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Extracellular Fibrils and Contact-Mediated Cell Interactions in *Myxococcus xanthus*

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Contact-mediated cell-cell interactions play an important role in the social life-style of *Myxococcus xanthus*. Previous investigations have demonstrated that fimbriae (also referred to as pili) and extracellular fibrils are involved in these social interactions (L. J. Shimkets, Microbiol. Rev. 54:473–501, 1990). We have used the relatively new technique of low-voltage scanning electron microscopy (an ultra-high-resolution scanning technique that allows for the nanometer resolution of biological materials) to observe the topological details of cell-cell interactions in *M. xanthus*. Our observations indicated that the fibrils (which measure approximately 30 nm in diameter) are produced most extensively by cells that are in close contact with each other and are aberrantly produced by the cohesion-deficient *dsp* mutants. Immunogold analysis identified an antigen which is located exclusively on the extracellular fibrils. Western blots (immunoblots) of this antigen (designated FA-1 for fibrillar antigen 1) indicated that it is composed of several immunoreactive bands (molecular size range, 90 to 14 kDa), all of which are sensitive to protease digestion. A technique for fibril isolation was developed by using FA-1 as a fibril-specific marker. Low-voltage scanning electron microscope observations of swarming cells demonstrated that the expression of fibrils is differentially regulated between adventurous (individual) and socially (group) motile cells. The differential expression of fibrils suggests the existence of a mechanism for the regulation of fibril biosynthesis that functions within the overall system governing social interactions in *M. xanthus*.

Myxococcus xanthus is a gram-negative prokaryote that belongs to a unique group of soil eubacteria, the myxobacteria. The myxobacteria belong to the delta subdivision of the *Proteobacteria* (31) and are distinguished from other bacteria by three characteristics. All myxobacteria translocate by gliding motility, exhibit complex social interactions, and when deprived of nutrients enter into a complex developmental cycle that results in the formation of a fruiting body containing thousands of resistant myxospores (23). It is this formation of a multicellular structure that makes the myxobacteria unique among the prokaryotes. Research in several laboratories indicates that contact-mediated cell-cell interactions play an important role in the biology of the myxobacteria. Since it has not been possible to determine that *M. xanthus* is able to respond chemotactically to a gradient (10), contact interactions and the exchange of signals may be the basis of their social behavior and directed motility.

During the vegetative phase, myxobacteria exhibit cooperative growth, a process in which the growth rate on insoluble macromolecules (i.e., protein, the primary nutrient source of *M. xanthus*) is dependent on cell density (25). This growth strategy may have been the evolutionary impetus for the formation of social behaviors in the myxobacteria (11). In addition, there are a number of other cell-cell interactions of *M. xanthus* that have been described (27), e.g., rippling (28), the motility-dependent interchange of the C signal (19), and social motility (16).

The gliding motility of *M. xanthus* is regulated by two genetically distinct systems, termed A (for adventurous) and S (for social) (16). The A motility is described as the motility exhibited by cells moving as individuals, and it has been noted that adventurous cells inevitably return to the swarm (27). The S motility system governs the movement of cells as groups (16). Kaiser and Crosby have demonstrated that cells must be in close (within 5 μm) proximity to express S motility (18). Because close apposition, rather than direct cell-cell contact, is sufficient to allow S motility, it has been suggested that extracellular appendages mediate these interactions (18).

The cells of *M. xanthus* possess two types of extracellular appendages, fimbriae (pili) and fibrils. Fimbriae were first described in *M. xanthus* by MacRae and McCurdy as 8.5-nm-thick structures observed by electron microscopy (20). Two classes of fimbriae (rigid and flaccid) were described and found to be composed of protein with an apparent subunit molecular size of 8,000 Da (9). Kaiser also observed fimbriae (which were referred to as pili) and described them as 10-nm appendages that were polarly oriented (17). Fimbriae have also been associated with S motility through the study of a group of contact-stimulated S motility (*tgl*) mutants (17).

Extracellular fibrils were first described by Fluegel (13) and later shown to be lateral, branching appendages with an average width of approximately 50 nm (2). Arnold and Shimkets were the first to demonstrate that the extracellular fibrils played a role in cell-cell cohesion and the social behaviors of *M. xanthus* (1). It was also demonstrated that the diazo dye Congo red prevented the formation of these fibrils (1). Congo red (and several other dyes) binds to the extracellular polysaccharides of several species of bacteria, indicating that the fibrils of *M. xanthus* might be composed of polysaccharides (27). A class of mutants was isolated (termed *dsp* for dispersed growing) that was shown to be incapable of S motility and which lack the ability to form cell-cell cohesions. Cells of this mutant class did not possess extracellular fibrils (1).

