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14. ABSTRACT
Rhodopseudomonas palustris is a photosynthetic bacterium that has good potential as a biocatalyst for the production of hydrogen gas, a biofuel. With this award we conducted basic studies to facilitate the development of a process where R. palustris cells grown on surfaces as biofilms, produce hydrogen gas using energy from the sun and electrons from agricultural waste. We characterized five new Rhodopseudomonas genome sequences and isolated and described R. palustris mutant strains that produce hydrogen constitutively at all times. We published

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Report Title

Biofilm formation by a metabolically versatile bacterium: final report

ABSTRACT

Rhodopseudomonas palustris is a photosynthetic bacterium that has good potential as a biocatalyst for the production of hydrogen gas, a biofuel. With this award we conducted basic studies to facilitate the development of a process where R. palustris cells grown on surfaces as biofilms, produce hydrogen gas using energy from the sun and electrons from agricultural waste. We characterized five new Rhodopseudomonas genome sequences and isolated and described R. palustris mutant strains that produce hydrogen constitutively at all times. We published papers describing features of biodegradation and photosynthesis that impinge on hydrogen production. In addition, we characterized hydrogen production by R. palustris biofilms. Taking a new direction, we unexpectedly found that the plant-derived carbon compound, p-coumarate is used by R. palustris to synthesize a novel chemical language of cell-to–cell communication in the form p-coumaryl-homoserine lactone. This language may not only allow Rhodopseudomonas cells to communicate with each other, but it may also be important for communication between bacteria and plants.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

1. Oda Y., F. W. Larimer, P. S. Chain, S. Malfatti, M. V. Shin, L. M., Vergez, L. Hauser, M. L. Land, S.Braatsch, J. T. Beatty, D. A. Pelletier, A. L. Schaefer and C. S. Harwood. 2008. Multiple genomes equences reveal adaptations of a phototrophic bacterium to sediment microenvironments. Proc. Natl.Acad. Sci. USA. 105:18543-18548. Epub 2008 Nov 1

2. Schaefer, A. L., E. P. Greenberg, C. M. Oliver, Y. Oda, J. J. Huang, G. Bittan-Banin, C. M. Peres, S. Schmidt, K. Juhaszova, J. R. Sufrin and C. S. Harwood. 2008. A new class of homoserine lactone quorum-sensing signals. Nature 454:595-599.

3. Pan, C., Y. Oda, P. K. Lankford, B. Zhang, N. F. Samatova, D. A. Pelletier, C. S. Harwood, and R.L. Hettich. 2008. Characterization of anaerobic catabolism of p-coumarate in Rhodopseudomonas palustris by integrating transcriptomics and quantitative proteomics. Mol. Cell. Proteomics. 7:938-948.

4. Rey, F. E., E. K. Heiniger and C. S. Harwood. 2007. Redirection of metabolism for biological hydrogen production. Appl. Environ. Microbiol. 73:1665-1671.

5. Braatsch, S., J. R. Bernstein, F. Lessner, J. Morgan, J. C. Liao, C. S. Harwood and J. T. Beatty. 2006. Rhodopseudomonas palustris CGA009 has two ppsR genes that each encode repressors of photosynthesis gene expression. Biochemistry 45: 14441-14451.

6. Peres. C. M. and C. S. Harwood. 2006. BadM is a transcriptional repressor and one of three regulators that controls benzoyl-CoA reductase gene expression in Rhodopseudomonas palustris. J. Bacteriol. 188:8662-8665.

7. Oda, Y., S. K. Samanta, F. Rey, L. Wu, X.-D. Liu, T.-F. Yan, J. Zhou, and C. S. Harwood. 2005. Functional genomic analysis of three nitrogenase isozymes in Rhodopseudomonas palustris. J. Bacteriol. 187:7784-7794.

8. Harrison, F.H. and C. S. Harwood. 2005. The pimFABCDE operon from Rhodopseudomonas palustris mediates dicarboxylic acid degradation and participates in anaerobic benzoate degradation. Microbiology 151:727-736.

9. Samanta, S. K. and C. S. Harwood. 2005. Use of the Rhodopseudomonas palustris genome to identify a single amino acid that contributes to the activity of a coenzyme A ligase with chlorinated substrates. Mol. Microbiol. 55:1151-1159.

Number of Papers published in peer-reviewed journals: 9.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

1. Harwood, C. S. 2008.. Nitrogenase-catalyzed hydrogen production by purple nonsulfur photosynthetic bacteria. p. 259-271. IN: A. Demain, C.S. Harwood and J. Wall (eds.) Bioenergy. American Society for Microbiology Press.

2. Harwood, C. S. 2008. Degradation of aromatic compounds by purple nonsulfur bacteria
IN: Hunter CN, Daldal, F,Thurnauer MC, and Beatty JT (eds) The Purple Phototrophic Bacteria.. Advances in Photosynthesis and Respiration, Vol. 28, pp. 577-594, Springer, Dordrecht, The Netherlands

Number of Papers published in non peer-reviewed journals: 2.00

(c) Presentations

1. Symposium “Putting microbes to work”; Annual ASM meeting, Atlanta, GA June 2005

2. First International Symposium on Syntrophic Microbiology, Los Angeles, CA, Dec 2005

3. Georgia Tech Environmental Systems Microbiology Symposium, Atlanta, GA, March, 2006

4. Joint Genome Institute Users meeting, Walnut Creek, CA, March, 2007

5. American Society for Microbiology Branch Meeting, Seattle, WA, March 2007

6. Society for Industrial Microbiology, Denver, CO, July 2007

7. US-EC Workshop on Metabolomics and Environmental Biotechnology, Palma de Mallorca, Spain, June, 2008.

Number of Presentations: 7.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

Number of Manuscripts: 0.00

Number of Inventions:

Graduate Students

NAME	PERCENT_SUPPORTED
Federico Rey	0.50
Faith H. Harrison	1.00
FTE Equivalent:	**1.50**
Total Number:	**2**

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Yasuhiro Oda	1.00
FTE Equivalent:	**1.00**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Caroline Harwood	0.10	No
FTE Equivalent:	**0.10**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Federico Rey
Faith H. Harrison
Total Number: 2

Names of other research staff

NAME	PERCENT_SUPPORTED	
Amy Schaefer	0.33	No
FTE Equivalent:	**0.33**	
Total Number:	**1**	

Sub Contractors (DD882)

Inventions (DD882)

Final report
3/18/09

Proposal : 48699-LS
W911NF-05-1-017

Biofilm formation by a metabolically versatile bacterium.
Caroline S. Harwood
Department of Microbiology
University of Washington

Abstract

Rhodopseudomonas palustris is a photosynthetic bacterium that has good potential as a biocatalyst for the production of hydrogen gas, a biofuel. With this award we conducted basic studies to facilitate the development of a process where *R. palustris* cells grown on surfaces as biofilms, produce hydrogen gas using energy from the sun and electrons from agricultural waste. We characterized five new *Rhodopseudomonas* genome sequences and isolated and described *R. palustris* mutant strains that produce hydrogen constitutively at all times. We published papers describing features of biodegradation and photosynthesis that impinge on hydrogen production. In addition, we characterized hydrogen production by *R. palustris* biofilms. Taking a new direction, we unexpectedly found that the plant-derived carbon compound, *p*-coumarate is used by *R. palustris* to synthesize a novel chemical language of cell-to–cell communication in the form *p*-coumaryl-homoserine lactone. This language may not only allow *Rhodopseudomonas* cells to communicate with each other, but it may also be important for communication between bacteria and plants.

Statement of the problem *Rhodopseudomonas palustris* is a photosynthetic bacterium that has good potential as a biocatalyst for hydrogen production by means of its nitrogenase enzymes. Hydrogen is produced concomitantly with ammonia as a product of nitrogen fixation. This process requires large amounts of ATP and electrons, which *R. palustris* can derive from plant biomass and sunlight, respectively. It should be possible to configure bioreactors where *R. palustris* cells illuminated by sunlight degrade agricultural waste and generate hydrogen as a product of nitrogen fixation. *R. palustris* forms biofilms - defined as multicellular communities enclosed in a self-produced extracellular matrix - on surfaces when it is grown with the green plant-derived aromatic compound, p-coumarate. This is an important characteristic because biofilms provide a means of exposing large surface areas of cells to light, a prerequisite for hydrogen production.

