MASTER COPY KEEP THIS CO

AD-A255 658

(2)

REPORT DOCUMENTATION

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	14 August 1992	Final Report - 6/15/89 - 6/14/92

4. TITLE AND SUBTITLE
Molecular Biology of Anaerobic Aromatic Biodegradation

5. FUNDING NUMBERS
DAAL03-89-K-0121

6. AUTHOR(S)
Caroline S. Harwood

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Microbiology
University of Iowa
Iowa City, IA 52242

8. PERFORMING ORGANIZATION
92-25575

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U. S. Army Research Office
P. O. Box 12211
Research Triangle Park, NC 27709-2211

AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Aromatic acids are intermediates in the biodegradation of structurally diverse aromatic compounds, including lignin monomers and environmental pollutants, by many metabolic types of anaerobic bacteria. They are also the starting compounds for central pathways of anaerobic benzene ring reduction and fission. We have identified and developed molecular tools that can be used to manipulate and clone genes for aromatic acid degradation from the bacterium, Rhodopseudomonas palustris. These tools have enabled us to identify genes specifying two enzymes that initiate the degradation of the compounds benzoate and 4-hydroxybenzoate, and we have also cloned, sequenced, and characterized a regulatory gene required for the expression of aromatic acid degradation enzymes. Thus, the first steps towards elucidating the molecular basis for benzene ring fission in the absence of oxygen have been accomplished.

92 9 22 006

14. SUBJECT TERMS	15. NUMBER OF PAGES
Biodegradation, Biotechnology, Aromatic Compounds	4
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

MOLECULAR BIOLOGY OF ANAEROBIC AROMATIC BIODEGRADATION

FINAL REPORT

CAROLINE S. HARWOOD

AUGUST 14, 1992

U. S. ARMY RESEARCH OFFICE
DAAL03-89-K-0121

UNIVERSITY OF IOWA

APPROVED FOR PUBLIC RELEASE;
DISTRIBUTION UNLIMITED

A. STATEMENT OF THE PROBLEM STUDIED.

Chlorinated aromatic compounds and aromatic hydrocarbons, including toluene and xylene, comprise a large proportion of the toxic wastes that have been released into the environment. Under anaerobic conditions the aromatic carboxylic acids, benzoate and 4-hydroxybenzoate, are formed as key intermediates during the biodegradation of aromatic pollutants. These acids then enter central pathways of anaerobic benzene ring reduction and fission, leading to complete mineralization.

Not a single catabolic pathway for the anaerobic degradation of any aromatic compound has yet been worked out in detail, and the molecular basis for aromatic compound degradation by bacteria is even less well explored. If the potential of bacteria to degrade benzene rings under anaerobic conditions is to be manipulated to realize their full detoxification potential, or to produce intermediary compounds that may have commercial value, it will be necessary to understand in detail the metabolic mechanisms involved, to know how the pathways are regulated, and to develop approaches for modifying the genes encoding key enzymes.

As an approach to achieving these goals we have been studying the anaerobic degradation of two selected aromatic acids - benzoate and 4-hydroxybenzoate - by one bacterial species - *Rhodopseudomonas palustris*. Our emphasis has been on developing tools to explore the genetic basis of aromatic acid degradation. Our expectation is that it will be possible to extend many of our conclusions to other bacteria and to related compounds.

B. SUMMARY OF THE MOST IMPORTANT RESULTS.

Our most important contribution during the last three years has been the identification and development of molecular tools that can be used to clone and manipulate genes in *R. palustris*. These tools have enabled us to identify a regulatory gene, termed *aadR* (for anaerobic aromatic degradation regulator) which is required for the expression of genes involved in anaerobic 4-hydroxybenzoate and benzoate degradation. We have also obtained partial clones of the genes for benzoate-CoA ligase and aromatic acid-CoA ligase II, enzymes that catalyze the first steps in the degradation of benzoate and 4-hydroxybenzoate, respectively. Finally, we have made extensive use of immunoblot assays to identify environmental factors that are required for the regulated expression of benzoate-CoA ligase. With this work we have accomplished the first steps in elucidating the molecular basis for benzene ring fission in the absence of oxygen.

C. PUBLICATIONS.

Papers:

Gibson, J., J. F. Geissler, and C. S. Harwood. 1990. Benzoate-coenzyme A ligase from *Rhodopseudomonas palustris*. Methods in Enzymology (Hydrocarbons and methylotrophy) 188:154-159.

Kim, M-K., and C. S. Harwood. 1991. Regulation of benzoate-CoA ligase in *Rhodopseudomonas palustris*. FEMS Microbiol. Letts. 83:199-204.

Dispensa, M., C. T. Thomas, M.-K. Kim, J. A. Perrotta, J. Gibson, and C. S. Harwood. 1992. Anaerobic growth of *Rhodopseudomonas palustris* on 4-hydroxybenzoate is dependent on AadR, a member of the cyclic AMP receptor protein family of transcriptional regulators. J. Bacteriol. 174: (in press).

Gibson, J., and C. S. Harwood. Anaerobic utilization of aromatic carboxylates by bacteria. IN: Biological Degradation and Bioremediation Technology of Toxic Chemicals, R. Chaudhry, ed. (in press).

Published abstracts:

Thomas, C., M. Dispensa, C. S. Harwood, and J. Gibson. 1990. Molecular analysis of anaerobic aromatic degradation by *Rhodopseudomonas palustris*. Abstr. Ann. Meet. Amer. Soc. Microbiol. 90:K136.

Thomas, C., M. Dispensa, M. K. Kim, J. A. Perrotta, J. Gibson, and C. S. Harwood. 1991. Molecular analysis of benzoate and 4-hydroxybenzoate photometabolism by *Rhodopseudomonas palustris*. VII International Symposium for Photosynthetic Prokaryotes.

Dispensa, M., and C. S. Harwood. 1992. Identification of *aadR*, a regulatory gene required for anaerobic 4-hydroxybenzoate degradation by *Rhodopseudomonas palustris*. Abstr. Ann. Meet. Amer. Soc. Microbiol. 92:K31.

D. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED.

Marilyn Dispensa (Technician)
Joseph Perrotta (Graduate Research Assistant)
Min-Kyung Kim (Graduate Research Assistant) - M. S. awarded

E. REPORT OF INVENTIONS:

None