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Microbial Observatories: Kilauea Volcano Observatory for Carbon Monoxide-Oxidizing Bacteria

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Annual Report for Period: 10/2006 - 09/2007 **Submitted on:** 07/10/2007
Principal Investigator: King, Gary M. **Award ID:** 0348100
Organization: University of Maine
Title:
Microbial Observatories: Kilauea Volcano Observatory for Carbon Monoxide-Oxidizing Bacteria

Project Participants

Senior Personnel

Name: King, Gary
Worked for more than 160 Hours: Yes
Contribution to Project:

Post-doc

Graduate Student

Name: Weber, Carolyn
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms. Weber is a Ph.D. student conducting dissertation research using the Observatory site and resources

Undergraduate Student

Technician, Programmer

Name: Yeung, Chieu
Worked for more than 160 Hours: Yes
Contribution to Project:
Mr. Yeung provides technical support for the research

Other Participant

Research Experience for Undergraduates

Organizational Partners

Other Collaborators or Contacts

I have collaborated with Dr. K. Nanba, Fukushima University, Japan, and Dr. Hiroyuki Ohta, Ibaraki University, Japan on CO oxidation on a volcanic system that parallels the system with which we are working on Kilauea.

Activities and Findings

Research and Education Activities:

Our major research activities have consisted of fieldwork on the Kilauea volcano, which has involved in situ assays of gas exchange, ex situ assays of the same plus a number of microbiological, physical and chemical analyses. We have also enriched and isolated several novel CO oxidizers that we are now characterizing, initiated a transplant
experiment (see below) and participated in an international exchange involving research on the volcanic island, Miyake-jima. This work has

contributed to our efforts on Kilauea.

We have developed a powerpoint presentation on volcanic systems that has been used in G7-8 science presentations. In addition, we have developed a field course in microbial ecology, the focus of which has been assays of CO oxidation, including molecular characterizations and enrichment and isolation. This course has been presented to a
group of 11 undergraduate students from Iowa and an undergraduate student from Japan.

Findings:

1. Isolation and characterization of CO-oxidizing bacteria. We have fully characterized and described a Hawaiian Stappia isolate, Stappia kahanamokuae, and obtained 3 novel CO-oxidizing mycobacteria plus three novel CO-oxidizing Burkholderia. We have also obtained a new, very slow-growing Bradyhrhizobium-like isolate that is important for several reasons, one of which is that it likely represents a very relevant physiotype and the second is that it may be a symbiont for one of the important pioneering trees.
We are continuing to develop and implement approaches to substantially expand our isolation and characterization efforts, and to broaden the phylogenetic distribution of cultured CO oxidizers as well as to obtain representatives of the numerous as yet uncultured forms that occur at our field sites. We have ongoing long-term enrichments
with low nutrient concentrations and using ground lava as a support for growth. Our goal is to obtain representatives of Acidobacter, Verrucomicrobia and Planctomyces. To this end, we have a new set of long-term enrichments underway using low carbon source concentrations and mineral substrates

2. CO oxidation by plant leaf epiphytes. We have demonstrated that the leaves of most major plant species in our research sites oxidize CO; in some cases, activity can be detected with atmospheric CO levels. We have used several approaches to show that the
activity is associtated with epiphytic bacteria. For example, antibiotics inhibit activity as does surface sterilization with ethanol swipes. We are currently initiating efforts to enrich and isolate CO oxidizers from the phyllosphere. No such isolates are known, and the ability of leaves to oxidize CO has been only poorly characterized.

3. Transect analyses. We established within the 1959 PuÆu Puai tephra deposit a transect from unvegetated tephra into neighboring tree islands. The latter have accumulated a distinct and often thick litter layer and are developing a peat-like soil. In contrast, the unvegetated material is little changed from its initial deposition. We have conducted in situ analyses of CO uptake at the site, and characterized maximum uptake potentials, activity distribution as a function of tephra size, biomass, and additional
biological, chemical and physical parameters.

We have also initiated analyses of water potential at the transect and additional sites. These assays have revealed extreme water stress (water potentials < -50 MPa at unvegetated sites. The results indicate that in addition to carbon and energy supplies, the
availability of water may be a critical limiting factor even in areas that receive significant rainfall. This suggests that the evolution of water holding capacity through the formation of biofilms could be a key factor in microbial community development.

