# UNIVERSITY OF WISCONSIN - LA CROSSE
# COLLEGE OF SCIENCE AND HEALTH

## TRAVEL & SUPPLIES GRANTS
## For Student Researchers

Date: 15Feb24 **Total funds requested** $500.00

Term for which funds are requested: 20 24 Fall
20 24 Spring
20 ___ Summer

Type of Grant:
**Type A: Supplies** Supplies
**Type B: Travel** ___

**Project Title** Using CRISPR to Explore the Influence the bolA Gene has on Cellulose Production in Komagataeibacter xylinus.

Conference Title (Travel Grants): ___

Destination/Dates of travel: ___

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Principle Student Author:

Print/type name: Lindsey Zimmerman Student ID: REDACTED
Local address: REDACTED Local phone: REDACTED
Signature: ___ E-mail: REDACTED

Other Student Authors:

Print/type name: ___ Student ID: ___
Local address: ___ Local phone: ___
Signature: ___ E-mail: ___

Print/type name: ___ Student ID: ___
Local address: ___ Local phone: ___
Signature: ___ E-mail: ___

**Faculty mentor 1:**

Name: Paul Schweiger Department: Microbiology
Signature: ___ E-mail address pschweiger@uwlax.edu

**Faculty mentor 2:**

Name: ___ Department: ___
Signature: ___ E-mail address ___

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(For office use only)

**Eligibility check for students:**

| | 1 | 2 | 3 |
|---|---|---|---|
| Enrolled? | x | | |
| SAH major | x | | |
| GPA > 2.5 | x | | |
| Previous reports on file | | | |

**Committee action:** Approval: Denial: Date: Amount:

**Dean's action:** Approval: Denial: Date:

UW-L CSH
TRAVEL AND SUPPLIES GRANTS FOR STUDENTS

# SUPPLIES GRANT APPLICATION

## Budget Form

Name: Lindsey Zimmerman

Project Title: Using CRISPR to Explore the Influence the bolA Gene has on Cellulose Production in Komagataeibacter xylinus.

Advisor: Paul Schweiger

Department: Microbiology

**Itemized list of required supplies :**

| Item | Quantity | | Unit Cost | | Total Cost |
|---|---|---|---|---|---|
| Q5 Site-Directed Mutagenesis Kit | 1 | x | $224.00 | = | $ 224.00 |
| 66-mer EXTREmer oligos | 4 | x | $70.00 | = | $ 280.00 |
| | | x | | = | $ |
| | | x | | = | $ |
| | | x | | = | $ |
| | | x | | = | $ |
| | | x | | = | $ |
| | | x | | = | $ |
| | | x | | = | $ |
| | | x | | = | $ |
| | | x | | = | $ |
| | | x | | = | $ |

**Total Cost:** $ 504.00

Please explain any unusual costs or circumstances. If the total cost is greater than $500, indicate where the balance of the funding will come from (your host department, advisor, yourself, etc.).

The cost is $4 over $500. This cost will be absorbed by either the department / advisor.

Using CRISPR to Explore the Influence the *bolA* Gene has on Cellulose Production in

*Komagataeibacter xylinus*.

Lindsey Zimmerman

**Abstract**:

Acetic acid bacteria are a group of bacteria that produce many industrially important products, such as vinegar (acetic acid), vitamin C, the antidiabetic drug miglitol, cosmetic self-tanning agents, and bacterial cellulose[1]. Bacterial cellulose has applications in the medical field as an artificial wound dressing for severe burns and as drug delivery system[2]. The production of bacterial cellulose requires the binding of a regulatory molecule called c-di-GMP. This molecule is known to be negatively regulated by the *bolA* protein in other organisms[3]. However, its regulation in cellulose producers, such as the acetic acid bacterium *Komagataeibacter xylinus*, is unknown. A predicted *bolA* gene was identified in *K. xylinus* but it was not able to be deleted, suggesting it is required for survival. The use of a gene expression interreference system called CRISPRi allows the knockdown of gene expression rather than deletion of gene function[4]. I propose to create a CRISPRi system for use in acetic acid bacteria and target the *bolA* gene to knockdown its expression. The knockdown of *bolA* expression is expected to increase cellular c-di-GMP levels and stimulate cellulose production. This study will help identify the physiological role of *bolA* in *K. xylinus,* as well as provide information for bioengineering increased bacterial cellulose yields for medical use.

