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# Carbon Monoxide Based Ecological Interactions Between Legumes and Their Rhizobial Symbionts

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**Annual Report for Period:**06/2006 - 05/2007**Submitted on:** 03/10/2007**Principal Investigator:** King, Gary M.**Award ID:** 0451597**Organization:** University of Maine**Title:**  
Carbon Monoxide Based Ecological Interactions Between Legumes and Their Rhizobial Symbionts**Project Participants****Senior Personnel****Name:** King, Gary**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** van Berkum, Peter**Worked for more than 160 Hours:** No**Contribution to Project:****Post-doc****Graduate Student****Undergraduate Student****Technician, Programmer****Other Participant****Research Experience for Undergraduates****Name:** Katzenberger, Jennifer**Worked for more than 160 Hours:** Yes**Contribution to Project:****Years of schooling completed:** Sophomore**Home Institution:** Other than Research Site**Home Institution if Other:** University of Colorado-Boulder**Home Institution Highest Degree Granted(in fields supported by NSF):** Doctoral Degree**Fiscal year(s) REU Participant supported:** 2006**REU Funding:** REU supplement**Organizational Partners****Other Collaborators or Contacts**

I have initiated a potential collaboration with a Japanese collaborator, Dr. Kiwamu Minamisawa, Tohoku University. I have assessed the CO oxidizing capability of one of his bradyrhizobial isolates, USDA 110, and we are currently considering options for using this organism in a survival analysis.

## Activities and Findings

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

Our research emphasizes the ecological impacts of carbon monoxide on interactions between rhizobia and their legume hosts. We are determining the potential for CO derived from plants and other sources to affect rhizobia survival, and thus impact the dynamics of their host plants, which impact plant succession.

### **Findings:**

We have made significant progress in our efforts to understand the ecological and ecosystem implications of carbon monoxide and hydrogen on relationships between legumes and their bacterial symbionts. We have largely accomplished one of our primary objectives, which was to determine the distribution of the capacity for CO oxidation among rhizobia. We have used activity and molecular methods to screen a diverse group of symbionts that are broadly representative of the rhizobia and their various hosts. Results indicate that about one-third of the isolates screened can be considered CO oxidizers. This capacity does not appear to be isolated to specific lineages of rhizobia or hosts, but is broadly distributed among taxa. We have also screened these same isolates for the presence of the large subunit gene (cbbL) of form 1 ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Only a small sub-set of the CO-oxidizing rhizobia possess cbbL, which strongly suggests that the capacity for CO (and hydrogen) oxidation is not for growth per se, but to promote survival and to facilitate interactions with hosts.

We have also begun to solve a conundrum involving the enzymes that oxidize CO. It appears that one type (form I) functions primarily as a CO dehydrogenase. This enzyme is likely responsible for most activity in situ that affects soil-atmosphere CO exchanges. A second enzyme (form II), may co-oxidize CO, but this enzyme appears kinetically much slower than the form I enzyme, and may have a different primary substrate. Interestingly, we have found that the form II enzyme occurs in all of the rhizobia we have screened thus far. Through a collaboration with a Japanese colleague, we will knock out a gene for the form II enzyme and use a phenotypic screen to attempt to assess its function.

In addition, we have developed the first molecular approach for assessing the distribution and diversity of aerobic uptake hydrogenases, the enzymes that are responsible for consuming atmospheric hydrogen and hydrogen from other sources (e.g., plant roots). Our approach is based on PCR amplification of a fragment of the large subunit of the hupL gene. Our near-term goal is to screen a library of rhizobia cultures and then to apply the method for the first time in an ecological context.

### **Training and Development:**

Ms. Jennifer Katzenberger worked as an REU student for this project and was introduced to a battery of microbiological methods and molecular ecological techniques. She participated in basic activity and gene surveys for CO oxidation in a collection of rhizobia, and developed an interest in microbial ecology that she intends to pursue at her home institution.

### **Outreach Activities:**

I have developed a field course in microbial ecology that will be offered to general biological science undergrads during April, 2007.

## Journal Publications

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

S. Cleave, K. Dunfield, G.M. King and J.C. Murrell, "Distribution and diversity of CO-oxidizing bacteria in bulk and rhizosphere agroecosystem soils", Appl. Environ. Microbiol., p. , vol. , ( ). Soon to be submitted,

### **Books or Other One-time Publications**

#### **Web/Internet Site**

#### **Other Specific Products**

#### **Product Type:**

**University of Maine Honors Thesis**

#### **Product Description:**

Ms. Podelnyk completed work culminating in an Honors thesis entitled, "ASSESSMENT OF MICROBIAL DIVERSITY IN LUPINUS PERENNUS NODULES BASED ON MOLECULAR COMPARISON OF THE COXL GENE". This was defended in April, 2006.

#### **Sharing Information:**

Kristy's thesis will be available generally for Honors students at UMaine and to others through UMaine's library.

#### **Contributions**

#### **Contributions within Discipline:**

Our work deals with fundamental aspects of the ecological interactions of legumes, the third most abundant family of flowering plants, and their bacterial symbionts. These interactions, which are based on production and consumption of carbon monoxide, have not been known or reported previously. Thus, our work adds a significant new dimension to what is known about a group of plants that provide a primary source of protein for about 1/3 of the world's population.

#### **Contributions to Other Disciplines:**

see previous

#### **Contributions to Human Resource Development:**

We have provided significant research experiences for several undergraduate students.

#### **Contributions to Resources for Research and Education:**

#### **Contributions Beyond Science and Engineering:**

See previous statement on contributions to discipline.

#### **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:**

Organizational Partners

Any Book

Any Web/Internet Site

Contributions: To Any Resources for Research and Education