UNIVERSITY OF WISCONSIN - LA CROSSE
COLLEGE OF SCIENCE AND HEALTH

TRAVEL & SUPPLIES GRANTS
For Student Researchers

Date: February 19, 2024 **Total funds requested** $ 500.00

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

Type of Grant:
Type A: Supplies X
Type B: Travel

Project Title Agrobacterium Mediated Mutagenesis as a Tool for Strain Creation and Gene Function Discovery in the Fungus Pleurotus eryngii

Conference Title (Travel Grants):

Destination/Dates of travel:

Principle Student Author:

Print/type name: Ben Graham 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: Todd Osmundson Department: Biology
Signature: E-mail address tosmundson@uwlax.edu

Faculty mentor 2:

Name: Department:
Signature: E-mail address

(For office use only)

Eligibility check for students:	1	2	3
Enrolled?			
SAH major			
GPA > 2.5			
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: Ben Graham

Project Title: Agrobacterium Mediated Mutagenesis as a Tool for Strain Creation and Gene Function Discovery in the Fungus Pleurotus eryngii

Advisor: Dr. Todd Osmundson

Department: Biology

Itemized list of required supplies :

Item	Quantity		Unit Cost		Total Cost
Plasmid pBHt2 for Agrobacterium transformation	1	x	$85.00	=	$ 85.00
Kanamycin antibiotic for transformant screening (1 g)	1	x	$30.47	=	$ 30.47
Minimal Medium salts for induction medium	1	x	$68.53	=	$ 68.53
2-(N-morpholino)ethanesulfonic acid (MES) for induction medium	1	x	$20.29	=	$ 20.29
Acetosyringone for inducing Agrobacterium cells	1	x	$29.40	=	$ 29.40
0.45-µm pore, 45-mm diameter nitrocellulose filters for growing transformed mycelium	1	x	$78.08	=	$ 78.08
cefotaxime for mutant screening	1	x	$20.00	=	$ 20.00
moxalactum for mutant screening	1	x	$62.72	=	$ 62.72
glycerol for growth of screened mutants	1	x	$40.50	=	$ 40.50
Whatman No. 1 filter paper for filtering of mycelial cultures	1	x	$14.50	=	$ 14.50
TAIL-PCR primers (6 border and 1 arbitrary primer)	7	x	$7.31	=	$ 51.17
		x		=	$

Total Cost: $ 500.66

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.).

Agrobacterium Mediated Mutagenesis as a Tool for Strain Creation and Gene Function Discovery in the Fungus *Pleurotus eryngii*

Abstract

Pleurotus eryngii, also known as the King Oyster Mushroom, is a commonly grown edible mushroom coveted for its taste, texture, and size. A variety of commercial *Pleurotus eryngii* strains are available and some have been isolated to perform well under temperature conditions outside of the normal range for the species, but none have been developed that have highly distinguishable morphologies or traits such as other pigments, sporelessness, or variations in stem shape. In previous research, I have used UV light mutagenesis to successfully alter *P. eryngii;* however, this method does not allow determining which genes are responsible for particular changes. The objective of the proposed research is to use *Agrobacterium*-mediated mutagenesis to extend this prior research, since this method allows mutated genes to be isolated and have their DNA sequenced, providing insight into the genetic mechanisms that govern commercially-relevant traits in edible mushrooms. Funding will be used to purchase necessary supplies and reagents for the project.

Narrative

Background - This study is part of an ongoing research project on the development of improved strains of the king oyster mushroom, *Pleurotus eryngii,* through the use of mutagenesis methods. The fungus *Pleurotus eryngii* is of significant culinary importance and is commonly cultivated across the world (Stamets 2000). Available commercial cultures of *P. eryngii* are available, but only differ in their optimal growth temperature. In contrast, mutagenesis of spores or mycelium (the fungal cells involved in growth and nutrient acquisition) in the relatives *Pleurotus ostreatus* (blue oyster mushroom) and *Pleurotus tuber-regium* have yielded a wide variety of strains that differ in shape, color, favored environmental conditions, fruiting speed, and yield (Sharma and Sharma 2014; Bamigboye et al. 2019). However, such efforts typically seek improvement as an end in itself, rather than determining which genes are responsible for particular changes in traits; furthermore, some of the most commonly used mutagenesis methods do not allow the effected genes to be determined. Our understanding of genetic mechanisms in mushroom growth could be improved considerably through *Agrobacterium*-mediated mutagenesis (Celis et al. 2017). In this method, the bacterium *Agrobacterium tumefaciens* is co-cultured with an organism of interest. *Agrobacterium* inserts a piece of its own genome randomly into the genome of the organism that it infects; because the infecting DNA sequence is known, molecular genetic techniques such as TAIL-PCR (thermally asymmetric interlaced polymerase chain reaction) can be used to isolate the infecting DNA to obtain the DNA sequence of the flanking DNA that represents the interrupted gene in the organism of interest.

