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Collaborative Research: Functional and Genomic Analysis of Polysymbiosis in the Wood-boring Bivalve Lyrodus pedicellatus

Daniel L. Distel
Principal Investigator; University of Maine, Orono

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Principal Investigator: Distel, Daniel L.
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Title:
Collaborative Research: Functional and genomic analysis of polysymbiosis in the wood-boring bivalve Lyrodus pedicellatus

Project Participants

Senior Personnel

Name: Distel, Daniel
Worked for more than 160 Hours: Yes
Contribution to Project:

Post-doc

Name: Ekborg, Nathan
Worked for more than 160 Hours: Yes
Contribution to Project:

Graduate Student

Name: Luyten, Yvette
Worked for more than 160 Hours: Yes
Contribution to Project:

Undergraduate Student

Technician, Programmer

Name: Spenlinhauer, Tania
Worked for more than 160 Hours: No
Contribution to Project:

Other Participant

Research Experience for Undergraduates

Organizational Partners

National Institutes of Health (NIH/OD/OER)

In this project we have also sought assistance from the National Resource for Imaging Mass Spectrometry (NRIMS) via a collaboration with its director Claude Lechene. As a result of this project, our laboratory was selected as a named collaborator in the NIH grant supporting this facility. This project receives no direct financial support from the NRIMS facility but does receive use of instrument time, software, and collaborative efforts of Dr. Lechene and his staff.

New England Biolabs

New England Biolabs has provided extensive in kind support (equipment use, computers

and chemical reagents), facilities support (use of laboratory space), collaborative research (extensive advice and assistance in protein biochemistry and molecular biology) and personnel exchanges (OGLF students and staff routinely work in NEB labs along side of NEB researchers in experiments related to this project).

Other Collaborators or Contacts

Dr. Joe Montoya is the named collaborator in this collaborative research proposal and has contributed to the stable isotope studies as described in the original proposal.

In addition Dr. Raymond W. Lee, of Washington State University has provided important assistance with GC-MS determinations of stable N and C isotope ratios and has contributed to experimental design in stable isotope experiments.

Activities and Findings

Research and Education Activities: (See PDF version submitted by PI at the end of the report)

Some Relevant History:
Funds for the current project were initially awarded to the University of Maine where I served as a faculty member until Spring 2004. Shortly thereafter, I resigned from the University of Maine and accepted a position as Director of Ocean Genome Legacy Foundation (OGL). At this time I requested transfer of this award to the Ocean Genome Legacy Foundation. The Ocean Genome Legacy Foundation is a new NSF awardee organization and as a result there has been some delay in completing the award transfer. As of this date, no funds have yet been received by Ocean Genome Legacy. Nonetheless, in anticipation of the transfer, the project was initiated at Ocean Genome Legacy according to the original schedule and supported by existing OGL funds.

Project Activities and Findings

In this project, we proposed to explore the physiological ecology of symbiotic xylotrophy in shipworms using molecular, biochemical and microbiological techniques. Three major questions were proposed;

1) What genetic variation occurs in symbiont populations within and between host species?

2) What is the quantitative contribution of symbionts to lignocellulose digestion and nitrogen fixation? and

3) What physical and biochemical mechanisms are involved in transfer of nutrients and enzymes between host and symbiont compartments?

This work extends prior research that has established that multiple symbiont species coexist within the bacteriocytes of shipworms, and complements ongoing research to elucidate evolutionary relationships among the diverse genera of wood boring bivalves.

Findings:

We have made considerable progress in each of the three areas described above.

1) What genetic variation occurs in symbiont populations within and between host species?

We have used a new method (CDCE-QPCR) of quantitative monitoring of abundance and distribution of specific symbiont rRNA alleles (identified in gene libraries) to explore variation in symbiont community structure and composition among members of a single laboratory reared shipworm population. Two small subunit rRNA libraries were generated from a single and four pooled specimens respectively of the shipworm L. pedicellatus. Screening by unidirectional partial sequencing revealed eighteen ribotypes in the two libraries, each of which was subsequently characterized by complete bidirectional sequencing. Phylogenetic analyses showed that the 18 ribotypes could be classified into five phylotypes that could be distinguished by short discrete signature sequences. Specific GC-clamped CDCE primers were designed to differentiate among the phylotypes and CDCE-QPCR was performed to enumerate and quantify the symbiont phylotype composition in the gills of 13 specimens of L. pedicellatus reared in laboratory culture. The results demonstrate large variation in symbiont community structure and composition among members of a single laboratory reared shipworm population. However, certain consistent patterns were observed. Only one identified phylotype was observed in all specimens and this ribotype accounted for only ~ 10% of the total symbiont population of each individual on average. This ribotype is identical to that of the cultivated symbiont of shipworms, T. turnerae. Two additional phylotypes were found to be numerically dominant but were nearly mutually exclusive in occurrence in individuals, i.e. when one was detected in a given host specimen, it was observed to account for the majority of the symbiont population in that individual while the second was either undetectable or present at very low levels. Each of the two dominant phylotypes occurred with approximately equal frequency among individual hosts. All host specimens had at least two and as many as five detectable phylotypes. This work is now in print in Applied and Environmental Microbiology (January 2006) v. 72(1) 412-17.

