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FINAL REPORT

DEPARTMENT OF ENERGY GRANT # DE -FG05-88ER13967

GRANT PERIOD: AUGUST 1988-NOVEMBER, 1989

DEPARTMENT OF MICROBIOLOGY-NORTH CAROLINA STATE UNIVERSITY

GENERAL

The third year of grant # DE-FG05-88ER13967 was transferred to North Carolina State University, Raleigh, North Carolina from the University of Georgia. This transfer required the training of a new technician and orientation of a new postdoctoral associate at this new site.

Seminars presented during this period:

1. University of Alberta-Alberta Research Council Biotechnology Series, "Microbial Biosurfactants-Genetics, Physiology and Applications. Edmonton, Canada, May, 1989

2. Batelle Pacific Northwest Laboratories, "Microbial Biosurfactants, Richland, WA. November, 1989.

3. University of Maryland, Eastern Shore Campus, "Biotechnology Perspectives in Hydrocarbon/Petroleum Microbiology" Princess Anne, MD, November, 1989.

Publications during this period:

1. Finnerty, W.R. 1989. Microbial Lipid Metabolism. IN: Microbial Lipids, Volume 2, C. Ratledge and J. Wilkinson, eds. Academic Press, London. pp. 525-566

2. Finnerty, W.R. 1989. Lipids of Acinetobacter. IN: Proceedings of World Congress on Biotechnology for the Fats and Oils Industry, American Oil Chemists Society, Champaign, IL. pp.

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3. Modzrakowski, M. and Finnerty, W.R. 1989. Intermediary Metabolism of Acinetobacter grown on dialkly ethers. Can. J. Microbiol. 35: 1031-1-36.

4. Finnerty, W.R. 1990. Primary Alcohol Dehydrogenases from Acinetobacter. Methods Enzymol. 188:14-17.

5. Finnerty, W.R. 1990. Aldehyde Dehydrogenases from Acinetobacter. Methods Enzymol. 188:18-20.

6. Finnerty, W.R. 1990. Assay Methods for Long Chain Alkane Oxidation in Acinetobacter. Methods Enzymol. 188:10-13.

7. Singer, M.E. and Finnerty, W.R. 1990. Physiology of Biosurfactant Synthesis by Rhodococcus species H13-A. Can. J. Microbiol. 36:741-745. (copy attached)

8. Singer, M.E., Finnerty, W.R., and Tunelid, A. 1990. Physical and chemical Properties of a Biosurfactant Synthesized by Rhodococcus species H13-A. Can. J. Microbiol. 36:746-750 (copy attached).

9. U.S. Patent #151.310 awarded for "Cloning Systems for Rhodococcus and Related Bacteria" in August, 1990. Patent assigned to the University of Georgia Research Foundation by inventors W.R. Finnerty and M.E. Singer. This patent was based on work performed totally at the University of Georgia and did not involve any research effort conducted at North Carolina State University.

TECHNICAL REPORT FOR LAST GRANT YEAR.

The research program developed four different areas of investigation to further analyze biosurfactant synthesis by Rhodococcus species H13-A.

1. Localization of genes encoding alkane utilization by Rhodococcus.

2. Identification of pMVS300 origin of replication.

3. Characterization of Rhodococcus sp H13-A pMVS100.

4. Miscellaneous

a. Electrophoration of Rhodococcus.

b. Transpositional Mutagenesis of Rhodococcus with Tn917.

1. Alkane Utilization Genes of Rhodococcus

The rationale for these studies resides in the fact that biosurfactant synthesis occurs only when cells are grown at the expense of alkanes as the sole source of carbon and energy. Accordingly, it is reasoned that biosurfactant synthesis is linked genetically to alkane oxidation and that the identification and characterization of the alk genes would provide information about the structural and regulator genes involved in biosurfactant synthesis. Prior evidence from this laboratory indicated that the genes encoding alkane oxidation and biosurfactant synthesis are chromosomal. The transfer and characterization of subject genes to Escherichia coli would expedite the study of these genes, since genetic systems for Rhodococcus is not well developed.

Rhodococcus H13-A chromosomal DNA was isolated and digested with the restriction endonuclease Sau3A. The shuttle vector, pMVS301, was single site cleaved with Bgl II, phosphorylated, and the chromosomal DNA fragments ligated into linearized pMVS301. The ligation mixture was used to transform competent E. coli HB101. Putative transformants were screened on LB agar containing 100 micrograms ampicillin per ml (LBA agar) and on chemically defined

agar medium containing hexadecane as the sole source of carbon and energy. Transformants which grew on LBA and/or hexadecane plates were screened for the presence of plasmid DNA larger than 10.1 kb (molecular size of pMVS301) on LBA agar by isolation of plasmid DNA. Isolated plasmid DNA was digested with Bgl II and the molecular sizes determined. The same experiments were performed with pMVS302.

RESULTS

The transformation frequency was extremely low in this series of experiments. Twelve putative transformants (L2, L6, L7, L8, L9, L11, L16, L21, L23, L31, L33, L34) were selected which contained pMVS301 with inserts, indicating the formation of a partial gene bank of Rhodococcus chromosomal DNA. Transformants L7, L8, L16, L23, and L33 were tested for growth on hexadecane-mineral salts agar medium containing 100 micrograms of ampicillin per ml(HMA agar). Repeated subculture on HMA agar resulted in the loss of L8, L16, L23, and L33 due to no growth. L7 yielded small colonies on HMA agar. Transfer of L7 to liquid medium containing hexadecane as the sole source of carbon and energy failed to yield growth of L7. Plasmid isolation from L7 grown on LBA agar indicated the presence of plasmid DNA larger than 10.1 kb, indicating the presence of chromosomal DNA insert into pMVS301.

Transformation of the plasmid derived from L7, which contained a chromosomal DNA fragment insert of approximately of 5.7 kb, into an alkane-negative mutant of Rhodococcus strain AS-50 yielded growth in the presence of hexadecane as the sole source of carbon

and energy. We were not able to determine the metabolic block in this mutant due to lack of time. Experiments employing ^{14}C-hexadecane indicated, however, the oxidation of alkane substrate to radioactive product(s). The identity of these product(s) have not been determined.

2. Characterization of p300 Origin of Replication

The 3.8 kb DNA insert of Rhodococcus H13-A pMVS300 DNA was derived from pMVS301 by restriction with HindIII and gel electrophoresis. Various enzymes were used to reduce the size of the DNA fragment, followed by religation into pIJ30 and transformation of the ligation mixture into E. coli HB101. The only transformants recovered were with religated pIJ30. Experiments unsuccessful.

3. Partial Characterization of Rhodococcus H13-A pMVS100.

pMVS100 was isolated from Rhodococcus strain E1A1 and purified by CsCl density gradient centrifugation. Restriction analysis of pMVS100 yielded the following fragmentation pattern:

HindIII-4 sites	Not I-5 sites
Nhe I-5 sites	Bgl II-7 sites

Attempted to clone the origin of replication (ori) of pMVS100. These experiments were unsuccessful in identifying a DNA fragment containing the ori. A restriction map of pMVS100 was not pursued further. The preliminary data does, however, indicate that pMVS100 has a different restriction pattern than pMVS300.

4. Miscellaneous Experiments

a. Electrophoration of Rhodococcus. A series of electrophoration studies were conducted to improve the time efficiency for transformation of Rhodococcus. These studies used

Rhodococcus H13-A strain AS-50 and pMVS301. Results of these studies showed that electrophoration of pMVS301 into strain AS-50 were successful. However, the numbers of transformants were highly variable. We were unable to routinely effect consistent transformation efficiencies and time ran out on the grant funds. These results need further development to determine the optimal conditions for consistent and efficient transformation frequencies. The electrophoration studies indicated that occassionally pMVS301 was altered in size by loss of unknown regions of pMVS301.

b. Transposons. We attempted to introduce the pTV1 ts containing Tn917 into Rhodococcus by transformation and electrophoration. Neither method was successful in introducing Tn917 into Rhodococcus H13-A.

CONCLUSIONS

1. A chromosomal DNA fragment has been cloned into pMVS301 which appears to contain gene(s) encoding the initial oxidation of alkane. Further studies are required the confirm this observation and to identify these genetic elements.
2. We were unable to develop further information on the origin of replication of pMVS301.
3. The preliminary characterization of pMVS100 indicates this native plasmid has a restriction pattern significantly different than that shown by pMVS300.
4. Electrophoration of Rhodococcus indicates transformation by this technique. Further studies are required to optimize conditions.

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