Significance Access to a diverse array of strains in commercial mushroom cultivation allows for different substrates to be utilized, contaminating organisms to be combated, and different culinarily-relevant phenotypes **t**o be produced. In addition, new strains can provide solutions to

serious problems that occur in industrial cultivation. The presence of massive amounts of spores in the air can lead to health problems if inhaled for long periods of time**,** necessitating respirators, air filtration, and additional cleaning in cultivation facilities. Researchers developed a sporeless strain of *P. eryngii* using ultraviolet (UV) mutagenesis, but this strain never became commercially available (Obatake et al. 2002). Because sporeless strains cannot, by definition, reproduce via spores, cultures cannot be stably reproduced over generations; consistent generation of new strains is therefore essential. Understanding the genetic mechanism of sporelessness would allow targeted interruption of the related genes.

Methods

Protoplast Production: Spores will be germinated on agar medium; individuals will be isolated, and then grown in malt yeast extract liquid culture for one week. Mycelium will then be rinsed with water and osmotic stabilizer, blotted dry using sterilized filter paper, and suspended in 2 mL of chitinase solution (15 mg/mL in 0.6 M) for cell wall digestion (Combier et al. 2003).

Agrobacterium Growth: *Agrobacterium tumefaciens* will be grown at 28‡C for 2 days in liquid LB medium supplemented with 50 ug/ml^-1 carbenicillin and 50 ug/ml^-1 kanamycin. One milliliter fresh culture will be transferred to 100 ml of YEB medium containing spectinomycin and streptomycin and grown overnight at 28 C to an optical density at 600 nm of 0.3–0.4.

Co-cultivation of *A. tumefaciens* and *P. eryngii*: 100 ml of bacterial culture induced with 200um acetosyringone will be added to each glass microfiber disc containing fungal macerated mycelium, before being placed on growth media. The plates will be incubated at 25c for 24-96 hours. Subculturing will be done on media without hygromycin, and then reintroduced to media with hygromycin to ensure stability of the hygromycin resistance. A hygromycin resistance gene

is incorporated into the infecting gene so that only successfully-mutagenized cells will grow in the presence of the fungicide hygromycin.

TAIL-PCR: TAIL-PCR (thermally asymetric interlaced polymerase chain reaction) will be used to amplify (preferentially make copies of) the infecting DNA. Primers that are complementary to the left and right border regions of the infecting DNA will be paired with a more general "arbitrary" primer to produce copies of the gene on either side of where the *Agrobacterium* DNA is inserted. Nested sets of three left-border and three right-border primers will be used to account for potential truncation of the border regions and to facilitate subsequent DNA sequencing (Mullins et al. 2001).

DNA sequencing: TAIL-PCR amplicons will be assessed for size and DNA quantity using agarose gel electrophoresis and fluorometric quantification, then prepared for sequencing using EXO-SAP IT enzymes to remove unincorporated nucleotides and PCR primers. The products will then be sent to Eurofins Genomics (Lexington, KY) for DNA sequencing. Funding for this portion of the project is available through my advisor's laboratory, so I am not requesting additional funding for that purpose in the present proposal.

Presentation of results: I will present the results of my research at the spring 2025 UWL Research and Creativity Symposium. Furthermore, if my advisor and I deem the results publishable, we will collaborate on preparation of a manuscript for submission to *Mycologia* or another appropriate scientific journal.

I will be taking the lead on all of the research methods described, with assistance from Dr. Todd Osmundson. I plan on continuously developing this project, with completion prior to my expected graduation in May, 2025.

Bibliography

Bamigboye C.O., Oloke J.K., Dames, J.F. 2019. Development of high yielding strain of *Pleurotus tuber-regium*: fructification, nutritional and phylogenetic studies. Journal of Food Science and Technology 56(8):3597-3608.

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Combier, J.P., D. Melayah, C. Rafier, G. Gay, R. Marmeisse. 2003 *Agrobacterium tumefaciens*-mediated transformation as a tool for insertional mutagenesis in the symbiotic ectomycorrhizal fungus *Hebeloma cylindrosporum*. FEMS Microbiology Letters 220: 141-148.

Mullins, E. D., Chen, X., Romaine, P., Raina, R., Geiser, D. M., and Kang, S. 2001. *Agrobacterium*-mediated transformation of *Fusarium oxysporum*: An efficient tool for insertional mutagenesis and gene transfer. Phytopathology 91:173-180.

Obatake Y., Murakami S., and Matsumoto T, Fukumasa-Nakai, Y. 2003. Isolation and characterization of a sporeless mutant in *Pleurotus eryngii*. Mycoscience 44(1): 33-40.

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