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11-4-2004

# LExEn: Role of Atmospheric Trace Gases in Microbial Colonization and Succession on Recent Lava Flows

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### Recommended Citation

King, Gary M., "LExEn: Role of Atmospheric Trace Gases in Microbial Colonization and Succession on Recent Lava Flows" (2004).  
*University of Maine Office of Research and Sponsored Programs: Grant Reports*. 74.  
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**Final Report for Period:** 10/2000 - 09/2004**Submitted on:** 11/04/2004**Principal Investigator:** King, Gary M.**Award ID:** 0085495**Organization:** University of Maine**Title:**

LExEn: Role of Atmospheric Trace Gases in Microbial Colonization and Succession on Recent Lava Flows

**Project Participants****Senior Personnel****Name:** King, Gary**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** Nuesslein, Klaus Rudolf**Worked for more than 160 Hours:** Yes**Contribution to Project:****Post-doc****Name:** Dunfield, Kari**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Dr. Dunfield worked as a postdoc employed by the award.

**Graduate Student****Name:** Gomez-Alvarez, Vicente**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Mr. Gomez-Alvarez is completing a Ph.D. supported in part by the award.

**Undergraduate Student****Technician, Programmer****Name:** Johnson, Kay**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** Yeung, William**Worked for more than 160 Hours:** Yes**Contribution to Project:****Other Participant****Research Experience for Undergraduates****Organizational Partners**

### Other Collaborators or Contacts

I have collaborated with Dr. Kenji Nanba from Tokyo University. Dr. Nanba participated in field studies and molecular analyses for a rubisco gene.

### Activities and Findings

#### **Research and Education Activities:**

The major research activities for the project have involved:

1. in situ and in vitro analyses of atmospheric trace gas uptake by recent volcanic deposits (about 25-300 yr old);
2. analyses of selected bulk chemical and microbiological parameters for recent volcanic deposits;
3. enrichment and isolation of trace-gas (CO) oxidizing bacteria from volcanic deposits;
4. application of molecular analyses (e.g., 16S rRNA gene, large sub-unit gene for CO dehydrogenase, large sub-unit gene for ribulose-bisphosphate-carboxylase gene) to determine general microbial community structure, population structure of specific microbial functional groups, and relationships between structure and characteristics of volcanic deposits.

The major educational activities for the project have involved:

1. support and training for a Ph.D. student;
2. support and training for a post-doctoral research associate;
3. support and training for a research assistant;
4. initiation of an outreach program for a K-8 schools in Hawaii and in Maine.

#### **Findings:**

The primary goal of this study has been to determine the role of atmospheric trace gases as carbon and energy sources for microbial colonization and succession on recent (0-300 yr old) volcanic deposits. These deposits initially lack fixed organic carbon and nitrogen, and represent extreme environments for bacteria. Regardless, ecosystem development occurs rapidly on the eastern and southern flanks of Kilauea Volcano, resulting in closed-canopy rainforests in < 200-300 yr. Work described below provides some of the first details regarding early microbial succession in these systems.

Analyses of trace gas uptake by deposits varying in age from 20-300 yr indicates that CO and H<sub>2</sub>, but not CH<sub>4</sub>, contribute significantly to respiratory energy flow (King, 2003a). Material from a 26-yr old lava flow, which contains < 0.1% organic carbon, consumes CO and H<sub>2</sub> at rates comparable to values observed for mature continental soils. These results suggest that in spite of low concentrations (0.1 and 0.5 ppm, respectively), CO and H<sub>2</sub> support microbial populations that are active early in ecosystem development.

Although atmospheric CH<sub>4</sub> does not contribute to initial microbial community

development, CH<sub>4</sub> uptake at elevated concentrations (100 ppm) reveals the presence of potentially active methanotrophs on material approximately 20 yr old. Comparisons of several vegetated sites show that methanotrophic activity develops in concert with plant communities. A 44-year old site with patchy stands of Ohia (*Metrosideros polymorpha*) and fire tree (*Myrica faya*) consumes atmospheric CH<sub>4</sub> intermittently, while 144 yr-old forested sites consume CH<sub>4</sub> consistently, with rates comparable to 300- and 50,000-yr old forested sites. Thus, the extent of plant colonization correlates with atmospheric CH<sub>4</sub> consumption, in contrast to CO and H<sub>2</sub> uptake, which are independent of plant development.

These observations help establish principles that govern biotic development in carbon- and energy-stressed systems. Specifically, results show that bacterial colonization does not depend strictly on plant-derived organics. Instead, the atmosphere provides suitable sources of inorganic reductants and carbon. Observations thus far also provide some of the first insights into the temporal development of microbial processes that play significant roles in the global budgets of critical tropospheric trace gases. While several published studies have documented CO and CH<sub>4</sub> uptake dynamics in mature systems, none have assessed these processes in systems evolving *de novo*.

In addition to trace gas exchange, phospholipid phosphate data, which reflect microbial biomass, have revealed another aspect of microbial community development. Results suggest that biomass accumulates approximately linearly over time, with a slower rate for drier sites north and southwest of Kilauea Caldera relative to wetter sites toward the southeast (King, 2003a). Cell number estimates show similar trends. Potential nitrogen fixation rates parallel patterns for biomass, as do rates of exoenzymatic activity (King, 2003a). In contrast, ammonia oxidation has been observed at a single site, a 300-yr old forest. This is consistent with results from efforts to amplify genes for ammonia monooxygenase (Gomez-Alvarez and N<sup>3</sup>sslein, 2002; Gomez-Alvarez et al., 2003).