**Narrative:**

**Background and Significance**

Acetic acid bacteria can convert ethanol, sugars, and polyols into various organic acids in the normal course of their metabolism. These biochemical reactions are used industrially to produce various fermented foods and beverages (e.g. vinegar and cocoa), vitamin C, cosmetic tanning agents, and the antidiabetic drug miglitol. Some of these bacteria can also produce a biofilm of cellulose[1]. Bacterial cellulose can be used as a wound dressing and can help deliver drugs during wound treatment, making it of industrial importance[2]. The production of bacterial cellulose requires the binding of a regulatory molecule called cyclic-di-guanine monophosphate (c-di-GMP) to bind the export machinery. In other bacteria, c-di-GMP levels are negatively regulated by the protein *bolA*, while nothing is known about the regulation c-di-GMP in acetic acid bacteria[3]. Analysis of the genome the model acetic acid bacteria, *Komagataeibacter xylinus*, revealed the presence of a predicted *bolA*. This led to the hypothesis that a decrease in *bolA* would increase the synthesis of cellulose. Attempts to delete this gene were unsuccessful (Schweiger Lab, unpublished results), suggesting that this gene is required. An alternative approach is to use a CRISPR interference (CRISPRi) system. This system uses an inactivated version of the Cas9 enzyme (dCas9) from *Streptococcus pyogenes* that binds DNA but does not cut, unlike the active version of Cas9 that cuts DNA[5,6]. This inactive dCas9 enzyme can be guided by a short piece of complementary RNA to a specific DNA region. Once bound to its target, dCas9 can partially block gene expression (i.e. knockdown expression). When engineered to target different regions of a gene, this system causes varying levels of reduced expression, allowing the function of required genes to be studied. I propose to use a CRISPRi

system to target multiple regions of the predicted *bolA* gene of *Komagataeibacter xylinus* and examine the effect of reduced gene expression on bacterial cellulose production. Reduced *bolA* protein levels is predicted to increase c-di-GMP levels and increase cellulose production, which would help characterize the role of *bolA* in *Komagataeibacter xylinus* and have medical applications.

**Aims/objectives:**

1. Construct an acetic acid bacterial CRISPRi vector that expresses inactive dCas9 and clone in *bolA* gene knockdown targets.
2. Examine the influence of decreased *bolA* gene expression on bacterial cellulose production in *Komagataeibacter xylinus.*

**Methods:**

**Aim 1**: To construct a CRISPRi system compatible in acetic acid bacteria, the required dCas9 and other required CRISPR elements will be amplified by PCR from the *E. coli* pCRISPathBrick plasmid and cloned into the acetic acid bacterial expression vector pBBR1p452 to create the plasmid pdCas9AABrick[5-7]. Site directed mutagenesis of a BsaI restriction site on pBBR1p452 will be done using the Q5 Site-Directed Mutagenesis kit (New England Biolabs) to allow cloning of two *bolA* gene knockdown targets into pdCas9AABrick. The two *bolA* knockdown targets will be cloned into the one remaining BsaI site using 66-mer oligonucleotide pairs following an established protocol[5,6]. DNA sequencing will be done to confirm plasmid construction and site directed mutagenesis is correct.

**Aim 2**: The pdCas9AABrick plasmids containing the *bolA* knockdown targets will be transformed into *K. xylinus* following established protocols[8]. Bacteria that take-up the CRISPRi plasmids will

be inoculated onto yeast-fructose (YF) medium for cellulose production assays. I will grow *K. xylinus* statically for 5 days in a 250 ml Erlenmeyer flask containing 25 ml YF medium and quantify the effect of *bolA* knockdown on cellulose production in comparison to wildtype by harvesting the cellulose biofilm, submerging in 1M NaOH for 1h at 80C, washing with distilled water until pH 7.0, and drying at 60C until a constant weight[8].

