Beta Beta Beta Research Foundation
Research Grant Application

Beta Beta Beta, through our Research Foundation, supports selected research activities by undergraduates who are regular members of TriBeta. Students interested in applying for a research scholarship must be registered as regular members at the National Office before the grant submission date.

The submission due date for academic year 2022-2023 is:
Friday, September 30th, 2022, 11:59pm PST.

Instructions for Submitting Research Grant Applications

- Please complete the Research Grant Application completely and save as a PDF file. All questions must be answered.

- Please save your application file with the following naming convention. If submitting multiple files, each file name must contain this information.

  Naming Convention: LastNameFirstName_GreekChapter_School.pdf
  Example: DalyIris_BetaZeta_UnivNorthAlabama.pdf

- Only Regular or promoted TriBeta members can be awarded a research grant. Associate members are not eligible for research grants.

- All research grant applications must be submitted via the JotForm upload link provided below.

  Jotform Upload

- Once submitted, the applicant(s), chapter advisor, and research advisor will be notified via email of the successful submission.

- Each chapter may submit a maximum of six (6) applications per year and no more than three (3) applications per research advisor.

- COVID-19 NOTICE: This 2021-2022 Beta Beta Beta Foundation Research Scholarship Application should be restricted to research that can be completed at applicant's home institution or nearby research facility with appropriate COVID-19 health measures in place.

For any questions, please contact the National Office via email: tribeta@una.edu.

Beta Beta Beta Research Grant Application
Academic Year: 2022-2023

Student Name(s): Iris Daly

Student Tribeta Membership #: 2221588

Student Email Address: ivdaly@una.edu

Chapter (Greek Name): Beta Zeta

School Name (no abbreviations please): University of North Alabama

District: SE-2

Chapter Advisor: Dr. Lisa Blankenship

Chapter Advisor Email: lblankenship@una.edu

Faculty Research Advisor Name: Dr. Terry Richardson

Faculty Research Advisor Email: trichardson@una.edu

Faculty Research Advisor Phone Number: 256-765-6220

Title of Research Project:

The Fruit Fly (Drosophila melanogaster) as a Suitable Model for Investigating the Infectious Process of Three Bacterial Pathogens of Humans

Amount Requested: $632.59

Did a student(s) in your chapter receive a grant(s) last year? If so, where did the student(s) present his/their work?

Yes, SE-1 & SE-2 District Convention with the ASB Convention, April 2022

Does the proposed research use human subjects? If yes, do you have your institution's approval? Include/Attach an explanation.

No

Does the proposed research use live vertebrates? If yes, do you have your institution's approval? Include/Attach an explanation.

Yes, approved by the Biology Dept Chair, Dr. Amy Crews in August 2022.

FUND DISBURSEMENT

Will your institution provide matching funds for this research project?
If Yes, describe source.

No

Will this be part of an ongoing funded project?
If yes, describe other source(s) of funding.

No

Will your institution require TriBeta funds to be administered through university disbursement procedures?

No

All research grant checks will be made payable to the Biology Department at your institution. If checks need to be made payable differently, please provide the specifics. Checks cannot be made payable to a student.

DESCRIPTION OF RESEARCH PROJECT

Your description should include the following: title, short abstract, and outline of your proposed project. Your outline should include your research plans with specific objectives, explanation of data, treatment, how the finding may relate to your hypothesis and literature cited or reviewed.

Included at end of document.

COMPLETION OF RESEARCH PROJECT

Location of research: Biology labs on campus

Projected time for completed of research: March 2023

Projected time for presentation of research: Spring 2023 at SE-1/SE-2 District Convention

PROPOSED BUDGET REQUEST SUMMARY

Complete the proposed budget request providing details and rationale of all expenditures. Travel (based on mileage) to conduct research may be included. Travel to present the research cannot be included. Please note, Beta Beta Beta Foundation Research Scholarships cannot be used for indirect costs or institutional overhead. Range of grants awarded are $250 - $1,500.

1. Supplies: $632.59
2. Equipment:
3. Travel to Conduct Research:
4. Other Costs:

TOTAL COSTS: $632.59

FINAL REPORT

Beginning 2003, a "**Final Report**" will no longer be required from recipients of the undergraduate "TriBeta Research Scholarship Foundation Grants". The research should be abstracted and presented, with funding acknowledgement, at a TriBeta District/Regional Convention and/or National Convention. The abstract must be submitted to the Convention Coordinators for documentation and program listing. The District Directors/Regional Vice Presidents will forward these abstracts to the editor of BIOS for inclusion in the convention news section.

Acknowledgment of Research Grant Funding Requirements

Please check next to each statement indicating you have read and agree to comply with all specifications of funding as outlined in this application.

☒ The research should be abstracted and presented, with funding acknowledgement, at a TriBeta District/Regional Convention and/or National Convention. The abstract must be submitted to the Convention Coordinators for documentation and program listing. The District Directors/Regional Vice Presidents will forward these abstracts to the editor of BIOS for inclusion in the convention news section.

☒ If the grant recipient is unable to present their research at a TriBeta convention the student may submit a written request seeking permission from their Regional Vice President to present their research at an alternate scientific meeting considered appropriate for undergraduate research. If the Regional Vice President grants this request the research to be presented must be abstracted, according to the BIOS format. Copies of the abstract and the meetings program, including a listing of the student's paper, must be forwarded to their Regional Vice President.

☒ In the event a student is unable to make a formal presentation of their research at a TriBeta convention or an alternate approved meeting, then a manuscript following the BIOS format should be submitted to BIOS for publication. Copies of this submission must be submitted to the Regional Vice President. In the event a student is unable to make a formal presentation of their research, the student must request permission to submit a Final Report to the Regional Vice President.

I have read and agree to comply with all specifications of funding as outlined in this application. Digital signatures are acceptable.

Student's Signature: ___Iris Daly_____ **Date:** ____**8/11/2022**_____

Faculty Sponsor's Signature: ____*Dr. Terry Richardson* _____ **Date:** ___**8/11/2022**_____

Chapter Advisor's Signature ___**Dr. Lisa Blankenship**_____ **Date:** __**8/11/2022**_____

The Fruit Fly (Drosophila melanogaster) as a Suitable Model for Investigating the Infectious Process of Three Bacterial Pathogens of Humans

A Proposal Submitted to the Tri-Beta Foundation Research Scholarship Program

By: Iris Daly, Beta Zeta Chapter, University of North Alabama

ABSTRACT

The innate immune system is the second line of defense against invasive microbial pathogens. It is an important prelude to activation of the adaptive system and in non-vertebrate animals it is the last line of defense against infection. Understanding how this system works in vertebrates is often complicated by the presence of the adaptive system. Hence, researchers have begun to study the basics of the system in fruit flies which lack an adaptive system. This study proposes to explore the role of the fruit fly's innate system in protection against three bacterial species that are known human pathogens. Specifically, the flies will be stressed modestly by diet limitation and infected with the bacteria via oral administration. They will then be monitored for mortality and/or sublethal infection. Results are expected to reveal the suitability of the fly as a model organism for studying the infectious process of these three pathogens in the face of a stressed host.

INTRODUCTION

The fruit fly, Drosophila melanogaster, has been demonstrated as a suitable model host for many different topics in scientific research and therefore, its characteristics are very well known. In fact results have shown that the innate system of fruit flies is very similar to that of their mammalian counterparts (Lemaitre and Hoffmann 2007). Since the genes of the innate system of humans and fruit flies are very similar, many conclusions about the human innate system can be drawn from implementing the fruit fly as a model organism of infection for Pseudomonas aeruginosa. The proposed study would determine whether a simpler mode of infection could be employed than what has already been published, and it would determine how effective the innate system combats an infection of P. aeruginosa when stressed on a nutritional basis. Based on preliminary studies, the principal investigator hypothesizes that when fruit flies are nutritionally deprived to some extent, the virulence of P. aeruginosa will become enhanced and cause an acute infection resulting in premature death of the fruit flies.

Two established methods have demonstrated that D. melanogaster is a model system of infection for P. aeruginosa. One such method involves pricking the abdomen and directly injecting the bacterium into the intestines of the fruit flies. This method of infection has the disadvantage that it produces injury of the fruit fly during pricking. Nonetheless, fruit flies pricked with P. aeruginosa exhibited much lower survival rates than fruit flies that had been pricked with no bacteria (D'Argenio et. al 2001). Another method more commonly employed because it is less tedious and does not physically harm the fruit fly is the Chugani method. In this method, P. aeruginosa is cultured, suspended in saline solution, and centrifuged. Next, the supernatant from the saline solution is collected, and it is assumed that the solution contains all products secreted from the bacterium. This suspension is added to filter paper that is placed on the surface of a 5% sucrose agar. By using this method it ensures that the flies will eat the bacteria's supernatant along with their food, thus causing the symptoms of infection (Chugani et. al 2000). This method has proven to be successful, evidenced by a decrease in percent survival rate of flies fed both bacteria and sucrose versus

those flies only fed sucrose. By using these two methods, scientists can evaluate many factors that influence the virulence of P. aeruginosa without having to compromise the health of human beings.

However, a preliminary study conducted by the principal investigator last year suggested that a simpler method could be used by simply feeding the bacteria to the flies. This method of feeding bacteria directly to the fruit flies has already been established using other bacteria such as Serratia marcescens, a known insect pathogen (Nehme et. al 2007). Feeding the bacterium P. aeruginosa to fruit flies resulted in a chronic infection that flourished within their bodies and replaced all natural flora originally inhabiting the fruit flies. However, it was undeterminable whether the fruit flies died of natural cause or because of the chronic infection.

One study conducted by Sibley et. al demonstrated the effects of polymicrobial interactions of P. aeruginosa and certain species of Streptococcus in the fruit fly using a modified version of the Chugani method (2008). The results strongly suggested that normally nonpathogenic Streptococcus species found in the oral cavity and in cystic fibrosis patients' sputum, when combined with P. aeruginosa create a synergistic effect that kills fruit flies much quicker than P. aeruginosa alone. Another bacterium known to create a synergistic effect with P. aeruginosa in the lungs of CF patients, Staphylococcus aureus, has also been demonstrated as pathogenic in fruit flies (Needham et. al 2004). However, whether or not they will create the same synergistic effect in fruit flies has yet to be determined conclusively. Research conducted by the principal investigator last year suggested that there was no synergistic effect with these two bacteria employing the method developed in our laboratory.

Not only have certain polymicrobial interactions been examined, but also a broad range of factors affecting the virulence of P. aeruginosa has been studied to some extent as well. Typically, most studies have tested the effects on the bacterium by altering the genetics of either the bacterium or fruit fly or by altering the environment in some way to induce stress or hypervirulence (Erickson et. al 2004). While several studies have shown that removing essential nutrients such as magnesium from the bacteria's environment will stress the bacteria, few studies have employed the opposite approach of stressing the fruit fly with physical factors such as starvation or desiccation. The current study proposed here seeks to determine whether the bacteria's virulence is affected by stress conditions imposed on the fruit fly, specifically starvation. Results from preliminary studies conducted by the principal investigator indicated that removing all food from the fruit flies' environment killed them quicker than the infection from bacteria, but when the fruit flies were given an adequate amount of food the fruit flies could withstand a chronic infection of P. aeruginosa. The proposed study will attempt to determine the level of nutrition at which death will occur due to infection rather than starvation or natural causes.

MATERIALS AND METHODS

Microbial cultures:

The fruit flies in this study will be challenged by three bacterial pathogens. These include Staphylococcus aureus (ATCC #43300), Pseudomonas aeruginosa (PA01) and a red pigmented strain of Serratia marcescens (ATCC #43861). These cultures will be obtained from Hardy Diagnostics (Santa Maria, California) except for P. aeruginosa PA01 which is a clinical lab isolate used in our research lab. Between experiments these cultures will be maintained and stored at refrigeration temperatures on TSA slants.

Fruit fly cultures:
The fruit flies used in this study will be purchased from The Fruit Fly Shop, San Diego, CA. They will be flightless mutants of the species Drosophila melanogaster that despite the mutant phenotype are otherwise normal. They will be maintained on the food and in the containers supplied by the company.

Assay of normal microflora of the fruit fly gastrointestinal tract:
An attempt will be made to isolate the bacterial microflora from the gastrointestinal tract of the fruit fly. This will be done by first decontaminating the surface of the fly in 70% isopropanol. Then, the body of the fly will be crushed and dilutions of the extract will be used to streak plates of tryptic soy agar (TSA) and MacConkey (Mac) agar for bacterial isolation. The plates will be incubated at room temperature for up to four days. If the resultant growth is indicative of the presence of a normal microflora (as evidenced by amount and species diversity), representative examples of each colony will be isolated and sub-cultured onto fresh TSA plates to isolate pure cultures. These will then be gram stained and examined microscopically. Oxidase reaction (Dryslide Oxidase, Fisher Scientific) will be determined. For each isolate that is an oxidase negative, gram negative rod and grew on Mac, identification to genus and species will be performed using the API 20E microtube system (BioMerieux). These experiments are necessary to be sure that the three species of bacteria used in the test experiments are not already a component of the microbial flora of the flies.

Fly Infection by Feeding: Mortality Experiments
Sterile glass vials containing 1.5% agar + 2% sucrose and fitted with air porous foam stoppers will be used to sustain the flies in the test vials during experimental trials. Overnight TSA grown bacterial cultures will be used to intentionally contaminate the vials. An isolated colony of an 18-24 hour TSA culture of the test bacterium will be used to contaminate a test vial. This will be done by using a sterile cotton swab to touch the center of the colony and then, using the swab, the bacteria will be transferred to the surface of the agar/sucrose in the test vial. Fifteen mature well-fed fruit flies (approximately 2-3 days after hatching) will be anesthetized using carbon dioxide and transferred to each test vial. The vials will then be placed at room temperature (approximately 23C) and examined at least once daily. The agar media will maintain adequate humidity for the duration of the experiment and there will be approximately 16 hours of fluorescent lighting and 8 hours of darkness. Daily counts of viable and non-viable fruit flies will be collected over a period of 1-2 weeks (depending upon the experiment). Each experimental run will involve 6 vials representing duplicates for each bacterium, plus another pair of control vials that will measure natural mortality in which the flies but not the bacteria will be present. To increase sample size and establish validity of the results, the entire experiments will be repeated at least 5-10 times.

