GRANT APPLICATION FORM

Check the Grant Award you are seeking.

_____Individual Development/Course Development Grant
_X_____Critchfield Research Grant
_____Ashforth Research Grant

Applicant Information:

Principal Researcher:	Kathryn Patterson Sutherland
Academic Rank:	Assistant Professor
Department:	Biology
Office Phone Number:	691-1075
E-Mail Address:	kpsutherland@rollins.edu

Grant Proposal:

Short Title of Grant Proposal:	Microcosm studies with a coral disease pathogen
Proposed Start Date:	July 1, 2008
Proposed End Date:	June 30, 2009

Description of Grant Proposal:

Objectives of grant proposal: Please list objectives that are clear, specific, concrete, and measurable.	**1) To conduct microcosm studies to better understand the survival and/or persistence of the white pox disease coral pathogen, *S. marcescens,* under various environmental conditions.** A microcosm is a small, simplified system maintained in a laboratory that contains the essential features of a natural ecosystem. In this experiment the microcosm will be a 1L glass flask containing artificial seawater. I will determine if environmental isolates of *S. marcescens* are capable of prolonged survival or growth in seawater alone (i.e., are they adapted to a marine existence) or if survival in a marine environment is improved by or requires a higher nutrient concentration relative to seawater (i.e., are *S. marcescens* capable of a marine existence only when exposed to nutrients excreted by marine organisms including corals and snails). I hypothesize that the association with nutrients (e.g., from marine organisms) will improve survival of most *S. marcescens* strains, especially strains isolated from corals and snails. I will also determine how *S. marcescens* are affected by varying salinity, nutrient, and temperature conditions. **Background:** In 2002, I identified a causal agent of the white pox disease of the Caribbean elkhorn coral, *Acropora palmata*, as the bacterium *Serratia marcescens* (Patterson et al., 2002). *S. marcescens* is a common enteric bacterium found in the intestines of humans, insects, and other animals, and in fresh water and soil. Prior to my research, *S. marcescens* was not commonly identified in marine environments. Since 2002, I have conduced targeted studies to elucidate the ecology and prevalence of this bacterium in offshore tropical and coral reef environments in the Florida Keys. Between June 2002 and August 2006, I cultured and identified 383 *S. marcescens* from samples collected from corals, coral-eating snails, seabirds, beach water, canal water, and human sewage (Sutherland et al. in prep). Genetic fingerprinting of 340 of these 383 *S. marcescens* identified 119 different fingerprints (i.e., 119 different strains of *S. marcecens*). One of these strains includes isolates collected from sewage,

	corals, and coral-eating snails. This strain, isolated from both reef and sewage sources establishes a definitive connection between human sewage and the reef ecosystem (Sutherland et al., in prep). Here I propose to determine if *S. marcescens* collected from various environments in the Florida Keys can survive for prolonged periods in the marine environment or if the persistence of this bacterium in marine systems requires a nutrient source supplied via an association with marine organisms (corals and/or snails). **Methods:** Flasks (1 L) will be prepared with sterile artificial seawater (Instant Ocean). For each microcosm, each test strain will be grown overnight, washed in sterile artificial seawater and diluted to ~10^6 cells ml^{-1} (determined by counting cells). One-ml of diluted cells will be transferred to flasks containing 500 ml of seawater or seawater with nutrient amendment. Up to 5 ml samples will be collected from each experimental flask at the time of inoculation (T_0), 4, 8, 12, 24, 48, 72 and 96 h post inoculation and then at day 7 and weekly for 1 month. Samples will be tested for the presence of *S. marcescens* using a two-step culture method for the growth and isolation of *S. marcescens*. The number of *S. marcescens* [i.e., colony forming units (CFU) ml^{-1}] will be determined. Survival in CFU will be determined for the duration of the experiments. Following the conclusion of experiments, the identity of a subset of the test strains will be confirmed with 16S rDNA sequencing. *Salinity Studies*: While *S. marcescens* is known to grow at high salt concentrations (Patterson et al. 2002), its ability to tolerate normal seawater salinities in the relatively nutrient poor conditions of coral reef ecosystems is not known. Therefore, microcosms will be manipulated to produce seawater of at least three salinities (by addition of sterile deionized water to Instant Ocean) ranging from 5 to 35 parts per thousand (35 parts per thousand is the salinity of seawater). *Nutrient Studies*: In individual microcosms, seawater will be amended with nutrients using nylon mesh bags filled with Osmocoat™ (time released fertilizer pellets). *Temperature Studies*: *S. marcescens*-inoculated seawater will be incubated at 'low' and 'high' temperatures, which will be determined empirically by the lowest and highest recorded sea surface temperatures in the Lower Keys. A total of eight *S. marcescens* test isolates will be used from the following sources: American Type Culture Collection (ATCC) type strain, elkhorn coral, massive starlet corals, smooth star corals, coral-eating snails, canal water, and sewage. All experiments will be performed in duplicate for each test isolate and experimental type (salinity concentration, nutrient concentration) at two temperatures. **References:** *indicates Rollins student Patterson, K.L., J.W. Porter, K.B. Ritchie, S.W. Polson, E. Mueller, E.C. Peters, D.L. Santavy, and G.W. Smith. 2002. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. *Proceedings of the National Academy of Sciences* 99:8725-8730. Sutherland, K.P., J.W. Porter, J.W. Turner, B.J. Thomas*, E.E. Looney, T. Luna*, V.A. Watson*, and E.K. Lipp. In prep. Prevalence, diversity and potential origin of the acroporid serratiosis pathogen in the Florida Keys, USA.
Describe how this project relates to your current expertise.	I have been studying coral reef ecosystems and the diseases affecting corals for over 10 years. Since discovering the cause of white pox disease as *Serratia marcescens* in 2002, I have been investigating the prevalence and ecology of this bacterium in marine environments of the Florida Keys. Most recently, I have determined that *S. marcescens* is common in human sewage and can be found in association with corals

