APPLICATION CHECKLIST
Research, Scholarship, and Creativity Grant

Deadline February 12th

Please print and complete this checklist and attach it as the cover page of your grant application.

Faculty information

Name: Kimberly Murphy Dept: Biology

Email: kmurphy@gustavus.edu Rank: Visiting Assistant Professor

Checklist

☑ **Description of previous projects (and outcomes) funded by RSC grants**

☑ **Complete project description, including separate statements of:**

1. **Purpose.** What are the intellectual, conceptual, or artistic issues? How does your work fit into other endeavors being done in this field?

2. **Feasibility.** What qualifications do you bring to this project? What have you done/will you do to prepare for this project? What is the time period, i.e. summer, summer and academic year, academic year only? Is the work's scope commensurate with the time period of the project?

3. **Project Design.** This should include a specific description of the project design and activities, including location, staff, schedules or itineraries, and desired outcomes.

☑ **RSC Budget Proposal Form attached as last page of application**

☑ **Nine (9) copies of completed application and budget (including this checklist) to be submitted to the John S. Kendall Center for Engaged Learning (SSC 119)**

If successful, my proposal can be used as an example to assist future faculty applications. This decision will not in any way influence the evaluation of my application. (Yes) / No **(please circle one)**

Identification and Characterization of Genes Involved in *Myxococcus xanthus* Motility and Fruiting Body Formation
A proposal to the Research, Scholarship and Creativity Fund

Kimberly Murphy
Biology

Statement of previous support
I have received no prior funding from the Research, Scholarship and Creativity Fund.

A. Purpose

Bacterial biofilms are large communities of cells that are attached to solid surfaces. Biofilms have been linked to a variety of health problems in humans, including many persistent infections that are resistant to traditional antibiotic therapy (Costerton et al., 1999, Costerton 2001). Therefore, there is a need for understanding the genetic and molecular basis for the formation of biofilms.

Myxococcus xanthus fruiting body formation is a well-studied model for bacterial biofilm formation. Under starvation conditions, *M. xanthus* initiates a complex developmental program in which large groups of cells migrate to aggregation centers and begin building multicellular fruiting bodies. Once a fruiting body is molded into its final shape, individual rod-shaped cells within this structure differentiate into dormant, spherical-shaped spores that are resistant to many forms of environmental stress. Many genes/proteins that are now known to be important for production of pathogenic biofilms were previously shown to be important for fruiting body formation. Therefore, fruiting body formation is a good model for understanding the genetics behind the formation of disease-causing biofilms. The long-term goal of this research is to expand our knowledge of the genetics behind bacterial biofilm formation using *M. xanthus* as a model organism. Specifically, the goals are to identify new genes important for *M. xanthus* fruiting body formation using mutational analysis, and to understand the roles of their corresponding protein products at a molecular level.

I propose to work with students (Dawn Comstock and Laura Leland) towards two specific aims: 1. to identify new genes that are important for *M. xanthus* fruiting body formation using phylogenomics and targeted mutational studies, and 2. to examine the phenotypes of *M. xanthus* insertion mutant. Our results will be presented by the students at a meeting or be written up for a paper to be submitted to a journal. Also, this support will allow for me to begin what will become a long-term research project for many undergraduate students at Gustavus Adolphus College.

B. Feasibility.

I have five years of experience working with *M. xanthus* and bacterial biofilms. Currently I collaborate with Dr. Roy Welch at Syracuse University. Dr. Welch's lab developed a genome-scale (phylogenomic) map that clusters *M. xanthus* genes based on similar evolutionary histories or coinheritance patterns (Srinivasan et al., 2005). Using this coinheritance map, I focused on several clusters of putative motility genes, since motility is known to be required for fruiting body development. Motility clusters that contain a large number of uncharacterized genes were

identified. Insertion mutations were generated in a subset of the uncharacterized genes. A large majority of these mutations were found to affect motility and/or fruiting body development. The goal for specific aim 1 is to complete this mutational analysis by inactivating the remaining uncharacterized genes in the motility gene clusters. The Garza Lab at Syracuse University has developed a procedure to rapidly make insertions in putative motility genes, which I have successfully used. This is the procedure we will use to mutate the remaining uncharacterized genes.

The goal of specific aim 2 is to examine the phenotypes caused by the targeted insertions that we generate in specific aim 1. I have experience constructing mutants and doing the phenotypic assays that are required to complete this research. In addition, both Dawn and Laura were in my J-term Biomolecular Research course. As part of this course, they worked on *M. xanthus* and successfully completed the phenotypic assays that are required to complete this research. Therefore, it should be possible to mutate the remaining uncharacterized genes in the motility gene clusters and examine the phenotypes of all the mutants within Spring and Fall semesters of 2010. As time permits, we will work our way through additional gene clusters that are predicted to be important for fruiting body formation.

During J-term of 2008, I taught a course entitled 'Biomolecular Research'. Both Dawn and Laura completed this course which provided students with a rich introductory experience in collaborative (student-faculty) research. The course offered an exploratory research experience for students early in their career and was focused on original research questions from my own laboratory. Research activity was augmented with weekly research update presentations and a final research presentation to emphasize the centrality of communicating results in the practice of scientific research. In addition, there were discussions of papers from the primary literature and regular discussions about how their experiences were informing their ideas about career and vocation.

The course had a huge impact on both Dawn and Laura. Both young ladies now exude enthusiasm for research. They have since sought out opportunities to present on their J-term experience and research results from the course and have approached me about continuing student-faculty research throughout the spring 2010 semester. This semester they will be presenting at undergraduate research symposia both at Gustavus Adolphus College and off campus. Also, Dawn and Laura will apply for the 2010 HHMI Summer Science Research Program allowing them the opportunity to do research with Gustavus professors this summer.

