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| PI: Pogliano, Kit J | Title: The chemical and genetic basis of interspecies interactions | |
| Received: 09/03/2010 | FOA: PA10-067 | Council: 01/2011 |
| Competition ID: ADOBE-FORMS-B | FOA Title: RESEARCH PROJECT GRANT (PARENT R01) | |
| 1 R01 AI095125-01 | Dual: | Accession Number: 3325306 |
| IPF: 577507 | Organization: UNIVERSITY OF CALIFORNIA AT SAN DIEGO | |
| Former Number: | Department: Div. of Biological Sciences | |
| IRG/SRG: ZRG1 GGG-B (03)M | AIDS: N | Expedited: N |
| <u>Subtotal Direct Costs (excludes consortium F&A)</u> | Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N | New Investigator: N Early Stage Investigator: N |
| Year 1: 391,234 | | |
| Year 2: 404,271 | | |
| Year 3: 417,828 | | |
| Year 4: 431,930 | | |
| | | |
| <i>Senior/Key Personnel:</i> | <i>Organization:</i> | <i>Role Category:</i> |
| Kit Pogliano | The Regents of the Univ. of Calif., U.C. San Diego | PD/PI |
| Pieter Dorrestein | The Regents of the Univ. of Calif., U.C. San Diego | MPI |

Appendices

Kappendi

**APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)**

| | | | |
|---|---|--|---|
| 1. * TYPE OF SUBMISSION | | 3. DATE RECEIVED BY STATE <input type="text"/> | State Application Identifier <input type="text"/> |
| <input type="checkbox"/> Pre-application <input checked="" type="checkbox"/> Application <input type="checkbox"/> Changed/Corrected Application | | 4. a. Federal Identifier <input type="text"/> b. Agency Routing Identifier <input type="text"/> | |
| 2. DATE SUBMITTED <input type="text" value="09/03/2010"/> | Applicant Identifier <input type="text" value="2011-0384"/> | | |
| 5. APPLICANT INFORMATION | | * Organizational DUNS: <input type="text" value="804355790"/> | |
| * Legal Name: <input type="text" value="The Regents of the Univ. of Calif., U.C. San Diego"/> Department: <input type="text" value="Office of C & G Administration"/> Division: <input type="text"/> * Street1: <input type="text" value="9500 Gilman Drive"/> Street2: <input type="text" value="0934"/> * City: <input type="text" value="La Jolla"/> County / Parish: <input type="text" value="San Diego"/> * State: <input type="text" value="CA: California"/> Province: <input type="text"/> * Country: <input type="text" value="USA: UNITED STATES"/> * ZIP / Postal Code: <input type="text" value="92093-0934"/> | | | |
| Person to be contacted on matters involving this application Prefix: <input type="text"/> * First Name: <input type="text" value="Derek"/> Middle Name: <input type="text"/> * Last Name: <input type="text" value="Jackson"/> Suffix: <input type="text"/> * Phone Number: <input type="text" value="858-534-1915"/> Fax Number: <input type="text" value="858-534-0280"/> Email: <input type="text" value="djackson@ucsd.edu"/> | | | |
| 6. * EMPLOYER IDENTIFICATION (EIN) or (TIN): <input type="text" value="1956006144A1"/> | | | |
| 7. * TYPE OF APPLICANT: <input type="text"/> H: Public/State Controlled Institution of Higher Education Other (Specify): <input type="text"/> Small Business Organization Type <input type="checkbox"/> Women Owned <input type="checkbox"/> Socially and Economically Disadvantaged | | | |
| 8. * TYPE OF APPLICATION: <input checked="" type="checkbox"/> New <input type="checkbox"/> Resubmission <input type="checkbox"/> Renewal <input type="checkbox"/> Continuation <input type="checkbox"/> Revision | | If Revision, mark appropriate box(es). <input type="checkbox"/> A. Increase Award <input type="checkbox"/> B. Decrease Award <input type="checkbox"/> C. Increase Duration <input type="checkbox"/> D. Decrease Duration <input type="checkbox"/> E. Other (specify): <input type="text"/> | |
| * Is this application being submitted to other agencies? Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> What other Agencies? | | | |
| 9. * NAME OF FEDERAL AGENCY: <input type="text" value="National Institutes of Health"/> | | 10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: TITLE: <input type="text"/> | |
| 11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT: <input type="text" value="The chemical and genetic basis of interspecies interactions"/> | | | |
| 12. PROPOSED PROJECT: * Start Date <input type="text" value="04/01/2011"/> * Ending Date <input type="text" value="03/31/2015"/> | | * 13. CONGRESSIONAL DISTRICT OF APPLICANT <input type="text" value="CA-053"/> | |
| 14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION Prefix: <input type="text"/> * First Name: <input type="text" value="Kit"/> Middle Name: <input type="text"/> * Last Name: <input type="text" value="Pogliano"/> Suffix: <input type="text"/> Position/Title: <input type="text" value="Professor"/> * Organization Name: <input type="text" value="The Regents of the Univ. of Calif., U.C. San Diego"/> Department: <input type="text" value="Div. of Biological Sciences"/> Division: <input type="text" value="General Campus"/> * Street1: <input type="text" value="9500 Gilman Drive"/> Street2: <input type="text" value="0934"/> * City: <input type="text" value="La Jolla"/> County / Parish: <input type="text" value="San Diego"/> * State: <input type="text" value="CA: California"/> Province: <input type="text"/> * Country: <input type="text" value="USA: UNITED STATES"/> * ZIP / Postal Code: <input type="text" value="92093-0934"/> * Phone Number: <input type="text" value="858-822-1314"/> Fax Number: <input type="text" value="858-822-5740"/> * Email: <input type="text" value="kpogliano@ucsd.edu"/> | | | |

| | | | |
|--|---|---|--|
| 15. ESTIMATED PROJECT FUNDING | | 16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS? | |
| a. Total Federal Funds Requested | <input type="text" value="2,462,924.24"/> | a. YES | <input type="checkbox"/> THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON: |
| b. Total Non-Federal Funds | <input type="text" value="0.00"/> | DATE: | <input type="text"/> |
| c. Total Federal & Non-Federal Funds | <input type="text" value="2,462,924.24"/> | b. NO | <input checked="" type="checkbox"/> PROGRAM IS NOT COVERED BY E.O. 12372; OR <input type="checkbox"/> PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW |
| d. Estimated Program Income | <input type="text" value="0.00"/> | | |
| <p>17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)</p> <p><input checked="" type="checkbox"/> * I agree</p> <p>* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.</p> | | | |
| <p>18. SFLLL or other Explanatory Documentation</p> <p><input type="text"/> <input type="button" value="Add Attachment"/> <input type="button" value="Delete Attachment"/> <input type="button" value="View Attachment"/></p> | | | |
| <p>19. Authorized Representative</p> <p>Prefix: <input type="text"/> * First Name: <input type="text" value="Derek"/> Middle Name: <input type="text"/> * Last Name: <input type="text" value="Jackson"/> Suffix: <input type="text"/> * Position>Title: <input type="text" value="Contract & Grant Officer"/> * Organization: <input type="text" value="The Regents of the Univ. of Calif., U.C. San Diego"/> Department: <input type="text" value="Office of C & G Administration"/> Division: <input type="text" value="General Campus"/> * Street1: <input type="text" value="9500 Gilman Drive"/> Street2: <input type="text" value="0934"/> * City: <input type="text" value="La Jolla"/> County / Parish: <input type="text" value="San Diego"/> * State: <input type="text" value="CA"/> CA: California Province: <input type="text"/> * Country: <input type="text"/> USA: UNITED STATES * ZIP / Postal Code: <input type="text" value="92093-0934"/> * Phone Number: <input type="text" value="858-534-1915"/> Fax Number: <input type="text" value="858-534-0280"/> * Email: <input type="text" value="djackson@ucsd.edu"/></p> <p>* Signature of Authorized Representative <input type="text"/> Derek Jackson</p> <p>* Date Signed <input type="text" value="09/03/2010"/></p> | | | |
| <p>20. Pre-application <input type="text"/> <input type="button" value="Add Attachment"/> <input type="button" value="Delete Attachment"/> <input type="button" value="View Attachment"/></p> | | | |

424 R&R and PHS-398 Specific Table Of Contents

| | Page Numbers |
|---|--------------|
| SF 424 R&R Face Page----- | 1 |
| Table of Contents----- | 3 |
| Performance Sites----- | 4 |
| Research & Related Other Project Information----- | 5 |
| Project Summary/Abstract (Description)----- | 6 |
| Public Health Relevance Statement (Narrative attachment)----- | 7 |
| Facilities & Other Resources----- | 8 |
| Equipment----- | 10 |
| Research & Related Senior/Key Person----- | 11 |
| Biographical Sketches for each listed Senior/Key Person----- | 12 |
| Research & Related Budget - Year 1----- | 19 |
| Research & Related Budget - Year 2----- | 22 |
| Research & Related Budget - Year 3----- | 25 |
| Research & Related Budget - Year 4----- | 28 |
| Budget Justification----- | 31 |
| Research & Related Budget - Cumulative Budget----- | 33 |
| PHS 398 Specific Cover Page Supplement----- | 34 |
| PHS 398 Specific Research Plan----- | 36 |
| Specific Aims----- | 37 |
| Research Strategy----- | 38 |
| Multiple PI Leadership Plan----- | 50 |
| Bibliography & References Cited----- | 51 |
| Letters of Support----- | 55 |
| Resource Sharing Plan----- | 57 |
| PHS 398 Checklist----- | 58 |
| Appendix | |

Number of Attachments in Appendix: 1

Project/Performance Site Location(s)

Project/Performance Site Primary Location I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The Regents of the Univ. of Calif., U.C. San Diego

DUNS Number: 8043557900000

* Street1: 9500 Gilman Drive

Street2: 0377

* City: La Jolla

County: San Diego

* State: CA: California

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 92093-0377

* Project/ Performance Site Congressional District: CA-053

Project/Performance Site Location 1

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City:

County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code:

* Project/ Performance Site Congressional District:

Additional Location(s)

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? Yes No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes No

If yes, check appropriate exemption number. 1 2 3 4 5 6

If no, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? Yes No

4.a. * Does this project have an actual or potential impact on the environment? Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? Yes No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? Yes No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract

8. * Project Narrative

9. Bibliography & References Cited

10. Facilities & Other Resources

11. Equipment

12. Other Attachments

Bacillus subtilis produces a wide array of extracellular metabolites that can inhibit the growth of bacteria and fungi or modify their behavior to attenuate the production of antibacterial products by potentially dangerous neighbors. We here propose to use the new technique of imaging mass spectrometry and classical analytical chemistry to systematically identify the extracellular metabolome of *B. subtilis*, with a focus on characterizing the interactive metabolome that is induced by other bacterial species. We will investigate the role these compounds play in two distinct outcomes of the interaction of *B. subtilis* with other species. The first is an impasse, in which *B. subtilis* forms closely abutting colonies with other species that produce a variety of antibacterial compounds (such as *P. aeruginosa*). The second, more frequent behavior is contact-dependent predation, in which *B. subtilis* moves towards, invades and destroys neighboring colonies, leading to death of the prey species and expanding the territory of the *B. subtilis* colony. These reproducible behaviors are conserved in different undomesticated *B. subtilis* strains. We will determine if these behaviors depend on the interactive metabolome and investigate the effects individual compounds have on target cell viability and behavior. We will further investigate the genetic requirements for interspecies interactions to identify stress responses, developmental and biosynthetic pathways that contribute to these distinct outcomes and we will use fluorescence microscopy to visualize the cellular consequences of interspecies interactions. These studies will illuminate the mechanistic basis for interspecies interactions and identify secondary metabolites that affect viability or behavior of other species that represent potential new antibacterial drugs.

Bacteria produce many extracellular metabolites that mediate their interaction with other species, many of which have antibacterial and antifungal activities. We will here elucidate the chemical, genetic and cellular mechanisms by which these molecules allow *Bacillus subtilis* to interact with other bacterial species, producing outcomes ranging from coexistence to the invasion and destruction of neighboring colonies. Interspecies interactions are critical in medicine and the metabolites that facilitate destruction of other species represent promising new pharmaceutical leads.

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

The K. Pogliano research suite is in the Natural Sciences Building, and consists of 2,100 square feet of laboratory and office space, including two enclosed microscope rooms, and a laboratory support room for media preparation. We have all the necessary facilities for growing *B. subtilis*, for standard molecular, genetic and biochemical techniques and for high resolution fluorescence microscopy.

Clinical: N/A

Animal: N/A

Computer:

The K. Pogliano lab has two PC computers, four MacIntosh Computers, and three computers dedicated to the microscopes. The Division of Biological Sciences also has Computer support services available.

Office:

The K. Pogliano lab includes three 120 square foot offices adjoining the laboratory, one for the PI and two for postdocs and graduate students.

Other:

The K. Pogliano research group is on the same floor as two conference rooms, a lunch room and dishwashers, autoclaves and warm rooms. A shared Beckman Ultracentrifuge located within the PIs lab and a Phosphoimager Storm is upstairs. Cryoelectron microscopy, DNA and protein sequencing and Mass spec. facilities are available on a fee for use basis.

The National Center for Microscopy and Imaging Resource (<http://ncmir.ucsd.edu/>) is also located on campus. This facility is devoted to developing new technologies and making them available to NIH funded research. They have cutting edge fluorescence and electron microscopes, and have the ability to perform correlative fluorescence and electron microscopy, electron tomography and 3D reconstructions of cells.

The Dorrestein laboratory space and computer facilities are located in the Biomedical Sciences building and the Skaggs School of Pharmacy and Pharmaceutical Sciences (~2000 sq ft total). With this space there are 16 offices for the Dorrestein lab members. These buildings are located next to each other. The main laboratory space and computer facilities are comprised of molecular biology equipment necessary for culture growth and mass spectrometers necessary for imaging experiments and metabolite structure elucidation including incubators, culturing rooms (mammalian and non mammalian cells), centrifuges for larger cultures with 4 and 10-liter capacity, shaker for culturing, cell culturing facilities, sonicators, lab computers used for data analysis, research and documentation of our work, an LTQFT- ICR-MS equipped with an Advion nanospray robot, a Prosilia DESI system and nanospray capabilities and a MALDI-TOF imaging system. In addition to the Dorrestein laboratory, additional facilities will be readily available for use including the

chemistry biomolecular mass spectrometry facility and chemistry small molecule mass spectrometry facility. These facilities offer additional mass spectrometry equipment including a variety of mass spectrometers for use during method development and structure elucidation. An additional 1000 sq ft. is being remodeled at this time to house two additional advanced mass spectrometers.