Chlorophylla contents indicate that microalgae occur at most sites. However, chlorophylla levels are relatively low and inconsistent with significant algal organic matter inputs. Moreover, two sites that support significant CO and H<sub>2</sub> uptake do not contain detectable chlorophylla. Thus, trace gas exchange does not appear to depend directly on allochthonous plant production.

Trace gas exchange rates and physical and chemical characterizations have been complemented by development and application of novel molecular methods for analyzing genes for two key enzymes in CO- and H<sub>2</sub>-oxidizing bacteria: CO dehydrogenase (*cox*) and ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*) (King, 2003b; Dunfield and King, submitted; Nanba et al., April 2004). Primers for *cox* and *rbc* large subunits have been successfully applied to a variety of cultures and to DNA extracts from four Kilauea field sites (King and Crosby, 2002; King, 2003b; Dunfield and King, 2003; Dunfield and King, submitted; Nanba et al., 2003; Nanba et al., April 2004). The primers developed in this study have not only facilitated an important research objective, but provide a promising tool for investigating the role of obligate and facultative lithotrophs in the structure and dynamics of microbial communities in other systems as described in this proposal.

A number of potentially novel heterotrophic isolates and CO oxidizers have been obtained from Kilauea deposits (King et al., in prep; Gomez-Alvarez and N<sup>3</sup>sslein, 2002). These efforts complement a series of molecular studies by K. N<sup>3</sup>sslein's group that emphasize nitrogenase, ammonia monooxygenase

and methane monooxygenase in addition to 16S rRNA gene analyses. Results from the latter parallel those for *coxL* in that they reveal distinct assemblages of organisms among the various sites, along with different patterns of dominance (Gomez-Alvarez et al., 2003). These and results from ECOPLATE and PLFA analysis strongly indicate that bacterial populations on recent deposits exist in distinct dynamic communities, the composition of which reflects age, water regimes, and a variety of biotic factors.

Briefly, studies conducted to date are among the first and most detailed analyses of microbial processes and diversity in association with recent volcanic deposits. The results greatly expand insights into these carbon stressed systems. In addition, use of *coxL* and *rbcL* PCR targets are not only novel in their application to volcanic systems, they are novel in a broader sense because neither has been a target in previous studies on the diversity of bacterial lithotrophs, broadly defined. Finally, results from this study have implications for understanding extreme environments more generally due to the ubiquity of CO and H<sub>2</sub> as substrates.

#### **Training and Development:**

Project participants learned new skill in gas analysis; analysis of microbial activity in situ and in vitro; techniques for isolation and characterization of trace-gas-utilizing bacteria; application of molecular approaches for understanding microbial community development and succession on volcanic deposits.

#### **Outreach Activities:**

A program to provide a synthetic understanding of volcanic ecosystems for G5-8 students at a K-8 school in Hawaii and in Maine has been initiated during the term of the project. This effort is now being developed further and will result in classroom presentations and web-based resources.

### **Journal Publications**

King, G.M. and H. Crosby, "Impacts of plant roots on soil CO cycling and soil-atmosphere CO exchange", *Glob. Change Biol.*, p. 1085-1093, vol. 8, (2002). Published

G.M. King, "Contributions of atmospheric CO and hydrogen uptake to microbial dynamics on recent Hawaiian volcanic deposits", *Appl. Environ. Microbiol.*, p. 4067-4075, vol. 69, (2003). Published

G.M. King, "Molecular and culture-based analyses of aerobic carbon monoxide oxidizer diversity", *Appl. Environ. Microbiol.*, p. 7257-7265, vol. 69, (2003). Published

G.M. King, "Uptake of carbon monoxide and hydrogen at environmentally relevant concentrations by mycobacteria", *Appl. Environ. Microbiol.*, p. 7266-7272, vol. 69, (2003). Published

Nanba, K., G.M. King and K. Dunfield, "Analysis of the distribution and diversity of lithotrophic bacterial populations on recent Hawaiian volcanic deposits", *Appl. Environ. Microbiol.*, p. 2245-2253, vol. 70, (2004). Published

Dunfield, K. and G.M. King, "Molecular analysis of carbon monoxide-oxidizing bacteria associated with recent Hawaiian volcanic deposits", *Appl. Environ. Microbiol.*, p. 4242-4248, vol. 70, (2004). Published

Dunfield, K. and G.M. King, "Analysis of the distribution and diversity in recent Hawaiian volcanic deposits of a putative carbon monoxide dehydrogenase large sub-unit gene", *Environ. Microbiol.*, p. , vol. , ( ). Accepted

**Books or Other One-time Publications****Web/Internet Site****Other Specific Products****Contributions****Contributions within Discipline:**

Results from the research have established for the first time the potential role of atmospheric trace gases for microbial colonization and succession on carbon-poor substrates.

Results have for the first time demonstrated the structure of bacterial lithotrophic communities using a molecular approach specific for bacterial rubisco; similarly, results demonstrate for the first time the unexpected large diversity of CO-oxidizing microbes based on a molecular approach.

**Contributions to Other Disciplines:****Contributions to Human Resource Development:**

The project has supported a Ph.D. student and a post-doctoral research associate. The post-doc has now assumed a faculty position.

**Contributions to Resources for Research and Education:****Contributions Beyond Science and Engineering:****Categories for which nothing is reported:**

Organizational Partners

Any Book

Any Web/Internet Site

Any Product

Contributions: To Any Other Disciplines

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering