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GLIDING MOTILITY AND VEGETATIVE CELL ULTRASTRUCTURE IN THE
MYXOBACTERIA

by

Thomas Henry MacRae

A Dissertation
submitted to the Faculty of Graduate Studies
through the Department of
Biology in Partial Fulfillment
of the requirements for the Degree
of Doctor of Philosophy at
The University of Windsor

WINDSOR, ONTARIO, CANADA

1976
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ABSTRACT

GLIDING MOTILITY AND VEGETATIVE CELL ULTRASTRUCTURE IN THE MYXOBACTERIA

by

Thomas Henry MacRae

Electron microscopy of sectioned, chemically fixed vegetative cells of *Chondromyces crocatus* revealed a microorganism with a typical gram-negative cell envelope. The cytoplasm contained, in addition to microtubules and two types of granules, a membrane-associated structure (MAS) that, although less extensive, bears some resemblance to polar membranes observed in flagellated bacteria. Examination of swarming cells negatively stained in situ, as well as thin sections, established that cell division occurs by septum formation and that well-defined mesosomes are associated with the process. Polar fimbriae and a compact, amorphous slime layer surrounding the cells were evident in shadowed and negatively stained preparations of in situ cells. Attempts to isolate nonmotile mutants of *C. crocatus* were unsuccessful.

Nonmotile and motility-altered mutants of *Myxococcus xanthus* were obtained by the use of chemical mutagens, ultraviolet irradiation and a procedure for selecting spontaneous mutants. As judged by their behaviour on a variety of growth media, in both plate and slide culture, the mutants were divided into four groups. One of these
groups contained mutants which are truly nonmotile. *M. xanthus* NM, previously described as a nonmotile mutant, may be similar to Type 3 mutants (described in text).

An electron microscopic examination of negatively stained preparations of whole cell lysates and *in situ* samples of *M. xanthus* demonstrated the presence of polar fimbriae, about 8.5 nm in diameter, on motile but not on nonmotile cells. Polar fimbriae have subsequently been demonstrated in all genera of myxobacteria except *Nannocystis*.

Partially purified fimbriae from *M. xanthus* were resistant to proteolytic enzymes, freezing and thawing and alkaline, neutral and slightly acid pH, but were depolymerized at pH 4.5 at 37°C.

Immune electron microscopy, employing anti-fimbriae serum, coupled with motility inhibition tests in plate and slide culture indicated that fimbriae may be required for cellular organization during swarming, rather than motility per se.

Also present in polar regions of *M. xanthus* and other myxobacteria, as well as in membrane fragments from these cells, were "holes" with an inside diameter of about 7.1 nm. The "holes" were surrounded by an electron transparent collar about 2.6 nm thick, from which 12 spike-like projections were sometimes observed to protrude. The relationship, if any, between the "holes" and fimbriae is, at present, obscure.
DEDICATION

To my wife, Cheryl, for her encouragement, patience and understanding.
ACKNOWLEDGEMENTS

I express my appreciation to Dr. H. D. McCurdy, Jr. for his intellectual and financial contributions during this study. My experiences with him have been both interesting and rewarding and it is hoped, that, in return, he also has gained from this association.

Thanks is extended to all committee members who reviewed this dissertation. I am grateful to Dr. R. G. E. Murray, University of Western Ontario, for the use of electron microscopic facilities, Mrs. Diane White and Mr. Berry Sears, University of Windsor, for technical assistance, Cheryl MacRae for typing this dissertation and all others who contributed to this study.

A special thanks is reserved for Mr. William C. Trentini, Mount Allison University. He prompted, through his ability as a teacher, my initial interest in Microbiology, and continued, throughout my tenure as a graduate student, to provide useful advice and encouragement.

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INTRODUCTION

The myxobacteria exhibit cellular co-operation throughout their complex life cycle. Such co-operation is expressed both in coordinated swarming in the vegetative state and in fruiting body formation. These activities require cell movement on a solid substrate and the myxobacteria meet this requirement by their ability to glide. Gliding is a type of surface translocation, exhibited by some nonflagellated bacteria, that is characterized by a relatively slow progression following the longitudinal axis of the cell. During movement cells may exhibit frequent changes in direction, but there is no evidence of cellular conformational change. (Doetsch and Hageage 1968; Henrichsen 1972).

Several hypotheses, variously invoking osmotic forces, surface tension phenomena, secretion of slime, and contractile waves propagated at the cell surface (Doetsch and Hageage 1968) have been advanced to explain the mechanism of gliding. Little evidence is available to support any of these ideas but the presence of fibrils, which may act as structural components required for the propagation of contractile waves at the cell surface, has been reported in the gliding bacteria, Oscillatoria princeps (Halfen and Castenholz 1970, 1971; Halfen 1973) and Cytophaga columnaris (Pate and Ordal 1967; Glaser and Pate 1973).
A comparison of nonmotile mutants and motile organisms at the molecular, ultrastructural and cellular level could provide information about the mechanism of gliding. Glaser and Pate (1973) isolated several nonmotile mutants of *C. columnaris* and Burchard (1970) reported the isolation of a nonmotile mutant of *Myxococcus xanthus*. Attempts to detect motility-related ultrastructural differences between the myxobacterial mutant, *Myxococcus xanthus* NM and the wild type, *M. xanthus* FB have been unsuccessful (Burchard 1970; Burchard and Brown 1973). An ultrastructural modification, the absence of fibrils from a *C. columnaris* nonmotile mutant, has been reported (Glaser and Pate 1973) but Burchard and Brown (1973) suggested the fibrils observed in motile *C. columnaris* were artifacts of preparation.

Grimm and Kühlwein (1973 a, b) compared spontaneous mutants of the myxobacter, *Archangium violaceum*, which they termed K (eubacterial) to wild-type or S (swarmer) cells. The former were poorly motile or largely nonmotile whereas the S cells were normally motile. They did not consider, however, that the K cells were damaged in their "Bewegungsapparat" or motility apparatus, but instead attributed their behavior to an increase in slime production. These authors expressed doubt that *M. xanthus* NM is a true motility mutant (Grimm and Kühlwein 1973a). Twitching, another type of surface translocation,
occurs in flagellated and nonflagellated bacteria (Henrichsen 1972). Twitching, as compared to gliding, is less organized. Movement is intermittent and jerky and the long axis of the cell is not regularly followed. Henrichsen (1975 a, b, c), Henrichsen and Blom (1975 a, b) and Henriksen and Henrichsen (1975) have presented considerable evidence that twitching motility is correlated with the presence of polar fimbriae in a variety of gram negative bacteria and one gram positive bacterium. This observation is of special interest in view of our results.

In the following report, procedures that resulted in the isolation and characterization of several motility mutants of *M. xanthus* are described. Some of these isolates appeared to be true nonmotile mutants. Electron microscopy, in addition to revealing several interesting ultrastructural features of *Chondromyces crocatus*, demonstrated an ultrastructural difference between motile and nonmotile cells of *M. xanthus*. An attempt was made by immunological and electronmicroscopic techniques to relate this structural change, in a causative way, to the loss of motility.
MATERIALS AND METHODS.

Microorganisms and Growth Conditions


Except as indicated, working cultures of all microorganisms were grown on SP (McCurdy 1963) agar plates or with shaking in 30 ml of SP in 125 ml screw-cap flasks at 30°C in the dark. *Polyangium cellulosum* M254 and *Chondromyces crocatus* M467 were grown on SP agar plates on which 0.1 ml of *Escherichia coli* extract (described below) had been spread. Dispersed growth of *C. crocatus* in liquid culture was obtained in SP containing 1 x 10⁻³ M disodiummethylenediaminetetraacetate (EDTA) and 4.0 g/l of casitone. Working cultures of *P. cellulosum* M222 and *N. exedens* M349 were grown on yeast-calcium (Y-Ca) agar (Reichenbach, personal communication). Y-Ca contained per litre of water: 10 g Baker's Yeast (from cake), 1 g CaCl₂ · 2H₂O, 15 g agar (pH 7.2).
Stock cultures of all microorganisms, except *C. crocatus*, were maintained at 18°C, in the dark, on the appropriate agars just described. *C. crocatus* was stored on SPE agar (McCurdy 1963) under the same conditions.

*E. coli* extract was prepared by sonicating 1 g, wet weight, of *E. coli* in 8 ml of H$_2$O for 5 min at full power with the Bronwill Scientific Sonicator (Rochester, New York). Sonication was followed by centrifugation at 12,250 g to remove cell debris. The resulting supernatant was filter-sterilized twice with a Seitz filter apparatus using positive pressure, distributed to vials, and stored at -20°C until used.

**Chemicals**

N-methyl-N'-nitro-N-nitrosoguanidine (NTG) was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. The frameshift mutagen, 2-chloro-6-methoxy-9-\( \text{[4-(2-chloro-ethyl) aminopropylamino]} \) acridine dihydrochloride (ICR-191E) was kindly supplied by Dr. H. J. Creech of the Chemotherapy Laboratory of the Institute for Cancer Research in Philadelphia. Hyanc throne methanesulfonate, 1-\( \text{[2-(diethylamino) ethyl]} \) amino\( \text{-4- (hydroxymethyl)} \) thioxanthene-9-one monomethanesulfonate, was kindly supplied by Dr. F. C. Nachod of the Sterling-Winthrop Research Institute, Rensselaer, New York.

Protease type VI (repurified from *Streptomyces*
griseus) and Protease type VIII (Subtilisin Carlsberg — from Bacillus subtilis — Crystallized and Lyophilized) were obtained from Sigma Chemical Co., St. Louis, Missouri. Trypsin 1:250 (from pancreas) was purchased from Difco Laboratories, Detroit, Michigan. Alpha amylase and lysozyme were from Nutritional Biochemical Corporation, Cleveland, Ohio.

Electron microscopic grade glutaraldehyde as an 8% solution in H2O stored under N2 and reagent osmium tetroxide as a 4% aqueous solution were obtained from Polysciences Inc., Warrington, Pennsylvania. Dithiothreitol was obtained from Sigma Chemical Co., St. Louis, Missouri. All other chemicals used were reagent grade.

**Mutant Isolation Techniques**

### Selection of Spontaneous *M. xanthus* Motility Mutants

Spontaneous motility mutants were selected by a modification of a method previously described by Armstrong *et al.* (1967) and Glaser and Pate (1973). Initially, a loopful of cells from a liquid culture of *M. xanthus* was spotted on SP and SP/10 agar (SP/10 is SP with a 10-fold reduction in casitone and carbohydrate concentrations). The plates were incubated at 30°C in the dark in humidity chambers (plastic refrigerator vegetable crispers) for 6 days, after which, a plug of cells was removed from the centre of the swarm with a Pasteur pipette and aspirated into 1 ml of SP in a screw-cap test tube. The culture was
incubated for 3 days at 30°C on a wrist action shaker. Again, a loopful of cells was removed, spotted on SP and SP/10 agar, and the cycle of growth on solid and liquid media was repeated. After 6 and 9 cycles of such transfers, the final liquid culture was dispersed by omnimixing, (see below), diluted in dilution medium (DM) (McCurdy 1963) and surface plated on SP for the isolation of motility mutants.

The standard procedure for dispersing cells here and for most other procedures was to transfer cells to a sterile Omnixer vessel containing 0.75 g of glass beads (-100 - +120 mesh size, Flex-O-Lite of Canada Ltd., St. Thomas, Ontario) and 1 ml of DM. The cells were then omnimixed for 60 sec at a rheostat setting of 80 with a Servall Omnirixer (Ivan Sorvall, Inc. Norwalk, Conn.) fitted with a Sorvall Micro-attachment.

Induction of M. xanthus Motility Mutants

For treatment with NTG a procedure similar to that described by Adelberg et al. (1965) was used. One hundred ml of SP in a 250-ml side-arm flask was inoculated with 1 ml of an early stationary phase culture of M. xanthus. The flask was incubated with shaking at 30°C in the dark until the culture, as determined turbidimetrically, was in the late exponential phase of growth. The cells were collected by centrifugation at 10,000 g for 15 min at 4°C and washed once by centrifugation with 100 ml of cold DM or 100 ml of SP. After washing, cells were dispersed in 30 ml of DM if washed with DM or 30 ml of SP if washed with SP, in a sterile
omnimixer vessel containing 2.5 g of glass beads.

The cell suspension, containing $1 \times 10^8 - 1 \times 10^9$ bacteria per ml, was divided into 4 ml aliquots in sterile test tubes and a filter sterilized aqueous solution of NTG was added to the following final concentrations: 25, 50, 100, 250 and 500 μg/ml. Following a 30 min exposure to NTG the bacteria were collected on 0.45 μm millipore filters and washed with 10 ml of cold DM. The filters were then placed in 30 ml of SP in 125 ml screw-cap flasks and the cells were removed from the filters by agitation and scraping. After aseptically removing the filters, the cultures were incubated at 30°C, in the dark, with shaking, for 20-50 h, i.e., until a significant increase in optical density of the culture had occurred. The length of incubation varied with the concentration of mutagen used. After growth, the cultures were diluted in DM and plated on SP for the isolation of motility mutants.

For mutant induction with frameshift mutagens late exponential phase cultures of M. xanthus containing $2 \times 10^8 - 5 \times 10^8$ cells/ml were diluted 1:10 in fresh SP in 250 ml side-arm flasks. Either ICR-191E (Oeschger and Hartman 1970) was added to final concentrations of 0.11, 0.55 and 1.10 μg/ml or hycanthone methanesulfonate (Hartman et al. 1971) was added to final concentrations of 5.0, 10.0 and 15.0 μg/ml. Both mutagens were dissolved in distilled
water and filter sterilized. The cultures were incubated on a shaker at 30°C in the dark until a significant increase in optical density occurred. Incubation periods ranged from 23-68 h depending on the type and concentration of mutagen employed. Following treatment, the cultures were washed by centrifugation, diluted in DM and surface plated on SP for the isolation of motility mutants. All manipulations with these two mutagens were done in dim light.

For ultraviolet irradiation, cells from 10 ml of a late exponential phase culture of *M. xanthus* containing $7.9 \times 10^8$ cells per ml were collected by centrifugation, resuspended in 10 ml of fresh SP and exposed, in a petri dish, to a General Electric 15 watt Germicidal lamp at a distance of 25 cm for 0, 15, 30, 60 or 120 sec. After treatment the cells were poured into 90 ml of SP in a 250 ml side-arm flask and incubated in the dark at 30°C with shaking until there was a significant increase in optical density. Incubation periods ranged from 20-116 h depending on the length of exposure to ultraviolet irradiation. Following incubation the cells were diluted in DM and plated on SP for isolation of motility mutants.

**Isolation and Characterization of *M. xanthus* Motility Mutants**

Plates inoculated with *M. xanthus* were examined with a dissecting microscope for colonies apparently formed by nonmotile mutants. The colonies chosen, here described
as "eubacterial", were smooth, convex, round or slightly amoeboid, with complete edges, and showing no displacement of cells away from the colony edge (Fig. 1 a, b). In contrast, motile cells form a spreading colony in which the colony edge is not clearly defined (Fig. 1 c, d). The "eubacterial" colonies were picked, streaked on SP and incubated at 30°C in the dark in humidity chambers. The streaks were examined with a dissecting microscope 1 week and 2 weeks after inoculation. Isolates not exhibiting spreading edges, and often those with limited spreading were further characterized.

Apparent motility mutants were restreaked on SP for a two day incubation following which cells were collected and omnimixed. After omnimixing, the cell suspension was serially diluted, tenfold, in DM and surface-plated on SP and SP/10. Cells were sometimes checked on CT-1 and P-T (Burchard 1970) dilution plates, but this was generally unnecessary. The plates were incubated at 30°C for 3 days in the dark, placed in humidity chambers, and the incubation allowed to continue for up to 4 weeks. Plates were examined at appropriate intervals with both a dissecting (32x) and a phase (200x) microscope to determine if cells were displaced from the colonies. Isolates were also streaked on SP, SP/10, CT-1 and P-T and examined after 2 weeks with the dissecting and phase microscopes. All nonmotile isolates were tested at least twice for cell displacement with the
Fig. 1a-d. Fig. 1a. Phase micrograph of a nonmotile (eubacterial-type) colony of NMM36-1C. Fig. 1b. The colony edge of a typical Type 4 nonmotile mutant.

Fig. 1c. A typical spreading colony of wild-type M. xanthus. Fig. 1d. The colony edge of wild-type M. xanthus. In contrast to NMM36-1C, the cells are scattered and the colony edge is not clearly defined.

The bar represents 100 \( \mu \text{m} \) in Fig. 1a, 20 \( \mu \text{m} \) in Fig. 1b and d, and 300 \( \mu \text{m} \) in Fig. 1c.
As a final test for motility, isolates were examined in slide culture. A glass microscope slide was dipped in melted agar (1 or 1.5%) twice, the agar was allowed to solidify and then removed from one side of the slide. The slide was inoculated with cells grown on agar plates, wrapped in Saran Wrap, (Dow Chemical of Canada Ltd., Toronto, Canada) and examined, using oil, with a phase microscope. An appropriate area was selected and photographed at hourly intervals for 7-8 h and after approximately 24 h of incubation at room temperature. The negatives were then examined for evidence of cell translocation. All isolates which are subsequently described in this report as nonmotile were tested, with replicates, on slide cultures of SP, SP/10 and CT-1 diluted 1:1 with distilled water.

For an isolate to have been considered nonmotile, it must not have exhibited translocation due to gliding in any of the above tests.

Preparation of Fibril-Containing Cell Lysates of M. xanthus

Cells of motile and nonmotile M. xanthus 48 h SP cultures grown at 30°C were harvested, washed and suspended in 10 mM Tris (hydroxymethyl)aminomethane (Tris) buffer (pH 7.5) containing 1 mM EDTA and 0.1 mM dithiothreitol (Clarke and Spudich 1974) in the proportion of 0.15 g cells
to 2 ml buffer. After 15 min preincubation in an ice bath, lysozyme was added to a final concentration of 20 μg per ml and the mixture incubated for an additional hour. Potassium chloride (final concentration 0.1 M) and magnesium chloride (final concentration 0.01 M) were added, the mixture incubated for 0.5 h, and then centrifuged at 10,000 g for 30 min. The resulting supernatant was centrifuged at 30,000 g for 1 h at 0°C (MSE Automatic "Superspeed 50") and the sediment, after resuspension in the same buffer, was negatively stained with 1% aqueous uranyl acetate (pH 3.9) and examined in the electron microscope.

Unless otherwise indicated all samples prepared for electron microscopy were negatively stained with 1% aqueous uranyl acetate (pH 3.9).

Myxobacterial Fimbriation

In situ Electron Microscopic Examination of Myxobacteria

To prepare in situ samples of motile myxobacteria, ultraviolet-sterilized, carbon-reinforced, formvar coated, nickel grids were placed in front of an advancing swarm of bacteria. The cells were allowed to glide onto the grids and the grids with adhering bacteria were removed and immediately processed for electron microscopy (see below).

Grids bearing nonmotile cells were prepared by spreading 0.1 ml of an early stationary phase culture on SP agar, allowing the plate to dry for 15-30 min at 30°C, and dropping the grids, formvar-side down on the agar surface.
After an incubation of 12-72 h, depending on the strain being examined, at 30°C, the grids were removed and prepared for electron microscopy (see below).

Negative staining – A drop of stain, 0.5% uranyl acetate in 50% ethanol, 1% aqueous uranyl acetate (pH 3.9 - 4.5) or 1% aqueous phosphotungstic acid (PTA) (pH 7.0), was placed on the grid for 20-30 sec, excess removed with filter paper, and the grid allowed to dry.

Replica making – Grids were removed from plates and either air-dried, dried under vacuum, or fixed in OsO₄ vapours for 30 min. The cells were platinum-shadowed and carbon-coated in a Balzer's freeze-etch unit (model BAS10 M Balzers AG, Lichtenstein), and replicas were washed as described by Bradley (1965) except that hydrochloric acid was replaced with sulfuric acid.