Shared facilities available at the Skaggs School of Pharmacy and Pharmaceutical Sciences.

The pharmacy school has an Agilent ion trap instrument with nanoinfusion and two 600Mhz NMRs with cryoprobes, including a 1.7 mm probe for the characterization of metabolites. To provide an indication of sensitivity, our laboratory has used this NMR instrument with this probe to elucidate complete structures using 2D-NMR methods with materials as low as 7 micrograms with a ~700 Da molecule and 20 microgram with a ~3kDa molecule. Most NMR's require 0.5 mg or more. Furthermore we have A CD instrument, a liquid handling system, autoclaves, dishwashers that are available in the School of pharmacy.

In summary, we have all the facilities to carry out the work.

Equipment

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Three microscopes essential for these studies are located in the K. Pogliano lab: 1. Two Applied Precision Spectris optical sectioning and deconvolution microscopes, which provide high-resolution images of fluorescently stained bacterial cells. One is equipped with a laser to allow photobleaching and TIRF and both also have an environmental chamber for timelapse experiments. Three workstations are available to allow quantitative image analysis and modeling. 2. an Olympus BX-60 microscope for screening purposes. The lab is equipped with a Beckman spectrophotometer, three PCR machines, a -70°C freezer, two -20°C freezers, several 4°C refrigerators, water baths and shakers, as well as standard equipment for molecular, biochemical and genetic experiments.

Shared equipment: The K. Pogliano lab is adjacent to Joe Pogliano's lab, and they share two large incubators (30°C and 37°C), an Alpha-Innotech Gel Documentation System, and a Sorvall RC5C Plus centrifuge. It is also adjacent to the Tim Baker lab, with whom we collaborate on a regular basis for obtaining high resolution electron micrographs of bacterial cells. Tim Baker has two electron microscopes, a 300KV Tecnai G2 Polara and 200KV Sphera, both of which are equipped with FEI tomography software for performing cryoelectron tomography. The 300KV Tecnai G2 Polara microscope is equipped with an ultra-low temperature stage allowing samples to remain frozen at liquid helium or liquid nitrogen temperatures. Within the building are biochemistry and biophysics labs that generously provide access to equipment for protein purification and structural studies, including an AVIV CD spectropolarimeter and a BIACore instrument. Dissecting microscopes equipped with fluorescence detection capabilities are available as part of the Division of Biological Sciences microscopy facilities.

Dorrestein laboratory equipment. The Dorrestein lab has about 2000 sq. ft. of office and lab space. The lab is equipped with incubators, culturing rooms (mammalian and non-mammalian cells), a microbiology clean hood, water baths, gel electrophoresis apparatus, PCR equipment, and power supplies, gel documentation systems for both UV and visible detection (Agarose and SDS-gel), small centrifuges, centrifuges for larger cultures with 4 and 10-liter capacity, shaker for culturing, cell culturing facilities, sonicators, 2 HPLC's, lab computers (13) used for data analysis, research and documentation of our work, an LTQ-FT-ICR-MS equipped with an Advion nanospray robot, a Prosilia DESI system and nanospray capabilities, this instrument is set up for secured remote access so that we can monitor the progress and control the progress of this instrument via the internet, a MALDI-TOF imaging system, pressure bomb for making our own capillary columns for proteomic studies to and an optical microscope. In addition through the marine biotechnology and the Moores cancer center we have access to screens against a variety of cell lines and organisms.

In summary, we have all the equipment to carry out the work proposed.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

| | | | | | |
|---|--|------------------------------|-----------------------------|-------------------|-----------------|
| Prefix: | * First Name: | Kit | Middle Name: | | |
| * Last Name: | Pogliano | | Suffix: | | |
| Position/Title: | Professor | Department: | Div. of Biological Sciences | | |
| Organization Name: | The Regents of the Univ. of Calif., U.C. San Diego | | | Division: | General Campus |
| * Street1: | 9500 Gilman Drive | | | | |
| Street2: | 0934 | | | | |
| * City: | La Jolla | County/ Parish: | San Diego | | |
| * State: | CA: California | Province: | | | |
| * Country: | USA: UNITED STATES | * Zip / Postal Code: | 92093-0934 | | |
| * Phone Number: | 858-822-1314 | Fax Number: | 858-822-5740 | | |
| * E-Mail: | kpogliano@ucsd.edu | | | | |
| Credential, e.g., agency login: | kpogliano | | | | |
| * Project Role: | PD/PI | Other Project Role Category: | | | |
| Degree Type: | | | | | |
| Degree Year: | | | | | |
| *Attach Biographical Sketch | | 1244-KPbiosketchSept1.pdf | Add Attachment | Delete Attachment | View Attachment |
| Attach Current & Pending Support | | | Add Attachment | Delete Attachment | View Attachment |

PROFILE - Senior/Key Person 1

| | | | | | |
|---|--|------------------------------|--------------------|-------------------|-----------------|
| Prefix: | * First Name: | Pieter | Middle Name: | | |
| * Last Name: | Dorresteijn | | Suffix: | | |
| Position/Title: | Asst Professor | Department: | School of Pharmacy | | |
| Organization Name: | The Regents of the Univ. of Calif., U.C. San Diego | | | Division: | General Campus |
| * Street1: | 9500 Gilman Drive | | | | |
| Street2: | 0934 | | | | |
| * City: | La Jolla | County/ Parish: | San Diego | | |
| * State: | CA: California | Province: | | | |
| * Country: | USA: UNITED STATES | * Zip / Postal Code: | 92093-0934 | | |
| * Phone Number: | 858-534-6607 | Fax Number: | | | |
| * E-Mail: | pdorresteijn@ucsd.edu | | | | |
| Credential, e.g., agency login: | pdorresteijn | | | | |
| * Project Role: | PD/PI | Other Project Role Category: | | | |
| Degree Type: | | | | | |
| Degree Year: | | | | | |
| *Attach Biographical Sketch | | 1245-PDbiosketchSept1.pdf | Add Attachment | Delete Attachment | View Attachment |
| Attach Current & Pending Support | | | Add Attachment | Delete Attachment | View Attachment |

Program Director/Principal Investigator (Last, First, Middle): Pogliano, Kit

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME Kit J. Pogliano | POSITION TITLE Professor of Biological Sciences | | |
|---|---|-------------------------|---|
| eRA COMMONS USER NAME (credential, e.g., agency login) kpogliano | | | |
| EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.) | | | |
| INSTITUTION AND LOCATION | DEGREE (if applicable) | MM/YY | FIELD OF STUDY |
| University of Washington, Seattle, WA Harvard Medical School, Boston, MA Harvard University, Cambridge, MA | Ph.D. | 06/86 09/93 10/96 | Biology Microbiology Molecular and Cellular Biology |

A. Personal Statement

I am best known as a microbial cell biologist who studies *Bacillus subtilis* sporulation, and also have expertise in microbial and phage genetics, protein secretion, membrane protein biogenesis and genomics. In addition to *B. subtilis*, I have worked on *Pseudomonas aeruginosa* and *E. coli*, with a focus on membrane protein biogenesis and pilus assembly.

This proposal entails the development and implementation of new genetic and cell biological methods, and I have a strong record of achieving technical advances in these areas. As a postdoctoral fellow in Richard Losick's lab I worked with Elizabeth Harry to apply immunofluorescence microscopy to *B. subtilis*, and in my own lab, I have continued to develop new methods to study bacterial cells. My lab was the first to use fluorescent membrane stains such as FM 4-64 to study *B. subtilis* sporulation and cell division, and we developed assays for membrane fission, and new timelapse methods that allow cell division and chromosome segregation to be followed with timelapse fluorescence microscopy. More recently, we have developed microculture and fluorescence microscopy assays to test the cellular effects of purified antimicrobial compounds on bacterial cell structure and viability and we have used these methods to test the role of the proposed cannibalism toxins Sdp and Skf. We implemented fluorescence recovery after photobleaching and total internal reflection microscopy and used these methods to study protein dynamics during both cell division and sporulation. We developed two powerful new genetic tools, cell specific GFP tagging for the specific localization of proteins in one or the other daughter cell during sporulation, and TAGIT, a transposon that allows the random insertion of *gfp* (and other genes) into chromosomal loci without disrupting operon structure or regulation. Thus, I have the technical expertise necessary to complete the proposed studies.

This grant proposal is product of an ongoing collaboration with Dr. Pieter Dorrestein, in which we are exploring the role of small molecules produced by *B. subtilis* on growth, development and interactions with other species. This interaction is mediated by the free exchange of students between the two labs, joint group meetings, and frequent conversations via email and phone. I have experience with interdisciplinary collaborations, as I work closely on my sporulation project with Dr. Carlos Bustamante (UC Berkeley), Dr. KC Huang (Stanford), Dr. Grant Jensen (Cal Tech) and Dr. Jonathan Dworkin (Colombia U) and with Dr. Ry Young (Texas A&M) on

Program Director/Principal Investigator (Last, First, Middle): Pogliano, Kit

bacteriophage lambda-induced host cell lysis. I manage these collaborations by frequent email, phone and Skype conferences and by occasional visits to perform or observe experiments and attend group meetings. I therefore have the experience required to manage the interdisciplinary and collaborative aspects of this grant.

B. Positions and Honors

Positions

- (1982-1984) Research assistant, Department of Genetics, University of Washington, with Dr. A.H. Doermann
- (1984-1986) Research assistant, Department of Microbiology and Immunology, University of Washington, with Dr. S. Lory
- (1986-1993) Graduate research, Department of Microbiology and Molecular Genetics, Harvard Medical School, with Dr. J. Beckwith
- (1993-1996) Postdoctoral fellow, Department of Cellular and Molecular Biology, Harvard University, with Dr. R. Losick
- (1996-2002) Assistant Professor, Division of Biological Sciences, University of California, San Diego
- (2002-2007) Associate Professor, Division of Biological Sciences, University of California, San Diego
- (2007-) Professor, Division of Biological Sciences, University of California, San Diego

Honors

- ALCOA Foundation Scholarship (1983)
- John and Dorothy Franco Award for undergraduate research (1985)
- National Science Foundation Predoctoral Fellowship (1986)
- Damon Runyon-Walter Winchell Foundation Postdoctoral Fellowship (1993)
- Beckman Young Investigator Award (1998)
- Searle Scholar (1998)
- Fellow, American Academy of Microbiology (2009)

C. Selected peer-reviewed publications (in chronological order)

1. Sharp, M.D. and K. Pogliano. (2002) Role of cell-specific assembly of SpolIIE in polarity of DNA transfer. *Science*, 295:137-139. PMID: 11778051
2. Abanes-de Mello, A., Y.-L. Sun, S. Aung and K. Pogliano. (2002) A cytoskeleton-like role for the bacterial cell wall during engulfment of the *Bacillus subtilis* forespore. *Genes & Dev.*, 16:3253-3264. PMC187501
3. Rubio, A. and K. Pogliano. (2004) Septal localization of forespore membrane proteins during engulfment in *Bacillus subtilis*. *EMBO J.*, 23:1636-1646. PMC391076
4. Blaylock, B., X. Jiang, A. Rubio, C.P. Moran, Jr. and K. Pogliano. (2004) Zipper-like interaction between proteins in adjacent daughter cells mediates protein localization. *Genes & Dev.*, 18:2916-2928. PMCID: PMC534652
5. Broder, D. and K. Pogliano. (2006) Forespore engulfment mediated by a ratchet-like mechanism. *Cell*, 126:917-928. PMID: 16959571

Program Director/Principal Investigator (Last, First, Middle): Pogliano, Kit

6. Chiba, S. K. Coleman and K. Pogliano. (2007) Impact of membrane fusion and proteolysis on SpolIQ dynamics and interaction with SpolIIAH. *J. Biol. Chem.* 282:2576-2586. PMID: 17121846
7. Becker, E.C. and K. Pogliano. (2007) Cell-specific SpolIIIE assembly and DNA translocation polarity are dictated by chromosome orientation. *Molecular Microbiology*, 66:1066-1079. PMID: 18001347
8. Ptacin, J., M. Nollmann, E.C. Becker, N.R. Cozzarelli, K. Pogliano and C. Bustamante. (2008) Sequence-directed DNA export guides chromosome translocation during sporulation in *Bacillus subtilis*. *Nature Structural and Molecular Biology* 15:485-493 PMID: 18391964
9. Gregory, J.A., E.C. Becker and K. Pogliano. (2008) *Bacillus subtilis* MinC destabilizes FtsZ rings at new poles and contributes to the timing of cell division. *Genes and Development*, 22:3475-3488. PMID: 19141479
10. Chiba, S. A. Lamsa and K. Pogliano. (2009) A ribosome nascent chain sensor of membrane protein biogenesis in *Bacillus subtilis*, *EMBO J.* 128:3461-3475. PMID: 19779460
11. Gregory, J.A., E.C. Becker J. Jung, I. Tuwatananurak and K. Pogliano. (2010) Tn5 assisted gene insertion technology (TAGIT), a tool for generating fluorescent fusion proteins, *PLoS One* 5:e8731. PMC2806921
12. Meyer, P., J. Gutierrez, K. Pogliano and J. Dworkin. (2010) Cell wall synthesis is necessary for membrane dynamics during sporulation of *Bacillus subtilis*. *Molecular Microbiology* 76:956-970. PMID: 20444098.
13. Gutierrez, J., R. Smith, and K. Pogliano. (2010) SpolID peptidoglycan hydrolase activity is required throughout engulfment during *Bacillus subtilis* sporulation. *J. Bacteriology*, 192:3174-3186.
14. Fleming, T., J.Y. Shin, S.H. Lee, E.C. Becker, K.C. Huang, C. Bustamante and K. Pogliano. (2010) Dynamic SpolIIIE assembly mediates septal membrane fission during *Bacillus subtilis* sporulation, *Genes and Development* 24:1160-1172.
15. Liu, W.T., Y.L. Yang, Y. Xu, A. Lamsa, N.M. Haste, J.Y. Yang, J. Ng, D. Gonzalez, C.D. Ellermeier, P.D. Straight, P.A. Pevzner, J. Pogliano, V. Nizet, K. Pogliano, P.C. Dorrestein (2010) Imaging mass spectrometry of intraspecies metabolic exchange revealed the cannibalistic factors of *Bacillus subtilis*. *Proc. Natl. Acad. Sci., USA*, in press.

D. Research Support

Ongoing research support

"Forespore engulfment during *B. subtilis* sporulation"

Principal Investigator: Kit Pogliano, Ph.D.

Agency: National Institute of Health, Institute of General Medical Sciences

Type: R01 (GM57045) Period: January 1, 1997 – December 31, 2014.

The objective of this grant is to achieve a detailed understanding of the phagocytosis-like process of engulfment during *B. subtilis* sporulation, with the primary focus on previously-identified engulfment-defective mutants. There is no overlap with the present application.

Role: PI

Program Director/Principal Investigator (Last, First, Middle): Pogliano, Kit

Completed research support

National Institutes of Health (GM-057045-S1) Supplement to promote diversity (8/1/08 – 12/31/10)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on the Sample Form.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| | |
|--------------------------------------|--|
| NAME Pieter C. Dorrestein | POSITION TITLE Associate Professor Skaggs School of Pharmacy and Pharmaceutical Sciences and Departments of Pharmacology, Chemistry and Biochemistry |
| eRA COMMONS USER NAME pdorrestein | |

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

| INSTITUTION AND LOCATION | DEGREE (if applicable) | YEAR(S) | FIELD OF STUDY |
|--|---------------------------|---------|---------------------|
| Northern Arizona University, Flagstaff, AZ | B.S. | 1998 | Chemistry |
| Cornell University, Ithaca, NY | M.S. | 2001 | Chemistry |
| Cornell University, Ithaca, NY | Ph.D. | 2004 | Chemical Biology |
| University of Illinois, Urbana-Champaign, IL | Postdoc. | 2006 | Bioanalytical Chem. |

A. Personal Statement.

Our work aims to develop new mass spectrometry based methods to observe adaptive metabolism. In this project we will apply imaging mass spectrometry approaches to understand the chemistry involved that make up biofilms and we aim to understand the chemistry that initiates predation activities of bacteria. This research requires the understanding of microbial genomics, proteomics, imaging mass spectrometry, natural product structure elucidation, bioactivity screening and an understanding of small molecule structure elucidation methods. My laboratory has all these capabilities. As a graduate student at Cornell University, I investigated the biosynthesis of thiamin and I discovered a new cysteine biosynthetic pathway in mycobacterium tuberculosis. The projects required advanced high resolution mass spectrometry approaches. As a post-doctoral that was co-sponsored by Neil Kelleher (University of Illinois) and Christopher Walsh (Harvard Medical School) advanced mass spectrometry approaches were employed to study the biosynthesis secondary metabolites of therapeutic value. My laboratory now has two mass spectrometers, an LTQ-FT-ICR-MS and a MALDI-TOF that will be used in the studies to investigate the interactions between bacteria.

B. Positions and Honors

Positons and Employment

- | | |
|------------|--|
| 1999-2004 | Research Assistant, Cornell University (08/1999 - 05/2004) |
| 2004-2006 | Postdoctoral NIH NRSA fellow, University of Illinois (6/2004 – 9/2006) |
| Sept. 2006 | Assistant Professor, University of California, San Diego (9/2006-6/2010) |
| July 2010 | Associate Professor, University of California, San Diego (starting 7/2010) |

Professional Memberships

- | | |
|--------------|---|
| 2001-present | American Chemical Society |
| 2005-present | American Society of Mass Spectrometry |
| 2006-present | Biomedical Science program |
| 2007-present | Center for Marine Biotechnology and Biomedicine |
| 2007-present | Moores Cancer Center |
| 2008-present | Society for Industrial Microbiology |
| 2008-present | Center for Algorithmic and Systems Biology |
| 2008-present | Center for Computational Mass Spectrometry |

Honors

- 2003 Vincent DuVineaud Award. Recognizing innovative and cutting-edge science
2004 Wentink Award recognizing the most outstanding graduating Ph.D. candidate in chemical biology
2004-2006 NIH NRSA Kirschstein fellowship. "Enzymology of NRPS and PKS proteins by mass spectrometry"
2008 Beckman young investigator.
2008 V-foundation scholar in cancer research.
2008 Lilly young investigator award in analytical chemistry.
2009 Hearst foundation award.

C. peer-reviewed publications

6 publications relevant to this work.

1. Yang, Y.L., Xu, Y., Straight, P., Dorrestein, P.C., Translating metabolic exchange with imaging mass spectrometry. **Nature Chemical Biology** (2009) 5(12):885-7. PMID: 19915536.
2. Esquenazi, E., Yang, Y-L., Watrous, J., Gerwick, W. H., Dorrestein, P. C., Imaging Mass Spectrometry of Natural Products. **Natural Product Reports**, (2009), 26(12):1521-34. PMID: 19936384 (cover article).
3. Esquenazi, E., Coates, C., Simmons, L., Gonzalez, D., Gerwick, W.H., Dorrestein, P.C. Visualizing the spatial distribution of secondary metabolites produced by marine cyanobacteria and sponges via MALDI-TOF imaging. **Molecular BioSystems**. (2008) 4, 562-570. PMCID: PMC2848974 (cover article).
4. Simmons, T. L., Coates R.C., Clark B. R., Engene N., Gonzalez D., Esquenazi, E., Dorrestein P. C., Gerwick W. H. Biosynthetic Origin of Natural Products Isolated from Marine Microorganism-Invertebrate Assemblages. **Proc. Natl. Acad. Sci. USA**. (2008) 25,105, 4587-94. PMCID: PMC2290810
5. Watrous J., Hendricks N., Meehan M., Dorrestein P.C., Capturing Bacterial Metabolic Exchange Using Thin Film Desorption Electrospray Ionization-Imaging Mass Spectrometry. **Anal. Chem.** (2010) 82, 1598-600. PMID: 20121185.
6. Wei-Ting Liu, Yu-Liang Yang, Yuquan Xu, Anne Lamsa, Nina M Haste, Jane Y. Yang, Julio Ng, David Gonzalez, Craig D. Ellermeier, Paul D. Straight, Pavel A. Pevzner, Joe Pogliano, Victor Nizet, Kit Pogliano, Pieter C. Dorrestein Imaging mass spectrometry of intraspecies metabolic exchange revealed the cannibalistic factors of *Bacillus subtilis* **Proc. Natl. Acad. Sci. USA**. (2010, accepted).

9 additional publications.

1. Calderone, C.T., Kowtoniuk, W.E., Kelleher, N.L., Walsh, C.T., Dorrestein, P.C. Convergence of isoprene and polyketide biosynthetic machinery: isoprenyl-S-carrier proteins in the pksX pathway of *B. subtilis*. **Proc. Natl. Acad. Sci. USA**. (2006) 103, 8977-8982. PMCID: PMC1482551
2. Zhang, J., Van Lanen, S. G., Ju, J., Liu, W. Dorrestein, P.C., Li, W., Kelleher, N. L., Shen, B. A. phosphopantetheinylating polyketide synthase producing a linear polyene to initiate enediyne antitumor antibiotic biosynthesis. **Proc. Natl. Acad. Sci. USA**. (2008) 5,105,1460-5. PMCID: PMC2234166
3. Burns K.E., Liu W.T., Boshoff H.I., Dorrestein P.C., Barry C.E. 3rd. Proteasomal protein degradation in mycobacteria is dependent upon a prokaryotic ubiquitin-like protein. **J. Biol. Chem.** (2009) 284, 3069-75. PMCID: PMC2631945
4. Lee S.W., Mitchell D.A., Markley A.L., Hensler M.E., Gonzalez D., Wohlrab A., Dorrestein P.C., Nizet V., Dixon J.E. Discovery of a widely distributed toxin biosynthetic gene cluster. **Proc. Natl. Acad. Sci. U S A**. (2008) 105, 5879-84. PMCID: PMC2311365
5. Ng,J., Bandeira, N.,Liu, W.T. Ghassanian, M., Simmons, T., Gerwick, W. Linington, R., Dorrestein P.C., Pevzner, P., Dereplication and De Novo Sequencing of Nonribosomal Peptides. **Nature Methods**, (2009), 6(8):596-9. PMCID: PMC2754211

- 6.** Esquenazia E., Dorrestein P. C., Gerwick W. H., Probing marine natural product defenses with DESI-imaging mass spectrometry. **Proc. Natl. Acad. Sci. USA.** (2009), 106, 7269-70. (comm.) PMCID: PMC2678597
- 7.** Pedro N. Leão, Alban R. Pereira, Wei-Ting Liu, Julio Ng, Pavel A. Pevzner, Pieter C. Dorrestein, Gabriele M. König, Vitor M. Vasconcelosa , and William H. Gerwick, Synergistic allelochemicals from a freshwater cyanobacterium. **Proc. Natl. Acad. Sci. USA.**, (2010, accepted)
- 8.** Wen Liu, Bodgen Tanasa, Oksana V. Tyurina, Yian Yuan Zhou, Reto Gassmann, Wei Ting Liu, Kenneth A. Ohgi, Chris Benner, Ivan Garcia-Bassets, Aneel K. Aggarwal, Arshad Desai, Pieter C. Dorrestein, Christopher K. Glass, Michael G. Rosenfeld. PHF8 mediates histone demethylation events involved in cell cycle progression. **Nature**, (2010, accepted).
- 9.** David J. Gonzalez, Shaun W. Lee, Mary E. Hensler, Samira Dahesh, Andrew L. Markley, Douglas A. Mitchell, Nuno Banderia, Victor Nizet, Jack E. Dixon, Pieter C. Dorrestein, Clostridiolysin S: a post-translationally modified biotoxin from Clostridium botulinum **J. Biol. Chem.** (2010, accepted).

D. Research Support

Ongoing Research Support.

| | |
|---|-------------------|
| R01 GM08283 Dorrestein (PI) New Approaches to sequencing of complex peptides In this 3 year non-renewable R01, we are developing methods to characterize complex peptides. | 9/25/08 – 8/31/11 |
| R01 GM08283 Tang (PI) 7/31/13 Characterization & Engineering of Fungal Megasynthesis in Escherichia coli I receive 20% or 48k/year of the R01 for the mass spectrometric detection of covalent intermediates of fungal polyketide synthases. | 8/1/08 – |
| Beckman Foundation Dorrestein (PI) 8/31/11 Harvesting Microbial Genomes for their therapeutic potential This grant aims to mine microbial genomes for new natural products. | 9/1/08 – |
| V-Foundation Dorrestein (PI) 12/31/11 Anti-Cancer Drugs from the Ocean This grant aims to overcome the supply issues of promising therapeutics isolated from the ocean. | 1/1/09 – |

Pending research support.

1R01GM094802-01 Dorrestein (PI) The program officer notified me a week ago that this grant will be funded
in the next few months.
Real-Time Imaging of Metabolic Communication
In this proposal we want to develop real-time mass spectrometry approaches to observe adaptive metabolism.

1S10RR029121-01 Score 22
Synapt ion mobility mass spectrometer
This proposal is requesting an ion mobility instrument for the observation of adaptive metabolism and natural products from complex samples.

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B. BUDGET PERIOD

* ORGANIZATIONAL DUNS: 8043557900000

*** Budget Type:** Project Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Ca

Delete Entry * Start Date: * End Date: Budget Period 1

A. Senior/Key Person

9. Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person

Additional Senior Key Persons:

Add Attachment

Delete Attachment

[View Attachment](#)

B. Other Personnel

| * Number of Personnel | * Project Role | Cal. Months | Acad. Months | Sum. Months | * Requested Salary (\$) | * Fringe Benefits (\$) | * Funds Requested (\$) |
|-----------------------|------------------------------|-------------|--------------|-------------|-------------------------|------------------------|------------------------|
| 4 | Post Doctoral Associates | 48.00 | 0.00 | 0.00 | 128,621.00 | 36,331.00 | 164,952.00 |
| 3 | Graduate Students | 13.50 | 0.00 | 0.00 | 57,648.00 | 34,191.32 | 91,839.32 |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
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| 7 | Total Number Other Personnel | | | | | Total Other Personnel | 256 791 32 |

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1* ORGANIZATIONAL DUNS: * Budget Type: Project Subaward/ConsortiumEnter name of Organization: **Delete Entry*** Start Date: * End Date: Budget Period 1**C. Equipment Description**

List items and dollar amount for each item exceeding \$5,000

| Equipment item | * Funds Requested (\$) |
|---|------------------------|
| 1. <input type="text"/> | <input type="text"/> |
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| 9. <input type="text"/> | <input type="text"/> |
| 10. <input type="text"/> | <input type="text"/> |
| 11. Total funds requested for all equipment listed in the attached file | <input type="text"/> |
| Total Equipment | <input type="text"/> |

Additional Equipment: **Add Attachment****Delete Attachment****View Attachment****D. Travel**

1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost**E. Participant/Trainee Support Costs**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other

Funds Requested (\$)**Number of Participants/Trainees** **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1**Next Period*** ORGANIZATIONAL DUNS: * Budget Type: Project Subaward/ConsortiumEnter name of Organization: **Delete Entry**Start Date: * End Date:

Budget Period 1

F. Other Direct Costs

1. Materials and Supplies
2. Publication Costs
3. Consultant Services
4. ADP/Computer Services
5. Subawards/Consortium/Contractual Costs
6. Equipment or Facility Rental/User Fees
7. Alterations and Renovations
8. Service Contracts
9. NGN/Communication Costs
10.

Funds Requested (\$)**Total Other Direct Costs** **G. Direct Costs****Funds Requested (\$)****Total Direct Costs (A thru F)** **H. Indirect Costs****Indirect Cost Type****Indirect Cost Rate (%)****Indirect Cost Base (\$)***** Funds Requested (\$)**

1.
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4.

Total Indirect Costs **Cognizant Federal Agency**

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs**Funds Requested (\$)****Total Direct and Indirect Institutional Costs (G + H)****J. Fee****Funds Requested (\$)****K. * Budget Justification** **Add Attachment****Delete Attachment****View Attachment**

(Only attach one file.)