Summary of most important results.

During the project period:

1) We published a study of nitrogenase gene expression and hydrogen production by *Rhodopseudomonas***.**

Oda, Y., S. K. Samanta, F. Rey, L. Wu, X.-D. Liu, T.-F. Yan, J. Zhou, and C. S. Harwood. 2005. Functional genomic analysis of three nitrogenase isozymes in *Rhodopseudomonas palustris*. J. Bacteriol. **187**:7784-7794.

R. palustris is one of just a few prokaryotes so far described that has *vnf* and *anf* genes for alternative vanadium cofactor (V) and iron cofactor (Fe) nitrogenases, in addition to

nif genes for a molybdenum cofactor (Mo) nitrogenase. Understanding the differential regulation of nitrogenase isozyme synthesis is an important step in the possible development of *R. palustris* as a biological catalyst for hydrogen production.

2) We finished up work initiated some years ago on the anaerobic degradation of aromatic compounds by *R. palustris* and on the regulation of this process. Aromatic compounds are good electron donors for hydrogen gas production.

Peres. C. M. and C. S. Harwood. 2006. BadM is a transcriptional repressor and one of three regulators that controls benzoyl-CoA reductase gene expression in *Rhodopseudomonas palustris*. J. Bacteriol. **188**:8662-8665.

Harrison, F.H. and C. S. Harwood. 2005. The p*imFABCDE* operon from *Rhodopseudomonas palustris* mediates dicarboxylic acid degradation and participates in anaerobic benzoate degradation. Microbiology **151**:727-736.

Samanta, S. K. and **C. S. Harwood**. 2005. Use of the *Rhodopseudomonas palustris* genome to identify a single amino acid that contributes to the activity of a coenzyme A ligase with chlorinated substrates. Mol. Microbiol. **55**:1151-1159.

3) We published a study describing the isolation of high hydrogen-producing mutants via directed evolution.

Rey, F. E., E. K. Heiniger and C. S. Harwood. 2007. Redirection of metabolism for biological hydrogen production. Appl. Environ. Microbiol. **73**:1665-1671

A description of this study was featured in Microbe, the News magazine of the American Society for Microbiology in May 2007.

4) We published a study of regulation of photosynthesis by *R. palustris*.

Braatsch, S., J. R. Bernstein, F. Lessner, J. Morgan, J. C. Liao, C. S. Harwood and J. T. Beatty. 2006. *Rhodopseudomonas palustris* CGA009 has two *ppsR* genes that each encode repressors of photosynthesis gene expression. Biochemistry **45**: 14441-14451.

5) In a study that is now being prepared for publication, we characterized hydrogen production by p-coumarate-induced biofilms.

6) We identified a new class of homoserine lactone quorum sensing signal that uses exogenously supplied p-coumarate as the substrate.

Schaefer, A. L., E. P. Greenberg, C. M. Oliver, Y. Oda, J. J. Huang, G. Bittan-Banin, C. M. Peres, S. Schmidt, K. Juhaszova, J. R. Sufrin and C. S. Harwood. 2008. A new class of homoserine lactone quorum-sensing signals. Nature 454:595-599.

7) We analyzed and reported the complete genome sequences of four additional *Rhodopseudomonas* strains.

Oda Y., F. W. Larimer, P. S. Chain, S. Malfatti, M. V. Shin, L. M., Vergez, L. Hauser, M. L. Land, S. Braatsch, J. T. Beatty, D. A. Pelletier, A. L. Schaefer and C. S. Harwood. 2008. Multiple genome sequences reveal adaptations of a

phototrophic bacterium to sediment microenvironments. Proc. Natl. Acad. Sci. USA. 105:18543-18548. Epub 2008 Nov 1