**Agar Extraction for Fimbriae**

Agar, from 5-8 plates, containing the outer centimeter of advancing swarms of motile myxobacteria was stirred 4 days at 4°C in 30 ml of 0.01 M phosphate buffer (PO₄ buffer) (pH 7.5) containing 0.2% sodium azide. The agar was removed by centrifugation at 10,000 g for 15 min, and the resulting supernatant was centrifuged at 100,000 g for 1.5 h. The 100,000 g sediment was resuspended in PO₄ buffer and negatively stained for examination with the electron microscope.

**Partial Purification of Fimbriae by Differential Centrifugation**

Cells grown on SP agar at 30°C for 48 h were harvested
from plate surfaces with PO₄ buffer. The cell suspension was agitated by hand for 1 min followed by centrifugation at 10,000 g for 20 min. The supernatant was retained, the cells resuspended in PO₄ buffer, agitated, recentrifuged and the supernatant combined with the initial supernatant. The combined supernatants were centrifuged at 10,000 g for 30 min to remove residual cells and slime. Fimbriae were collected by centrifugation at 100,000 g for 1.5 h and resuspended in PO₄ buffer. Residual cells and membrane contamination were partially removed by centrifugation at 20,000 g for 45 min. When necessary, fimbriae were concentrated by recentrifugation at 100,000 g for 1.5 h and suspended in a smaller volume of PO₄ buffer. Protein concentrations were determined by the Lowry method (Lowry et al. 1951).

Attempts to purify fimbriae by ammonium sulfate or pH precipitation and by centrifugation on discontinuous sucrose and cesium chloride gradients were unsuccessful.

Production of Fimbriae in Liquid Culture

*M. xanthus* grown at 30°C with shaking in SP was sampled at early and late log and stationary phases. Cells were removed by centrifugation at 10,000 g for 20 min and the resulting supernatant was centrifuged at 100,000 g for 1.5 h.
Both cells and pellets were resuspended in PO₄ buffer, negatively stained, and examined in the electron microscope.

**Effect of Chemical and Physical Agents on Fimbriae**

Partially purified fimbriae (100,000 g sediment centrifuged at 20,000 g for 45 min) at a concentration of 120 µg protein/ml was mixed in a 1:1 ratio with the following enzymes: 15 µg/ml of trypsin, protease type VI and protease type VIII in 0.05 M Tris (pH 7.5) containing 2 x 10⁻³ M MgCl₂; 15 µg/ml of alpha amylase in 0.05 M sodium citrate (pH 5.7 - final pH 6.2). The reaction mixtures were incubated at 37°C for 20 h.

Fimbriae (100,000 g sediment) were resuspended in 0.05 M phosphate buffer at various pH's and incubated at either 30 or 37°C for up to 23 h to test the effect of pH on fimbriae structure.

The effect of freezing (-20°C) and thawing on fimbriae structure was determined.

The effects of the various agents on fimbriae structure were observed electron microscopically.

**Immunological Methodology**

**Production of Rabbit Anti-Fimbriae Serum**

A rabbit was inoculated intravenously with 0.5 ml of a partially purified fimbriae preparation (530 µg protein/ml) and subcutaneously with 0.7 ml of the same preparation
mixed with 0.7 ml of complete Freund's adjuvant. After 2 weeks the rabbit was injected intravenously at weekly intervals with approximately 0.85 ml of partially purified fimbriae (approximately 1 mg protein/ml) for 3 weeks, followed by weekly bleedings and a 1 ml booster of fimbriae (0.48 mg protein/ml) 2 weeks after the last injection.

Adsorption of Serum

Three ml samples of serum, heated at 57°C for 30 min to destroy complement, were adsorbed three times for 1 h at room temperature with M. xanthus wild type cells grown on SP agar or in SP liquid or with nonmotile mutants grown on SP agar. Cell numbers per adsorption ranged from $7.0 \times 10^{10}$ - $7.0 \times 10^{12}$. Adsorption was terminated by centrifugation at 10,000 g for 15 min with the third adsorption followed by recentrifugation to remove residual debris.

Anti-motility activity of the serum was tested by the slide culture method, by spreading and drying one drop of the appropriate serum on the agar surface before inoculation with motile M. xanthus. Serum effects upon the migration of cells from colonies was determined by the degree of inhibition of colony spreading toward serum-filled wells (made with a #1 cork borer) in agar plates. Appropriate controls with nonimmune serum were performed.

Immune Electron Microscopy

Attempts were made to label fimbriae (0.64 mg
protein/ml) with adsorbed and unadsorbed anti-fimbriae serum by a modification of the method of Almeida and Waterson (1969). Essentially, 0.1 ml of fimbriae and 0.1 ml of serum, either undiluted or diluted 1:10 or 1:100 were mixed at R.T. with 0.3 ml of 0.85% NaCl. The tubes were incubated at 37°C for 1.5 h and then at 4°C for 19.5-22 h. The precipitates were collected by centrifugation at 10,000 g for 20 min, washed with 0.5 ml of 0.85% NaCl, resuspended in 0.5 ml of distilled H₂O and negatively stained for electron microscopy.

**Immunoelectrophoresis**

Immunoelectrophoresis was performed in 0.05 M sodium barbital buffer (pH 8.2) containing 0.2% sodium azide and solidified with 1.5% Difco purified agar. Immunelectrophoresis slides were run at 12.5 mA per slide for 1 h with 0.05 M sodium barbital (pH 8.2) as buffer. Slides were stained with 0.1% aqueous Buffalo Black for 30 min followed by destaining in 70% acetic acid for 10 min.

To determine the location of fimbriae after electrophoresis, agar from appropriate locations was removed from slides and extracted by the agar extraction method previously described in this report.

**Electron Microscopy of Chondromyces crocatus Thin Sections**

For electron microscopy of *C. crocatus* thin sections, plates were incubated for 3–4 days and only those
cells in the outer 0.5-1.0 cm portion of the swarm were used. The cells, as outlined below, were chemically fixed by different methods.

(a) Three ml of a cold prefixative mixture, prepared immediately before use, containing 0.5% osmium tetroxide and 4% glutaraldehyde in H₂O at pH 7.2, were poured onto a plate on which C. crocatus was growing, swirled gently, immediately poured off, and the plate was dried on the slant in the cold for 5 min. The cells were scraped into piles, enrobed in H₂O agar containing 4% glutaraldehyde, cut into small blocks, and placed in a vial containing 4 ml of a mixture of 1% OsO₄ and 4% glutaraldehyde in H₂O at pH 7.2. The cells were fixed 4 h in the cold. Cells were also scraped into piles without fixation, placed directly into the fixative for 4 h and then enrobed in agar.

(b) Cells were prefixed on plates as described above but with 3 ml of 4% glutaraldehyde in 0.02 M sodium cacodylate buffer at pH 7.5 instead of a glutaraldehyde-osmium mixture. The cells were scraped into piles, enrobed in agar containing 4% glutaraldehyde, cut into small blocks and fixed in cold 4% glutaraldehyde for 3.5 h. The blocks were rinsed once with sodium cacodylate buffer and then fixed in cold 1% OsO₄ in the same buffer for 4 h. Cells scraped into piles before fixation were placed directly in 4% glutaraldehyde, fixed for the required time, and enrobed in agar before fixation in 1% OsO₄.
(c) Variations of the above fixatives involving changes in buffers, in buffer concentrations, and the addition of Mg++, Ca++, casamino acids, silver nitrate, and/or p-chloromercuribenzoic acid were tested but cell preservation was best under conditions described.

After fixation, the blocks were rinsed once with water or sodium cacodylate buffer, and dehydrated through a graded series of ethanol concentrations. The blocks were embedded in Spurr low viscosity embedding medium (Spurr 1969) and polymerized at 70°C for 10 h.

Thin sections were cut with glass knives on a Reichert Om U2 ultramicrotome, picked up on 200-mesh, carbon-reinforced, formvar-coated grids and stained with 7.5% uranyl acetate in 50% ethanol for 1 h followed by lead citrate (Reynolds 1963) for 4 min.

All thin sections and other samples prepared for electron microscopy were examined at an acceleration of 75 kV in a Hitachi Hu-12 electron microscope fitted with a double condenser.

**Light Microscopy**

Photomicrographs were taken with a Carl Zeiss (Jena) Nfpk microscope with either a Carl Zeiss (Jena) or Nikon AFM automatic exposure device.
EXPERIMENTAL RESULTS

PART I

STUDIES OF CHONDROMYCES CROCATUS

Initial studies of gliding motility in the myxobacteria involved an ultrastructural examination of *C. crocatus* in concert with attempts to isolate nonmotile mutants of this organism.

**General Ultrastructural Characteristics of Chondromyces crocatus**

*C. crocatus*, the only myxobacter for which thin sections were prepared, exhibited a typical gram negative cell envelope consisting of two unit membranes as well as a thin peptidoglycan layer (Fig. 2). The cytoplasmic membrane, which was about 6 nm thick, was separated from the peptidoglycan layer by an electron-translucent space. The periplasmic space was exterior to the cytoplasmic membrane, and bounded by the 7-8 nm thick outer membrane. Occasionally, some blebbing of the outer layer of the cell envelope was apparent (Fig. 3, 12, 13).

In addition to abundant dark-staining ribosomes and nuclear material, the cytoplasm contained at least two types of granules (Fig. 2). One type consisted of numerous electron-opaque deposits at polar locations, while the second appeared as electron-transparent areas with definite edges which were located at random in the cell.
Fig. 2. Thin section of *C. crocatus*. CM, cytoplasmic membrane; PL, peptidoglycan layer; OM, outer membrane; OG, electron-opaque granules; TG, electron-transparent granules; F, filaments, 7.5 - 10 nm in diameter. Fixation was in glutaraldehyde mixed with osmium in H₂O. The bar represents 100 nm.

Fig. 3. Electron micrograph of *C. crocatus* fixed in glutaraldehyde followed by OsO₄ in H₂O. Note microtubules, 15 - 19 nm in diameter, a membrane-associated structure with fibrillar extensions to the cytoplasmic membrane and surface blebs. T, microtubules; MAS, membrane-associated structure; B, bleb. The bar represents 100 nm.

Fig. 4. Thin section of *C. crocatus*. Note the membrane-associated structure with two double tracks parallel to the cytoplasmic membrane and with extensions to the cytoplasmic membrane. Cell fixed as described for Fig. 2. MAS, membrane-associated structure; CM, cytoplasmic membrane. The bar represents 50 nm.

Fig. 5. Thin section of *C. crocatus* disrupted during electron microscopic fixation. Cells fixed in glutaraldehyde mixed with OsO₄ in H₂O to which was added 4 x 10⁻⁴ M AgNO₃. MAS, membrane-associated structure; CM, cytoplasmic membrane; OM, outer membrane. The bar represents 100 nm.
Parallel arrays of filaments 7.5 - 10 nm in diameter and of varying length were often observed in longitudinal sections of polar regions of the cells (Fig. 2). In other cross and longitudinal sections, numerous 15-19 nm microtubules which frequently had 4 nm peripheral particles associated with them along their lengths were also seen (Fig. 3).

Membrane-Associated Structure

An unusual structure, termed here the membrane-associated structure (MAS), was observed in many thin sections, including those of disrupted cells (Figs. 3, 4, 5, 6). It consisted of two parallel double tracks 7.5 - 11 nm wide which varied in length from about 55 - 130 nm. The two double tracks were occasionally in contact (Fig. 3) but were usually separated by a space up to 3 nm wide (Fig. 4). Serial sections through MAS indicated that its other dimension was less than 250 nm (Fig. 6). MAS appeared, therefore, to be more or less disc-shaped. Extending from MAS to the cytoplasmic membrane were a number of apparently fibrillar extensions about 11-15 nm long and 7.5-15 nm apart.

Septum Formation

*Chondromyces crocatus* formed a well-defined septum (Figs. 7-10). The initial invagination of the cytoplasmic membrane (Fig. 7a) was followed by its progressive inward growth and the appearance within the invagination of the peptidoglycan layer (Fig. 7b). Formation of
Fig. 6 a-d. Serial sections of *C. crocatus* showing presence of the membrane-associated structure in two cross sections. (The reported thickness of MAS was based on estimation of the thickness of silver to gold colored sections.) Fixation as described for Fig. 2. MAS, membrane-associated structure. The bar represents 100 nm.
Fig. 7 a-d. Electron micrographs of septum formation in *C. crocatus*. Fig. 7a. Initial invagination of the cytoplasmic membrane. Fig. 7b. Progressive inward growth of the septum. Fig. 7c. Complete septum. Note the thickened peptidoglycan layer bounded on both sides by the cytoplasmic membrane and the outer membrane, which has yet to grow inward. Fig. 7d. Cell separation.

CM, cytoplasmic membrane; S, septum; N, nucleoid; PL, peptidoglycan layer; OM outer membrane; TG, electron-transparent granule. Fixation was in glutaraldehyde followed by OsO₄ in 0.02 M sodium cacodylate for Fig. 7a. Cells in Fig. 7b were fixed as described for Fig. 2. The cell in Fig. 7c was fixed in glutaraldehyde followed by OsO₄ in 0.02 M sodium cacodylate buffer to which was added 0.01% CaCl₂ and 0.01% casamino acids. Glutaraldehyde mixed with OsO₄ in 0.02 M sodium cacodylate buffer was the fixative used in Fig. 7d. The bar represents 200 nm in all micrographs.
Fig. 8. *C. crocatus* negatively stained with 0.5% uranyl acetate in 0.014 M veronal acetate buffer containing 0.01 M CaCl$_2$. S, septum. Upon drying, the cell flattened, showing the septum as a ring around the cell. The bar represents 300 nm.

Fig. 9. Platinum-carbon replica of *C. crocatus* vacuum dried *in situ*. S, septum. Micrographs of all replicas have been reversed so that shadows appear dark. The bar represents 400 nm.

Fig. 10a, b. Fig. 10a. Electron micrograph of *C. crocatus* fixed as described for Fig. 7a. Fig. 10b. *C. crocatus* negatively stained *in situ* with 1% PTA in H$_2$O at pH 7.0. M, mesosome; S, septum; CM, cytoplasmic membrane; OM, outer membrane. The bar represents 200 nm in both micrographs.
the complete septum (Fig. 7c) was followed by the invagination of the outer cell envelope layer and cell separation (Fig. 7d). Septum formation was observed in fixatives either with or without the addition of 0.10% CaCl₂ and 0.01% casamino acids. In addition to their presence in chemically fixed cells, septa were observed in in situ preparations that had been negatively stained or replicated (Figs. 8, 9, 10b).

Complex mesosomes of the type present in gram positive microorganisms were often located near the septum. These were seen in both thin sections (Fig. 10a) and cells negatively stained in situ (Fig. 10b).

**Electron Microscopy of In Situ Chandromyces crocatus**

*In situ* replicas revealed that cells in a swarm were surrounded by an amorphous slime layer. Polar fimbriae, 6 - 8 nm in diameter, 0.4 - 2.2 μm long and numbering up to six per cell, were observed on cells in replicated (Fig. 11) and negatively stained preparations (Fig. 12).

Air or vacuum-dried cells had little surface structure (Fig. 11). The cells were often in close contact with one another with some cellular connections apparently mediated by the slime. On the other hand, replicas of fixed cells often exhibited an abundance of bleb-like structures between the cells (Fig. 13) as well as many granular areas on the surface of the cell (Fig. 14). These areas were of two types, one having a finer granulation than the other.
Fig. 11. Electron micrograph of a platinum-carbon replica of *in situ* *C. crocatus*, air-dried. F, fimbriae; C, cells; SL, slime layer. The bar represents 200 nm.

Fig. 12. Electron micrograph of *C. crocatus* negatively stained *in situ* with 0.5% uranyl acetate in H₂O at pH 4.5. B, bleb; F, fimbriae. The bar represents 200 nm.
Figs. 13 - 14. Electron micrographs of *C. crocatus* cell surfaces replicated *in situ* with carbon-platinum. OsO₄ vapor fixed cells. B, bleb; FG, area of fine granulation on the cell surface; CG, area of coarse granulation on the cell surface. The bar represents 500 nm in Fig. 13 and 100 nm in Fig. 14.
Mutagenesis of Chondromyces crocatus

Attempts to isolate nonmotile mutants of *C. crocatus* were unsuccessful. Failure was due to the unpredictable growth of *C. crocatus* in agar pour plates and the inability to easily distinguish the compact colonies formed by the motile dispersed variant of *C. crocatus* from colonies of nonmotile variants, if such were present. The absence of nonmotile mutants of this organism prohibited an ultrastructural comparison of wild type and nonmotile variants and necessitated a search for nonmotile mutants among other genera of myxobacteria.
EXPERIMENTAL RESULTS

PART 2

STUDIES OF MYXOCOCUS XANTHUS AND OTHER MYXOBACTERIA

The second part of this study involved mostly *Myxococcus xanthus*, a widely studied and easily manipulated myxobacter. Other myxobacterial strains, including at least one representative from each genus, were examined. Many *Myxococcus xanthus* motility-altered mutants, some of which were nonmotile, were isolated. In an effort to relate an observed ultrastructural modification to the loss of motility, nonmotile and motile cells were examined by both ultrastructural and immunological techniques. Results from these experiments are described in this section of the dissertation.

Isolation of Motility Mutants of *Myxococcus xanthus*

Nonmotile variants of *M. xanthus* were initially selected on the basis of the "eubacterial" appearance of their colonies (Fig. 1a, b). This procedure was not without difficulty, since platings of *M. xanthus* even without treatment, usually yielded mixtures of colony types which in varying proportions consisted of typical spreading colonies (motile) and nonspreading colonies. Whether the latter were in fact of the "eubacterial" type described above could only be ascertained by close microscopic scrutiny. Many colonies were excluded from further study when they
were found to have narrow bands of displaced single cells and groups of cells at their peripheries (i.e. resembled the colony in Fig. 15a). Additionally, other colonies which were definitely "eubacterial" upon initial examination, exhibited evidence of peripheral swarming after subsequent transfers to other media and hence were discarded.

Thus, as not all colonies could be examined and since the definitive determination of the motility characteristics of the variants obtained required exhaustive study, it was not possible to estimate the frequency or mutation rates of either induced or spontaneous variants.

It should also be noted that in the determination of whether cell displacement observed on slide cultures was due to gliding, it was necessary to exclude a type of displacement that was occasionally observed when pairs of cells were in contact along a portion of their length. In such instances, one cell would slide along the other until they were in nearly complete lateral contact after which no further movement occurred. Since this phenomenon was observed in live as well as heat killed preparations (60°C, 0.5 hr) it was attributed to a physical cause, perhaps slime contraction, but not gliding.

Although a much larger number of isolates was examined, only fourteen were fully characterized with respect to motility. These isolates, which are listed in Table 1, were of four types:

Type 1 - Colonies of this isolate were initially of
<table>
<thead>
<tr>
<th>Name of Mutant</th>
<th>Type of Mutant</th>
<th>Method by Which Mutant Was Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIM36-2</td>
<td>1</td>
<td>Spontaneous - Cells spotted on SP/10 for swarming</td>
</tr>
<tr>
<td>MCM36-4g</td>
<td>2</td>
<td>Hycanthone - 5 µg/ml</td>
</tr>
<tr>
<td>MCM36-4q</td>
<td>2</td>
<td>Hycanthone - 10 µg/ml</td>
</tr>
<tr>
<td>MIM36-1*</td>
<td>3</td>
<td>Spontaneous - Cells spotted on SP for swarming</td>
</tr>
<tr>
<td>MIM36-2a*</td>
<td>3</td>
<td>ICR-191E - 0.11 µg/ml</td>
</tr>
<tr>
<td>MIM36-1g</td>
<td>3</td>
<td>Spontaneous - From plate counts</td>
</tr>
<tr>
<td>MIM36-1e</td>
<td>3</td>
<td>Hycanthone - 5 µg/ml</td>
</tr>
<tr>
<td>MIM36-2e</td>
<td>3</td>
<td>ICR-191E - 1.11 µg/ml</td>
</tr>
<tr>
<td>MIM36-3</td>
<td>3</td>
<td>Spontaneous - From plate counts</td>
</tr>
<tr>
<td>MIM36-5M</td>
<td>3</td>
<td>ICR-191E - 1.11 µg/ml</td>
</tr>
<tr>
<td>NMM36-1C</td>
<td>4</td>
<td>NTG in DM - 100 µg/ml</td>
</tr>
<tr>
<td>NMM36-3d</td>
<td>4</td>
<td>NTG in SP - 25 µg/ml</td>
</tr>
<tr>
<td>NMM36-5L</td>
<td>4</td>
<td>ICR-191E - 1.11 µg/ml</td>
</tr>
<tr>
<td>NMM36-6J</td>
<td>4</td>
<td>UV irradiation - 120 sec</td>
</tr>
</tbody>
</table>

*Note: These Type 3 mutants exhibited cell displacement on plates, whereas other Type 3 mutants did not.

All mutants were isolated from different experiments.
the "eubacterial" type with no evidence of cell migration around the colonies. Upon further incubation, however, the appearance of cellular tongues and isolated cells at the colony peripheries, indicative of gliding, were observed. The extent of movement depended upon the colony density on the plates and the time of incubation. On densely crowded plates (i.e., more than about 1000 colonies) cell displacement occurred after one week of incubation. Colonies on less densely crowded plates had cells separated from their edges beginning in about two weeks, while plates with very few colonies often did not exhibit cell translocation until after 3-4 weeks of incubation (Fig. 15a, b). The results were essentially the same on SP, SP/10 and CT-1, although CT-1 tended to suppress motility.

When examined in slide culture, the Type 1 mutants showed extensive cell displacement attributable to gliding (Fig. 15c, d).

**Type 2** — Colonies of these isolates were initially selected as nonmotile mutants because of their colonial morphology on plates. Subsequent platings of these colonies, however, yielded both wild type and "eubacterial" colonies as well as colonies with both "eubacterial" and wild-type sectors. Repeated attempts to isolate stable clones from colonies of this isolate were unsuccessful. Neither did slide cultures prepared from various platings of these isolates yield consistent results as cell translocation
Fig. 15a-d.  Fig. 15a. Light micrograph of an MIM36-2 colony with spreading edges. Fig. 15b. Phase micrograph of MIM36-2 showing the displacement of many cells from the colony edge. Fig. 15c, d. Phase micrographs of the same field taken one hour apart of MIM36-2 in slide culture. Cell movement is extensive. The bar represents 200 μm in Fig. 15a, 20 μm in Fig. 15b, and 5 μm in Fig. 15c-d.
was sometimes observed and sometimes not and when it did occur, involved varying numbers of cells.

**Type 3** - Representatives of this group showed little evidence of motility as indicated by colonial morphology and behaviour in slide culture. The colonies were in all instances largely of the "eubacterial" type although rarely in old cultures, tongues of cells were observed projecting from, but still attached to, the colony edge (Fig. 16a). In only two cases, MIM36-1 and MIM36-2a, were cells observed separated from the colony edge, but these were rare and involved only a few cells and colonies (Fig. 16b).

In slide culture, all of the Type 3 mutants did evidence some cell displacement attributable to gliding, but this was also rare and usually involved only one or two cells (Fig. 16c, d).

*M. xanthus* NM (Burchard 1970) most closely resembled mutants of Type 3 except that cell tongues and the occurrence of isolated cells and small groups of cells beyond colony edges were more frequently observed on older (4 weeks) more crowded plates (Fig. 17a). Similarly, in slide culture, while only a few cells were ever seen to have changed position (Fig. 17b, c, d), evidence of some movement was observed on most slides.
Fig. 16a-d. Fig. 16a. Phase micrograph of MIM36-1 showing tongues of cells (arrows) which appear to have resulted from outgrowth rather than gliding motility.

Fig. 16b. Phase micrograph of MIM36-1 showing the displacement of a few cells from the colony edge.

Fig. 16c, d. Phase micrographs of MIM36-5M taken one hour apart. The arrow indicates the cell that has moved. The bar represents 30 μm in Fig. 16a, b and 5 μm in Fig. 16c, d.
Fig. 17a-d. Fig. 17a. Phase micrograph of an *M. xanthus* NM colony edge showing single cells and clumps of cells (arrows) displaced from the colony edge. Fig. 17b-d. Phase micrographs taken at hourly intervals of *M. xanthus* NM in slide culture. The arrows indicate some of the cells which have changed position during observation. The bar represents 30 μm in Fig. 17a, and 5 μm in Fig. 17b-d.
During these studies three spontaneous motile revertants of *M. xenothus* NM were isolated, all of which exhibited, in plate culture, the swarming behaviour of motile cells. Swarming, as indicated by the rate of colony expansion on plates, was not, however, as extensive as for wild type cells, although after transfer from either liquid or solid growth medium to a slide culture, the movement exhibited by the revertants was comparable to wild type movement.

**Type 4** - Mutants of this type never exhibited evidence of motility with any of the test conditions employed. The colonies were always of the "eubacterial" type (Fig. 1a, b) on all media and repeated observation in slide culture over long time periods yielded no evidence of cell displacement. The phase micrographs of NMM36-5L shown in Figures 18a and b, taken over a four hour interval, are typical of the results obtained for all the Type 4 isolates.

While the other Type 4 mutants were of normal cellular morphology, NMM36-3d when first inoculated into liquid media yielded spirillum-like cells (Fig. 19a). On subsequent transfers to liquid media or upon growth on agar media, the cell morphology became less irregular but was still distinctly different from normal (Fig. 19b). Growth on all media appeared somewhat slower than that of the other Type 4 mutants and the wild type. NMM36-3d
Fig. 18a, b. Phase micrographs, taken four hours apart of the same field of NMM36-5L in slide culture. No cells have changed position during observation. The bar represents 5 μm in both figures.

Fig. 19a, b. Phase micrographs of NMM36-3d. Fig. 19a shows spirillum-like cells. Fig. 19b shows cells with slightly irregular morphology. The bar represents 5 μm in both figures.
also differed from all other strains in producing less slime.

**Fibril Isolation from *Myxococcus xanthus***

Fibrils with an approximate diameter of 8 - 5 nm and of varying length (Fig. 20) were originally observed in all-lysates of wild type cells but only very rarely seen in any of a number of replicate samples of the non-motile mutants.

Attempted purification of these fibrils by actin purification methods (Clarke and Spudich 1974; Spudich 1974; Abramowitz et al. 1975), decoration of the fibrils with heavy meromyosin (HMM) (Pollard and Korn 1971; Forer and Behnke 1972; Forer and Jackson 1975; Palevitz et al. 1974) obtained from Dr. A. Forer of York University, Toronto, and demonstration of a deoxyribonuclease inhibitory activity (Lazarides and Lindberg 1974) in *M. xanthus* cell lysates were unsuccessful. It was noted, however, that the fibrils bore a resemblance to fimbriae earlier observed on *C. crocatus* (Figs. 11, 12).

**Myxobacterial Fimbriae**

**Survey of Myxobacteria for Fimbriae**

Examination of negatively stained *in situ* samples of motile *M. xanthus*, grown on agar, consistently revealed fimbriae, usually on one cell pole only and present in varying numbers (Fig. 21). These fimbriae, which were
Fig. 20. An electron micrograph of a negatively stained fibril from *M. xanthus*. The bar represents 100 nm. All subsequent samples prepared for electron microscopy were negatively stained with 1% aqueous uranyl acetate (pH 3.9).

Fig. 21. An electron micrograph of negatively stained motile *M. xanthus* cells showing polar fimbriae (arrows). The bar represents 200 nm.

Fig. 22. An electron micrograph of negatively stained nonmotile *M. xanthus* cells showing the absence of polar fimbriae. Thin, probably slime, fibers (arrows) are present. The bar represents 100 nm.
identical in appearance to the fibrils previously detected (Fig. 20), were never observed in any in situ preparations of the nonmotile mutants (Fig. 22). Very rarely, fibrils of similar structure to the fimbriae were observed in preparations of nonmotile cells but they were never attached to cells. In addition, attempts to purify fimbriae from nonmotile mutants by differential centrifugation yielded only a small number of fibrils similar to fimbriae. Much finer fibrils perhaps attributable to slime were, however, commonly observed in all in situ preparations of motile and nonmotile M. xanthus grown on agar (Fig. 22).

Extension of these methods revealed fimbriae on all other genera of motile myxobacteria examined, except Nannocystis (Table 2) and (Fig. 23). For Nannocystis exedens, repeated attempts to demonstrate fimbriae by in situ and agar extraction methods were unsuccessful. Fimbriae were either not observed or observed in very low numbers in in situ samples of various motility-altered mutants including: MIM36-1, MIM36-1g, MIM36-2e, MCM36-4q and MIM36-2. Further, motile M. xanthus failed to produce fimbriae in liquid culture, a condition in which gliding does not occur.

Fimbriae were either not detected or were present in very low numbers when in situ and differential centrifugation procedures were applied to M. xanthus NM. Fibers, similar to fimbriae, but usually not attached to cells, and
Table 2
Genera of Motile Myxobacteria Examined for Fimbriae

<table>
<thead>
<tr>
<th>Myxobacteria Examined</th>
<th>Average Fimbriae Diameter (nm)</th>
<th>Polar Holes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. xanthus</em> - M36</td>
<td>8.5</td>
<td>+</td>
</tr>
<tr>
<td><em>M. fulvus</em> - M7</td>
<td>8.2</td>
<td>+</td>
</tr>
<tr>
<td><em>M. virescens</em> - M22</td>
<td>8.7</td>
<td>+</td>
</tr>
<tr>
<td><em>A. gephyra</em> - M58</td>
<td>8.1</td>
<td>+</td>
</tr>
<tr>
<td><em>A. violaceum</em> - M210</td>
<td>7.8</td>
<td>+</td>
</tr>
<tr>
<td><em>C. fusca</em> - M29</td>
<td>6.9</td>
<td>+</td>
</tr>
<tr>
<td><em>M. lichenicola</em> - M155</td>
<td>8.5</td>
<td>+</td>
</tr>
<tr>
<td><em>S. aurantiaca</em> - M341</td>
<td>8.5</td>
<td>+</td>
</tr>
<tr>
<td><em>P. cellulosum</em> - M209</td>
<td>7.5</td>
<td>+</td>
</tr>
<tr>
<td><em>P. cellulosum</em> - M222</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td><em>P. cellulosum</em> - M254</td>
<td>7.5</td>
<td>-</td>
</tr>
<tr>
<td><em>H. exedens</em> - M349</td>
<td>No Fimbriae</td>
<td>-</td>
</tr>
<tr>
<td><em>C. crocatus</em> - M467</td>
<td>6 - 8</td>
<td>-</td>
</tr>
</tbody>
</table>

* Note: *A. violaceum* exhibited the greatest number of fimbriae.

"+" - Holes observed.

"-" - Holes not observed.

"+-" - Uncertain if holes were observed.
Fig. 23a-f. Electron micrographs of negatively stained, in situ myxobacteria. Polar fimbriae are present in all genera except Nannocystis which instead often exhibited thin fibrils. Fig. 23a. Archangium violaceum (M210); Fig. 23b. Cystobacter fuscus (M29); Fig. 23c. Mellittangium lichenicola (M155); Fig. 23d. Stigmatella aurantiaca (M341); Fig. 23e. Polyangium cellulosum (M209); Fig. 23f. Nannocystis exedens (M349). The bar represents 200 nm in all micrographs.
in only small numbers were observed when the three motile revertants of *M. xanthus* NM were examined by *in situ* differential centrifugation and agar extraction methods.

Present in polar regions of many myxobacteria, as well as in membrane fragments from cells, were what we referred to as "holes" (Fig. 24a, b) and (Table 2). *M. xanthus*, the only species for which a thorough study was made, had "holes" with an inside diameter of about 7.1 nm, surrounded by an electron transparent collar, about 2.6 nm thick, from which 12 spike-like projections were sometimes observed to protrude (Fig. 24c).

**Partial Purification of Fimbriae**

Fimbriae were partially purified by differential centrifugation resulting, as measured by total protein concentration, in a relatively low yield (Table 3). The final fimbriae product possessed residual membrane contamination (Fig. 25).

**Effect of Chemical and Physical Agents on Fimbriae**

Trypsin, protease type VI, protease type VIII, alpha amylase, and freezing and thawing numerous times had no effect on fimbriae. Fimbriae were indifferent to alkaline, neutral and slightly acid pH's at 30 and 37°C but were almost completely disrupted after 23 hr at pH 4.0 and 3.0 at 30°C and at pH 4.5 and 4.0 at 37°C.
Fig. 24a-c. Fig. 24a. Negatively stained *M. xanthus* with "holes" (arrows) evident at the cell pole. Fig. 24b. Membrane fragment of *M. xanthus* showing "holes", some of which (arrows) exhibited evidence of protrusions from the collar. Fig. 24c. Electron micrograph of a negatively stained, isolated "hole" showing 12 spike-like protrusions from the collar. The bar represents 100 nm in Fig. 24a, b and 50 nm in Fig. 24c.
Table 3

Partial Purification of Fimbriae*

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Step</th>
<th>Volume (ml)</th>
<th>Protein (μg/ml)</th>
<th>Total Protein (μg)</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10,000 g supernatant</td>
<td>935</td>
<td>85</td>
<td>79,475</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>100,000 g sediment</td>
<td>23.6</td>
<td>271</td>
<td>63,956</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>20,000 g supernatant</td>
<td>22</td>
<td>126</td>
<td>2,772</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>from fraction 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100,000 g sediment</td>
<td>1.1</td>
<td>1,213</td>
<td>1,334</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>from fraction 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Representative result from a typical purification.
Fig. 25. Electron micrograph of a negatively stained partially purified (100,000 g sediment-concentrated 20 X) fimбриae preparation showing residual membrane contamination (arrows). The bar represents 200 nm.
Immunology

Production and Adsorption of Anti-Fimbriae Serum

Control serum obtained before immunization of rabbits failed to inhibit the migration of cells from colonies and the movement of single cells in slide culture (Table 4). Serum raised against partially purified fimbriae inhibited the migration of cells from colonies and the movement of cells in slide culture. The anti-fimbriae serum did not, however, as indicated by the continued reproduction of immobilized cells in slide culture, prevent the growth of M. xanthus (Fig. 26). Following adsorption, all anti-fimbriae sera, except that adsorbed with M. xanthus grown on agar, retained the ability to inhibit cellular migration from colonies. The ability to inhibit single cell movement, as indicated by slide culture techniques, varied (Table 4).

Immune Electron Microscopy

Extensive fimbriae decoration by anti-fimbriae antibodies was indicated by fuzziness along the entire length of the fimbriae (Fig. 27a). As the serum was progressively diluted large clumps of loosely joined fimbriae with pairs of parallel fimbriae joined by antibody molecules (Fig. 27b) or isolated pairs of antibody-linked fimbriae (Fig. 27c) were present. The distance between parallel fimbriae was about 9.5 - 13.0 nm.

As shown by Table 4, control serum failed to decorate fimbriae. The ability of anti-fimbriae serum
Table 4

Ability of Unadsorbed and Adsorbed Anti-fimbriae Serum to Inhibit Motility and Decorate Fimbriae of *Mycoplasma xanthus*

<table>
<thead>
<tr>
<th>Serum Tested</th>
<th>Ability of Serum to Inhibit Motility</th>
<th>Ability of Serum to Decorate Fimbriae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plate Culture</td>
<td>Slide Culture</td>
</tr>
<tr>
<td>Unimmunized</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-fimbriae serum, Unadsorbed</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Anti-fimbriae serum adsorbed with <em>M. xanthus</em> grown on agar (fimbriated cells)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-fimbriae serum adsorbed with <em>M. xanthus</em> grown in liquid (nonfimbriated cells)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Anti-fimbriae serum adsorbed with NMM36-1C (nonmotile mutant)*</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Anti-fimbriae serum adsorbed with NMM36-3d (nonmotile mutant)</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Anti-fimbriae serum adsorbed with NMM36-5L (nonmotile mutant)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Anti-fimbriae serum adsorbed with NMM36-6J (nonmotile mutant)</td>
<td>+++</td>
<td>†</td>
</tr>
</tbody>
</table>

"+" - Inhibition or labelling
"-" - No inhibition or no labelling
* - All nonmotile mutants lack fimbriae
Fig. 26a-c. Phase micrographs of motile *M. zanthus* in slide culture demonstrating that cell movement but not cell growth is inhibited by unadsorbed, anti-fimbriae serum. Fig. 26a. 0 min; Fig. 26b. 240 min post inoculation; Fig. 26c. 420 min post inoculation. The bar represents 5 μm in all figures.
Fig. 27a-c. Electron micrographs of mixtures of fimbriae and anti-fimbriae serum negatively stained with uranyl acetate. Fig. 27a. Heavily labelled fimbriae (Undiluted serum adsorbed with NMM36-1C). Fig. 27b. Large clump of fimbriae with parallel pairs of fimbriae, apparently connected by antibody molecules (arrows), projecting from the clump edge (Unadsorbed serum diluted 1:10). Fig. 27c. An isolated, small group of fimbriae showing pairs of parallel fimbriae, apparently connected by antibody molecules (arrows) (Serum adsorbed with NMM36-1C and diluted 1:10). The bar represents 100 nm in all figures.
adsorbed with *M. xanthus* grown on agar to decorate fimbriae was greatly reduced when compared to other sera, both adsorbed and unadsorbed, all of which labelled fimbriae about equally (Table 4).

**Immuonelectrophoresis**

Upon immunoelectrophoresis of a partially purified fimbriae preparation using control serum, a compact band formed by a negatively charged antigen(s) was evident (Fig. 28a). This band probably consisted of fimbriae that were not removed from the agar during washing. Using unadsorbed anti-fimbriae serum, two relatively diffuse bands with opposite charges were observed (Fig. 28b). Antibody against the positively charged band disappeared or almost completely disappeared after adsorption with all strains of *M. xanthus* listed in Table 4 (Fig. 28c, d). The only significant reduction in antibody against the negatively charged antigen(s) came after serum adsorption with fimbriated *M. xanthus* (i.e. motile *M. xanthus* grown on agar) (Fig. 28c). Adsorption with nonfimbriated strains of *M. xanthus* (i.e. either *M. xanthus* grown in liquid or nonmotile *M. xanthus*) did not cause a significant reduction in antibody against the negatively charged antigen(s) (Fig. 28d).

Electron microscopy of the oppositely charged antigens, extracted from the agar in P04 buffer, revealed that the fimbriae were in the negatively charged precipitin band.
Fig. 28a-d. Photographs of immunoelectrophoresis slides. The wells contained partially purified fimbriae (636 µg protein/ml). The troughs contained:
a. control serum, b. unadsorbed anti-fimbriae serum, c. anti-fimbriae serum adsorbed with motile *M. xanthus* grown on agar, d. anti-fimbriae serum adsorbed with motile *M. xanthus* grown in liquid (these results are representative of anti-fimbriae serum adsorbed with all nonmotiles).
DISCUSSION

Examination of C. croatus thin sections revealed that ultrastructurally, this microorganism is, in most respects, identical with other gram negative bacteria. The cell envelope is typical in appearance (Glaubert and Thornley 1969; Costerton et al. 1974a) except that it has a very thin peptidoglycan layer. A thin peptidoglycan is, however, not unique to C. croatus (Costerton et al. 1974b).

The cytoplasm contained many dense-staining ribosomes as well as at least two types of granular inclusions with unknown chemical identities. The electron transparent areas morphologically resemble poly β-hydroxybutyric acid (PHB) (Wang and Lundgren 1969; Vela et al. 1970; Craig et al. 1973), but freeze etching (Mccurdy, unpublished data) and chemical analysis (Dworkin, personal communication; McCurdy, unpublished data) have so far failed to reveal PHB in the myxobacteria. The electron dense granules may consist of glycogen. Since the electron microscopic appearance of glycogen varies (Ellar and Lundgren 1966; Voelz 1967; Craig et al. 1973) identification of glycogen granules on morphological grounds is difficult. Positive identification of these two inclusions awaits histochemical analysis.
At present, little may be said about the filaments. They were not observed in negatively stained preparations and may be simply preparation artifacts. It is unlikely that the microtubules are artifacts for they have been observed in thin sections of other myxobacteria (Schmidt-Lorenz and Kühlwein 1968; McCurdy, unpublished data). Schmidt-Lorenz and Kühlwein ascribed to them a function in motility. Van Iterson et al. (1967) considered the possibility that similar structures in *Proteus mirabilis* may have a role in swarming. In eukaryotic cells, microtubules are frequently associated with actin filaments (van Iterson et al. 1967; Goldman 1971; Forer 1974) and as such may have some role in cell movements and locomotion. The microtubules observed in sections are somewhat smaller in diameter than rhapidosomes. Rhapidosomes are structures seen in negatively stained preparations from various myxobacteria (Reichenbach 1965, 1967; McCurdy and MacRae 1974) and are thought to be defective bacteriophage components.

Abadie (1971), examining *C. crocatus* from mixed cultures, reported a structure similar to MAS. Since the cultures involved were not pure, an unambiguous determination of whether or not the structure was in *C. crocatus* or the contaminant was not possible. In contrast to our observations, he observed the structure only in longitudinal
sections and only at the poles of the cells. He speculated that the structure may be involved with gliding motility and slime secretion, as a chemoreceptor organelle, or as all three. The structure morphologically has certain resemblances to polar membranes which are found in certain flagellated bacteria and which are thought to have some role in movement (Cohen-Bazire and Kunisawa 1963; Murray and Birch-Andersen 1963; Remsen et al. 1968; de Boer and Hazeu 1972; Chalcroft et al. 1973; Hammill and Germano 1973).

It is interesting to speculate whether the structure seen in C. crocatus might represent a remnant of an ancestral flagellar apparatus. As such it could be completely vestigial or it could have some yet undetermined role in gliding. Obviously such possibilities make further study of MAS of extraordinary interest in relation to the evolution and mechanism of gliding motility and justify a continued search for nonmotile mutants of C. crocatus.

Many gram-negative microorganisms, including two myxobacteria (Voelz 1965; Zhilina 1968), are said to divide by constriction (Poindexter and Cohen-Bazire 1964; Steed and Murray 1965; de Boer and Hazeu 1972; Hammill and Germano 1973; Burdett and Murray 1974b). Constriction involves a simultaneous inward growth of all layers of the gram negative cell envelope and the pinching
apart of daughter cells without the formation of a septum. Septa have, however, been clearly demonstrated in a number of gram-negative bacteria (Kaiser and Starzyk 1973; Rodolakis et al. 1973; Burdett and Murray 1974a, b; Costerton et al. 1974b; Sleytr et al. 1974) including the myxobacteria (McCurdy, unpublished data). Burdett and Murray (1974a, b), after extensive examination of various strains of *E. coli*, stated that division by constriction in this organism is an artifact of electron microscopic preparation probably resulting from inadequate cellular fixation. *C. acroca tus* has been shown here to form a well-defined septum during cell division.

Like septa, complex mesosomes, so conspicuous in gram positive bacteria (Kakefuda et al. 1967; Burdett and Rogers 1972) are often difficult to demonstrate in gram negative microorganisms even though they have been observed in an increasing number of species (Poindexter and Cohen-Bazire 1964; Voelz 1965; Cohen-Bazire et al. 1966; Pate and Ordal 1967; Hoffman et al. 1973; Rucinsky and Cota-Robles 1974). *C. acroca tus* possesses complex mesosomes that, since they are frequently seen in proximity to septa, probably have a role in septum formation.

Why is it possible to preserve septa and mesosomes in *C. acroca tus* whereas in other gram negative bacteria this is extremely difficult or impossible? The growth and fixation of *C. acroca tus* on agar, as opposed to liquid culti-
vation, may favour the preservation of septa and mesosomes, as centrifugation, a potentially disruptive manipulation during fixation, is unnecessary. The use of \( \text{OsO}_4 \) and glutaraldehyde combined and of buffers with low osmolarities may favour preservation of septa and mesosomes in gram-negative bacteria. The very slow growth rate of \( C. \text{crocatu} \text{s} \) (generation time: 20 to 30 h, unpublished data) may be correlated with a relatively slow process of septum formation and therefore, considerable stability of associated structures. In more rapidly dividing cells the balance between synthesis of necessary components and hydrolytic enzymes involved in septum formation may be quite precarious. Thus, attempts to demonstrate septa may be prevented by premature separation of the septal wall or membranes during fixation. According to this view constriction is a fixation artifact but this view is not universally accepted (Hammill and Germano 1973).

Morphological variation between fixed and unfixed cells of \( C. \text{crocatu} \text{s} \) examined in situ may be an artifact of preparation, or the well defined blebs and granular areas of fixed cells may represent a slime secretion mechanism. Blebbing, as a mechanism of secretion, has been indicated for other bacteria (Chatterjee and Das 1967; Henderson and Hodgkiss 1973) but it is possible that blebbing is a response to
unfavourable environmental conditions (Knox et al. 1966; Bayer 1967) or is a preparation artifact. The slime, as for Streptococcus mutans (Bozzola et al. 1973; Johnson et al. 1974) and marine bacteria (Corpe 1970; Fletcher and Floodgate 1973), may play a role in attachment to solid surfaces. Others have postulated that the slime functions in gliding (Doetsch and Hageage 1968). Pimbriae, and their possible functions, will be discussed later in this report.

C. crocatus was the myxobacter initially chosen for studies of gliding. Several interesting structures, possibly associated with motility, were observed but the failure to isolate nonmotile mutants of C. crocatus prevented further study of the relationship of these structures to the motility mechanism. For this reason, a search for nonmotile mutants was begun using the more easily manipulated myxobacter, Myxococcus xanthus.

Experiments described in this report, using M. xanthus, have resulted in the isolation of four types of variants with altered motility. It should be noted, when characterizing these mutants, that a variety of environmental factors including nutrient concentration, surface moisture, humidity, colony density, culture age and physiological condition may affect gliding (Henrichsen 1972; Glaser and Pate 1973; Grimm and
Kühlein 1973a; Burchard 1974). Also changes in the properties of the slime produced by myxobacteria may lead to an apparent loss of motility (Grimm and Kühlein 1973a, b). For these reasons, putative non-motile mutants require extensive study using a variety of conditions with particular attention to the behaviour of cells in slide cultures.

Consideration of the factors that may be involved in the behaviour of motile cells in colonies is useful in attempting to explain the behaviour of the Type l mutant. Burchard (1974) has noted that cells of M. xanthus tend to move up a nutrient concentration gradient. Depletion of nutrients in the immediate neighbourhood of the colony would tend to create just such a gradient. On the other hand, there is evidence that myxobacterial cells secrete a chemotactic substance(s) that causes the attraction of single cells or smaller groups of cells to larger masses in their proximity. This is dramatically demonstrated in the time lapse motion pictures of Reichenbach (1966). One hypothesis explaining the behaviour of the Type l mutant cells is that they fail to respond, as does the wild type, to the nutrient gradient at the periphery of the
colony. Depending on the degree of crowding of the plate and the time of incubation, however, the chemotactic substance(s) secreted by neighbouring colonies eventually exerts an influence either by "flooding" the gradient tending to hold the cells within the colony or by occasioning a countervailing external gradient, perhaps in concert with some enzyme action. In any case the movement of cells away from the colony would be permitted.

The Type 1 mutant is interesting, then, in that it offers possibilities for the study of both gliding and chemotaxis. The latter subject is of particular interest for the myxobacteria as they exhibit cellular co-operation, probably mediated by chemical messengers, throughout their complex life cycle (Dworkin 1971, 1973).

Type 2 mutants were unstable and may represent another example of the phase variation previously reported in M. xanthus by Wireman and Dworkin (1975) and A. violaceum by Grimm and Kühlwein (1973a, b).

The Type 3 isolates may represent mutants in which the motility mechanism has been affected, but the rare occurrence of motile cells in slide culture and evidence of translocation from an occasional colony prevents an unambiguous judgement. It may be that what was observed was the appearance of revertants, although
isolated swarmer colonies were never observed. On the other hand, the lack of motility may be due to secondary factors such as modification in the character of the slime as suggested by Grimm and Kühlwein (1973a, b) rather than an alteration in the "Begwegungsapparat" or motility apparatus.

*M. xanthus* NM resembled the Type 3 isolates most closely. Cell movement was, however, more consistently observed in slide culture and was more consistently suggestive of motility. The three revertants of *M. xanthus* NM might suggest that the indications of motility which were observed were due to their presence in the population, but this is doubtful.

Of the isolates examined only four, the Type 4, failed to exhibit evidence of movement under any of the experimental conditions. It is these mutants which appear most likely to be affected at the level of the motility mechanism rather than in some secondary characteristic such as slime composition and for this reason this group of mutants was chosen for further study. That there were at least two types of nonmotile mutants, as indicated by the morphological abnormality exhibited by NMM36-3d, was of some interest as each type of nonmotile mutant may be altered in different functions or components of the motility mechanism. This could be of considerable value
in attempting to construct, by comparison with the wild
type, an idea of the nature of the motility mechanism.

Actin and/or myosin are found in several
eucaryotic systems including: the slime mold, Dicyo-
etrium discoideum (Clarke and Spudich 1974), Amoeba
proteus (Pollard and Korn 1971), the green alga, Nitella
(Palevitz et al. 1974), the higher plant Haemanthus
katherinae Baker (Forer and Jackson 1975) and human
platelets (Abramovitz et al. 1975). The seemingly
ubiquitous distribution of these contractile proteins
and their involvement in various cellular movements
suggests that they form a common molecular basis for
motility. For this reason whole cell lysates of motile
and nonmotile M. xanthus were examined for actin-like
filaments.

Fibrils, similar in diameter to actin (Pollard
and Korn 1971; Palevitz et al. 1974; Forer and Jackson
1975) were found in motile but not nonmotile M. xanthus.
These structures could not be concentrated by actin
purification methods nor labelled with HMM thus
indicating that they were either not actin or were
sufficiently different from eucaryotic actin to preclude
interaction with HMM. That a search for actin-like
contractile proteins should be continued, however, is
indicated by the recent report of such a protein in
E. coli (Minkoff and Damadian 1976) where it is thought to have a role in ion exchange.

Due to the resemblance of the fibrils from M. xanthus to fimbriae previously seen on C. crocatus, in situ samples of wild type and nonmotile mutants of M. xanthus were examined for fimbriae. The observation that all nonmotile mutants of M. xanthus lacked the polar fimbriae so conspicuously displayed by motile cells indicated a possible role for these structures in gliding. This was supported by the presence of fimbriae on all genera of myxobacteria except Nannocystis. Nannocystis was, however, difficult to manipulate. It swarmed well only on Y-Ca agar and fimbriae were more difficult to demonstrate, even on M. xanthus, when grown on this agar. In addition, swarming cells of Nannocystis tended to depress the agar during growth and hence did not readily attach to grids, making it difficult to obtain good in situ samples. Bradley (1972a, b) has shown that negative staining, chemical fixation and shadow-casting can cause loss (retraction?) of Pseudomonas aeruginosa fimbriae. Thus fimbriae of N. exedens, if present, could have been adversely affected in the same manner during electron microscopic preparation. If this were the case, methodology other than that already employed may demonstrate fimbriae on this microorganism.
Twitching, which can be considered as a disorganized type of gliding, is inhibited by specific anti-fimbriae serum in *Neisseria gonorrhoea* (Brinton, personal communication). Evidence presented by Henrichsen (1975a, b, c), Henrichsen and Blom (1975a, b) and Henriksen and Henrichsen (1975) has also indicated that twitching is correlated with fimbriae in a variety of gram negative and one gram positive bacterium. Henrichsen and Blom (1975b) were, however, unable to find a correlation between gliding and fimbriaation in *Cytophaga* and *Vitreoscilla* but they did not examine *in situ* preparations.

In order to characterize the fimbriae and as an aid in determination of motility-related functions these structures were partially purified by differential centrifugation. This procedure resulted in a relatively low yield (about 2%) with residual membrane contamination. That attempts to increase fimbriae yield by ammonium sulfate and pH precipitation were unsuccessful was probably due to the low concentration of fimbriae protein in the initial suspension.

Fimbriae from *Proteus vulgaris* (Weibull and Hedvall 1953) are similar to those from *M. xanthus* in their resistance to proteolytic enzymes. On the other hand, *E. coli* fimbriae vary in their resistance to proteolytic enzymes such as trypsin, pepsin and chymotrypsin
Polar fimbriae from *Pseudomonas aeruginosa* (Weiss and Raj 1972) are depolymerized at approximately the same pH and temperature as fimbriae from *M. xanthus* whereas peritrichous fimbriae from *P. vulgaris* (Weibull and Hedvall 1953) and *E. coli* (Brinton 1959, 1965) can tolerate extremes of pH for long periods of time without depolymerizing. A method for depolymerization and subsequent repolymerization of myxobacterial fimbriae, such as that employed by Brinton (1965), especially in concert with an initial concentration of fimbriae, would facilitate fimbriae purification.

As one approach to determining if fimbriae possessed motility-related functions, antibody was raised against the partially purified fimbriae preparation. This serum inhibited movement of *M. xanthus* in both plate and slide culture. Upon adsorption with cells lacking fimbriae the anti-fimbriae serum always retained the ability to inhibit migration of cells in plate culture, but sometimes lost the ability to inhibit single cell movement in slide culture. The loss of anti-motility activity, as demonstrated by the slide culture technique, suggested that fimbriae may not have a role in motility *per se*.

To test further the role of fimbriae in gliding, immune electronmicroscopy was employed. It would be expected if fimbriae are the organelles of motility in
*M. xanthus*, and if binding of antibody molecules to fimbriae inhibits fimbria function(s) (Bradley 1972a; Novotny and Pives-Taylor 1974), then adsorbed serum failing to inhibit movement of single cells in slide culture would also fail to decorate fimbriae.

*M. xanthus* fimbriae, decorated similarly to other fimbriae (Lawn 1967; Lawn and Maynell 1970; Novotny and Turner 1975), flagella (Elek et al. 1964; Lawn 1967; Kagawa et al. 1973) and viruses (Hoglund 1967, 1968; Almeida and Waterson 1969) were obtained by immune electron microscopy using adsorbed anti-fimbriae serum. The distance separating antibody labelled fimbriae corresponds to some published results (Feinstein and Rowe 1965; Valentine and Green 1967; Hoglund 1968; Novotny and Turner 1975) but controversy exists regarding the size and shape of antibody molecules attached to antigens (Almeida et al. 1965; Almeida and Waterson 1969; Feinstein and Rowe 1965; Valentine and Green 1967; Green 1969; Hoglund 1968). That anti-fimbriae antibody was present in adsorbed serum was further confirmed by immunoelectrophoresis combined with agar extraction of immunoelectrophoresis slides.

The decoration of fimbriae correlated well with the effect on motility as judged by the plate culture method as in all cases when labelling of fimbriae occurred,
movement was inhibited. On the other hand, adsorbed antiserum which failed to inhibit movement of single cells in slide culture did nevertheless decorate fimbriae.

These results cast further doubt upon the unequivocal conclusion that fimbriae have a direct role in motility per se. If fimbriae lack this function, there is the possibility that they have a role in the coordination of movement such as that exhibited in the migration of cells, in groups, from colonies. The absence of fimbriae on the three motile revertants of *M. xanthus* NM and MIM36-2, all of which fail to swarm normally, is in agreement with this possibility. Contrary to this are other strains of motile *M. xanthus* which possess fimbriae but exhibit reduced swarming on agar plates (unpublished observations). Modification of slime causing increased cellular adhesiveness could be responsible for reduced swarming in these fimbriated strains.

Discrepancies in experimental results concerning the role of fimbriae in gliding may have been caused by variations in the experimental systems. Test organisms are placed in direct contact with the serum in the slide culture test, whereas in the plate test the serum must diffuse through the agar to the bacteria. Thus, serum titre experienced by cells in the two procedures is certain
to vary. In the same way, the titre, and subsequently the degree of fimbriae labelling in the plate and slide culture test could vary when compared to the labelling of fimbriae in the immune electronmicroscopy technique.

Determination of the extent of fimbriae labelling upon exposure to anti-fimbriae serum, in the plate and slide culture test, would facilitate interpretation of these results. Also repetition of these experiments using a more highly purified fimbriae preparation as antigen, would help to clarify the function(s) of fimbriae.

Ottow (1975) and Brinton (1965) have suggested that fimbriae may function as organs of transport, either for nutrients and other metabolites or for bacterial and viral nucleic acids. Should fimbriae mediate the transfer of informational molecules, they would confer an ecological advantage upon fimbriated cells as the organization of single cells into informational syncitia would be permitted. Such an organized condition is exhibited by the myxobacteria, both during nutrient gathering and fruiting body formation.

Brinton (1965) has also shown that E. coli fimbriae, due to their large number of amino acids with nonpolar side chains, are distinctly hydrophobic and therefore can escape from an aqueous environment. Henrichsen (1975b, c) having noted that twitching occurs at the colonial air-water interface, and assuming that the polar fimbriae of twitching bacteria are hydrophobic, has argued that the hydrophobicity of these fimbriae
confers upon twitching bacteria an affinity for the colonial air-water interface. In this manner fimbriae influence twitching.

Myxobacteria concentrate at air-water interfaces (McCurdy, unpublished data) and gliding has been reported to occur at such interfaces (Henrichsen 1975c). The fimbriae of gliding microorganisms may affect motility by conferring upon cells an affinity for the air-water interface known to occur at the edge of colonies (Henrichsen 1975 b, c). This proposed, gliding-related, fimbrial function could be tested using experiments similar to those done by Henrichsen (1975b, c) in his studies of twitching.

Perhaps related to the polar fimbriae are the "holes" observed in polar regions of many different myxobacteria. Similar structures were observed in Neisseria gonorrhoeae (Swanson et al. 1971; Swanson 1972; Novotny et al. 1975), N. meningitidis (Swanson and Goldschneider 1969), Bacteroides nodosus (Hamilton et al. 1975), and Pseudomonas aeruginosa (Bradley 1972a). These structures were correlated in all cases with fimbriae, except in N. meningitidis, an organism which has subsequently been shown to possess fimbriae (Devoe and Gilchrist 1974). It has been stated by Bradley (1972a) and Hamilton et al. (1975) that the "holes" represent locations where fimbriae are inserted into the cells. Hamilton et al. (1975)
further suggest that the collars may act as local reinforce-
ments of the cell envelope to accommodate fimbriae insertion.
Reports of fimbriae retraction and extrusion from cells
(Bradley 1972a, b; Jacobson 1972; Lawn and Maynell 1975;
Lotz and Pfister 1975; Novotny and Fives-Taylor 1974),
are consistent with these ideas. On the other hand,
Novotny et al. (1975) have stated that these structures
are not holes, but rather rings. Swanson (1972), although
seemingly convinced that the structures are holes, suggests
they may be membrane foci that bind larger numbers of
stain molecules by virtue of their chemical composition
rather than because of their depressed contour.

As the "holes" of *M. xanthus*, according to available
information, are smaller in diameter than the fimbriae, it
would seem unlikely that they act as insertion loci for
fimbriae, but this obviously needs further investigation
before a definitive conclusion is reached. Indeed, the
relationship of the fimbriae to the "holes", and of both
these structures to gliding requires a great deal more
work.
SUMMARY

An ultrastructural study of *Chondromyces crocatus* revealed that this gram negative microorganism divides by septum formation. Mesosomes, often in association with septa, and thus possibly involved in cell division, were observed.

Several other interesting structures, including filaments, microtubules, a membrane-associated structure and fimbriae were evident upon continued electron microscopic examination. Attempts to isolate nonmotile mutants of *C. crocatus* and thereby determine the rôle, if any, of these structures in gliding were unsuccessful.

Subsequent experiments with *Myxococcus xanthus* resulted in the isolation of four types of motility-altered mutants, one of which was nonmotile. *M. xanthus* NM, a previously reported nonmotile mutant, exhibited evidence of cellular translocation in both plate and slide culture and thus may be motile. Nonmotile mutants lacked polar fimbriae observed on motile cells of all genera of myxobacteria, except *Nannocystis*.

Antibody raised against a partially purified fimbriae preparation from *M. xanthus* possessed an antimotility activity. Adsorption of this serum with nonmotile mutants, and subsequent testing of the ability of this serum to inhibit motility and decorate fimbriae

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indicated that fimbriae may be involved in cellular co-operation rather than motility *per se*.

Structures that were termed "holes" were observed at the cell poles of many myxobacteria, but their relationship to fimbriae and gliding is at present obscure.
BIBLIOGRAPHY


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