SEMESTER/EXPLORATORY GRANT APPLICATION
Cover Sheet

Amount Requested: $201.60

Project Information

Santana, Darian
Student Participant (Last, First)

Precipitation of Carbonates by Viral Lysis of Cyanobacteria in GSL
Project Title (10 words or less)

Frantz, Carie — **2507**
Faculty Mentor Name (last, first) — Mail Code

Science — **Geosciences/Microbiology**
College (Weber State is the University, NOT college) — Department

This project ___ DOES/_**X**_ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Student Signature — 24 Sep 2018 Date

Project Mentor Signature — 9/7/18 Date Received by Mentor. Must be 10 business days before final deadline.

2507 Campus Mail — 6181 Phone Ext.

Undergraduate Research Committee Representative — Date Received by URC Rep. Must be 5 business days before final deadline.

Faculty Mentor Department Chair — Date

verbally approved, but not available for signature

Please check if attended Research Proposal Workshop:

☒ Date Workshop attended October 2017
(Please fill in the date of attendance)

SEMESTER/EXPLORATORY GRANT APPLICATION
Budget Worksheet

BUDGET ITEM	Department or College Funds	Outside Agency Funds	Personal Funds	Undergrad. Research Funds	GRAND TOTAL
Materials	Fast-Growing Cyanobacteria: $125	N/A	N/A	N/A	**$125.00**
Equipment	External Calcium Analysis: $48.40	N/A	N/A	External Calcium Analysis: $201.60	**$250.00**
Mileage to gather Data (.38 per mile)	70 Miles: $26.60 Microbiology Dept.	N/A	N/A	N/A	**$26.60**
GRAND TOTAL	$200.00	N/A	N/A		**$401.60**

NOTES:

- Maximum request not to exceed $1000 and may not include a Research Scholarship.
- Equipment and left-over materials purchased with this grant will remain the property of WSU.
- You may not request money for gas purchases for travel. WSU reimburses travel expenses at a set mileage rate only.
- Grant money cannot be used retroactively on previously existing expenses. Requests for reimbursements will be denied. All purchases must be made after receiving funding and clearance from the OUR office.

SEMESTER/EXPLORATORY GRANT APPLICATION
Body of Proposal

DIRECTIONS: The instructions within each section should be removed and replaced with your proposal text. Do not exceed 4 double-spaced pages. Supporting documents or materials should be included as addenda. Proposals should be written clearly and simply. Depending on your specific discipline, your proposal should contain a research question or purpose statement.

Project Description

(Approximately 2 pages)

Precipitation of Carbonate Minerals in the Great Salt Lake by Phage-Mediated Lysis of Autotrophic Bacteria

Introduction

Microbialites (rocks built by microbes) have been on the Earth for about 3.5 billion years and give scientists a look into past environments and how our planet has changed over time (Grotzinger & Knoll, 1999). However, how they form is not fully understood. Observational and experimental work with modern marine microbialites and microbial communities have elucidated some mechanisms, but a thorough understanding of what different microbialite forms, chemistry, and features tell us about past ecosystems and environments does not yet exist. Great Salt Lake (GSL) offers a nearby laboratory to study microbialites, as it hosts extensive microbialite deposits with active microbial communities. In our project, we will investigate one hypothesized formation mechanism: that bacteriophage lysis of cyanobacteria releases stored intracellular carbonate, which when released facilitates calcium carbonate precipitation, which makes up the majority of the GSL microbialites.

Background

Atmospheric carbon dioxide naturally dissolves in bodies of water and exists as dissolved inorganic carbon (DIC) primarily in the form of carbonate ions. Carbonate in modern seawater, for instance, exists at a supersaturated concentration. Cations, particularly Ca^{2+}, can serve to precipitate free carbonate, yielding sediment, though spontaneous precipitation rarely occurs due to a lack of the energy required for initial nucleation.

Most photosynthesizing microorganisms utilize a variety of mechanisms to increase cytoplasmic concentrations of DIC. Often these mechanisms lead to conversion of carbonates to CO_2 to be used by RuBisCO in photosynthetic carbon fixation. The result of this trait is a concentration of intracellular carbonate that is significantly higher than that of the surrounding water. Previous work (Lisle et al., 2016; Xu et al., 2018) has indicated that phage-mediated lysis of photosynthetic cells may release a high enough concentration of carbonate ions to shift the equilibrium in the immediate vicinity such that the energy required for nucleation is overcome and calcium carbonate can be spontaneously precipitated. This mechanism may in part elucidate the process of formation of biogenic structures in the Great Salt Lake known as microbialites.

Question/Hypothesis/Purpose

Does the mechanism of calcium carbonate release by viral lysis contribute to the formation of the microbialites in the Great Salt Lake? Our hypothesis is that if we introduce lytic cyanophage to cyanobacteria species in the lake, when the cyanobacteria cells burst they will release measurable levels of carbonate ions. This will facilitate a carbonate precipitation reaction:

$$Ca^{2+}(aq) + CO_3^{2-}(aq) \rightarrow CaCO_3(s)$$

Over time we predict to see a decrease in calcium ion levels in solution as carbonate precipitation progresses. This result indicates that viral lysis is contributing to calcium precipitation in the lake and introduces a possible mechanism of microbialite formation.

Project Methods & Timeline

(Approximately 1 page)

Experimental Design

We will begin this experiment by preparing our BG-11 approximately 48 hours prior to our field outing, which is on September 29, 2018. We will then take a trip to the south arm of the Great Salt Lake via Antelope Island. We will bring our collection tools for the designated sample gathering; most importantly bringing the BG-11 media and sterile tubes. We will collect the cyanobacteria using a metal spatula from the microbialites directly. After returning to the laboratory, we will inoculate the cyanobacteria on the media. The media is selective to cyanobacteria and we can increase selectivity by imitating the salinity of the Salt Lake. The salinity will be measured using a refractometer.

If we are unable isolate an active sample of cyanobacteria, we will have ordered a fast-growing lab strain of cyanobacteria from a vendor that will be used for its rapid growth and the isolation of the cyanophage. These cyanobacteria are expected to have lawn growths in under a week for the use of cyanobacteria isolation in the form of plaque formations. We will collect water samples from the area near the microbialites that we harvested to increase our chances of finding a cyanophage.

This will be done in a matter of hours and will begin the process of culturing the cyanobacteria which can take several days. Once completed, we will begin the filtration of the Great Salt Lake water sample. This will include centrifuging the sample at a high velocity then filtering the water using a filtration system with a 0.2 μL filter. This will ensure that no residual bacteria invade our filtered sample. Once the cyanophage is filtered, we can begin testing by using spot plates and observing for plaque formation.

This process and the culturing of the cyanobacteria will be the lengthiest aspect to our experiment. However, once the sample has been cultured and the phage is isolated amplification and concentration of the phage will begin to produce the expected results of the experiment. Overall, we anticipate that it will take three weeks for the lab strain of the fast growing and cyanophage to be completed. We also anticipate it will take approximated 6 weeks for the slow growing wild type Cyanobacteria to be finished. We are also planning on 2 weeks for the final data analyses to return. The entire experiment should be completed with our results in approximately 8 to 10 weeks from the beginning. See Addendum B for detailed processes.

Conclusion

The production of a mineral precipitate and a decrease in the calcium levels in solution would indicate that phage-mediated lysis of cyanobacteria could be contributing to the formation of microbialites in the Great Salt Lake. The purpose of this experiment is to discover if lysis of these photosynthetic bacteria can cause spontaneous precipitation of calcium carbonate. This would be an important discovery. If we do see calcium carbonate precipitating, it may bring us one step closer to understanding the mystery of how these microbialites form.

Budget Explanation

(Approximately 1 page)

Item	Purpose	Quantity	Vendor	Price
Synechoccus elongatus	Fast-growing Cyanobacterium	1	UTEX 2973	$125.00
External Calcium Analysis	Confirmation of Results	8 Tests	USU	$250.00
Gas Mileage	Traveling Expenses	~70 Miles	Local Gas Station	$26.60
			Grand Total	**$401.60**

As outlined previously, we will be purchasing fast-growing Cyanobacteria (*Synechoccus elongatus*) from UTEX 2973. We will also be sending our samples to Utah State University for External Confirmation of Results. We have been permitted to use field sampling materials that have been purchased via previous grants. Care has been taken to ensure that we have located the most precise yet budget-friendly results for calcium analysis. We will also be travelling approximately 70 miles to and from the location to collect our samples. The Department of Microbiology has granted us $200, so we are requesting $201.60 from the Undergraduate Research Fund.

Addendum A

BG-11 Medium Recipe

#	Components	Amount	[Stock Solution]	Final Concentration
1	$NaNO_3$ (Fisher BP360-500)	10 mL/L	30 g/200 mL dH_2O	17.6 mM
2	K_2HPO_4 (Sigma P 3786)	10 mL/L	0.8 g/200 mL dH_2O	0.23 mM
3	$MgSO_4 \cdot 7H_2O$ (Sigma 230391)	10 mL/L	1.5 g/200 mL dH_2O	0.3 mM
4	$CaCl_2 \cdot 2H_2O$ (Sigma C-3881)	10 mL/L	0.72 g/200 mL dH_2O	0.24 mM
6	Citric Acid·H_2O (Fisher A 104)	10 mL/L	0.12 g/200 mL dH_2O	0.031 mM
7	Ferric Ammonium Citrate	10 mL/L	0.12 g/200 mL dH_2O	0.021 mM

8	Na2EDTA·2H2O (Sigma ED255)	10 mL/L	.02 g/200 mL dH2O	.0027 mM
9	Na2CO3 (Baker 3604)	10 mL/L	0.4 g/200 mL dH2O	0.19 mM
10	BG-11 Trace Metals Solution	1 mL/L		
11	Sodium Thiosulfate Pentahydrate (agar media only, sterile) (Baker 3946)	1 mL/L	49.6 g/200 mL dH2O	1 mM
12	NaCl (optional for salinity)	mL/L	g/200 mL dH2O	mM

Directions
(Improved recipe as of March 2009)

For 1 L Total
For liquid media:
1. To approximately 900 mL of dH2O add the first 9 components in the order specified while stirring continuously.
2. Bring total volume to 1 L with dH2O.
3. Cover and autoclave medium.
4. Allow to cool then store at refrigerator temperature.

For agar media:
1. To approximately 400 mL of dH2O add the first 9 components in the order specified while stirring continuously.
2. Bring total volume to 500 mL with dH2O.
3. In a separate container add 15 g of agar to 500 mL of dH2O.
4. Cover and autoclave both solutions.
5. In a water bath allow both solutions to cool to 45-50°C.
6. Add sterile Sodium Thiosulfate (Component 10) to agar solution and mix well.
7. Combine both agar and liquid solutions, mix well. Note the agar can solidify quickly.
8. Allow to cool then store at refrigerator temperature.

Addendum B

Preparation of *Synechococcus*:

1. For the process of preparing the BG- 11 and saline BG-11 media see *Addendum A*
2. Inoculate from the ordered slant containing the *Synechococcus elongatus* UTEX 2973 cyanobacteria, to a BG-11 broth.
3. Micropipette 1 mL of broth solution to 4 mL of DI and then 1mL to a prepared BG-11 agar plate using a spread plate technique to form a lawn of growth.
4. Repeat twice for both media.
5. Incubate both samples at 41°C with a 12-hour day and 12-hour night cycle under clear-white fluorescent light for three days or until a healthy lawn is present.
6. These plates will later be used for further inoculations of broths and plates used in the isolation of cyanophages.

Isolation of Cyanobacteria from GSL:

1. For the procedural preparation of saline BG-11 see *Addendum A*.
2. Use a refractometer to measure the saline concentration of the water where the cyanobacteria are being harvested. This will be used to determine the saline content of the saline BG-11 agars and broths for culturing.
3. Bringing back a microbialite from the Great Salt Lake (GSL), to obtain samples for culturing of cyanobacteria.
4. Using a scoopula, scrape off a part of the microbialite, weigh out about 1-2 grams of the scrapings.
5. Place the scrapings into a saline BG-11 broth to dissolve and allow the cyanobacteria to begin reproducing for culturing. Repeat three more times.
6. Incubate at 37°C with a 12-hour day and 12-hour night cycle under fluorescent white lights for one week and re-evaluate growth.
7. Keep in incubator.

Isolation of Cyanophage:

1. Collect water sample from surrounding areas of cyanobacteria collection. Including site of cyanobacteria sample and from the shoreline along with sediment using Jugs provided by Dr. Matthew Domek.
2. Water samples will then be centrifuged in 50 ml tubes to remove large particles in the pellet. The supernatant can then be filtered in the 0.2 μm filtration system (Figure 1.1) to rid the sample of microorganisms and potentially isolate the phage.
3. Using the filtered sample, we will then be able to test for phage activity against *Synechococcus elongatus* using soft agar spot plates on the media described in *Addendum A*.
4. Soft agar will be melted then inoculated with the cyanobacteria and poured onto a media plate. Once allowed to solidify, using the template in Figure 1.2, the spots will be inoculated with 0.2 μl of filtered phage water.
5. Once the spot plates are indicative of phage presence then we can amplify and concentrate the Cyanophage.

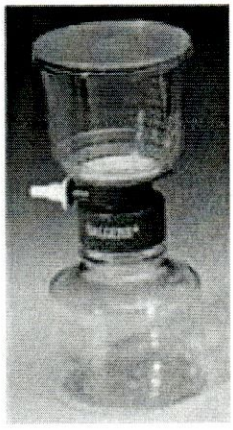

Figure 1.1

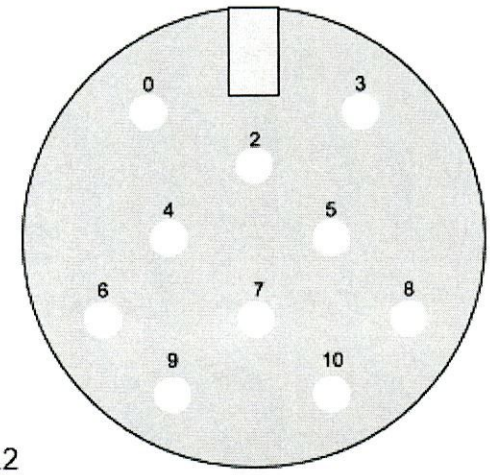

Figure 1.2

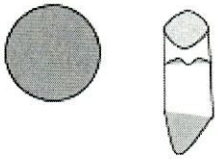

Figure 1.3
BG-11 broth and agar.

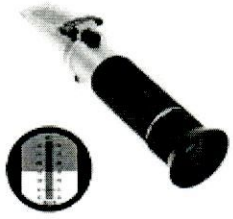

Figure 1.4 Refractometer

Figures

Using phage-mediated lysis we expect to observe cyanobacteria to precipitate carbonate. We expect the use of a synthesized bacteriophage form the Great Salt Lake in the presence of the cyanobacteria will result in phage-mediated lysis. Phage-mediated lysis with cyanobacteria should release high levels of carbonate ions causing precipitation amongst the cyanobacteria. GSL water is slightly undersaturated for the carbonate minerals calcite and aragonite, which will not precipitate spontaneously (abiogenically) under normal environmental conditions. However, the phage-mediated lysis should allow for the equilibrium to shift enough to allow precipitation. As precipitation occurs, we expect to observe decreasing calcium levels, and possibly evidence of sediment developments.

References

Grotzinger, J.P., & Knoll, A.H. (1999). Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annual Review of Earth & Planetary Sciences*, 27:313-358. doi: 10.1146/annurev.earth.27.1.313

Lisle, J. T., & Robbins, L. L. (2016). Viral Lysis of Photosynthesizing Microbes As a Mechanism for Calcium Carbonate Nucleation in Seawater. *Frontiers in Microbiology, 7.* doi:10.3389/fmicb.2016.01958

Xu, H., Peng, X., Bai, S., Ta, K., Yang, S., Liu, S., . . . Guo, Z. (2018). Precipitation of Calcium Carbonate Mineral Induced by Viral Lysis of Cyanobacteria. *Biogeosciences Discussions,* 1-21. doi:10.5194/bg-2018-194

SEMESTER/EXPLORATORY GRANT APPLICATION
Additional Questions

1. What funding have you received from OUR in the past, where has your previous project been disseminated. **N/A**

2. Is this project part of a required course? If so, please indicate the support (monetary and in-kind) provided for this project by the academic department. **No**

3. What additional sources of funding have been solicited? Is your department willing/able to fund any equipment they will be retaining? **The Departments of Geosciences and Microbiology are funding the equipment, consumables, and mileage for the field work. We are requesting funds for the fast-growing strain of cyanobacteria and for calcium analyses.**

4. Where do you plan to disseminate the results of this project? **Research and results will be presented in Seminar at the end of the semester, written up as a final report to be submitted to ERGO, and presented at the OUR symposium in Spring, 2019.**

5. If you are requesting a stipend, please list all significant time commitments (5+ hours per week) that you expect to maintain over the duration of your project including, for example, class and work schedules. **N/A**

SEMESTER/EXPLORATORY GRANT APPLICATION

Faculty Recommendation Form

Student Name (last, first): Santana, Darian

Project Title: Precipitation of Carbonates by Viral Lysis of Cyanobacteria in GSL

Mentor Directions: After carefully reviewing the proposal and assessing both the viability of this project and the qualifications of the student requesting funding, answer the questions found below. Please expand the sections as necessary (**do not attach separate letter**). If the project involves the use of human subjects or protected animals, be sure the student secures IRB or ACUC approval. If the project receives funding, it is your responsibility to work closely with the student, monitor the ongoing progress of the project and budget, and evaluate the project's results. Failure to do so will jeopardize funding for this project and any future projects.

1. How long and in what capacity have you known this student?

The students are taking my GEO/MICR 3753 Geomicrobiology course, and I have known them since the start of the Fall semester.

2. Briefly describe the proposed project. Is this part of a larger research project? Is this part of a course? If so, how is the project apart from the nature and scope of activities normally taken for the course (Please attach a copy of your course syllabus)?

Students in my Geomicrobiology course are asked to design a geomicrobiology research project related to Great Salt Lake. They spend the first few weeks developing and discussing ideas with their peers, then prepare proposals for submission to OUR to be completed during the semester. This year, the students divided into two groups with related, but distinct research focuses. This group has come up with a novel and intriguing hypothesis about a biological mechanism for carbonate production in GSL and has designed an experiment to test it.

3. Give an assessment of the project's significance to the student's discipline and of the project's educational and/or professional benefit to the student.

This project was entirely the idea of the project lead. This is the second year in a row that a microbiology student has taken an idea from a recent course and sought to apply it to a question in Geomicrobiology. In this project, the students will investigate the potential for viral-induced cell lysis to release intracellular carbonate stored by cyanobacterial primary producers in Great Salt Lake. This could provide a concentrated source of carbonate and facilitate calcium carbonate precipitation, potentially providing a mechanism for building the calcium carbonate deposits in GSL. I am very excited to see what they find.

4. Comment on the qualifications of the student to successfully complete this project, both in terms of the project's scope and its time frame.

The students involved in this project are microbiology majors with a variety of advanced coursework between them, including coursework in virology, microbial ecology, and advanced laboratory techniques. They have worked well together to develop their ideas and write this proposal. Their experiment is well-designed and novel and I am confident in their ability to carry out this experiment.

5. Comment on the justification and appropriateness of the project budget, including the necessity of a stipend (if requesting one).

The students are asking for funds for a culture collection culture of a fast-growing cyanobacterium and for external chemical analyses. The costs are reasonable, and no stipend is requested.

6. Describe your role in the project.

I will mentor the students, help them with the field sample collection, media development and troubleshooting, and experiment setup, make my laboratory facilities available to them, and help them make connections with other faculty as needed. I will be able to provide them with contextual information, background, and literature suggestions. I will also review their results and interpretations, advise them as they prepare their presentations, and encourage them to more broadly disseminate their results.

7. Include anything else that you think will be helpful to the committee in evaluating this application.
This project will give the students hands-on experience with geochemical and geomicrobiological techniques, and complement the material they are learning in my Geomicrobiology course. Undergraduate research is a High-Impact Practice, and course-based undergraduate research is a means of involving more students (particularly non-trad, low-income, and underrepresented students) in high-impact experiences. These projects help students develop scientific independence. In the past, projects in this class have led to longer-term independent research projects that have resulted in exciting discoveries.

This project ____ DOES _X_ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

9/26/18

Project Mentor Signature Date

2507 Campus Mail Code

6181 Phone Extension