	and a coral predator (the coral-eating snail). This proposed research builds on these previous studies.
Describe the relationship of this project to your long term professional goals.	My long term professional goals include the continued study of the white pox disease pathogen and the impact of this bacterium on coral reef ecosystems. My goal is to definitively resolve whether the *S. marcescens* coral pathogen can be linked to a human source. The identification of *S. marcescens* as a coral pathogen marked the first time that a common inhabitant of the human gut was shown to be a marine invertebrate pathogen. My recent research in collaboration with Rollins Students indicates that sewage contamination is affecting coral reefs in the Florida Keys. My goal is to determine if human inputs cause or exacerbate disease, and therefore whether human inputs should be managed to protect coral reefs.
Describe why this project is important.	White pox disease exclusively affects the federally threatened Caribbean elkhorn corals. This study will increase the scientific knowledge of the coral pathogen that has contributed to the decimation of populations of elkhorn coral in recent years. This research is one of the few cases where a definite demonstration of human related causes could have a real and significant conservation and management effect on coral reefs. Understanding the factors that drive the emergence and maintenance of coral disease outbreaks is critical to protecting the remaining coral reefs of Florida and the wider Caribbean, where diseases are changing the structure of reef frameworks.
Describe the expected outcome(s) for this project (publication, performance, exhibit, paper)	Results of this research will be presented at national scientific meetings and published in a peer-reviewed scientific journal.
Describe the contribution this project will make to Rollins College.	This grant will contribute to the education of Rollins students. I plan to collaborate with students this summer through the Student-Faculty Summer Scholarship Program. Through collaborative research in my laboratory, students will learn microbiological and molecular techniques that can be applied to future classroom learning, laboratory research, and careers in any scientific discipline.

Proposed Budget:

(Please review Permitted Expenditures section and provide as much detail as possible. Be specific about what costs will be incurred for travel, telephone, staff support, photocopying, etc. This budget will be for one year only).

Item	Justification	Amount
Equipment/Supplies		
Tryptic Soy Broth	For pre-test culture of test strains	$150.00
15ml sterile tubes	For pre-test culture of test strains	$203.00
MCSA Agar	For post-test culture of test strains	$140.00
DTC Agar	For post-test culture of test strains	$118.00
Cephalothin Sodium Salt	For post-test culture of test strains – antibiotic for DTC agar	$235.00
Colistin Sulfate Salt	For post-test culture of test strains – antibiotic for MCSA agar	$235.00
Petri Plates	For post-test culture of test strains	$150.00
Erlenmeyer Flasks (1L)	For Microcosms	$200.00
Instant Ocean	For artificial seawater in microcosms	$100.00
Osmocoat	For nutrient amendment in microcosms	$100.00
Nylon Mesh Bags	For nutrient amendment in microcosms	$150.00
Aluminum Foil	To cover microcosms	$25.00
Personnel Support		$ 0
Travel		$ 0

Other 16S rDNA Sequencing Publishing	Funds are requested for 16S rDNA sequencing. Commercial companies charge $10/sample for this service. Funds are also requested to cover the cost of a publication in a peer-reviewed scientific journal. Cost of publication is estimated using *Applied and Environmental Microbiology* publication costs ($65.00/page; $325 for a 5 page publication).	$500.00 $325.00
TOTAL		$2631.00

Previous Funding from Rollins College:

1. Have you received previous funding for a Critchfield/Ashforth Research, Individual Development, Course Development, or Cornell Research Grant?	**YES** **X**	**NO**
If yes, list all grants received: Genetic profiling of *Serratia marcescens*: tracking the source of a coral pathogen. 2007		
2. Have you applied for other grants from Rollins this year?	**YES**	**NO** **X**
If yes, list all grants applied for, grants awarded, and amounts to be received:		
3. Have you received any other grants or funding from Rollins during the previous five years?	**YES**	**NO** **X**
If yes, list all grants awarded and amounts received over the last five years:		

Other Support for Current Proposal

1. Have you applied for or been granted any external or other internal sources of funding for this proposal?	**YES**	**NO** **X**
If yes, clearly identify all other requests which duplicate this proposal, indicating the periods and amounts of all support requested and/or received, along with the status of the support.		
If you are requesting funds for a second or third year of support for one project, attach a progress report.		

Attach to this application:

Abbreviated Vita (3 pages maximum) listing all papers published and/or presented that are related to this project. If this is a new area for you, list your most recent publications/ presentations.

Department Approval Statement: Proposals requiring departmental approval (new course development, curriculum redesign, etc.) or support (supplies, space, supporting personnel, etc) should be accompanied by a statement from the department head, director, or appropriate dean that affirms support for the project. This statement is particularly important when the proposal involved the design of a new course or changes to the department's curriculum changes.

Research Review Board Approval: If the proposed research involves human subjects or vertebrates, a letter of approval from the requisite board should be attached to this grant application (IRB for human subjects or IACUC for vertebrates).

Signature of Principal Researcher ____Kathryn P. Sutherland_____

Date ____1-18-08_____

Send this application electronically to the Dean of the Faculty. In addition, make 10 hard copies of your application, including 10 copies of your vita and deliver them to the Dean of the Faculty by the application due date.