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#3

Final Report for Period: 09/2001 - 08/2005
Submitted on: 08/09/2006
Principal Investigator: DiChristina, Thomas J.
Award ID: 0110915
Organization: GA Tech Res Corp - GIT
Title:
Outer Membrane-Targeted Secretion of Respiration-Linked Fe(III) Terminal Reductases

Project Participants

Senior Personnel

Name: DiChristina, Thomas
Worked for more than 160 Hours: Yes
Contribution to Project:

Post-doc

Graduate Student

Name: Moore, Charles
Worked for more than 160 Hours: Yes
Contribution to Project:
Fifth year Ph.D. student . This NSF Project was a major component of his Ph.D. thesis. Part of his Graduate research assistantship supported by this project. Will graduate in May 2003 with a Ph.D. in Biology.

Name: Haller, Carolyn
Worked for more than 160 Hours: Yes
Contribution to Project:
Fifth year Ph.D. student . This NSF Project is a major component of her Ph.D. thesis. Part of her Graduate research assistantship supported by this project. Graduated in December 2002 with a Ph.D. in Biology.

Name: Graziano, Sara
Worked for more than 160 Hours: Yes
Contribution to Project:
Second year Ph.D. student . This NSF Project is a major component of her Ph.D. thesis. Part of her Graduate research assistantship supported by this project.

Name: Schmitt, Karen
Worked for more than 160 Hours: Yes
Contribution to Project:
First year Ph.D. student . This NSF Project is a major component of her Ph.D. thesis. Part of her Graduate research assistantship supported by this project.

Name: Bates, David
Worked for more than 160 Hours: Yes
Contribution to Project:
Ph.D. student supported by this NSF grant.

Name: Burns, Justin
Worked for more than 160 Hours: Yes
Contribution to Project:
PhD student supported by this NSF grant.

Undergraduate Student

Name: Taylor, Lloyd

Worked for more than 160 Hours: Yes

Contribution to Project:

Undergraduate research technician working on project for course credit under direction of Ph.D. student Graziano. Graduated with a B.S. in Biology in May 2002.

Name: Nelson, Rebecca

Worked for more than 160 Hours: Yes

Contribution to Project:

Undergraduate research technician working on project for course credit under direction of Ph.D. student Moore. Graduated with a B.S. in Biology in May 2002.

Name: Pritchard, Jessica

Worked for more than 160 Hours: Yes

Contribution to Project:

Undergraduate research technician working on project for course credit under direction of Ph.D. student Haller. Graduated with a B.S. in Biology in May 2002.

Name: Lubarsky, Michael

Worked for more than 160 Hours: Yes

Contribution to Project:

Undergraduate research technician working on project for course credit under direction of Ph.D. student Graziano.

Technician, Programmer

Other Participant

Name: Maxwell, David

Worked for more than 160 Hours: Yes

Contribution to Project:

Howard Hughes Medical Institute (HHMI) summer intern from North Carolina working on project under direction of Ph.D. student Graziano.

Research Experience for Undergraduates

Organizational Partners

Western Michigan University

Professor Johnson R. Haas of the Chemistry Department at Western Michigan University visited our laboratory and worked with my Ph.D. students at Georgia Tech on several aspects of our NSF-funded project. This collaborative arrangement resulted in a publication in Environmental Science and Technology (see publication list).

Other Collaborators or Contacts

No other collaborators to date.

Activities and Findings

Research and Education Activities:

Our research and education activities have included laboratory-based experiments for Ph.D. students and undergraduates, presentation of research findings at national and local meetings and educational outreach activities with local high schools (see below). Our laboratory research has focused on testing the hypothesis that Fe(III)-respiring bacteria employ a type II secretion system to target respiration-linked, Fe(III) terminal reductases to the bacterial outer membrane where they contact and transfer electrons to solid Fe(III) substrates.

Our research team has included a broad range of levels including middle school students, high school students, high school science teachers, undergraduate students, graduate students and visiting professors. The middle school and high school science teachers are participants in the Georgia Industrial Fellowship for Teachers (GIFT) program that provides funds for teachers and their students to participate in the day-to-day activities of a university research lab. The undergraduate students are part of the Howard Hughes Medical Institute (HHMI) program which provides stipends for academic and summer undergraduate research fellowships at Georgia Tech.

INVITED NATIONAL PRESENTATIONS (YEAR TWO):

DiChristina, T. 2003. New insights into the molecular mechanism of bacterial metal respiration. Gordon Research Conference: Applied and Environmental Microbiology, New London, CT.

DiChristina, T. 2003. New insights into the molecular mechanism of bacterial metal respiration, Department of Microbiology, University of Georgia, Athens, GA.

Graziano, S. and T. J. DiChristina. 2003. Molecular mechanism of bacterial Fe(III) respiration. American Society for Microbiology National Meeting, Washington, DC.

INVITED LOCAL PRESENTATIONS (YEAR TWO):

Graziano, S. and T. J. DiChristina. 2002. Molecular mechanism of bacterial Fe(III) respiration. Biology Graduate Student Association Annual Symposium, Georgia Institute of Technology.

Findings:

We have reported three major research findings in the Year Two funding period:

1. In Year One of the project, we demonstrated that the type II secretion gene gspE was required for Fe(III) and Mn(IV) reduction by S. putrefaciens strain 200: gspE insertional mutants were unable to respire anaerobically on Fe(III) or Mn(IV), yet retained the ability to respire anaerobically on all other electron acceptors. A 91 kDa Fe(III)-reducing protein was present in the peripheral proteins loosely attached to the outside face of the S. putrefaciens wild-type outer membrane, but was missing from this location in the gspE insertional mutants. This finding provided the first genetic evidence linking dissimilatory Fe(III) and Mn(IV) reduction and type II protein secretion. In Year Two of the project, we determined that type II secretion was also required for Fe(III)

reduction by other Shewanella strains. We carried out an analogous set of insertional mutagenesis experiments to generate type II secretion mutants of S. oneidensis strain MR-1. The type II secretion gene gspD was PCR-amplified from the S. oneidensis chromosome and insertionally inactivated with the 2.2 kb suicide plasmid pKNOCK-Gm. As observed with the S. putrefaciens gspE insertional mutants, the S. oneidensis gspD insertional mutants were unable to reduce or grow anaerobically on solid Fe(III) oxides as terminal electron acceptor. The peripheral proteins loosely attached to the outside face of the S. putrefaciens wild-type and gspD insertional mutants were isolated and subsequently tested for Fe(III) reduction activity after separation in a native gel. Five Fe(III)-reducing bands were detected in the peripheral protein fraction of the wild-type strain, while only four of the five bands were detected in the peripheral protein fraction of the gspD insertional mutant. We hypothesize that the Fe(III)-reducing band present in the wild-type strain (and missing in the gspD insertional mutant) contains the outer membrane-targeted, respiration-linked Fe(III) terminal reductase of S. oneidensis.

2. We also used MALDI-TOF mass spectrometry analysis of trypsin degraded proteins to identify two polypeptides associated with the Fe(III) terminal reductase of S. oneidensis MR-1. The polypeptides displayed signficant sequence homology to 1) an outer membrane c-type cytochrome, OmcB and 2) a component of the Paracoccus denitrificans sulfur oxidation system, SoxB. The entire set of Paracoccus denitrificans Sox homologs has been identified in S. oneidnesis MR-1 whole genome scans. These findings indicate that the P. denitrificans sulfur oxidation and S. *oneidensis Fe(III) reduction pathways* may utilize similar respiratory chain components, an unexpected yet potentially important finding with respect to evolution of bacterial respiratory systems.

3. Unexpectedly, the gspD insertional mutant of S. oneidensis MR-1 expressed wild-type Fe(III) respiration activity when exposed to the exogenous electron shuttle AQDS. These findings indicate that the anaerobic electron transport pathways terminating with the reduction of Fe(III) and AQDS utilize separate respiration-linked reductases. The findings also provide a novel research strategy to identify the AQDS reductase of S. oneidensis: carry out a second round of mutagenesis with the gspD (Fe(III) reduction-deficient) mutant, identify respiratory mutants lacking the ability to respire on a combination of Fe(III) and AQDS, and identify the genes involved in AQDS reduction via complementation analysis with a S. oneidensis MR-1 gene clone bank.

Training and Development:

Our project has trained seven Ph.D. students and four undergraduate students in the use of molecular biology techniques. The students have developed an ability to think critically about environmental microbiology problem solving. The project has also fostered a sense of teamwork among the Ph.D. students, including the ability to interact with visiting faculty, undergraduate students, high school teachers and high school students. The project has exposed a set of high school teachers and students from the Atlanta metro area to the excitement of molecular microbiology and environmental science.

Outreach Activities:

We have provided undergraduate students and high school teachers and high school students (several of whom are from groups traditionally

underrepresented in science) with hands-on research experience in a university setting. We hope to recruit several of these students to attend Georgia Tech as undergraduate or Ph.D. students in the near future.

Journal Publications

DiChristina, T. J., Moore, C. M. and C. A. Haller, "Dissimilatory Fe(III) and Mn(IV) reduction by Shewanella putrefaciens requires ferE, a homolog of the pulE (gspE) Type II protein secretion gene.", Journal of Bacteriology, p. 142, vol. 184, (2002). Published

Moore, C. M. and T. J. DiChristina, "A Shewanella putrefaciens cytochrome c3 insertional mutant retains the ability to respire anaerobically on all terminal electron acceptors.", Journal of Bacteriology, p. , vol. , ( ). In preparation

Graziano, S. L., Haller, C. A. and T. J. DiChristina, "Dissimilatory Fe(III) and Mn(IV) reduction by Shewanella oneidensis requires ferD, a homolog of the pulD (gspD) Type II protein secretion gene.", Journal of Bacteriology, p. , vol. , ( ). In preparation

Haas, J. R. and T. J. DiChristina, "Effects of Fe(III) chemical speciation on dissimilatory Fe(III) reduction by Shewanella putrefaciens.", Environmental Science and Technology, p. 373, vol. 36, (2002). Published

Graziano, S. and T. DiChristina, "Exogenous electron shuttle AQDS overcomes the Fe(III) reduction deficiency of a type II secretion mutant of Shewanella oneidensis MR-1", Applied and Environmental Microbiology, p. , vol. , ( ). Submitted

DiChristina, T., J. Fredrickson and J. Zachara, "Enzymology of electron transport: Energy generation with geochemical consequences.", Reviews in Mineralogy and Geochemistry, p. 27, vol. 59, (2005). Published

Books or Other One-time Publications

Haller, C. A. and T. J. DiChristina, "Genetic Approaches in Bacteria with No Natural Genetic Systems", (2002). Book, Published
Editor(s): Streips, U. N. and R. E. Yasbin
Collection: Modern Microbial Genetics II
Bibliography: John Wiley and Sons, New York, pp. 581-602

Moore, C. M. and T. J. DiChristina, "Metal Cycling", (2002). Book, Published
Editor(s): G. Bitton
Collection: The Encyclopedia of Microbiology
Bibliography: John Wiley and Sons, New York, pp. 1902-1913

Haller, C. A., "Dissimilatory Fe(III) Reduction by Shewanella putrefaciens: Biochemical and Genetic Analyses", (2002). Thesis, Published
Bibliography: Georgia Institute of Technology

Moore, C. M., "Molecular Mechanism and Biogeochemical Controls of Microbial Fe(III) Reduction", (2002). Thesis, Published
Bibliography: Georgia Institute of Technology

DiChristina, T., D. Bates, J. Burns, J. Dale and A. Payne, "Microbial metal reduction by members of the genus Shewanella: novel strategies for anaerobic respiration", (2006). Book, Published
Editor(s): L. Neretin
Collection: Biogeochemistry of Anoxic Marine Basins
Bibliography: Kluwer Publ. Co., Dordrecht, NL

Web/Internet Site

URL(s):
http://www.biology.gatech.edu/dichristina.html
http://www.biology.gatech.edu/announcements.html#grants
Description:

Other Specific Products

Product Type:
Physical collection (samples, etc.)
Product Description:
Generation of a set of Type II secretion-deficient mutants of S. putrefaciens.
Sharing Information:
Available for public use.

Product Type:
Data or databases
Product Description:
4,369 nucleotide sequence of S. putrefaciens chromosomal region containing Type II secretion gene homologs D, E, F and G.
Sharing Information:
Available through GenBank (accession no. AF188713)

Product Type:
Graduate sttudent presentations at international meetings
Product Description:
Graduate student presentations at international meetings
Sharing Information:
Research findings presented at:

Bates, D and T. DiChristina. 2006. Microbial Fe(III) reduction by Shewanella oneidensis MR-1. American Society for Microbiology National Meeting, Orlando, FL.

Burns, J. and T. DiChristina. 2006. Molecular mechanism of metal respiration by Shewanella oneidensis MR-1. American Society for Microbiology National Meeting, Orlando, FL.

Bates, D and T. DiChristina. 2005. Microbial Fe(III) reduction by Shewanella oneidensis MR-1. IUMS International Meeting, SanFrancisco, CA.

Burns, J. and T. DiChristina. 2005. Molecular mechanism of metal respiration by Shewanella oneidensis MR-1. IUMS International Meeting, SanFrancisco, CA.

Bates, D and T. DiChristina. 2005. Microbial Fe(III) reduction by Shewanella oneidensis MR-1. American Society for Microbiology National Meeting, Atlanta, GA.

Burns, J. and T. DiChristina. 2005. Molecular mechanism of metal respiration by Shewanella oneidensis MR-1. American Society for Microbiology National Meeting, Atlanta, GA.

Adiga, M. and T. DiChristina. 2004. Dissimilatory Fe(III) and Mn(IV) reduction by

Shewanella oneidensis requires ferD, a homolog of the pulD (gspD) Type II protein secretion gene. American Society for Microbiology National Meeting, New Orleans, LA.

