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PLASMID-MEDIATED HYDROCARBON UTILIZATION IN ACINETOBACTER PHOSPHADEVORUS

THE UNIVERSITY OF ARIZONA M.S. 1983

PLASMID-MEDIATED HYDROCARBON UTILIZATION IN *ACINETOBACTER PHOSPHADEVORUS*

by

Mickey H. Stewart

A Thesis Submitted to the Faculty of the

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STATEMENT BY AUTHOR

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ABSTRACT

The bacterium Acinetobacter phosphadevorus contains a single plasmid (pYG1) that mediates growth on the n-alkanes C_8 through C_{10}. This study determined that the molecular weight of the plasmid (pYG1) is approximately 17.8 Md. A cured strain (pYG1$^-$) of A. phosphadevorus was shown to be unable to grow on the C_{10} alcohol, 1-decanol, but was able to utilize the C_{10} fatty acid, n-decanoic acid. Furthermore, loss of the plasmid (pYG1) caused changes in the outer surface hydrophobic nature of A. phosphadevorus, as determined by Hydrophobic Interaction Chromatography. There was also a concommitant change in outer membrane protein content of the cured strain. The significance of these observations is discussed.

CHAPTER 1

INTRODUCTION

The bacterium, unofficially designated as Acinetobacter phosphadevorus (isolate P7), is a pleomorphic gram negative rod, 1.0 um by 1.5-2.0 um, occurring in pairs or clusters when grown on lab media. The characteristics of being gram negative, non-motile, non-spore forming, oxidase and catalase negative classify this organism under the genus Acinetobacter. While similar, in some characteristics, to the type species Acinetobacter calcoaceticus, P7, however, can metabolize hydrocarbons as large as C-40, and has the property of volutin granule formation.

A. phosphadevorus was originally isolated from a sewage treatment plant in San Antonio, Texas. The microbiological flora of the activated sludge at this treatment plant was screened to determine what organism, if any, was responsible for the reported removal of large amounts of phosphate. Russ (57) originally isolated P7 and determined that the low phosphate levels observed in the sewage effluent was primarily due to this organism's high affinity for luxury phosphate uptake. Roinestadt (54) further determined that P7 formed volutin on over 20 different types of laboratory media. Subsequent work on P7's nutritional needs indicated that the primary carbon source utilized was acetate (57).

Polyphosphate granule formation in P7 was studied by Kutzer (38) to determine under what optimum growth conditions that P7, unlike many organisms demonstrating volutin formation, could take up luxury amounts of phosphate without extreme deprivation of any needed nutrient.

Some of the initial work on the characterization of metabolic pathways was performed by Ferguson (17). This work presented supportive evidence for the existence of a serine pathway and the tricarboxylic acid cycle. The possibility of these pathways existing in A. phosphadevorus was first proposed by Kutzer (38). Ferguson (17) further classified P7's ability to grow on various hydrocarbons including crude oil preparations.

Early work indicated that P7 could metabolize several substrates ranging from one carbon compounds, such as formate and methanol, to long chain aliphaphitics, such as hexadecane (17, 38, 57). The existence of the serine and TCA cycles for the degradation of these metabolites had been proposed by Kutzer and Ferguson (38, 17). An enzymatic analysis of a C-1 assimilatory pathway, the serine pathway, a C-2 assimilatory pathway, the glyoxylate cycle and tricarboxylic acid cycle was performed by Jones (31). The results of Jones' work gave conclusive evidence for the operation of the TCA cycle, glyoxylate by-pass and the serine pathway in A. phosphadevorus.

In light of Ferguson's preliminary characterization of P7's hydrocarbon growth abilities, Nash (43) undertook a detailed study of the mechanisms used by P7 in hydrocarbon degradation. Electron

micrographs revealed the presence of inclusion bodies within the cells when grown on hexadecane. Subsequent work showed that this inclusion body consisted of unmodified hexadecane and appeared to resemble inclusion bodies occurring in other Acinetobacter species (59). The incorporation of hexadecane into these inclusion bodies was found to be rapid within the first 24 hours of growth. Examination of the cells under starvation conditions using electronmicrographs indicated the disappearance of these hexadecane inclusions. The conclusion drawn was that A. phosphadevorus removed hexadecane from the outside medium in large quantities or in a matter indicative of luxury uptake and utilized this stored component under starvation conditions (43).

Hexadecane, after transport into the cell, was in part placed into storage bodies and also directed into metabolic processes. The particular pathway for hydrocarbon degradation was not elucidated. The most common pathway for utilization of fatty acids is that of beta oxidation (25, 30, 41). A comparison of ^{14}C labeled palmitic acid and ^{14}C labeled hexadecane revealed that the utilization of palmitic acid may be accomplished through beta-oxidation, and that utilization of hexadecane is not similar to that of palmitic acid (43).

A. phosphadevorus was observed to grow on aliphatic hydrocarbons ranging from 12 to 40 carbons in length (43). Challenged with a mixture of hydrocarbons of varying lengths, the organism showed a propensity for dodecane over long chain compounds such as tetracontane. When exposed to acetate, the carbon source most abundant in its natural habitat, and hexadecane, A. phosphadevorus preferentially

utilized the hydrocarbon. This suggested that hexadecane either inhibited the uptake or metabolism of acetate. Nash's work provided data which was subsequently used in this laboratory to study the molecular mechanism by which P7 utilized hydrocarbons.

DNA hybridization studies of Kronland (unpublished data), revealed a plasmid in A. phosphadevorus. Garvey (21) subsequently characterized the plasmid (designated as pYG1) and determined the molecular weight. Other related strains of Acinetobacter spp. were examined for plasmid presence. This study revealed that two of the related Acinetobacter sp. contained plasmids.

Plasmid function was analyzed by creating a strain devoid of pYG1. This was accomplished by exposing A. phosphadevorus to ethidium bromide. The strain developed from this procedure was designated as EB22 (designated as $P7P^-$ in this study). Strain EB22 ($pYG1^-$) was then examined for loss of drug resistance, metal ion resistance, and luxury phosphate uptake. The results indicated that pYG1 played no role in any of these activities (21). When analyzed for growth on a series of substrates, however, the strain could not grow on the n-alkanes C_8 through C_{10}, although it grew well on other alkanes tested (C_{11}-C_{18} and C_{20}). Further analysis of a series of hydrocarbon mutants generated from both strains of A. phosphadevorus ($pYG1^+$ and $pYG1^-$) established a pattern for hydrocarbon degradation mechanisms. Garvey (21) postulated that A. phosphadevorus utilized at least two chromosomally-mediated n-alkane catabolic pathways, differing by one or more enzymes. It was suggested that the pathways may have an overlap in function,

however the specific interaction has yet to be determined. The role of pYG1 in hydrocarbon degradation and its interaction with chromosomally mediated mechanisms was not resolved.

The term plasmid was originally used by Lederberg (39) to describe all extrachromosomal hereditary determinants. More precisely defined, plasmids are considered extrachromosomal elements which are physically distinct from the chromosome of the cell and are able to be perpetuated stably in this condition (9). Plasmids are generally considered non-essential for the growth of the cell, but provide genetic properties conferring upon the host bacterium selective advantages under varying environmental conditions (10).

Plasmids occur in a wide variety of bacteria, and can be transferred from one bacterium to another within the same genus or even into bacteria of different genera (9, 15). The sizes of plasmids vary greatly and range from 0.5 to 300 megadaltons (10). The functions coded for by plasmids consist of antibiotic resistance, resistance to metal ions, the production of bacteriocins, hemolysins, and degradation of a wide variety of substrates (12, 45, 46).

Pseudomonas species have long been known to utilize a wide range of organic compounds including hydrocarbons as sources of carbon and energy (61). Indeed, Stanier (61) observed that pseudomonads were able to degrade over 160 various compounds. While many of the compounds were catabolized by action mediated at the chromosomal level, it was also shown that some of the catabolite activities were directed by plasmid-specified enzymes (23, 67).

Chakrabarty (5) discovered the SAL plasmid, responsible for salicylate utilization, in a strain of Pseudomonas . It was later noted that CAM (camphor); OCT (octane); NAH (naphthalene); and TOL (benzoate and toluate) were also plasmid-borne characteristics. The SAL and NAH plasmids carried genes coding for complete degradative pathways while CAM acted in conjunction with chromosomal gene products to effectively catabolize their respective substrates (5, 14, 53). It was also observed that not only did plasmid presence confer unique abilities beyond chromosomal capabilities for substrate degradation but also often coded for similar enzymes. Williams and Murray showed that some strains of Pseudomonas (TOL^+) had the ability to oxidize benzoate to catechol by a plasmid-specified enzyme. Other strains (TOL^-), however, used a chromosomally specified enzyme for the same function (68).

Many strains of Pseudomonas have been shown to grow on n-alkanes and oxidize these compounds by the following pathway: $R\text{-}CH_3 \rightarrow R\text{-}CH_2OH \rightarrow R\text{-}CHO \rightarrow R\text{-}COOH$ beta oxidation (Grund et al., 22). Pseudomonas putida strains can grow on n-alkanes hexane through decane due to the action of a transmissible OCT plasmid (22). These authors have shown that the OCT plasmid acts in concert with the chromosome to effect complete oxidation of n-alkane compounds. In particular, it was shown that this OCT plasmid codes for an inducible alkane-hydroxylating and primary alcohol-dehydrogenating activity while the chromosome codes for the constitutive oxidizing activities of the primary alcohols.

Chakrabarty (7) has shown that the genes coding for hydrocarbon degradation in many plasmids are transposable. A study using the organisms Escherichia coli, Salmonella typhimurium, Agrobacterium tumefaciens and Agrobacterium vinelandii showed that the same transposable genes did not express themselves equivalently in these strains. He proposed that these genes were under regulatory control by chromosomal genes.

Other degradative functions that are plasmid mediated in Pseudomonas sp. include chlorinated aromatics such as 2-methyl-4-chlorophenoxyacetic acid; 3,5-xylenol; pseudocumene and 3-ethyltoluene; and steroid catabolism (36, 37, 63). Organisms such as Flavobacterium sp., Alcaligenes paradoxus, Arthrobacter crystallopoietes, and E. coli have been shown to degrade 6-aminohexanoic acid, pesticides, 2-hydroxypyridine, and ribonucleic acids, respectively, through plasmid mediated mechanisms (19, 44, 47, 66).

Plasmids occurring in Acinetobacter spp. were first described by Christiansen (8) in 1973. Two strains of Bacterium anitritum (currently Acinetobacter calcoaceticus) were shown to contain two plasmids, which coded for resistance to the antibiotics sulfonamide and ampicillin. Later work on Acinetobacter spp. has shown one species to contain a plasmid that codes for mercury resistance (48). Work by Hinchcliffe and Vivian (27, 28) has shown that a strain of Acinetobacter calcoaceticus contained a plasmid (pAV1) which coded for resistance to sulphonamides and mediated chromosome transfer. A second plasmid (pAV2) has been determined to code for entry

restriction of RP_4, R388, and S-A plasmids into Acinetobacter calcoaceticus (29). The plasmid pAV2 was also noted to affect the frequency of pAV1 transmissibility (26).

Organisms that grow on hydrocarbons are often observed to have a notable hydrophobic nature of the outer membrane (55). This property has also been cited in this paper as a factor in the partitioning of microorganisms at interfaces (55), in the adherence of bacteria to nonwettable plastic surfaces (55), in the attachment of bacteria to phagocytes (55), and other mammalian cells (55). One method used to assess outer surface hydrophobicity of bacteria relied upon the attachment of these bacteria to polystyrene surfaces (20). Work by Rosenberg with A. calcoaceticus indicated that a mutant which lacked cell surface hydrophobicity was unable to adhere to polystyrene plates (55).

The polystyrene plate assay can only characterize outer surface hydrophobicity in general terms. Hydrophobic interaction chromatography (HIC) is a method which examines the interaction of hydrophobic moities of the cell surface to specific compounds affixed to a gel matrix. HIC involves the use of either agarose or sepharose gels with conjugated side chains of varying hydrophobic nature (62). Separations by HIC are dependent on interactions between three principle components: the hydrophobic matrix, the solvent water and the hydrophobic solute (52). The use of HIC is a simple and useful means of discriminating between bacterial strains with respect to the hydrophobicity of their surfaces (62). This paper suggested that the

pilus-like K88 antigen, with a proponderance of apolar side chains, was responsible for binding to the various derivatized side chains in HIC.

It has been shown that fimbriae are a major factor in adherence to polystyrene and hydrocarbons (56). The authors of this paper examined various strains of A. calcoaceticus and observed that strains lacking thin fimbriae were unable to adhere to polystyrene or grow on hexadecane. Various hydrophobic properties have been associated with bacterial fimbriae. Fimbriae contribute to the ability of various bacterial cells to be retained on hydrophobic gels (16, 62). Analysis of amino acid residues of fimbriae from species of Neisseria, Moraxella, and Pseudomonas have been shown to contain a high proportion of hydrophobic groups (50).

The purpose of this study was to further characterize properties mediated by the plasmid pYG1. Equivalent carbon length hydrocarbons, alcohols, and fatty acids were used as growth substrates to resolve degradative pathways coded for by pYG1. Analysis of hydrophobic properties conferred by plasmid action was examined by HIC and polystyrene plate adherence. The production of outer membrane proteins as regulated by pYG1 was assayed by use of the Triton-X100 procedure (1).

CHAPTER 2

MATERIALS AND METHODS

Bacterial Strains and Plasmids

Acinetobacter phosphadevorus containing the plasmid pYG1 (P7) and its isogenic derivative cured of pYG1 ($P7P^-$) were obtained from Irving Yall, Ph.D. *Acinetobacter calcoaceticus* (originally ATCC #19606), *Herella vaginicola* (originally ATCC #12359), *Acinetobacter anitritus* (originally ATCC #12381) were obtained from the stock culture collection at the Department of Microbiology, University of Arizona. *Yersinia enterocolitica* and *Escherichia coli* strains designated at TAMU 70, TAMU 72, TAMU 146, and JD 071 were obtained from Jacqueline Dubel, Department of Microbiology, University of Arizona.

Media

Carbon-free basal medium contained per liter of double distilled water: 1.5 g sodium ammonium phosphate ($NaNH_4HPO_4$), 1.0 g monobasic potassium phosphate (KH_2PO_4), and 0.2 g magnesium sulfate-7-hydrate ($MgSO_4 \cdot 7H_2O$).

Peptone and peptone plus Tween 80 contained per liter of basal medium: 30 g Bacto-peptone (Difco) and 1.0 ml of Tween 80 (Atlas Chemical Industries), respectively.

L-Broth consisted of: 10 g tryptone (Difco), 5.0 g yeast extract (Difco), 5.0 g sodium chloride (NaCl), and 1.0 g of glucose per liter of distilled water.