2) What is the quantitative contribution of symbionts to lignocellulose digestion and nitrogen fixation?

With our collaborators at the National Resource for Imaging Mass Spectrometry (NRIMS) we are developing new methods of multiple imaging mass spectrometry (MIMS) and transmission electron microscopy (TEM) that allow a single thin section of epoxy embedded tissue to be visualized by both methods successively. This gives us the unique ability to quantitatively measure isotope composition of individual cells and subcellular components and to measure up to four masses simultaneously. This gives unprecedented ability to measure isotopes quantitatively at sub-micron resolution and to map the distribution of isotope ratios to structures visualized by transmission electron microscopy. Using this method we can observe changes in nitrogen isotope composition of individual bacterial symbiont cells in situ in host tissues. Using 15N2 tracer gas we are able to show that symbionts fix nitrogen within bacteriocytes in the gill tissue of the host and that this fixed nitrogen is subsequently used by host cells in locations remote from the symbionts for growth and biosynthesis. This is the first direct demonstration and observation of nitrogen fixation at the cellular or subcellular scale and the first direct demonstration of intracellular bacterial diazotrophy in an animal tissue. This work will be submitted for publication in 2006.

We also continue work to clone and characterize cellulases produced by the cultivated symbiotic bacterium, Teredinibacter turnerae. Efforts in this project have focused on a unique bifunctional cellulase dubbed celAB. To date we have successfully expressed celAB in both bacterial and yeast expression systems, however, work continues to achieve sufficient expression levels and to develop appropriate purification protocols to allow thorough functional characterization of this gene and to generate polyclonal antibodies for planned immunohistochemical investigations.

We hope to show that celAB has the unique characteristic of containing both functional endo- and exoglucanase catalytic domains in a single polypeptide. Such an enzyme is a good candidate for a functional role in symbiotic cellulose digestion since it obviates the

need for expression, regulation and transport of two independent polypeptides normally needed to accomplish complete hydrolysis of cellulose to its monomer cellobiose.

3) What physical and biochemical mechanisms are involved in transfer of nutrients and enzymes between host and symbiont compartments?

The MIMS method described above will be instrumental in exploring the physical and biochemical mechanisms of nutrient transfer between hosts and symbionts. The appropriate experiments are now being devised and implemented.

Training and Development:

This grant has provided funding for the training of one graduate student and one high school student and has contributed to the professional development of one postdoctoral researcher. Graduate student research supported by this award was selected for oral presentation at the American Society for MicrobiologyÆs (ASM) Conference on Beneficial Microbes (April, 2005, Lake Tahoe, NV) and for poster presentation at the ASM 105th General Meeting (June 2005, Atlanta, GA). This award also supported the participation of one graduate student in the ASM 2005 Graduate and Postdoctoral Summer Institute in Preparation for Careers in Microbiology, a 5-day intensive workshop including discussion and practice in grant writing, scientific presentations, teaching and mentoring. Additionally, research sponsored by this award has supported the training one graduate student and one postdoctoral researcher in advanced technologies via collaborations at Massachusetts Institute of Technology and the National Resource of Imaging Mass Spectrometry (NRIMS). This award also supported one high school student intern whose duties include maintenance of shipworm cultures in the laboratory. This involved training in routine preparation of various solutions (artificial sea water, supplemental nutrients and feeds) and performing chemical analyses of seawater (specific density, nitrate, nitrite, calcium, pH, phosphate, etc) as well as record keeping and interpretation of results.

Outreach Activities:

The Ocean Genome Legacy Foundation participates in several outreach efforts to the local community. We are currently in the process of developing mentoring programs for K-12 teachers in the Ipswich School District. On January 23, 2006, OGLF, in conjunction with New England Biolabs, hosted 15 representatives from the Ipswich primary, middle and high schools, including teachers and administrators, for a three-hour development workshop to formulate a working plan for educational collaboration with the Ipswich school system. OGLF also maintains one part-time intern position for students from the Ipswich High School (position currently held by Sam Wilbur, sophomore).

Journal Publications

Yvette A. Luyten, Janelle R. Thompson, Wendy Morrill, Martin F. Polz, and Daniel L. Distel, "Extensive Variation in Intracellular Symbiont Community Composition among Members of
a Single Population of the Wood-Boring Bivalve Lyrodus pedicellatus (Bivalvia:Teredinidae)", Applied and Environmental Microbiology, p. 412, vol. 72, (2006). Published

Books or Other One-time Publications

Web/Internet Site

URL(s):

www.oglf.org

Description:

Research supported by this award is briefly described under the "Research" link at www.oglf.org. The site is undergoing development and will soon contain more detailed findings and descriptions of current projects.