Sankovich, K. and T. DiChristina. 2004. Mn(III) reduction by Shewanella oneidensis MR-1. American Society for Microbiology National Meeting, New Orleans, LA.

Graziano, S. and T. DiChristina. 2003. Dissimilatory Fe(III) and Mn(IV) reduction by Shewanella oneidensis requires ferD, a homolog of the pulD (gspD) Type II protein secretion gene. American Society for Microbiology National Meeting, Washington, DC.

Adiga, M. and T. DiChristina. 2003. Dissimilatory Fe(III) and Mn(IV) reduction by Shewanella oneidensis requires ferD, a homolog of the pulD (gspD) Type II protein secretion gene. American Society for Microbiology, Southeastern Branch Meeting, Athens, GA.

Sankovich, K. and T. DiChristina. 2003. Mn(III) reduction by Shewanella oneidensis MR-1. American Society for Microbiology, Southeastern Branch Meeting, Athens, GA.

Contributions

Contributions within Discipline:

We discovered that type II protein secretion was required for anaerobic Fe(III) respiration by the gamma-proteobacteria S. putrefaciens strain 200, and that the respiration-linked Fe(III) reductases are loosely attached to the outside face of the S. putrefaciens outer membrane. This finding opposes the widely accepted view that bacterial terminal reductases are embedded or associated with the inner (cytoplasmic) membrane. *Identification of homologs of type II secretion genes in all Fe(III)*-respiring gram-negative bacteria whose genomes have been sequenced (i.e., Shewanella oneidensis MR-1, Geobacter metallireducens, G. sulfurreducens, Aeromonas hydrophila) suggests that type II secretion may be required for electron transport to Fe(III) in all Fe(III)-respiring, gram-negative bacteria.

We generated a type II secretion insertional mutant of a second Shewanella strain (Shewanella oneidensis MR-1) and confirmed that type II secretion was required for Fe(III) reduction by S. oneidensis MR-1 as well as S. putrefaciens strain 200. The Fe(III) terminal reductase was identified in the S. oneidensis wild-type outer membrane, yet was missing from the outer membrane of the type II secretion mutant. These findings suggest that outer membrane-targeted protein secretion is required for Fe(III) respiration by members of the genus Shewanella.

We also identified two polypeptides associated with the Fe(III) terminal reductase of S. oneidensis MR-1. The polypeptides displayed signficant sequence homology to 1) an outer membrane c-type cytochrome, OmcB and 2) a component of the Paracoccus denitrificans sulfur oxidation system, SoxB. These findings indicate that the sulfur oxidation and Fe(III) reduction pathways may utilize similar respiratory chain components.

We also discovered that the S. oneidensis MR-1 Fe(III) respiration-deficiency is overcome by providing an exogenous electron shuttle (AQDS) to type II secretion mutants that are otherwise unable to respire on Fe(III). This finding suggests that electrons are transported to Fe(III) and AQDS via separate respiration-linked terminal reductases.

Contributions to Other Disciplines:

Our findings will impact a wide variety of other biological and non-biological disciplines, including general protein secretion, membrane physiology, microbial physiology, environmental microbiology, microbial ecology, bioremediation, biogeochemistry, geochemistry, mineralogy, aquatic chemistry, oceanography (chemical and biological) and evolution. For example, several hyperthermophilic Archaea most closely related to the last common ancestor are able to respire anaerobically on Fe(III) as sole terminal electron acceptor and at least one contains several putative Type II protein secretion genes. Microbial Fe(III) respiration may therefore provide a model system with which to study the evolutionary history of respiratory processes in all microorganisms.

Contributions to Human Resource Development:

We have provided students from groups traditionally underrepresented in science with the opportunity to participate in the day-to-day operations of a university research lab.

Contributions to Resources for Research and Education:

The research project has fostered the development and implementation of a new graduate level course at Georgia Tech entitled 'Geomicrobiology' (BIOL 6765).

Contributions Beyond Science and Engineering:

Nothing yet to report.

Categories for which nothing is reported:

Recent genetic and biochemical studies have indicated that anaerobic Fe(III) respiration by S. putrefaciens requires outer-membrane targeted secretion of respiration-linked Fe(III) reductases. Thus, the availability of Fe(III) to S. putrefaciens may be governed by equilibrium chemical speciation both in the solution phase andâ€¦ CONTINUE READING. View on PubMed. 1999. Outer membrane-targeted secretion of respiratory-linked Fe(III) reductases. Science. 12. Friedheim, E., and L. Michaelis.Â 1990. Respiration-linked proton translocation coupled to anaerobic reduction of manganese(IV) and iron(III) in Shewanella putrefaciens MR-1. Journal of Bacteriology 172:6232-6238. 44. Nealson, K. H., and B. Little.