refinement on the data, and external data on the structure and atom positions of Na and B was attained in order to determine the atom positions of hydrogen (in this case its isotope deuterium) within the unit cell. Volume vs. Pressure data was also collected in order to attain an appropriate equation of state and from it determine the compound's bulk modulus.
Why is your project worth researching? NaBH4 is a hydride with possible applications as a hydrogen storage material for future renewable energy technologies.
What relevance will it have on the community, society, and in your research field?
What did you find? When NaBD4 is studied at high pressures up through 12.2 GPa, shifting of peaks to a higher 2θ in neutron diffraction patterns indicates a smaller d-spacing as the unit cell is compressed. The appearance of new peaks also indicates at least one phase transition to lower symmetry somewhere between 6.3 and 7.9 GPa, determined to be from a cubic Fm-3m space group to an orthorhombic Pnma one. The resulting pressure vs. volume data was used to fit a 3rd order Birch-Murnaghan equation of state to the cubic phase and a 2nd order one to the orthorhombic phase, with the determined values of B, B', and V0.
What is the future of your research project? To continue researching potential applications.

Neutron Diffraction of $NaBD_4$: Phase Transition, Rietveld Structure Refinements, and Equation of State

Guillermo Esparza, Patricia Kalita, Professor Andrew Cornelius
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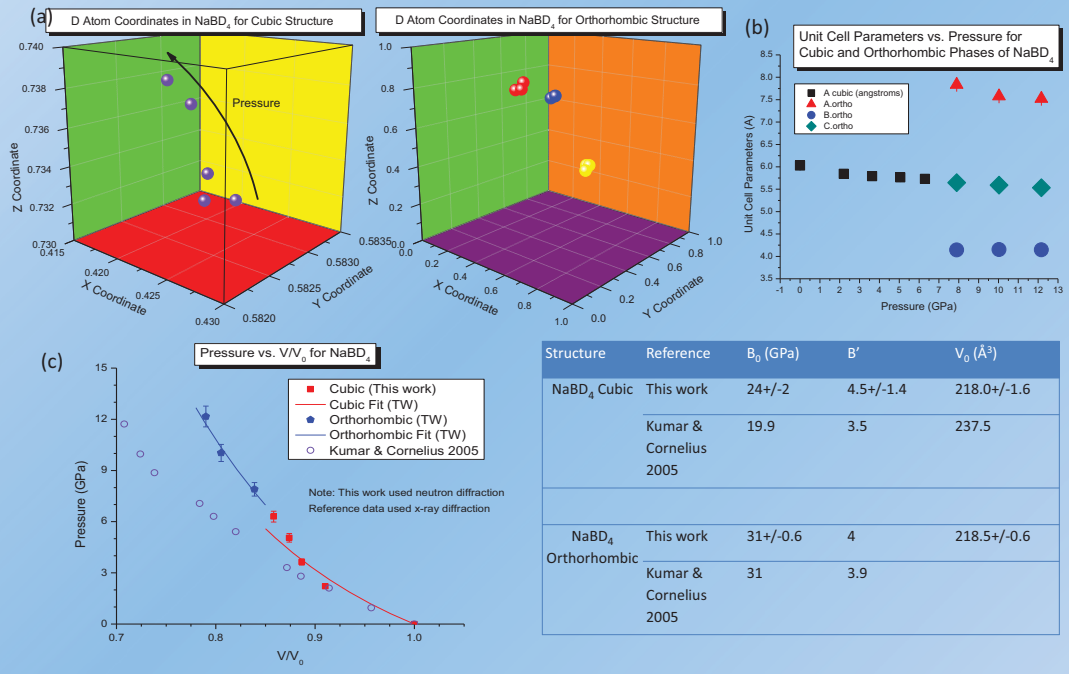
University of Nevada Las Vegas, NV 89154

BACKGROUND

$NaBH_4$ is a hydride with possible applications as a hydrogen storage material for future renewable energy technologies. It's dehydrogenation properties are enhanced with the mixture of particular catalysts through ball-milling techniques during which local pressures may exceed several GPa's. It is for this reason that understanding the behavior of pressure induced phase changes of its crystalline unit cell is an area of interest.

RESULTS

Analysis of the neutron diffraction data using Rietveld Refinement showed a phase transition occurring into the orthorhombic phase between 6.3 and 7.9 GPa. However, this appeared to occur from the cubic phase, as the intermediate tetragonal structure mentioned in other literature to appear between 6.3 and 8.9 GPa was not observed. Atom coordinates were determined for deuterium, demonstrating only a small amount of drift, and are showcased in Fig.1 (a) for the cubic and orthorhombic structures. The Pressure vs. Volume data is also shown

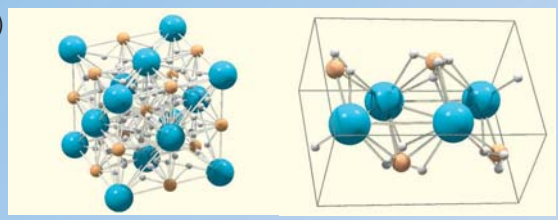


Structure	Reference	B_0 (GPa)	B'	V_0 (Å^3)
$NaBD_4$ Cubic	This work	24+/-2	4.5+/-1.4	218.0+/-1.6
	Kumar & Cornelius 2005	19.9	3.5	237.5
$NaBD_4$ Orthorhombic	This work	31+/-0.6	4	218.5+/-0.6
	Kumar & Cornelius 2005	31	3.9	

Fig.2 (a). 3D plots displaying the shift of deuterium atom positions within the unit cell. (b). A plot of unit cell parameters with a dependence on pressure for both observed phases of $NaBD_4$. (c) Plot of Pressure vs. volume data along with equations of state for both phases and a table displaying the determined values for B, B', and V_0 .

EXPERIMENTAL PROCEDURE & DATA ANALYSIS

This study makes use of neutron diffraction data collected from $NaBD_4$ up to about 12 GPa. The sample was held in a Paris-Edinburgh cell in non-hydrostatic pressure conditions. The program Topaz was used to perform Rietveld Refinement on the data, and external data on the structure and atom positions of Na and B was attained in order to determine the atom positions of hydrogen (in this case its isotope deuterium) within the unit cell. Volume vs. Pressure data was also collected in order to attain an appropriate equation of state and from it determine the compound's bulk modulus.



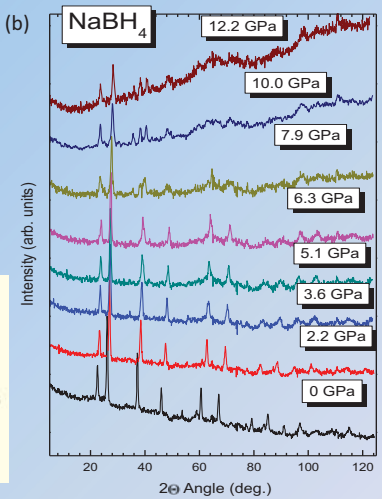


Fig.1 (a) Unit cell structure for the cubic phase (left) and orthorhombic phase (right). (b) Representative neutron diffraction patterns at various pressures up to 12.2 GPa for $NaBD_4$ in non-hydrostatic pressure conditions.

CONCLUSIONS AND SUMMARY

When $NaBD_4$ is studied at high pressures up through 12.2 GPa, shifting of peaks to a higher 2θ in neutron diffraction patterns indicates a smaller d-spacing as the unit cell is compressed. The appearance of new peaks also indicates at least one phase transition to lower symmetry somewhere between 6.3 and 7.9 GPa, determined to be from a cubic Fm-3m space group to an orthorhombic Pnma one. The resulting pressure vs. volume data was used to fit a 3rd order Birch-Murnaghan equation of state to the cubic phase and a 2nd order one to the orthorhombic phase, with the determined values of B, B', and V_0 displayed in Fig. 2 (c).

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Educational Institute of Project: UNLV
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Title: Correlation Between Grain Dislocation Density and Orientation for Naturally Deformed Mantle Xenolith from the Jagersfontein Mine

Abstract: Determining the reaction of poly-crystalline structures to induced stress is an extremely difficult problem in contemporary engineering and geology. The main challenge lies in the inhomogeneity of the grains inside of the poly-crystalline structures. To predict the response of a certain polycrystalline structure to a specific stress, you must resort to one of two views on grain interaction, an orientation or propagation based model. For every material there may be certain correlations between the prediction model used and the actual deformation that occurred. Our work centers around describing the correlation of these prediction models with a sample of naturally deformed mantle xenolith from the Jagersfontein Mine in South Africa. In order to correlate the models with the sample, we needed to calculate the orientation and dislocation density of the individual grains. To measure the dislocation density, the ratio of the area of the dislocations to the total area of the grain itself, the sample needed to go through a decoration process. The sample was heated to accelerate oxidization and highlight the sample's dislocations. This "decorating" process allows us to easily discern the dislocations on the surface of the sample using a Scanning Electron Microscope (SEM). Dislocation density can then be calculated by using an open-source image analysis software called ImageJ on the image of the grain. Additionally, the other calculation, the orientation of the grain, is measured by an Electron Backscatter Diffraction (EBSD) analysis of the sample. The EBSD is a process of firing electrons at the sample and reading the diffractions produced. These diffractions create a "picture" of Kikuchi Bands, simply the diffraction lines produced by the electrons, which can be analyzed by proprietary software resulting in a calculation of the orientation of each grain. We were then able to look for correlations between the dislocation density and the orientation of each grain and identify which model describes the deformation results most accurately.

Why are you doing this project? We are doing this project in order to better understand how rocks deform.
What problem are you trying to solve? We're trying to see if the deformation of crystals within the rock can be predicted by the orientation of the crystals in the rock.
What tools or equipment are you using? Scanning Electron Microscope, Polishing Pad, Diamond Slurries, and Electron Backscatter Device.
Why is your project worth researching? The rock deformation process is still an unsolved mystery, but with a better understanding of it, we could engineer materials better and understand mantle rheology more completely. Moreover, with better understanding of rock deformation, buildings could be safer and long-standing geological problems could be closer to being answered.
What relevance will it have on the community, society, and in your research field? Better understanding of rock deformation could revolutionize construction and the building process. Also, there is a possibility that one could better predict how earth materials react under pressure, like in an earthquake.
What did you find? We were able to look for correlations between the dislocation density and the orientation of each grain and identify which model describes the deformation results most accurately.
What is the future of your research project? In the future we will most likely perform the same experiment on different materials in order to draw general conclusions about deformation.

Correlation Between Grain Dislocation Density and Orientation for Naturally Deformed Mantle Xenolith from the Jagersfontein Mine

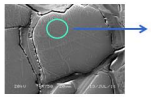
Quinton Guerrero (Monmouth College) and Quinlan Smith (California Lutheran University)
UNLV Physics REU Advisor: Pamela Burnley
Dept. of Physics, University of Nevada Las Vegas, Las Vegas NV 89119

Abstract

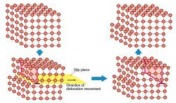
Determining the reaction of poly-crystalline structures to induced stress is an extremely difficult problem in contemporary engineering and geology. The main challenge lies in the inhomogeneity of the grains inside of the poly-crystalline structures. To predict the response of a certain polycrystalline structure to a specific stress, you must resort to one of two views on grain interaction, an orientation or propagation based model. For every material there may be certain correlations between the prediction model used and the actual deformation that occurred. Our work centers around describing the correlation of these prediction models with a sample of naturally deformed mantle xenolith from the Jagersfontein Mine in South Africa. In order to correlate the models with the sample, we needed to calculate the orientation and dislocation density of the individual grains. To measure the dislocation density, the ratio of the area of the dislocations to the total area of the grain itself, the sample needed to go through a decoration process. The sample was heated to accelerate oxidization and highlight the sample's dislocations. This "decorating" process allows us to easily discern the dislocations on the surface of the sample using a Scanning Electron Microscope (SEM). Dislocation density can then be calculated by using an open-source image analysis software called ImageJ on the image of the grain. Additionally, the other calculation, the orientation of the grain, is measured by an Electron Backscatter Diffraction (EBSD) analysis of the sample. The EBSD is a process of firing electrons at the sample and reading the diffractions produced. These diffractions create a "picture" of Kikuchi Bands, simply the diffraction lines produced by the electrons, which can be analyzed by proprietary software resulting in a calculation of the orientation of each grain. We were then able to look for correlations between the dislocation density and the orientation of each grain and identify which model describes the deformation results most accurately.

Introduction

Our sample rock is a piece of Kimberlite, a poly-crystalline rock which is usually created in the upper mantle of the earth's core. This rock, being poly-crystalline, is composed of many grains of crystals. In a deformation process, such as one that occurs naturally in the Earth's upper mantle, the grains of the poly-crystalline rock are subjected to pressures and forces that are great enough to displace and move atoms in the crystal structures of the grains. The results of these movements and displacements of atoms in the crystals are what we refer to as dislocations. There are two major types of dislocations, edge dislocations and screw dislocations. Both of these types can be highlighted through an oxidation process, which can occur because of iron naturally present in the rock. The iron can be oxidized by subjecting it to extremely high temperatures for an extended period of time. This oxidation process creates a visible contrast between the dislocations and the grain itself. This contrast is what we are examining in our SEM images.



Picture of Grain 13 with a few dislocations circled



This is a diagram showing how dislocations can occur on the atomic level

The orientation of a grain is the calculated orientation of the plane of the atoms of the crystalline structure. For poly-crystalline rocks every grain has its own orientation, and these orientations affect the way that a stress field is transferred through the rock. Different orientations cause different reactions to a specific stress field. Every orientation has a slip system which can be conducive to a stress field or non-conducive. If the orientation is conducive to a stress field, then the slip system will be aligned with the stress field and a dislocation can be formed and propagated through the crystal. If the orientation is non-conducive, then the slip system will not be aligned with the stress field and no dislocations can be formed. In essence, orientation is the basis for the formation of dislocations, and attempting to combine these two ideas experimentally is the basis of our research.

Experiment

- A thorough polishing of our sample was done, ranging from 400 grit down to ¼ micron.
- The sample was then decorated (oxidized) in a lab furnace at 900 degrees Fahrenheit for 45 minutes.
- Dislocation density data was collected by using the SEM to take photographs of the grains.
- Orientation data was collected by using the EBSD function of the SEM.
- EBSD data was taken by hand using optical and SEM images of the sample's surface to locate our SEM grains.
- Orientation data was converted into pole figures, allowing a visual display of orientation for all the grains.
- The SEM pictures were analyzed using the open source image analysis software, ImageJ, which allowed us to calculate both dislocation density area and total area of every grain.
- The dislocation density was divided into ranges, then colored, and finally mapped to the pole figures, allowing for a qualitative analysis of correlation.

SEM Results

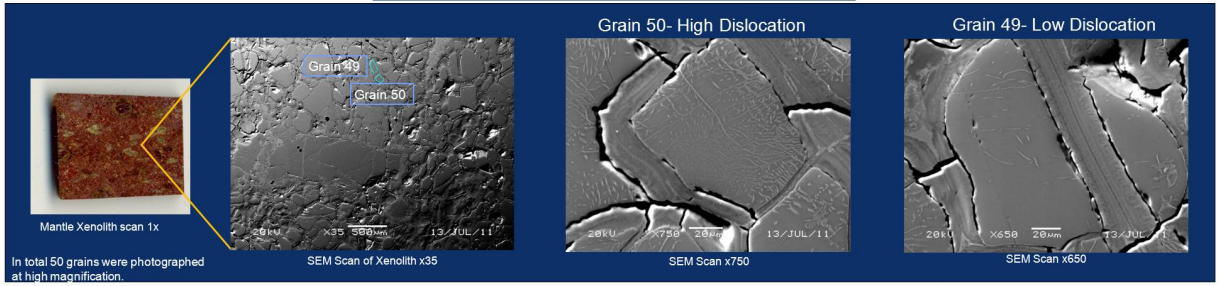
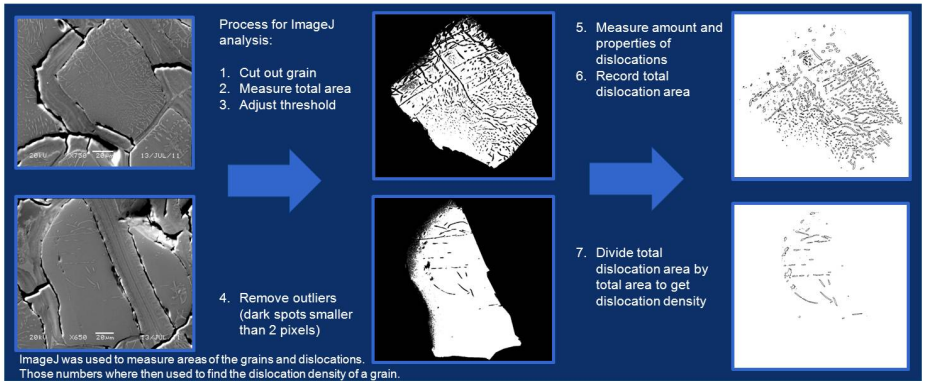


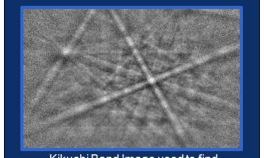
Image Analysis



EBSD Analysis

Electron Back Scatter Diffraction was used to determine the crystal orientation in all 50 grains.

EBSD Map 4 85x



Kikuchi Band Image used to find Euler Angles (crystal orientation)

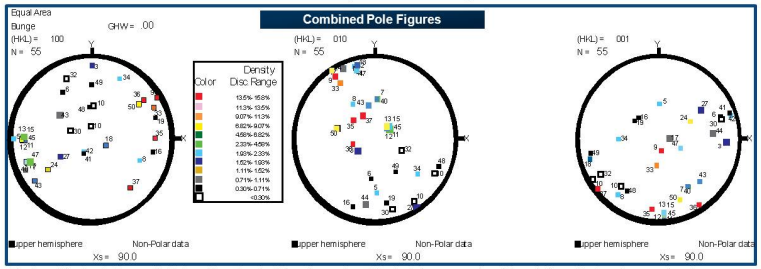
Test Pt.	Euler1(°)	Euler2(°)	Euler3(°)
11	101.8462	105.1199	36.14105
12	125.6644	117.2289	30.28106

Euler Angles produced by software processing of he Kikuchi Bands

Results

Grain	EBSD Map	Test Point	Euler1(°)	Euler2(°)	Euler3(°)	Dislocation Type	Total Area	Total Dis. area	Dis. Density
49	4	12	101.8462	105.1199	36.14105	Low	5.653	.044	0.78%
50	4	11	125.6644	117.2289	30.28106	High	5.49	0.517	9.4%

Data table for Grains 49 and 50 shown above. Data was compiled like this for all 50 grains.



Final combined pole figure with high and low density dislocations colored. Each circle represents a different plane. Each grain we analyzed Has three different Euler angles describing its orientation. As can be seen in the above diagram grains with similar dislocation densities seem to cluster around the same areas in the plane.

Conclusions

- Evaluation of the pole figures revealed a noticeable trend between orientation and dislocation density
- High dislocation dense grains clustered on the right side of the 100 direction and they clustered on the top of the 010 direction.
- Low dislocation dense grains clustered in the middle of the 100 direction and the bottom of the 010 direction.
- In the end its easy to see a trend between dislocation density and orientation, but, because of systematic error in the image analysis of ImageJ, definite conclusions cannot be drawn.

Acknowledgements

Support from the REU program of the National Science Foundation under grant DMR-1005247, and support from the High Pressure Science and Engineering Center (HPSEC), funded by the US Department of Energy, is gratefully acknowledged. Thanks would like to be extended to the SEM Lab and to the Rock Deformation Lab, for the use of their equipment, and a special thank you to our research advisor, Dr. Pamela Burnley.

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Educational Institute of Project: UNLV
Department: Physics and Astronomy
Research Site: BPB 142

Title: Crystal Structural Behavior of CoCu□O□ at High Temperatures

Abstract: High temperature structure of CoCu□O□
The spin ladder compounds have received much attention recently due to their relation to the high transition temperature superconductivity. Also the study of spin ladder compounds is of great interest to explore the specific characteristics that result in their behavior. The CoCu□O□ spin ladder crystal structure is similar to SrCu□O□, which is apparent composition for many high temperature superconductors. The effects of temperature on structural change are investigated for this system. High temperature x-ray diffraction patterns were collected up to 1000□C and the variation of lattice parameters as a function of temperature up to decomposition is studied.
The thermal stability of CoCu□O□, has been studied at elevated oxygen pressures beyond a high temperature of 1000□C [1]. Temperatures at which CoCu□O□ undergoes decomposition reactions were studied along with the products of the reactions. The study introduced here provides structural details and the linear coefficient of thermal expansion (CTE) before progressive decomposition.

Why are you doing this project? To explore specific characteristics that may result in the high transition temperature for superconductivity.
What problem are you trying to solve? Inefficiency in electronic transport (reducing waste energy in transport of electricity).
What tools or equipment are you using? D8 Advance X-Ray Diffractometer, high temperature stage.
Why is your project worth researching? Research in this area may lead to more efficiency in transport of electricity.
What relevance will it have on the community, society, and in your research field? An increase in efficient electronic transport will lead to less and even no waste energy. This can reduce costs of electricity and make electricity more accessible and economical to remote areas. Also high temperature superconductors may be used in improving speed in computer circuits and innovations in transportation.
What did you find? Linear coefficient of thermal expansion; the change of lattice parameters as a function of temperature. The decomposition of CoCu2O3 from 30C to 1000C.
What is the future of your research project? Further researching characteristics of the material to understand which characteristics may lead to high transition temperature superconductivity.

Crystal Structural Behavior of $CoCu_2O_3$ at High Temperatures

April Jeffries*, Ravhi Kumar, and Andrew Cornelius
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Abstract:

High temperature structure of $CoCu_2O_3$

The spin ladder compounds have received much attention recently due to their relation to the high transition temperature superconductivity. Also the study of spin ladder compounds is of great interest to explore the specific characteristics that result in their behavior. The $CoCu_2O_3$ spin ladder crystal structure is similar to $SrCu_2O_3$, which is apparent composition for many high temperature superconductors. The effects of temperature on structural change are investigated for this system. High temperature x-ray diffraction patterns were collected up to 1000°C and the variation of lattice parameters as a function of temperature up to decomposition is studied.

The thermal stability of $CoCu_2O_3$, has been studied at elevated oxygen pressures beyond a high temperature of 1000°C [1]. Temperatures at which $CoCu_2O_3$ undergoes decomposition reactions were studied along with the products of the reactions. The study introduced here provides structural details and the linear coefficient of thermal expansion (CTE) before progressive decomposition.

Experimental Details:

Sample Preparation:

Powder samples of $(Ca_{1-x}Co_x)Cu_2O_3$ (x=0.05) were prepared by the solid state reaction method as described elsewhere [2]. The phase purity of the sample was verified by powder XRD measurements on polycrystalline samples and found to be in single phase. The chemical compositions of the synthesized samples were determined [2].

High Temperature X-Ray Diffraction:

Figure 1.
D8 Advance X-Ray Diffractometer

A circular corundum sample stage was loaded with fine powdered $CoCu_2O_3$ and loaded into the vacuumed high temperature stage in the Bruker D8 Advance X-Ray Diffractometer (Figure 1). Sample was heated at a rate of 0.5°C/sec from 30°C to 400°C. XRD data was collected at temperatures of 30 ° C, 100 ° C, 200 ° C, 300 ° C, and 400 ° C, then cooled at a rate of 1 °C/sec back to 30 °C for a final collection of data. Another run from 30 °C to 1000 °C was conducted to track the decomposition of the sample. XRD data was collected starting at 30 °C, and every 200 °C up to 1000 °C, and back to 30 °C. TOPAS was used to analyze the x-ray diffraction patterns and the track percent compositions of the sample at various temperatures. Origin8 was used to analyze the data and obtain the linear coefficient of thermal expansion.

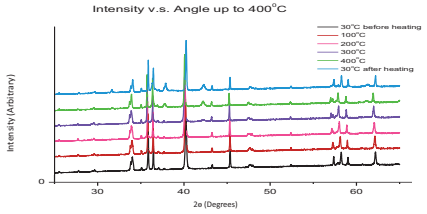

Figure 2.
Waterfall plot of XRD data, heating from 30° to 400°C, and cooling back to 30°.

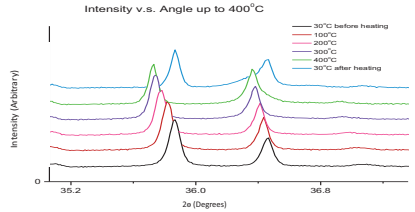

Figure 3.
Zoom of Figure 2 to show a shift to lower angle as temperature increases. A decrease in angle corresponds to an expansion of the cell.

A coefficient of thermal expansion (CTE)is indicative of the amount a structure expands or contracts in response to a temperature change. Linear CTE, α, is determined by the following equation [3]:

$$\alpha = \frac{1}{L_o}\frac{DL}{DT}$$

Where L_o is the initial length, and DL/DT is the slope of the tangent to the length vs. temperature line. Linear CTE has units of 1/ °C.

Figure 4a. Lattice Parameter "a" as a Function of Temperature

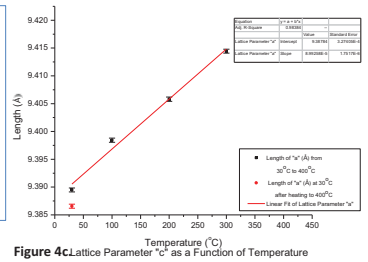

Figure 4b. Lattice Parameter "b" as a Function of Temperature

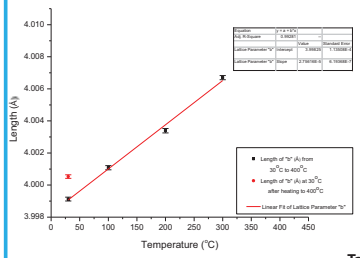

Figure 4c. Lattice Parameter "c" as a Function of Temperature

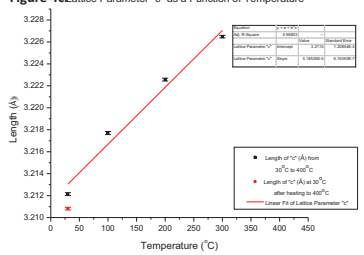

Table 1

Lattice Parameter	1/L_o (1/Å)		DL/DT (Å / °C)		Linear CTE, α (1/°C)	
A	9.38949±	0.00035	8.99E-05±	1.75E-06	8.44E-04±	1.64E-05
B	3.99913±	0.00012	2.76E-05±	6.19E-07	1.10E-04±	2.48E-06
c	3.21214±	0.00013	5.19E-05±	6.16E-07	1.67E-04±	1.98E-06

Results:

- TOPAS was used to display the intensity peaks which are characteristic of the material (Figure 2 and Figure 3) from 30°C to 400°C. As the temperature increases the shift of the peaks to the lower 2θ shows an expansion of the cell.
- Length of lattice parameter vs. temperature data is linear only to 300°C, so linear CTE was determined using data from 30°C to 300°C using Origin8 (Figure 4 a-c).
- Linear CTE for each lattice parameter can be seen in Table 1.
- In the study by Buchner, at 557°C a decomposition of $CoCu_2O_3$ into CuO and CoO occurs [1].
- Reactions observed in the previous study [1] occur at 900°C to form CoO and O_2 from Co_3O_4, and at 952°C $CoCu_2O_3$ begins to reform.

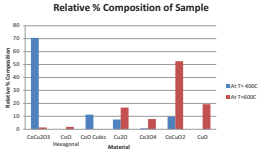

Figure 5a.
The relative percent compositions of the sample between 400°C and 600°C are shown. The relative percent composition of CuO and CoO increases from 400°C to 600°C, as the percent composition of $CoCu_2O_3$ decreases.

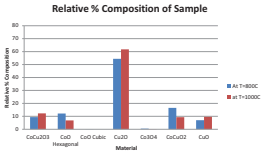

Figure 5b.
Above is the relative percent compositions between 800°C and 1000°C. The relative percent composition of CoO does not increase and $CoCu_2O_3$ does not appear to significantly reform from 800°C to 1000°C.

Conclusions:

- Lattice parameters do not have the same coefficient of thermal expansion, therefore the volume expansion is not isotropic.
- Products of the reaction between 400°C and 600°C are in good agreement with previous TGA/DTA study [1].
- $CoCu_2O_3$ decomposes at 557°C [1]. There are no temperature induced phase changes observed before decomposition.

References:

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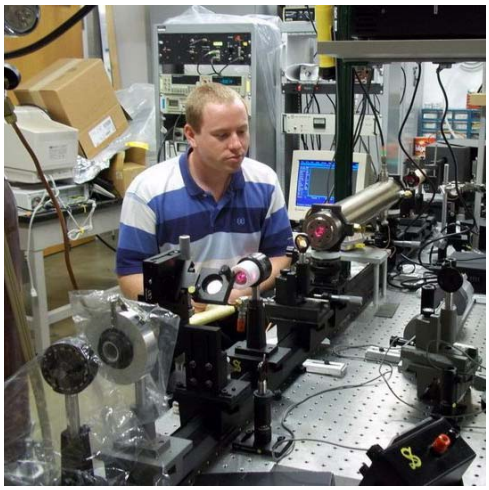
Acknowledgements:

Support from the REU program of the National Science Foundation under grant DMR-1005247 is gratefully acknowledged. The authors thank Dr. Thomas Hartmann and Jerry Egland for assistance with x-ray diffraction equipment and software. Help in data collection and analysis is acknowledged for Daniel Antonio, Patricia Kalita and Jason Baker.



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Title: Second Hyperpolarizability of Carbon Tetrachloride

Abstract: Although present theories of nonlinear optics for second-harmonic generation agree with observed behavior in simple atoms such as Helium, more complex molecules containing many electrons cannot consistently be described by theory. Carbon tetrachloride gas was used to produce electric field induced second-harmonic generation because of its complex structure. The resulting second-harmonic signal can be used to determine the hyperpolarizability of carbon tetrachloride by comparing it with a reference of Nitrogen gas. The hyperpolarizability value, an intrinsic property of molecules, can serve as a benchmark to compare with future work in the theory of nonlinear optics.

What problem are you trying to solve? Although present theories of nonlinear optics agree with observed behavior in simple atoms such as helium, more complex molecules containing many electrons, such as carbon tetrachloride (CCl4), cannot consistently be described by theory. Through experimental analysis of nonlinear materials, a new, more sophisticated model for describing their properties could be realized.
What tools or equipment are you using? We performed our experiments by taking second-harmonic signal measurements for triplets of N2 gas, a mixture of N2 and CCl4 gases, and N2 gas. The decision to measure the N2 signal before and after the CCl4 signal allowed us to monitor the power fluctuations and possible mode changes of the laser. Since the second- harmonic signal varies with the square of the power, changes in the power output can significantly alter the signal.
Why is your project worth researching? The purpose of our experiment was to measure the nonlinear behavior of the second harmonic signal generated from CCl4 and to compare the results with the prediction by the CCSD(T) molecular model.
What relevance will it have on the community, society, and in your research field?
What did you find? The value we obtained for agrees with previous experiments, which provides further evidence that these results are within their quoted margins of error.
What is the future of your research project? To further the study of the nonlinear properties of CCl4 and other molecules, one would like to eliminate error due to power and mode fluctuations by testing with a more stable laser. Also, performing the experiment at multiple fundamental frequencies will give a more accurate best fit line for w2 versus . A more accurate γs value would provide a better foundation for refining current molecular models.

Second Hyperpolarizability of Carbon Tetrachloride

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Background

Although present theories of nonlinear optics agree with observed behavior in simple atoms such as helium, more complex molecules containing many electrons, such as carbon tetrachloride (CCl_4), cannot consistently be described by theory. Through experimental analysis of nonlinear materials, a new, more sophisticated model for describing their properties could be realized. The purpose of our experiment was to measure the nonlinear behavior of the second harmonic signal generated from CCl_4 and to compare the results with the prediction by the CCSD(T) molecular model.

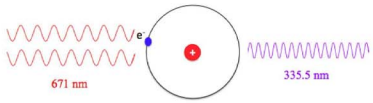

Figure 1: Second harmonic generation occurs when two fundamental frequency photons are incident upon and absorbed by a molecule with nonlinear properties. The resulting emitted photon has twice the frequency of the fundamentals.

Materials with nonlinear properties can convert light from one frequency to another, allowing for generation of many different frequencies from a single source. We measured second-harmonic generation, a nonlinear process in which two light waves of equal frequency are combined into a single light wave with double the frequency of either initial wave. The experiment utilized static electric field induced second-harmonic generation (ESHG) to produce a measureable second-harmonic signal from CCl_4. The signal was compared with the signal from nitrogen, a gas of known second hyperpolarizability γ, to determine the value of γ for CCl_4. Our γ_{CCl_4} value was compared to previously measured values at varying frequencies and with the prediction by the CCSD(T) mathematical model using the 31G(3d)+pd basis set, the highest approximation by reference 2.

The relevant equation for the second hyperpolarizabilty is given by the Taylor series expansion of the total electric dipole moment with respect to the local electric field E.

$$\mu_{total} = \mu + \alpha E + \beta E^2/2 + \gamma E^3/6 + \ldots$$

In the presence of a static electric field E_0 and an electric field E_w varying with frequency w, the γ term is given by:

$$\frac{\gamma E^3}{6} = \gamma[E_0 + E_w\cos(wt)]^3 = \gamma[E_0^3 + 3E_0^2E_w\cos(wt) + 3E_0E_w^2\cos^2(wt) + E_w^3\cos^3(wt)]$$

$3E_0E_w^2\cos^2(wt) = \frac{3E_0E_w^2}{2}[\cos(2w)+1]$ is responsible for the second harmonic signal generated in our experiment.

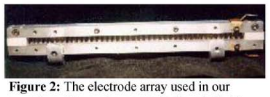

Figure 2: The electrode array used in our experiment to produce a static electric field.

Typically, the second-harmonic wave and the fundamental wave travel at different speeds through the nonlinear medium. As the length of the medium is increased, the second-harmonic waves generated at the beginning and the end of the sample get progressively farther out of phase, interfering perfectly destructively at the coherence length of the sample. ESHG allows for periodic phase matching in which the static field reverses in sign at length intervals equal to the coherence length of the sample, causing a phase shift of π radians in the generated second-harmonic wave, and therefore causing all second harmonic waves to be generated in phase with each other. The coherence length of the sample is adjusted to match the spacing of the electrodes by changing the density of the sample.

Apparatus

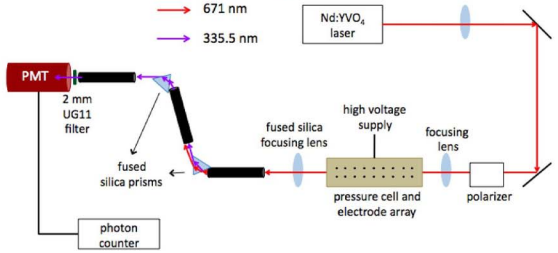

Figure 3: A schematic diagram of our experimental setup. Fused silica optics allow for transmission of UV light.

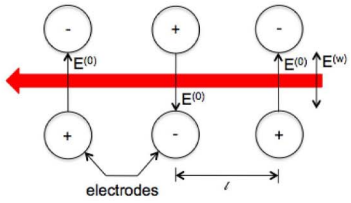

Figure 4: The static electric field generated by the electrode array is determined by the high voltage input. The periodic reversal of the sign of the field allows for optimal phase matching when the pressure is set to give a coherence length comparable to the length of the spacing between the electrodes. The enhanced amplitude of the signal due to phase matching varies by the square of the number of pairs of electrodes. The array used in our experiment contained 150 pairs of electrodes separated by 2.692 mm.

Data & Analysis

We performed our experiments by taking second-harmonic signal measurements for triplets of N_2 gas, a mixture of N_2 and CCl_4 gases, and N_2 gas. The decision to measure the N_2 signal before and after the CCl_4 signal allowed us to monitor the power fluctuations and possible mode changes of the laser. Since the second- harmonic signal varies with the square of the power, changes in the power output can significantly alter the signal. A change in mode affects the intensity profile of the beam, which also effects the second-harmonic signal. To reduce the error from changes in power and mode, triplets in which the peaks for N_2varied by amounts greater than counting statistics could account for, were discarded.

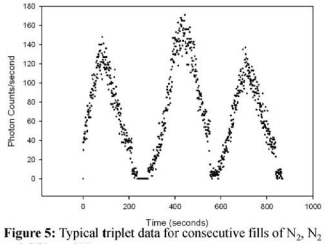

Figure 5: Typical triplet data for consecutive fills of N_2, N_2 and CCl_4, and N_2.

The peak signal value and pressure were approximated by a quadratic equation. Because each peak was not perfectly parabolic, a better approximation was obtained by only analyzing the top fifty percent of the data. This method also accounted for short power fluctuations of the laser by averaging over a range of pressures.

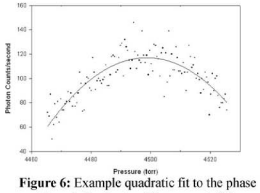

Figure 6: Example quadratic fit to the phase match peak for the top fifty percent of the second-harmonic signal from N_2.

Results

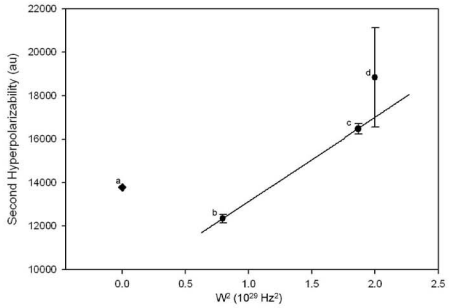

Figure 7: A linear relationship was observed between the second hyperpolarizability and the square of the frequency. The large error bar for our data d was caused primarily by fluctuations in laser beam propagation. Data point a was computed using the CCSD(T) model with basis set 31G(3d)+pd and was not included in the linear regression. [a]Reference 2. [b]Reference 3. [c]Reference 4. [d]This work.

A linear relationship is expected between the square of the frequency and the second hyperpolarizability. Therefore, a linear regression, weighted by the error bars, was obtained for all experimentally measured points. The static second hyperpolarizability determined by our fit was γ_s = 9268 375 au.

Assuming ideal gas behavior for both nitrogen and carbon tetrachloride, the ratio of phase match densities was calculated to be ρ_{N_2}/ρ_{CCl_4} =9.8375 0.1127. The observed phase match pressure for nitrogen was 4506 2 torr.

Conclusion

The value we obtained for γ_{CCl_4} agrees with previous experiments, which provides further evidence that these results are within their quoted margins of error.

The value of γ_s derived from the best fit line is not consistent with the value expected from the CCSD(T) method using a 31G(3d)+pd basis set. The two values of γ_s differ by 33%. The 4% error in our calculated value for γ_s does not account for this discrepancy, suggesting that current modeling techniques for nonlinear optics are unreliable.

To further the study of the nonlinear properties of CCl_4 and other molecules, one would like to eliminate error due to power and mode fluctuations by testing with a more stable laser. Also, performing the experiment at multiple fundamental frequencies will give a more accurate best fit line for w^2 versus γ_{CCl_4}. A more accurate γ_s value would provide a better foundation for refining current molecular models.

Acknowledgements

We would like to thank Dr. David P. Shelton for his extensive help with all aspects of the experiment. Support from the REU program of the National Science Foundation under grant DMR-1005247 is gratefully acknowledged.

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Title: Investigation of Raman Active Modes of MgxZn1-xCr2O4

Abstract: Using Raman spectroscopy, vibrational modes of the spinel structure MgxZn1-xCr2O4 were experimentally examined. The spinel compounds were synthesized by producing solid solutions via combustion method, of MgxZn1-xCr2O4 in the range x=0 to x=1 in 0.1 intervals. The purpose of which was to experimentally verify gradual shifts of Raman peaks as the samples transitioned between the two different compounds and gain information about the dependencies of the lattice vibrations on the tetrahedral and octahedral cations. X-ray diffraction was also used to verify spinel structure, and track the changes in lattice parameter of the samples.

Why are we doing this project? We are doing this project to better understand the vibrational modes in certain series of spinel structures. Which until this point have only been solved theoretically.
What problem are you trying to solve? We are trying to verify the accuracy of the theoretically predicted vibrational modes in the series MgxZn1-xCr2O4
What tools/equipment are you using? We are using a box furnace to synthesize our samples by combustion, and Ramon Spectroscopy, as well as X-ray diffraction is being used to analyze the samples.
What relevance will it have on the community, society and in your research field? We will gain a better understanding of vibrational modes in spinel structures. This updated knowledge in material properties can lead to advancements in the production of technology. For example, the spinel structure LiMn2O4 is commonly used as the cathode in Li-ion batteries.
What did you find? Results pending.
What is the future for your research topic? Upon completion of this particular series, there are others that can be experimentally verified. Such as ZnCr2-xFexO4

Investigation of Raman-Active Modes of $Mg_xZn_{1-x}Cr_2O_4$

Nick Macholl - Loyola University Chicago :: Tyler Mosher - Clarkson University

Advisors
Dr. John Farley :: Dr. Brian Hosterman

UNLV UNIVERSITY OF NEVADA LAS VEGAS PHYSICS DEPARTMENT

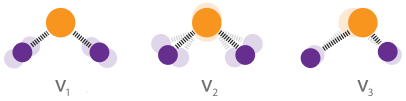
RAMAN SPECTROSCOPY

Raman spectroscopy is a spectroscopic technique for studying the vibrational modes of molecules. Typically, the object of study is illuminated with a laser beam. Light scattered by the sample is collected and examined. The vast majority of photons are scattered elastically, and have the same energy as the incident laser photons. However, a small fraction of the photons can give up some of their energy to cause vibrational excitation of the molecule. This results in a scattered photon with less energy than the incident photons. By comparing the energies of the incident and scattered photons, we can determine the vibrational behavior of molecules and crystals.

Elastic Scattering

Inelastic Scattering

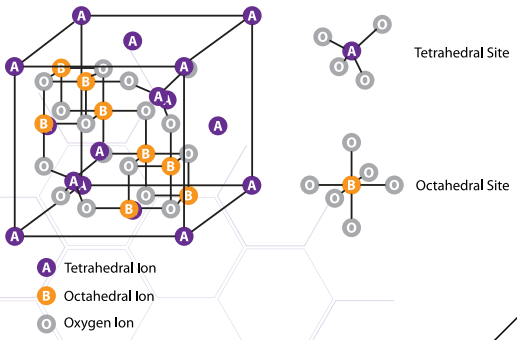
Vibrational Modes of Water



SPINEL STRUCTURE

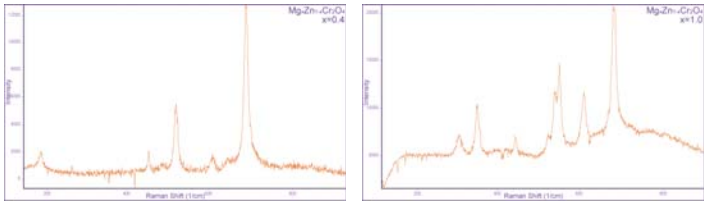
The spinel structure is a cubic close-packed mixed metal oxide of the form AB_2O_4 with eight tetrahedral sites and sixteen octahedral sites occupied by the cations. Spinel is versatile in that it can incorporate many different cation species in its structure. There are over one hundred known spinel compounds. A common example is magnetite (Fe_3O_4). Many spinels have industrial applications, such as lithium manganese oxide ($LiMn_2O_4$) for use as a cathode material in lithium ion batteries.

Spinel Structure Diagram

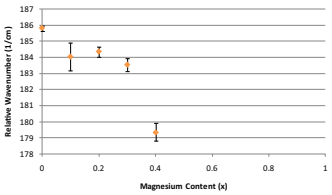


RESULTS

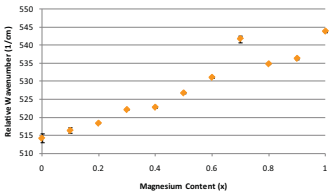
Micro-Raman Spectra



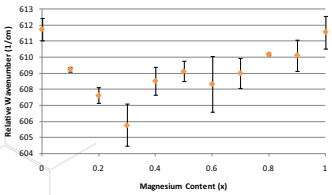
F_{2g} (1) Mode of $Mg_xZn_{1-x}Cr_2O_4$



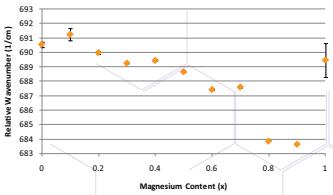
F_{2g} (2) Mode of $Mg_xZn_{1-x}Cr_2O_4$



F_{2g} (3) Mode of $Mg_xZn_{1-x}Cr_2O_4$



A_{1g} Mode of $Mg_xZn_{1-x}Cr_2O_4$



CONCLUSIONS

Our data shows that some of the observed Raman-active modes depend upon the magnesium content. Our $F_{2g}(1)$ mode disagrees with literature[2] in that we observed this mode at lower energies than reported in every data acquisition. Additionally, our $F_{2g}(1)$ mode was no longer visible at x>0.4. The $F_{2g}(2)$ mode showed a linear dependence upon magnesium content with the largest percent change of any other observed mode at 5.56%. The $F_{2g}(3)$ mode exhibited the most peculiar behavior in that the two end points were equivalent, though the solid solutions showed a variation in the mode's energy. This behavior requires more investigation. Lastly, the A_{1g} mode showed the most independent behavior with respect to magnesium content. However, the mode's energy decreased slightly through the series. This may be due to a small increase in the lattice parameter between zinc chromite (8.327Å)[3] and magnesium chromite(8.3344Å)[4].

MOTIVATION

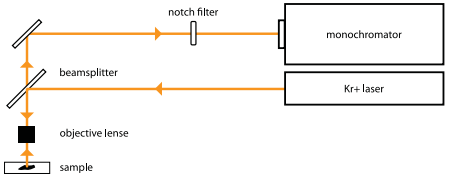
With the vibrational understanding relatively unknown for many spinel oxides, experimental data are needed to test theory and to gain a better understanding of the dependence of these modes on the tetrahedral and octahedral cations. This experimental data allows theorists to validate theory with experiment, which leads to a better understanding of this structure. Additionally, unpredicted material properties could be found that have use in industrial applications.

EXPERIMENT

Solid solution spinel oxides of the form $Mg_xZn_{1-x}Cr_2O_4$, were synthesized via combustion reaction in x=0.1 intervals from 0 to 1. Eleven samples were generated using magnesium nitrate $Mg(NO_3)_2 \cdot 6H_2O$, zinc nitrate $Zn(NO_3)_2 \cdot 6H_2O$, and chromium nitrate $Cr(NO_3)_3 \cdot 9H_2O$ with urea $CO(NH_2)_2$ as fuel. The stoichiometric composition of each sample was found based on the total oxidizing and reducing valencies of the oxidizer and fuel[1]. Resulting in a combined molar proportion of x:1-x:2:6.67 of magnesium nitrate, zinc nitrate, chromium nitrate and urea respectively. The reactions were performed in a box furnace, at approximately 350°C for 20-25 minutes.

For the first time, micro-Raman spectral studies were performed on this series to observe its vibrational spectrum. The micro-Raman microscope system consisted of a Lexel RamanIon krypton ion laser tuned to 647.1 nm as the excitation source. The laser light was passed through a spatial filter containing a 10 µm pinhole, and then sent to the entrance port of a Nikon MM-40 Measuring Microscope. The laser light is redirected by a beam splitter through the microscope and focused onto the sample. A 50X objective was used for all data acquisition. The light is then directed to a Horiba Jobin Yvon TRIAX 550 monochromator, and a Princeton Instruments liquid nitrogen cooled Spec 10 CCD detector, which was used for photon counting and sending the information to the computer software. This setup can be seen below. Multiple data acquisitions were taken for all eleven samples in the series, in which we tracked the gradual shifts in the Raman peaks as the sample transitioned from zinc chromite (x=0), to magnesium chromite (x=1). This allowed us to gain information about the dependence of the lattice vibrations on the tetrahedral and octahedral cations. In all, four modes were tracked through the transition.

Micro-Raman Apparatus



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We would also like to acknowledge Daniel Sneed for his work in the laboratory.

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Research Site: BPB 110

Title: Alkali Halide Alkanalates as Pressure-Media in High-Pressure DAC Experiments

Abstract: The goal of the experiment is to determine if a series of alkali halide alkanalates would make good pressure-media for high-pressure experiments utilizing diamond-anvil cells. In a diamond-anvil cell, the pressure transmitting medium transforms uniaxial pressure supplied by the cell into uniform hydrostatic pressure. The brunt of the experimental work involved setting up and aligning the optical equipment, as well as calibrating the grating spectrometers. To test the potential pressure-media, we measured the fluorescence of the media with a 457 nm laser and TriVista triple-grating spectrometry apparatus. We also measured the stiffness of the Raman modes of the media as a function of the pressure in the cell. The pressures will range to a maximum of 20 GPa.

Why are you doing this project? We are doing this project to determine new techniques in high-pressure diamond anvil cell experiments.
What problem are you trying to solve? We are trying to determine if certain organic liquids serve as better pressure-media in a diamond anvil cell.
What tools or equipment are you using? In order to determine this, we are using an optical system with a 457 nm blue laser and a TriVista spectrometry system, along with a diamond-anvil cell.
Why is your project worth researching? Different high-pressure diamond-anvil cell experiments require different pressure-media – we hope to illuminate a new media that can be used by high-pressure researchers.
What relevance will it have on the community, society, and in your research field? If it is found that the Alkali Halide Alkanalates serve as good pressure-media in diamond-anvil cells, then other researchers will be able to use them as needed in future experiments.
What did you find? Are results are still pending at this time.
What is the future of your research project? To provide other researchers Alkali Halide Alkanalates as a good pressure-media in diamond-avil cells to be used in researchers future experiments.

Ionic Alkalihalides as Pressure Media in DAC Experiments

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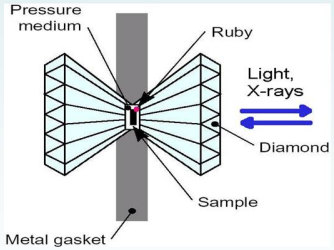
Abstract

In Diamond Anvil Cells (DACs), usually a pressure transmitting medium functions to transform the uniaxial pressure supplied by the opposing diamond anvils into uniform hydrostatic pressure acting on the sample. Conventionally, a 4-1 methanol-ethanol solution, or a 16-3-1 methanol-ethanol-water solution is used as pressure transmitting medium. However, these two solutions transform into a glass with high elastic shear strength at pressures around 12-14 GPa and no longer function as hydrostatic medium. Our goal was to determine if liquid ionic alkalihalide alkanolate complexes will provide more uniform pressure in the cell up to 20 GPa. Ruby (Cr-doped Al_2O_3) produces two Cr^{3+} fluorescence lines when exposed to sufficiently energetic radiation(457.9 nm in our case). These two fluorescence lines shift toward the IR with increasing pressure. We used the splitting of the two fluorescence lines as well as the width of the peaks in order to measure the shear strength of the alkalihalide-alkanolate as a function of pressure.

Introduction

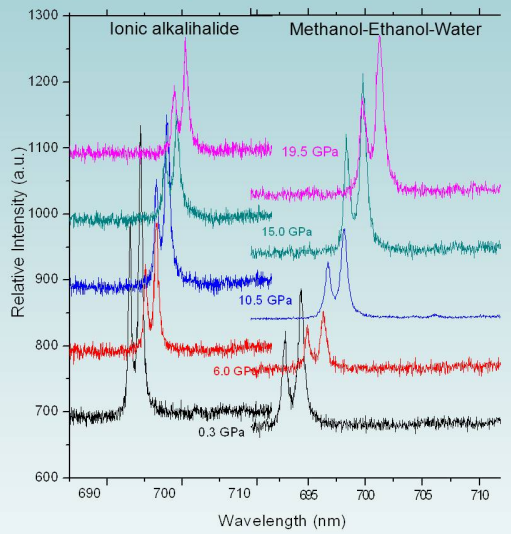
Fig. 1: Diagram of a Diamond Anvil Cell



Basic diagram of a DAC. The opposing diamond anvils provide uniaxial pressure onto the pressure medium, the sample, and the ruby. The pressure medium converts the uniaxial pressure from the anvils into uniform hydrostatic pressure. Since we were simply testing the pressure medium, no sample was inserted for our trials.

Data and Conclusions

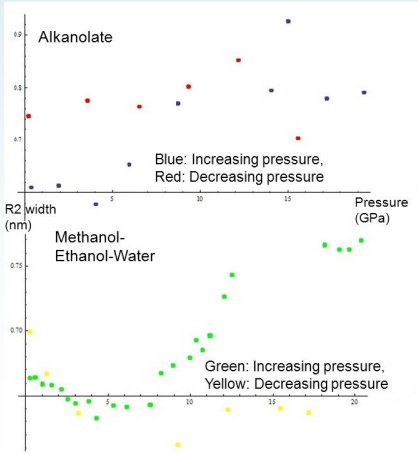
Fig. 2: Wavelength vs. Pressure for both samples



While the alkalihalide-alkanolate may be superior to the standard methanol-ethanol-water pressure medium, it is not preferable to gas pressure media such as helium. While the alkalihalide-alkanolate exhibits modest shear strength after the glass-transition visible as a pressure gradient between different points in the chamber of ~1 GPa. Gas-media like helium crystallize but maintain lower shear strength to much higher pressures. The question might arise: why not always use helium as a pressure medium, since it maintains near hydrostatic conditions at higher pressures? Since helium is a gas, loading it into the DAC is a markedly more difficult task. Additionally, different media are needed for different samples, given that reactions between the media and the sample are unwanted.

The standard pressure medium in DACs, a 16-3-1 methanol-ethanol-water solution, begins to transition into a glass at approximately 9-14 GPa. In our case we saw a glass transition as pressure neared 10 GPa. The alkalihalide-alkanolate undergoes a glass transition at approximately 7 GPa. It is in the glass transition where the alkalihalide complex is superior to the methanol-ethanol-water solution. As pressure increases to above 15 GPa, the alkalihalide-alkanolate maintains a relatively constant R2 peak width, whereas the methanol-ethanol-water solution experiences a drastic increase in peak width (fig 3). Broader ruby fluorescence peaks imply a greater shear strain on the sample, in other words: non-hydrostatic conditions. Thus, at high pressures, the alkalihalide-alkanolate provides conditions closer to hydrostatic pressure.

Fig. 3: R2 Width vs. Pressure for both samples



Experimental Setup

Fig. 4: Experimental Setup

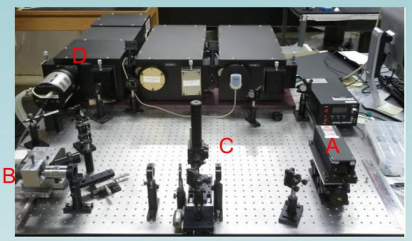


Figure 3 shows our experimental setup. A 457.9 nm laser (labeled A in the above picture) is reflected such that it travels through a focusing lens and hits the DAC (B). The excited fluorescence from the DAC travels through a collecting lens, reflects off a translatable mirror. If the mirror is in position, then the sample can be viewed in the microscope (C). Otherwise, when the mirror is moved out of position, then the light passes the microscope and travels through a condensing lens and into the spectrometer (D). We used the spectroscopy program WinSpec32 to image the peaks from the pressure media, and Origin 8 to subsequently analyze the peaks.

References

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2 http://upload.wikimedia.org/wikipedia/commons/c/c9/DiaAnvCell1.jpg

3 W. Bassett, High Pres. Research, **29** (2), 163 (2009).

Support from the REU program of the National Science Foundation under grant DMR-1005247 is gratefully acknowledged. I would also like to thank my research advisors: Dr. Oliver Tschauner and Dr. Valentin Iota for their help this summer.

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Title: Investigation of Ramon Active Modes of MgxZn1-xCr2O4

Abstract: Using Raman spectroscopy, vibrational modes of the spinel structure MgxZn1-xCr2O4 were experimentally examined. The spinel compounds were synthesized by producing solid solutions via combustion method, of MgxZn1-xCr2O4 in the range x=0 to x=1 in 0.1 intervals. The purpose of which was to experimentally verify gradual shifts of Raman peaks as the samples transitioned between the two different compounds and gain information about the dependencies of the lattice vibrations on the tetrahedral and octahedral cations. X-ray diffraction was also used to verify spinel structure, and track the changes in lattice parameter of the samples.

Why are we doing this project? We are doing this project to better understand the vibrational modes in certain series of spinel structures. Which until this point have only been solved theoretically.
What problem are you trying to solve? We are trying to verify the accuracy of the theoretically predicted vibrational modes in the series MgxZn1-xCr2O4
What tools/equipment are you using? We are using a box furnace to synthesize our samples by combustion, and Ramon Spectroscopy, as well as X-ray diffraction is being used to analyze the samples.
What relevance will it have on the community, society and in your research field? We will gain a better understanding of vibrational modes in spinel structures. This updated knowledge in material properties can lead to advancements in the production of technology. For example, the spinel structure LiMn2O4 is commonly used as the cathode in Li-ion batteries.
What did you find? Results pending.
What is the future for your research topic? Upon completion of this particular series, there are others that can be experimentally verified. Such as ZnCr2-xFexO4

Investigation of Raman-Active Modes of $Mg_xZn_{1-x}Cr_2O_4$

Nick Macholl - Loyola University Chicago :: Tyler Mosher - Clarkson University

Advisors
Dr. John Farley :: Dr. Brian Hosterman

UNLV UNIVERSITY OF NEVADA LAS VEGAS PHYSICS DEPARTMENT

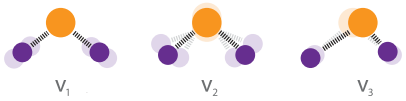
RAMAN SPECTROSCOPY

Raman spectroscopy is a spectroscopic technique for studying the vibrational modes of molecules. Typically, the object of study is illuminated with a laser beam. Light scattered by the sample is collected and examined. The vast majority of photons are scattered elastically, and have the same energy as the incident laser photons. However, a small fraction of the photons can give up some of their energy to cause vibrational excitation of the molecule. This results in a scattered photon with less energy than the incident photons. By comparing the energies of the incident and scattered photons, we can determine the vibrational behavior of molecules and crystals.

Elastic Scattering

Inelastic Scattering

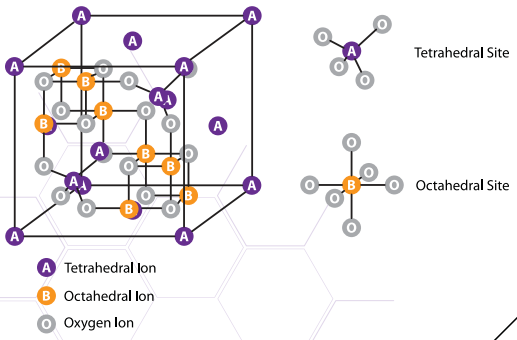
Vibrational Modes of Water



SPINEL STRUCTURE

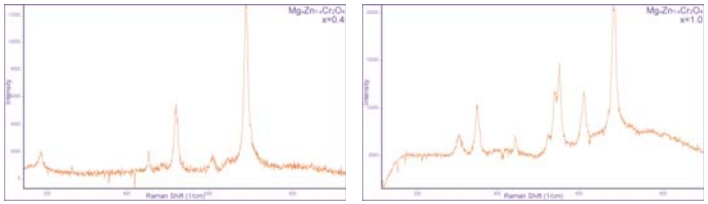
The spinel structure is a cubic close-packed mixed metal oxide of the form AB_2O_4 with eight tetrahedral sites and sixteen octahedral sites occupied by the cations. Spinel is versatile in that it can incorporate many different cation species in its structure. There are over one hundred known spinel compounds. A common example is magnetite (Fe_3O_4). Many spinels have industrial applications, such as lithium manganese oxide ($LiMn_2O_4$) for use as a cathode material in lithium ion batteries.

Spinel Structure Diagram

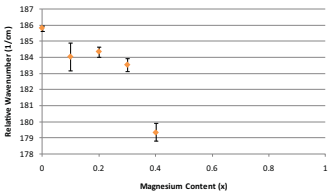


RESULTS

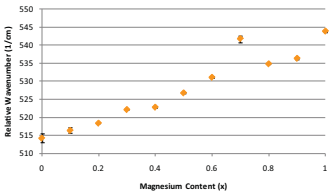
Micro-Raman Spectra



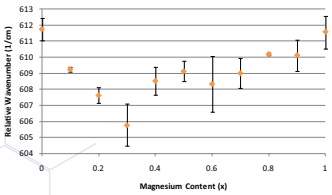
F_{2g} (1) Mode of $Mg_xZn_{1-x}Cr_2O_4$



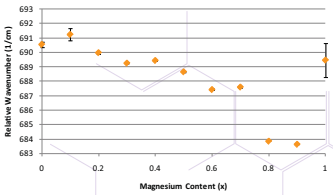
F_{2g} (2) Mode of $Mg_xZn_{1-x}Cr_2O_4$



F_{2g} (3) Mode of $Mg_xZn_{1-x}Cr_2O_4$



A_{1g} Mode of $Mg_xZn_{1-x}Cr_2O_4$



CONCLUSIONS

Our data shows that some of the observed Raman-active modes depend upon the magnesium content. Our $F_{2g}(1)$ mode disagrees with literature[2] in that we observed this mode at lower energies than reported in every data acquisition. Additionally, our $F_{2g}(1)$ mode was no longer visible at x>0.4. The $F_{2g}(2)$ mode showed a linear dependence upon magnesium content with the largest percent change of any other observed mode at 5.56%. The $F_{2g}(3)$ mode exhibited the most peculiar behavior in that the two end points were equivalent, though the solid solutions showed a variation in the mode's energy. This behavior requires more investigation. Lastly, the A_{1g} mode showed the most independent behavior with respect to magnesium content. However, the mode's energy decreased slightly through the series. This may be due to a small increase in the lattice parameter between zinc chromite (8.327Å)[3] and magnesium chromite(8.3344Å)[4].

MOTIVATION

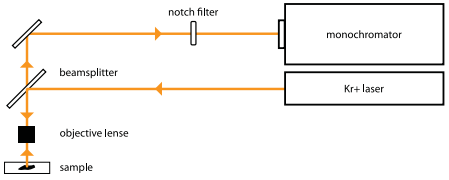
With the vibrational understanding relatively unknown for many spinel oxides, experimental data are needed to test theory and to gain a better understanding of the dependence of these modes on the tetrahedral and octahedral cations. This experimental data allows theorists to validate theory with experiment, which leads to a better understanding of this structure. Additionally, unpredicted material properties could be found that have use in industrial applications.

EXPERIMENT

Solid solution spinel oxides of the form $Mg_xZn_{1-x}Cr_2O_4$, were synthesized via combustion reaction in x=0.1 intervals from 0 to 1. Eleven samples were generated using magnesium nitrate $Mg(NO_3)_2 \cdot 6H_2O$, zinc nitrate $Zn(NO_3)_2 \cdot 6H_2O$, and chromium nitrate $Cr(NO_3)_3 \cdot 9H_2O$ with urea $CO(NH_2)_2$ as fuel. The stoichiometric composition of each sample was found based on the total oxidizing and reducing valencies of the oxidizer and fuel[1]. Resulting in a combined molar proportion of x:1-x:2:6.67 of magnesium nitrate, zinc nitrate, chromium nitrate and urea respectively. The reactions were performed in a box furnace, at approximately 350°C for 20-25 minutes.

For the first time, micro-Raman spectral studies were performed on this series to observe its vibrational spectrum. The micro-Raman microscope system consisted of a Lexel RamanIon krypton ion laser tuned to 647.1 nm as the excitation source. The laser light was passed through a spatial filter containing a 10 µm pinhole, and then sent to the entrance port of a Nikon MM-40 Measuring Microscope. The laser light is redirected by a beam splitter through the microscope and focused onto the sample. A 50X objective was used for all data acquisition. The light is then directed to a Horiba Jobin Yvon TRIAX 550 monochromator, and a Princeton Instruments liquid nitrogen cooled Spec 10 CCD detector, which was used for photon counting and sending the information to the computer software. This setup can be seen below. Multiple data acquisitions were taken for all eleven samples in the series, in which we tracked the gradual shifts in the Raman peaks as the sample transitioned from zinc chromite (x=0), to magnesium chromite (x=1). This allowed us to gain information about the dependence of the lattice vibrations on the tetrahedral and octahedral cations. In all, four modes were tracked through the transition.

Micro-Raman Apparatus



ACKNOWLEDGEMENTS

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We would also like to acknowledge Daniel Sneed for his work in the laboratory.

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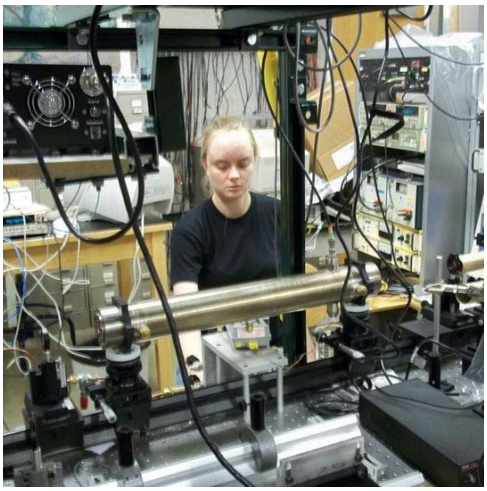
[2] Z.Wang, H. S. C. O'Neill, P. Lazor and S. K. Saxena. Journal of Physics and Chemistry of Solids **63**, 2057-2061 (2002)
[3] A. Wold. Journal of Chemical Education **57**, 531-536 (1980)
[4] B. D. Hosterman 2010. Ph.D. Thesis, University of Nevada Las Vegas, 55pp.



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Mentor/Advisor: David Shelton
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Title: Second Hyperpolarizability of Carbon Tetrachloride

Abstract: Although present theories of nonlinear optics for second-harmonic generation agree with observed behavior in simple atoms such as Helium, more complex molecules containing many electrons cannot consistently be described by theory. Carbon tetrachloride gas was used to produce electric field induced second-harmonic generation because of its complex structure. The resulting second-harmonic signal can be used to determine the hyperpolarizability of carbon tetrachloride by comparing it with a reference of Nitrogen gas. The hyperpolarizability value, an intrinsic property of molecules, can serve as a benchmark to compare with future work in the theory of nonlinear optics.

What problem are you trying to solve? Although present theories of nonlinear optics agree with observed behavior in simple atoms such as helium, more complex molecules containing many electrons, such as carbon tetrachloride (CCl4), cannot consistently be described by theory. Through experimental analysis of nonlinear materials, a new, more sophisticated model for describing their properties could be realized.
What tools or equipment are you using? We performed our experiments by taking second-harmonic signal measurements for triplets of N2 gas, a mixture of N2 and CCl4 gases, and N2 gas. The decision to measure the N2 signal before and after the CCl4 signal allowed us to monitor the power fluctuations and possible mode changes of the laser. Since the second- harmonic signal varies with the square of the power, changes in the power output can significantly alter the signal.
Why is your project worth researching? The purpose of our experiment was to measure the nonlinear behavior of the second harmonic signal generated from CCl4 and to compare the results with the prediction by the CCSD(T) molecular model.
What relevance will it have on the community, society, and in your research field?
What did you find? The value we obtained for agrees with previous experiments, which provides further evidence that these results are within their quoted margins of error.
What is the future of your research project? To further the study of the nonlinear properties of CCl4 and other molecules, one would like to eliminate error due to power and mode fluctuations by testing with a more stable laser. Also, performing the experiment at multiple fundamental frequencies will give a more accurate best fit line for w2 versus . A more accurate γs value would provide a better foundation for refining current molecular models.

Second Hyperpolarizability of Carbon Tetrachloride

Phillip C. Lotshaw[1], Anna M. Smith[2], and Dr. David P. Shelton[3]

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[2]Department of Physics, Davidson College, Davidson, NC 28035, USA

[3]Department of Physics, University of Nevada-Las Vegas, Las Vegas, NV 89154, USA, NSF-REU Program Summer 2011

Background

Although present theories of nonlinear optics agree with observed behavior in simple atoms such as helium, more complex molecules containing many electrons, such as carbon tetrachloride (CCl_4), cannot consistently be described by theory. Through experimental analysis of nonlinear materials, a new, more sophisticated model for describing their properties could be realized. The purpose of our experiment was to measure the nonlinear behavior of the second harmonic signal generated from CCl_4 and to compare the results with the prediction by the CCSD(T) molecular model.

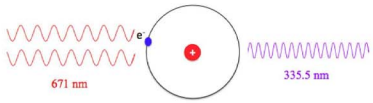

Figure 1: Second harmonic generation occurs when two fundamental frequency photons are incident upon and absorbed by a molecule with nonlinear properties. The resulting emitted photon has twice the frequency of the fundamentals.

Materials with nonlinear properties can convert light from one frequency to another, allowing for generation of many different frequencies from a single source. We measured second-harmonic generation, a nonlinear process in which two light waves of equal frequency are combined into a single light wave with double the frequency of either initial wave. The experiment utilized static electric field induced second-harmonic generation (ESHG) to produce a measureable second-harmonic signal from CCl_4. The signal was compared with the signal from nitrogen, a gas of known second hyperpolarizability γ, to determine the value of γ for CCl_4. Our γ_{CCl_4} value was compared to previously measured values at varying frequencies and with the prediction by the CCSD(T) mathematical model using the 31G(3d)+pd basis set, the highest approximation by reference 2.

The relevant equation for the second hyperpolarizabilty is given by the Taylor series expansion of the total electric dipole moment with respect to the local electric field E.

$$\mu_{total} = \mu + \alpha E + \beta E^2/2 + \gamma E^3/6 + \ldots$$

In the presence of a static electric field E_0 and an electric field E_w varying with frequency w, the γ term is given by:

$$\frac{\gamma E^3}{6} = \gamma[E_0 + E_w\cos(wt)]^3 = \gamma[E_0^3 + 3E_0^2E_w\cos(wt) + 3E_0E_w^2\cos^2(wt) + E_w^3\cos^3(wt)]$$

$3E_0E_w^2\cos^2(wt) = \frac{3E_0E_w^2}{2}[\cos(2w)+1]$ is responsible for the second harmonic signal generated in our experiment.

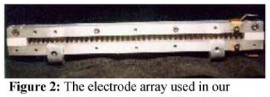

Figure 2: The electrode array used in our experiment to produce a static electric field.

Typically, the second-harmonic wave and the fundamental wave travel at different speeds through the nonlinear medium. As the length of the medium is increased, the second-harmonic waves generated at the beginning and the end of the sample get progressively farther out of phase, interfering perfectly destructively at the coherence length of the sample. ESHG allows for periodic phase matching in which the static field reverses in sign at length intervals equal to the coherence length of the sample, causing a phase shift of π radians in the generated second-harmonic wave, and therefore causing all second harmonic waves to be generated in phase with each other. The coherence length of the sample is adjusted to match the spacing of the electrodes by changing the density of the sample.

Apparatus

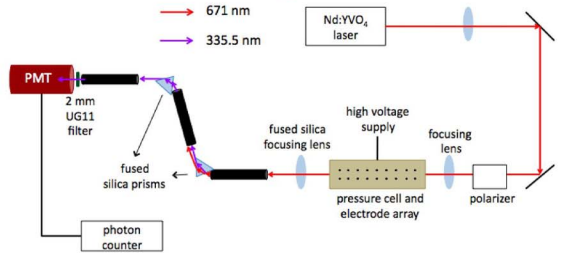

Figure 3: A schematic diagram of our experimental setup. Fused silica optics allow for transmission of UV light.

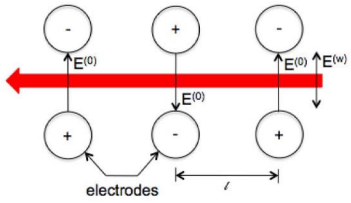

Figure 4: The static electric field generated by the electrode array is determined by the high voltage input. The periodic reversal of the sign of the field allows for optimal phase matching when the pressure is set to give a coherence length comparable to the length of the spacing between the electrodes. The enhanced amplitude of the signal due to phase matching varies by the square of the number of pairs of electrodes. The array used in our experiment contained 150 pairs of electrodes separated by 2.692 mm.

Data & Analysis

We performed our experiments by taking second-harmonic signal measurements for triplets of N_2 gas, a mixture of N_2 and CCl_4 gases, and N_2 gas. The decision to measure the N_2 signal before and after the CCl_4 signal allowed us to monitor the power fluctuations and possible mode changes of the laser. Since the second- harmonic signal varies with the square of the power, changes in the power output can significantly alter the signal. A change in mode affects the intensity profile of the beam, which also effects the second-harmonic signal. To reduce the error from changes in power and mode, triplets in which the peaks for N_2varied by amounts greater than counting statistics could account for, were discarded.

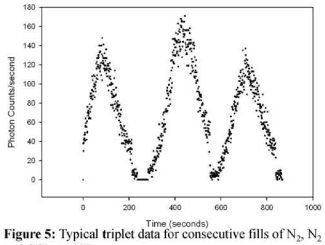

Figure 5: Typical triplet data for consecutive fills of N_2, N_2 and CCl_4, and N_2.

The peak signal value and pressure were approximated by a quadratic equation. Because each peak was not perfectly parabolic, a better approximation was obtained by only analyzing the top fifty percent of the data. This method also accounted for short power fluctuations of the laser by averaging over a range of pressures.

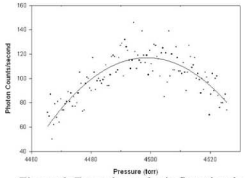

Figure 6: Example quadratic fit to the phase match peak for the top fifty percent of the second-harmonic signal from N_2.

Results

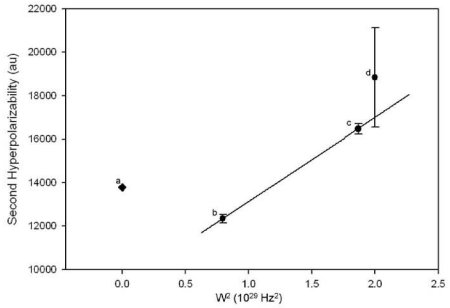

Figure 7: A linear relationship was observed between the second hyperpolarizability and the square of the frequency. The large error bar for our data d was caused primarily by fluctuations in laser beam propagation. Data point a was computed using the CCSD(T) model with basis set 31G(3d)+pd and was not included in the linear regression. [a]Reference 2. [b]Reference 3. [c]Reference 4. [d]This work.

A linear relationship is expected between the square of the frequency and the second hyperpolarizability. Therefore, a linear regression, weighted by the error bars, was obtained for all experimentally measured points. The static second hyperpolarizability determined by our fit was γ_s = 9268 375 au.

Assuming ideal gas behavior for both nitrogen and carbon tetrachloride, the ratio of phase match densities was calculated to be ρ_{N_2}/ρ_{CCl_4} =9.8375 0.1127. The observed phase match pressure for nitrogen was 4506 2 torr.

Conclusion

The value we obtained for γ_{CCl_4} agrees with previous experiments, which provides further evidence that these results are within their quoted margins of error.

The value of γ_s derived from the best fit line is not consistent with the value expected from the CCSD(T) method using a 31G(3d)+pd basis set. The two values of γ_s differ by 33%. The 4% error in our calculated value for γ_s does not account for this discrepancy, suggesting that current modeling techniques for nonlinear optics are unreliable.

To further the study of the nonlinear properties of CCl_4 and other molecules, one would like to eliminate error due to power and mode fluctuations by testing with a more stable laser. Also, performing the experiment at multiple fundamental frequencies will give a more accurate best fit line for w^2 versus γ_{CCl_4}. A more accurate γ_s value would provide a better foundation for refining current molecular models.

Acknowledgements

We would like to thank Dr. David P. Shelton for his extensive help with all aspects of the experiment. Support from the REU program of the National Science Foundation under grant DMR-1005247 is gratefully acknowledged.

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Title: Correlation Between Grain Dislocation Density and Orientation for Naturally Deformed Mantle Xenolith from the Jagersfontein Mine

Abstract: Determining the reaction of poly-crystalline structures to induced stress is an extremely difficult problem in contemporary engineering and geology. The main challenge lies in the inhomogeneity of the grains inside of the poly-crystalline structures. To predict the response of a certain polycrystalline structure to a specific stress, you must resort to one of two views on grain interaction, an orientation or propagation based model. For every material there may be certain correlations between the prediction model used and the actual deformation that occurred. Our work centers around describing the correlation of these prediction models with a sample of naturally deformed mantle xenolith from the Jagersfontein Mine in South Africa. In order to correlate the models with the sample, we needed to calculate the orientation and dislocation density of the individual grains. To measure the dislocation density, the ratio of the area of the dislocations to the total area of the grain itself, the sample needed to go through a decoration process. The sample was heated to accelerate oxidization and highlight the sample's dislocations. This "decorating" process allows us to easily discern the dislocations on the surface of the sample using a Scanning Electron Microscope (SEM). Dislocation density can then be calculated by using an open-source image analysis software called ImageJ on the image of the grain. Additionally, the other calculation, the orientation of the grain, is measured by an Electron Backscatter Diffraction (EBSD) analysis of the sample. The EBSD is a process of firing electrons at the sample and reading the diffractions produced. These diffractions create a "picture" of Kikuchi Bands, simply the diffraction lines produced by the electrons, which can be analyzed by proprietary software resulting in a calculation of the orientation of each grain. We were then able to look for correlations between the dislocation density and the orientation of each grain and identify which model describes the deformation results most accurately.

Why are you doing this project? We are doing this project in order to better understand how rocks deform.
What problem are you trying to solve? We're trying to see if the deformation of crystals within the rock can be predicted by the orientation of the crystals in the rock.
What tools or equipment are you using? Scanning Electron Microscope, Polishing Pad, Diamond Slurries, and Electron Backscatter Device.
Why is your project worth researching? The rock deformation process is still an unsolved mystery, but with a better understanding of it, we could engineer materials better and understand mantle rheology more completely. Moreover, with better understanding of rock deformation, buildings could be safer and long-standing geological problems could be closer to being answered.
What relevance will it have on the community, society, and in your research field? Better understanding of rock deformation could revolutionize construction and the building process. Also, there is a possibility that one could better predict how earth materials react under pressure, like in an earthquake.
What is the future of your research project? In the future we will most likely perform the same experiment on different materials in order to draw general conclusions about deformation.

Correlation Between Grain Dislocation Density and Orientation for Naturally Deformed Mantle Xenolith from the Jagersfontein Mine

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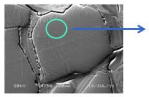
Abstract

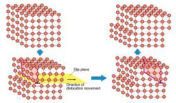
Determining the reaction of poly-crystalline structures to induced stress is an extremely difficult problem in contemporary engineering and geology. The main challenge lies in the inhomogeneity of the grains inside of the poly-crystalline structures. To predict the response of a certain polycrystalline structure to a specific stress, you must resort to one of two views on grain interaction, an orientation or propagation based model. For every material there may be certain correlations between the prediction model used and the actual deformation that occurred. Our work centers around describing the correlation of these prediction models with a sample of naturally deformed mantle xenolith from the Jagersfontein Mine in South Africa. In order to correlate the models with the sample, we needed to calculate the orientation and dislocation density of the individual grains. To measure the dislocation density, the ratio of the area of the dislocations to the total area of the grain itself, the sample needed to go through a decoration process. The sample was heated to accelerate oxidization and highlight the sample's dislocations. This "decorating" process allows us to easily discern the dislocations on the surface of the sample using a Scanning Electron Microscope (SEM). Dislocation density can then be calculated by using an open-source image analysis software called ImageJ on the image of the grain. Additionally, the other calculation, the orientation of the grain, is measured by an Electron Backscatter Diffraction (EBSD) analysis of the sample. The EBSD is a process of firing electrons at the sample and reading the diffractions produced. These diffractions create a "picture" of Kikuchi Bands, simply the diffraction lines produced by the electrons, which can be analyzed by proprietary software resulting in a calculation of the orientation of each grain. We were then able to look for correlations between the dislocation density and the orientation of each grain and identify which model describes the deformation results most accurately.

Introduction

Our sample rock is a piece of Kimberlite, a poly-crystalline rock which is usually created in the upper mantle of the earth's core. This rock, being poly-crystalline, is composed of many grains of crystals. In a deformation process, such as one that occurs naturally in the Earth's upper mantle, the grains of the poly-crystalline rock are subjected to pressures and forces that are great enough to displace and move atoms in the crystal structures of the grains. The results of these movements and displacements of atoms in the crystals are what we refer to as dislocations. There are two major types of dislocations, edge dislocations and screw dislocations. Both of these types can be highlighted through an oxidation process, which can occur because of iron naturally present in the rock. The iron can be oxidized by subjecting it to extremely high temperatures for an extended period of time. This oxidation process creates a visible contrast between the dislocations and the grain itself. This contrast is what we are examining in our SEM images.



Picture of Grain 13 with a few dislocations circled



This is a diagram showing how dislocations can occur on the atomic level

The orientation of a grain is the calculated orientation of the plane of the atoms of the crystalline structure. For poly-crystalline rocks every grain has its own orientation, and these orientations affect the way that a stress field is transferred through the rock. Different orientations cause different reactions to a specific stress field. Every orientation has a slip system which can be conducive to a stress field or non-conducive. If the orientation is conducive to a stress field, then the slip system will be aligned with the stress field and a dislocation can be formed and propagated through the crystal. If the orientation is non-conducive, then the slip system will not be aligned with the stress field and no dislocations can be formed. In essence, orientation is the basis for the formation of dislocations, and attempting to combine these two ideas experimentally is the basis of our research.

Experiment

- A thorough polishing of our sample was done, ranging from 400 grit down to ¼ micron.
- The sample was then decorated (oxidized) in a lab furnace at 900 degrees Fahrenheit for 45 minutes.
- Dislocation density data was collected by using the SEM to take photographs of the grains.
- Orientation data was collected by using the EBSD function of the SEM.
- EBSD data was taken by hand using optical and SEM images of the sample's surface to locate our SEM grains.
- Orientation data was converted into pole figures, allowing a visual display of orientation for all the grains.
- The SEM pictures were analyzed using the open source image analysis software, ImageJ, which allowed us to calculate both dislocation density area and total area of every grain.
- The dislocation density was divided into ranges, then colored, and finally mapped to the pole figures, allowing for a qualitative analysis of correlation.

SEM Results

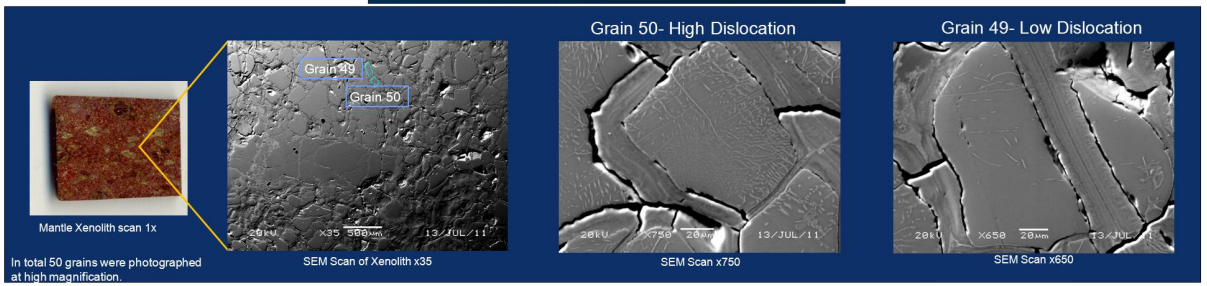
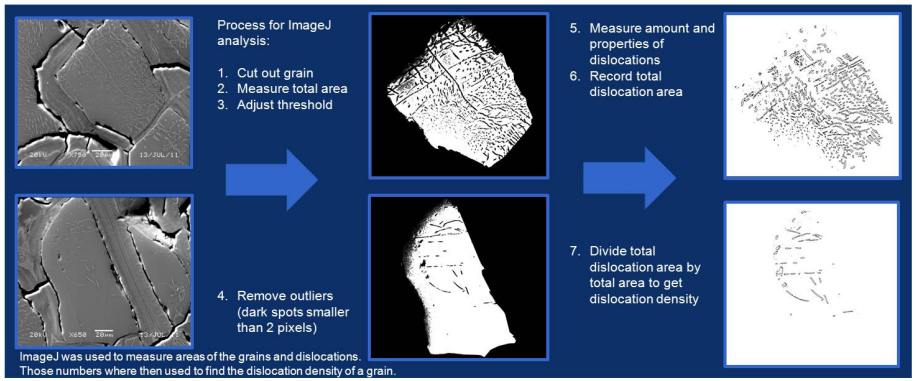
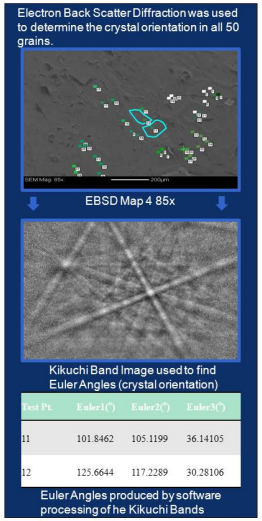


Image Analysis



EBSD Analysis

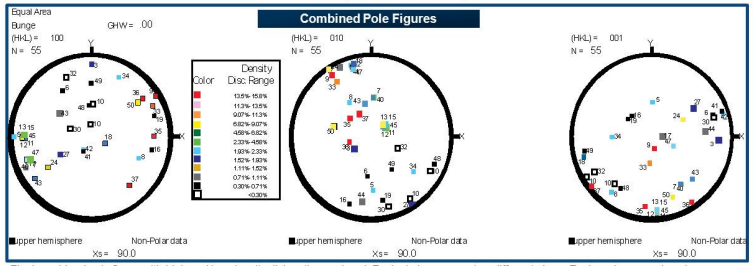


Test Pt.	Euler1(°)	Euler2(°)	Euler3(°)
11	101.8462	105.1199	36.14105
12	125.6644	117.2289	30.28106

Results

Grain	EBSD Map	Test Point	Euler1(°)	Euler2(°)	Euler3(°)	Dislocation Type	Total Area	Total Dis. area	Dis. Density
49	4	12	101.8462	105.1199	36.14105	Low	5.653	.044	0.78%
50	4	11	125.6644	117.2289	30.28106	High	5.49	0.517	9.4%

Data table for Grains 49 and 50 shown above. Data was compiled like this for all 50 grains.



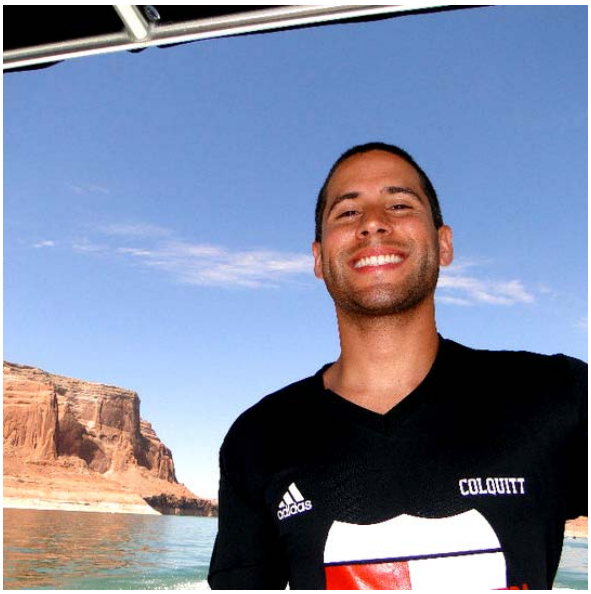
Final combined pole figure with high and low density dislocations colored. Each circle represents a different plane. Each grain we analyzed Has three different Euler angles describing its orientation. As can be seen in the above diagram grains with similar dislocation densities seem to cluster around the same areas in the plane.

Conclusions

- Evaluation of the pole figures revealed a noticeable trend between orientation and dislocation density
- High dislocation dense grains clustered on the right side of the 100 direction and they clustered on the top of the 010 direction.
- Low dislocation dense grains clustered in the middle of the 100 direction and the bottom of the 010 direction.
- In the end its easy to see a trend between dislocation density and orientation, but, because of systematic error in the image analysis of ImageJ, definite conclusions cannot be drawn.

Acknowledgements

Support from the REU program of the National Science Foundation under grant DMR-1005247, and support from the High Pressure Science and Engineering Center (HPSEC), funded by the US Department of Energy, is gratefully acknowledged. Thanks would like to be extended to the SEM Lab and to the Rock Deformation Lab, for the use of their equipment, and a special thank you to our research advisor, Dr. Pamela Burnley.



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Location of High School: Las Vegas, NV
Mentor/Advisor: Ravhi S. Kumar
Educational Institute of Project: UNLV
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Title: Pressure Induced Structural Phase Changes in Nb5Si3

Abstract: Refractory metal silicides are a important class of materials, they are used in high temperature applications such as turbines and aerospace modules. Having a higher melting temperature and lower density, both which are desirable, Nb5Si3 is considered to be a superior alloy in comparison to nickel super-alloys. Even though the behavior of this alloy is under investigation at high temperature by several groups, there are no studies available on high pressure properties. Here, we focus on the high pressure crystal structure of Nb5Si3. High pressure powder x-ray diffraction was performed on Nb5Si3 using synchrotron radiation at Argonne National Laboratory; up to 35 GPa was applied and the preliminary data analysis shows a pressure induced structural transition below 10 GPa. The results will be discussed in detail.

Why are you doing this project? I chose Ravhi as the mentor I wished to work with because he currently works with superconductors, thermoelectrics and phase changing alloys. I intend to gain experimental knowledge and laboratory experience. Why this project: I love physics.
What problem are you trying to solve? We are showing the benefit of working with refractory metal silicides (in this case, Nb5Si3), integrating, plotting data sets and fitting the equation of state (Birtch-Murnghan).
What tools or equipment are you using? I have used (and/or am currently using): many software programs including, EOSfit, fit2D, OriginPro8, Jade7, Igor, PowderCell2.4, high powered microscopes, EDM drills, laser drills, the synchrotron (at Argonne National Laboratory), Scopus, ISI Web of Knowledge and, of course, the Internet.
Why is your project worth researching? The discovery of new properties of compounds, the practice of applying math and science, the expanse of our knowledge based, the experience of working with and meeting great people. And without a doubt, the research experience.
What relevance will it have on the community, society and in your research field? The finding of higher preforming alloys can have application in various forms of propulsion, improving electronics and/or enhancing structural integrity in extreme environments.
What did you find? I found data is quite difficult to ascertain in an experimental setting. The setting up and gathering of data takes patience and appreciation for detail. Nb5Si3 has a higher melting temperature and lower density and is considered to be a superior alloy in comparison to nickel super-alloys. We can use electrical current and a dielectric and drill 100 micrometer holes in metals. Lasers look nothing like I thought they would.
What is the future for your research project? The studying Nb5Si3 will continue past the REU internship program. For myself, this project will result in a poster presentation, possibly a paper and help me gain a better understanding of how science can be applied in a laboratory. I am doing another research program (hopefully, the next one might last longer, time wise) and I can not wait until the next internship, where ever that may be.

High Pressure Structural Studies on Nb_5Si_3 up to 26.2 GPa

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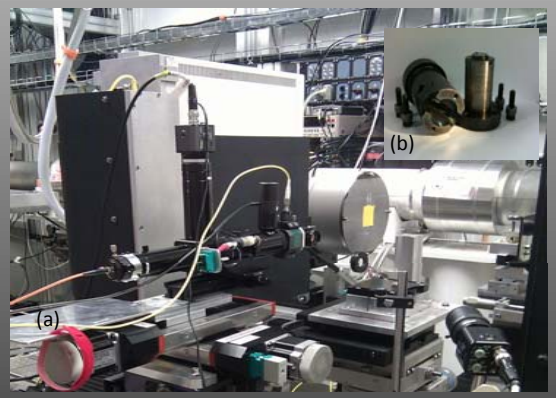
BACKGROUND

With the use of synchrotron techniques, we can better understand how crystalline structures behave under extreme conditions. This yields the opportunity to resolve complex crystal structures [1]. Here, we focus on the high pressure crystal structure of Nb_5Si_3. Refractory metal silicides are an important class of materials as they are used in high temperature applications such as turbines and aerospace modules. As an example, the performance of a jet engine is highly influenced by the maximum internal pressure and temperature possible. Obtaining higher levels of thrust is dependent upon the material's ability to remain structurally sound under extreme temperatures and pressures; Nb_5Si_3 has a higher melting temperature and lower density achieving better performance under said conditions [2].

EXPERIMENTAL

The Nb_5Si_3 sample was prepared by Dr. Ravhi Kumar at the University of Nevada, Las Vegas. A stainless steel gasket with a 130 µm centered circular hole drilled out was placed on top of one of two 300 µm diamond culets in a Mao-Bell diamond anvil cell (DAC), shown in Fig. 1(b). Inside the hole, ruby grains and powder sample are placed, along with a 4:1 mixture of methanol and ethanol as a pressure transmitting medium. The DAC was then placed into the offline ruby system, where the initial pressure and subsequent pressures of the cell were measured. The offline ruby system was used to fluoresce the ruby grains determining the various pressures. It was then loaded into the x-ray diffraction (XRD) system at HPCAT Sector 16, beam-line BM-D at Argonne National Laboratory and room temperature XRD measurements were performed; the x-ray wavelength was 0.422450 Å and pressures ranging from 0.42 GPa up to 26.2 GPa were reached. The crystalline structure was examined using the MDI Jade software package and those integrated patterns are shown in Fig. 1(c).



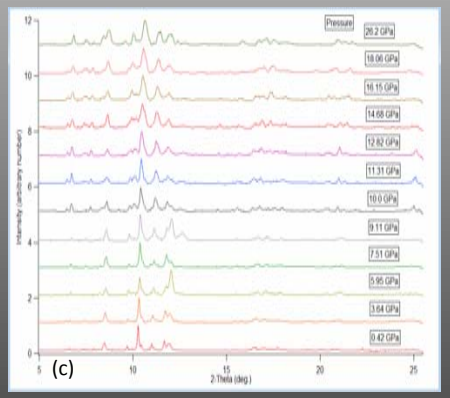
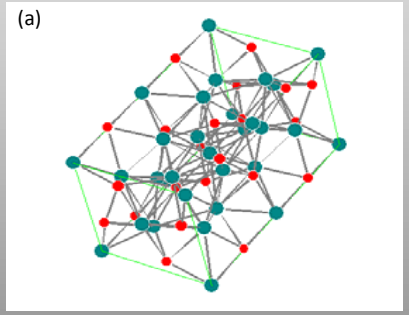


Fig.1 (a) Synchrotron x-ray diffraction set up in BM-D hutch at HPCAT, Argonne National Laboratory (b). Mao-Bell diamond anvil cell. (c). X-ray diffraction pattern(s): from pressures near ambient, up to 26.2 GPa on Nb5Si3.

RESULTS

The lowest pressure pattern, 0.42 GPa, was indexed to the tetragonal I4/mcm(140) space group with lattice parameters a = b = 6.566±.001 Å and c = 11.88±.004 Å from this experiment. The literature quotes a = b = 6.557 Å and c = 11.86 Å which agrees reasonably well with data collected [3]. Up to 10 GPa, the XRD patterns were fit to the I4/mcm(140) structure and volumes were obtained. The ambient volume, as determined from this experiment was 515.12 $Å^3$, and agreed with the literature value of 509.91 $Å^3$ [3]. The bulk modulus from the literature is 191.0 GPa while the bulk modulus for the data collected is 210±10 GPa [4]. Evidence of a structural phase transition can be seen in the XRD patterns of Fig. 1(c); this evidence occurs at 10.0 GPa where peaks appear and change in the two-theta range of 6-9°, as well as in other regions. Fig. 2(a) shows the ambient tetragonal structure of Nb_5Si_3 and Fig. 2(b) shows the pressure vs. volume plot corresponding with the third order Birch-Murnaghan equation up to the phase transition at 10.0 GPa. The error bars in Fig. 2(b) were calculated from the Jade fit.



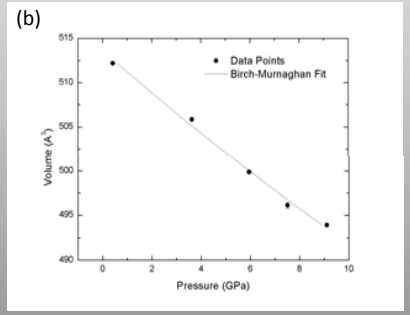


Fig.2 (a). Tetragonal crystal structure at ambient pressure (b). P-V plot of Nb_5Si_3

CONCLUSIONS AND SUMMARY

The preliminary data analysis shows a pressure induced structural transition at 10 GPa with peaks appearing in the two-theta range of 6-9°. The data retrieved from the XRD of Nb_5Si_3 matches the reading: confirming both the bulk modulus of 191.0 GPa and ambient cell volume 509.91 $Å^3$ [4,5]. Nb_5Si_3 has proven to hold structural integrity up to 10 GPa. The material shows much promise in delivering a higher level of structural integrity under extreme conditions. The phase change structure is undetermined at this time and further investigation is currently underway.

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Title: Motif Logic

Abstract: Cells are the most basic building blocks of life and they carry out complex chemical and physical functions in order to survive and make decisions. In turn proteins are building blocks for cells. Proteins are known to interact with each other and help cells carry out sophisticated processes, but the mechanisms are largely unknown. Large databases and networks of protein interactions have been established, but these give little inference into how more complex protein signaling networks work toward building complex cell processes. The overarching hypothesis is that cells make decisions through gated protein interactions and we can assign logic to these reactions in order to understand how they build larger cell "thought" processes. Directional protein gates can be represented as one of sixteen Boolean logics depending on the possible inputs and outputs, which when used in combination create simple circuits such as a double inverter, which in turn build into higher functionality. By using computers to assign digital logic and gating to protein-protein interactions, our goal is to relate the pathway created within the network to electrical circuits with known functions such as counting, by starting from the individual gates and building upward. This would allow for a far more complete understanding of how cells work and "think," and could transform how we think about signal transduction. Eventually understanding these pathways would allow investigation into treatment of diseases such as cancer and auto-immune diseases, where it appears that there is an error in cell logic, with examples including cancer cells starting to grow when they should not divide, or beta-cells being.

Why are you doing this project? We want to have a more complete knowledge about how cells work because they are the building blocks of life.
What problem are you trying to solve? We are trying to understand how cells think and make decisions that are necessary to cell and human survival.
What tools or equipment are you using? We are using powerful computers and extremely large databases or protein interactions to analyze and solve the problem.
Why is your project worth researching? Through this project we hope to learn how our body works at a level previously unexplored, and through this understanding to develop new drugs to combat disease.
What relevance will it have on the community, society, and in your research field? The largest relevance would be in the scientific community where we would create a completely new understanding of how cells function, while in society it would result in new cures to diseases and more efficient and safe medication.
What did you find? So far we have found that Boolean logic can be assigned to protein pathways and that massive protein networks exist with large amounts of crosstalk and large variability in communication.
What is the future of your research project? The next step is to search for larger circuits in the pathways and from there to develop more unifying rules for cell thought processes.

Proposed Mechanistic Basis of Cellular Decision Making

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Introduction

A cell exhibits sophisticated behaviors that exhibit decision-making capabilities. For example, phagocytic cells will chase a bacterial cell, which requires integration of many processes such as motility, sensory, and navigation systems. Understanding such complex cell behaviors is likely essential for treating human system failure as exemplified by type 2 diabetes where cells decide to stop eating glucose. We hypothesize that there are logical circuits within protein networks that are used by cells to make decisions. Like protein interactions in cells, electrical circuits are engineered with a series of interconnected transistors capable of making decisions.

Protein interaction networks can be modeled as a network of logic gates. For any two input and one output logic gate there are sixteen possible logical truths (e.g AND, OR, XOR). Some logical functions can be represented by biological terms such as inhibitor, constitutionally active, coincidence detector, and dominant negative, supporting our modeling approach. For Example, Figure 2 shows the presence of a double inverter circuit in the Insulin signaling pathway diagram.

To begin to test our hypothesis, we modeled known human protein-protein interactions as a network where nodes are proteins and edges are their interactions. Protein nodes were combined into units of 4 protein chains based on previously known interactions. By searching for and matching common chain components, larger circuits were built. We built structures for electrical circuits such as half-adders, latches, flip-flops, XORs, implication circuits, and encoders. These principal components of many electrical circuits are often used to construct complex functional circuits that one would also expect to find in cell logic processing.

Due to vast combinatorial complexity, we thoroughly examined 69 total proteins in a ~10,000-node protein interaction network constructed from several databases. Each protein is the start protein and has on average half a million chains associated with it. We identified structures for millions of flip-flops, latches and half-adders. We also identified 26 proteins with encoders. We searched for two different implementations of the XOR logic and found 25 with one type of XOR and only 3 proteins with the other type of XOR. This result suggest that circuitry may be inherited and modularized as protein domains are in proteomes. This analysis can be extended, refined and experimentally tested.

Hypothesis

Protein interaction networks contain the structure of electrical circuits

Sample Circuit pathway

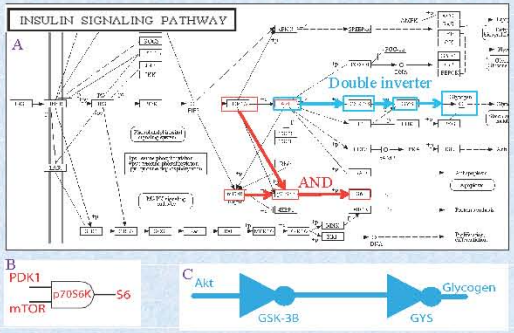


Figure 2. Components of electrical circuits as represented in a signal transduction diagram. The Insulin Signaling Pathway has a double inverters (blue) and a classic AND logic gate (red).(A) The AND gate requires two inputs to produce an output, otherwise there is no output (B). The double inverter is a combination of two NOT logic gates (C). The double inverter is used for amplification of signals.

Strategy for finding electrical circuits

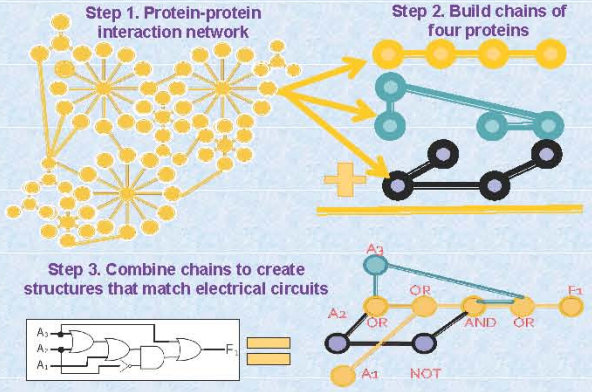


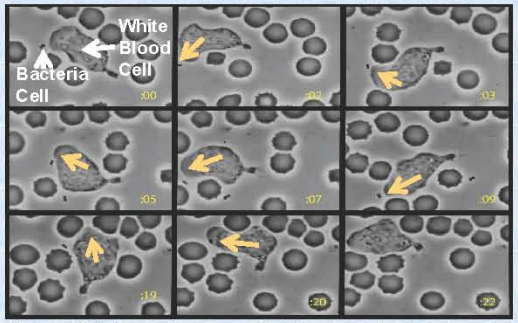
Figure 3. Strategy for identifying circuits in cell networks. 1. A human protein-protein interaction network was built from several databases. 2. All possible four protein chain assemblies (chains) were extracted from the network. 3. We identified overlap in components of those chains to search for the structures of the seven different electrical circuits.

Circuit Structures Identified

Simple Circuits		Complex Circuits	
1/2 Adder	100%	Encoder	59%
Flip Flop	95%	1 implies 2	80%
Latch	57%	XOR 1	57%
* % of 1ST proteins with identified circuits in search (out of 69 proteins)		XOR 2	7%

Conclusions:
1. Cells contain structures for common electrical circuits
2. One type of XOR was observed much more often than another type.
These results suggest that cells contain complex logical circuitry and that the XOR logic may have evolved once and then used in a modular way.

Cells exhibit complicated behaviors



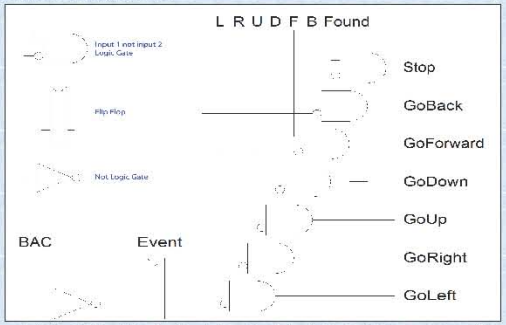


Figure 1. White blood cells use complicated logic to catch bacteria: navigation might look like this electrical circuit. In the 1950's David Rogers made a video of a white blood cell chasing a bacterium. Sequential frames of the video are shown. The salmon arrow shows the direction the white blood cell movement. This task of chasing a bacterium requires an integration of complex logical decisions. A possible navigation/sensory system might look something like the electrical circuit on the right. Others have suggested the presence of complex cellular circuits but the data has never been sufficient to test for the circuits (Dueber et al., 2003).

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Programs used: Navicat, Eclipse, Excel, PowerPoint ModelSim, Quartus II

Funding: (NSF:DBI 1005223 REU Site: A broad view of environmental microbiology) and grants from NIH (GM07689, LM010101)

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Title: BIOLOGICAL EFFECTS ON SERPENTINITE WEATHERING

Abstract: Serpentinites, perhaps more than any other rock type, control the composition and evolution of the development of the surrounding ecosystems. The bulk chemistry of serpentinite rocks, high in Mg and trace elements, and low in nutrients such as Ca, K, P, and N, causes an extreme and stressful environment for ecosystems. However, the role that those serpentine ecosystems play in development of serpentine soils has not been examined.

Due to the unusual chemistry of serpentine soils, serpentine ecosystems have deeper and better-developed root systems than other ecosystems. The rhizosphere of serpentine systems, documented to produce abundant organic acids and siderophores, is also likely to impact serpentine soils. In order to test the effects of biological impacts on serpentine soil formation, soil pore waters will be analyzed for siderophores and organic acids. Furthermore, Fe-oxidizing bacteria have been detected using Biological Activity Reaction Tests (BARTs) and cultures of such bacteria will be grown. In addition to directly measuring the biological factors including organic acids, siderophores, and Fe-oxidizing bacteria, the impact of such weathering on soils and rock was examined using XRF, XRD, and SEM.

Why are you doing this project? To determine a future field and potential school/advisor for graduate school.
What problem are you trying to solve? Measuring the biological factors including organic acids, siderophores, and Fe-oxidizing bacteria.
What tools or equipment are you using? XRD, XRF, SEM, UV-VIS, GC/MS, BET
Why is your project worth researching? Serpentinites, perhaps more than any other rock type, control the composition and evolution of the development
of the surrounding ecosystems. The bulk chemistry of serpentinite rocks, high in Mg and trace elements, and low in nutrients such as Ca, K, P, and N, causes an extreme and stressful environment for ecosystems. However, the role that those serpentine ecosystems play in development of serpentine soils has not been examined.
What relevance will it have on the community, society, and in your research field? It will determine the biological effects on the weathering of serpentinite for future studies.
What did you find? Nothing yet, I am still waiting on chemicals to run most tests.
What is the future of your research project? Impacts specific biological factors have on the weathering rate of serpentinite. Looking into carbon sequestration .

Biological Effects on Serpentinite Weathering

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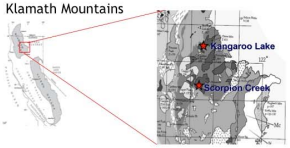
ABSTRACT

Serpentinites, perhaps more than any other rock type, control the composition and evolution of the development of the surrounding ecosystems. The bulk chemistry of serpentinite rocks, high in Mg and trace elements, and low in nutrients such as Ca, K, P, and N, causes an extreme and stressful environment for ecosystems. However, the role that those serpentine ecosystems play in development of serpentine soils has not been examined.

Due to the unusual chemistry of serpentine soils, serpentine ecosystems have deeper and better-developed root systems than other ecosystems. The rhizosphere of serpentine systems, documented to produce abundant organic acids and siderophores, is also likely to impact serpentine soils. In order to test the effects of biological impacts on serpentine soil formation, soil pore waters were analyzed for organic acids. Furthermore, Fe-oxidizing bacteria have been detected using Biological Activity Reaction Tests (BARTs) and such bacteria were investigated by enrichment cultures. In addition to directly measuring the biological factors including organic acids, siderophores, and Fe-oxidizing bacteria, the impact of such weathering on soils and rock was examined using XRF, XRD, and SEM.

FIELD AREA

Klamath Mountains



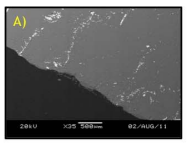
Serpentinite derived samples were collected on ridge tops of the Trinity Alps of the Klamath Mountains.

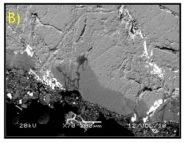
To avoid areas that have been glaciated more recently than 10 ka (> 6800 ft), samples were collected between elevations of 4500 - 6000 ft to confirm a glaciation date of approximately 12 ka.

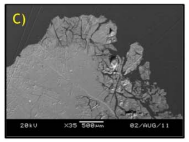
METHODS

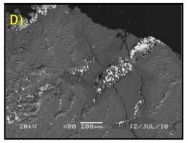
Sample Collection	Samples were stored in the refrigerator after collecting from soil pits and rock cores.
Mineral Content	XRD: Analyzed from powdered samples.
	XRF: Analyzed for major elements from powdered and heated samples.
	SEM/EDS: Analyzed from epoxy embedded and polished samples.
Siderophores	UV-VIS: Pore water samples will be mixed with a CAS assay and analyzed.
Bacteria	Biological Activity Reaction Tests (BARTs): Mixed samples with a phosphate buffered saline solution and observed over time.
	Cultures: suspended rock samples in vitamin rich gel with a iron sulfide base and observed over time.
Organic Acids	UV-VIS: Detection of organic acids by absorbance at 253.7 nm.

SEM



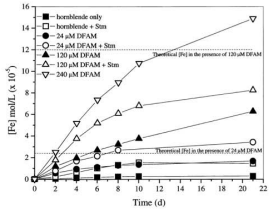






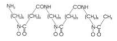
SEM pictures of samples: In each case the image is of a cross-section through a characteristic weathered surface of the rock into the unweathered interior. Light grey mineral in all samples was identifed as lizardite. White minerals were primarily iron oxides.A) Scorpion Creek rock 70-76 cm. B) Scorpion Creek Soil 0-10 cm. C) Kangaroo Lake rock 57-66 cm showing visible signs of weathering D) Kangaroo Lake soil 60 cm.

SIDEROPHORES

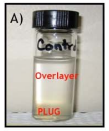


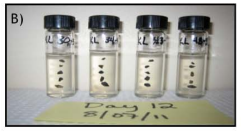
$$FeDye + L \rightarrow FeL + Dye$$

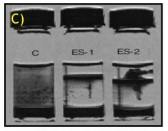
Left: Concentration of iron over time from dissolution experiments of the mineral hornblende with and without the siderophore desferrioxamine B. (DFAM) (Liermann et al., 2000). Below: structure of DFAM (Liermann el al., 2000). Above: Schematic of the test for siderophores (Schwynn and Neilands, 1987), in which the siderophore (L) reacts with the dye Chrome Azurol S, resulting in a color change which allows the concentration of the siderophore to be measured.



BACTERIA

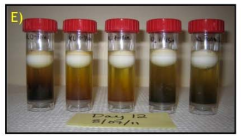


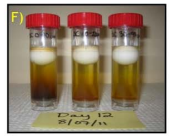




(Emerson and Moyer 1997)

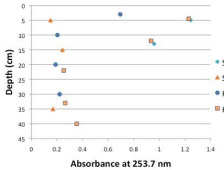
Iron oxides from oxidized ferrous iron adhere to surface silicate clays decreasing the weathering rate. Iron oxidizing bacteria would most likely be found in the low oxygen environment in deep rock samples. A) Control. B) Kangaroo Lake rock deeper set of samples. C) Representation of iron-oxidizing bacteria growth.



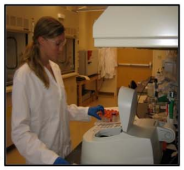


Iron related Biological Activation Reaction Tests (BARTs), in which 0.5 g of rock or soil are combined with phosphate-buffered saline solution and a nutrient pellet. D) Control. E) Kangaroo Lake soil samples. F) Scorpion Creek samples. Growth of iron related bacteria would result in a brown ring, brown gel, or brown cloud.

ORGANIC ACIDS



Left: Absorbance at 253.7 nm of pore waters versus depth. SC and KL refer to Scorpion Creek and Kangaroo Lake sites respectively. Right: UV-VIS analysis for organic acids in sample pore waters.



CONCLUSIONS

- Bulk chemistry and bulk minerology analysis indicated lizardite as the main mineral in the rock samples.
- SEM analysis indicates weathered surfaces.
- In order to confirm the presence of iron related bacteria previously detected, Biological Activation Reaction Tests and Fe-oxidizing samples will be allowed to culture for an extended period of time. Previous tests have implied longer time needed for growth.
- Ultraviolet absorbance of porewaters suggests decreasing concentrations of UV-absorbing compounds with increasing depth.
- Organic acids are likely to be present but other organic compounds such as lignin, tannin, humic substances, and aromatic compounds also absorb UV light.

FUTURE WORK

Siderophores	What type of siderophores are present in weathered serpentinite?
	What are the effects on weathered serpentinite?
Iron Oxidizing Bacteria	How many bacteria are present?
	Does the number of bacteria have a major impact on the weathering rate?
	How does the abundance of iron affect the bacteria?
Organic Acids	Which organic acids impact the weathering rate the most?
	Does the depth of the soil have an impact on the amount of organic acids present?
	What are the controlled effects of specific organic acids on the weathering of serpentinite?
Overall	What is the biological effect on serpentinite weathering?

ACKNOWLEDGEMENTS

We would like to thank the National Science Foundation Research Experience for Undergraduates program A Broad View of Environmental Microbiology at the University of Nevada, Las Vegas (DBI1005223) for funding and UNLV Department of Geoscience and UNLV School of Life Sciences for their support. We would also like to thank Chris Adcock, Kurt Regner, Jaci Batista, Jared Gore, and Dennis Bazylinski for their assistance with materials and lab use.

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Title: Identification of Nitrifying Bacteria in a Commercial Inoculant Using Enrichment and Molecular Biology Techniques

Abstract: Nitrifying bacteria play an important role in aquatic and terrestrial environments through the nitrogen cycle. Nitrification, one of the processes of the nitrogen cycle, refers to the oxidation of ammonia to nitrate. This process requires two types of chemoautotrophic bacteria, ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB). These bacteria are essential in maintaining an optimal environment for plants and aquatic organisms, such as fish. Current applications of nitrifiers include: inoculants for aquariums, biofertilizers, and nitrogen removal in wastewater treatment plants. This study wants to identify a consortium of nitrifers that can be used to produce sufficient nitrate for plants in a hydroponic system. Previous studies have shown that Fritz-zyme turbostart 700, a commercial freshwater inoculant has had success in a semi-hydroponic system, zeoponics. Our lab's preliminary data has shown that Fritz-zyme contains more than the specific nitrifying bacteria. In order to create the optimal consortium, it would be mandatory that we know exactly what bacteria we are working with. Using 16s rDNA universal primers and PGEM-T easy vector cloning kit, this study will amplify the 16s rDNA present in different enrichment samples and clone it into the PGEM-T easy vector E. coli plasmid. The cloned plasmids are transformed into competent E. coli cells and sequenced to identify the bacteria present in each sample. This study will determine whether the current enrichment techniques being used are sufficient to eliminate the heterotrophic and spore-forming bacteria present in the original Fritz-zyme.

Why are you doing this project? I am doing this project to learn more about nitrifying bacteria and their applications in society.
What problem are you trying to solve? Our problem involves identifying the specific nitrifying bacteria present in a commercial aquarium inoculant. Our preliminary data on the product suggest that it contains more than nitrifying bacteria. We want to identify what other organisms are in the product and find ways to specifically isolate the nitrifying bacteria.
What tools or equipment are you using? Some of the many equipment that we are using for this project includes: PCR thermocycler, heating blocks, spectrophotometers, aquarium test strips, and phylogenetic analysis software. We are also using the services of UNLV Genomic Center for sequencing.
Why is your project worth researching? Learning what types of organisms are present in the commercial inoculant will be vital in helping to create a consortium of microbes capable of providing the necessary nutrients to support plant growth in a hydroponic system.
What relevance will it have on the community, society, and in your research field? This project will hopefully lead to a commercialized product that can be used to grow plants in a hydroponic system using only microbes.
What did you find? We are still awaiting results at this time.
What is the future of your research project? Determining if we can successfully isolate the necessary nitrifiers from the product and characterizing their oxidation rates of certain nitrogen compounds. The step after that will be finding out how well they will respond with a nitrogen fixing microorganism.

Identification of Nitrifying Bacteria Contained in a Commercial Inoculant Using Molecular Biology Techniques

By Anthony Harrington, John Perry, and Penny S. Amy
University of Nevada, Las Vegas-School of Life Sciences

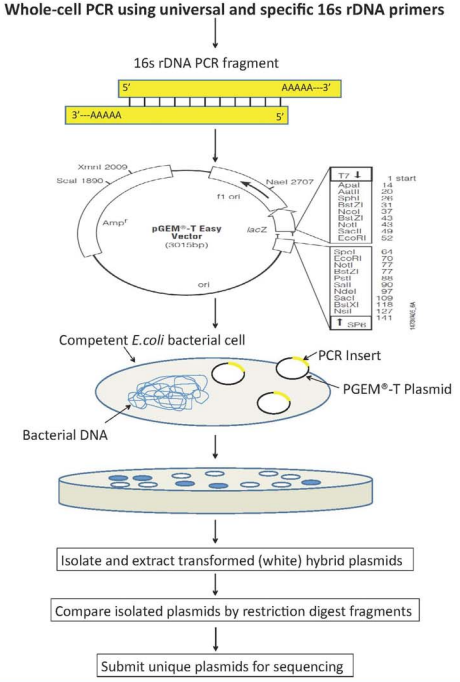
Introduction

Nitrifying bacteria play an important role in the aquatic and terrestrial nitrogen cycle. Nitrification, one of the processes of the nitrogen cycle, refers to the oxidation of ammonia to nitrate. This process requires two types of chemoautotrophic bacteria: ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). These bacteria are essential as they supply nitrate for the growth of plants and aquatic organisms.

Current applications of nitrifiers include: inoculants for aquaria, biofertilizers, and nitrogen removal in wastewater treatment plants. Previous studies have shown that Fritz-zyme Turbostart 700, a commercial freshwater inoculant, has been successfully used in a semi-hydroponic system, i.e., zeoponics. In our laboratory, preliminary data have shown that Fritz-zyme contains more than the specific nitrifying bacteria. In order to determine an optimal consortium for zeoponics, it is necessary that we know exactly what bacteria are present. Using 16s rDNA universal primers and pGEM®-T Easy Vector Cloning Kit (Promega), we amplified the 16s rDNA genes from Fritz-zyme and cloned them into the pGEM®-T Easy *E. coli* vector plasmid. The cloned plasmids were transformed into competent *E. coli* cells and sequenced to identify the bacteria present in each sample. In this study, we determined whether the current enrichment techniques being used are sufficient to eliminate the heterotrophic and spore-forming bacteria present in Fritz-zyme.

Materials and Methods

Whole-cell PCR using universal and specific 16s rDNA primers



Whole-Cell PCR followed by PCR Purification using QIAquick PCR Purification Kit (Qiagen)

TA Cloning via Ligation using pGEM-T Easy Vector Kit (Promega)

Transformation using Monserate MON1 competent cells

Blue/white colony screen on Luria Bertani agar containing Ampicillin, X-Gal and IPTG

Plasmid Prep using Qiagen Mini-Prep Kit

Restriction Digest using SAC I

Sequencing using primers T7 and SP6 at UNLV Genomics Center

Materials and Methods (continued)

Primer	5'→ 3'	Specificity
27f	AGAGTTTGATCCTGGCTCAG	Bacterial 16s rDNA gene
1492r	ACGGCTACCTTGTTACGACTT	Bacterial 16s rDNA gene
EUB338f	ACTCCTACGGGAGGCAGC	Bacterial 16s rDNA gene
Nso1225r	CGCCAATTGTATTACGTGTGA	AOB 16s rDNA gene
NIT3r	CCTGTGCTCCATGCTCCG	Nitrobacter 16s rDNA gene
Ntspa685r	CGGGAATTCCGCGCTC	Nitrospira 16s rDNA gene

Results

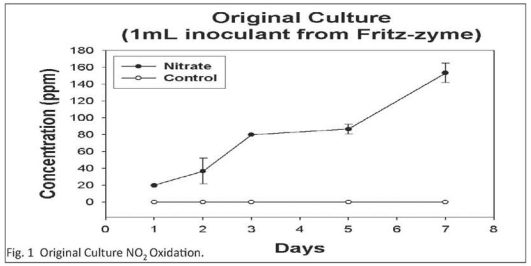


Fig. 1 Original Culture NO_2 Oxidation.

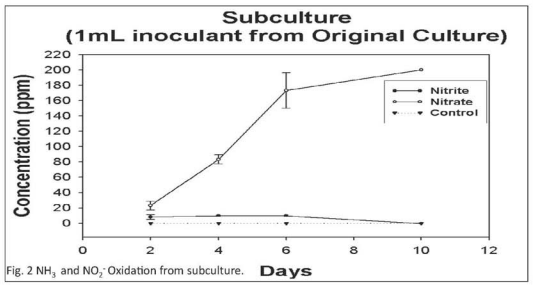


Fig. 2 NH_3 and NO_2^- Oxidation from subculture.

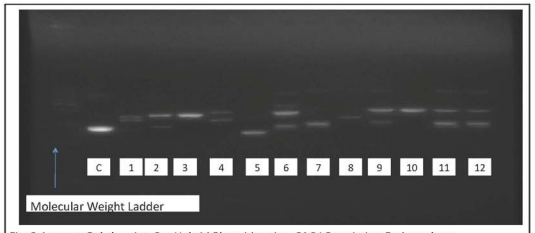


Fig. 3 Agarose Gel showing Cut Hybrid Plasmids using SAC I Restriction Endonuclease

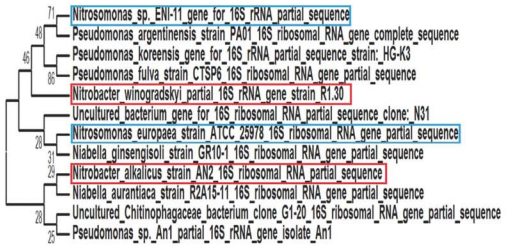


Fig. 4 Phylogenetic tree of cloned inserts created in MEGA 5.0 using results from BLASTn from NCBI Database. Contigs were created in DNA Baser Software. Values next to nodes represent percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates). Blue boxes represent Ammonia-oxidizing bacteria. Red boxes represent Nitrite-oxidizing bacteria.

Conclusion

1. We confirmed the activity of nitrifying bacteria based on NH_3 and NO_2^- oxidation using test strips.
2. Sequencing data showed the presence of Ammonia-oxidizing and Nitrite-Oxidizing bacteria in Fritz-zyme.
3. Sequencing data also showed the presence of non-nitrifying bacteria from the genera *Pseudomonas* and *Niabella*, which could indicate the presence of denitrifying bacteria as well as nitrifying bacteria.

Future Research

- Measure oxidation of NH_3 and NO_2^- using Ion-Selective Electrodes to gain a more accurate measurement of oxidation.
- Find Primers capable of amplifying 16s rDNA from sub-culture samples. Possible candidates include EUB338f and EUB338r along with specific 16s rDNA primers.
- Determine if sub-culturing techniques are suitable for isolating pure nitrifiers.

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Research Site: DRI

Title: The Use of Chloramines to Exterminate Quagga Mussel Veligers

Abstract: Quagga Mussels, Dressenia bugensis, were discovered in Lake Mead Nevada in 2007 and have since spread to other western waterways (CA, NV, AZ, UT, CO); the highest densities being in Lake Mead and Lake Mojave. Once the mussels have established themselves in an aquatic system they obstruct underwater infrastructures such as dams and can clog water intake pipes and screens reducing pumping capabilities for power and treatment plants, costing companies, industries and communities millions. Control of this invasive species is not only important for the anthropogenic uses of the bodies of water but also the overall ecology of the lake. Quagga mussels are prodigious water filterers that have the ability to reduce the phytoplankton causing changes in water transparency ultimately resulting in trophic level changes in the food web. Quagga Mussels are highly polymorphic and have the ability to adapt rapidly to extreme environmental conditions. Veligers (planktonic larval form of quagga mussels) allow them to easily infest new areas. Most of the research thus far has gone into researching the physiological processes, ecology and biology of this species; little research has gone into developing methods to control the species and stopping them from spreading to other systems. One of the commonly used treatments includes the use of chlorine. Chlorine is effective in treating veligers but it leaves a residue called trihalomethanes, which at higher concentrations can be carcinogenic. Recently, scientists have hypothesized that chloramines (chlorine and ammonia) instead of chlorine can be used as an effective alternate disinfectant with little to no harmful residual trihalomethane. The goal of this research is to test effectiveness of various dosages of chloramines on the treatment of veligers in laboratory conditions with no residual trihalomethanes. The results will not only provide important information on the use of chloramines but also have potential to develop a new economical and safer way of treating veligers in water treatment facilities.

Why are you doing this project? This research is being conducted to study viability of using cholarmines to treat quagga mussel veligers (planktonic larvae).
What problem are you trying to solve? Water treatment facilities have been using chlorine to treat veligers at their intake system. Chlorine is effective in treating veligers but it leaves a residue called trihalomethanes, which at higher concentrations can be carcinogenic. There is a need for research to find alternate chemical treatments that leave no such residues.
What tools or equipment are you using? Veligers are collected from Lake Mead using plankton tows and brought to the laboratory. The samples are then filtered through a 120µm mesh filter to filter out the larger zooplankton. The samples are then put into 500mL Imhoff settlement cones so the veligers settle at the bottom while other zooplanton smaller than 120 um remain in the upper water column. The veligers are then dosed with various concentrations of the chloramines, and the chlorine residue in the water water is measured. Number of veligers living and dead are counted under the microscope using a gridded slide.
Why is your project worth researching? This research is important because quagga mussels are causing many problems specifically at intake plants of water treatment plants. Veligers could attach at the intake pipes and block water flow costing the treatment plants millions of dollars.
What relevance will it have on the community, society, and in your research field? Finding an effective dose of chloramines to kill the veligers will not only save the treatment plants money but, will also eliminate the use of chlorine to get rid of the veligers. This leads to less trihalomethanes in the treated water and safer drinking water for the public.
What did you find? Preliminary research suggests that the quagga mussel veligers are effectively killed by chloramines, but the lowest and most efficient dose has yet to be determined.
What is the future of your research project? Our study involves finding the lowest dose that will effectively kill all the mussels while leaving little harmful residues in water.

The Use of Chloramines to Eradicate Quagga Mussel Larvae

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Abstract

Quagga Mussels, *Dressenia bugensis*, are a growing problem in the western United States, particularly in their ability to infest underwater infrastructures and clog water intake pipes and screens of power and treatment plants. Chlorine has been found to be the most effective chemical to get rid of veligers (planktonic larval form of quagga mussels) in the pipes. However, chlorine leaves a residue called trihalomethane, which is a carcinogen at higher concentrations. The purpose of this project is to test the effectiveness of an alternate chemical, chloramines (chlorine and ammonia), which leaves behind little to no residual trihalomethane. Upon experimentation with various dosages of chloramines, it was found that 1.0 mg/l effectively kills approximately 97% of the veligers after an exposure time of approximately 4 hours. Our results provide critical information needed to replace the use of harmful chlorines in drinking water systems.

Introduction

Quagga Mussels were discovered in Lake Mead Nevada in 2007 and have since spread to other western waterways (CA, NV, AZ, UT, CO); the highest densities being in Lake Mead and Lake Mojave. Once the mussels have established themselves in an aquatic system they obstruct underwater infrastructures such as dams and can clog water intake pipes and screens reducing pumping capabilities for power and treatment plants, costing companies, industries and communities millions. Quagga mussels are prodigious water filterers that have the ability to reduce the phytoplankton causing changes in water transparency ultimately resulting in trophic level changes in the food web.

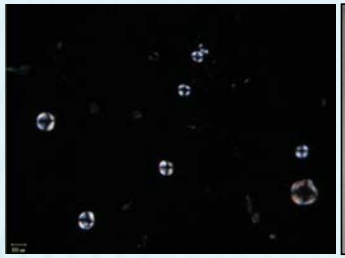
Quagga Mussels are highly polymorphic and have the ability to adapt rapidly to extreme environmental conditions. Veligers (planktonic larval form of quagga mussels) allow them to easily infest new areas. Recently, scientists have hypothesized that chloramines (chlorine and ammonia) instead of chlorine can be used as an effective alternate disinfectant with little to no harmful residual trihalomethane. Results of this project will not only provide important information on the use of chloramines but also have potential to develop a new economical and safer way of treating veligers in water treatment facilities.

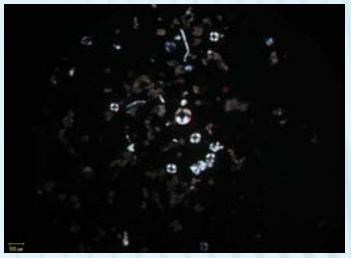
Methods

Three dosages of chloramines (0.50 mg/L, 0.75 mg/L and 1.0 mg/L) were tested on veligers These dosages were chosen through communication with Southern Nevada Water Authority (SNWA), the agency providing water services to the city of Las Vegas, and preliminary trial and error experiments. There were three replicates of each dosage and 3 controls (with veligers) without the chemical addition. There were also two chemical controls for each dosage (6 total) without veligers.

The first experiment focused mainly on a short term exposure time (5 and 10 minutes). This is due to the fragile nature of the animals, and unstable nature of chlorine for long periods of time in carbonate rich Lake Mead water. The 24 hour reading was taken to ensure that the system displayed 100% mortality. The second experiment looked at an intermediate exposure time (4 hours) to see the difference after a longer time period compared to the shorter exposure.

Veligers were sampled from the Lake Mead Marina in the mornings of the day of the experiments. Vertical tows were conducted using a 63 µm plankton net by submerging the net approximately 10 meters below the surface of the water from the dock. Approximately 15-20 tows were performed depending on veliger density on the sampling day. The samples were then placed in 250 ml sample bottles and transported back to the lab in a cooler. The lights were turned off while handling the animals and for the duration of the experiment to reduce stress on the animals. Water from the tows was then filtered with a 120 µm mesh filter, to eliminate larger zooplankton. The filtered water with veligers was then pooled into a large container to homogenize the sample and then was placed in 12 500 ml imhoff settlement cones, each filled to 250 ml. The cones were then treated with chloramines with desired residual chlorine concentration. A Hach pocket colorimeter was used to measure Cl_2 residuals after the addition of the chemical at initial, 5 minutes, 10 minutes, 4 hours (Exp. 2) and 24 hours after exposure. The Number of live and dead veligers are counted using a gridded slide 5 and 10 minutes after exposure to allow the veligers to become comfortable enough to swim on the slide. They are also counted after 4 hours and 24 hours to check for survivability over time. Each treatment is staggered to ensure that each is exposed for the same amount of time.





Results

A one-way analysis of variance (ANOVA) was performed to test overall effect of treatments on veligers. Tukey's HSD pair wise test was used to compare the effect of different dosages on veliger activities. Statistical analysis was done separately for each treatment (5 min, 10 min and 4 hrs). Values < 0.05 (95% confidence level) were considered significant. The significant differences are indicated with different letters (capital for 5 min, bold capital for 10 min and small for 4 hrs) in the figures 1 and 3. Percentages of veligers swimming were calculated on the basis of number of swimming out of the total number present. This percentage was then corrected with the average control percentage for each of the time periods. Statistical analyses were carried out using JMP software (SAS Institute, Cary, North Carolina).

Experiment # 1			
Dose mg/L	% Swimming		
	5 minutes	10 minutes	24 hours
Control	62%	60%	52%
0.50	22%	28%	0%
0.75	17%	12%	0%
1.00	9%	9%	0%

Table 1: Percent of swimming veligers for experiment #1.

Experiment # 2				
Dose mg/L	% Swimming			
	5 minutes	10 minutes	4 hours	24 hours
Control	61%	66%	56%	60%
0.50	40%	28%	26%	0%
0.75	34%	28%	10%	0%
1.00	10%	5%	3%	0%

Table 2: Percent of swimming veligers for experiment # 2.

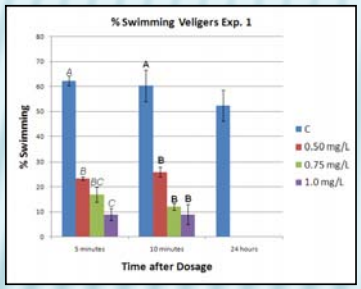


Figure 1: Number of swimming veligers for experiment #1. Experiment #1 had 5 min and 10 min exposure times.

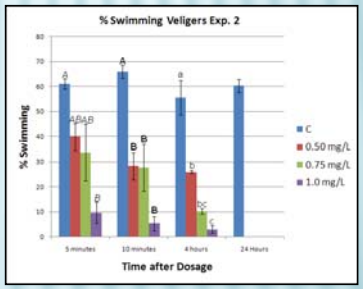


Figure 3: Number of swimming veligers for experiment #2. Experiment #2 had 5 min, 10 min, and 4 hour exposure times.

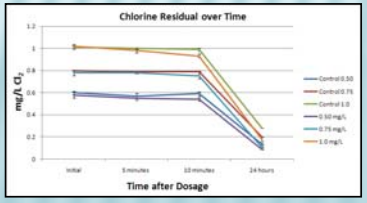


Figure 2: Residual chlorine concentration through the duration of experiment #1.

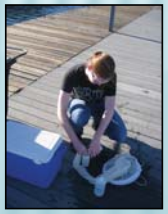


Figure 4: Residual chlorine concentration through the duration of experiment #2.

Discussion

- Both experiments display differences in percentages between the control and the dosed veliger water indicating that the chloramines are having a negative impact on the survivability of the veligers (tables 1 and 2).
- In the first experiment the statistical analysis of the percentages swimming shows that there is a significant ($p < 0.05$) effect of the treatment, however effects varied between the dosages (figure 1). 1.0 mg/L had the highest effect after 5 minute exposure time.
- In the second experiment both 0.50 mg/L and 0.75 mg/L display no significant difference between the control and the dosed water after 5 minutes.
- All three dosages are significantly different than the control in 10 minute exposure does.
- 4 hours after exposure, all three dosages are significantly different from the control, 0.75 is similar to both 0.50 and 1.0, but 0.50 and 1.0 are significantly different from each other.
- Most effective treatment dose in both experiments proved to be 1.0 mg/L. Each replicate either had 0 swimmers or only 1 swimmer which was of larger size and was clearly negatively affected by the chemical because swimming was reduced and only the movement of cilia was observed.
- After 24 hours there was a complete mortality in all dosages.
- 4 hour exposure time was chosen in the second experiment because the residence time from intake to final treatment in water treatment plants is approximately 4 hours.
- The chlorine residues for the control and dosed veliger water follow a similar trend (figures 2 and 4). Chlorine concentrations in dosed water was stable for both short exposures (5 and 10 min). The concentration slightly declined in 4 hours but there was very little chlorine left after 24 hours (< 0.3 mg/L).

Conclusions

With the significant differences in swimming between the controls and the dosed water it can be concluded that chloramines are toxic to veligers. It can also be concluded that 0.50 mg/L, 0.75 mg/L and 1.0 mg/L will cause complete mortality after a time period of 24 hours however this is not viable in treatment plants. After a time period of 4 hours 1.0 mg/L is very close to causing full mortality and may be the threshold value to cause complete mortality. It would be advisable to dose above 1.0 mg/L to achieve complete mortality of veligers in a system.

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Title: Alternate User of D-amino Acid Lies in Algae

Abstract: The recent discovery of an abundance of D-amino acid in water and soil raises the question of which organisms are able to utilize this normally toxic form. It has been shown that bacteria can uptake D-amino acids using racemases in reverse. Current research is inconclusive as to other organisms that have a racemase gene. This study was performed to determine the effect of D-amino acids on algal growth under sterile conditions in the absence of bacteria. The effects of D-glutamic acid on Micromonas pusilla, D-proline and D-leucine on Chlorella sorokiniana, and D-proline, D-alanine, and D-aspartic acid on Euglena gracilis were studied to determine if the algae would prosper, die, or display no change upon addition of the foreign nitrogen source. Preliminary results based solely upon spectrophotometer readings show the presence of D-amino acid retards and/or has a negligible effect on the growth of algae. Pending HPLC analysis, which will determine the decrease of D-amino acid in the supernatant, conclusions will be drawn in regards to whether the D-amino acid form is useable by algae.

Why are you doing this project? The D-form of amino acids is thought to be toxic to most living things, although it is known from another project in our laboratory that bacteria can grow on D-amino acids by running their racemases in reverse. This work was motivated by the recent discoveries of D-amino acids in soils and waters, which are derived primarily from cell wall (peptidoglycan) remnants of bacteria.

What problem are you trying to solve? Algae, which are composed exclusively of L-amino acids (like most organisms on Earth), may help mop up the toxic Damino acid from our environment so that we are not exposed. However, racemases are not known to be present in algae. So the question arose as to whether algae can detoxify D-amino acids or if they rely on associated bacteria to remove D-amino acids for them.

What tools or equipment are you using? Different algal strains (Chlorella sorokiniana and Euglena gracilis) were allowed to grow in their selective mediums before distribution into 24 mL test tubes.

Why is your project worth researching? A better understanding of relationships between bacteria and plants/animal have implications in agriculture and medicine. For example, it is true that gut bacteria are responsible for removing D-amino acids for us, then a patient who is taking antibiotics may have to be careful about what to eat, because some food, such as yogurt and cheese, contain bacteria and D-amino acids.

What relevance will it have on the community, society, and in your research field?

What did you find? The findings give reason to believe that certain D-amino acids can be used by algae, given the decrease in concentration in the samples. The level of aspartic acid did not display signs of decrease even though Euglena continued to grow.

D-Amino Acid Utilization in Algae

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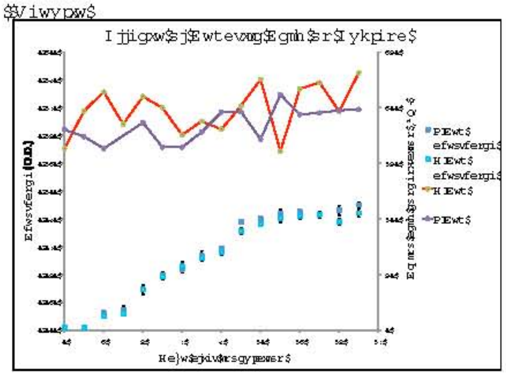

Figure 1- This graph shows amino acid concentration (shown in purple and red) while also monitoring the growth of algae (shown in blue and green). The algae continue to grow in D-Asp even though the main quantity of NaRS$_3$ is not present in the media with the level of amino acid remaining constant, giving rise to Euglena utilizing the trace amount of NaRS$_3$ found in the Trace Minerals solution.

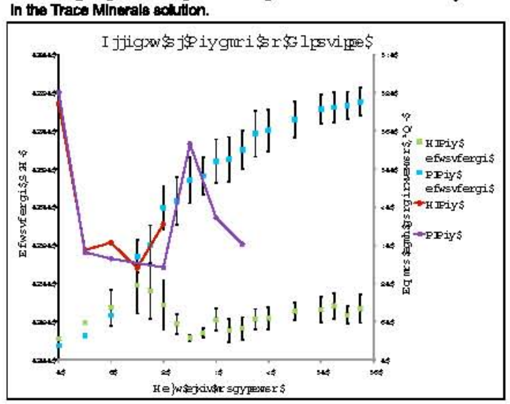

Figure 2- This graph shows amino acid concentration (shown in purple and red) while also monitoring the growth of algae (shown in blue and green). The increase of D-Leucine in solution while the absorbance of algae present in D-Leucine can be associated with algal death and the release of D-Leu into solution.

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Title: Regulation of the *Shigella flexneri icsP* gene and H-NS dependent repression

Abstract: The gram negative bacterium Shigella flexneri is known to cause dysentery in humans and primates. In order to help prevent the spread of shigellosis, gene regulation must be understood. Studies show that the virulence genes in S .flexneri are thermo regulated. At 30°C histone-like nucleoid structuring protein (H-NS) represses transcription of virulence genes and at 37°C VirB derepresses virulence genes. One of the genes that contribute to the virulence of S. flexneri is icsP. My project focuses on the regulation of the S. flexneri icsP gene and has two main goals. The first is to identify the sequence of H-NS dependent repression. Previous studies suggest that the DNA sequence necessary for the H-NS dependent repression is located between -1058 and -523 upstream of the icsP promoter. We are going to create a series of promoter truncations to positions -637, -601,-550, and -436 relative to the icsP promoter. We anticipate this will pin-point which sequences are required for H-NS dependent repression. My second goal is to further characterize the PicsP-lacZ reporter plasmid. Specifically we want to know if icsP promoter activity is being affected by an upstream chloramphenicol cassette. Our hypothesis is that the chloramphenicol cassette is not affecting icsP promoter activity. To test this we will insert a transcription terminator after the chloramphenicol cassette stopping all transcription before the icsP promoter. Promoter activity will be measured by beta-galactosidase assays. These studies will improve our understanding of the regulation of the icsP promoter, and this knowledge may be helpful when studying the regulation of other virulence genes in S. flexneri.

Why are you doing this project? I am doing this project in order to understand gene regulation in virulent bacteria.
What problem are you trying to solve? I am trying to identify the DNA sequence of H-NS dependent repression.
What tools or equipment are you using? I will be cloning genes and making plasmids. I will also be measuring promoter activity by beta-galactosidase assays.
Why is your project worth researching? My project is worth researching because it will help us prevent virulence diseases from spreading.
What relevance will it have on the community, society, and in your research field? Over one million people around the world die from shigellosis therefore it is important to understand these genes in order to prevent shigellosis.
What did you find? We have not yet determined the region where H-NS dependent repression occurs.
What is the future for your research project? Our future research project will be to better understand the gene regulation mechanism in bacteria.

Regulation of the *Shigella flexneri icsP* gene and H-NS dependent repression

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Introduction

Shigella flexneri is a gram negative bacterium which is known to cause dysentery in humans and primates. *S. flexneri* is the cause of over a million deaths worldwide especially in children and the elderly. When Shigella enters its host it is able to recruit actin from the host's cell, forming an actin based tail that allows it to move. The outer membrane protein which recruits actin is IcsA, and the protein which regulates IcsA is IcsP, where IcsP cleaves excess IcsA. The *icsP* gene is regulated by VirB and the histone-like nucleoid structuring protein (H-NS) which are two transcription factors. At 30°C H-NS represses transcription of virulence genes and at 37°C VirB derepresses virulence genes. Our goal is to identify the DNA sequences required for the H-NS dependent repression of the *icsP* promoter. These studies will improve our understanding of the regulation of the *icsP* promoter, and this knowledge may be helpful when studying the regulation of other virulence genes in *S. flexneri*.

Previous studies tell us H-NS dependent repression of the *icsP* promoter requires sequences located between -893 and -351 with respect to the transcription start site (TSS) (Fig. 1 & 2)

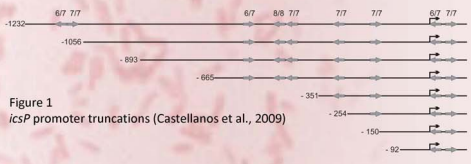

Figure 1
icsP promoter truncations (Castellanos et al., 2009)

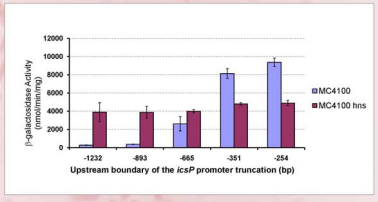

Figure 2
Experiment to identify regions of the *icsP* promoter required for H-NS dependent repression

Hypothesis 1

H-NS dependent repression requires sequences between -637 and -351 with respect to TSS.

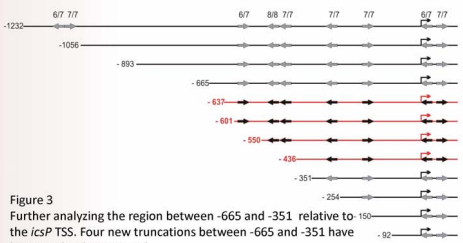

Figure 3
Further analyzing the region between -665 and -351 relative to the *icsP* TSS. Four new truncations between -665 and -351 have been made, shown in red

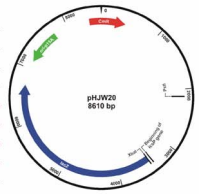

Figure 4
Promoter fragments were cloned into pHJW20 to measure Beta Galactosidase activity.

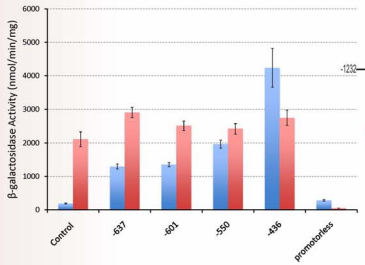

Figure 5
icsP promoter activity is less repressed near the -436 truncation

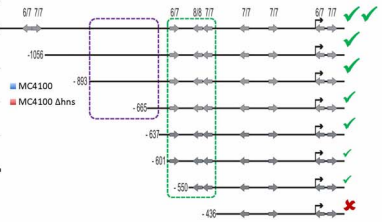

Figure 6
Sequences -893 to -637 and -601 to -436 are required for H-NS dependent repression

My second goal is to further characterize the *PicsP-lacZ* reporter plasmid. Specifically we want to know if *icsP* promoter activity is being affected by an upstream gentamycin cassette.

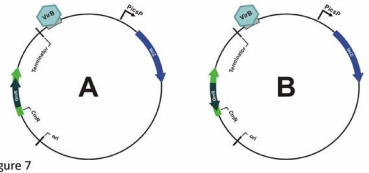

Figure 7
In plasmid A the gentamycin cassette is transcribed in the same direction as P*icsP*. In plasmid B the gentamycin cassette is transcribed in the opposite direction as P*icsP*.

Hypothesis 2

The gentamycin cassette is affecting *icsP* promoter activity.

Material and Methods

To test this we will insert a transcription terminator after the gentamycin cassette stopping all transcription before the *icsP* promoter.

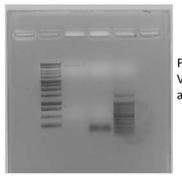

Figure 8
Vector for terminator (8573 bps) and insert (241 bps) amplification by PCR

Conclusion

1. H-NS dependent repression of the *icsP* promoter requires two sequences between -893 to -637 and -601 and -436.
2. Insert lambda *oop* (transcription terminator) was unable to be amplified by PCR, possible hairpin occurring.

Future Directions

1. Zone in on the two sequences between -893 to -637 and -601 to -436 to determine if H-NS binds within these regions.
2. Further analyze regions -893 to -637 and -601 to -436 using protein experiments
3. Assay these truncations in the presence of VirB
4. Instead of PCR amplification of transcription terminator, we will isolate the terminator DNA from a plasmid using restriction enzymes.

Acknowledgements

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• Dr. Wing, Dr. Zavala, Dr. Medh, Dr. Hogue, Nick Egan , Pashtana Usufzy, Juan Duhart, Holly Martin, Denisse Reyes , Amanda Wigley, Hiro Park and Anthony Daulo

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Title: Proposed Mechanistic Basis of Cellular Decision Making

Abstract: A cell exhibits sophisticated behaviors that exhibit decision-making capabilities. For example, phagocytic cells will chase a bacterial cell, which requires integration of many processes such as motility, sensory, navigation, and phagocytic systems. Understanding such complex cell behaviors is likely essential for treating human system failure as exemplified by type 2 diabetes where cells decide to stop eating glucose. We hypothesize that there are logical circuits within protein networks that are used by cells to make decisions. Like a cell, electrical circuits are engineered with a series of interconnected transistors capable of making decisions. At most fundamental level we can model protein interaction networks as a series of connected transistors where proteins are logic gates take two inputs and produce one output. For any two inputs, and one output logic gate there are sixteen possible logical truths (e.g AND, OR, XOR). Surprisingly, some truths are represented by biological terms such as inhibitor, constitutionally active, coincidence detector, and dominant negative, supporting our modeling approach. Furthermore our hypothesis was supported by the identification of a double inverter in examination of a AKT signaling pathway diagram. To begin to test our hypothesis, we modeled known human protein-protein interactions into a network where nodes are proteins and edges are their interactions. Here, we first looked for the structure of common electrical circuits within this protein-protein interaction network. Protein nodes were combined into units of 4 protein chains based on previously known interactions. By searching for and matching common chain components, larger circuits were built. We built structures for simple electrical circuits such as half-adder, latch, and flip-flop, as wells as more complex circuits such as several types of XORs one-then-two, and encoders. These principal components of many electrical circuits and used to construct functions that one would expect to find in cell logic processing. Due to combinatorial complexity we first through examined 50 total protein in a ~10,000 node protein interaction network constructed from several databases. We identified structures for millions of flip-flops, latches and half-adders. More complex circuits were more variable where we identified ~10 proteins with encoders, and two proteins with one type of XOR. XOR can be engineered in many ways and we searched for two types. One type of XOR was observed only twice, whereas another type of XOR was observed in ten proteins. This result suggest that circuitry may be inherited and modularized as protein domains are in proteomes.

Why are you doing this project? The human cell has always been fascinating in its complexity. The cell is made up of so many different proteins that it is hard sometimes to understand it all. I wanted to come up with a different way to look at the cell so that I could understand it better.

What problem are you trying to solve? A cell exhibits sophisticated behaviors that exhibit decision making capabilities. For example, leukocyte cell will chase a bacteria cell which requires a variety of different behaviors like stop, go, and a whole navigation system to name a few.

What tools or equipment are you using? ModelSim, Quartus II, Eclipse (java compiler), Navicat (SQL GUI), Microsoft Excel, Microsoft Powerpoint, SVN

Why is your project worth researching? Understanding complex cell behaviors is essential for treating human system failure as in the case of diabetes II where there is a common condition cells decide to stop eating glucose.

What relevance will it have on the community, society, and in your research field? The understanding of microbiology is based on the cell. The better our understanding of the cell, the better anything that relies on that knowledge like in the study of Alzheimer's disease, cancer, and all metabolic diseases.

What did you find? The cell can be represented as digital circuits. Each protein node in the network is readily labeled. As an example we found a double inverter which in computers can be used in some counters as a delay component to resolve data races.

What is the future of your research project? Hopefully we will be able to find more and more complicated circuits in the cell. If we do find some very complicated things we could certainly manufacture pieces that are missing or replace old parts. There is also the idea that a certain kind of circuit evolved and can be followed and a genetics tool.

Proposed Mechanistic Basis of Cellular Decision Making

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Introduction

A cell exhibits sophisticated behaviors that exhibit decision-making capabilities. For example, phagocytic cells will chase a bacterial cell, which requires integration of many processes such as motility, sensory, and navigation systems. Understanding such complex cell behaviors is likely essential for treating human system failure as exemplified by type 2 diabetes where cells decide to stop eating glucose. We hypothesize that there are logical circuits within protein networks that are used by cells to make decisions. Like protein interactions in cells, electrical circuits are engineered with a series of interconnected transistors capable of making decisions.

Protein interaction networks can be modeled as a network of logic gates. For any two input and one output logic gate there are sixteen possible logical truths (e.g AND, OR, XOR). Some logical functions can be represented by biological terms such as inhibitor, constitutionally active, coincidence detector, and dominant negative, supporting our modeling approach. For Example, Figure 2 shows the presence of a double inverter circuit in the Insulin signaling pathway diagram.

To begin to test our hypothesis, we modeled known human protein-protein interactions as a network where nodes are proteins and edges are their interactions. Protein nodes were combined into units of 4 protein chains based on previously known interactions. By searching for and matching common chain components, larger circuits were built. We built structures for electrical circuits such as half-adders, latches, flip-flops, XORs, implication circuits, and encoders. These principal components of many electrical circuits are often used to construct complex functional circuits that one would also expect to find in cell logic processing.

Due to vast combinatorial complexity, we thoroughly examined 69 total proteins in a ~10,000-node protein interaction network constructed from several databases. Each protein is the start protein and has on average half a million chains associated with it. We identified structures for millions of flip-flops, latches and half-adders. We also identified 26 proteins with encoders. We searched for two different implementations of the XOR logic and found 25 with one type of XOR and only 3 proteins with the other type of XOR. This result suggest that circuitry may be inherited and modularized as protein domains are in proteomes. This analysis can be extended, refined and experimentally tested.

Hypothesis

Protein interaction networks contain the structure of electrical circuits

Sample Circuit pathway

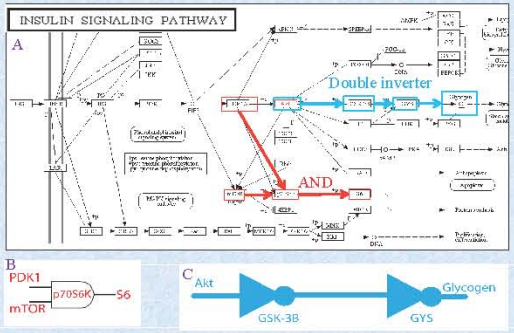


Figure 2. Components of electrical circuits as represented in a signal transduction diagram. The Insulin Signaling Pathway has a double inverters (blue) and a classic AND logic gate (red).(A) The AND gate requires two inputs to produce an output, otherwise there is no output (B). The double inverter is a combination of two NOT logic gates (C). The double inverter is used for amplification of signals.

Strategy for finding electrical circuits

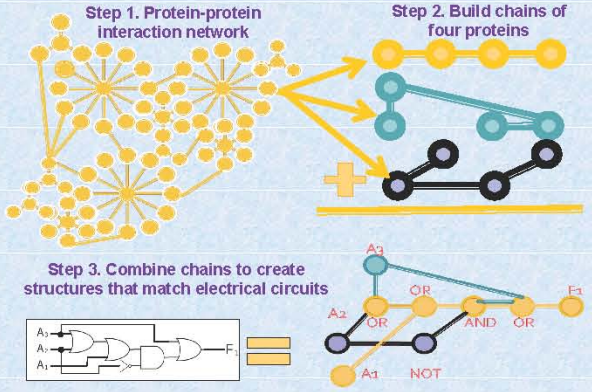


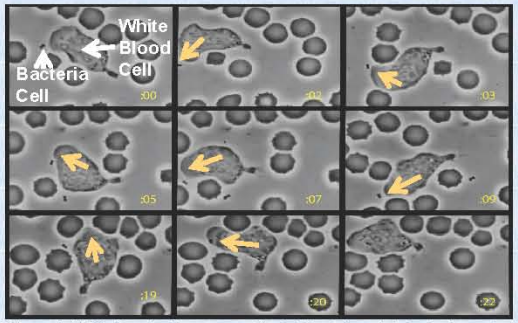
Figure 3. Strategy for identifying circuits in cell networks. 1. A human protein-protein interaction network was built from several databases. 2. All possible four protein chain assemblies (chains) were extracted from the network. 3. We identified overlap in components of those chains to search for the structures of the seven different electrical circuits.

Circuit Structures Identified

Simple Circuits		Complex Circuits	
1/2 Adder	100%	Encoder	59%
Flip Flop	95%	1 implies 2	80%
Latch	57%	XOR 1	57%
* % of 1ST proteins with identified circuits in search (out of 69 proteins)		XOR 2	7%

Conclusions:
1. Cells contain structures for common electrical circuits
2. One type of XOR was observed much more often than another type.
These results suggest that cells contain complex logical circuitry and that the XOR logic may have evolved once and then used in a modular way.

Cells exhibit complicated behaviors



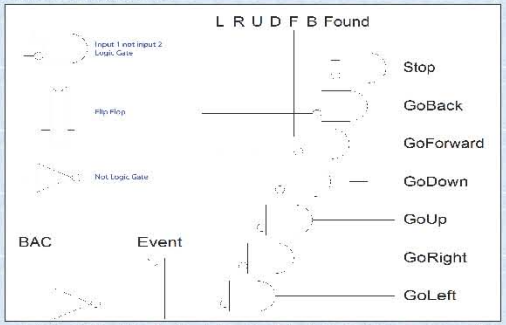


Figure 1. White blood cells use complicated logic to catch bacteria: navigation might look like this electrical circuit. In the 1950's David Rogers made a video of a white blood cell chasing a bacterium. Sequential frames of the video are shown. The salmon arrow shows the direction the white blood cell movement. This task of chasing a bacterium requires an integration of complex logical decisions. A possible navigation/sensory system might look something like the electrical circuit on the right. Others have suggested the presence of complex cellular circuits but the data has never been sufficient to test for the circuits (Dueber et al., 2003).

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Rogers, D. Neutrophil Chases and Eats a Staphylococcus aureus Bacterium 1950's. Vanderbilt University, Nashville. July 29, 2011 <http: //www. biochemweb.org/ neutrophil. shtml>.

Programs used: Navicat, Eclipse, Excel, PowerPoint ModelSim, Quartus II

Funding: (NSF:DBI 1005223 REU Site: A broad view of environmental microbiology) and grants from NIH (GM07689, LM010101)

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Title: Novel Thermophilic Cellulolytic Isolates Belonging to the Phylum Chloroflexi

Abstract: The major technological hurdle limiting second-generation biofuels technology is the difficulty in efficiently converting structurally complex lignocellulose feedstocks to fermentable sugars or directly to biofuels. Corn stover and aspen shavings were incubated in Great Boiling Spring in Gerlach, Nevada and used as inocula for 72 enrichments designed to support the growth of strongly cellulolytic microorganisms. One enrichment generated a biofilm capable of degrading insoluble cellulose, and this consortium was found to contain a mixture of rods and filaments. Rods belonging to the genera Geobacillus and Rhodothermus were isolated by plating and found to have weak cellulolytic activity on insoluble cellulose. It was hypothesized that the filaments were responsible for the strong cellulolytic activity displayed by the community, but the filaments were not observed to grow on plates.

Why are you doing this project? I am working on this project because I would like to confirm if the bacterium that I am studying is from a novel class or order. Above this, I would like to know how this bacterium degrades cellulose and the potential applications that it has on the biofuel industry.
What problem are you trying to solve? The main challenge of this project is to cultivate the bacterium efficiently for sequencing and phylogenetic analysis.
What tools or equipment are you using? We utilized 24 different kinds of media in order to determine the most suitable one. We are also growing them in incubators of five different temperatures to find out the best temperature where they grow in. Also, the liquid medium where the bacterium is grown is tested everyday for its pH and number of filaments present per ml. The medium is also tested for fermentation products by employing gas chromatography. DNA was extracted using the FastDNA Spin Kit for Soil and was amplified through PCR. The amplified DNA samples were then sent for sequencing. The sequences were then utilized to identify the identity of the bacterium. This was done by utilizing a database called BLAST which stands for Basic Local Alignment Search Tool.
Why is your project worth researching? This project is necessary to be conducted because it is a novel species of bacterium which branches off from the earliest photosynthetic bacteria in the phylogenic tree. Moreover, because the bacterium degrades cellulose, it has the potential to produce ethanol which is currently a popular subject in fuel production. Lastly, this species of bacterium can be exploited to reduce waste products that contain cellulose.
What relevance will it have on the community, society, and in your research field? This project will greatly benefit the community and society in general because if the bacterium turns out to ferment ethanol from agricultural waste products, the bacterium can be genetically modified to enhance its ethanol yield. This ethanol can then be used to run vehicles in a less expensive cost. Moreover, in the field of research, this bacterium will be an addition to the list of thermophilic bacteria ever discovered and studied. Also, further research will be needed to understand the structure and metabolism of this bacterium. By doing this, genetic modification can be done to improve its fermentative capability.
What did you find? We found out from its 16S rDNA sequence that it belongs to the Phylum Chloroflexi, a photosynthetic group of filamentous bacteria. Moreover, this new bacterium was discovered to grown in two different strains (Novel Strains JKG1 and JKG2) in the Hedlund Lab. Also, the bacterium grew in long filaments of 500-700 microns while in the R2A Agar Medium. It also grew in lesser concentration and length in the Castenholz Medium D with 1% Filter Paper (Cellulose). The fermentation metabolites are currently still in the process of being identified.
What is the future of your research project? Extensive studies must be contacted in finding out the identity and structure of the bacterium's fermentative metabolites. Moreover, this microorganism must be tested to grow with a light source to find out if it will grow better. Its genome must also be identified. Lastly, genomic and phenotypic analyses must be applied to further characterize the various strains.

Novel Thermophilic Cellulolytic Isolates Belonging to the Phylum *Chloroflexi*

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Introduction

Current biofuel technologies utilize valuable foodstuffs, such as corn kernels and cane sugar, as sources of easily metabolized sugars. Microbes are used to ferment these sugars into bioethanol, a first-generation biofuel. However, in order to avoid diverting foodstuffs from the food supply, the development of second-generation biofuels technology is necessary. Second-generation biofuels are produced by converting structurally complex lignocellulosic biomass, such as agricultural and municipal wastes, to fermentable sugars or directly to biofuels (1).

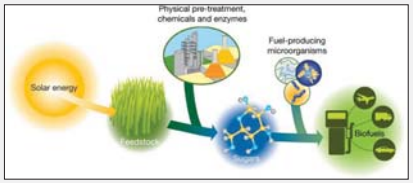


Fig. 1. Second generation biofuel processing. Solar energy is converted to lignocellulose by photosynthesis. Chemically and physically pre-treated plant material is de-polymerized into sugar monomers via microorganisms or cellulases. Simple sugars are then fermented into biofuels by microbes. (Illustration from Rubin, 2008).

The major technological hurdle limiting the mass production of second-generation biofuels is the difficulty in efficiently converting structurally complex lignocellulosic materials to fermentable sugars or directly to biofuels. The discovery of novel thermophilic microorganisms and enzymes that have high activities or broad substrate ranges on plant polymers addresses this challenge.

Research Objectives

Objective I: To obtain pure cultures of thermophilic microbes capable of cellulose degradation

Objective II: To characterize the morphology and physiology of the isolates

Objective III: To identify isolates through DNA sequencing of the 16S rRNA gene and phylogenetic analysis

Experimental Design

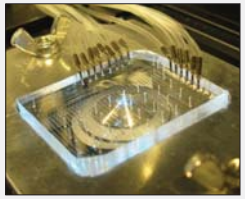
Objective I:

Corn stover and aspen shavings were incubated in Great Boiling Spring in Gerlach, Nevada (Fig. 2) and used as inocula for 72 enrichments designed to support the growth of strongly cellulolytic microorganisms. The enrichments were tubes containing Filter Paper Medium I (Castenholz Medium D with filter paper as the sole organic carbon source and additional vitamins and nitrogen).

Fig. 2. Photograph of Great Boiling Spring.

One enrichment generated a biofilm capable of degrading insoluble cellulose, and this consortium was found to contain a mixture of rods and filaments. Rods belonging to the genera *Geobacillus* and *Rhodothermus* were isolated by plating and found to have weak cellulolytic activity on insoluble cellulose. It was hypothesized that the filaments were responsible for the strong cellulolytic activity displayed by the community, but the filaments were not observed to grow on plates. Hence, optical tweezers and a microfluidic cell sorting device (Fig. 3) were employed to physically isolate the filaments from the community. 33 single filaments, six small groups of filaments, and one negative control were sorted into Filter Paper Medium I and incubated at 55 C.

Fig. 3. Photograph of a microfluidic cell sorting device.



Objective II:

Isolates were tested for growth on 12 different media known to support growth of related organisms (2-4). The media were prepared with different combinations of organic carbon sources, redox environments, pH values, and use of agitation to give a total of 28 different conditions. Gram staining was employed to characterize the cell wall of the isolates.

Objective III:

The DNA of two pure cultures, JKG1 and JKG2, was isolated, the 16S rRNA genes was amplified and the PCR products were sent for DNA sequencing. The resulting near full-length 16S rRNA genes sequences were submitted to NCBI BLAST to find and compare regions of local similarity to other microorganisms. These sequences were aligned in mothur, and then imported into ARB, where neighbor-joining and maximum likelihood trees were produced.

Results and Discussions

Objective I:

Six cultures inoculated with single filaments grew to high cell density and efficiently degraded the filter paper at temperatures ranging from 45-65 C (Fig. 4).

Fig. 4. Photograph depicting the extent of filter paper degradation performed by isolates after 16 days of incubation in Filter Paper Medium I at 50 C.

Objective II:

All six isolates were unbranched multicellular filaments ranging from 20 to >600 µm in length and 0.7-0.8 µm in width (Fig. 5A,B). All isolates were capable of gliding motility but were not motile in liquid medium.

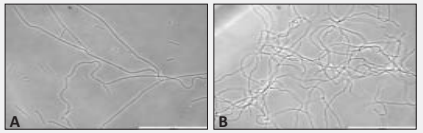


Fig. 5. Phase-contrast micrographs of the isolates in Filter Paper Medium I (A) and in R2A Medium (B).

Of the 28 different conditions tested, the best growth was observed in R2A Medium while shaking at 65 RPM. Filaments sometimes aggregated into spherical (Fig. 6A) or thread-like (Fig. 6B) structures and were observed to cling to glass surfaces. The isolates stained Gram-negative. In addition, some dense cultures were visibly pink or red and all cell pellets produced through centrifugation were pink or red.

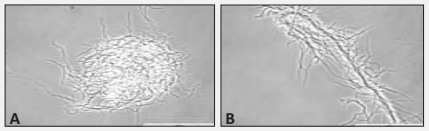


Fig. 6 Phase-contrast micrographs displaying isolates aggregating into spherical (A) and thread-like (B) structures in R2A medium.

Objective III:

BLAST results showed that the isolates are members of the Phylum *Chloroflexi*, with 84% sequence identity similar to their nearest cultured relative, *Roseiflexus castenholzii*, indicating that the isolates are only distantly related to cultivated microorganisms. The evolutionary distance tree (Fig. 7) places the isolates within the Class *Chloroflexi*, branching between *Herpetosiphon aurantiacus* (non-photosynthetic) and *Roseiflexus castenholzii* (photosynthetic).

If photosynthetic, the isolates could be the most phylogenetically "ancient" phototrophs, while if not photosynthetic, the isolates could shed light on the evolution of photosynthesis.

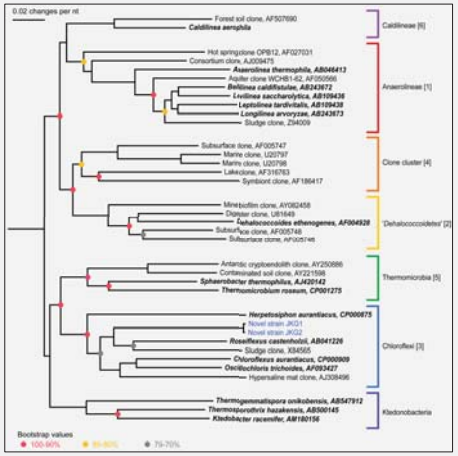


Fig. 7. Neighbor-joining tree of the Phylum *Chloroflexi*. 10,000 bootstrap replicates were performed and node support of at least 70% is displayed. Outgroups used were *Escherichia coli* (X80725), *Bacillus subtilis* (D26185), and *Corynebacterium diphtheriae* (X84248), after Hugenholtz et al (5).

Future Directions

Detailed phylogenetic and phenotypic characterization is underway to determine whether the organisms represent a novel order or family within the *Chloroflexi*, further resolve the branching pattern of the phylogenetic tree, and to delineate isolate activities against specific biopolymers. Future enzymatic and genomic characterization will determine whether these organisms or their enzymes may be useful for increasing the efficiency of second-generation biofuels technology.

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Acknowledgements

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Title: Inactivation of spo0A gene increases Stationary Phase Mutagenesis in Bacillus subtilis

Abstract: Stationary phase mutagenesis occurs when a population of cells acquires mutations conferring escape from non-growing or stress conditions. This type of mutations is observed in nutritionally starved cells. Because the mutations occur after the onset of stress and in cells that are in non-replicative conditions, elucidating the underlying mechanisms contributes novel views to the process of evolution and apply to the formation of cancer in human cells and antibiotic resistance in microbial pathogens. Studies have shown that in Bacillus subtilis, the Mfd protein which is a transcription repair coupling factor is necessary for this phenomenon to occur. Here, we investigate the effect of expression of heterologous Mfd proteins on stationary phase mutagenesis in Escherichia coli and B. subtilis. Mfd from both bacterial sp. was cloned under the transcriptional control of IPTG. Results and their implication on how Mfd mediates the formation of stationary-phase mutations are discussed.

Why are you doing this project? To gain further understanding of how the Mfd protein affects stationary phase mutagenesis in protein from different bacterial sp.
What problem are you trying to solve? To determine how cells generate mutations when they are not dividing. This process applies to the development of many human diseases.
What tools or equipment are you using? PCR machine, gel electrophoresis, Klett meter, molecular and microbial techniques such as cell culturing, plating, preparation of media, and cloning.
Why is your project worth researching? The process under study is central to evolution and is unraveling novel mechanisms in molecular genetics. We can relate our research to antibiotic resistance and cancer in humans.
What relevance will it have on the community, society, and in your research field? We can relate the concept of stationary phase mutagenesis with cancer.
What did you find? Currently in the works
What is the future of your research project? The results of my research will inform on how and what parts of Mfd are important for stationary-phase mutagenesis.

Inactivation of *spo0A* gene increases Stationary Phase Mutagenesis in *Bacillus subtilis*

Denisse Reyes[1], Amanda Prisbrey[2], Holly Martin[2], and Eduardo A. Robleto[2]

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Abstract

Stationary phase mutagenesis occurs when a population of cells acquires mutations conferring escape from non-growing or stress conditions. This type of mutations is observed in nutritionally starved cells. Because the mutations occur after the onset of stress and in cells that are in non-replicative conditions, elucidating the underlying mechanisms contributes novel views to the process of evolution and apply to the formation of cancer in human cells and antibiotic resistance in microbial pathogens. In *B. subtilis*, entry into stationary phase activates the development of differentiated cell subpopulations that confer competence, cannibalism, antibiotic production, biofilm formation and sporulation. Here we investigate whether the development of endospore formation influences the ability of cells to generate stationary-phase mutations. One key factor in the development of sporulation is the Spo0A transcription factor. We examined the accumulation of mutations in two chromosomal markers in cells that were either Spo0A proficient and Spo0A deficient. Cells that are deficient in Spo0A were significantly increased in accumulation of mutations that confer leucine or methionine prototrophy compared to cells proficient in Spo0A. These results then suggest that activation of the program for the development of endospores prevents the formation of mutations in stationary-phase cells.

Background

- Stationary phase mutagenesis, also known as stress-induced mutagenesis, increases genetic diversity in cells under stressful or non-dividing conditions (first observed in the 1950s by Francis J. Ryan).
- In 1990 this concept was reintroduced and studied in conditions where a *lac⁻ E. coli* strain was starved for a carbon source and observed for its accumulation of lac⁺ mutations in stress conditions. It was observed that cells accumulated more mutations in conditions of stress than in growth conditions .
- Studies have shown that the *E. coli lacI* system has two mutational pathways, the adaptive point mutation and the adaptive amplification pathway (Rosenberg and Hastings, 2004).
- In *Bacillus subtilis*, studies have shown that the Mfd protein influences the generation of stationary phase mutations, whereas in *E. coli* Mfd does not seem to affect the generation of adaptive mutations.
- Despite the different mechanisms between *B. subtilis* and *E. coli* there are some similarities; error-prone DNA replication influences stress-induced mutations in both systems (Robleto et al. 2007).
- Sporulation by *B. subtilis* is a developmental process that is responsible for the conversion of a growing cell into a dormant cell type known as an endospore. This developmental program gets activated during conditions of stress and results in the production of a cell subpopulation.
- Entry into stationary phase in *B. subtilis* is activated by the Spo0H sigma factor and results in the formation of different cell subpopulations; one of these subpopulations consists of those cells that form spores.
- The master regulator for entry into sporulation in *Bacillus subtilis* is the DNA-binding protein Spo0A, which has been found to influence the expression of over 500 genes during the early stages of development (Molle et al. 2003).
- Here, we investigate the effect of Spo0A on stationary phase mutations that confer methionine or leucine biosynthesis.

Hypothesis

From a germline perspective, the formation of endospores requires high fidelity DNA replication to ensure genome integrity. Thus, inactivating the sporulation gene spo0A increases the number of mutations in stationary phase mutagenesis in *Bacillus subtilis*.

Methods

Strains used:

- **AAP101 (MF476→YB955)**
- **YB955 (*hisC952 metB5 leuC 427 xin spβ^{SENS}*)**

Strain AAP101 was constructed by isolating DNA from strain MF476 and transforming strain YB955 as previously described in Boylan et al. paper in 1972. Transformants were selected on plates containing erythromycin at concentration of 1µg/mL)

Stationary Phase assay

References

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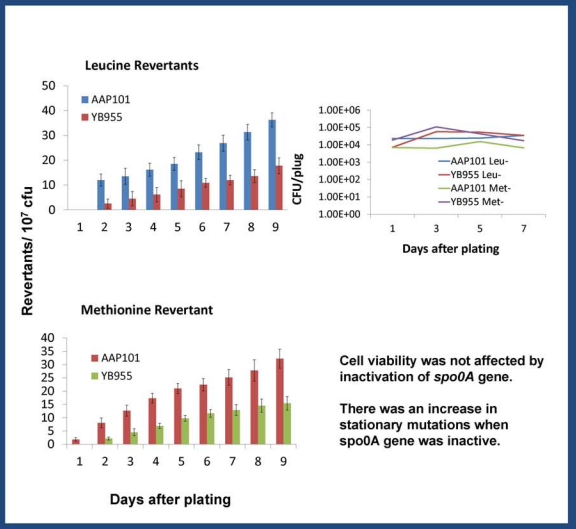
Acknowledgements

This project was supported by NSF grants DBI 1005223 (REU Site: A Broad View of Environmental Microbiology) and NSF MCB0843606.

Strain MF474 were a kind gift from The laboratory of Dr. Masaya Fujita at the University of Houston.

Results



Cell viability was not affected by inactivation of *spo0A* gene.

There was an increase in stationary mutations when spo0A gene was inactive.

Conclusions

- Inactivation of the sporulation gene *spo0A* increases stationary phase mutagenesis in *Bacillus subtilis*.
- Activation of the sporulation program inhibits development of stationary phase mutations.

Future Directions

- Examine revertants at the Histidine allele.
- Examine the effect of single inactivation of Spo0H and in combination with Spo0A on stationary phase mutagenesis.

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Title: Magnetosome Genes in the Gammaproteobacterium Strain BW-2

Abstract: Magnetotactic bacteria (MTB) biomineralize intracellular nanometer-sized, magnetic crystals surrounded by a lipid bilayer membrane known as magnetosomes. These crystals, which consist of magnetite (Fe3O4) or greigite (Fe3S4), cause the cell to align along the geomagnetic field lines as they swim, a phenomenon known as magnetotaxis. Strain BW-2 is a magnetite-producing magnetotactic bacterium isolated from Badwater Basin, Death Valley National Park (California) and is one of only two species of MTB that are known to phylogenetically belong to the Gammaproteobacteria class of the Proteobacteria phylum. The biomineralization of magnetite in magnetotactic bacteria is mediated by a series of genes that include the mam, mms, and mtx genes that presumable control the production of and the size and shape of the magnetite crystal within the magnetosomes. Magnetosome genes have not yet been found in the genomes of magnetotactic Gammaproteobacteria. In this study, we use the polymerase chain reaction with degenerate primers designed from mam genes in other magnetotactic bacteria, and DNA sequencing to search for and amplify possible mam genes in the Gammaproteobacterium strain BW-2. In addition, with enough DNA sequence, we may be able to find evidence of the presence of a magnetosome gene island in this organism. Positive results from this study will be instrumental in determining evidence for lateral gene transfer of the magnetosome gene island to the Gammaproteobacteria and the evolution of magnetotaxis based on magnetite biomineralization in general.

Why are you doing this project? I am doing this project in order to find evidence of the presence of the magnetosome gene island which will also determine evidence for lateral gene transfer of these islands and the evolution of magnetotaxis.
What problem are you trying to solve? By using polymerase chain reactions, I was able to determine if the organism had the possible mam genes that code for the biomineralization of these crystals and with enough DNA sequencing determine the presence of the magnetosome island.
What tools or equipment are you using? So far, I have been able to amplify two mam genes: mamk and mamO from the strain BW-2.
Why is your project worth researching? Studying the biomineralization of these crystals in magnetotactic bacteria is important in many commercial and medical applications.
What relevance will it have on the community, society, and in your research field? An example would be the separation of cells and the diagnosis and treatment of cancerous tumors.
What did you find? Our results make strain BW-2 an excellent candidate for a whole genome sequencing.
What is the future of your research project? To further our research.

Magnetosome Genes in the *Gammaproteobacterium* Strain BW-2

Lucero Rivera[1], Denis Trubitsyn[2] and Dennis A. Bazylinski[2]
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Abstract

Magnetotactic bacteria (MTB) biomineralize intracellular nanometer-sized, magnetic crystals surrounded by a lipid bilayer membrane known as magnetosomes. These crystals, which consist of magnetite (Fe_3O_4) or greigite (Fe_3S_4), causes the cell to align along the geomagnetic field lines as they swim, a phenomenon known as magnetotaxis. Strain BW-2 is a magnetite-producing magnetotactic bacterium isolated from Badwater Basin, Death Valley National Park (California) and is one of only two species of MTB that are known to phylogenetically belong to the *Gammaproteobacteria* class of the *Proteobacteria* phylum. The biomineralization of magnetite in magnetotactic bacteria is mediated by a series of genes that include the *mam*, *mms*, and *mtx* genes that presumably control the production of and the size and shape of the magnetite crystal within the magnetosomes. Magnetosome genes have not yet been found in the genomes of newly discovered magnetotactic *Gammaproteobacteria*.

In this study, we use polymerase chain reaction with degenerate primers designed from *mam* genes found in other MTB, and DNA sequencing to search for and amplify possible *mam* genes in the *Gammaproteobacterium* strain BW-2. In addition, with enough DNA sequence, we may be able to find evidence of the presence of a magnetosome gene island in this organism. Positive results from this study will be instrumental in determining evidence for lateral gene transfer of the magnetosome gene island to the *Gammaproteobacteria* and the evolution of magnetotaxis based on magnetite biomineralization in general.

Introduction

Magnetotactic bacteria (MTB) are Gram-negative prokaryotes that display a phenomenon known as magnetotaxis where these organisms align along geomagnetic field lines as they swim (Lefevre *et al.* 2011). MTB experience a torque in a magnetic field that causes them to passively align along magnetic field lines: cells are not being pulled in any direction (as shown on Figure 1) and even dead cells align but don't swim. This phenomenon is caused by the ability of MTB to biomineralize intracellular magnetic crystals (Figure 2) that consist of either an iron oxide or iron sulfide surrounded by a lipid bilayer known as magnetosomes (Bazylinski and Frankel, 2004). Most of these organisms organize magnetosomes in one or more chains (Bazylinski and Frankel, 2004). The control of production and specific characteristics that these crystals display are mediated by a series of genes known as the *mam*, *mms*, and *mtx* genes. These genes are organized in the genomes of MTB of the *Alpha-* and *Deltaproteobacteria* within a genomic island, known as the magnetosome island. Genomic islands are typically surrounded by transposable elements such as insertion sequences, transposases, and pseudogenes (Bazylinski and Frankel, 2004). Because of this, magnetosome genes and the trait of magnetotaxis is thought to have been transmitted to many different bacteria through horizontal gene transfer.

The purpose of this study is to search for and amplify *mam* genes in the newly-isolated *Gammaproteobacterium* strain BW-2. If enough DNA sequence is obtained, it may be possible to show evidence for the existence of the magnetosome island in this organism thus also providing evidence for horizontal gene transfer of the magnetosome island to the *Gammaproteobacteria*.

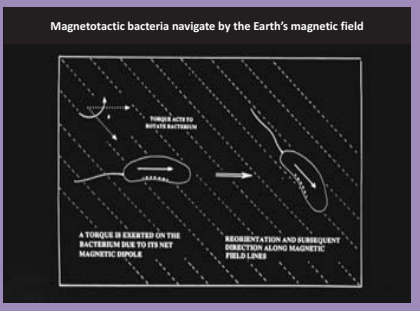


Figure 1. The effect of the Earth's inclined geomagnetic field lines on a magnetotactic bacterium. Cells experience a torque that tends to align them along the field lines while they swim, as if it were a compass needle. Cells are not being pulled in any direction.

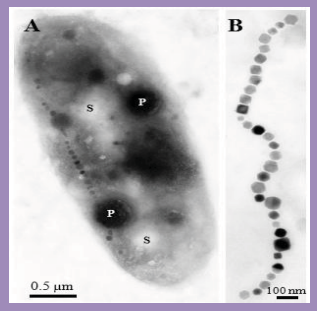


Figure 2. Transmission electron micrograph of a cell of strain BW-2 (A) and a magnetosome chain from the organism (B).

Results

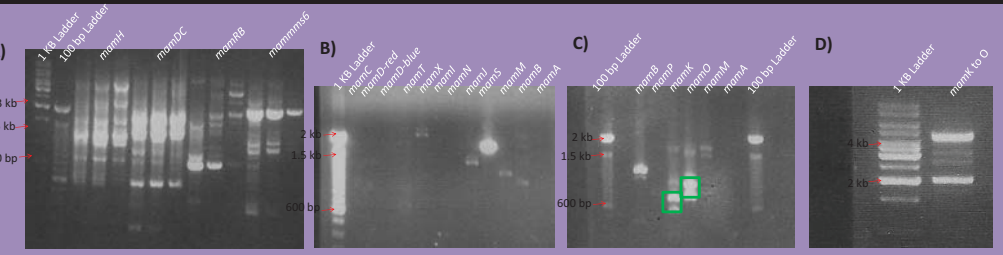
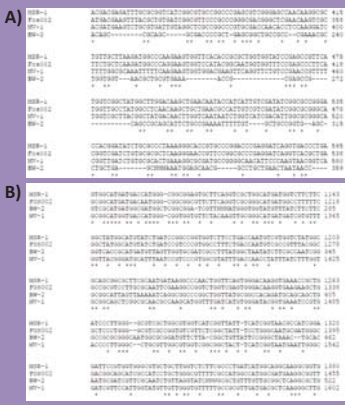


Figure 3 A-E. A) PCR products using primers for the genes *mamH, mamD, mamC, mamR through mamB, mamS*, and *mms6*. Each primer was tested at the temperatures of 47, 50, 54° C. Sizes did not coincide with expected values. **(Top Right) B)** Amplified products using primers specifically designed for strain MV-1, none with products at expected values. **(Top Right) C)** DNA gel suggests the presence of genes *mamK* and *mamO*, that is, there are PCR products with predicted sizes of 586 bp and 1 kb. **(Top) D)** DNA gel of PCR products using primers specifically designed using authentic DNA sequence of BW-2 genes *mamK* and *mamO* in order to amplify the region between these two genes which, based on the arrangement of *mam* genes in other magnetotactic bacteria, should contain several *mam* genes and PCR product should have an expected size of approximately 4 kb. **(Top) E)** Molecular organization of magnetosome genes from previously described MTB. **(Left)**

**DNA markers used are GeneRuler™ 1 kb DNA Ladder (Fermentas) and TrackIt 100 bp DNA Ladder (Invitrogen)*

Figure 4. Alignment of DNA sequence of the amplified fragments *mamK* **(A)** and *mamO* **(B)** for the strain BW-2 compared with the *mamK* and *mamO* genes of *Magnetospirillum gryphiswaldense (MSR-1), Candidatus* Magnetovibrio blakemorei (MV-1) and an uncultivated bacterium (Fos002).



Methods

The polymerase chain reaction (PCR) using degenerate primers (Table 1) for specific magnetosome genes was employed to amplify possible *mam* genes in strain BW-2. Primers were designed from *mam* genes found in magnetotactic *Alpha-* and *Deltaproteobacteria*. PCR products were sequenced (Functional Biosciences, Inc., Madison WI) while some PCR products were cloned into pGEM-T Easy Vector (Promega) prior to sequencing.

Gene	Sequence	Approximate size of amplified DNA fragment(bp)
MV-1 *mamA*	5'-cggaatagcttttcgcgta-3' 5'-cgttggaaatgtacgcac-3'	354
MV-1 *mamC*	5'-cgacttccatcgcatcaa-3' 5'-gcttttcagcttgtaacg-3'	322
MV-1 *mamD-blue*	5'-gcatccgctcaacgttga-3' 5'-gcggctacttttggtaacc-3'	367
MV-1 *mamD-red*	5'-ctcgttggcaagaccgtaa-3' 5'-caccgcaagttcgttct-3'	406
MV-1 *mamB*	5'-agacaagcttcaagggca-3' 5'-ccaagcgttggccataat-3'	389
MV-1 *mamI*	5'-ggattgcttgccgatttcc-3' 5'-cgtcctcgatgaggtctt-3'	181
MV-1 *mamM*	5'-gtgtgttgatggtgctca-3' 5'-cgcagcgcgaataaaagt-3'	354
MV-1 *mamN*	5'-acttgtccttaacgtgcc-3' 5'-gtgccgatgatgttaacg-3'	374
MV-1 *mamS*	5'-cttacctaccctaccct-3' 5'-gaatgtcacgggcttgat-3'	370
MV-1 *mamT*	5'-taatccaggtaaaccgc-3' 5'-tttacggtgcgcaaggca-3'	403
MV-1 *mamX*	5'-ccgaagaatggatcatcg-3' 5'-cggcttcaaaatcacatg-3'	323
MSR-1 *mamI*	5'-atgcaaaaaaccggcgt-3' 5'-tttattcttatcttcagcatcac-3'	1278
mamA	5'-wtysgtggygaaraagc-3' 5'-ctgcactraagtcaggcc-3'	319
mamB	5'-acgarvtgatgatcgcytac-3' 5'-gagsgtccatcayscgc-3'	245
mamK	5'-tccgarccgttywtggtc-3' 5'-ccaatacygsggcggmag-3'	586
mamM	5'-tsgcsgatgccatgtattcg-3' 5'-sgcgccgatgatcrccag-3'	398
mamO	5'-ctgggarcaracccggcg-3' 5'-ctgttggcratggcgtthtgc-3'	1000
mamP	5'-catggcagggyatggacg-3' 5'-tggcaatyggtgcakgcg-3'	401
BW-2 *mamK-O*	5'-cagcagttatcgatatagtcaccc-3' 5'-actagtggtgcgttaattgagg-3'	3992
BW-2 *mamK-O*	5'-taaattgatagcgtggtttgat-3' 5'-tcaattaacgccaccactaag-3'	3992
mamH	5'-ggcasggghgcbgtvtggc-3' 5'-tcrgccaartagatgaaccac-3'	395
mamD-C	5'-cgtgrmtggtyggmaagacccc-3' 5'-acvaccarvccrccrccgac-3'	1000
LM-1 *mamRB-S*	5'-tccgacgtgatcaatctgctgc-3' 5'-aatgtcgagttcgttcctgaccac-3'	1130
mms6- mamD	5'-cggttccggyctggc-3' 5'-cgtgrmtggtyggmaagacccc-3'	2700

Table 1. Degenerate PCR primers used to amplify magnetosome genes in strain BW-2.

Discussion

Here we show strong evidence that the magnetosome genes, *mamK* and *mamO*, are present in the genome of BW-2. Although, no evidence for the presence of a large number of other magnetosome genes was found in strain BW-2, the genes may still be present. This is most likely due to the fact that the degenerate primers we used were designed from gene sequences from magnetotactic *Alpha-* and *Deltaproteobacteria*. The magnetosome genes from *Gammaproteobacteria* may be significantly different enough that most of the primers we used were not effective. We also expected to amplify a fragment approximately 4 kb long, based on data from magnetotactic *Alpha-* and *Deltaproteobacteria*, between the genes *mamK* and *mamO* with primers designed directly from strain BW-2. However, we did not, and it may be that the fragment could not be amplified for a number of reasons, one likely possibility being that the organization of the magnetosome genes is different in strain BW-2 compared to other MTB (Figure 3E).

Our results make strain BW-2 an excellent candidate for a whole genome sequencing.

Future Directions

Studying magnetite biomineralization in magnetotactic bacteria is important because magnetite magnetosomes have been shown to have unique magnetic, physical and optical properties that can be exploited in a very large number of scientific, commercial and medical applications (Lang and Schüler, 2006) including magnetic cell separation and the diagnosis and treatment of cancerous tumors.

Acknowledgements

This project was funded by the U.S.National Science Foundation (NSF) grant DBI 1005223; REU Site: A Broad View of Environmental Microbiology. Research in the Bazylinski lab is supported by U.S. NSF grant EAR-0920718.

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Title: Investigating the origin of coprolites from three great basin caves

Abstract: The study of coprolites (fossilized feces) in the scientific community is proving to be a controversial topic, altering the currently accept beliefs of Clovis people migration into North America. In previous years, the oldest dated remains of human artifacts were no more than 11,000 14C yr B.P (Gilbert et al. 2008). However, recently human coprolites found in Oregon have dated 12,300 14C years B.P, about 1,000 14C years earlier than the currently accepted Clovis complex (Gilbert et al. 2008). During a recent archeological dig of the Bonneville Estates Rockshelter, coprolite remains dating 6,000 14C years were found and collected. The source of most of these 19 samples is currently unknown and some are thought to be of human origin. To test this hypothesis, DNA from samples was extracted and PCR amplified using various primers. Primers used amplified regions of mitochondrial DNA (mtDNA) specific for humans. Positive results have been seen with mtDNA primers and DNA will be sent for sequencing to detect homology of Native American origin. Primers specific for Bacteroides strains will also be used in conjunction with mtDNA, seeing as some species of Bacteroides are specific to human fecal matter (Dick et al. 2005). The biggest obstacle in this process will be avoiding contamination. All participants who may have come in contact with the extracted DNA will provide a sample of mtDNA to be sequenced and compared to coprolite sequences. If samples found are of human origin, it could provide an interesting lens into the colonization of the Bonneville Estates Rockshelter area.

Why are you doing this project? To determine if humans inhabited caves at The Bonneville Estates Rockshelter 6,000 years ago
What problem are you trying to solve? The origin of coprolites
What tools or equipment are you using? PCR (primers, LA taq, DNA extractions), gel electrophoreses (agar, TAE buffer, PCR products), sequencing, BLAST
Why is your project worth researching? Helps us understand migratory and living patterns of ancient humans
What relevance will it have on the community, society, and in your research field? Look at d, also could open up new opportunities to other scientists. Many archeologists are mostly interested in the tools they find in digs, this would spark interest in microbiologists to take on similar projects.
What did you find? Human mitochondrial DNA and prevalence of bacteria in the coprolites, in the midst of sequencing now.
What is the future of your research project? Test coprolites from other caves in the United States, perhaps may test coprolites from South Africa that date back to 160,000 14C years.

Investigating the origin of coprolites from three great basin caves

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University of Minnesota[1]; Desert Research Institute, Las Vegas, Nevada[2]; Desert Research Institute, Reno, Nevada[3]

Introduction

The study of coprolites (mummified feces) is a relatively new endeavor, which enables investigations of the health and diet of ancient people and provides some of the oldest evidence to date for the human habitation in North America (2). In this project, 18 coprolites were examined from archeological digs at three Great Basin caves: the Bonneville Estates Rockshelter (UT), Hidden Cave (NV), and Top of the Terrace Rockshelter (UT). The main objectives were: 1) to verify human origin through the presence of mitochondrial DNA (mtDNA) and 2) assuming human origin, characterize intestinal microflora of Native Americans prior to European contact. Primer sets specific for human mtDNA were employed to obtain products and establish human origin in general and Native American origin specifically (through SNP analysis). Initial microbiological efforts targeted the bacterial genus, *Bacteroides*, which tend to dominate gut flora in modern humans and thus is considered an ideal indicator for human fecal contamination (1,6). Primers targeting human-associated *Bacteroides* spp. strains were used in conjunction with human mtDNA results to further verify human origin. A major obstacle in this project, as might be expected, was damage to ancient DNA (aDNA). aDNA from coprolite samples is usually degraded into short fragments due to hydrolytic or oxidative damage, greatly reducing the possibility of long polymerase chain reaction (PCR) amplifications (4). The suggestion is that if large fragments are obtained from PCR, that the sample is most likely contaminated (3). To repair the fragmented aDNA, a technique termed reconstructive polymerization (RP) developed by Golenberg et al. (3) was applied. If these samples are found to be of human origin, it could provide an interesting lens into not only humans, but also the colonization of Western North America and beyond.

Archeological dig at Bonneville Estates Rockshelter

Archeological dig at Hidden Cave

Materials and Methods

Dr. Dave Rhode (DRI) provided 18 samples, 13 suspected to be of human origin and 5 known to be wood rat coprolites. Through carbon dating performed by Dr. Rhode, the samples are predicted to be between 1,500-6,000 years old. DNA from coprolite samples, modern human and non-human (horse) fecal matter and human mtDNA were extracted using MoBio Ultraclean Soil DNA isolation kit. The presence of bacterial 16S RNA was found using universal primers: 27F YM+3, 926R and 1496R. Prior to PCR, 1:40 dilutions of some of the templates were needed to negate the activity of inhibitors which co-purified with the DNA. Damaged aDNA was repaired using the method of reconstructive polymerization adapted from Golenberg et al. (3). *Bacteroides-Prevotella* primers Bac32F/Bac708R (Field 2000) amplified specific bacterial DNA. Primers L00654 and H00686 (Gilbert et al. 2008) were used to amplify human mtDNA.

Results

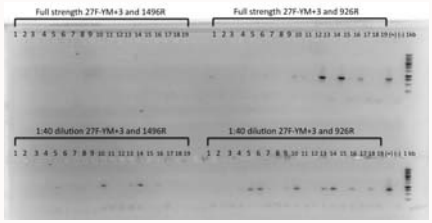

Figure 1. PCR amplification using universal 16S RNA bacterial primers 27F-YM+3 and 926R/1496R. Dilutions performed in second row to determine presence of inhibitors. Refer to figure 5 for specific sample information.

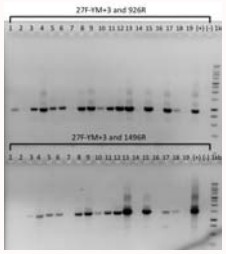

Figure 2. PCR amplification using universal 16S RNA bacterial gene primers 27F-YM+3 and 926R/1496R after reconstructive polymerization. Refer to figure 5 for specific sample information.

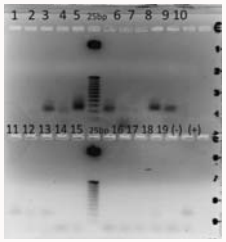

Figure 3. PCR amplification using human mtDNA primers L00654 and H00686. Refer to figure 5 for specific sample information.

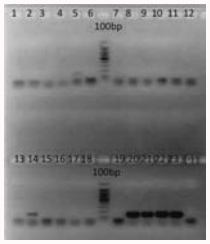

Figure 4. PCR amplification using *Bacteroides* spp. primers specific to *Bacteroides* human fecal matter. Refer to figure 5 for specific sample information.

#	Sample	12	HC 449
1	BER N7W23 Acc 24433 FS 14C0	13	HC 914
2	BER N7W23 Acc 24433 FS 14C1	14	TOT 2.8 Rat Dung not processed for DNA
3	BER N6W16 Acc 19665 FA 389	15	TOT 4 Rat Dung not processed for DNA
4	BER Acc 4482 FS 98	16	TOT 4 Rat Dung not processed for DNA
5	BER Acc 5725 FS 26	17	TOT 5 Rat Dung not processed for DNA
6	BER Acc 3425 FS 20	18	TOT 5 Rat Dung not processed for DNA
7	BER Acc 6698 FS 15	19	Blank
8	BER Acc 9588 FS 104	20	Human fecal sample
9	HC 65	21	Human Fecal sample
10	HC 342	22	Horse fecal sample
11	HC 363	23	Bacteroides DNA

Figure 5. Description of coprolite samples. BER represents samples collected from Bonneville Estates Rockshelter, HC represents samples collected from Hidden Cave and TOT represents samples collected from Top of the Terrace.

Conclusions

Original amplification using 16S universal bacterial primers showed 6 positive results at full strength and 9 positive results at a dilution of 1:40 (figure 1). This is probably due to the dilution of inhibitors that are common with aDNA (5). Coprolite DNA amplification with 16S rRNA gene universal bacterial primers after reconstructive polymerization yielded 15 positive amplifications (figure 2). Repairing the aDNA allowed for a more effective amplification for future reactions and reconstructive polymerization product was successfully used as a template for said reactions. The amplification of mtDNA as seen is promising but does not guarantee human origin (figure 3). It will be necessary to properly sequence these products to verify Native American origin and to show lack of contamination with scientists who have handled the coprolites. Samples 5 and 14 were positive for mtDNA and *Bacteroides* spp., making these samples more likely to be of human origin (figure 4).

Future Directions

- Clone mtDNA fragments into a vector to be sequenced and compared to humans of Native American origin, compare to scientist's mtDNA who have come in contact with samples
- Create 16S rRNA gene libraries of bacteria in samples
- Cultivate spore forming bacteria from samples

Acknowledgments

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Title: Synthesis of Chimeric Receptors Essential for Spore Germination

Abstract: Various species of bacteria have been reported to form an endospore, a metabolically dormant cell, during times of nutrient deficiencies and extreme stress. These said structures are outstandingly resistant to harsh chemicals, extreme temperatures, and can revert back to a metabolically active cell, through a process known as germination, when the necessary conditions are met. The rigid membrane of the endospore contains various germination (Ger) receptors which sense the external environment for necessary metabolites and germinants. Ger receptors are encoded by tricistronic operons that produce three distinct membrane proteins, the A, B, and C subunits. Although the function of the Ger receptor has been established by genetics, no information is currently available for germinant binding site. Bioinformatic and genetic approaches has predicted that the C-terminus of the B subunit is the most likely candidate to contain the germinant binding site. B. Subtilis and B. Megaterium, two species of the Bacilli genus, germinate in response to different germinants; B. Subtilis germinates in response to L-alanine by activation of the GerA receptor, while B. Megaterium germinates in response to L-leucine by the activation of its GerU receptor. The focus of this study is to construct chimeric genes in which fragments of B. Subtilis GerA receptors and B. Megaterium receptors are fused together. These B. Subtilis::B. Megaterium chimeric receptors will be introduced into the B. Subtilis genome and the mutant B. Subtilis spores will then be tested for the ability to germinate with leucine in order to establish the leucine binding site of GerUB. During the initial pilot studies, the regions coding for the N-terminus of the GerA receptor from B. Subtilis and the C-terminus of the GerU receptor from B. Megaterium were amplified using polymerase chain reaction with primer ends complementary to each other in order to further produce the desired hybrid genes without the use of restriction enzymes.

Why are you doing this project? Spores need to germinate in order to transform into a metabolically active cell. Before the cell can germinate, it must sense the environment for necessary metabolites. By transforming a spore's germination receptors, we can change what it can germinate to.
What problem are you trying to solve? Although the function of the germination receptors of spores are established via genetics, there's currently no information available on the binding site of the germinants.
What tools or equipment are you using? Polymerase chain reaction is the main tool using to achieve the focal point of this project. By amplifying various regions of the genome where the germination receptors are coded, the products can then be transformed into an organism and tested for whether or not it has the associated phenotype.
Why is your project worth researching? In addition to isolating where the germinants bind, the intermediate steps will produce methods of mutants with varying degrees of germination. If a germination receptor that binds to copper, for example, is isolated, the receptor can then be transformed into a host and the host can be used as an environmental probe.
What relevance will it have on the community, society, and in your research field? The various germinant binding sites of the Bacillus genus can be isolated. Using this method of isolation, amplification, and transformation, environmental probes can be created.
What did you find? The various genes coding for the germination receptors can be isolated and recombined, but it is not known yet whether or not the host organism will accept the plasmid containing the chimeric genes or express the phenotype.
What is the future of your research project? Various species of Bacilli all germinate to different metabolites. The binding site of the germinants in all of the species is not known and will take more than one summer or semester to decipher where these sites lie on the genome. In addition, mutants can further be created which can germinate to specific germinants, assuming that they uptake the desired plasmids.

Synthesis Of Chimeric Receptors Essential For Spore Germination

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Abstract

Various species of bacteria have been reported to form an endospore, a metabolically dormant cell, during times of nutrient deficiencies and extreme stress. Endospores are outstandingly resistant to harsh chemicals, extreme temperatures, and can revert back to a metabolically active cells, through a process known as germination, when the necessary conditions are met. The rigid membrane of the endospore contains various germination (Ger) receptors which sense the external environment for necessary metabolites and germinants. Ger receptors are encoded by tricistronic operons that produce three distinct membrane proteins, the A, B, and C subunits. Although the function of the Ger receptor has been established by genetics, no information is currently available for germinant binding site. Bioinformatic and genetic approaches has predicted that the C-terminus of the B-subunit is the most likely candidate to contain the germinant binding site. *B. subtilis* and *B. megaterium*, two species of the Bacilli genus, germinate in response to different germinants; *B. subtilis* germinates in response to L-alanine by activation of the GerA receptor, while *B. megaterium* germinates in response to L-leucine by the activation of its GerU receptor. The focus of this study is to construct chimeric genes in which fragments of *B. subtilis* GerA receptor and *B. megaterium* GerU receptor are fused together. These *B. subtilis*::*B. megaterium* chimeric receptors will be introduced into the *B. subtilis* genome and the mutant *B. subtilis* spores will then be tested for the ability to germinate with leucine in order to establish the leucine binding site of GerUB. During the initial pilot studies, the regions coding for the N-terminus of the GerA receptor from *B. subtilis* and the C-terminus of the GerU receptor from *B. megaterium* were amplified using polymerase chain reaction with primer ends complementary to each other in order to further produce the desired hybrid genes without the use of restriction enzymes.

Background

Endospores, metabolically dormant cell structures, are extremely resistant to harsh chemicals, high temperatures, and are formed when a vegetative cell is under stress or senses nutrient deficiencies. Conversely, these structures can revert back to a vegetative cell once germinants, such as amino acids, nucleosides, sugars, salts, and other extraneous nutrients are readily available (Christie and Lowe, 2008). Ger receptors, receptors located on the surface of endospores, vary from species to species and recognize various germinant compounds (Ross and Abel-Santos, 2010). In previous studies, the B- subunit of the tricistronic operons coding the Ger receptors has been reported to contain the germinant binding site (Ross and Abel-Santos, 2010) . Under the assumption that the B- subunit produces the receptor that can bind a specific germinant, the B- subunit of the Ger receptor in *B. subtilis* was amplified with an interconnecting sequence from *B. megaterium*. The focus of this study is to determine whether spore germination specific to a species can be altered and possibly amplified to germinate in the presence of foreign molecules.

Methods

Polymerase Chain Reaction (PCR)
The genomic DNA from *B. megaterium* QMB1551 (ATCC 12872) and *B. subtilis* YB955 was extracted using the Wizard Genomic DNA Purification Kit and used as a template for initial PCR reactions. Appropriate primers were designed to produce complementary ends, which would allow for the engineering of chimeric genes without the use of restriction enzymes (Horton, 1988). Primers 1 and 2, noted in fig. 2, amplifies the front end of the *B. subtilis* GerAB receptor. Primers 3 and 4 amplifies the tail end of the *B. subtilis* GerAB receptor. Primers 5 and 6 amplifies the GerUB receptor of *B. megaterium*. Samples containing the appropriate primers, PCR master mix (Promega), water, and template were subjected to 30 cycles of PCR amplification. Each cycle ran with a denaturing temperature of 94°C, annealing temperature of 50°C, and elongation temperature of 68°C. The resulting products were amplified using overhang PCR containing the appropriate primers.

Bacterial Transformations
Selected reactions containing the correct size PCR product were inserted into the Blunt II-TOPO vector (Invitrogen) following the manufacturer's protocol. The vector was then transformed into One Shot Top10 Chemically Competent E. coli (Invitrogen) following the manufacturer's protocol. Transformants were grown on Luria Bertani agar plates containing a selective antibiotic and incubated overnight at 37°C. Resulting colonies grown in the presence of the selective media were screened for the desired insert. Colonies were picked and finally added into a PCR tube to act as the template for sequential PCR reactions as previously described.

Methods (Con't)

Restriction Digest and Ligation of Products
Plasmids containing the desired insert were isolated using the QIAprep Spin Miniprep Kit (Qiagen). The insert was digested with EcoRI and incubated at 37°C for 2 hours. The PMutin4 plasmid, a plasmid that integrates directly into the *B. subtilis* genome, was digested and incubated similarly. The plasmid reaction was dephosphorylated, incubated at 37°C for 1 hour, and then 65°C for 15 minutes. Both reactions were purified and ligated together using T4 Ligase (New England Biolabs) following the manufacturer's protocol.

***B. subtilis* Transformations**
10mL of *B. Subtilis* YB955 cells resuspended in a growth medium was grown until 90 minutes after the transition between the cell's exponential and stationary phase was measured. The cells were then transferred to minimal growth media and incubated at 37°C for an hour shaking. Varying concentrations of plasmid DNA were added to the cells and incubated at an hour. The transformants were grown on tryptose blood agar base plates containing a selective antibiotic.

Results

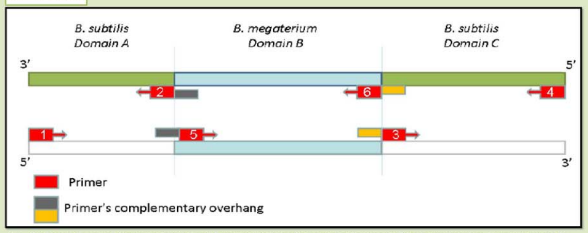

Fig. 1. Strategy used for amplifying and recombining the genes that code for germination Receptors in *B. subtilis* YB955 and *B. megaterium* QMB1551. Primers were designed to be complementary to each other; sequential PCR reactions using initial PCR products will act As templates after the first denaturing and annealing cycle. Figure was adapted from Patel et al., 2010.

Primer	Primer Sequence (5' -> 3')
1) B. Subtilis 1 Forward Primer	ATCATCGGCCTTTGATCTG
2) B. Subtilis 1 Reverse Primer	TATGGCAAAAACAGCATCACT
5) B. Megaterium Forward Primer	TGCTGTTTTTGCCATATTTAAA
6) B. Megaterium Reverse Primer	GTTATGCCTAAAGGATACGAC
3) B. Subtilis 2 Forward Primer	GTATCCTTTAGGCATAACGGT
4) B. Subtilis 2 Reverse Primer	TGCTTTAATGCAGAGGTTCTC

Fig. 2. Primers designed to amplify the genes coding for the germination receptors in *B. subtilis* YB955 and *B. megaterium* QMB1551. The highlighted regions signify the complementary overhang as depicted in Figure 1.

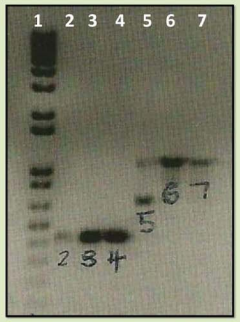

Fig. 3. PCR products of initial and sequential chimeric reactions. Lane 1 contains a molecular weight marker. Lanes 2, 3, and 4 contain the amplified region of the tail end of the B. Subtilis GerAB receptor (422bp). Lane 5 contains the initial chimeric PCR reaction consisting of the front end of the *B. subtilis* GerAB receptor and *B. megaterium* GerUB receptor (732bp).Lanes 6 and 7 contains the PCR product of the final chimeric product consisting of the *B. megaterium* GerUB receptor flanked by the Front and tail ends of the *B. subtilis* GerAB receptor (1127bp)

Results (Con't)

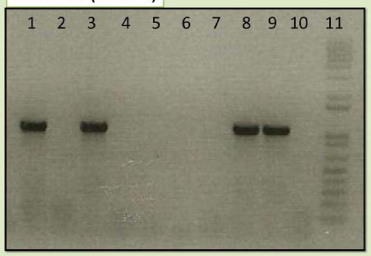

Fig. 4. Colony screen for insert into the PMutin4 Plasmid. Lanes 1, 3, 8, and 9 were positive for colonies containing the desired insert (1127bp). Lane 11 contained a molecular weight marker.

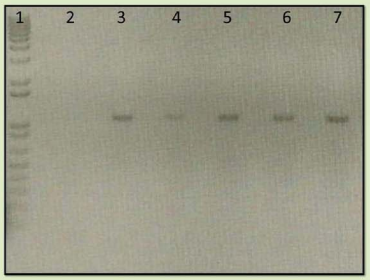

Fig. 5. Colony screen for the PMutin4 plasmid containing the chimeric germination receptor insert into *B. subtilis* YB955. Lane 1 contains a molecular weight marker. Lanes 3 through 7 were positive for *B. subtilis* colonies containing the desired insert (1127bp).

Discussion

The initial gene that codes for the proposed chimeric germination receptor was successfully generated and confirmed based on the addition of base pairs from subsequent PCR reactions when analyzed on an agarose gel. The synthesized chimeric product was then inserted into the PMutin4 vector. The PMutin4 vector was chosen due to its ability to integrate directly into the *B. subtilis* genome. The PMutin4 vector needed to be subsequently cloned into a strain that would reproduce the vector, but with homologous recombination due to *B. subtilis*' ability to first cleave circular DNA, then uptake it as a linear strand (Kaufenstein et al., 2011). B. Subtilis has been transformed and successfully screened to contain the insert. The general outline of introducing foreign germination receptor genes has been outlined in this project.

Future Direction

Having created only one mutant of wild type *B. subtilis* YB955, other mutants will be created using the same methods. Once this mutant has been tested for germination and produces desirable results, other genes coding for various other germination receptors can hopefully be integrated into the *B. subtilis* genome and then tested for. Using this strategy as a model spores can be created as used as environmental probes, germinating only when inquired germinant or compound is present.

Acknowledgements

I would like to thank Dr. Abel-Santos, Dr. Ross, and Dr. Robleto for constant guidance throughout the entire project while working on it. I would also like to thank the Abel-Santos lab, and the Robleto lab for helping me understand the various mechanisms of sporulating organisms, such as *Bacillus*.

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Title: Nitrite oxidation in the Great Boiling Spring system in the US Great Basin

Abstract: While recent advancements have contributed greatly to our current knowledge of the nitrogen biogeochemical cycle in high temperatures, there is still much more that remains unknown. In this study, we focused our attention on the second step of nitrification, chemolithotrophic nitrite oxidation. We combined in situ measurements of dissolved inorganic nitrogen (DIN) species in two hot spring outflow systems in the Great Boiling Spring (GBS) geothermal system, and characterization of nitrite-oxidizing enrichment cultures form Great Boiling Spring. Measurements of DIN showed that source water ammonia concentrations decreased in the outflow channels with concomitant increases in nitrite and nitrate concentration at >70°C and >50°C, respectively. Enrichments for thermophilic nitrite-oxidizing bacteria (NOB) inoculated with sediment/water slurries from Great Boiling spring yielded nitrite-oxidizing cultures at 50°C after 12 weeks of incubation. No successful cultures were obtained at 65°C or 80°C. Mixed cultures contained an organism with high 16SrRNA gene homology to Nitrospira calida, a thermophilic NOB from Gorjachinsk Hot Spring, Russia. Nitrite oxidation activity was more stable and reproducible under a suboxic headspace (3:1 N2:air) as compared to a full air headspace. Nitrite oxidation activity in the culture was highest at 45°C (2.4 μM/h) and had a maximum temperature of 55°C. The optimal initial nitrite concentration was 500-750 μM. The work presented here suggest a high temperature limit for chemolithotrophic nitrite oxidation somewhere between 65°C and 55°C, suggesting that a temperature-driven decoupling of ammonia oxidation and nitrite oxidation exists, consistent with the accumulation of nitrite observed in the GBS system above 60°C. Nitrospira spp. may be important nitrite oxidizers in geothermal springs worldwide.

Nitrite oxidation in the Great Boiling Spring system in the U.S. Great Basin

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Introduction

Nitrification is an important two-part step in the nitrogen cycle that leads to the production of NO_3^- (Figure 1). In the first part of nitrification, ammonia oxidation, NH_4 is oxidized to NO_2^-, and in the second step, nitrite oxidation, NO_2^- is oxidized to NO_3^-. Since no known organism can catalyze both steps of the reaction, it is useful to consider these parts separately.

Until recently, little was known about nitrification at high temperatures. Ammonia oxidation has been demonstrated up to 74°C by the thermophilic archaeon *Candidatus* Nitrosocaldus yellowstonii (2), and more recently, a thermophilic isolate, *Nitrospira calida*, was shown to oxidize NO_2^- in temperatures up to 50°C (3). While NH_4 oxidation is generally considered to be the rate-limiting step of nitrification, this may not be the case at high temperatures since accumulation of NO_2^- has been reported in some hot springs where NH_4 is the dominant form of inorganic nitrogen (1).

This project focuses on NO_2^- oxidation. To understand this process in geothermal environments, it is necessary to characterize the biogeochemistry of the hot springs and then cultivate and characterize the nitrite-oxidizing microbes that are involved in the Great Boiling Spring (GBS) geothermal system.

$$NO_2^- + O_2 \rightarrow NO_3^-$$

Figure 1. The conversion of nitrite to nitrate, the second step of nitrification.

Methods

Biogeochemistry:
Samples were collected from the outflow of two different hot springs at sites that had a difference of approximately 10°C in temperature. They were filtered, put on ice, and transported back to the lab to be analyzed using a Lachat QuikChem 8000 FIA nutrient analyzer.

Optimal Temperature and Concentration:
To quantitatively measure the optimal environment for NO_2^- oxidizers from GBS, 60 bottles designed to enrich for NO_2^- oxidizers were inoculated with 1 mL from an enrichment incubated at 55°C with a NO_2^- concentration of 300μM. For the optimal temperature experiment, 6 inoculated enrichments and 3 uninoculated controls were spiked with 300 μM NO_2^- and incubated at each temperature. For the concentration experiment 6 inoculated enrichments and 3 uninoculated controls were spiked with NO_2^- at each concentration and incubated at 45°C the NO_2^- was monitored weekly starting 2 weeks after inoculation. After six weeks of incubation the 3 inoculated enrichments with the fastest rate at each condition were chosen for the rate experiment. Samples were taken every 3 hours for the optimal temperature experiment and every 6 hours for the optimal concentration experiment. The NO_2^- levels were tested using spectrophotometry with the Lamotte kit.

Phylogenetic analysis:
16S rRNA gene was amplified and sequenced using a *Nitrospira* specific primer. The 16S rRNA gene homology was compared with known nitrite oxidizers. Sequences were obtained from the NCBI database and aligned using RDP multiple alignment.

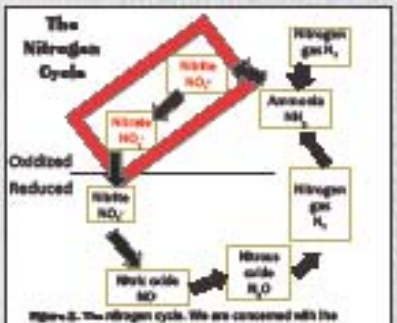

Figure 2. The nitrogen cycle. We are concerned with the second step of nitrification, which is the oxidation of nitrite indicated by the red box.

Results

Biogeochemistry

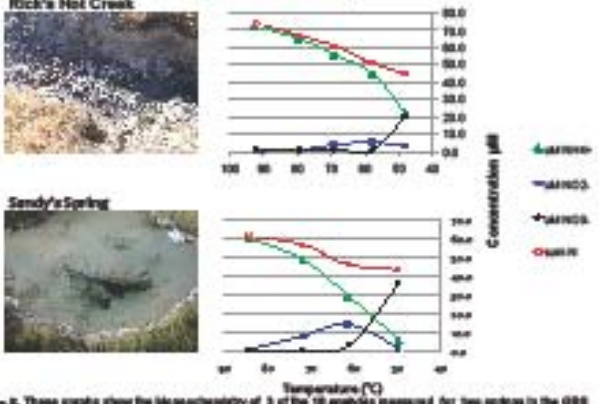

Figure 3. These graphs show the biogeochemistry of 3 of the 10 analytes measured for two springs in the GBS system. As shown above, similar patterns were observed for NH_4^+, NO_2^-, and NO_3^- for both springs. NO_2^- first became detectable between 70°C and 80°C as the amount of NH_4^+ decreased. NO_2^- first became detectable around 69°C as the amount of NO_2^- decreased.

Optimal Temperature

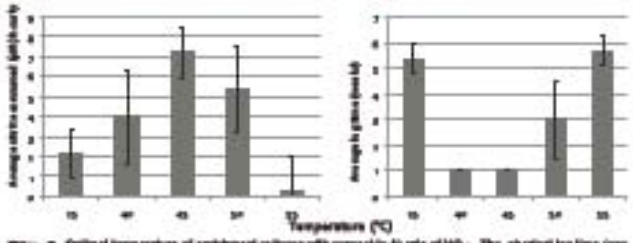

Figure 4. Optimal temperature of enrichment cultures with respect to A) rate of NO_2^-. The shortest lag time (one week) for NO_2^- oxidation occurs when the cultures are incubated at 40°C and 45°C. Error bars represent standard deviation of the mean.

Optimal Concentration

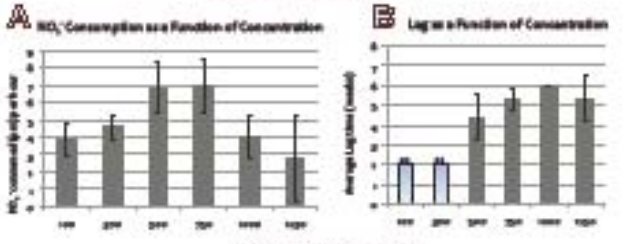

Figure 5. Optimal initial NO_2^- concentration for enrichment cultures with respect to A) rate of NO_2^- consumption and B) time of lag. These results show that the highest rate of nitrite consumption by the nitrogenous in mixed culture occurs when the initial NO2- concentration is 600μM and 700μM. However, the lag time is shortest at lower initial NO_2^- concentrations (100 μM and 300 μM). Error bars represent standard deviation of the mean.

Phylogenetic analysis

Figure 6. Phylogenetic analysis of microorganism from enrichment culture (M5501).

Discussion

While previous literature indicates that NH_4 oxidation may occur at temperatures up to 90°C (3,4,5), measurements of dissolved inorganic nitrogen showed that source water ammonia concentrations decreased in the outflow channels with concomitant increases in nitrite and nitrate concentrations at >70°C and >80°C, respectively. From our optimal temperature experiment, we found that nitrite oxidation activity in the culture was highest at 48°C (3.4 μM/h) and had a maximum temperature of 55°C. These findings suggest a high temperature limit for chemolithotrophic nitrite oxidation between 68°C and 80°C, indicating that a temperature-driven decoupling of ammonia oxidation and nitrite oxidation exists, consistent with the accumulation of nitrite observed in the GBS system above 68°C. From the optimal concentration experiment, the initial NO_2^- concentration that resulted in the fastest rate of NO_2^- oxidation (600-700 μM) was different from the concentration that produced the shortest lag time (100-300 μM). This difference may be explained by the fact that the enrichments were inoculated from an enrichment maintained at 300 μM NO_2^-. From our phylogenetic analysis, we found that mixed cultures inoculated from GBS contained an organism with high 16S rRNA gene homology to *Nitrospira calida*, a thermophilic nitrite-oxidizing bacterium from Gorjachinsk Hot Spring, Russia. Lebedeva et. al found that *Nitrospira calida* was able to oxidize NO_2^- at temperatures ranging from 37-59°C with a maximum nitrite oxidation rate at between 49-53°C (3), similar to the results presented here. All together, these results indicate that *Nitrospira* may be important NO_2^- oxidizers not only in the GBS geothermal system, but also in geothermal springs worldwide.

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Acknowledgements

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Title: Isolation, characterization, and genome sequence of the first representative of a novel class within the Chloroflexi that is abundant in some US Great Basin hot springs and may play important roles in N and C cycling

Abstract: A thermophilic, facultatively microaerophilic, heterotrophic bacterium, designated strain JAD2, was isolated from sediments of Great Boiling Spring (GBS), an ~80°C, cirumneutral hot spring in the US Great Basin (GB). The strain grew anaerobically on yeast extract or peptone with an optimal growth temperature of 70-75°C. Growth was stimulated by addition of 0.01 atm O2 to the culture vessel headspace, but was inhibited by higher concentrations (0.2 atm). Cells of JAD2 formed non-motile filaments ranging from 10 to >300 μm in length, which typically decreased in length during stationary phase. 16S rRNA gene-targeted pyrotag sequencing and clone library data suggest that close relatives of this isolate are prominent members of the sediment communities in GBS. Shotgun sequencing of the JAD2 genome produced an assembly consisting of ~3.2Mbp with an average G+C content of 67.3%. Phylogenies inferred from the 16S rRNA gene and predicted amino acid sequences of various conserved proteins indicate that JAD2 is the first cultivated representative of the GAL35 group, a new class within the Chloroflexi. Predicted genes in the draft genome encoding a putative carbon monoxide dehydrogenase (coxMSL), nitrite reductase (nrfHA) and nitrous oxide reductase (nosZ) suggest that this isolate may play important roles in N and C cycling in GBS sediments.

Isolation, characterization, and genome sequence of the first representative of a novel class within the *Chloroflexi* that is abundant in some US Great Basin hot springs and may play important roles in N and C cycling

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Abstract

A thermophilic, facultatively microaerophilic, heterotrophic bacterium, designated strain JAD2, was isolated from sediments of Great Boiling Spring (GBS), an ~80ºC, circumneutral hot spring in the US Great Basin (GB). The strain grew anaerobically on yeast extract or peptone with an optimal growth temperature of 70-75ºC. Growth was stimulated by addition of 0.01 atm O_2 to the culture vessel headspace, but was inhibited by higher concentrations (0.2 atm). Cells of JAD2 formed non-motile filaments ranging from 10 to >300 µm in length, which typically decreased in length during stationary phase. 16S rRNA gene-targeted pyrotag sequencing and clone library data suggest that close relatives of this isolate are prominent members of the sediment communities in GBS. Shotgun sequencing of the JAD2 genome produced an assembly consisting of ~3.2 Mbp with an average G+C content of 67.3%. Phylogenies inferred from the 16S rRNA gene and predicted amino acid sequences of various conserved proteins indicate that JAD2 is the first cultivated representative of the GAL35 group, a new class within the *Chloroflexi*. Predicted genes in the draft genome encoding a putative carbon monoxide dehydrogenase (*coxMSL*), nitrite reductase (*nrfHA*) and nitrous oxide reductase (*nosZ*) suggest that this isolate may play important roles in N and C cycling in GBS sediments.

Introduction

Recent studies have shown that novel phylum- and class-level bacterial and archaeal lineages are abundant in various hot springs in the US Great Basin (GB) (Costa *et al.*, 2009; Dodsworth *et al.*, 2010; Dodsworth *et al.*, 2011; Vick *et al.*, 2010). Among these, Great Boiling Spring (GBS), a circumneutral, ~80ºC spring, has been shown to have a robust biogeochemical nitrogen (N) cycle (Dodsworth *et al.*, 2011; Hedlund *et al.*, 2011). One overarching goal of our studies of hot springs in the GB and elsewhere is to understand the metabolic capabilities of microbial novel lineages and how they contribute to biogeochemical and energy cycling in these environments. Here we describe the isolation, initial characterization and draft genome sequence of strain JAD2, the first cultured representative of the "GAL35" group (Hugenholtz phylogeny, Greengenes), which likely represents a new class within the phylum *Chloroflexi*.

Methods

Ultrafiltered spring water or a synthetic medium mimicking GBS water chemistry, supplemented with $NaCO_3$, NH_4Cl, and a trace element mixture, was made anaerobic by sparging with N_2:CO_2 (80:20) for 1 hour, portioned in an anaerobic chamber, and autoclaved. Solid medium was supplemented with gelrite (0.8%) and $MgCl_2$ (0.4%). Subsequent addition of a vitamin mixture, phosphate, and complex organics from sterile, anaerobic stocks were made by needle and syringe. Liquid cultures (5 ml) were grown in 18x150 mm tubes sealed with butyl rubber stoppers with 1 atm overpressure of N_2:CO_2 in the headspace. Growth was monitored by phase contrast microscopy. 16S rRNA gene pyrotag sequencing and genome sequencing (Illumina paired end, assembled using Velvet) were performed at the United States Department of Energy Joint Genome Institute (US DOE-JGI).

Isolation and characterization

- Isolated from GBS sediment using solid media designed to enrich for heterotrophic nitrate reducers (5 mM NO_3^- and complex organics, anaerobic conditions)
- Samples of colonies were observed by microscopy; those containing filaments were streaked for isolation (5x).
- Anaerobic growth on GBS spring water medium with yeast extract and/or peptone as C and energy sources, no added e^- acceptor.
- Growth stimulated by 0.01 atm O_2, inhibited by 0.2 atm.
- Generation time of ~1 day with yield of 10^6-10^7 filaments/ml.
- Pretreatment of butyl rubber stoppers by boiling in 1% Na_2S was necessary for consistent growth in liquid culture.
- Filamentous morphology (Fig. 1); no obvious motility.
- Draft genome sequence is ~3.2 Mbp in ~80 contigs with N_{50} of 139873, G+C content of 67.3%.
- Growth on synthetic medium with added $FeCl_3$; most other trace elements appeared to prevent growth in this synthetic medium.

Figure 1. Phase contrast micrograph (400x) of a late log-phase culture of JAD2.

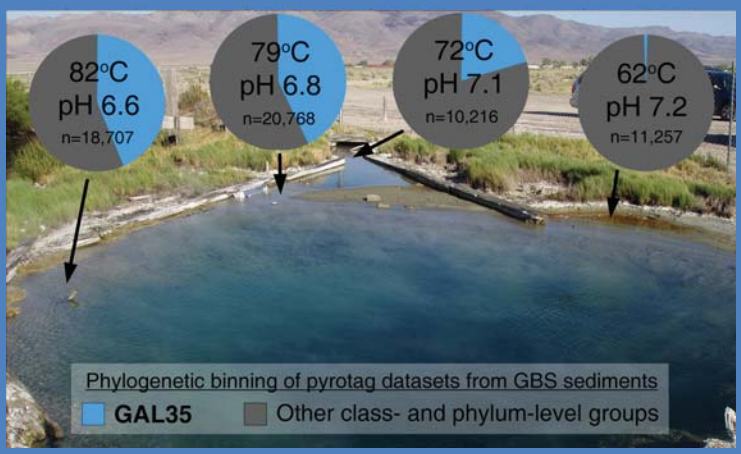

Figure 2. Close relatives of JAD2 in the GAL35 lineage (Fig. 4) are abundant in GBS sediments. DNA was extracted from sediment collected at various locations in GBS in Feb. 2010 (corresponding to sites A, B, C and E in Guy *et al.*, 2011). 16S rRNA gene fragments from bacteria and archaea were amplified by PCR using primers 926F and 1392R and the products were sequenced at JGI on a Roche 454 FLX pyrosequencer using Titanium chemistry. Resulting sequences (n) were classified using PyroTagger (Kunin and Hugenholtz, 2010). Members of the GAL35 group are prominent in 16S rRNA gene clone libraries in a variety of GB hot springs (Costa *et al.*, 2009; Dodsworth et al., 2011; Vick *et al.*, 2010).

Phylogeny of strain JAD2 and the GAL35 group

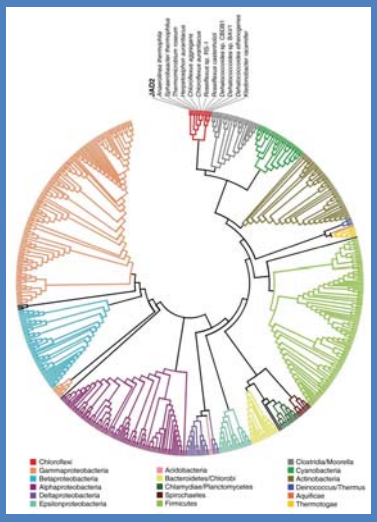

Figure 3. Strain JAD2 is a member of the phylum *Chloroflexi*. The cladogram shows the phylogeny inferred from concatenated amino acid sequences of 31 housekeeping genes from taxa representing major phyla within the Bacteria (Wu and Eisen, 2008). Strain JAD2 and other members of the *Chloroflexi* form a monophyletic lineage with high bootstrap support (100% of 500 replicates). Sequences were aligned using ClustalW and the tree was constructed and visualized using the MEGA5 software package (http://www.megasoftware.net).

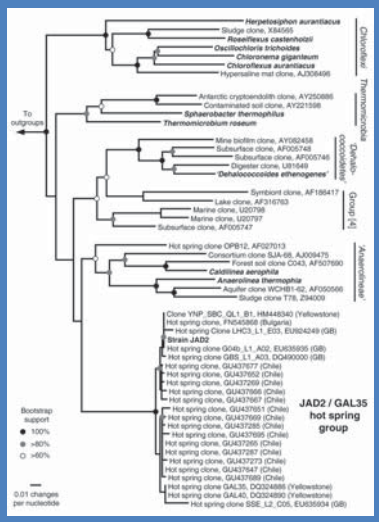

Figure 4. Phylogeny of 16S rRNA genes demonstrate that strain JAD2 and relatives (>90% identity) in the GAL35 group form a distinct clade within the phylum *Chloroflexi*. Phylogeny was inferred using distance matrix and neighbor joining methods (Felsenstein, 2004) with near-full-length sequences from named classes and class-level groups within the *Chloroflexi* (Hugenholtz *et al.*, 2004), using *E. coli*, *B. subtilis*, and *C. diphtheriae* as outgroups. Named species or isolates are in bold. Regions where GAL35 clones were obtained are indicated. GB, Great Basin.

Potential roles of JAD2 in N and C cycling in GBS

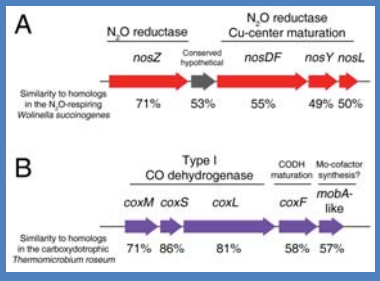

Figure 5. Loci in the JAD2 genome encoding a putative N_2O reductase (A) and CO dehydrogenase (B). *CoxDE* are elsewhere in the genome. Homologs of the NH_4^+-forming nitrite reductase (*nrfHA*), catalyzing respiratory nitrite ammonification in *W. succinogenes*, are also present.

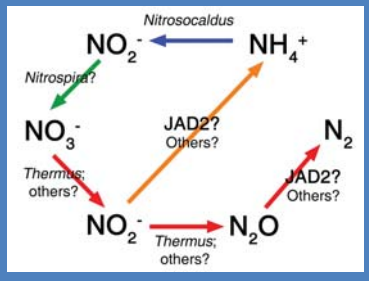

Figure 6. Groups potentially catalyzing steps in the N cycle in GBS: ammonia (blue) and nitrite (green) oxidation, denitrification (red), and dissimilatory nitrite reduction to ammonium (orange). Based on JAD2 genome and other studies (Dodsworth *et al.*, 2011; Hedlund *et al.*, 2011).

Summary and future directions

Strain JAD2 is a member of the GAL35 group, which likely represents a new class-level group within the phylum *Chloroflexi* (Figs. 3,4). Close relatives of JAD2 are abundant in GBS and other GB hot springs (Fig. 2) and have been found in terrestrial geothermal systems worldwide (Fig. 4), and may play important roles in N and C cycling in these environments (Figs. 5,6).

Further characterization of JAD2 will include:
- Full physiological characterization, formal proposal of class- through species-level taxonomic groups.
- Compare cell envelope structure to that of other members of the *Chloroflexi* and other bacterial phyla.
- Determine whether proposed pathways for CO oxidation, N_2O reduction and nitrite reduction to ammonium are functional and linked to respiration, and their importance in GBS and other hot springs.
-Assess the physicochemical habitat and biogeography of the GAL35 lineage in hot springs globally by qPCR.

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Title: A Spatial and Temporal Analysis of Microbial Communities in Great Boiling Spring, Nevada, by 16S rRNA Gene Pyrotag Analysis

Abstract: Great Boiling Spring (GBS) is a large, circumneutral, long residence time geothermal spring in the US Great Basin. Twelve samples were taken from four different sediment sites and the planktonic community in the bulk water of GBS on up to four different dates. Microbial community composition and diversity was assessed by using a barcoded, improved universal primer set targeting the V8 portion of the 16S rRNA gene and PCR. Over 200,000 products were sequenced using the Roche 454 GS FLX Titanium System. Sediment and planktonic microbial communities were distinct with very little overlap, regardless of the sampling location or temperature. Planktonic communities were extremely uneven and were dominated by a single phylotype related to Thermocrinis in the Aquificales. Benthic microbial communities grouped according to temperature and sampling location. Two locations, Site A (80-87°C) and Site B (79°C), were predominantly composed of the crenarchaeal class Thermoprotei, the novel archaeal lineage pSL4, and the novel bacterial lineage GAL35. Populations of the ammonia oxidizing archaeon "Candidatus Nitrosocaldus yellowstonii" comprised 5-15% of all samples when Site A was cooler than normal (80°C) and at cooler sites throughout the spring (76-62°C). At cooler temperature sites (76-62°C), the phylum-level diversity and evenness were significantly higher, and bacteria made up a significantly higher percentage of the population. To our knowledge, this is the most detailed study of the spatial and temporal variation in any geothermal spring. The study underscores the distinctness of planktonic and benthic communities and the importance of temperature in driving the spatial variation of microbial phylotypes throughout the mineralogically homogenous source pool.

A Spatial and Temporal Analysis of Microbial Communities in Great Boiling Spring, Nevada, USA

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Objectives

1. To describe the spatial distribution of the bacteria and archaea inhabiting Great Boiling Spring (GBS).
2. To examine the temporal variability of the microbial community at the different sites throughout GBS.
3. To compare and contrast the water and sediment-bourne microbial communities of GBS.

Introduction

GBS is a large, circumneutral, long residence time geothermal spring located in the US Great Basin. It is mineralogically homegenous, with analyses showing major solutes of Na^+ and Cl^- and an active nitrogen cycle [1,2]. Twelve samples were taken from four different sediment sites and the bulk water of GBS on up to four different dates. Microbial community composition and diversity were assessed through the analysis of more than 300,000 16S rRNA gene pyrotags. To our knowledge, this is the most detailed study of the spatial and temporal variation in any geothermal spring. This study underscores the distinctness of water and sediment-borne communities and the importance of temperature in driving the spatial and temporal variation of microbial phylotypes throughout the source pool.

Methods

Eight sediment samples were collected using sterile coring devices and four water samples were collected using either normal or tangential flow filtration, as previously described. Sediment samples were collected from four sites at the edge of the hot spring, ranging in average temperature from 61.9 - 82.6 °C (Fig 1). Samples were stored on dry ice and transported to the laboratory, where DNA was extracted using a slightly modified version of the Joint Genome Institute's (JGI) CTAB protocol [3]. Extracted DNA was shipped on dry ice to the JGI, where DNA sequencing of the the V6 portion of the 16S rRNA gene was performed using the Roche 454 GS FLX Titanium System. The resulting reads were run through the PyroTagger pipeline [4], where they were filtered for quality, requiring a minimum length of 200 bp and Phred values of at least 27 for 90% of bases, and clustered at 97% sequence identity. Sequnces identified by PyroTagger as potential chimeras, along with additional low abundance sequences that did not align properly, were confirmed as chimeras by BLASTing in NCBI and discarded from the analysis. The Quantitative Insights Into Microbial Ecology pipeline [5] was then used to perform cluster and principal coordinate analyses (PCoA), utilizing the Morisita-Horn index and abundance-weighted and unweighted UniFrac distance metrics. The Morisita-Horn index is a similarity index that nearly independent sample size [6] and the UniFrac metric is a method of comparing microbial communities based on phylogentic information [7].

Results and Conclusions

Water and sediment-borne microbial communities were distinct with very little overlap, regardless of the sampling location or temperature (Fig 2). Water-borne communities were extremely uneven and were dominated by a single phylotype related to *Thermocrinis* in the *Aquificales*. Sediment-borne microbial communities grouped according to temperature and sampling location. Two locations, Site A (80-87°C) and Site B (79°C), were predominantly composed of the crenarchaeal class *Thermoprotei*, the novel archaeal lineage pSL4, and the novel bacterial lineage GAL35. Populations of the ammonia-oxidizing archaeon "*Candidatus* Nitrosocaldus yellowstonii" comprised 5-15% of all samples when Site A was cooler than normal (80°C) and at cooler sites throughout the spring (76-62°C). At cooler temperature sites (76-62°C), the phylum-level diversity and evenness were significantly higher, and bacteria made up a significantly higher percentage of the population. Cluster analysis results from weighted UniFrac and Morisita-Horn showed the water-borne samples clustering together and distinct from the sediment-borne samples, with jackknife values of 100% separating the water-borne clusters from the rest of the trees (Figs 3a,b). The result of the unweighted UniFrac cluster analysis had lower jackknife values overall and less well-defined clustering (Fig 3c) than the Weighted UniFrac and Morisita-Horn trees; this suggests that relative OTU abundance has a greater influence on the sample clusters in the weighted metrics (weighted UniFrac and Morisita-Horn) than the specific OTUs observed in each sample. Clusters of high temperature sample sites (0812.A/1002.B/1002.A and 0906.A/1007.A) were maintained in all three trees, with jackknife values of at least 70% at the main cluster node. This suggests that with or without abundance weighting, temperature is a driver of community composition. Weighted UniFrac and Morisita-Horn PCoA results showed clustering similar to the respective cluster analysis, with water-borne samples (Fig 4a,b) grouping tightly. Site type, water or sediment, was the major principal coordinate in both weighted UniFrac and Morisita-Horn, explaining 74.45 and 53.59% of variation between samples, respectively; temperature was responsible for much of the remaining variation, explaining 13.70 and 22.76% of weighted UniFrac and Morisita-Horn, respectively. This amounts to an explanation of 88.15 and 76.35% variation in the weighted UniFrac and Morisita-Horn analyses, respectively. The results of the unweighted UniFrac analysis (Fig 4c) showed a less defined grouping, with only 19.77% of variation explained by P1, temperature, and 15.80% by P2, site type, resulting in a total variation explained of 35.57%. This result is similar to the unweighted UniFrac cluster analysis, again suggesting that relative OTU abundance has a greater influence on the sample clusters in the weighted metrics than the specific OTUs observed in each sample.These results show that the water and sediment-borne communities of GBS are dominated by different organisms in different relative abundances, and that between sediment-borne communites, temperature has a significant influence on community composition.

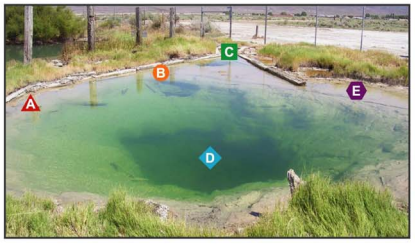

Figure 1 Photograph of GBS with sample sites, A-E, indicated. Average recorded temperatures (°C) at each site: A , 82.6, B, 79.2, C, 73.8, D, 81.0, and E, 61.9. Samples from site D are of the water-borne microbial community and samples from sites A, B, C, and E are sediment communities.

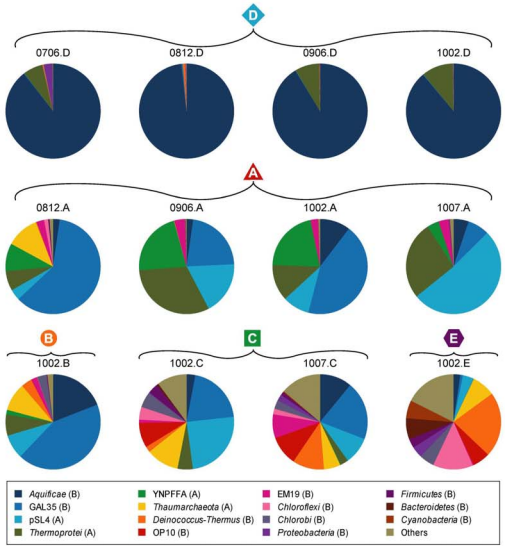

Figure 2 Pie charts describing community composition of four water and eight sediment samples, including 15 most abundant taxa of all 12 samples, with remaining taxa grouped as "Others". "(A)" or "(B)" after taxon name in pie chart legend designates archaeal or bacterial taxon, respectively. Sample names indicate date and site of sampling, formatted as YYMM.site (Ex. 0706.D, sampled June 2007 at site D).

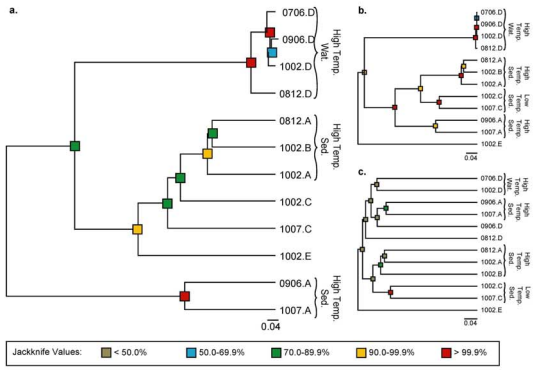

Figure 3 Trees resulting from cluster analysis using three different algorithms, (a) Abundance-weighted UniFrac (b) Morisita-Horn (c) Unweighted UniFrac. Jackknife value ranges are shown at each node. Clusters grouped by site type (water or sediment) or temperature (high or low) are indicated by a bracket. Wat., water sed., sediment.

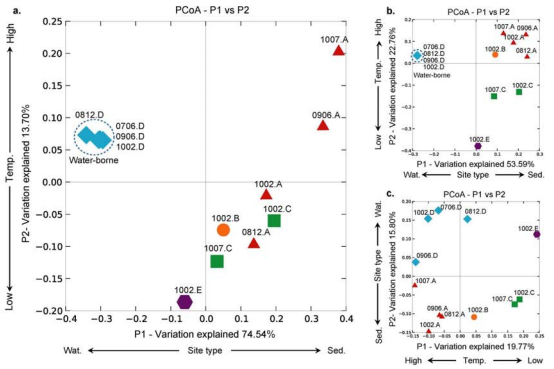

Figure 4 Results of PCoA using three different algorithms, (a) Weighted UniFrac (b) Morisita-Horn (c) Unweighted UniFrac. Interpretations of the environmental factors responsible for variation explained, either temperature or site type, by each coordinate, P1 or P2, are indicated. Clusters of water-borne samples are circled. Wat., water; sed., sediment; temp., temperature.

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Acknowledgements

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Thank you to Chris Ross for helpful strategic conversations.

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Department: Healthcare Administration

Title: HIV Patients' Rights

Abstract: HIV is a sexually transmitted disease that develops into AIDS. There is no cure for it, only treatment. In this poster, we look at the pros and cons of disclosing this type of information. People who decide to disclose their HIV status may have various reasons for doing so, but most do it for emotional support and for prevention of spreading the disease. Those who decide to keep it private primarily do it to not face rejection, discrimination, degradation, and loss of respect. (Petronio Page 72) The problem with creating a public database is that many organizations are against the idea of disclosing personal information. Through documents as old as the Constitution, government officials have deemed a public database to be unethical. It confronts with individuals' basic rights. Others still argue that creating such a database would lower the spread of HIV, saving hundreds of lives. The balance between patient's privacy and the well-being of society has yet to be found. We want to prevent the spreading of AIDS, so disclosure should be mandatory. By knowing who has the disease the chances of spreading it become minimal. In exchange, patients should be treated just as any other person.

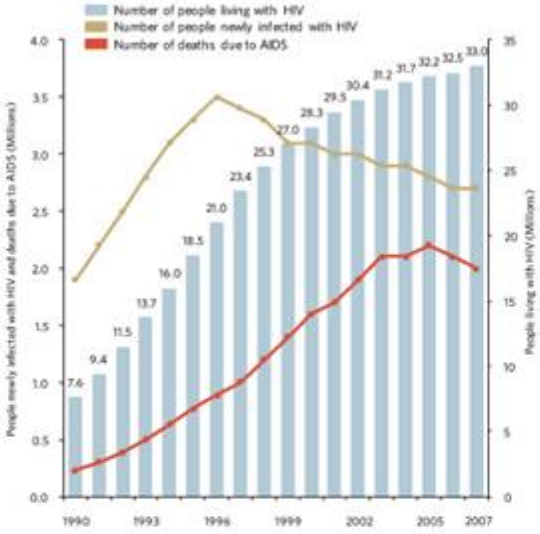
Why are you doing this project? I am doing this project because HIV is a topic that is worth researching. It is such a deadly virus, that it deserves to be researched closely in hopes to find ways to stop the spread.
What problem are you trying to solve? I am trying to resolve whether or not an HIV public database is an ethical solution to the HIV epidemic.
What tools or equipment are you using? I used scholarly journal entries along with books and articles to compile information.
Why is your project worth researching? My project is worth researching because HIV has killed so many people already, there is no reason why it should continue. Creating a database is a proposed solution to the problem.
What relevance will it have on the community, society, and in your research field? It will help individuals who are struggling with forming an opinion on whether or not an HIV database is ethical.
What did you find? I found many strong arguments for the public database, along with strong arguments against. Some arguments even reference to the U.S. Constitution.
What is the future of your research project? I hope my research will be examined by many and it will help uncertain peoples decide that a HIV database would indeed be an ethical decision.

AIDS/HIV DENIALISM:
Patients' Privacy Rights

FADIA "FAY" ABDULLAH
GUADALUPE "LUPE" MEDRANO
UNLV: SCIENCE 101- SPRING 2011

Abstract
HIV is a sexually transmitted disease that develops into AIDS. There is no cure for it, only treatment. In this poster, we look at the pros and cons of disclosing this type of information. People who decide to disclose their HIV status may have various reasons for doing so, but most do it for emotional support and for prevention of spreading the disease. Those who decide to keep it private primarily do it to not face rejection, discrimination, degradation, and loss of respect. (Petronio Page 72) The problem with creating a public database is that many organizations are against the idea of disclosing personal information. Through documents as old as the Constitution, government officials have deemed a public database to be unethical. It confronts with individuals' basic rights. Others still argue that creating such a database would lower the spread of HIV, saving hundreds of lives. The balance between patient's privacy and the well being of society has yet to be found. We want to prevent the spreading of AIDS, so disclosure should be mandatory. By knowing who has the disease the chances of spreading it become minimal. In exchange, patients should be treated just as any other person.

Introduction
HIV/AIDS weakens a person's ability to fight off infections and cancer, thus creating an extremely weak body that is susceptible to many diseases. Because it is such a serious issue, the idea of creating a public database has been circulating. The database would be similar to a state's sex offender registry. But along with the idea of publicizing AIDS victims have come many arguments. The main argument against the database is that it would go against patients' privacy rights. Others argue it would cause public humiliation to the peoples infected, and possibly cause riots to break out against them. Although the thought of innocent people being ridiculed over a virus is unfortunate, the fact that these people may be spreading HIV to unaware citizens is even more unfortunate. That is why some believe that taking the risk of public humiliation of victims is well worth the possibility of lowering the spread of the fatal virus. This forces HIV and gay advocacy groups to confront the burning question: Which do you value more — your privacy or the chance to halt this plague? (Eskenazi Paragraph 1)



Number of people living with HIV, number of people newly infected with HIV and number of AIDS deaths in the world (Millions), 1990-2007
Source:
<http://www.who.int/hiv/topics/mdg/info/en/index.html>

Pros of Public Database
Compiling a database of the infected makes it easier to track (and prevent) the spread of the disease. (Eskenazi Paragraph 2)
The youth will be made more aware of the commonness of the HIV virus and how easily it can be spread.
People will be aware if their partners are infected. (Emlet Page 4)
It will possibly lower the possibility of people spreading the disease with a criminal intent.
It is the public 's responsibilities to use data for social goods. (Gostin Paragraph 2)

Cons of Public Database
Person with HIV is 'discreditable' and becomes 'discredited' through disclosure. (Petronio Page 71)
Results in loss of employment, housing, access to public education, insurance, and health care. (Petronio Page 72)
Loss of friends, family, spouses, and the emotional and instrumental support those relationships might otherwise provide. (Petronio Page 72)
Despite 'duty to treat' many providers prefer to avoid patients with HIV. (Petronio Page 76)
Rejection of potential sexual partners. (Emlet Page 4)
Potential risk of information being abused and used in ways not appreciated. (Emlet Page 4)
Face anxiety over the potential of unintentional disclosure. (Emlet Page 5)

Conclusion
Since the beginning of the HIV/AIDS epidemic, well over half a million people have died of AIDS in the United States. A virus as deadly as cancer should be given more extreme solutions. It may not be considered ethical in some eyes, but anyway of reducing the increase of peoples infected should be taken into consideration. Simply reinforcing safe behavior and urging HIV testing is not enough to end the epidemic.
Yes, the thought of publicly humiliating individuals is terrible, but if serious measures are not taken, the HIV virus will continue to spread. And because the main ways to spread it are through unprotected sex and needle sharing, this means that youth are very much at risk. With young age comes ignorance, and going as extreme as a public database will more than likely open the eyes of teens who engaging in these practices. AIDS is one of the most serious, deadly diseases in human history, and if a public database is what it takes to reduce the spread, then it should be attempted.

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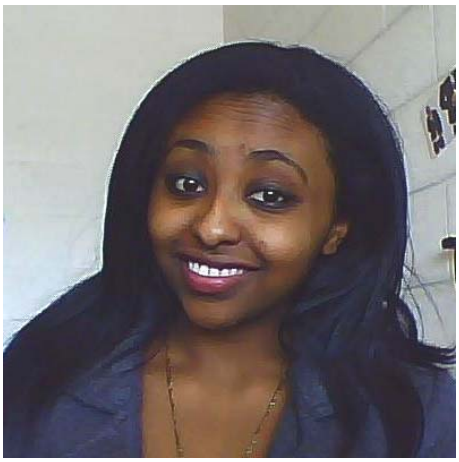

Mekdelawit Mezgebu and Victor Trinh

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Location of High School:
Mentor/Advisor:
Educational Institute of Project: UNLV
Department:
Research Site:

Title: Intelligent Design and its Place in the Classroom

Abstract: Creationism, is the dominant belief held by the public. Evolution, on the other hand, is the competing theory of the mechanisms of creation. The recent dispute among the scientific and political field that has furthermore complicated the question of intelligent design being integrated into the curriculum of public schools is addressed here. We attempt to give both sides of the argument, along with analyzing the components of each theory. Intelligent design advocates are for the idea of accommodating what they have coined "intelligent design" into the classrooms of American schools. The opponents of intelligent design (evolution supporters); however, claim that this is preposterous because intelligent design is not even considered a real science. Science is based on the fact that hypotheses can be tested empirically and proven a number of times before they become theories. According to evolution proponents, intelligent design does not complete such task. Intelligent design supporters believe that even if evolution explained creation of man, there would have to be a creator since humans are complex beings. This and other questions will be covered throughout the poster.

Why are you doing this project? We completed this project in order to become more familiar with the place of religion in the classroom. The connection between science and religion has always been of interest to us, and we wanted to explore its limits in the public sphere.
What problem are you trying to solve? In the poster, we attempted to answer the question of whether or not intelligent design should be integrated into the public school curriculum along with evolution.
What tools or equipment are you using? Microsoft Powerpoint to complete our poster.
Why is your project worth researching? Religion and science are always going to be conflicting, and it is better to be aware of both points of view before having an opinion. This can be done by researching. Not only will it make one aware of other views but provide insight into current events concerning the topic, as well.
What relevance will it have on the community, society, and in your research field? It gives the ability to draw a public course of action. Conflicts can bring interest, and in becoming more aware of this topic, people can become entrenched in finding a solution or a middle ground to the question of religion v. science.
What did you find? We found that there is a middle ground to reach in the argument between evolution supporters and intelligent design advocates. It is acceptable to believe the mechanisms of evolution are what make life possible on earth, along with the help of a creator.
What is the future of your research project? We have not prepared in thinking about that yet.

Naturalized Mexican Immigrants & their Voting Behavior

By Cynthia Hernandez

Special Thanks
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John Tuman, Ph.D

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PURPOSE

Explain determinants of voting with a main focus on the effects of membership in Mexican hometown associations. In particular, does membership make it more or less likely that Mexican immigrants who have naturalized will vote.

INTRODUCTION

Despite the increasing population size of Latinos (individuals who are of Hispanic descent) in the United States, the number of Latino voters is not increasing. This is based on the ratio of Latino residents and those who are registered to vote.

Between 2000 and 2004, Latinos accounted for 50 percent of the population growth; however, they represented only 24 percent of new voters. When compared to white and black voters, only 59 percent of Latinos were eligible to vote in 2004 compared to the 97 percent of white eligible voters and 94 percent of black eligible voters. Even though Latinos rose to 41.3 million.

in 2004, Mexicans represented the largest documented immigrant group in the United States, but they also represent ed the largest group with the lowest voter turnout rates. Thus, it is important to understand the reasons why Mexican immigrants who have naturalized decide to vote.

DATA & METHODS

The Latino National Survey (LNS) contains 8,634 un-weighted and completed interviews of self-identified Latino/Hispanic residents of the United States. Interviewing began on November 17, 2005, and continued through August 4, 2006. The survey covered several cities
and states with a high number in Latino population.
The sample was stratified by geographic designation, meaning that each state sample was a valid, stand-alone representation of that state's Latino population. The survey asked questions regarding their demographics.

The primary independent variable of interest is membership in hometown associations. Controlling for other variables that are commonly associated with voting, the influence of membership in these associations will be produced.

Log Likelihood=280.723
N = 449
P < .01

Dependent Variable
*Voting 2004 Elections
- Binary variable

Independent Variable
*Hometown Association Involvement

Control Variables
*Political Interest
*Union Membership
*English Proficiency
*Income Level
*Education Level
*Birthdate
**the higher the year of birth, the younger the person, the less likely she or he will vote

LOGISTIC REGRESSION ANALYSIS

VOTE 2004 Election	Hypothesized Results	Coefficient	Standard Error	Z Value	Level of Statistical Significance	Direction of Influence
Political Interest	Positive influence	0.797	0.265	3.00	0.003 (99%)	Positive & Statistically Significant
Hometown Association Involvement	Negative influence	0.834	0.465	1.79	0.073 (90%)	Positive & Statistically Significant
Union Membership	Positive influence	0.795	0.384	2.07	0.038 (95%)	Positive & Statistically Significant
English Proficiency	Possible influence	0.456	0.135	3.38	0.001 (99%)	Positive & Statistically Significant
Income Level	Possible influence	0.054	0.045	1.18	0.237	Positive but Not Significant
Education Level	Positive influence	-0.009	0.061	-0.15	0.880	Negative but Not Significant
Birthdate	Negative influence	0.000	0.000	2.02	0.044 (95%)	Positive & Statistically Significant

PRELIMINARY FINDINGS

The logistic regression analysis shows that having an interest in politics has a positive effect on voting for Mexican immigrants who have naturalized. This is result is not necessarily novel. However, the positive effect that hometown association membership has on voting behavior is. This shows that membership in hometown associations is a primary determinant in voting behavior among Mexican immigrants who have naturalized.

Further research is necessary to explain this effect due to the contradicting research that exists on the impacts of hometown association membership. This project will attempt to reconcile this contradiction.

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Stuart Allen

Academic Institution: Georgia Institute of Technology
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Department: Chemistry
Research Site: HRC 245

Title: Analysis of Uranium Fuel Pellets for Forensic Applications

Abstract: Potential forensic signatures in nuclear fuels were explored using uranium dioxide (UO2) to make fuel pellets with varying percent binder of total mass as well as pellets varying percent U3O8 of total uranium content. Ammonium uranyl carbonate was precipitated from the mixing of ammonium carbonate and uranyl nitrate. The precipitate was calcined overnight at 800 ˚C to make U3O8. The U3O8 was reduced to UO2 in a tube furnace with H5 gas overnight at 600 ˚C. X-ray diffraction was performed on the UO2 to verify the composition. Aluminum stearate and ethylene stearamide were used as binders, and binder percentages ranging from 0.25 to 1.5 weight percent, were tested to determine the effect on pellet density. The criterion for determining the optimal percent binder was the highest sintered density due to having the highest energy to mass ratio. The optimal binder content was determined to be 0.66 weight percent for aluminum stearate and 0.5 weight percent for ethylene stearamide. Using the optimal percent binder, pellets were made with the U3O8 percentage of uranium content varying from 5 to 20 weight percent to determine the effects on pellet density. Over the range studied, the impact on density of varying the U3O8 content was less than 5 %. The fuel pellets were analyzed under an optical microscope to study the microstructure and other features. By determining the dependence of density on percent binder and percent U3O8 and the microstructure, information was gained that can be used to determine forensic signatures in nuclear fuels.

Kyle Childs

Academic Institution: South Carolina State
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Department: Radiochemistry

Title: Evaluation of metals Rhenium and Technitium with ligands Acetohydroxamic acid and Ethylenediaminetetraacetic acid

Abstract: Evaluation of the metal-ligand interaction of Rhenium and Technetium with Acetohydroxamic acid (AHA) and Ethylenediaminetetraacetic acid (EDTA) was performed by use of UV-Visible spectroscopy. Complexation was performed through ligand exchange. All Re-AHA complexes were carried out in aqueous solutions of Rhenium oxycitrate obtained from reductive synthesis procedure, but Re-EDTA was complexed in an alkaline environment as EDTA only becomes miscible in water at a pH of 10 or greater. Technetium complexation was carried out in an acidic medium of methanol for AHA and basic media of ethanol. Technetium metal compound was obtained from a multi-step reduction synthesis to obtain Tc(V) from Tc(VII). Based upon results obtained by molar absorbtivity measurements of Re-AHA complex, it was concluded the Rhenium does complex and the same was observed for the TcO-AHA complex. UV-visible spectra showed no shift for Re-EDTA complex which neither confirmed nor denied the presence of a complex. Initial Tc-EDTA complex was not evaluated as solid precipitate was produced during complexation and was hypothesized to be the result of the oxidation of technetium in the presence of a sodium hydroxide. A second complex synthesis was performed in acidic medium for Tc-EDTA which yielded a UV-Vis active complex. For all four complexes produced, crystallization was performed through complex ion exchange with hexaflourophosphate. These samples will be analyzed by X-ray diffraction to confirm if the proposed complexes are forming. Crystal yield was varied by the amount of metal compound added to the complex.

Briana Clanton

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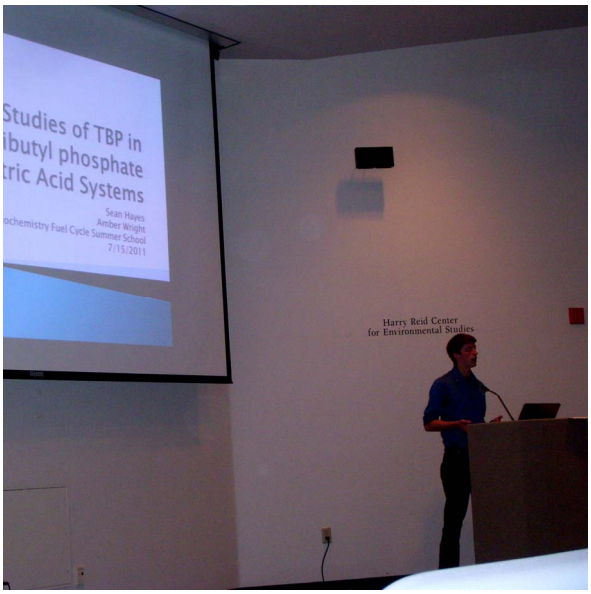
Title: Comparison of Two Actinide Separation Methods Using Extraction Chromatography Resins

Abstract: The mobility of radionuclides in the environment is of great interest to gain a better understanding of the effect on the specific environment. One such method that can provide information on contaminant mobility is sequential extraction. The resulting leachates from a sequential extraction procedure must be separated and purified before counting by alpha spectroscopy. Extraction chromatography is an efficient separation tool that produces less waste than other methods.
This research involves investigating two separation methods using extraction chromatography; Maxwell (2006) and Thakur (2011). Tracers were used to monitor the recovery of the samples. The samples were first prepared by adding a known amount of activity of each isotope; 229Th, 232U, 242Pu, 243Am. Samples were then subjected to a ferric hydroxide co-precipitation to pre-concentrate the actinides before separation. Triplicates of each sample were run through the Maxwell procedure and another set of triplicates was run through the Thakur procedure. The samples were then prepared for alpha spectroscopy by CeF3 micro precipitation. Each sample was counted until counting error was less than 1 %. The total recovery was calculated for each sample.



Sean Hayes

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Department: Chemistry
Research Site: HRC 245

Title: Solubility Studies of TBP in Various Tributylphosphate and Nitric Acid Systems

Abstract: Data on the solubility of tributylphosphate in bi-phasic nitric acid systems with and without uranium was collected using a Parr pressure vessel and density meter. Equa-portions of either pure TBP or 30% TBP in n-dodecane were used as the organic phase and 1 M, 5 M and 10 M nitric acid was used as the aqueous phase. UO2(NO3)2 was used in trials studying uranium. Runs were completed at 50 °C and 75 °C with the phases separated manually after 10 minutes. Separations were performed quickly in order to minimize phase separations after heating was removed. Temperature data over the heating period was collected to look for signs of a runaway reaction and to create a temperature profile of each mixture. Nitrate ions were detected using ion chromatography and quantified using a peak area method based on a generated calibration curve. From the concentrations of nitric acid and a referenced constant describing water concentration in TBP systems, a model was generated to predict the density of the resulting aqueous phase. With a predicted density and a known mole fraction of both water and nitric acid, an estimate of TBP concentrations could be generated. These results agreed to within 10% of the measured densities of the aqueous phases.

Nieka Jackson

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Title: Separation of Neighboring Lanthanides

Abstract: Lanthanide separations techniques using ion chromatography methods had been studied since the early 1950s. There has been limited research performed on the separation of neighboring lanthanides and the extraction of the specific lanthanide fraction of interest. Experiments have been in progress to explore an efficient separation method of thulium and erbium with respect to various factors such as flow rate, temperature, pH of the elution phase and use of different complexing agents for elution off of a cation exchange resin. An automated ion chromatography system (DIONEX ICS-3000) has been used to analyze for trace levels of the lanthanides by the use of a post column reagent for the detection of the lanthanides with a UV-VIS detector. Unfortunately, using a post-column reagent setup prevents the possibility of differentiating between the different lanthanides since the absorption of light is a result of PAR-lanthanide interaction, independent of the specific lanthanide. Therefore, differentiating one lanthanide from another in a fraction cannot be determined using the post-column reagent method. The use of a fraction collector can be used to collect fractions of interest for elemental analysis with an inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Experiments were performed using a mock solution of a fraction collected from the IC run which included trace amounts of thulium and erbium and the complexing agent (α-HIBA) ,to determine an efficient method of separation of lanthanides from the (α-HIBA) by column purification using the TRU resin. IR spectroscopy was utilized to observe the presence/absence of the organics before and after the purification and the lanthanides were analyzed with ICP-AES to determine the yield of the entire procedure.

Charles Loelius

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Title: Investigation of Uranium-Zirconium Fuel Pellets for TerraPower Traveling Wave Reactor

Abstract: The objective of this project is to discover the lowest reasonable percentage of zirconium that could be used in the fuel pellets of TerraPower's traveling wave nuclear reactor. The traveling wave reactor in development by TerraPower is intended to maximize the energy obtained and prevent proliferation by burning through the products created over the period of operation. The fuel being looked at currently is a uranium zirconium alloy, where the zirconium suffices to stabilize the phase of the fuel and to prevent interactions between the fuel and the cladding. The production of fuel pellets was begun by testing only pure zirconium. Another pellet was then made similarly but made out of zirconium metal foil. This was tested and was found to show less oxidation. Two pellets were then created, made of depleted uranium with 10% zirconium, where the constituents were washed in 70% nitric acid. One was coated in mineral oil and one was washed in a hexane bath. These were then both heated to 1500 °C for 2 hours in a furnace, with argon flowing over. These formed alloys, which were characterized using microscopy and XRD analysis. This suggests a method of producing pellets for testing the minimum amount of zirconium in the pellet.

Kristin McConnell

Academic Institution: University of Texas at Austin
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Research Site: HRC 245

Title: Analysis of Uranium Fuel Pellets for Forensic Applications

Abstract: Potential forensic signatures in nuclear fuels were explored using uranium dioxide (UO2) to make fuel pellets with varying percent binder of total mass as well as pellets varying percent U3O8 of total uranium content. Ammonium uranyl carbonate was precipitated from the mixing of ammonium carbonate and uranyl nitrate. The precipitate was calcined overnight at 800 ˚C to make U3O8. The U3O8 was reduced to UO2 in a tube furnace with H5 gas overnight at 600 ˚C. X-ray diffraction was performed on the UO2 to verify the composition. Aluminum stearate and ethylene stearamide were used as binders, and binder percentages ranging from 0.25 to 1.5 weight percent, were tested to determine the effect on pellet density. The criterion for determining the optimal percent binder was the highest sintered density due to having the highest energy to mass ratio. The optimal binder content was determined to be 0.66 weight percent for aluminum stearate and 0.5 weight percent for ethylene stearamide. Using the optimal percent binder, pellets were made with the U3O8 percentage of uranium content varying from 5 to 20 weight percent to determine the effects on pellet density. Over the range studied, the impact on density of varying the U3O8 content was less than 5 %. The fuel pellets were analyzed under an optical microscope to study the microstructure and other features. By determining the dependence of density on percent binder and percent U3O8 and the microstructure, information was gained that can be used to determine forensic signatures in nuclear fuels.

Tenisa Meadows

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Research Site: UNLV HRC 245

Title: Evaluation of metals Rhenium and Technitium with ligands Acetohydroxamic acid and Ethylenediaminetetraacetic acid

Abstract: Evaluation of the metal-ligand interaction of Rhenium and Technetium with Acetohydroxamic acid (AHA) and Ethylenediaminetetraacetic acid (EDTA) was performed by use of UV-Visible spectroscopy. Complexation was performed through ligand exchange. All Re-AHA complexes were carried out in aqueous solutions of Rhenium oxycitrate obtained from reductive synthesis procedure, but Re-EDTA was complexed in an alkaline environment as EDTA only becomes miscible in water at a pH of 10 or greater. Technetium complexation was carried out in an acidic medium of methanol for AHA and basic media of ethanol. Technetium metal compound was obtained from a multi-step reduction synthesis to obtain Tc(V) from Tc(VII). Based upon results obtained by molar absorbtivity measurements of Re-AHA complex, it was concluded the Rhenium does complex and the same was observed for the TcO-AHA complex. UV-visible spectra showed no shift for Re-EDTA complex which neither confirmed nor denied the presence of a complex. Initial Tc-EDTA complex was not evaluated as solid precipitate was produced during complexation and was hypothesized to be the result of the oxidation of technetium in the presence of a sodium hydroxide. A second complex synthesis was performed in acidic medium for Tc-EDTA which yielded a UV-Vis active complex. For all four complexes produced, crystallization was performed through complex ion exchange with hexaflourophosphate. These samples will be analyzed by X-ray diffraction to confirm if the proposed complexes are forming. Crystal yield was varied by the amount of metal compound added to the complex.

Birdy Phathanapirom

Academic Institution: University of Texas, Austin
Mentor/Advisor: Ken Czerwinski
Educational Institute of Project: UNLV
Department: Chemistry
Research Site: HRC 245

Title: Investigation of Pentavalent Uranium Oxide via Reduction of [UO2]2+ Under Hydrothermal Reaction Conditions

Abstract: The synthesis of [UV(H2O)2(UVIO2)2O4(OH)](H2O)4 (1) via hydrothermal reaction of UO22+ with Zn and hydrazine dihydrochloride at 120 °C for three days was investigated. Yields of Compound 1 in the form of a dark red crystalline solid were not observed. Hydrothermal reaction of UO22+ with Zn and hydrazine dihydrochloride at 120 °C under normal atmosphere and H2 atmosphere via addition of Na(BH3) for three days yielded UIVO2+x, determined by powder X-ray diffraction (XRD). A lattice parameter of 5.4126(43) Å was measured using XRD, which disagrees with the known lattice parameter for UO2 of 5.467 Å, suggesting cation deficiency in the UO2 structure. An unknown peak in XRD data was observed at 3.3 Å.

Zachary Schriver

Academic Institution: Purdue
Mentor/Advisor: Ken Czerwinski
Educational Institute of Project: UNLV
Department: Chemistry
Research Site: HRC 245

Title: Solid Solution Diffusion Couples for Analyzing Fuel/Cladding Interactions

Abstract: Diffusion couples were designed and created for the purpose of examining the effectiveness of novel production methods for Uranium-Zirconium alloys. The diffusion couple designs and material choices were derived from previous work done by Dan Koury, Ph.D. and Gerald Egeland, Ph.D. of the University of Nevada Las Vegas in addition to previous experiments by D. D. Keiser of Idaho National Laboratories. Solid solution diffusion between uranium and zirconium, and uranium and molybdenum were both chosen to be examined for their applicable nature towards the study of interactions between nuclear fuels and their cladding. uranium, zirconium, and molybdenum samples were cut, polished, clamped together, and exposed to a heat of 700 °C for 100 hours. The resulting couples (U-Zr & U-Mo) were then polished, leaving only the original uranium pieces with their newly formed diffusion zones. These diffused Uranium samples were then clamped on either side of a polished iron sample and heated to 650 °C for 65 hours, allowing a new diffusion layer to form. This final sample was then mounted and polished for scanning electron microscopy. Analysis of the final couple intends to find the extent of the metals' diffusion into one another, and thus their effectiveness at alloy creation.

Alan Stebbins

Academic Institution: University of Massachusetts — Amherst
Mentor/Advisor: Ken Czerwinski
Educational Institute of Project: UNLV
Department: Chemistry
Research Site: HRC 245

Title: Characterization of TcO3S

Abstract: 99Tc is an important radionuclide in fuel waste because it is long lived, and in non-reducing conditions the most common form is the pertechnetate ion, TcO4−, which is highly soluble and environmentally mobile. This project began by trying to synthesize TcS4- to obtain a comparison with TcO4-, in hopes of finding a more desired waste form of 99Tc. Past work using rhenium, a good surrogate for technetium, successfully synthesized ReS4-. The same synthesis was unsuccessful in producing TcS4-; the product seemed to be Tc2S7. While attempting the synthesis of TcS4- under various other conditions, an unknown compound was detected and determined to be TcO3S-. The goal of the project became to characterize TcO3S-. Previous work has developed a procedure, reacting TBATcO4 and HMDST, producing TcO3S- and various other species. Ultraviolet-visible spectroscopy (UV-Vis) and electrospray ionization mass spectrometry (ESI/MS) kinetic experiments were performed on the reaction, allowing the synthesis of TcO3S- to be observed and characterized. The UV-Vis exhibits a short lived peak at a wavelength of 364 nm, while the ESI/MS displays a short lived peak at 178 m/z, which both confirm the presence of TcO3S- . Various reaction ratios of TBATcO4 and HMDST were performed to determine the optimal conditions, and the longest appearance with the highest absorbance and corresponding intensity occurred at a 1 Tc : 200 S mole ratio. Using rhenium as a surrogate, a reaction was performed under nitrogen gas atmosphere to produce ReO3S-. ReO3S- has been widely characterized, and using the literature data, confirmation of the ReO3S- synthesis was deduced through ESI/MS and UV-Vis. Optimizing the conditions of this reaction to produce optimized amounts of ReO3S- may become beneficial to the isolation or production of TcO3S-. Future work includes isolating, optimizing production of, and further characterization of the TcO3S- compound.

Douglas Wood

Academic Institution: Oregon State University
Mentor/Advisor: Ken Czerwinski
Educational Institute of Project: UNLV
Department: Chemistry
Research Site: HRC 245

Title: Precipitating and Characterizing Hexamminecobalt(III) Chloride

Abstract: Technetium is a long-lived radioisotope that poses a problem for spent nuclear fuel reprocessing. There are several methods being considered to remove technetium from the fuel cycle. This experiment explored the availability of alloying ammonia pertechnetate with hexamminecobalt(III) chloride. This alloy was precipitated by mixing the two solutions and characterized by the following: powder XRD, single-crystal XRD, sorption onto several graphites, analyzed on the SEM, and UV-Visible spectophotometer. A sample of the alloy was reduced in the tube furnace under H5 gas and compared to a sample heated under argon gas by powder XRD. The crystal grown for the single-crystal XRD was not large enough to receive immediate results and requires further attention. Untreated, heat-treated, and activated graphite samples were prepared to characterize the sorption of pertechnetate onto each type of graphite. All samples showed low amounts of sorption, activated having the most, and untreated having the least amount of sorption. There was not enough pertechnetate in the graphite samples to see any phases under the SEM. The UV-Visible spectra indicates a large amount of cobalt and technetium in the alloy and will be further analyzed to determine relative concentrations compared to the spectra of stock hexamminecobalt (III) chloride. Formation of the CoTc3 alloy is easily achievable. It does not absorb well onto graphite in general and could become pertinent information in Generation IV reactors or methods of long-term storage for spent nuclear fuel. The SEM images and data have yet to be analyzed but should indicate certain phases under normal and reducing conditions.

Kimberly Gray

Academic Institution: Department of Energy
Mentor/Advisor: Ken Czerwinski
Educational Institute of Project: UNLV
Department: Chemistry
Research Site: HRC 245

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