**Student Involvement:**

I started my independent research project work on this project in mid-Fall ’23. I will continue to work on this project until I graduate in May ’25. I have been involved in the planning and execution of the project. My role is to create the dCas9 expression vector and clone our *bolA* targets into the plasmid. I will also be responsible for the bacterial cellulose production assays.

**Dissemination:**

We will target publication in high-impact journals such as Applied Microbiology and Biotechnology or Applied and Environmental Microbiology, as well as the UWL Journal of Undergraduate Research. The data from this study will be presented at the UWL Research & Creativity Symposium and the Annual ASM North Central Branch Conference.

**Resources:**

The project will be done in the lab of Dr. Paul Schweiger in PSSC 4020, which has all the necessary equipment and resources needed to complete the proposed work (e.g. incubators, electrophoresis equipment, thermocyclers, refrigerators/freezers, centrifuges, etc.).

**Other funding:**

This project has no other sources of current or pending funding. The money requested will go directly to research supplies that are needed to complete the molecular work of this project.

**Budget:**

The requested funds ($500) will be used to purchase the supplies needed for site directed mutagenesis and to construct two *bolA* CRISPRi targets needed to knockdown gene expression. This requires a Q5 Site-Directed Mutagenesis kit (New England Biolabs) and 2 pairs of long synthetic 66-mer oligonucleotides (4 total single-stranded DNA fragments) of high purity. All other supplies are already available in Dr. Schweiger's lab in PSSC 4020.

| **Item** | **Supplier** | **Cat#** | **Purpose** | **Unit Price** | **Units** | **Total Price** |
|---|---|---|---|---|---|---|
| Q5 Site-Directed Mutagenesis Kit | NEB | E0554S | Site Directed mutagenesis | $224.00 | 1 | $224.00 |
| 66-mer EXTREmer oligos | Eurofins Genomics | n/a | Cloning *bolA* CRISPRi targets | $70.00 | 4 | $280.00 |
| **Total** | | | | | | $504.00 **($500 requested)** |

**References:**


1. Yassunaka Hata, N. N., Surek, M., Sartori, D., Vassoler Serrato, R. & Aparecida Spinosa, W. Role of Acetic Acid Bacteria in Food and Beverages. *Food Technol Biotechnol* **61**, 85-103 (2023). https://doi.org/10.17113/ftb.61.01.23.7811
2. Lahiri, D. *et al.* Bacterial Cellulose: Production, Characterization, and Application as Antimicrobial Agent. *Int J Mol Sci* **22** (2021). https://doi.org/10.3390/ijms222312984
3. Moreira, R. N. *et al.* BolA Is Required for the Accurate Regulation of c-di-GMP, a Central Player in Biofilm Formation. *mBio* **8** (2017). https://doi.org/10.1128/mBio.00443-17
4. Azam, M. W., Zuberi, A. & Khan, A. U. bolA gene involved in curli amyloids and fimbriae production in E. coli: exploring pathways to inhibit biofilm and amyloid formation. *J Biol Res (Thessalon)* **27**, 10 (2020). https://doi.org/10.1186/s40709-020-00120-7
5. Kirtania, P. *et al.* A single plasmid based CRISPR interference in Synechocystis 6803 - A proof of concept. *PLoS One* **14**, e0225375 (2019). https://doi.org/10.1371/journal.pone.0225375
6. Cress, B. F. *et al.* CRISPathBrick: Modular Combinatorial Assembly of Type II-A CRISPR Arrays for dCas9-Mediated Multiplex Transcriptional Repression in E. coli. *ACS Synth Biol* **4**, 987-1000 (2015). https://doi.org/10.1021/acssynbio.5b00012
7. Kallnik, V., Meyer, M., Deppenmeier, U. & Schweiger, P. Construction of expression vectors for protein production in Gluconobacter oxydans. *J Biotechnol* **150**, 460-465 (2010). https://doi.org/10.1016/j.jbiotec.2010.10.069
8. Anguluri, K., La China, S., Brugnoli, M., Cassanelli, S. & Gullo, M. Better under stress: Improving bacterial cellulose production by Komagataeibacter xylinus K2G30 (UMCC 2756) using adaptive laboratory evolution. *Front Microbiol* **13**, 994097 (2022). https://doi.org/10.3389/fmicb.2022.994097