All of the facilities needed to conduct this research are present in Nobel Hall. Once we have constructed the mutant *M. xanthus* strains, we will make freezer stocks of each strain. A backup copy of each strain should be housed at a separate college or university. Dr. Roy Welch at Syracuse University has agreed to keep our backup stock.

C. Project Design.

To produce the mutants, internal fragments of each gene will be generated using PCR. PCR fragments will be cloned into a version of the pCR2.1-TOPO vector (Invitrogen) conferring kanamycin (kan) resistance. Then, plasmids carrying the gene fragments will be electroporated into wild-type *M. xanthus* cells. These plasmids can integrate into chromosomal copies of the target genes by homologous recombination of the PCR fragments. A single cross over yields kan resistant electroporants with two truncated copies of the target gene separated by vector DNA. Thus, it is likely that the target motility gene will be inactivated by the plasmid insertion. Mutants carrying a single plasmid insertion are identified by PCR.

Descriptions of the phenotypic assays that we plan to use to complete specific aim 2 are as follows:

Monitoring fruiting body formation. *M. xanthus* strains are inoculated into flasks containing CTTYE broth, and the cultures are incubated at 32°C with vigorous swirling. After each culture reaches a density of 5 X 10^8 cells/ml, the cells are pelleted, the supernatant removed, and the cells are resuspended in TPM buffer to a density of 5 X 10^9 cells/ml. Aliquots of this cell suspension are spotted onto TPM agar plates and incubated at 32°C. The progress of the fruiting body development will be monitored visually using microscopy. Images are captured with a digital camera.

Monitoring sporulation. The final stage of *M. xanthus* fruiting formation is the production of spherical-shaped, stress-resistant spores, therefore we will determine whether our mutants are defective for the process of sporulation using standard assays (Caberoy et al., 2003). Mutant and wild-type cells will be harvested after 5 days on TPM plates. Harvested cells will be resuspended in TPM buffer, the cell suspension will be sonicated, and the sonicated cells will be incubated at 50°C for 2 hours. The number of heat- and sonication-resistant spores that germinate into colonies will be determined.

Motility assays. The motility of our mutants will be examined using swarm expansion assays (Kaiser and Crosby, 1983). Cells from the mutants as well as wild-type cells are grown in CTTYE nutrient broth to a density of 5 X 10^8 cells/ml, the cells are concentrated 10-fold, and aliquots are spotted on CTTYE plates containing 0.4 or 1.5% agar. Two different agar percentages are used because two motility systems control *M. xanthus* swarming (or gliding) motility on a solid surface, the A and S systems. A-motility appears to be favored on relatively firm and dry surfaces (1.5%), while S-motility seems to be favored on soft and wet surfaces (0.4%). After the plates are incubated for 3 to 5 days at 32°C, the diameters of the mutant colonies are compared to the diameters of the wild-type colonies. To be classified as a motility mutant, the mean diameters of the mutant colonies have to be less than 80% of those of wild-type colonies (Caberoy et al., 2003).

Budget

I am asking for $1500 for this research project. This will cover molecular biology supplies for constructing mutants such as Taq Polymerase, dNTPs, and a pCR2.1-TOPO kit as well as disposables and media components for the phenotypic assays (casitone, yeast extract, agar, tips, tubes, Petri plates, media components).

Literature Cited

Caberoy NB, Welch RD, Jakobsen JS, Slater SC, and AG Garza. 2003. A global mutational analysis of NtrC-like activators in *Myxococcus xanthus*: identifying activator mutants defective for motility and fruiting body development. *J Bacteriol* 185: 6083-6094.

Costerton JW, Stewert PS, and EP Greenberg. 1999. Bacterial biofilms: a common cause of persistent infections. *Science* 284: 1318-1322.

JW Costerton. 2001. Cystic fibrosis pathogenesis and the role of biofilms in persistent infection. *Trends Microbiol* 9: 50-52.

Kaiser D and C Crosby. 1983. Cell movement and its coordination in swarms of *Myxococcus xanthus*. *Cell Motil* 3: 227-245.

Srinivasan BS, Caberoy NB, Suen G, Taylor RG, Shah R, Tengra F, Goldman BS, Garza AG, and RD Welch. 2005. Functional genome annotation through phylogenomic mapping. *Nat Biotechnol* 23: 691-698.

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BUDGET INFORMATION

Faculty Stipend
($500 professor; $600 associate professor; $700 assistant professor)

Expenses
Faculty may apply for up to **$1500** to pay for the cost of equipment, materials, personnel, and travel associated with the project to be funded by the RSC Grant. All expenses must be necessitated by the project to be funded by the RSC Grant.

ITEM		AMOUNT
Equipment (e.g., transcription machine, camera, cassette recorder—but not to include computer hardware):		$
1:	Cost:	
2:	Cost:	
3:	Cost:	
Materials (e.g., books, printing, software, lab supplies)		$1500.00
1:Molecular biology supplies	Cost:$900.00	
2:lab supplies (media components)	Cost:$300.00	
3:lab supplies (disposables)	Cost:$300.00	
Personnel (e.g., typist, transcriptionist, student assistant)		$
1:		
2:		
Travel Costs (cannot include conference travel, see http://gustavus.edu/finance/travel.php for allowable travel expenses)		$
Airfare:		
Mileage: Number of miles_____ @ $0.55/mile		
Lodging:		
Meals:		
Other Expenses:		$
1:	Cost:	
2:	Cost:	
3:	Cost:	
		$
TOTAL EXPENSES		$1500.00
AMOUNT REQUESTED (not to exceed $1500 + stipend commensurate with rank)		$1500.00 + stipend

Have you applied for, or received funding from, another source to help support this project? NO
Funding Source:
Amount:
Please explain how the RSC will be used in addition to the other funding.