Other Specific Products

Product Type:

Oral Presentation and Abstract

Product Description:

Poster Presentation and Published Meeting Abstract:
Y.A. Luyten, Q. Nie, K. Dirrig, W. Morrill, and D.L. Distel. Culture-Independent Identification of Nitrogenase Reductase (nifH) Genes in the Symbiont Community of the Wood-Boring Bivalve Lyrodus pedicellatus (Bivalvia:Teredinidae). American Society for Microbiology's Conference on Beneficial Microbes, Lake Tahoe, NV; April 17 -21, 2006

Sharing Information:

This poster was presented at a national scientific meeting. The abstract is published and is available in print form and through various electronic databases.

Product Type:

Poster Presentation and Abstract

Product Description:

Poster Presentation and Published Meeting Abstract:
Y.A. Luyten, R.W. Lee, Q. Nie, J. Montoya, and D.L. Distel. Diversity of diazotrophic intracellular symbionts and contribution of symbiotic nitrogen fixation to dietary requirements of the shipworm Lyrodus pedicellatus (Bivalvia:Teredinidae). 105th General Meeting of the American Society for Microbiology, Atlanta, GA; June 5-9, 2005

Sharing Information:

This poster was presented at a major international scientific meeting. The abstract is published and is available in print form and through various electronic databases.

Product Type:

Oral Presentation, Poster and Abstract

Product Description:

Oral and Poster Presentation and Published Meeting Abstract: D.L. Distel, Y.A. Luyten, and C.P. Lechene. Multi-Isotope Imaging Mass Spectrometry (MIMS) of a Nitrogen-Fixing Bacterium. 15th International Conference on Secondary Ion Mass Spectrometry, Manchester, UK; September 12-16, 2005.

Sharing Information:

This talk and poster was presented at a major international scientific meeting. The abstract is published and is available in print form and through various electronic databases.This abstract is also available electronically at http:// www.nrims.hms.harvard.edu/about_nrims.php

Contributions

Contributions within Discipline:

(1) We have worked in collaboration with colleagues at Massachusetts Institute of Technology to develop new applications for constant denaturant capillary electrophoresis (CDCE) and end-point quantitative polymerase chain reaction (QPCR) in microbial ecology and environmental microbiology. Originally used for detection of single nucleotide polymorphisms (SNPs), CDCE-QPCR allows the simultaneous separation and detection DNA

molecules differing by as few as one base pair.

Using CDCE-QPCR we have demonstrated extensive variation in the intracellular symbiont community composition of a single shipworm population (Lyrodus pedicellatus). These findings have added to the growing body of evidence that rather than being monocultures, microbial endosymbiont populations may be both complex and dynamic. These results indicate that many symbiotic associations must be re-evaluated with respect to co-existing genetic variability and the potential correlation of such variability with symbiotic function or developmental state, condition, or environmental fitness of the host. Such insights may have broad implications for ecologically important symbioses, e.g. rhizobial/root nodule symbioses in leguminous plants.

(2) With our collaborators at the National Resource for Imaging Mass Spectrometry (NRIMS) we are developing new methods that use multiple imaging mass spectrometry (MIMS) and transmission electron microscopy (TEM) to measure and visualize 15N/14N (and other isotope) ratios in single cells and subcellular structures. These methods promise to provide powerful new, never before possible, means of dissecting the physical, physiological and biochemical relationships between host animals and their microbial symbiotic associates. This research also demonstrates a type of symbiosis (intracellular diazotrophic endosymbiosis) never observed previously in an animal host.

Contributions to Other Disciplines:

The CDCE-QPCR techniques described above provide a way to simultaneously detect and measure multiple DNA biomarkers that differ by as little as a single nucleotide in a highly sensitive, quantitative and reproducible manner. The method will permit scientists to examine fine-scale genetic variation in microbial communities in many important contexts and will be valuable in many fields including infectious disease, soil microbiology, plant science, biogeochemistry, population biology and evolution. Similarly, MIMS-TEM provides a means to explore elemental composition and dynamics of individual cells and sub-cellular structures and has broad applicability in medicine, cell biology, biogeochemistry and natural sciences.

Contributions to Human Resource Development:

This grant has provided support for the training of a graduate student, a high school student, a post-doctoral student, and partial support for a laboratory technician.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

This project explores the function of a symbiosis of fundamental scientific, ecological, and economic importance. Investigation of the shipworm symbioses will provide insight into other important marine symbioses and specific human and animal pathogenic infections to which they bear a strong resemblance. In marine environments, shipworms are the principal mineralizers of cellulose (the most abundant organic polymer on earth) and so are key to understanding the ecological fate of this important resource. Shipworms cause an estimated $1 billion in damage to wooden vessels and structures annually, so knowledge of shipworm biology is essential to design of ecologically benign control measures. Additionally, cellulases discovered here may have significant value; the commercial market for cellulase exceeds $100 million annually. Finally, the project will contribute to the training and education of graduate, undergraduate, high school, and minority students.

Special Requirements

Special reporting requirements: None

Change in Objectives or Scope: None

Unobligated funds: $ 0.00

Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

Any Book

Contributions: To Any Resources for Research and Education