To further understand the evolution of microbial communities on volcanic deposits, we have established what we intend to be a long-term manipulative experiment. We have collected lava ôfrassö from a coastal site with recent flows from the ongoing PuÆu OÆo
eruption and transferred this material to our site at PuÆu Puai. The material has been deposited in two sets of triplicate 1 m diameter rings, about 7 cm deep. One set of rings is located in an unvegetated area and the other set is deployed beneath the canopy of Metrosideros polymorpha trees. Our goal is to follow the development of CO uptake
capacity and communities of CO-oxidizing colonists over time to determine how plant communities influence colonization, community structure and succession. We have sampled at roughly 3 month intervals from initiating this effort. Our results show detectable CO oxidation activity within 6 months for the canopy tranplants, but that more than 18 months is required for activity on the unvegetaged transplants. We also see dramatic differences in biomass, enzymatic activity and growth on Ecoplate substrates. In addition, we have initiated an analysis of diversity on 1-yr old samples. The preliminary results show that unvegetated lavas are colonized primarily by Acidobacteria with a modest number of OTU's. In contrast, the canopy material is colonized by a much greater diversity of phyla and species.

We have also initiated molecular analyses based on the coxL gene for the large sub-unit of CO dehydrogenase. Initial results suggest a very rich diversity of divergent (but we believe authentic) coxL sequences indicative of a broad phylogenetic range of CO- oxidizing
bacteria colonizing bare tephra deposits. We anticipate that CO oxidizer communities will be substantially different at neighboring tree island sites, with dominance by Proteobacteria.

Training and Development:

Ms. Weber, a Ph.D. student, has continued her work on the project and gained substantial experience with several aspects of field work and lab

work, including enrichment and isolation of CO oxidizers. Her dissertation work in in full stride at present and producing novel insights.

We also supported an REU student, Ms. Jennifer Katzenberger. Ms. Katzenberger was introduced to a wide range of molecular and physiological approaches for characterizing CO oxidizers, and completed a summer project using these tools. Ms. Katzenberger has just completed her senior year at the University of Colorado and is preparing for med school.

Outreach Activities:

We presented a unit on volcanic ecosystems and their biology to students in grades 5-8 at South Bristol Elementary School and conducted a 3-week intensive field course in microbial ecology for undergraduates.

The field course consisted of a set of lectures in general and ecological microbiology and was complemented by field and lab work designed to enumerate, enrich and isolate CO oxidizers, estimate activity under near in situ conditions, and conduct a molecular characterization of community structure. The results were very good on all counts and we anticipate a poster presentation at ASM 08.

Journal Publications

G.M. King and C.F. Weber, "Physiological, ecological and phylogenetic characterization of Stappia, a marine CO-oxidizing bacterial genus.", Appl. Environ. Microbiol., p. 1266, vol. 73, (2007). Published,

G.M. King and C.F. Weber, "Distribution, diversity and ecology of aerobic CO-oxidizing bacteria.", Nature Rev. Microbiol., p. 107, vol. 5, (2007). Published,

G.M. King and C.F. Weber, "Distribution and activity of CO-oxidizing bacteria along an ecosystem successional gradient", ISME Microbial Ecology, p. , vol. , ( ). Submitted,

G.M. King, "Roles and significance of lithotrophs in volcanic environments", Microb. Environ., p. , vol. , (2007). Accepted,

Books or Other One-time Publications

Web/Internet Site

URL(s):

http://server.dmc.maine.edu/KilaueaSite/index.htm

Description:

Other Specific Products

Contributions

Contributions within Discipline:

We have developed the first insights into associations of CO oxidizing communities with plants and now have the first molecular analyses of early microbial colonization on volcanic substrates. In addition, we have documented for the first time microbial CO oxidation associated with plant leaves.

Contributions to Other Disciplines:

Our work has substantially expanded the understanding of aerobic CO-oxidizing bacteria. As a result, we are now entering a variety of collaborations with other investigators who have different research programs that include pathogenesis and bioremediation.

Contributions to Human Resource Development:

We are training a new Ph.D. student and have provided training broadly in microbial ecology for a group of undergraduates with no field experience.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Special Requirements

Special reporting requirements: None

Change in Objectives or Scope: None

Unobligated funds: less than 20 percent of current funds

Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

Organizational Partners

Any Book

Any Product

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering