REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

1. AGENCY USE ONLY (Leave Blank)

2. REPORT DATE
11/17/00

3. REPORT TYPE AND DATES COVERED
FINAL 25 Sep 95 - 31 Mar 99

4. TITLE AND SUBTITLE
Use of Genetic Engineering to Produce a Mutated Cytochrome P450 Enzyme Capable of Both Oxidizing & Reductivity Dechlorinating Hazardous Organic Chemicals

5. FUNDING NUMBERS
DAAH04-95-1-0639

6. AUTHOR(S)
Dr. William L. Alworth
Dr. David Mullin

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Tulane University

8. PERFORMING ORGANIZATION REPORT NUMBER

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

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
ARO 34209.1-LS-AAS

11. SUPPLEMENTARY NOTES
The views, 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.

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

12 b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The goal of this project is to develop new catalysts and organisms that will be capable of enhancing in situ bioremediation. Our approach is to prepare site-specific mutants of the P450 monooxygenase enzyme P450 102 (BM-3) from the soil bacterium Bacillus megaterium, that possess the abaility to catalyze transformations of recalcitrant organic compounds that are hazardous environmental contaminants. In this investigation we focused our effort on designing, producing. Purifying, and studying site-specific mutant of the wild type (nonmutated) P450 102 that can catalyze the oxidation of polycyclic aromatic compounds (PAHs) such as benzo[a]pyrene and mutants that can catalyze the reductive dechlorination of organochlorine compounds such as pentachloroethane.

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

TITLE

Use of Genetic Engineering to Produce a Mutated Cytochrome P450 Enzyme Capable of Both Oxidizing and Reductively Dechlorinating Hazardous Organic Chemicals

Final Report

William L. Alworth, Ph. D.
P. I.

11/17/2000

U.S. Army Research Office

ASSERT Grant No: **34209LA-AAS**

Tulane University
Department of Chemistry
Department of Cell and Molecular Biology
and
Tulane/Xavier Center for Bioenvironmental Research

4. Body of the report

A. Problem Studied:

The goal of this project is to develop new catalysts and organisms that will be capable of enhancing *in situ* bioremediation. Our approach is to prepare site-specific mutants of the P450 monooxygenase enzyme P450 102 (BM-3) from the soil bacterium *Bacillus megaterium* that possess the ability to catalyze transformations of recalcitrant organic compounds that are hazardous environmental contaminants. In this investigation we focused our effort on designing, producing. Purifying, and studying site-specific mutants of the wild type (nonmutated) P450 102 that can catalyze the oxidation of polycyclic aromatic compounds (PAHs) such as benzo[a]pyrene and mutants that can catalyze the reductive dechlorination of organochlorine compounds such as pentachloroethane.

B. Summary of Most Important Results:

- We produced a site-specific mutant of the soluble P450 102 from *Bacillus megaterium* (F87G) that converts the P450 102 from a fatty acid hydroxylase into a PAH hydroxylase capable of metabolizing pyrene and benzo[a]pyrene.

- We demonstrated that when the gene for the P450 102 F87G mutant ins transfected into the aquatic bacterium *Caulobacter crescentus* this organism becomes capable of metabolizing an aqueous solution of the PAH pyrene.

- We discovered that the P450 102 F87G mutant initially catalyzes the conversion of pyrene to 1-hydroxypyrene and then catalyzes the further conversion of 1-hydroxypyrene to oxidation products that generate autocatalytic oxidation of NADPH.
- We produced a second site-specific mutant of P450 102 from *Bacillus megaterium* (A328V) that catalyzes the rapid oxidation of pentachloroethane. It is significant that this P450 102 mutant, in contrast to mammalian forms of P450 that metabolize chloroalkanes, is not inactivated by the pentachloroethane oxidation

products.

- We produced double site-specific mutants of P450 102 that manifest additional catalytic activities, for example, the ability to oxidize organic molecules such as phenanthrene. It was also learned that the catalytic properties of double mutants of P450 102 such as F87G, A328G can not be predicted from the catalytic properties of the individual mutants.

The most important result of this research is that we successfully demonstrated the validity of our approach for generating new catalysts and genetically engineered organisms capable of enhancing the in situ bioremediation of hazardous environmental contaminants such as PAHs and organochlorine compounds.

C. List of Publications and Technical Reports:

While not research papers or technical reports were published describing our research results, the co-P.I. Dr. David A. Mullin, will present an invited talk at the Combined Southeast/Southwest Regional Meeting of the American Chemical Society in New Orleans Dec. 8, 2000 at a Symposium on New Cytochrome P450 Chemistry.

D. List of Participating Scientific Personnel:

P. I., William L. Alworth, Department of Chemistry and Center for Bioenvironmental Research, Tulane University

Co-P. I., David A. Mullin, Department of Cell and Molecular Biology and Center for Bioenvironmental Research, Tulane University

Support from this ASSERT grant was used to support the graduate research of the following students at Tulane University:

Qiuwen Xia, Ph. D. Tulane University, 1997

Matthew Ranson, M. S., Tulane University, 2000

In addition the Ph. D. dissertation of Weiqiang Zhao, Ph. D., 1999 from Tulane University in Cell and Molecular Biology, is based upon the research financially supported this project but Dr. Zhao did not receive stipend support from this grant.

5. Inventions

None to report.

6. Bibliography

None required