Fly Infection by Feeding: Sub-lethality Experiments
At the termination of the mortality experiments, surviving flies will be sacrificed by immersing in 70% isopropanol to kill them and remove external microbes. The flies will then be crushed and extracts will be plated onto TSA plates and the appropriate selective medium for each of the test bacteria to determine if the gastrointestinal flora has changed from that seen in the control flies to that of the intentionally introduced species. Pseudosel agar will be used to detect P. aeruginosa, mannitol salts agar will be used to detect S. aureus and because Serratia marcescens forms a distinct red colony, TSA plates will suffice for detecting its presence. The results of these experiments will demonstrate if rather than causing mortality, the bacterial species can infect the flies without killing them.

Statistical Treatment of Data:
Kaplan-Meier survival curves will be constructed as the primary statistical treatment of the data. This is one of the more common methods used to analyze data involving mortality over time as a function of some imposed condition. In the present experiments, the difference in mortality of the flies in response to the three different microbial pathogens will be monitored.

LITERATURE CITED

Chugani, S. A., M. Whitely, K. M. Lee, D. A. D'Argenio, C. Manoil, and E.P. Greenberg. 2000. QscR, a modulator of quorum-sensing signal synthesis and virulence in Pseudomonas aeruginosa. PNAS 98:2752-2757.

D'Argenio, D. A., L. Gallagher, C. A. Berg, C. Manoil. 2001. Drosophila as a model host for Pseudomonas aeruginosa infection. J. Bacteriol. 183:1466-1471.

Erickson, D. L., J. L. Lines, E. C. Pesci, V. Venturi, and D. G. Storey. 2004. Pseudomonas aeruginosa relA contributes to virulence in Drosophila melanogaster. Inf. Imm. 72:5638-5645.

Lemaitre, B. and J. Hoffmann. 2007. The host defense of Drosophila melanogaster. Annu. Rev. Immunol. 25:697-743.

Needham A.J., M. Kibart, H. Crossley, P.W. Ingham, and S.J. Foster. 2004. Drosophila melanogaster as a model host for Staphylococcus aureus infection. Microbiology 150:2347-2355.

Nehme, N.T., S. Lie´geois, B. Kele, P. Giammarinaro, E. Pradel, J.A. Hoffmann, J. J. Ewbank, and D. Ferrandon. 2007. A model of bacterial intestinal infections in Drosophila melanogaster. PLos Pathog. 3:1694-1709.

Sibley, C.D., K. Duan, C. Fischer, M.D. Parkins, D.G. Storey, H.R. Rabin, and M.G. Surette. 2008. Discerning the complexity of community interaction using a Drosophila model of polymicrobial infections. PLoS Pathog. 4:1-10.

Attachment B

1. One bottle (1 lb) of Tryptic soy agar (Justification: This is the medium that will be used for the general growth of normal microflora of the GI tract of the fruit flies prior to identification)
   $67.17 (Source: Hardy Diagnostics, Santa Maria, CA)

2. API Strips 20E (Justification: To correctly identify the microflora isolated on the surface of the frog; I expect that gram negative rods will be isolated & this is a standard clinical lab product for identifying these cultures)
   $200.12 (Source: BioMerieux Vitek, Durham, North Carolina)

3. Reference Cultures - Staphylococcus aureus (ATCC 43300), and Serratia marcescens (ATCC 43861). (Justification: To use two known pathogenic reference cultures, one (S.a.) that is a species often found in the lungs of cystic fibrosis patients and one (S.m.) that is a known pathogen of insects including fruit flies)
   $81.66 total for the two (Source: Hardy Diagnostics, Santa Maria, CA)

4. One bottle (1 lb) of Pseudosel agar (Justification: This medium will be used to select for Pseudomonas from the GI tract of the flies as a means for determining it's survival in the body of the fly).
   $89.86 (Source: Hardy Diagnostics, Santa Maria, CA)

5. One bottle (1 lb) of MacConkey agar (Justification: This medium will be used to select for enteric bacteria from the GI tract of the flies to determine the identification of normal microflora of the fly gut)
   $69.88 (Source: Hardy Diagnostics, Santa Maria, CA)

6. Fruit Fly Culture Kit (Justification: In order to conduct research on fruit flies year round, a continual source of the fruit flies will be needed along with the food to maintain them and containers to house them)
   $73.90 (Source: The Fruit Fly Shop, San Diego, CA)

7. CO2 Refills (Justification: Fruit flies will need to be anesthetized in order to put the correct number of fruit flies into each vial with ease)
   $50.00 ($2.50 each X 20) (Source: Academy, San Angelo, TX)

Total requested = $632.59