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 8043557900000

*** Budget Type:** Project Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Ca

Delete Entry * Start Date: * End Date: Budget Period

A. Senior/Key Person

9. Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person

Additional Senior Key Persons:

Add Attachment

Delete Attachment

[View Attachment](#)

B. Other Personnel

| * Number of Personnel | * Project Role | Cal. Months | Acad. Months | Sum. Months | * Requested Salary (\$) | * Fringe Benefits (\$) | * Funds Requested (\$) |
|-----------------------|------------------------------|-------------|--------------|-------------|-------------------------|------------------------|------------------------|
| 4 | Post Doctoral Associates | 48.00 | 0.00 | 0.00 | 131,145.00 | 37,034.00 | 168,179.00 |
| 3 | Graduate Students | 13.50 | 0.00 | 0.00 | 58,991.00 | 37,546.85 | 96,537.85 |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
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| | | | | | | | |
| 7 | Total Number Other Personnel | | | | | Total Other Personnel | 264,716.85 |

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2* ORGANIZATIONAL DUNS: * Budget Type: Project Subaward/ConsortiumEnter name of Organization: **Delete Entry*** Start Date: * End Date: Budget Period 2**C. Equipment Description**

List items and dollar amount for each item exceeding \$5,000

| Equipment item | * Funds Requested (\$) |
|---|------------------------|
| 1. <input type="text"/> | <input type="text"/> |
| 2. <input type="text"/> | <input type="text"/> |
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| 8. <input type="text"/> | <input type="text"/> |
| 9. <input type="text"/> | <input type="text"/> |
| 10. <input type="text"/> | <input type="text"/> |
| 11. Total funds requested for all equipment listed in the attached file | <input type="text"/> |
| Total Equipment | <input type="text"/> |

Additional Equipment: **Add Attachment****Delete Attachment****View Attachment****D. Travel**

1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost**E. Participant/Trainee Support Costs**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other

Funds Requested (\$)**Number of Participants/Trainees** **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2**Next Period*** ORGANIZATIONAL DUNS: * Budget Type: Project Subaward/ConsortiumEnter name of Organization: **Delete Entry**Start Date: * End Date:

Budget Period 2

F. Other Direct Costs

1. Materials and Supplies
2. Publication Costs
3. Consultant Services
4. ADP/Computer Services
5. Subawards/Consortium/Contractual Costs
6. Equipment or Facility Rental/User Fees
7. Alterations and Renovations
8. Service Contracts
9. NGN/Communication Costs
10.

Funds Requested (\$)**Total Other Direct Costs** **G. Direct Costs****Funds Requested (\$)****Total Direct Costs (A thru F)****H. Indirect Costs****Indirect Cost Type****Indirect Cost Rate (%)****Indirect Cost Base (\$)***** Funds Requested (\$)**

| | | | |
|---|------------------------------------|---|---|
| 1. <input type="text" value="Modified Total Direct Costs"/> | <input type="text" value="54.50"/> | <input type="text" value="91,913.00"/> | <input type="text" value="50,092.00"/> |
| 2. <input type="text" value="Modified Total Direct Costs eff 7/1"/> | <input type="text" value="55.00"/> | <input type="text" value="275,738.00"/> | <input type="text" value="151,656.00"/> |
| 3. <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 4. <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |

Total Indirect Costs **Cognizant Federal Agency**

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs**Funds Requested (\$)****Total Direct and Indirect Institutional Costs (G + H)****J. Fee****Funds Requested (\$)****K. * Budget Justification** **Add Attachment****Delete Attachment****View Attachment**

(Only attach one file.)

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 8043557900000

*** Budget Type:** Project Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Ca

Delete Entry * Start Date: * End Date: Budget Period

A. Senior/Key Person

9. Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person

Additional Senior Key Persons:

Add Attachment

Delete Attachment

[View Attachment](#)

B. Other Personnel

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3* ORGANIZATIONAL DUNS: * Budget Type: Project Subaward/ConsortiumEnter name of Organization: **Delete Entry*** Start Date: * End Date: Budget Period 3**C. Equipment Description**

List items and dollar amount for each item exceeding \$5,000

| Equipment item | * Funds Requested (\$) |
|---|------------------------|
| 1. <input type="text"/> | <input type="text"/> |
| 2. <input type="text"/> | <input type="text"/> |
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| 8. <input type="text"/> | <input type="text"/> |
| 9. <input type="text"/> | <input type="text"/> |
| 10. <input type="text"/> | <input type="text"/> |
| 11. Total funds requested for all equipment listed in the attached file | <input type="text"/> |
| Total Equipment | <input type="text"/> |

Additional Equipment: **Add Attachment****Delete Attachment****View Attachment****D. Travel**

1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost**E. Participant/Trainee Support Costs**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other

Funds Requested (\$)**Number of Participants/Trainees** **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3[Next Period](#)* ORGANIZATIONAL DUNS: * Budget Type: Project Subaward/ConsortiumEnter name of Organization: [Delete Entry](#)Start Date: * End Date:

Budget Period 3

F. Other Direct Costs

1. Materials and Supplies
2. Publication Costs
3. Consultant Services
4. ADP/Computer Services
5. Subawards/Consortium/Contractual Costs
6. Equipment or Facility Rental/User Fees
7. Alterations and Renovations
8.
9.
10.

Funds Requested (\$)**Total Other Direct Costs** **G. Direct Costs****Funds Requested (\$)****Total Direct Costs (A thru F)** **H. Indirect Costs****Indirect Cost Type****Indirect Cost Rate (%)****Indirect Cost Base (\$)***** Funds Requested (\$)**

1.
2.
3.
4.

Total Indirect Costs Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)**I. Total Direct and Indirect Costs****Funds Requested (\$)****Total Direct and Indirect Institutional Costs (G + H)****J. Fee****Funds Requested (\$)**K. * Budget Justification [Add Attachment](#)[Delete Attachment](#)[View Attachment](#)

(Only attach one file.)

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B. BUDGET PERIOD

*** ORGANIZATIONAL DUNS:** 8043557900000

*** Budget Type:** Project Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Ca

Delete Entry * Start Date: * End Date: Budget Period

A. Senior/Key Person

9. Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person

Additional Senior Key Persons:

Add Attachment

Delete Attachment

[View Attachment](#)

B. Other Personnel

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4* ORGANIZATIONAL DUNS: * Budget Type: Project Subaward/ConsortiumEnter name of Organization: **Delete Entry*** Start Date: * End Date: Budget Period 4**C. Equipment Description**

List items and dollar amount for each item exceeding \$5,000

| Equipment item | * Funds Requested (\$) |
|---|------------------------|
| 1. <input type="text"/> | <input type="text"/> |
| 2. <input type="text"/> | <input type="text"/> |
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| 10. <input type="text"/> | <input type="text"/> |
| 11. Total funds requested for all equipment listed in the attached file | <input type="text"/> |
| Total Equipment | <input type="text"/> |

Additional Equipment: **Add Attachment****Delete Attachment****View Attachment****D. Travel**

1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost**E. Participant/Trainee Support Costs**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other

Funds Requested (\$)**Number of Participants/Trainees** **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4**Next Period*** ORGANIZATIONAL DUNS: * Budget Type: Project Subaward/ConsortiumEnter name of Organization: **Delete Entry**Start Date: * End Date:

Budget Period 4

F. Other Direct Costs

1. Materials and Supplies
2. Publication Costs
3. Consultant Services
4. ADP/Computer Services
5. Subawards/Consortium/Contractual Costs
6. Equipment or Facility Rental/User Fees
7. Alterations and Renovations
8. Service Contract
9. NGN/Communication Costs
10.

Funds Requested (\$)**Total Other Direct Costs** **G. Direct Costs****Funds Requested (\$)****Total Direct Costs (A thru F)****H. Indirect Costs****Indirect Cost Type****Indirect Cost Rate (%)****Indirect Cost Base (\$)***** Funds Requested (\$)**

1. Modified Total Direct Costs
2.
3.
4.

Total Indirect Costs **Cognizant Federal Agency**

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs**Funds Requested (\$)****Total Direct and Indirect Institutional Costs (G + H)****J. Fee****Funds Requested (\$)****K. * Budget Justification** **Add Attachment****Delete Attachment****View Attachment**

(Only attach one file.)

Personnel

Pogliano lab

Kit Pogliano, Ph.D., Principal Investigator (2 summer months) will be responsible for the administration and direction of the project.

To be named, Postgraduate researcher, (12 calendar months). A new postdoctoral fellow will be recruited to direct the experiments on the requirements for the interaction between *B. subtilis* and *P. aeruginosa*. Support is requested for this postdoctoral fellow.

To be named, Postgraduate researcher (12 calendar months). I will recruit a new postdoctoral fellow to direct the experiments on the genetic requirements for *B. subtilis* colony invasion. This postdoctoral fellow is expected to obtain a fellowship, so no support is requested.

Anne Lamsa. Graduate student (6 calendar months). Ms. Lamsa will expand her studies on SDP to include its affect on other bacterial species and to test the cellular effects of other antimicrobial compounds identified in the proposed studies. Ms. Lamsa is a senior student no longer supported by departmental training grants. Her research effort is devoted to this project.

To be named. Graduate student (1.5 calendar months). I request funding to support the efforts of an additional graduate student to identify and quantify different types of interactions between *B. subtilis* and other species. This student will be partially supported by graduate training grants, so only partial support is requested. His or her research effort will be devoted to this project.

Dorrestein lab

Pieter C. Dorrestein, PhD. Dorrestein will be responsible for the chemistry of this project. He will be involved in the experimental design of the imaging mass spectrometry, the isolation and structure elucidation of the molecules and the chemical modification of the molecules.

Christopher Michael Rath. Postgraduate researcher. (12 calendar months) Chris will be arriving in the Dorrestein laboratory 02/01/2011 from the University of Michigan. He will be responsible for the isolation and structure elucidation of the isolated molecules.

To be named, Postgraduate researcher. (12 calendar months) This person will be recruited to take over David Gonzalez's efforts. This person will be using imaging mass spectrometry to understand the chemistry of predation with emphasis of pathogen interactions.

David Gonzalez, Graduate student. (6 calendar months). David is the person responsible for the analysis of the interaction of *B. subtilis* with pathogens. He will be graduating in 12 months therefore there are no funds requested for him.

Wei-Ting Liu. Graduate student. (6 calendar months). Wei-Ting has been purifying the cannibalistic factors from *B. subtilis*. She is currently developing the purification schemes to isolate sufficient quantities for chemical derivatization of the cannibalistic factors and other metabolites for fluorescence studies and will expand her efforts to the predation of *B. subtilis* with other microbes (e.g. *Pseudomads*).

Other expenses

Support (12K) is requested for the service contract for one of our two microscopes. The service contract fully covers travel of the service engineers and replacement parts and is essential to keep our microscope in top working order.

Support for half the service contract of the MALDI-TOF mass spectrometer is requested (13K) and 5K for the structure elucidation which mostly involves a large amount of NMR time.

We request funds to cover the cost of publications. Much of our data must be presented in color; associated charges are often \$2,000/publication.

RESEARCH & RELATED BUDGET - Cumulative Budget

| | | Totals (\$) |
|---|------------|--------------------|
| Section A, Senior/Key Person | | 189,304.00 |
| Section B, Other Personnel | | 1,076,512.52 |
| Total Number Other Personnel | 28 | |
| Total Salary, Wages and Fringe Benefits (A+B) | | 1,265,816.52 |
| Section C, Equipment | | |
| Section D, Travel | | 31,488.00 |
| 1. Domestic | 31,488.00 | |
| 2. Foreign | | |
| Section E, Participant/Trainee Support Costs | | |
| 1. Tuition/Fees/Health Insurance | | |
| 2. Stipends | | |
| 3. Travel | | |
| 4. Subsistence | | |
| 5. Other | | |
| 6. Number of Participants/Trainees | | |
| Section F, Other Direct Costs | | 347,958.72 |
| 1. Materials and Supplies | 279,702.00 | |
| 2. Publication Costs | 8,367.00 | |
| 3. Consultant Services | | |
| 4. ADP/Computer Services | | |
| 5. Subawards/Consortium/Contractual Costs | | |
| 6. Equipment or Facility Rental/User Fees | | |
| 7. Alterations and Renovations | | |
| 8. Other 1 | 39,913.72 | |
| 9. Other 2 | 19,976.00 | |
| 10. Other 3 | | |
| Section G, Direct Costs (A thru F) | | 1,645,263.24 |
| Section H, Indirect Costs | | 817,661.00 |
| Section I, Total Direct and Indirect Costs (G + H) | | 2,462,924.24 |
| Section J, Fee | | |

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: * First Name:
 Middle Name:
 * Last Name:
 Suffix:

2. Human Subjects

Clinical Trial? No Yes
 * Agency-Defined Phase III Clinical Trial? No Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: * First Name:
 Middle Name:
 * Last Name:
 Suffix:
 * Phone Number: Fax Number:
 Email:

* Title:

* Street1:
 Street2:
 * City:
 County/Parish:
 * State:
 Province:
 * Country: * Zip / Postal Code:

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? No Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: <http://stemcells.nih.gov/research/registry/>. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

New Resubmission Renewal Continuation Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application

(for RESUBMISSION or REVISION only)

| | | | |
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| | Add Attachment | Delete Attachment | View Attachment |
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2. Specific Aims

| | | | |
|------------------------------|--------------------------------|-----------------------------------|---------------------------------|
| 1241-KPSpecificAimsSept1.pdf | Add Attachment | Delete Attachment | View Attachment |
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3. *Research Strategy

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| 1242-KPScienceSept2.pdf | Add Attachment | Delete Attachment | View Attachment |
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4. Inclusion Enrollment Report

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5. Progress Report Publication List

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Human Subjects Sections

6. Protection of Human Subjects

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7. Inclusion of Women and Minorities

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8. Targeted/Planned Enrollment Table

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9. Inclusion of Children

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Other Research Plan Sections

10. Vertebrate Animals

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11. Select Agent Research

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12. Multiple PD/PI Leadership Plan

| | | | |
|-------------------------------|--------------------------------|-----------------------------------|---------------------------------|
| 1246-KPMULTIPLE PI LEADERSHIP | Add Attachment | Delete Attachment | View Attachment |
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13. Consortium/Contractual Arrangements

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14. Letters of Support

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| 1247-KPlettersSupportSept2.pdf | Add Attachment | Delete Attachment | View Attachment |
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15. Resource Sharing Plan(s)

| | | | |
|------------------------------|--------------------------------|-----------------------------------|---------------------------------|
| 1248-ResourceSharingPlan.pdf | Add Attachment | Delete Attachment | View Attachment |
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16. Appendix

[Add Attachments](#)

[Remove Attachments](#)

[View Attachments](#)

Specific Aims

A key aspect of the successful interaction of bacterial cells with their environment is mounting an appropriate response to neighboring cells of the same or different species. Many of these interactions depend on secreted metabolites. For example, quorum sensing molecules allow bacteria to sense population density, inducing behaviors more effective at high cell density, such as secretion of toxins, antibiotics and hydrolytic enzymes. Recent data suggests that bacteria also use extracellular metabolites to modify the behavior of neighboring species in a manner that promotes their own survival in a multispecies environment. Two such interactions have been described in *B. subtilis*, the use of secreted toxins to induce lysis of neighboring colonies and the use of surfactin to inhibit antibiotic production by *S. coelicolor*, thereby killing or disarming potentially dangerous neighbors.

Progress in studying metabolites that affect other species has been hindered by a lack of methods for their study on solid medium, where interspecies interactions are best characterized. The recent development of mass spectrometry based imaging methods allows characterization of the spatial and temporal distribution of extracellular metabolites in interacting bacterial colonies. We have used this method to identify, purify and structurally characterize the *B. subtilis* cannibalism toxin, and here propose to use this method to study the interaction of *B. subtilis* with other species. We will focus on two distinct outcomes of interaction, the impasse produced when *B. subtilis* interacts with *P. aeruginosa*, and a new, apparently predatory behavior in which *B. subtilis* invades and destroys colonies of neighboring species. We will capitalize on the complimentary expertise of the two PIs to elucidate the chemical and genetic basis for these behaviors.

Aim 1: Characterize the interactive metabolome of *B. subtilis* 3610

- 1a. Identify the extracellular metabolome of *B. subtilis* using mass spectrometry and imaging mass spectrometry, to identify metabolites produced by *B. subtilis* in pure culture.
- 1b. Identify and purify interactive metabolites (extracellular metabolites that change during interspecies interactions) that are produced by *B. subtilis*, *P. aeruginosa* and prey species.
- 1c. Test the purified metabolites for biological activity, to determine if they affect viability, cellular architecture or motility of the target species.
- 1d. Identify *B. subtilis* genes required for production of interactive metabolites. We will use a candidate gene approach and screen a transposon library for those that disrupt specific metabolites with biological activities that cannot be identified by the first approach.

Aim 2: Identify genes involved in interspecies interactions

- 2a. A candidate gene approach will be used to determine if genes involved in production of extracellular metabolites, motility, development or stress responses are involved in interactions.
- 2b. A genetic screen will be used to identify genes involved in interspecies interactions, using either a signature-tagged mutant library for high-throughput screens or transposon mutagenesis.

Aim 3: Visualize interspecies interactions using timelapse fluorescence microscopy

- 3a. *B. subtilis* and the target species will be fluorescently tagged to allow visualization of interactions between these species using fluorescence microscopy. We will track interspecies interactions at the population and single cell level, to visualize movement towards other species and cell lysis.
- 3b. Fluorescent reporters of genes involved in interspecies interactions will be constructed, to visualize their induction during interactions at high and low magnification. This experiment will provide a more complete view of when and where the metabolites, stress responses and developmental pathways are required.

(a) Significance

Interactions between different bacterial species are prevalent in nature and critical for medicine, where bacteria in the normal flora can reduce or enhance infections by opportunistic pathogens, yet little is known about how these various outcomes are determined (reviewed by [1]). *Bacillus subtilis* is an ideal organism for studying inter- and intra-species interactions, as it has well documented interactions with other bacteria and is capable of protecting plants against a variety of bacterial and fungal pathogens [2-5]. There are three morphologically distinct outcomes of interactions between *B. subtilis* and other species: coexistence with neighboring colonies (Fig. 1A), lysis of neighboring colonies (Fig. 1B) and a newly described outcome involving invasion and destruction of the neighboring colony that we call contact dependent predation (Fig. 1C; preliminary data section). We here propose to determine how *B. subtilis* achieves these distinct outcomes of interactions with other species.

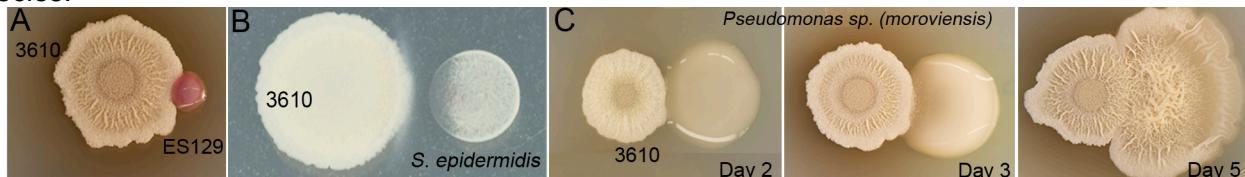


Figure 1: Outcomes of interaction of *B. subtilis* with different species on MSGG medium that supports robust biofilm formation. (A) An impasse, in which the two species form closely abutting colonies (shown after 72 hr at 37°C). (B) Lysis at a distance, during the interaction with *S. epidermidis* (36 hr at 37°C). The *B. subtilis* colony later expands to occupy the former site of the target colony. (C) Invasion of environmental strain ES97 colonies proceeds over several days. Colony invasions often result in death of the target species (Fig. 3). rRNA of ES129 is 98% identical to *Serratia marcescans*, ES97 is 96.4% identical to *Pseudomonas moroviensis*.

B. subtilis strains produce a wide array of extracellular metabolites such as fatty acids, polyketides, ribosomally and non-ribosomally produced peptides that are likely involved in interspecies interactions, since many inhibit growth of various bacteria and fungi [6-10]. One of the best-studied secondary metabolites is the lipopeptide surfactin, which is produced by most undomesticated *B. subtilis* strains and plays several roles, including inhibiting growth of bacterial species such as *Pseudomonas syringae* and stimulating *B. subtilis* to produce the robust biofilm characteristic of undomesticated *B. subtilis* strains [7, 11, 12]. Surfactin also mediates the dramatic interaction between *B. subtilis* and *S. coelicolor*, inhibiting *S. coelicolor* development and the consequent production of antimicrobial products that would otherwise inhibit *B. subtilis* growth [13, 14]. In this case, surfactin effectively disarms a potentially dangerous neighbor, allowing continued growth in a multi-species environment. Our preliminary data indicate that *B. subtilis* also produces unidentified extracellular metabolites whose roles remain unclear and that some extracellular metabolites are essential for determining if *B. subtilis* can coexist with neighboring species or if its colonies are invaded and destroyed.

One factor limiting studies of the role of extracellular metabolites in microbial interactions is the lack of methods to monitor their production during culture on solid medium, which is usually required for maximal production. This limitation has recently been overcome by the application of MALDI-TOF imaging mass spectrometry to growing bacterial colonies (Fig. 2; recently reviewed by [15,16]). This powerful new method provides spatial and temporal data on secondary metabolite production and allowed characterization of the interaction between *S. coelicolor* and *B. subtilis* [13]. We recently used this method to demonstrate the first directional secretion of antimicrobial agents towards a competing colony, in the directional secretion of plipastatin and surfactin from *B. subtilis* towards MRSA strains of *S. aureus* (Fig. 2A). We have also used MALDI-imaging to study *B. subtilis* cannibalism [17,18], a process in which biofilm forming cells kill non-biofilm forming and Spo0A⁻ siblings to release nutrients that promote biofilm growth (Fig. 2B). We used classical analytical chemistry to purify and structurally characterize both cannibalism toxins (SKF and SDP) and growth assays and fluorescence microscopy to demonstrate that purified SDP lyses *B. subtilis*, *S. aureus* and *S. epidermidis* cells (Fig. 2B-D, Appendix 1). These results indicate that SDP is capable of mediating both cannibalism and predation.

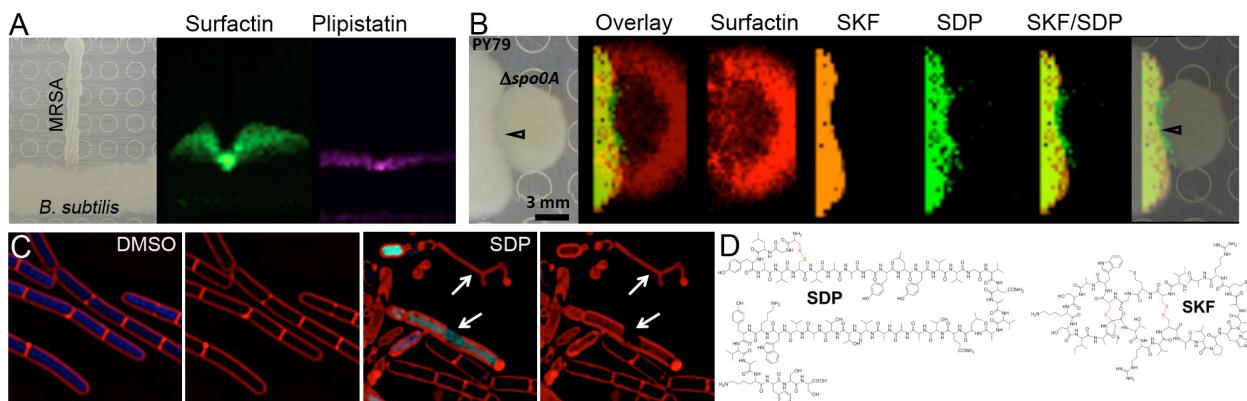


Figure 2: Extracellular metabolite mediated interactions visualized by MALDI imaging (described in Fig. 5) and fluorescence microscopy. (A) Interaction between MRSA *S. aureus* and *B. subtilis* 3610, showing a photograph of the coculture grown on a MALDI plate (left) and MALDI imaging showing surfactin (green) and plipastatin (purple) are secreted from the MRSA-proximal side of the *B. subtilis* colony. (B) The cannibalistic interaction between wild type *B. subtilis* strain PY79 and a *spo0A* mutant, which is lysed by PY79 (arrowhead). MALDI imaging demonstrates that the *spo0A* strain produces low levels of surfactin (red), and that the cannibalistic factor SDP diffuses into the zone of clearing, while SKF does not. (C) Fluorescence microscopy of SDP-treated *B. subtilis* cells stained with the red membrane stain FM 4-64, the membrane impermeable nucleotide stains Sytox green and DAPI (blue). SDP induces membrane tubules and holes (arrows) and increases Sytox permeability. (D) The structure of SDP and SKF. See Appendix 1 for details regarding identification of surfactin, plipastatin, SDP and SKF.

We here propose to extend these findings to test the hypothesis that many extracellular metabolites synthesized by *B. subtilis* determine the outcome of interactions with other species: coexistence or predation of one or the other species. We propose to further test the hypothesis that *B. subtilis* produces specialized cell types that are critical for interaction with other species and that might be induced by specific extracellular metabolites secreted by the target species.

Our studies will take advantage of the complimentary expertise of the two PIs, Pieter Dorrestein, a chemist who specializes in natural products and has developed methods to use imaging mass spectrometry to visualize metabolic exchange between bacterial species, and Kit Pogliano, a microbial cell biologist who specializes in *B. subtilis* development and has developed methods to investigate the effects of small molecules and antibiotics on bacterial cell structure. We propose to use MALDI imaging, genetics and fluorescent microscopy to elucidate the chemical, genetic and physical mechanisms by which *B. subtilis* uses small molecules to govern development and interactions with different species.

(b) Innovation

This proposal involves several innovative new technologies. The first is MALDI-TOF imaging mass spectrometry (IMS), a powerful new method that provides a spatial and temporal view of small molecule production during interspecies interactions. This method rapidly provides a detailed view of the metabolic responses to other organisms, allowing high throughput analysis of interactive metabolism. We will combine this analysis with high-resolution fluorescence microscopy to visualize interactions at the population and single cell levels and to assess the effects of purified secondary metabolites on cell structure and behavior. Integrating these approaches allowed us to identify the cannibalism toxin and demonstrate that it kills cells by inducing an autolytic mechanism that is clearly distinct from other treatments often described as autolysis (Appendix 1 and Fig. 8).

B. subtilis produces many extracellular metabolites whose role and biosynthetic pathways remain unclear. We will elucidate the role these molecules play in interactions with other species, with a focus on two behaviors, predation, in which *B. subtilis* moves towards, invades and overwhelms neighboring colonies (Fig. 1C), and an impasse (Fig 1A), in which *B. subtilis* can form adjacent colonies with other potentially dangerous bacteria, but only if it is capable of producing extracellular metabolites. We have developed quantitative measures to distinguish between these two behaviors.

(c) Approach

The overall goal of the experiments described here is to identify the developmental and chemical processes that mediate the interaction between *B. subtilis* and other species. We seek to identify small molecules that mediate inter- and intra-species interactions, elucidate the mechanisms by which these molecules act on cells and colonies and determine which *B. subtilis* developmental states are required for interspecies interactions. We will first describe preliminary data that documents three distinct outcomes of the interaction of *B. subtilis* with other species, then our specific approach for elucidating the chemical and genetic determinants of these interactions.

Preliminary data

We first set out to characterize the outcomes of the interaction of two undomesticated *B. subtilis* strains, 3610 and ES73 (a new environmental isolate), with other bacterial species including human pathogens (such as *P. aeruginosa* and *S. aureus*) and newly isolated environmental strains. We tested if co-culture affected either growth or colony architecture on plates that support robust biofilm formation by *B. subtilis* or motility on plates that support swarming motility. These experiments are ongoing, but thus far, each of the outcomes we here describe was observed with both undomesticated *B. subtilis* strains, suggesting that they are conserved responses to other species.

Impasse: keeping potentially deleterious bacteria at bay

The first behavior we propose to call an **impasse**. In this case, *B. subtilis* can form colonies immediately adjacent to some species, such as *Pseudomonas aeruginosa* (PA01 and PA103; Fig. 3A). This behavior depends on the ability of *B. subtilis* 3610 to produce extracellular metabolites, since strains with a mutation in *sfp*, which encodes the phosphopantethenyltransferase (PPTase) that activates enzymes that synthesize surfactin, plipastatin, bacillibactin and bacillaene [19,20], allows PA01 to lyse 3610 colonies (Fig. 3A). This demonstrates that *P. aeruginosa* is a potential predator of *B. subtilis* and suggests that *B. subtilis* uses an *sfp* dependent pathway to produce a small molecule that prevents its colonies from being invaded by *P. aeruginosa*. We propose experiments to identify the *B. subtilis* and *P. aeruginosa* molecules and genes involved in producing an impasse.

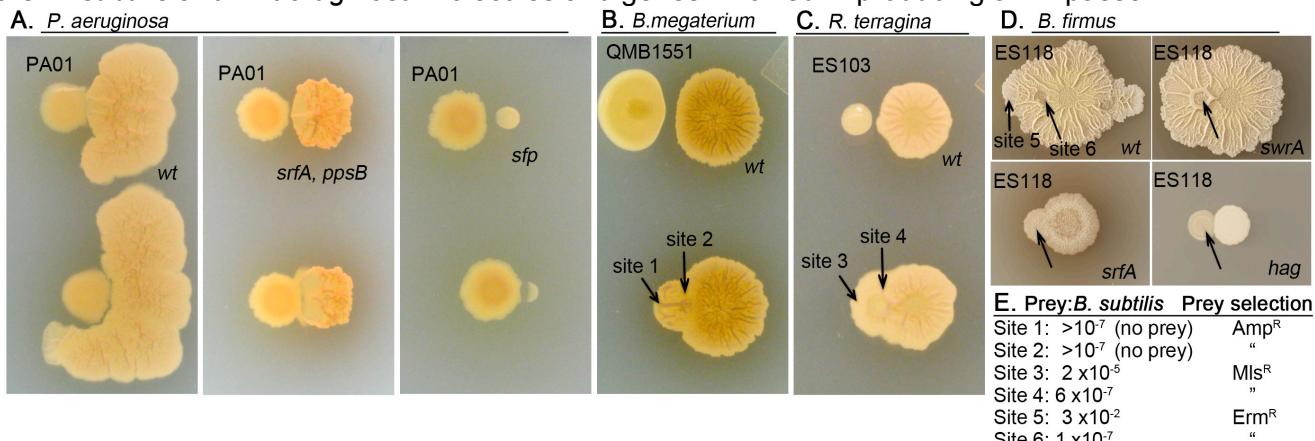


Figure 3: The co-colony assay for interspecies interactions. Co-culture of *B. subtilis* strain 3610 with other species on MSGG medium that supports robust biofilm production, after inoculation of liquid cultures of the test species (left) and *B. subtilis* (right) either 12 mm (top) or 5 mm apart (bottom). **(A) An impasse** is produced with *P. aeruginosa* strains PA01 (shown) and PA103. *Sfp* mutants are lysed by PA01, but a *srfA,ppsB* double mutant strain that cannot produce two *Sfp*-dependent metabolites (Surfactin and Plipastatin) are partially PA01 resistant. This suggests that another *Sfp*-dependent metabolite participates in this interaction. **(B-D) Contact-dependent predation** of three species: (B) *B. megaterium* (C) *Raoultella terrigina* and (D) *B. firmus*. After contact, *B. subtilis* engulfs and grows over the colony. Movement over colonies of ES118 (as well as QMB1551 and ES103) is independent of surfactin and swarming motility since the *srfA* and *swrA* mutants still move across the prey colony, but appears to depend on flagellar motility, since the *hag* mutant prevents colony invasion. **(E) Survival of the target species is assessed by determining the colony forming units (CFU) of each species plating under the indicated conditions to select for the prey species; a chloramphenicol resistant 3610 derivative is used to select for *B. subtilis*.**

Deleterious effects on other species: Colony lysis and predation

We noted two distinct types of interactions that produced deleterious effects on the target species. First, many species showed lysis, either in colonies near a *B. subtilis* colony (Fig. 1) or in spot test assays (Appendix 1), a classical outcome of antimicrobial compounds that diffuse away from the colony and inhibit growth of other bacterial species. We also noted a second type of predatory behavior in which an apparently intact target colony was invaded and subsequently taken over by *B. subtilis*. During subsequent days of incubation, the *B. subtilis* colony moved across the prey colony and ultimately there were 10^2 to $>10^5$ fold more *B. subtilis* cells than prey cells at the site of prey inoculation (Fig. 3C). This surprisingly frequent behavior allowed *B. subtilis* to colonize a much larger area of the plate than in the absence of prey. Motility assays demonstrated that *B. subtilis* showed directed, flagellum-dependent movement towards many of these prey species including *B. megaterium* strains ES108 and QMB1551 and other environmental isolates in the genera *Bacillus*, *Lysinibacillus*, *Pseudomonas*, and *R. terragina* (Fig. 4 and not shown); engulfed colonies also showed reductions in CFU in a variety of different growth media (Fig. 3 and not shown). Together the above results demonstrate that *B. subtilis* displays cooperative predatory behavior that involves the movement towards and ultimate destruction of a prey species to promote continued growth and expansion of the *B. subtilis* colony. This behavior, which we here call contact dependent predation, is strikingly similar to the “wolf pack” hunting behavior of *Myxococcus xanthus* [21-23].

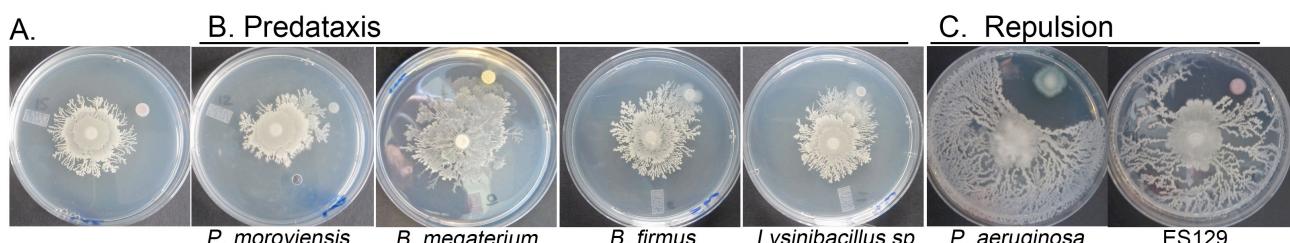


Figure 4: Affect of other species on the flagellum-dependent surface motility of *B. subtilis* strain 3610. Various species were inoculated into MSGG plates containing 0.7% agar, which prevents swimming motility (through the medium) and confines bacteria to the surface. Under these conditions, *B. subtilis* motility depends on surfactin, flagella, and SwrA, demonstrating that it is classical swarming motility not sliding motility. Thus, although swarming motility is not required for colony invasion (Fig. 3) *B. subtilis* is capable of swarming towards prey colonies. (A) No interaction versus (B) directed motility towards various prey species after 18 hours of incubation at 37°C. (C) Repulsion, in which 3610 avoids *P. aeruginosa* strains PA01 (shown) and the environmental strain ES129 even after 36 hours of incubation. Note that the co-colony assay in Fig. 3 shows that colonies of the latter two species abut *B. subtilis* colonies, suggesting that at high cell density and/or in a biofilm the species can occupy adjacent niches, although at low cell density they avoid one another. This might be related to the increased resistance to antimicrobial compounds provided by cells at high cell density and in biofilms [24].

We hypothesize that predation depends on extracellular metabolites produced by both *B. subtilis* and the prey species and on the ability of *B. subtilis* to differentiate into specialized cell types such as swarmer cells that move on solid surfaces (and perhaps across colonies) and miner cells that secrete high levels of hydrolytic enzymes. Extracellular metabolites produced by the prey species are likely required to elicit movement towards the prey species, whereas metabolites produced by *B. subtilis* are likely involved in killing prey cells during colony invasion. It is tempting to speculate that contact-dependent predation differs from lysis at a distance in its dependence on extracellular metabolites that show limited diffusion and must therefore be physically delivered to prey cells. Such molecules would have been missed in traditional screens for antibacterial compounds but are promising leads for new classes of antibacterial compounds.

Overview of the experimental plan

We propose to test the hypothesis that extracellular metabolites, even if they do not exert antibiotic activity, determine if *B. subtilis* acts as predator or prey in interspecies interactions. This hypothesis predicts that there should be a specific small molecule response to prey species and that mutations that disrupt the production of required extracellular metabolites or developmental pathways affect predatory behavior. In Aim 1, we will characterize the extracellular metabolome of *B. subtilis* strain 3610, alone and in co-culture with organisms that produce either an impasse or a predatory response.

In Aim 2, we will explore the small molecules and genetic pathways required for the various steps in contact dependent predation (predataxis, colony invasion and killing of prey species). In Aim 3, we will explore the impasse generated by the interaction of 3610 and *P. aeruginosa*, identifying extracellular metabolites and genes in both species that are required to produce an impasse rather than destruction of one colony by the other.

Aim 1: Characterize the extracellular metabolome of *B. subtilis* 3610

Our preliminary data indicates that the relatively well-studied *B. subtilis* strain 3610 produces several unknown secondary metabolites under various conditions (Fig. 5, Appendix 1 and not shown). We estimate that there are about five additional reproducible small molecules/peptides of unknown origin that have not been structurally characterized or the biosynthetic pathway identified, bringing the number of detected secondary metabolites involved in interactions with the environment to eighteen. Most of these molecules act on neighboring organisms through distinct mechanisms. We here propose to systematically identify the unidentified small molecules produced by 3610, those that are induced by *P. aeruginosa* PA01, which reaches an impasse with *B. subtilis*, and by species whose colonies are invaded and destroyed by 3610, such as *B. megaterium* QMB1558 and *R. terragina* ES103 (Gram positive and Gram negative respectively). We will elucidate the chemical structure of these metabolites and identify and inactivate the genes encoding the biosynthetic enzymes. The mutants will be tested for effects on production of other metabolites, and interaction with other species.

Aim 1a. Define the extracellular metabolome of *B. subtilis*

We will use two different mass spectrometry approaches to discover secondary metabolites and peptides that belong to the extracellular metabolome, together with analytical chemistry to identify the structure of these molecules and genetics to identify biosynthetic genes. We will initially focus on extracellular metabolites whose production is altered in the presence of prey species of *B. subtilis* strain 3610, which is readily genetically manipulated, but these approaches ultimately will be applied to species that induce changes in *B. subtilis* metabolites or behavior (Aims 2-3).

The first mass spectrometry approach uses whole cell MALDI mass spectrometry (WC-MS) of cells grown in different media to observe metabolite production [25-27]. We have modified this method to obtain mass spectrometry data from colonies at different stages of growth, providing temporal data on secondary metabolite production. We also display data from these time courses using a heat map representation of the peak height rather than the mass-to-charge ratio (m/z) normally used to visualize mass spectrometry data. This enables us to observe trends in molecules that are changing (Fig. 5). This is advantageous because MALDI mass spectrometry is inherently noisy in the low m/z region due to the presence of the matrix and because we can visualize changes in the levels of individual metabolites during colony growth and biofilm formation. This approach has revealed several additional unidentified metabolites in *B. subtilis* strains 3610 and PY79 (m/z of 715, 824, 920, 2106 and 3840; Fig. 5). It further reveals that the two related strains are in different small molecule states: PY79 is unable to produce high levels of plipastatin or surfactin, due to a frameshift mutation in *sfp*. (Fig 5, Appendix 1). The low levels of surfactin and plipastatin produced is likely due to the promiscuous activity of the only other phosphopantetheinyl transferase in *B. subtilis* involved in fatty acid biosynthesis. This is in contrast to 3610, which produces both molecules. PY79 also over-expresses SKF and SDP relative to 3610, for reasons that are not clear. WC-MS allows us to analyze 10,000 samples per week, with few restrictions as to the types of media used. It is therefore well suited for high throughput screens, since the main rate-limiting step is culturing the bacteria.

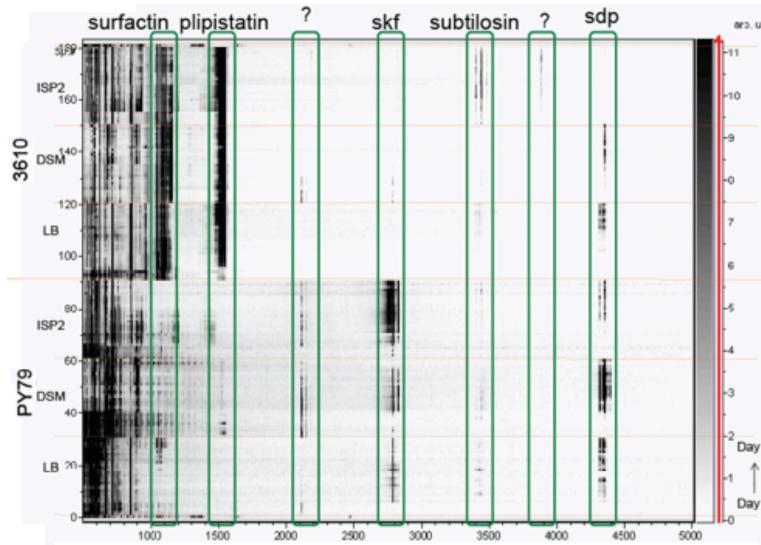


Fig. 5: Whole cell mass spectrometry (WC-MS). *B. subtilis* strain 3610 and its domesticated descendant PY79 over 6 days of growth on three different media (LB, DSM and ISP2). Several unidentified metabolites are produced and differences are noted between the two strains. PY79 is unable to produce high levels of plipastatin or surfactin, due to a frameshift mutation in *sfp*; it also overproduces SKF and SDP relative to 3610.

The second approach is imaging mass spectrometry (IMS), which allows MALDI imaging of colonies that are grown directly on the MALDI target plate or on agar that is transferred to the plate after growth [13] (Fig. 5). This powerful method allows the spatial distribution of various metabolites within and around bacterial colonies to be directly assessed, and we can readily observe the spatial distribution of various metabolites. For example, the lipopeptides surfactin and plipastatin spread away from *B. subtilis* 3610 colonies, while other ions, such as 715 m/z remain closely associated with the colonies. IMS provides the unique opportunity to directly visualize changes in the interactive metabolome upon interaction with other species. This ability has allowed us to document the directional release of plipastatin and surfactin towards MRSA (Fig. 2A), the *B. subtilis*-mediated inhibition of antibiotic production by *S. coelicolor* [13], and the specific induction of various secondary metabolites when *B. subtilis* strain SIO1 is cultured adjacent to *Promicrobium* strain SIO11 (Fig. 7).

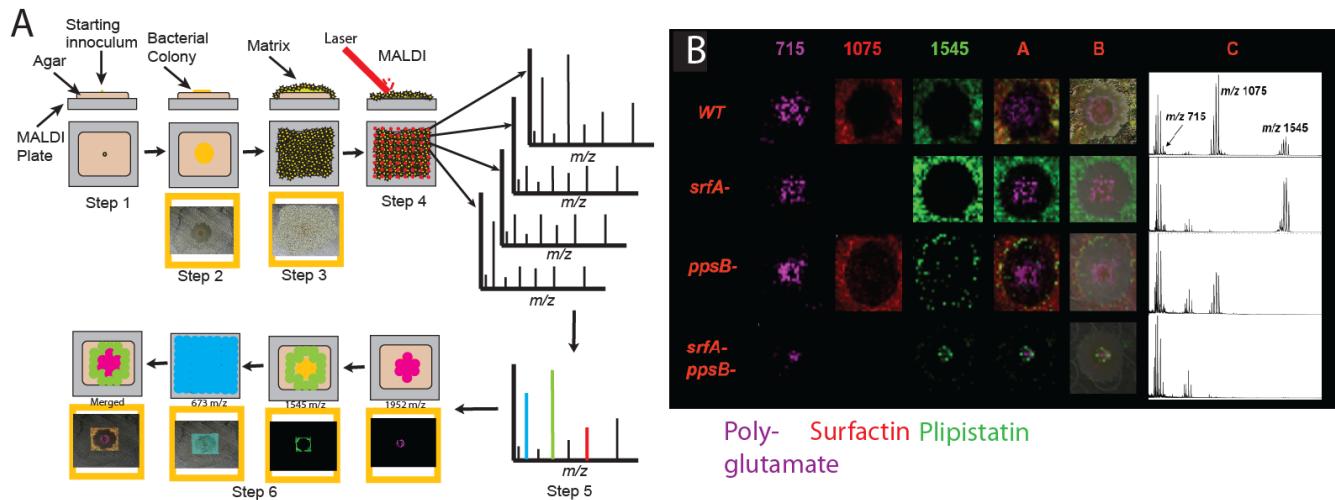


Figure 6: MALDI imaging mass spectrometry. (A) A thin layer of culture medium is overlayed on the MALDI plate, inoculated with a bacterial culture and allowed to form a colony. The matrix is overlayed and MALDI-TOF data collected at various positions on the plate. Each ion is color-coded and a composite image produced. (B) IMS of *B. subtilis* 3610, the *srfAA*, *ppsB* single and double mutants, which demonstrates that ions m/z 1075 as surfactin and m/z 1545 as plipastatin. Subpanel a shows the merged signal of m/z 715, 1075 and 1545; b the merged signal with an optical image of the colony, c the average signal from m/z 600–1,600 of all spectra obtained in the imaging runs.

These two mass spectrometry methods provide complimentary views of the quantity and spatial distribution of secondary metabolites; the following methods will be used to determine the structure of molecules of interest. First, the mass will be compared to that of known molecules produced by the

strain in question and the identity confirmed by its fragmentation pattern after tandem mass spectrometry and by testing if mutations in the biosynthetic genes (if they have been identified) abolish the metabolite with few changes to other metabolites. We used this approach to establish that 3440 m/z is subtilosin [13]. Second, if the observed mass does not correspond to a known molecular entity, the molecule will be subject to fragmentation, since many molecules have diagnostic mass shifts in their fragmentation behavior. For example, peptides have mass shifts that correspond to amino acids, sugars display 164 or 178 Da losses, while phosphorylated molecules display a characteristic 80 Da loss. We used this approach to identify ion 715 m/z as a polyglutamate of unknown structure (Fig. 6B). Third, we are developing new algorithms that allow us to extract structural and sequence information from mass spectrometry data of complex molecules such as cyclic non-ribosomal peptides without purifying the molecule [28, 29]. We routinely obtain and analyze hundreds of tandem mass spectrometry data on a daily basis.

Molecules that cannot be assigned by these methods or by the candidate gene approach described below (Section 1d), such as the unknown ions 2106 and 3840 m/z (Fig. 5), will be purified via mass spectrometry guided isolation for full structural assignment. We routinely utilize state-of-the-art NMR facilities in the UCSD Pharmacy School, including a new 600 MHz NMR equipped with a Bruker TCI ^1H - ^{13}C cryoprobe, which is currently the most mass-sensitive probe available at any field strength. We have successfully employed 2D NMR approaches to elucidate the structure of a variety of molecules with as little as 2-20 μg of material, including SKF and SDP (Appendix 1).

Prioritization: Although we are developing new mass spectrometry based algorithms that facilitate dereplication and structural characterization of complex secondary metabolites, the full structure elucidation is still a bottleneck. We will therefore focus our structure elucidation efforts on molecules that are hypothesized to be involved in interspecies interactions based on IMS data in the next section.

Impact: These experiments to characterize the extracellular metabolome of *B. subtilis* in pure culture are a critical prelude to Aim 1b, in which we will identify metabolites induced by other species.

Aim 1b: Identify the interactive metabolome

We seek to identify extracellular metabolites that change when *B. subtilis* interacts with other species, which we here call the interactive metabolome. These molecules likely play key roles in mounting an appropriate response to neighboring species. We will culture *B. subtilis* adjacent to organisms that elicit different behavioral responses, initially focusing on the interactions that produce an impasse (*S. marcescens* ES129 and *P. aeruginosa*) and two that result in contact dependent predation, *B. megaterium* QMB1558 and *R. terragina* ES103 (Fig. 3C, 4B). This will be accomplished either on a Petri dish or directly on the MALDI plate (Fig. 7A; [13], appendix 1) and imaging mass spectrometry will be performed. This method allows us to rapidly identify metabolites that control *B. subtilis* behavior, and we have used the approach to identify small molecules that are induced by co-culture of *B. subtilis* strain SIO1 and *Promicromonospora* sp. strain SIO11, which has a remarkable ability to repel *B. subtilis* swarms and thereby defend its territory (Fig. 7B). Mass spectrometry guided isolation followed by tandem mass spectrometry and NMR, showed that m/z 2869 is a peptide produced by SIO11 that when added to *B. subtilis* SIO1 (Fig. 7C-D) inhibits motility by a mechanism that does not involve death of the *B. subtilis* cells.

We will monitor the interaction of *B. subtilis* with environmental organisms and with human pathogens such as *S. aureus* (Fig. 1A) and *P. aeruginosa*. We will first focus on interactions with species that elicit clearly distinct behaviors from *B. subtilis* (impasse versus contact dependent predation, predataxis versus repulsion), to determine if these outcomes elicit characteristic small molecule responses and to determine the timing of any such response. We will then expand our studies to the libraries of environmental strains available in the Dorrestein and Pogliano labs and a library of pathogens available in the laboratory of Dr. Victor Nizet. We will simultaneously characterize outcomes of the interactions of these species with strain 3610, using the assays shown in Figures 3 and 4 to determine if they elicit predatory behaviors or reach an impasse with 3610. We routinely use imaging mass spectrometry to analyze 50 such interactions a week, so we can analyze the interaction

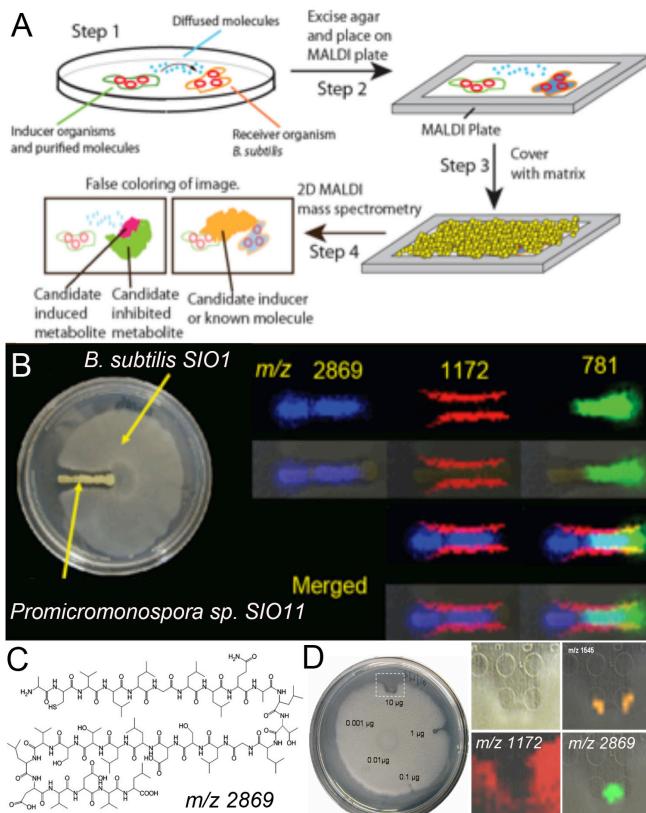


Figure 7. Connecting chemistry with phenotypes using imaging mass spectrometry of the interactive metabolome. (A) A thin film of agar is deposited on top of the MALDI target plate, an inoculum of bacteria is allowed to grow and is then covered by matrix and dried. The sample is subjected to MALDI-IMS and all of the spectra are averaged. Distinctions observed by MALDI-IMS are displayed with different colors and superimposed on a picture of the sample. (B) An example of an environmental *B. subtilis* SIO1 interacting with SIO11 and that a hydroxamate siderophore with a mass of 781 is upregulated but only when the two organisms are in contact. (C) This interaction also shows that the peptide inhibits *B. subtilis* motility (D).

between 3610 and a starting set of 300 species in less than two months. Molecules that are produced by either *B. subtilis* or the target species when the two species are interacting will be purified, tested for biological activity (as described below) and the structure elucidated as described above.

Impact: These experiments will provide a global view of the interactive metabolome of *B. subtilis*, and allow us to identify molecules that are induced when this species interacts with *P. aeruginosa*, and other prey species.

Aim 1c. Test purified interactive metabolites for biological effects

Purified molecules produced by *B. subtilis* or the target species will be tested for their effects on cell viability using the microculture methods developed for our studies of SDP and SKF (Appendix 1). Briefly, various concentrations of compounds are added to 50 μ l cultures and tested for effects on colony forming units/ml at different treatment times and concentrations. Fluorescent stains are then used to monitor the effects of compounds at 2-5X the minimal inhibitory concentration on cell morphology and permeability using epifluorescence microscopy (Fig. 8A) and timelapse epifluorescence microscopy (Fig. 8B). These methods can readily discriminate between different mechanisms by which compounds kill bacterial cells. For example nisin causes rapid increases in Sytox and DAPI permeability leading to increased fluorescence in most cells, while vancomycin causes internal membrane spheres with lysis and increased permeability observed in a subset of cells, and SDP causes the production of membrane tubules and spheres (Fig. 2C, 8B). These viability assays will also be applied to target species produced compounds that deter movement of a *B. subtilis* swarm towards target cells, to discriminate between those that directly inhibit motility or chemotaxis and those that inhibit growth or cause cell lysis. Compounds that effect motility but not viability will be tested to see if they affect flagellar rotation or chemotaxis using in tethering and capillary assays, respectively [30,31].

We anticipate identifying target species produced compounds that do not directly affect *B. subtilis* viability or motility and rather inhibit differentiation into cell types required for interspecies interactions. For example, compounds that repel swarms might reduce flagellar or surfactin gene expression, which could impact motility under the slow moving swarm conditions. This possibility will be revisited

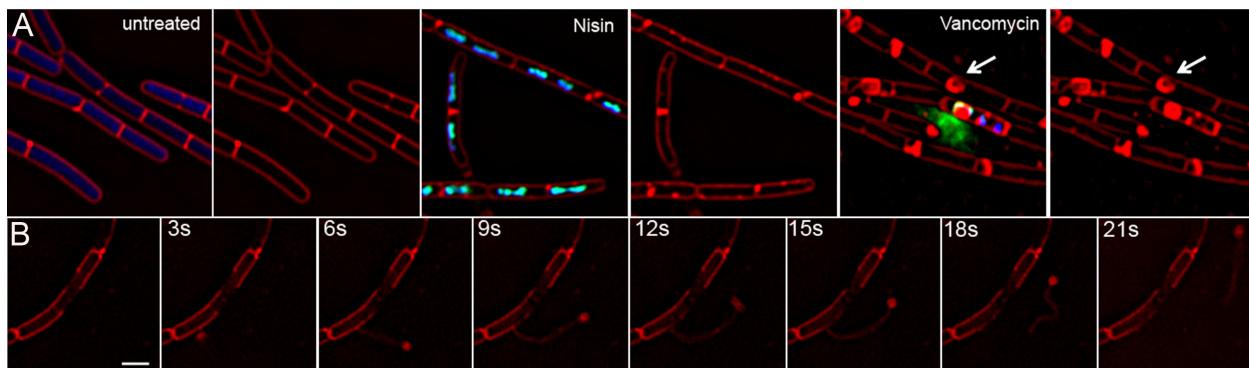


Figure 8. Fluorescence micrographs showing the effects of nisin, vancomycin and SDP on *B. subtilis* cells. **(A)** Permeability assay. The red shows FM 4-64, a fluorescent membrane stain; the blue and green show DAPI and sytox green, two membrane impermeable DNA stains. Sytox provides the greatest increase in fluorescence in permeabilized cells. All nisin-treated cells are rapidly permeabilized, while vancomycin treated cells rapidly show membrane deformations (arrow) but only a subset are permeabilized. **(B)** Timelapse microscopy showing formation and release of a membrane tubule in SDP treated cells. See appendix 1 for additional details.

in Aim 3b, when we will use timelapse fluorescence microscopy of interacting species to follow induction of GFP fusions to genes required for these interactions.

Impact: These experiments will allow us to identify metabolites produced by *B. subtilis* and the prey species that affect viability and compounds that might alternatively impact gene expression that will be retested in aim 3.

Aim 1d. Identify genes required for extracellular metabolite production

We will use complementary approaches to identify genes required for biosynthesis of unknown secreted small molecules. First, in the case of ribosomally-encoded peptides such as SKF and SDP, the structure data alone will facilitate identification of the biosynthetic gene. Second, we will continue our candidate gene approach to determine if mutations in identified genes for secreted small molecules eliminate molecules observed by mass spectrometry. These will include genes encoding CSF and its 8 functionally related pentapeptides (CSF, ComX, Phr peptides), as well as bacillibactin, bacillaene, bacilysin, bacilysin, subtilosin and various PKS molecules. This approach has allowed us to unambiguously identify ions corresponding to SDP, SKF, plipastatin, subtilosin and surfactin. Many of the required mutations exist and have been characterized by our laboratories (Appendix 1 and NCB 2009 paper); others will be made using long-flanking homology PCR and moved into 3610 by transduction. Third, we anticipate that several biosynthetic genes will remain unidentified by the directed approach above, such as the poly-glutamate ion that is produced by many *Bacilli* whose genomes lack homologues of enzymes involved in the identified poly-glutamate biosynthesis pathway. In these cases, we will use transposon mutagenesis to isolate mutations that abolish secondary metabolite production. Briefly, a transposon library will be constructed in strain 3610 using a temperature sensitive plasmid to deliver a Mariner based transposon into this strain [32]. The resulting library will be screened by IMS (or WC-MS if IMS requires too much time or optimization) to identify mutants that eliminate the molecules of interest. The insertion sites will be sequenced after amplification by random-primed PCR (a method we routinely employ; [33,34]). The insertion mutations will be tested for their ability to invade and kill prey colonies and to deter *P. aeruginosa*. Those that affect interspecies interactions will be further studied after constructing in frame deletions and performing complementation studies to identify genes in the region responsible for the phenotype. The insertion library will also be used in Aim 2b.

Impact: These experiments will identify genes required to synthesize extracellular metabolites and produce mutations in these genes for use in Aim 2a.

Aim 2: Identification of *B. subtilis* genes involved in interspecies interactions.

We will here use a genetic approach to identify *B. subtilis* produced extracellular metabolites and developmental processes that contribute to interspecies interactions. We will use two independent

approaches towards identifying these genes, a candidate gene approach (Aim 2a) and a forward genetic screen (Aim 2b). These studies will be greatly facilitated by our ongoing collaboration with Roberto Kolter (letter of support), whose studies on the impact of interspecies interactions and extracellular metabolites on *B. subtilis* biofilm formation will complement our proposed experiments.

Aim 2a. A candidate gene approach to identify *B. subtilis* genes and extracellular metabolites involved in interspecies interactions

We will assess the impact of mutations on the interaction with *P. aeruginosa* PA01 and with two strains that are invaded and destroyed by 3610, *B. megaterium* QMB1558 and *R. terrigena* ES103 (Gram positive and Gram negative respectively). Interspecies interactions will be assessed using the co-colony assay (on MSGG plates) and the motility assay (on swimming and swarm plates of different media). The motility assay has the advantage that it can be performed on a variety of different media therefore reducing the possibility that the behaviors we observe are dependent on particular features of growth on MSGG medium. Experiments will be quantified to determine the CFU of each species at their respective inoculation sites and at the site of initial colony contact at various times after inoculation. Mutants that affect the interaction with any one of these three species will subsequently be tested against our library of environmental and pathogenic strains. The goal of expanding our test species is to determine if either coexistence or colony invasion depends on a conserved set of products or behaviors, which would be consistent with the outcome being a developmental response to neighboring species.

We will test mutants affecting the following processes for the impact on interspecies interactions:

i. Extracellular metabolites. Mutations in genes required to synthesize extracellular metabolites identified in Aim 1 and previously identified metabolites will be tested for effects on interspecies interactions. Our top priorities are to test mutations in genes required to synthesize surfactin (*srfAA*;[35]), plipastatin (*ppsB*;[36]), bacilysin (*bacB*;[37]), bacillaene (*pksX*;[38]), subtilosin A (*albA*;[39]), bacillibactin (*dhbF*; [40]), bacilysocin (*ytpA*; [41]) and the cannibalism toxins SDP and SKF [17]. We are excited by the possibility that SDP might be involved in colony invasion, since it kills *Staphylococcus* species, suggesting it might also target other Gram-positive bacteria. We will use mass spectrometry (Aim 1) to test if the mutations have pleiotrophic effects on the *B. subtilis* extracellular metabolome, as might be predicted for mutations in genes whose products either are required to synthesize several metabolites or regulate *B. subtilis* development. One gene for which such pleiotrophic effects are expected is *sfp*, which encodes the Sfp phosphopantetheinyl transferase that is essential for the biosynthesis of surfactin, plipastatin, bacillibactin and bacillaene. Thus any phenotype associated with the *sfp* mutation will be tested with single and multiply mutant strains affecting each of these pathways. A second gene with pleiotrophic effects is *srfAA*, which is required to produce surfactin, a molecule that is not only required for swarming motility but also for differentiation of *B. subtilis* into biofilm and cannibalism toxin producing cells. We will attempt to rescue the *sfp* phenotype by adding purified surfactin, which is commercially available, or purified plipastatin or bacillibactin (at this time it is not possible to purify bacillaene). We will screen each molecule to see if they can rescue the severe colony morphology phenotype of *sfp*, or *srfAA* mutants on MSGG medium [18]. These mutations will also be tested to see if they affect the extracellular metabolome of the species with which they interact.

ii. Motility. We hypothesize that once colonies touch, *B. subtilis* might either swim or swarm through the target colony to mediate colony invasion. To test this hypothesis, we will test effects of mutations in genes required for both forms of motility (such as the *hag* gene that encodes flagellin) and genes required only for swarming motility (such as the *srfA* gene that produces surfactin and the *swrA* gene required to produce swarming cells; [42]). Our preliminary data indicates that *hag* is essential for colony invasion, while *srfA* and *swrA* are dispensable for colony invasion of three different target species (*B. firmus*, *P. moroviensis* and *R. terrigena*). This suggests that swimming but not swarming motility is required for colony invasion, a conclusion we will further test by inactivating other genes in required for flagellar assembly or rotation. We will also test the role of these mutations in the

interaction with *P. aeruginosa*, since it is possible that motility is required to define the boundary between the species in the co-colony assay.

iii. Chemotaxis. *B. subtilis* moves towards prey species on swarm plates that restrict *B. subtilis* to movement over the surface (swarming motility). Movement towards prey might reflect a chemotactic response towards prey or their release of surfactin-like molecules that could allow more rapid movement towards prey. We can distinguish between these two mechanisms by testing if mutations that abolish chemotaxis but not motility (i.e.: *cheA*, *cheY*) are capable of moving towards prey species in swarm plates. Continued movement towards prey would suggest a surfactin-like mechanism; abolished movement would suggest a chemotactic response. In the latter case, we will identify the chemoreceptor required for this behavior by testing *mcpA*, *mcpB* and *mcpC* mutants [43]. We will also explore the basis for the repelling interaction between *B. subtilis* and *P. aeruginosa* in motility plates, which suggests that although the two species can occupy adjacent niches in the high cell density and biofilm conditions of a colony, they cannot at the low cell density characteristic of the leading edge of a moving swarm. There are three possible explanations for these repelling interactions in motility plates, killing of cells at the leading edge of the swarm as it nears the other species [24], direct inhibition of motility and chemotaxis away from a repellent. We will test the latter possibility by testing if *cheA*, *cheY* and *mcp* mutations affect the ability of *B. subtilis* to avoid *P. aeruginosa*, and we will subsequently test the effects of purified *P. aeruginosa* compounds on growth, flagellar rotation and swimming.

iv. Development and stress responses. *B. subtilis* has various specialized cell types and stress response pathways that might be involved in interspecies interactions, a possibility we will test by determining if mutations in genes encoding transcription factors impact interspecies interactions. The top priority mutations are underlined and include those that affect development of minor cells that secrete hydrolytic enzymes (*degS*, *degU*) and the switch between motility and biofilm production (*slrR*, *sinR*, *sinI*;[44]). We will also test mutations affecting sigma factors that mediate responses to antibiotics, cell envelope and general stresses (*sigB*, *sigH*, *sigM*, *sigW*, *sigV*, *sigX*, *sigY*, *sigZ*, *ylaC*, *sigI*;[45-48]) and regulatory proteins that govern antibiotic production and stationary phase fates (*scoC*, *abrB*, *abh*, *comA*, *comP*, *comK*; [49-51]).

Impact: These experiments will allow us to rapidly test candidate genes involved in producing extracellular metabolites, controlling developmental and stress response pathways to get a system-wide view of molecules involved in interspecies interactions. Aim 3 will use fluorescence microscopy to visualize induction of required pathways during interactions between *B. subtilis* and other species.

Aim 2b: An unbiased screen for genes involved in interspecies interactions

We will complement the candidate gene approach described above with two forward screens for mutants that abolish the ability of *B. subtilis* to invade or coexist with different species. First, we will screen the library of Mariner insertions constructed in Aim 1d to identify mutants that reduce the ability of 3610 to invade colonies of *B. megaterium* QMB1558 and *R. terragina* ES103 or its ability grow adjacent to *P. aeruginosa* strain PA01. We have experience with similar labor-intensive genetic screens [33, 34] and can screen >300 mutants for interaction defects/week. Second, we will use the library of signature-tagged deletion mutants that is under construction in laboratory of Dr. David Rudner, a recipient of a GO grant to construct research tools. Such libraries allow high throughput screens to identify mutations that fail to survive under particular conditions and are widely used to identify genes required for virulence or growth in population [52-55]. If the library is constructed and made available as anticipated, we will screen pools of mutants for those that fail to invade a prey colony, identifying insertions that are absent from the prey-proximal side of the interaction site. We will also screen for mutants that fail to survive in co-culture with *P. aeruginosa*, identifying mutants that fail to survive on plates containing *P. aeruginosa*. The benefit of signature tagged mutagenesis is that it allows the direct and rapid comparison of genes required for interactions with different species, to determine if these interactions require a core set of genes.

Impact: These experiments will provide a more complete and unbiased view of the genes required for interspecies interactions and they have the potential to allow us to rapidly determine if similar or different genes are required for interactions with different bacterial species.

Aim 3: Use fluorescence microscopy to visualize interspecies interactions

We will also use timelapse fluorescence microscopy to visualize interspecies interactions at high and low magnification. Our goal is to visualize movement of *B. subtilis* towards other species, invasion of the colony, prey cell lysis, and induction of genes required for interspecies interactions. GFP and mCherry fusion proteins will be used to unambiguously identify and sensitively detect *B. subtilis* cells during these interactions and to follow the expression of particular genes and differentiation into different cell fates during interactions with other species. We will focus on the interaction of 3610 with two species whose colonies it invades, *R. terragina* and *B. megaterium*.

Aim 3a. Use of fluorescence microscopy to follow individual cells

We will fluorescently label *B. subtilis*, *R. terragina* and *B. megaterium* cells with two fluorescent proteins: GFP, which is very bright, allowing sensitive detection of a few bacterial cells and it rapidly folds into the mature fluorescent state, providing a rapid readout of gene expression, and mCherry which folds more slowly, but can be used with Sytox Green to assess cell lysis. These proteins will be expressed from promoters active at various stages of growth; we will initially test *rrnO*[56] and *repO*[57]. For *B. subtilis*, the genetic constructs will be integrated into the chromosome, while for the prey cells, mCherry will be inserted into broad host range plasmids that can be moved into for Gram negative or Gram positive species by conjugation (pRK2 and pLS20[58-61]). The fluorescently tagged bacteria will be used for the following experiments. First, we will track individual motile *B. subtilis* cells during colony invasion using mixtures of GFP labeled and unlabeled *B. subtilis* cells. This will allow us to determine if *B. subtilis* shows evidence of *M. xanthus*-like predataxis[21]. Second, we will mCherry label the prey cells and perform microscopy on medium containing Sytox green, which shows greatly increased fluorescence in lysed cells, allowing visualization of cell lysis. These experiments will be performed at low magnification using a dissecting microscope to provide an overview of the interaction and at higher magnification using our deconvolution fluorescence microscope. We routinely use timelapse microscopy over both long and very short timescales [62, 63] and will adopt recent methods that for high-resolution studies of swarming motility [64, 65] if warranted by data indicating that small molecules or species directly affect *B. subtilis* swarming.

Impact: These studies will allow us to sensitively determine when *B. subtilis* cells reach the prey colony, to directly visualize prey cell lysis and allow high-resolution studies of interspecies interactions, motility and lysis.

Aim 3b. Visualize induction of genes required for interaction with other species

The experiments in Aims 1 and 2 will identify genes involved in interspecies interactions. We will here determine when and where these genes are expressed, in order to determine if they are involved in the initial stages of the interaction (movement towards prey species) or in later stages of invasion or host cell destruction. We will also determine if any of the secondary metabolites produced by prey species affect the expression of these genes, since those that do not affect *B. subtilis* viability or motility might instead prevent expression of genes required for colony invasion. We will construct GFP fusions to the promoters of genes involved in interspecies interactions and use the dissecting fluorescence microscope to determine if these genes are expressed immediately after inoculation of the adjacent colonies or during later stages of colony invasion; with deconvolution microscopy used to provide a higher resolution view. We anticipate that some small molecules and developmental pathways will be specifically required for initial steps in the interaction, such as those involved in colony expansion or biofilm production, while others might be required for later steps such as degradation of the prey cell biofilm, resistance to antimicrobial products, or prey cells destruction.

Impact: These studies will allow us to determine where (and when) the genes required for interspecies interactions are expressed, providing insight into the precise roles they play, and they will allow us to visualize the cellular response of *B. subtilis* to other species.

MULTIPLE PI LEADERSHIP PLAN

The proposed work will be undertaken jointly by the laboratories of Dr. Pieter Dorrestein and Dr. Kit Pogliano at the University of California San Diego.

For this project to succeed, chemists, mass spectrometrists, microbiologists have to work together. This grant will support students and post-docs who will move freely between the Dorrestein and Pogliano laboratories. Each PI will be responsible for directing and overseeing the students or post-docs progress on this project. Although there is a strong interaction between the laboratories, each laboratory is responsible for the development of specific areas.

The Dorrestein laboratory is responsible for all the mass spectrometry work and chemistry described in this proposal while the Pogliano lab is responsible for the microbiology and microscopy. The next section highlights some of the strong interactions that the two groups have. Because the Dorrestein is not a microbiology laboratory, it is of utmost importance that they coordinate the mass spectrometry experiments with the biology students. For example, Anne Lamsa often walks over to the Dorrestein laboratory to assist Jane Yang in the culturing of the microbes for mass spectrometry. At the same time Wei-Ting Liu assists Anne Lamsa with the handling of the isolated natural products that she has isolated for proper handling in the microscopy experiments. Recently, David Gonzalez has been spending 20-25% of his time on the microscope together with the Pogliano lab members to analyze the effects of the microbe-microbe interactions.

To summarize, Dorrestein, Pogliano and their associates interact on a regular basis since they all are at UCSD. They have open access to each other's laboratories and there is a free exchange of research materials and information. This is why this collaboration is so successful. We also hold joint project meetings, text, skype, e-mail to effectively communicate on a daily basis. Furthermore, collaborating students and postdocs attend group meetings in each lab. Thus the PIs have already developed a very effective communication plan that will form the basis for this new project.

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Dear Pieter,

I will be happy to provide a letter of support for your and Kit's R01 on "The chemical and genetic basis of interspecies interactions". The scanning mass spectrometry based approaches that you have developed in your laboratory are great for the characterization of the molecular signals that control microbial interactions. The tools developed in your lab has enabled the molecular characterization of such interactions in just hours as opposed to years thus enabling the investigation of many such interactions in a short time. My post-docs Beth and Matt, during their three week visit to your lab, have already generated a great set of data and has provided unprecedented insight into the signals that microbes use. I look forward to a continued and productive collaboration.

Sincerely,

Roberto Kolter

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Dear Kit,

As you know, I am leading an effort to construct an ordered library of signature-tagged ("bar-coded") in-frame deletion mutations in *Bacillus subtilis*. This library of mutations will allow high throughput deep-sequencing based screens to identify mutations required for survival under a variety of conditions, including during the interspecies interactions that is the topic of your present proposal.

The mutations are initially being constructed in laboratory strain 0168 and we will ultimately move them into the undomesticated strain 3610 either at the end of the funded GO grant project, or by a collaborative effort involving laboratories, such as yours, that routinely study strain 3610.

The mutant collection will be made available to the research community as soon as they are available, which I currently estimate to be in August of 2011.

Sincerely,

A handwritten signature in black ink, appearing to read "David Rudner".

David Rudner

Resource sharing plan

We will adhere to the NIH Grant Policy on Sharing of Unique Research Resources including the [Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources](#) issued December 23, 1999. All 'model organisms' generated by this project will be distributed widely or deposited into a repository/stock center making them available to the broader research community, either before or immediately after publication, in accordance with University policies. If we assume responsibility for distributing the newly generated model organisms, we will fill requests in a timely fashion. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. Material transfers will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with University policies and the NIH Principles and Guidelines document.

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

New Resubmission Renewal Continuation Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

* First Name:

Middle Name:

* Last Name:

Suffix:

Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes No

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes No

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

Yes No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

| *Budget Period | *Anticipated Amount (\$) | *Source(s) |
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5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

Yes No