Trypticase soy broth (TSB, BBL) plus yeast extract (TSB+YE) had the following composition per liter of double distilled water: 17 g tryptone peptone, 3.0 g phytone peptone, 5.0 sodium chloride (NaCl), 2.5 g dipotassium phosphate (K_2HPO_4), and 0.6 g yeast extract.

The pH of all media was adjusted to 7.5 with 1 M KOH, unless otherwise indicated. Solid media was prepared by adding 15 g of agar (Difco) to one liter of the appropriate broth.

Chemicals

All chemicals were of reagent grade. Hydrocarbons, alcohols, and fatty acids used for growth studies and as supplements for selective media had a purity exceeding 97 percent and were purchased from the Sigma Chemical Company (St. Louis, Missouri). TEMED (N,N,N',N'-Tetramethylethylene diamine), sodium dodecyl sulfate (SDS), ammonium persulfate, beta-mercaptoethanol (B-ME), acrylamide, and bis-acrylamide were of electrophoresis quality and were obtained from Bio Rad (Richmond, California). Hydrophobic interaction chromatography gels consisting of CL-4B sepharose, and CL-4B sepharose with conjugated side groups of either octyl of phenyl, and dodecyl-agarose were purchased from Sigma Chemical Company.

Growth Conditions

Bacterial strains P7, P7P$^-$, A. calcoaceticus, H. vaginicola, and A. anitritus were grown in 500 ml of peptone media. Broth cultures for mini-lysate preparations were grown in 3.0 ml of peptone broth contained in a 10 ml screw cap tube. Aeration for broth cultures was provided by continuous shaking on a New Brunswick Rotary Shaker (model C.S.) at 200 rpm. Plate and broth cultures were incubated at 25 C, unless otherwise indicated.

For transformation studies, strain P7P$^-$ was grown in 25 ml of L-Broth contained in a 125 ml Erlenmeyer flask. Aeration and incubation conditions were as described above.

Strains JD 071, TAMU 70, TAMU 72, and TAMU 146 were grown in 3 ml of TSB+YE media contained in 10 ml screw cap tubes. Incubation was at 37 C without aeration in a FREAS (Model 815) Precision Scientific Company) incubator.

Yersinia enterolitica used in the Rapid Polystyrene Plate method was grown in TSB+YE medium (25 ml per 125 ml Erlenmeyer flask) to mid log phase and then transferred to peptone solid medium and incubated at 25 C.

Centrifugation Conditions - Cell Harvesting

Cells were harvested in a refrigerated centrifuge (Beckman Model J2-21) equipped with a Beckman JA 20 rotor, at 10,000 rpm (12,000 x g) at 4 C for 15 min., unless otherwise indicated.

Growth Substrate Conditions

Hydrocarbon and alcohol growth substrates were tested by streaking a carbon free Basal Medium agar plate. A 2 cm x 2 cm square of Gelman Saturation pad (Gelman Instrument Company) was taped to the top of the petri dish and saturated with approximately 0.1 ml of the appropriate hydrocarbon or alcohol. The plates were sealed with parafilm and incubated at 25 C for three days or until growth was observed.

Fatty acid growth substrates were tested by solubilizing the fatty acid to be tested in diethyl ether and then streaking the surface of a carbon free Basal Medium agar plate. Bacterial strains were then streaked onto the plate surface and incubated as described above.

Mini-Lysate Preparation for Plasmid DNA Analysis

Cells were grown in 10 ml screw cap tubes in 4 ml of TSB+YE or peptone broth, as appropriate for strain type, to late-log phase (1). Approximately one ml cell aliquots were transferred to 1.5 ml polypropylene tubes, and sedimented in a Microcentrifuge (Beckman, Microfuge B) at approximately 8,000 x g, for 5 min. at room temperature. The cells were washed once with 1.0 ml of TE buffer (50 mM Tris-hydrochloride, pH 8.0, 10 mM Na_2EDTA), recentrifuged, and resuspended in 40 ul of TE buffer. Cells were lysed and chromosomal DNA denatured by adding 0.6 ml of Lysis buffer (TE + 4% SDS, pH 12.42) and incubating at 37 C for 20 min. After denaturation, the samples were neutralized by adding 30 ul of 2 M Tris-HCl (pH 7.0) and gently mixed. To this was added 240 ul of 5 M NaCl and the mixture was incubated at 0 C for

at least 60 min. The precipitated chromosomal DNA was pelleted (5 min. at 25 C in the microcentrifuge), the supernatant fractions transferred to another polypropylene tube, and 550 ul of cold isopropanol (-20 C) was added. The supernatant fraction was incubated for 30 min. at -20 C, and the precipitate pelleted by centrifuging for 5 min. in the microcentrifuge. The pellet was air-dried and resuspended in 30 ul of TES buffer (30 mM Tris-hydrochloride, pH 8.0, 5 mM EDTA, 50 mM NaCl). The samples were analyzed immediately by agarose gel electrophoresis.

Agarose Gel Electrophoresis of Plasmid DNA

Mini-lysates were electrophoresed in 0.7% agarose (Bethesda Research Laboratories), dissolved in Tris-borate buffer (89 mM Tris base, 2.5 mM Na_2EDTA and 89 mM boric acid). A 15 ul aliquot of each plasmid DNA sample was mixed with 5 ul tracking dye solution (0.07% bromphenol blue (Kodak), 75% SDS, 33% glycerol) and loaded onto the gel. Electrophoresis was conducted on a horizontal gel apparatus (BRL, Model HO), using an LKB power supply (Biochrom, Model 2103) at 60 mA, 125 V, until the dye reached the bottom of the gel (approximately 12 h.). The gel was then stained in Tris-borate buffer containing 1 ul/ml of ethidium bromide (Cal Biochem, stock solution consisted of 10 ug/ml in distilled water) for 30 min. Excess ethidium bromide was removed prior to photography by rinsing the gel for 30 min. in tap water. The gel was illuminated for purposes of viewing and photography on a transilluminator (Ultra-Violet Products, Inc., Chromato-Vue,

Model C-63). The gel was photographed with a 35 mm camera (Canon, Model A1) on Plus X Pan film (Kodak) using a combination of red 23A and orange 15 filters to enhance contrast. The distance of migration of the plasmid DNA bands were measured on prints of the photographic negative.

Plasmid DNA Molecular Weight Determination

Four bacterial strains containing plasmids of known molecular weight were used to determine the rate of migration with respect to molecular size. Strains used were: JD 071, containing plasmids of 36, 3.7, 3.4, 2.6, 2.0, 1.8, and 1.4 Md; TAMU 70, containing a plasmid of 25 Md; TAMU 72, with a plasmid of 36 Md; and TAMU 146, with a single plasmid of 65 Md.

A plot of the log of relative mobility vs. the log of molecular weight yielded a linear curve. This relationship was used to determine the size of the plasmid contained within Acinetobacter phosphadevorus.

Outer Membrane Protein Isolation

A modification of the procedure as described by Achtman et al. (1) was used to isolate outer membrane proteins. Strains P7 and P7P$^-$ were inoculated into one liter of peptone broth and grown to stationary phase. The cultures were then transferred to pre-cooled (0 C) 250 ml polypropylene centrifuge bottles and centrifuged at 10,000 x g for 10 min. at 4 C. The supernatant phase was discarded and the cell pellets were resuspended in 10 ml of 10 mM Tris-HCl (pH 8.0) containing 5 mM $MgCl_2$ by using a vortex mixer. The cell suspensions were

transferred to 15 ml polypropylene beakers and sonicated on a sonifier (Branson, Cell Disruptor Model 200), at the maximum setting, for two 45 second bursts. The beakers were maintained in an ice bath during this procedure. The resulting suspensions were placed in 30 ml Corex centrifuge tubes and centrifuged at 750 x g at 4 C for 20 min. The supernatants were collected and respun at 48,000 x g for 60 min. at 4 C. The supernatant layers were discarded and the pellets resuspended in 500 ul of sterile distilled water. From the 500 ul solution, 300 ul was removed and stored at -20 C for future analysis. The remaining 200 ul portion was maintained in the Corex tube to which was added 400 ul of extraction buffer (final concentration of 2% v/v Triton X-100, 10 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$). The mixtures were vortexed and incubated at room temperature for 20 min. The Triton insoluble outer membrane proteins were pelleted by centrifuging at 48,000 x g for 60 min. at 4C. The supernatants were discarded and the pellets resuspended in 100 ul of sterile distilled water and 100 ul of 2X electrophoresis sample buffer (62.5 mM Tris, pH 6.8, 10% glycerol, 0.001% bromphenol blue, 5% B-ME, and 2% SDS). The samples were boiled for approximately 5 min. prior to being analyzed by SDS-polyacrylamide gel electrophoresis.

SDS-Polyacrylamide Gel Electrophoresis of Outer Membrane Proteins

Outer membranes isolated from P7 and P7P$^-$ were analyzed by SDS-PAGE. The protocol is a modification of the procedure as described in the manufacturer's instructions (Hoefer Scientific Instruments).

Gel Preparation

A vertical slab gel electrophoresis unit (Hoefer Scientific Instruments, Model SE520) was used. The running gel consisted of 15.3 ml of distilled water, 18.8 ml of 1.5 M Tris-HCl, pH 8.8, 0.75 ml of 10% SDS, 40 ml of acrylamide-bis (30:0.8), and 0.11 ml of ammonium persulfate. This solution was mixed and degassed. Lastly, 0.038 ml of TEMED was added, combined solutions were rapidly mixed and placed into a 60 ml syringe and applied carefully into the gel apparatus. After solidification of the running gel (approximately 1 h.), a staking gel consisting of 6.1 ml distilled water, 2.5 ml 0.5 M Tris-HCl, pH 6.8, 0.1 ml of 10% SDS, 1.3 ml acrylamide-bis (30:0.8), 0.06 ml ammonium persulfate and 25 ul TEMED was prepared and applied to the gel apparatus. Sample wells were formed by the use of a Teflon comb with 20 teeth inserted into the stacking gel. After gel solidification (1 h.), the Teflon comb was removed resulting in sample wells of approximately 25 ul capacity. Prior to sample application, 5.0 liters of running buffer (containing per liter of distilled water: 3.03 g Tris base, 14.4 g glycine, 1.0 g SDS) were added to the electrophoresis unit.

Sample Application

Samples were applied to wells using a 10 ul syringe (Hamilton). Quantities ranging from 5 ul to 15 ul were used depending upon respective protein concentrations.

Gel Electrophoresis

Electrophoresis was carried out using a power supply (LKB, Model 2103) set at 20 mA, 125 V. The gel was electrophoresed for 10 h. The electrophoresis unit was cooled by use of a recirculating water bath (Brinkman, Model Lauda K 2/R) at a temperature of 10 C.

Staining of Gel

The gel was stained overnight in a solution containing 0.25% Coomassie Blue (Bio-Rad, electrophoresis grade), 45.4% methanol and 9.2% acetic acid in distilled water. The gel was destained for approximately one h. in a solution consisting of 30% ethanol and 10% acetic acid in distilled water. The resulting protein bands were visualized using a fluorescent light source. The gel, if not immediately examined after destaining, was stored in a Preserving Solution consisting of 30% ethanol, 10% acetic acid, and 10% glycerol in distilled water.

Photography of Gel

The gel was photographed with Tech Pan film (Kodak) by use of a Polaroid MP4 camera equipped with a Nikor 135 mm lens and a number 15 yellow filter.

Rapid Polystyrene Plate Assay

Test strains were examined for their ability to adhere to polystyrene plates. The method used is a modification as described by Rosenberg (55). The procedure consisted of incubating the organisms overnight in peptone broth or TSB+YE and then swabbing onto a peptone

agar plate (four strains were tested per plate). The plates were incubated at 25 C for 24 h. or until a confluent lawn was observed. A polystyrene petri dish (Falcon) was then pressed onto the surface of the agar containing the bacteria. The replica of the lawns obtained on the polystyrene surface was then washed for 2 min. under a vigorous stream of tap water. After all loose cells were removed, the plate was fixed by addition of methanol and stained with Gentian Violet to enhance visualization.

Hydrophobic Interaction Chromatography

Bacterial strains P7 and P7P$^-$ were analyzed for their ability to adhere to hydrophobic gels.

Growth Conditions

Strains were grown in peptone medium to stationary phase and swabbed onto peptone plus Tween 80 medium agar plates. Plates were then incubated at 25 C for approximately 24 h. or until a confluent lawn of growth was observed. Organisms were then resuspended into Basal Medium at a concentration of approximately that of a MacFarland standard #3.

Chromatography

The procedure used is a modification of that used by Cyril Smith (62). Gels consisting of CL-4B sepharose, CL-4B sepharose with conjugated side groups of octyl or phenyl, and dodecyl-agarose were washed thoroughly with 3% SDS followed by several rinses with the

Hydrophobicity Eluant (50 mM Tris, pH 8.0, 10 mM NaCl) to remove any residual contaminating material. Toluene (0.2%) was added to the gel suspensions as a preservative. Gel suspensions were allowed to equilibrate at approximately 23 C and chromatographed at the same temperature. Columns were obtained from Bio-Rad (Standard Econocolumns #5) with dimensions of 1.0 x 4 cm. A gel bed volume of 0.6 ml was used for all experiments. Bacterial suspensions of 100 ul were allowed to drain into the gel matrix. The gel beds were then washed with the Hydrophobicity Eluant and 1.0 ml fractions were collected.

Absorbance Measurements of Bacterial Fractions

All measurements were made using a spectrophotometer (Beckman, Model DU-5) at an absorbance of 600 nm with a one cm light path. A sample of the Hydrophobicity Eluant run through the control column CL-4B was used to establish a baseline reading.

Transformation Assay

Transformation of P7P$^-$ using purified plasmid from P7 was attempted using a modification of the method by Sawula and Crawford (58). Modifications were as follows: P7P$^-$ (recipient strain) was grown to mid log in a 250 ml Erlenmeyer flask containing 50 ml of peptone medium. The cells were harvested and washed twice in Basal Medium. The pellet was resuspended in 10 ml of Basal Medium. From this suspension, one ml samples were evenly spread over the surface of five agar plates of Basal Medium plus decane. To each of these plates was added purified plasmid DNA in quantities ranging from 5-10 ul.

Plates were incubated at 25 C for 48 h. or until visible colonies were observed. An additional method used consisted of growing the recipient strain to mid-log phase in L-Broth (25 ml of broth in 125 ml Erlenmeyer flasks) and adding 25 ul of purified plasmid DNA. The cells were harvested and placed on Basal Medium plates plus decane, without addition of plasmid DNA directly to plate, and treated as above. Colonies were picked and restreaked to Basal Media agar plates plus decane to avoid possible auxotrophs. After two streak isolations, colonies were placed in 5.0 ml screw cap tubes containing 3.0 ml of peptone broth, grown overnight and assayed for plasmid presence using the Mini-Lysate procedure.

CHAPTER 3

RESULTS

The results presented in this section represent an examination of the plasmid pYG1 contained in P7. Previous work concentrated on the plasmid's role in antibiotic sensitivity, metal cation and oxyanion resistance, and luxury phosphate uptake abilities (21). This study is concerned with characterizing pYG1 at a molecular level, as well as defining several physical properties mediated by its action.

Initial descriptions of the strain P7P$^-$ indicated that the organism had lost its ability to utilize the hydrocarbon decane (21). The present study further elucidates on the metabolic capabilities of P7 with respect to plasmid involvement. Equivalent hydrocarbon, alcohol, and fatty acid derivatives were screened to determine plasmid function in the recognition and subsequent use of similar carbon length compounds. Hydrophobicity properties conferred by pYG1 were tested using the rapid polystyrene plate assay and the procedure of hydrophobic interaction chromatography. Molecular and physical properties of pYG1 were determined by use of mini-lysate plasmid analysis. In light of the differential substrate utilization and altered surface states between P7 (pYG1$^+$) and P7 (pYG1$^-$), Triton X-100 analysis of outer membrane proteins was performed. The findings of this study suggest that there are significant differences between strains P7 (pYG1$^+$) and P7 (pYG1$^-$).

Growth Characteristics of Acinetobacter phosphadevorus

Growth characteristics of P7 and P7P$^-$ were compared by observing colony formation on basal medium agar plates supplemented with the appropriate substrate. The two strains were screened for their ability to utilize equivalent carbon length derivatives of either hydrocarbon, alcohol, or fatty acid. This study focused on a comparison of the abilities of P7 and P7P$^-$ to distinguish variations between compatible carbon length substrates.

It was observed that the wild-type strain P7 grew on all of the substrates examined (Table 1). P7 showed no distinct preference for carbon length of C_{10}, C_{12}, or C_{16}. There was, however, a preference for hydrocarbons over alcohol and fatty acid derivatives. The fatty acid compounds were utilized more slowly than either hydrocarbons or alcohols. Work by Nash (43) indicated that the rate of uptake for C_{10} was much greater than that for C_{16}. However, the method used in this study was strictly qualitative, examining only for the presence of growth and not the specific rate of uptake.

Strain P7P$^-$ did not grow on decane or 1-decanol, the alcohol equivalent of decane. Examination of growth on the C_{10} fatty acid (decanoic acid) revealed that the organism was capable of utilizing this compound. P7P$^-$ was shown to grow on all of the other substrates tested (Table 1).

Mini-Lysate Analysis

Strains P7, P7P$^-$, and A. calcoaceticus were screened for the presence of plasmids using the SDS-alkaline pH procedure. P7P$^-$

Table 1. Growth Characteristics of Acinetobacter phosphadevorus

Derivative	Substrate	Strain P7	Strain P7P⁻
HYDROCARBON	N-decane (C_{10})	+	-
	N-dodecane (C_{12})	+	+
	N-hexadecane (C_{16})	+	+
ALCOHOL	1-decanol (C_{10})	+	-
	Dodecanol (C_{12})	+	+
	Hexadecanol (C_{16})	+	+
FATTY ACID	N-decanoic acid (C_{10})	+	+
	Dodecanoic acid (C_{12})	+	+
	Hexadecanoic acid (C_{16})	+	+

contains no plasmids while P7 contains a band representing one single plasmid species (Figure 1). These results are in agreement with those reported by Garvey (21). The type species A. calcoaceticus contained three plasmids of smaller molecular weight.

Analysis of plasmid molecular weight was performed using four standard marker strains containing plasmids of known molecular weight. Figure 2 illustrates the results of the four marker strains as compared with P7 and A. calcoaceticus. Figure 2 is a graphic representation of the molecular weight determinations. A linear relationship between plasmid molecular sizes (marker strains) and relative mobility in the agarose gel was observed. The rate of migration of the plasmids contained in P7 and A. calcoaceticus was measured and when plotted on this graph respective weights were derived. A. calcoaceticus contained three plasmids with weights of 10.0, 9.4, and 6.4 Md., respectively. P7 contained a single plasmid with a molecular weight of 17.8 Md.

The original molecular weight as reported by Garvey (21) was 29 Md. This discrepancy in results may be attributed to either differential electrophoresis conditions or perhaps an erroneous measurement of an open circular form of the plasmid, which runs at a much slower rate.

Outer Membrane Protein Analysis

Proteins occurring in the outer membrane region of strains P7 and P7P$^-$ were examined by the Triton X-100 procedure and SDS

Figure 1. Agarose Gel Electrophoresis of Partially Purified Plasmid DNA

Lane A. TAMU 70, 15 ul sample containing a single plasmid with a molecular weight of 25 Md

Lane B. JD 071, 15 ul sample, containing 5 plasmids with molecular weights of 36 Md, 3.7 Md, 2.6 Md, 1.8 Md, and 1.4 Md

Lane C. TAMU 72, 15 ul sample, containing a single plasmid with a molecular weight of 36 Md

Lane D. TAMU 146, 15 ul sample, containing a single plasmid with a molecular weight of 65 Md

Lane E. A. phosphadevorus (P7), 20 ul sample

Lane F. A. phosphadevorus plasmid minus ($P7P^-$), 20 ul sample

Lane G. A. phosphadevorus (P7), 20 ul sample

Lane H. A. calcoaceticus, 15 ul sample

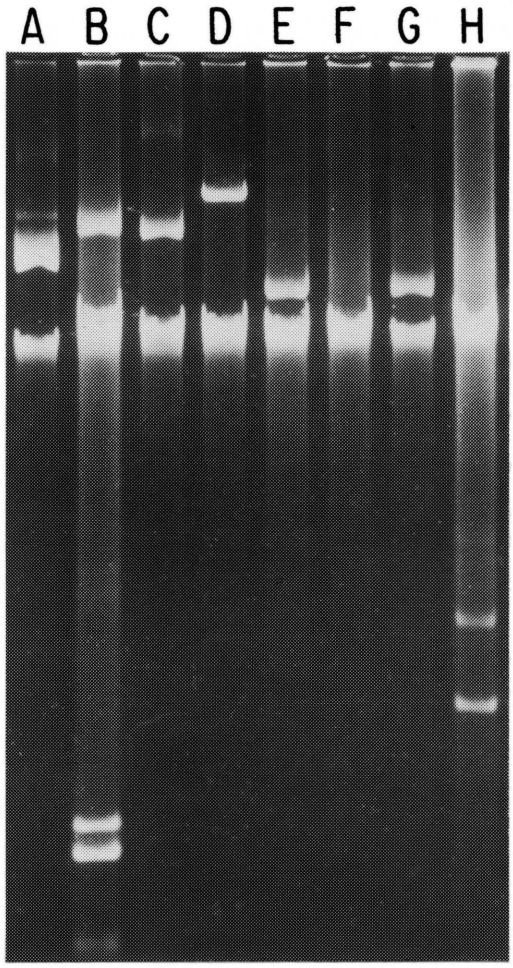

Figure 1. Agarose Gel Electrophoresis of Partially Purified Plasmid DNA

Figure 2. Representative Plot of the Log of Molecular Weight Versus the Log of the Relative Mobility of Plasmid DNA Standards and Plasmid DNA from A. phosphadevorus (P7) and A. calcoaceticus

Plasmids from:

◇ , P7 (17.8 Md)

△ , A. calcoaceticus (containing plasmids of 10.1 Md, 9.4 Md, and 6.4 Md)

● , JD 071 (containing plasmids of 36 Md, 3.7 Md, 2.6 Md, 1.8 Md, and 1.4 Md)

 , TAMU 70 (25 Md)

 , TAMU 72 (36 Md)

□ , TAMU 146 (65 Md)

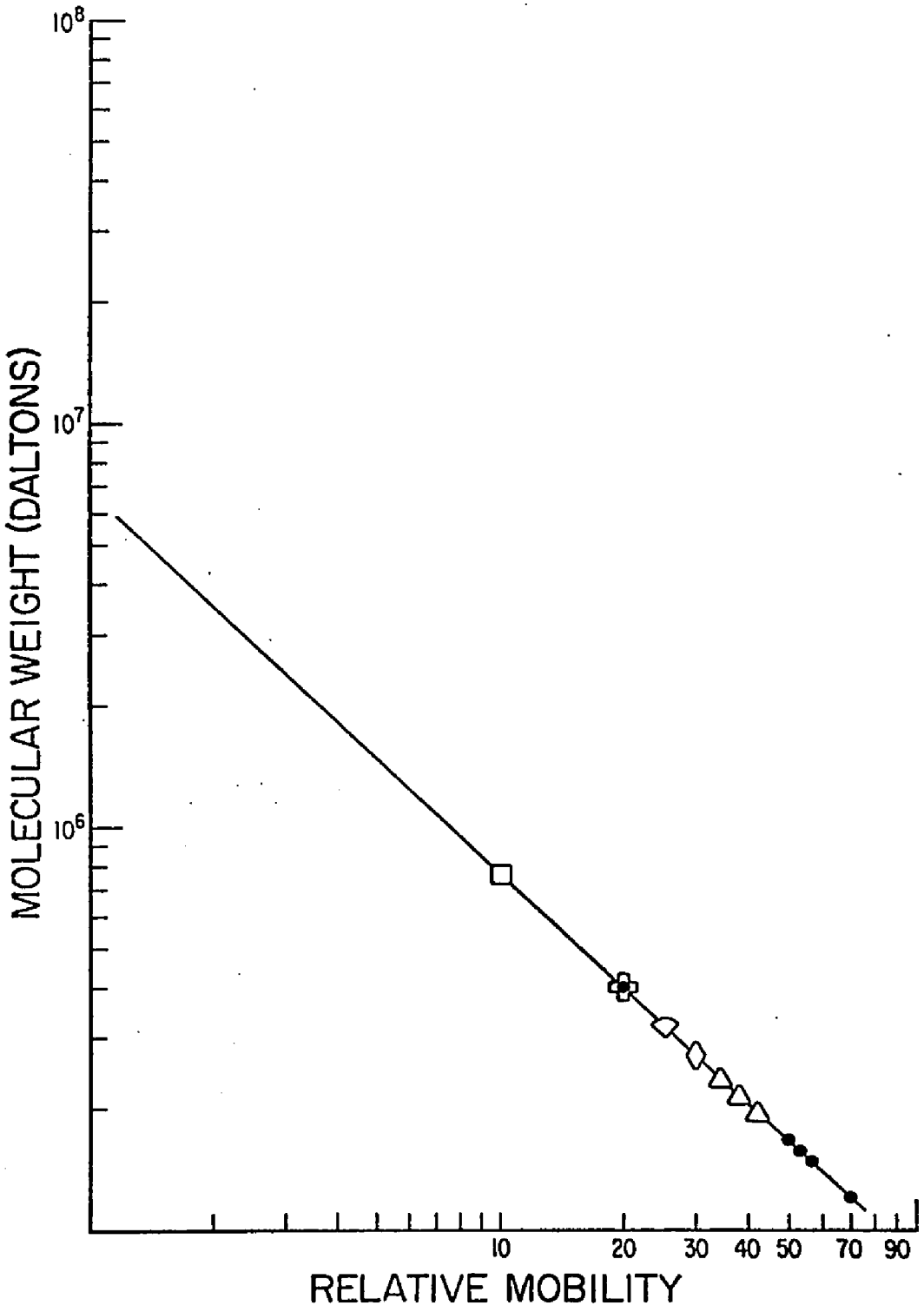

Figure 2. Representative Plot of the Log of Molecular Weight Versus the Log of the Relative Mobility of Plasmid DNA Standards and Plasmid DNA from A. phosphadevorus (P7) and A. calcoaceticus

polyacrylamide gel electrophoresis. Results are shown in Figure 3.

Lane A shows the relative number of polypeptides occurring in the outer cell membrane of P7, and Lane B shows the polypeptide profile of P7P$^-$. The latter strain contained fewer proteins, in particular proteins of lower molecular weight. High molecular weight proteins present in both strains were similar, but with several bands absent for P7P$^-$. Since similar heavy-weight proteins were of comparable intensity, it is suggested that the absence of small proteins in the P7P$^-$ lane is not a result of insufficient sample concentration but rather the lack of production of these proteins by P7P$^-$.

Rapid Polystyrene Plate Assay

Adherence to polystyrene is a property often associated with organisms that interact with hydrophobic compounds (56). This test gives some indication of the nature of the outer surface structure of an organism, particularly fimbrae and/or pili arrangement. Strains P7 and P7P$^-$, along with related species and control organisms, were assayed for their ability to adhere to polystyrene plates.

Both P7 and P7P$^-$ had the ability to bind to the polystyrene surface of the petri dish (Figure 4). Thus, the absence of the plasmid in strain P7P$^-$ appears to play no significant role in adherence of the organism to polystyrene. *A. calcoaceticus*, *H. vaginicola*, and *A. anitritus* are strains that are related to *A. phosphadevorus*. Figure 4 clearly indicates that each of these strains had the ability to bind to the polystyrene surface. These three organisms were also tested for their ability to grow on a series of hydrocarbons ranging from C_{10}

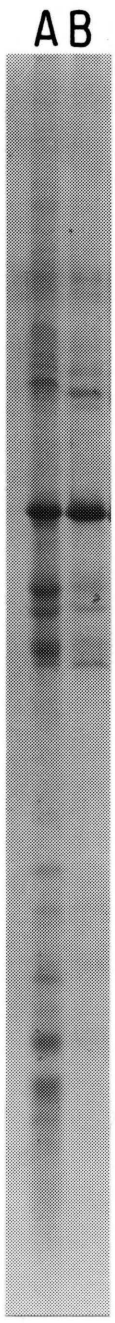

Figure 3. SDS-Polyacrylamide Gel Electrophoresis of Outer Membrane Proteins from Strains P7 and P7P$^-$

Lane A. Outer Membrane Protein bands of P7
Lane B. Outer Membrane Protein bands of P7P$^-$

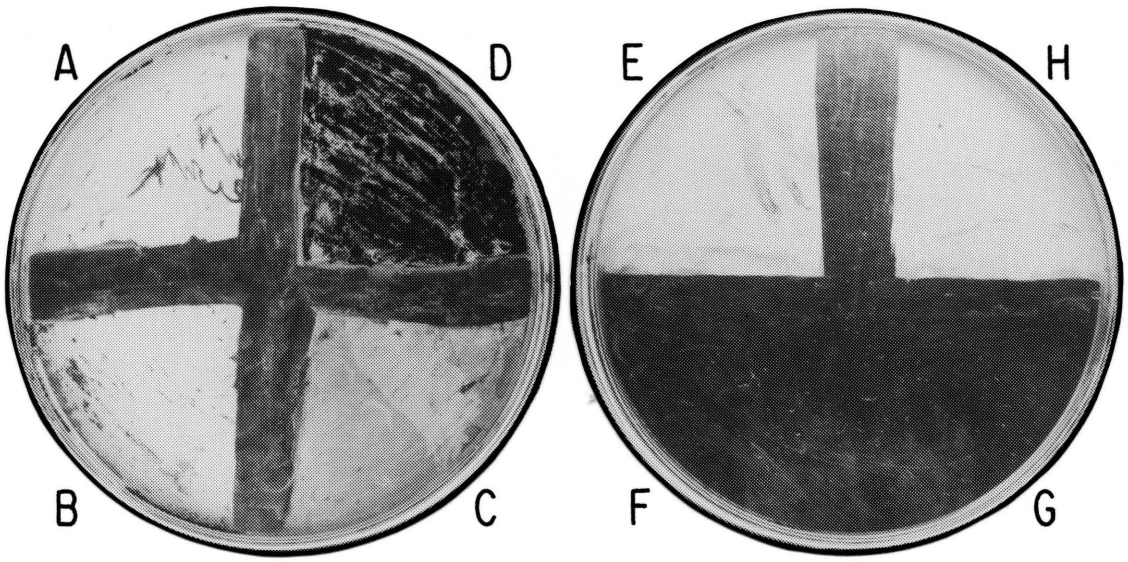

Figure 4. Rapid Polystyrene Plate Assay

Replica imprint of quadrant:

A - P7
B - P7P$^-$
C - Acinetobacter calcoaceticus
D - Herella vaginicola
E - Acinetobacter anitritus
F - TAMU 70
G - Yersinia enterocolitica
H - P7

to C_{17} in length (author's unpublished data). A. calcoaceticus showed minimal growth on C_{10} and C_{11}, while H. vaginicola showed minimal growth on C_{10}, C_{11}, and C_{12}. A. anitritus did not grow on any of the hydrocarbons tested. Thus, the ability to grow on the hydrocarbons had little correlation to the organisms' ability to bind to polystyrene surfaces.

Y. enterocolitica and TAMU 70 were used as negative controls. Figure 4 clearly shown that neither Y. enterocolitica nor TAMU 70 could bind to the petri dish under the experimental conditions used in this test.

Hydrophobic Interaction Chromatography

In order to characterize the hydrophobic properties of the strains P7 and P7P$^-$ on a more critical basis than the rapid polystyrene plate analysis, the procedure of hydrophobic interaction chromatography was employed. This latter method involves the use of either agarose or sepharose gels with conjugated side chains of varying hydrophobic nature. Binding to a particular side chain is mediated through charge interaction and the binding to a particular conjugated group aids in the elucidation of the outer surface properties of the organism.

Table 2 presents the results from the hydrophobic interaction chromatography of P7 and P7P$^-$. It may be observed that P7 had little affinity for any of the columns. A maximum retention of 4.3 percent occurred on the CL-4B-octyl column. Strain P7P$^-$, however, had a marked ability to bind to each of the conjugated columns and gel retention exceeded 90 percent in each case. Comparison of strains with respect

Table 2. Hydrophobic Interaction Chromatography of Strains P7 and P7P⁻

Strain	Column	Absorbance[1]	% Retained on Gel[2]
P7	CL-4B	1.62	-
	-octyl	1.55	4.3
	-phenyl	1.60	1.2
	-dodecyl	1.60	1.2
P7P⁻	CL-4B	1.40	-
	-octyl	0.015	98.9
	-phenyl	0.02	98.5
	-dodecyl	0.13	90.7

[1]Sample size of 1.0 ml aliquots were collected from the column and absorbance determined by optical density measurements at 600 nm. Hydrophobicity eluant served as baseline control (1.0 ml was eluted through column CL-4B and subsequently read at 600 nm).

[2]Percent retention was calculated as the ratio of either octyl, phenyl, or dodecyl to that of CL-4B column for each organism.

to particular hydrophobic side chains showed no particular preference, but rather P7P$^-$ was appreciably more hydrophobic over all than P7 with regard to the three tested hydrophobic ligands.

It should be noted that both strains used in this study were grown on peptone plates containing Tween 80. Strain P7P$^-$ without the plasmid self-clumped to such an extent that resuspension in the basal media buffer was impossible unless harsh physical means, such as ultrasonification, were used. Tween 80 provided an environment that satisfactorily reduced cell-cell interaction, without inducing severe physical stress to the cells.

CHAPTER 4

DISCUSSION

Microbial degradation of hydrocarbons has been well documented (2, 3, 24, 51). The variety of organisms capable of hydrocarbon utilization is quite extensive. Zobell, in an initial review of this topic, indicated that approximately 100 species of bacteria, yeasts, and molds posessed this property (69). The number of organisms known to degrade hydrocarbons has no doubt increased since Zobell's publication in 1946. Chromosomally mediated hydrocarbon metabolism via the beta-oxidative pathway has often been described as the predominant mechanism for bacteria (30, 34, 42). However, plasmid function in hydrocarbon degradation pathways has also been reported (6).

Ferguson (17) originally observed that P7 could grow on an extensive array of hydrocarbons, including crude oil. Nash (43), in subsequent studies, examined aliphatic hydrocarbon use with respect to co-oxidative metabolism, as well as the eventual fate of hydrocarbons once inside the cell. Work by Garvey (21) consisted of a characterization of the plasmid contained in P7 as originally observed by Kronland (unpublished data). Garvey concluded that the plasmid pYG1 was responsible, in part, for the utilization of n-alkanes C_8 through C_{10}. Growth on the alkanes C_{11}-C_{20} appeared to be unrelated to the presence or absence of pYG1 . The specific level at which the plasmid functioned in regard to C_8-C_{10} utilization has not been determined.

With respect to Garvey's findings, P7 and P7P$^-$ were retested for their growth abilities on a series of hydrocarbons. As previously reported, P7P$^-$ could not grow on decane. P7 and P7P$^-$ were then assayed for their ability to utilize equivalent carbon length derivatives of either hydrocarbon, alcohol, or fatty acids. It was observed that P7P$^-$ had also lost the ability to grow on 1-decanol, the alcohol equivalent of decane. It was interesting to note, however, that P7P$^-$ could still grow on decanoic acid, the C_{10} fatty acid derivative. Acinetobacter sp. have been reported to degrade alkanes by use of the beta-oxidative pathway (34). This pathway involves the conversion of the alkane to the alcohol and finally to the fatty acid equivalent where it undergoes terminal oxidation. The fact that P7P$^-$ cannot grow on decane or 1-decanol, but can grow on decanoic acid suggests several possibilities. Due to the interruption of the production of needed enzymes or other related factors, the organism may have lost the essential binding proteins needed in the recognition and subsequent transport across the membrane. In addition, the organism may have lost the ability to transform the alkane into the alcohol and thus into the fatty acid. This contention is supported by the work of Grund et al. (22), who showed that alkane oxidation in Pseudomonas putida is mediated by both plasmid and chromosomal genes. The plasmid codes for the alkane hydroxylating and alcohol-dehydrogenating activities, whereas the chromosome is responsible for oxidation of the primary alcohols.

Nash's (43) work with hydrocarbon metabolism suggested that hexadecane and hexadecanoic acid did not follow the classical pathway

of terminal oxidation. Labeled compounds of alkane fatty acids were not incorporated into similar cellular pools. In contrast to the terminal oxidation pathway, Finnerty (18) has described a species of Acinetobacter which cleaves alkanes at the tenth position, thus resulting in the formation of a hydroxy acid and an alcohol. Garvey, using various mutants of either P7 or P7P$^-$, has postulated that A. phosphadevorus (pYG1$^+$) may possess two chromosomally mediated n-alkane catabolic pathways. One pathway may be responsible for degradation of low and high carbon-containing compounds (e.g., C_{11}, C_{12}, and C_{17}), while the other pathway may code for catabolism of carbon lengths of C_{13} and C_{14}. Work by Dostalek (13) supports the contention that many organisms possess multiphasic degradation pathways. The specific role of pYG1 in hydrocarbon degradation is still unclear at this time. It should be noted that even the mechanism of hydrocarbon utilization at the chromosomal level has yet to be elucidated.

A. phosphadevorus has the ability to grow on a large variety of hydrocarbons, and therefore was tested for its ability to adhere to polystyrene. Bacterial strains that interact with hydrophobic compounds usually show an increased affinity for binding to polystyrene surfaces (55). Both P7 and P7P$^-$ were tested for their ability to bind to polystyrene plates, and no differences were observed. Related strains of A. phosphadevorus, including A. calcoaceticus, H. vaginicola, and A. anitritus, were also examined for their ability to adhere to polystyrene surfaces. Although these bacteria produced limited growth on only three or fewer of the hydrocarbons tested, they adhered quite

readily to the polystyrene surfaces. The properties of hydrocarbon utilization and polystyrene adherence, therefore, do not exhibit an exclusive correlation. Loss of the plasmid pYG1 would appear not to effect attachment to polystyrene surfaces. Specific attachment of bacterial cells to the polystyrene plate has been suggested to be mediated by fimbriae located on the surface of the bacterial cell (56). The rapid polystyrene plate method does not take into account other factors that are significant in hydrophobic interaction, such as extracellular solubilizing agents (70).

Hydrophobic interaction chromatography was used to evaluate the ability of P7 and P7P$^-$ to interact with hydrophobic compounds on a more quantitative basis than that possible by the rapid polystyrene plate assay. P7P$^-$ had a much greater affinity for all conjugated side groups tested than did strain P7. P7P$^-$ bound to all the derivatized sepharose gels tested, and gel retention exceeded 90 percent in each case. P7, however, adhered only to the CL-4B octyl column with only 4.3 percent retention. Therefore, P7P$^-$ exhibited greater hydrophobic nature than that of P7. There were no significant differences between binding to the various conjugated side groups. This test indicates the outer surface characteristics of P7P$^-$ differ from those of P7. It has been reported that bacterial fimbriae play a major role in hydrophobic interactions and constitute the predominant structure that adheres to hydrophobic gels (16, 62). An assumption may be made that the plasmid pYG1 is involved in the regulation of fimbriae constituents. Amino acid analysis of proteins contained in bacterial fimbriae of

Neisseria, Moraxella, and Pseudomonas spp. have shown a high degree of hydrophobic amino acids (50). The exact relationship of plasmid to fimbriae production has not been resolved in this organism, but the results suggest some possible involvement.

In light of P7P$^-$ loss of the ability to utilize decane and decanol and associated outer surface alterations, an analysis of outer membrane proteins was accomplished by use of the Triton X-100 procedure. Strains P7 and P7P$^-$ exhibit a notable difference in relative protein number. A comparison of the two strains' outer membrane proteins revealed significant differences. The isogenic strain cured of the plasmid showed a drastic reduction in outer membrane protein production, especially with respect to polypeptides of lower molecular weight. Proteins of higher molecular weight remained relatively consistent for both strains with the absences of only a few large proteins in P7P$^-$. Analysis of comparable protein fragments of P7 and P7P$^-$ revealed a similar staining density, indicating that the absence of proteins in P7P$^-$ was a function of P7P$^-$ protein production and not insufficient sample concentration.

Proteins located in the outer membrane region of bacteria have been known to function in many cellular events, most notably transport processes (35). These proteins may function as binding proteins or associated transport complexes allowing movement of metabolizable substrates into the cell (49, 64). The inability of P7P$^-$ to grow on decane or 1-decanol could be attributed to a non-functional transport system. Plasmids have been reported to play a role in the production

of various polypeptides (60). The specific involvement of the plasmid pYG1 in the production or regulation, or both, of transport proteins in this organism has as yet to be studied. Work by Nash (43) has indicated that A. phosphadevorus transports alkanes and fatty acid moities as whole molecules. Exposing P7P$^-$ to radioisotopically-labeled compounds, such as decane or 1-decanol, may provide a clearer understanding of the plasmid's possible involvement in transport processes.

Analysis of the role of pYG1 has been confined to a comparison of two isogenic strains differing only by the absence of pYG1 in the ethidium bromide-cured strain (P7P$^-$). To conclusively state that pYG1 was specifically involved in the loss of functions observed in P7P$^-$, a transformation assay was attempted. The experimental procedure relied upon growth on decane, presumably coded for by plasmid-mediated processes. The procedure consisted of exposing the cured strain P7P$^-$ to a preparation of purified plasmid DNA. The cells were then grown under conditions which would select for transformants which expressed the selected function. Unfortunately, a transformant could not be constructed by the method employed. Successful transformation is dependent upon achieving maximum conditions of competency for the recipient cell population (11). Competency, or the ability of a recipient strain to transport DNA from the culture medium into the cell, depends upon several factors, including: stage of growth cycle; nutritive value of the culture medium; degree of aerobiosis or anaerobiosis; pH; presence

of appropriate concentrations of divalent cations; and phenotypic properties of recipient cells, such as structure of cell surface (11).

Transformation in several strains of bacteria has been studied and is well-documented (40, 65). Transformation of A. calcoaceticus has been successfully performed and reported (32, 33). The procedure used in this study is a modification of that used for A. calcoaceticus as described by Sawula and Crawford (58). This procedure was used in the expectation that the conditions required for competency in A. calcoaceticus would be similar for A. phosphadevorus. The inability to transform A. phosphadevorus, however, indicates that other physical conditions must be required to affect competency. This study has shown that P7P$^-$ has altered outer surface properties. While it is difficult to assess what effect this condition has upon DNA uptake, it must be considered as a possible predisposing factor. The transformation conditions used in this study were developed for chromosomal DNA or whole cell DNA content. Optimal conditions may not be present to transfer plasmid DNA into the cell. The maximum efficiency of plasmid transformation has been reported to be approximately 10^{-3} per recipient cell, using techniques particular for plasmid DNA transformation in E. coli (4). Hopefully, a transformation system for A. phosphadevorus will be developed, with continued analysis of procedures exclusively for plasmid transformation.

The plasmid pYG1 contained in A. phosphadevorus has been shown to be involved in hydrocarbon utilization and hydrophobic interactions. Original work by Garvey (21) has determined that the plasmid was not

active in either antibiotic or metal cation resistance, classical roles for many plasmids. The present study further characterized pYG1's involvement in the mediation of hydrocarbon usage. pYG1 has been implicated in mediating hydrophobic properties associated with fimbriae structure. The hydrophobic nature of outer membrane structures has been shown to facilitate interaction with nonpolar compounds, such as hydrocarbons. The significance of microbial degradation of petroleum products cannot be overlooked, especially in light of efficient industrial as well as environmental applications of these organisms. Further work on A. phosphadevorus at a molecular level as well as continued examination of physiological processes is needed. Additional research on this organism should provide better insight into the process of microbial degradation of hydrocarbons.

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