In this study of *M. xanthus* and its fibrils, we report

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observations obtained with ultra-high-resolution scanning electron microscopy, a technique that allows for nanometer resolution of the surfaces of biological materials (22). We have demonstrated that fibril expression by cells undergoing A or S motility is differentially regulated. Procedures have been developed for the isolation of the extracellular fibrils, and an antigen, recognized by monoclonal antibody 2105 (MAb 2105) (14), has been localized to the fibrils. This antigen has been named FA-1 (fibrillar antigen 1). This article reports the beginning of our attempts to understand the nature of the fibrils and the role they play in mediating the contact interactions of *M. xanthus*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *M. xanthus* was grown in CT broth (24) at 32°C with shaking at 300 rpm. The medium contained kanamycin (70 μg/ml) when required for Tn*5*-containing strains. Two strains of *M. xanthus* were used throughout the investigation, the wild-type strain MD 207 (DK 1622) and the *dsp* mutant strain MD 1000 (DK 3468, provided by L. J. Shimkets) which contains a Tn*5* marker linked to the *dsp* locus (26).

Electron microscopy. Cells were deposited on glass chips (4 by 8 mm; cut from a standard microscope slide) placed on the bottom of the wells of 24-well tissue culture dishes. Each glass chip was covered with 1 ml of a suspension of cells at a density of 5×10^8 cells per ml of CT broth and incubated for times that varied from 15 to 90 min. The *dsp* strains, which are not adherent to glass, required that the glass chips be coated with a 0.1% solution of poly-L-lysine (Sigma Chemical Co., St. Louis, Mo.) according to the manufacturer's directions. For the observation of swarm edges, 10^7 cells were spotted in 2-μl drops onto CTT agar (24) next to strips of sterile dialysis membrane (3 by 7 mm) that had been dried onto the agar surface. The resultant swarms were allowed to glide and grow onto the dialysis tubing for 36 to 48 h.

Samples (cells on either the glass chips or the dialysis membrane) were rinsed twice in 0.1 M K_2HPO_4 (pH 7.6) and then fixed for 90 min with 2.5% glutaraldehyde in 0.1 M K_2HPO_4. The samples were then dehydrated through a graded series of increasing concentrations of ethanol and dried at the critical point of CO_2 by use of a Tousimis Samdri-780A critical-point drier. After the critical-point drying, the dialysis membrane samples were affixed to glass chips by use of a cyanoacrylate adhesive. The dried samples were coated with platinum by using an Ion-Tech model 705 Microsputterer ion beam sputtering unit at 10 kV and 4 mA for 6 min, roughly calibrated to give a thickness of 10 Å (1 nm) of platinum. Samples were viewed with a Hitachi S-900 low-voltage, high-resolution scanning electron microscope (LV-SEM [22]).

For immunogold visualization of antigens with the LV-SEM, cells were deposited on glass chips and fixed as described above. Samples were blocked for 45 min with 0.1% bovine serum albumin (BSA; 99% globulin-free; Sigma) in 0.1 M K_2HPO_4 and then probed for 30 min with the primary antibody (MAb 2105 [14]). The primary antibody was washed off with three changes of 0.1 M K_2HPO_4, and 19 μl of a 1:50 dilution of the immunogold conjugate (15 nm of gold conjugated to protein A; Biocell Research Laboratories, Cardiff, United Kingdom) was placed directly on the chip. Samples were washed three times in 0.1 M K_2HPO_4 and then processed as described above. Visualization of the gold particles was accomplished by use of a molecular-mass backscatter detector on the Hitachi S-900 which was modified for low-voltage operation by the findings of Autrada (4).

SDS-PAGE and immunoblots. The procedures for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotting to nitrocellulose are described elsewhere (15). In all experiments, 10% gels were used. Probing of Western blots (immunoblots) and dot blots with monoclonal antibodies was performed by the method of Harlow and Lane (15). The conjugate for these experiments was horseradish peroxidase linked to a goat anti-mouse immunoglobulin G (Jackson Immunoresearch, Westgrove, Pa.). The production of hybridomas and characterizations of monoclonal antibodies used in this study were described previously (14). The protein content of samples was assayed by the BCA method (29).

Isolation of extracellular fibrils. Shaken liquid cultures of the wild-type strain of *M. xanthus* were grown to high density in CT broth and transferred to CTT agar (24). The agar cultures were incubated at 32°C for 48 h which allowed for the formation of dense lawns of cells. Cells were harvested by gently scraping them into TNE (10 mM Tris [pH 7.5], 100 mM NaCl, 5 mM EDTA) and resuspended by stirring on ice. The cell suspension was adjusted to a density of approximately 10^9 cells per ml by the addition of TNE buffer. The cells were solubilized by the addition of SDS at a final concentration in the cell slurry of 0.5%. Stirring was continued at room temperature until the solution had cleared. The cell lysate was centrifuged at 12,000 × *g* for 10 min at 20°C, and the supernatant fluid was discarded. The soft pellet was washed by resuspension in 1 volume of TNE and by centrifugation at 12,000 × *g* for 10 min; washes were repeated three times.

The amount of FA-1 relative to total protein present in the different fractions was determined by using endpoint dilution dot blots (see Fig. 8). SDS-PAGE and Western blots were performed as described above. For the observation of isolated fibrillar material by LV-SEM, samples were deposited on poly-L-lysine-coated glass chips for 90 min at 25°C. Samples were prepared for immunoelectron microscopy as described above, with the exception that the fixation step was eliminated.

RESULTS

High-resolution observation of the surface of *M. xanthus*. The Hitachi S-900 LV-SEM allows for ultrahigh resolution of the surface details of biological materials. With this microscope, the surface topology of prokaryotic cells can be observed with a level of resolution comparable to that obtained with thin sections viewed in a transmission electron microscope (22). Figure 1 shows wild-type cells of *M. xanthus* from a submerged stationary culture observed with an LV-SEM (for comparison to images of *M. xanthus* by use of conventional SEM, see reference 27).

Figure 1A shows a single cell with fibrillar connections to neighboring cells and to the substrate. The dimensions of this representative cell (0.4 by 5.6 μm) are slightly less than the 0.5 by 7 μm normally taken as the actual size of *M. xanthus*; some cells were observed to be as short as 4 μm. This apparent discrepancy may be due to shrinkage during critical-point drying, a factor that may also account for the rugose appearance of the cell surface. The dimensions of the fibrils observed ranged from several nanometers to tens of micrometers in length, with a relatively constant width of 30 nm. When the LV-SEM was used, we did not see fimbriae on *M. xanthus*. However, fixation and drying procedures dif-

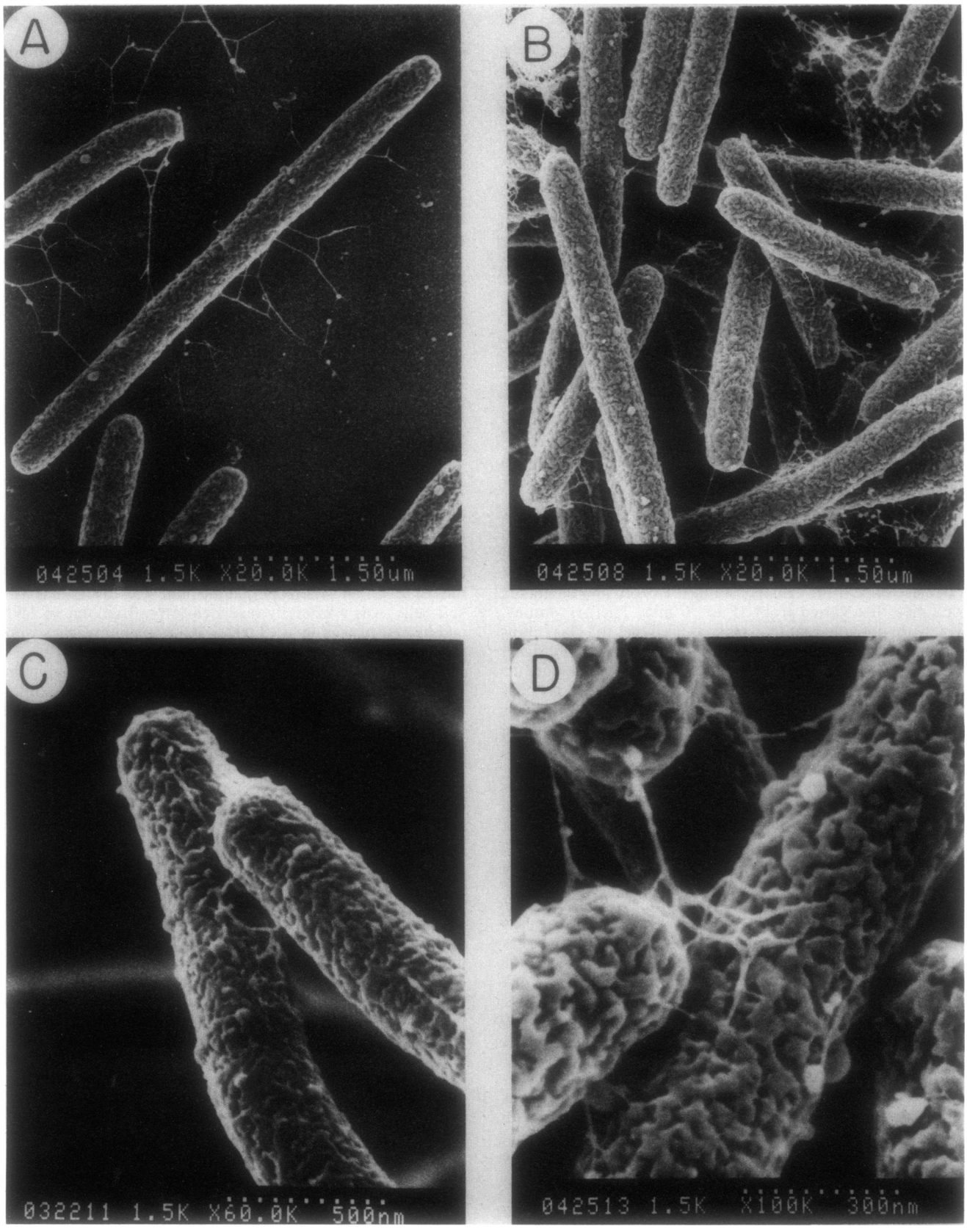

FIG. 1. LV-SEM images of vegetative cells of *M. xanthus* grown on a solid surface in submerged culture. (A) Individual cells and fibrils. (B) A cluster of cells with extensive fibrillar interconnections. (C) Cells from submerged culture. These cells are anchored to the substrate at only one end, the free end is shown. (D) High magnification of the cell surface and fibril anchoring points. Note the branched nature of the fibrils.

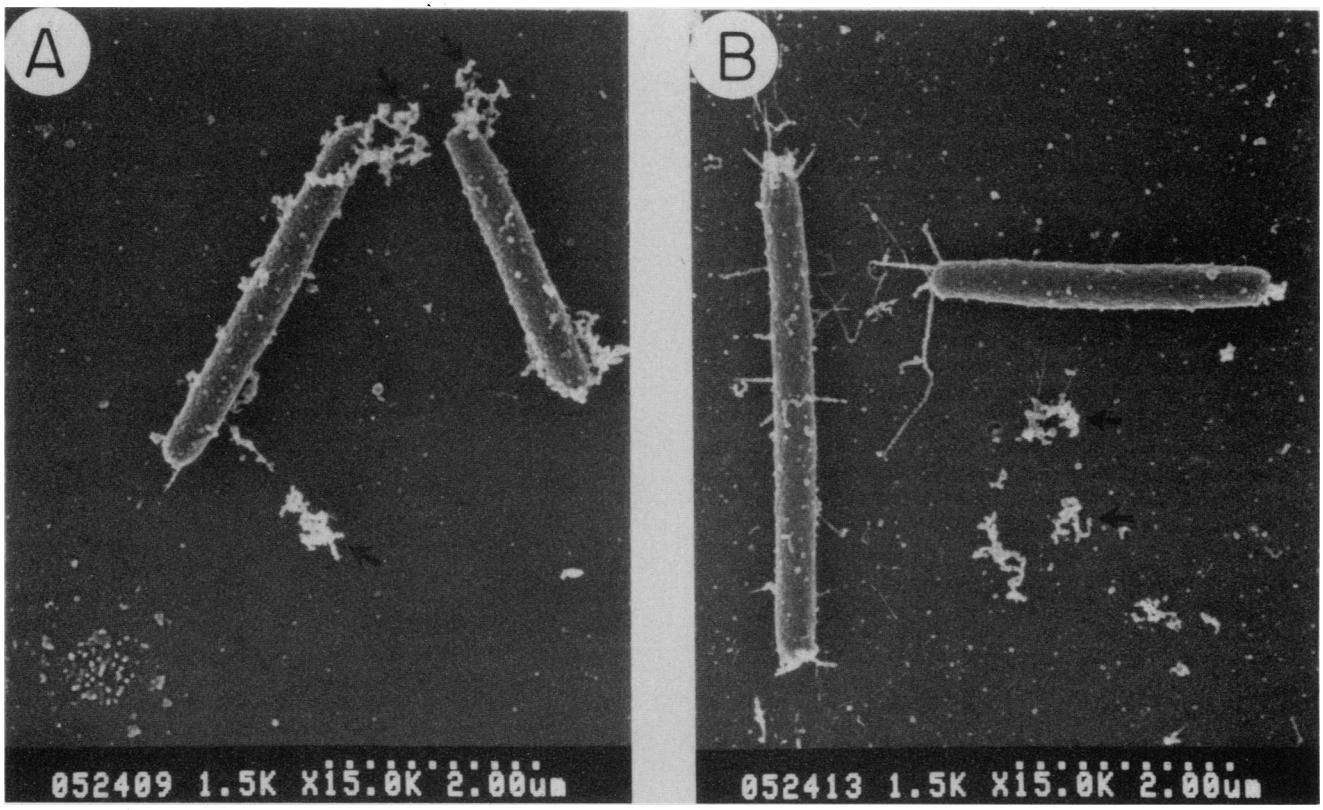

FIG. 2. LV-SEM images of *dsp* mutants displaying what appear to be aberrant fibrils. Only rarely were such fibrils observed to connect two cells. Cells were never seen in clusters. The arrows point to debris that might be the aberrant fibrillar material that had dissociated from the cells.

fered from those used to demonstrate fimbriae (20). In addition, the original observations of fimbriae were done with negatively stained material, a technique that we have not used.

In Fig. 1B, a cluster of cells is shown, demonstrating the extensive networks of fibrils produced by the cells. These networks are most extensive when cells are in close contact with each other. We also noted that the amount of fibrillar material increased with the length of time that the cells spent on the surface (before fixation) and with the number of cells. Cells fixed before deposition had no fibrils, indicating that the fibrils were not produced during growth in shaken liquid culture (micrographs not shown). High-magnification (×100,000) observation of the cell surface (Fig. 1D) demonstrates the frequently branched nature of the fibrils and the attachments of fibrils to the cell surface. Observation of numerous samples has indicated that there is no apparent polar orientation of the fibrils on cells grown in a submerged culture.

The extracellular appendages of *M. xanthus* have been suggested as the mediators of cell-cell cohesion (1), primarily on the basis of the apparent absence of these structures on the cohesion-deficient *dsp* mutants (1). Because we are interested in the potential role played by the extracellular fibrils in cell-cell interactions, *dsp* mutants were observed by using LV-SEM to determine if they possessed the fibrils observed on wild-type cells. Ultra-high-resolution analysis of the *dsp* mutant strain (MD 1000) indicated that these cells produced what appeared to be aberrant fibrils. Debris repeatedly found near *dsp* cells (Fig. 2, arrows) appears to be material that had dissociated from the cells. Consistent with the inability of this strain to form cell-cell cohesions (1), no clusters of cells were observed in any of the samples prepared. Because the *dsp* strains were anchored to the glass chips by using poly-L-lysine, there was a possibility that fibril production was affected by this compound. As a control, wild-type cells were anchored in the same fashion and were found to produce a normal complement of extracellular fibrils (data not shown).

Observation of cells displaying individual and group motility. The micrographs of cells among densely populated areas (Fig. 1) indicated that the cells are interconnected to the rest of the population by the extracellular fibrils. The degree of fibrillar interconnections might form the physical basis for the difference between A (individual) and S (group) motility. To determine the difference in fibril expression between individually motile cells and cells moving in groups, samples from an active swarm edge (containing both individual and grouped cells) were prepared for observation by LV-SEM. The results of one such analysis are shown in Fig. 3: panel A is a low-magnification view of a swarm flare and panels B to D are fields of higher magnification from regions of that flare.

Figure 3B shows adventurously motile cells from the most distal portion of the flare. Very few fibrils are expressed on such cells even when they are in clusters of up to three cells. Those fibrils that are evident do not interconnect cells but rather are in contact with the substrate. Figure 3C shows what is an apparent transition zone between nonfibrillated and fibrillated cells. The cells that are located at the center of

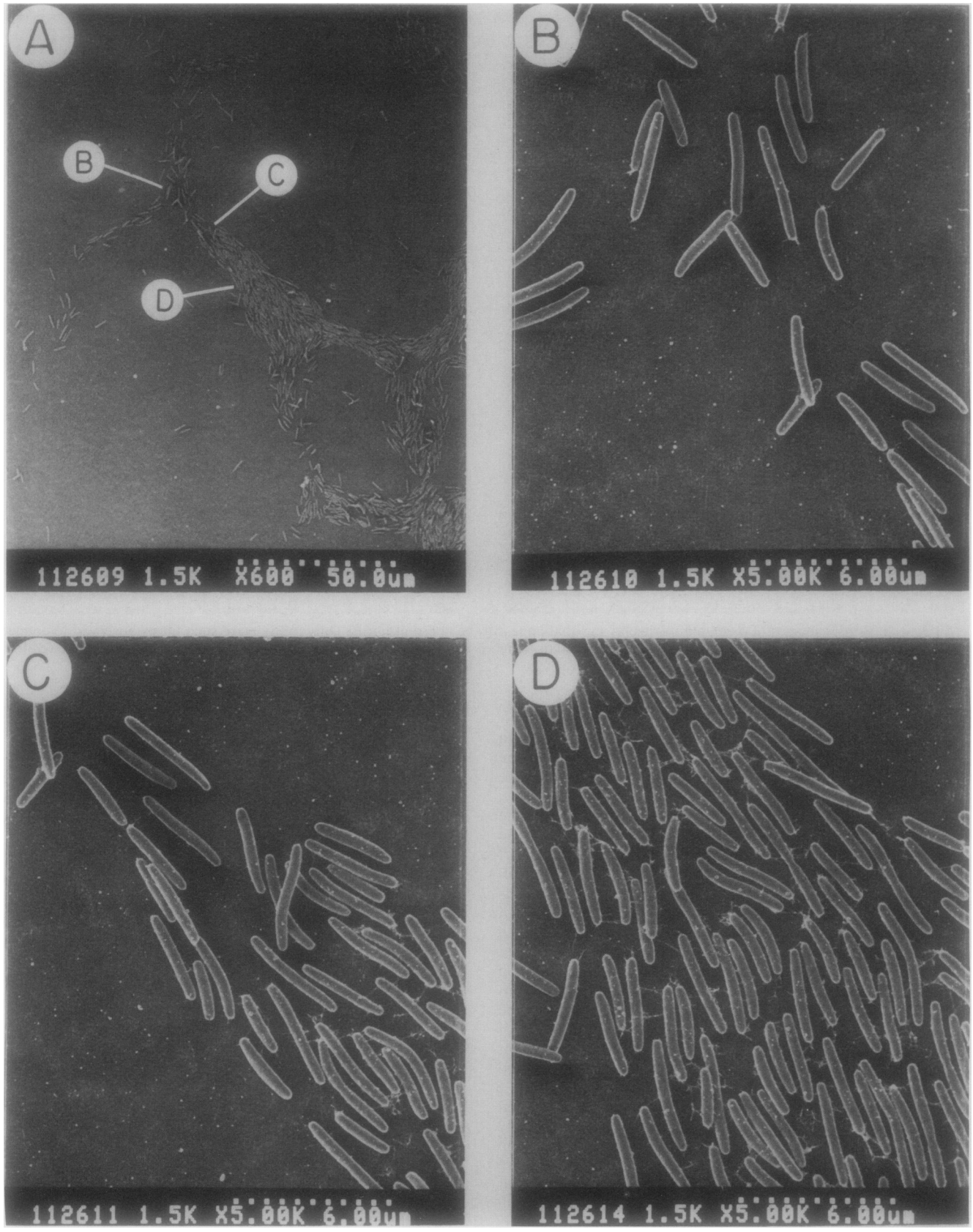

FIG. 3. A swarm of wild-type *M. xanthus* cells showing both adventurous and socially motile cells. (A) Low-magnification image of a flare from the swarm edge. Arrows denote cells near the center of the three subsequent panels. (B) Adventurously motile cells from the portion of the flare distal to the population. (C) Cells from the flare at a transition point between low (upper left) to higher (lower right) cell density. Note the sharp line at which fibrillar interconnections begin. (D) Cells from the most densely populated region of the flare.

TABLE 1. Fibril expression in socially (grouped) motile and adventurously (ungrouped) motile cells of *M. xanthus*

Type of cells	No. of cells[a]	% of total
In groups[b]		
With fibrils	441	85
With no fibrils	78	15
Individual[c]		
With fibrils	0	0
With no fibrils	21	100

[a] A total of six fields were counted from two different sample sets.
[b] Cells not separated from other cells by more than one cell length.
[c] Cells separated from other cells by more than one cell length.

the most densely populated region of the flare (Fig. 3D) express a number of fibrillar connections.

To quantitate the observed trend in fibril expression between the two cell types, cells from six different LV-SEM fields were counted. Each cell was counted as either individuals (cells separated by more than one cell length) or grouped (cells not separated by more than one cell length). The cells from each group were then scored as fibrillated or nonfibrillated. The results (Table 1) confirm the observation that while grouped cells were usually fibrillated, individual cells were never found to possess fibrils. The increase in fibrillar interconnections from the low-density region to the high-density region indicates that cell density plays a role in fibril expression.

To further test the notion that cell density, and not simply surface contact, was required for fibril expression, the following experiment was conducted. Cells were deposited on dialysis membrane strips on CTT agar at densities that would yield cells that were either in close proximity (permitting S motility) or well isolated from their nearest neighbor (allowing only A motility). Cells were incubated for 12 h to insure sufficient time for fibril production. If cell-cell contact or close apposition was required for fibril expression, then those cells at low plating density should not have produced fibrils, even after being on a solid surface for times determined to be sufficient for fibril production in submerged culture. The results indicated that cells plated at a low density (Fig. 4A) did not produce fibrils even after prolonged contact with a solid surface, while those plated at a high density produced extensive amounts of fibrils and apparently began to align themselves in active swarms (Fig. 4B).

Analysis and localization of a fibril-specific antigen. The *dsp* mutants, which lack the ability to cohere and do not produce intact fibrils, were noted to react weakly with MAb 2105, 1 of 40 monoclonal antibodies generated against the surface of *M. xanthus* (14). MAb 2105 was used as the probe in Western blots of total cellular protein from both wild-type and *dsp* cells (Fig. 5). In the wild-type strain, several immunoreactive bands were found, ranging in molecular size from 90 to 14 kDa (Fig. 5, lane A). However, in the cohesion-deficient *dsp* strain, essentially only the 66-kDa band could be detected and it was present at a substantially reduced amount. All of the different bands were shown to be sensitive to protease digestion (Fig. 5, lanes C and D),

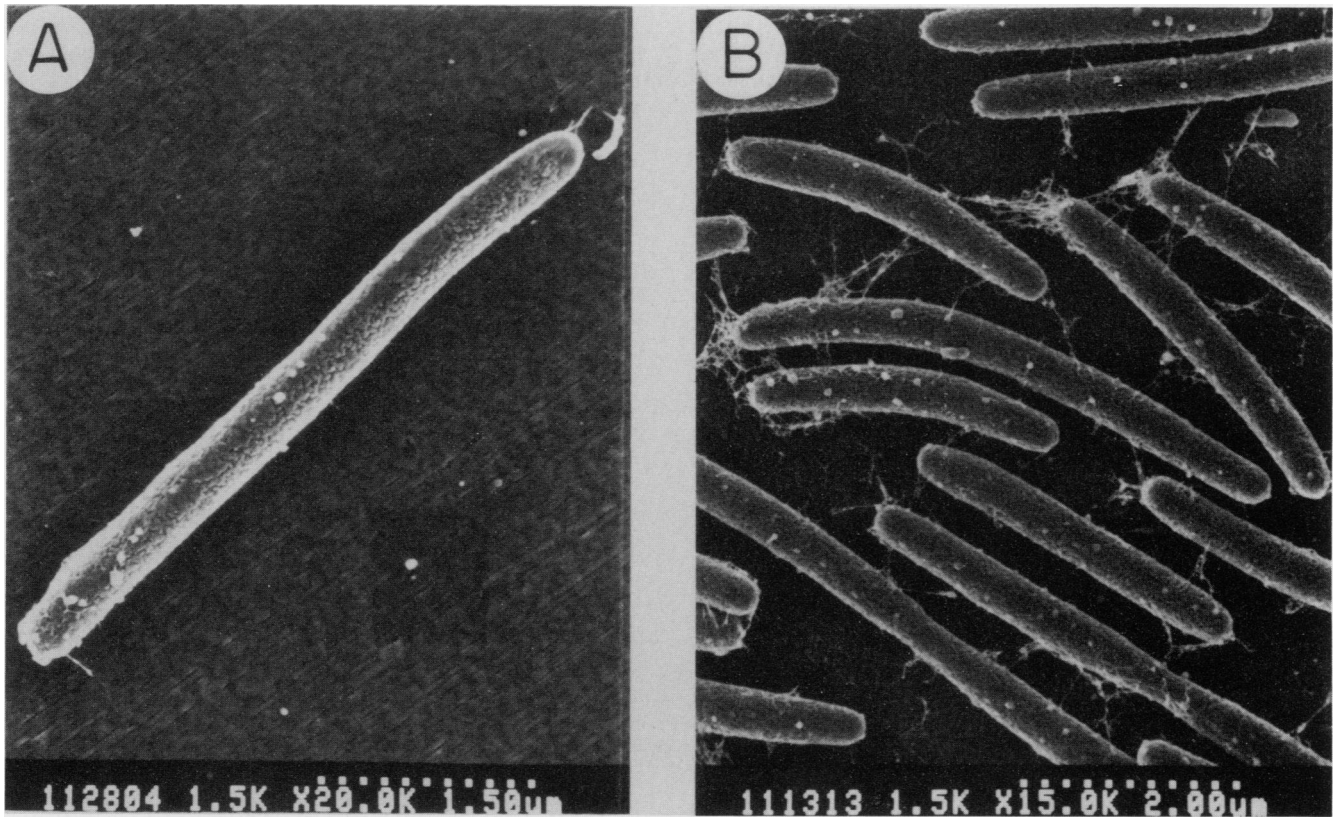

FIG. 4. Cell density influences fibril production. Cells were deposited on dialysis membrane strips on nutrient agar at 1×10^7 (A) and 5×10^8 (B) cells per ml and incubated at 32°C for 12 h. Cells at high density began to produce fibrils and apparently began to align themselves into swarms. Cells at low density produced very few fibrils.

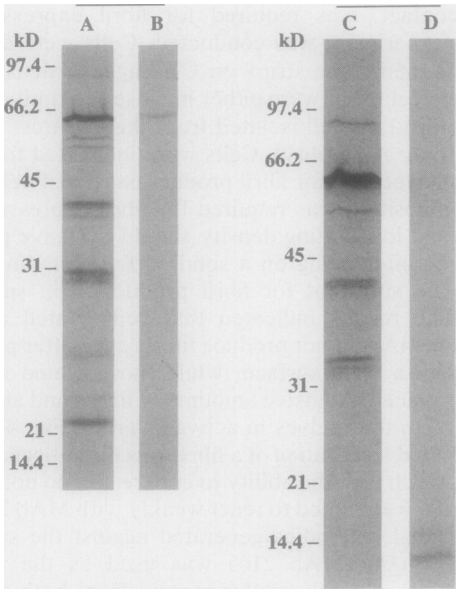

FIG. 5. Western blots of whole-cell extracts (probed with MAb 2105) from cells of wild-type *M. xanthus* (MD 207; lane A) and a *dsp* mutant (MD 1000; lane B). Cell extracts of the wild-type strain were digested with a protease cocktail containing pronase E (1 mg/ml), proteinase K (0.2 mg/ml), trypsin (0.01 mg/ml), and chymotrypsin (0.1 mg/ml). These samples were incubated at pH 7.5 and 37°C for 0 and 60 min (lanes C and D, respectively). Digestions were stopped by the addition of SDS-PAGE sample buffer (15).

indicating that the antigen recognized by MAb 2105 is composed, at least in part, of protein. Interestingly, the 90-kDa band was present only on cells grown in shaken liquid culture and was lost when cells were grown on a surface (Fig. 6).

To localize the antigens recognized by MAb 2105, cells were labeled with the antibody and a 15-nm gold-labeled protein A conjugate for analysis with the LV-SEM. The Hitachi S-900 LV-SEM is equipped with a backscatter detector that permits the detection of metals of a different molecular mass than the primary coating metal (i.e., the gold

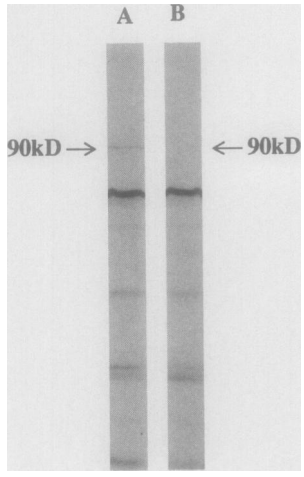

FIG. 6. Western blots of whole-cell extracts (probed with MAb 2105) grown in liquid culture (lane A) or on a solid surface (lane B). The arrow indicates the 90-kDa band.

of the conjugate versus the platinum coating metal). Figure 7 shows that MAb 2105 reacts almost exclusively with the extracellular fibrils. What appears to be background labeling in the backscatter image is predominantly the labeling of fibrillar fragments anchored to the substratum, as can be seen in the secondary electron image. Control samples, which were probed only with the conjugate, indicated very little background labeling. Because MAb 2105 labels only fibrils and not the cell surface, we have named these antigens FA-1.

Isolation of extracellular fibrils from *M. xanthus*. From the immunogold localization results, it was apparent that MAb 2105 could be used as a marker in the isolation of the extracellular fibrils from *M. xanthus*. The scheme developed, essentially the centrifugal sedimentation of solubilization-resistant material, has proven a useful and reproducible means of fibril isolation.

The diazo dye Congo red had been shown to inhibit cell-cell interactions in *M. xanthus*, essentially causing wild-type strains to behave as *dsp* mutants (2). It was also shown that Congo red (5 μg/ml) inhibited the formation of fibrils (1), suggesting that the binding site of Congo red was on the fibrils. The dye was also used in attempts to isolate the Congo red receptor (fibrils) of *M. xanthus* (3). In light of these findings, initial fibril isolations were performed with Congo red in the wash buffers at a concentration of 5 μg/ml. When the suspended fibrillar material was then centrifuged, the resulting pellet had bound most of the dye, effectively removing it from the supernatent fluids. This effect was not quantitated, the Congo red dye was found not to be required for fibril isolation, and, accordingly, the dye was not added in later isolations.

Dot blots of whole cells and isolated fibrils probed with MAb 2105 as a fibril-specific marker showed that there is a 16-fold enrichment of the fibril-specific antigens recognized by MAb 2105 in the material isolated from the whole cells (Fig. 8). SDS-polyacrylamide gels stained for total protein and Western blots showed that, of the 14 protein bands present in the isolated fibrils, 10 reacted with MAb 2105 (Fig. 9, lanes C and D). These data indicated that the isolated fraction was indeed an enrichment of fibrillar material.

Isolated fibrils were probed with MAb 2105 and subjected to immunogold analysis with the Hitachi S-900 LV-SEM. The results (Fig. 10) showed quite clearly that the fibrils remained intact and that reactivity with MAb 2105 was maintained. Controls, in which fibrils were probed with conjugate only (omitting MAb 2105), showed an extremely low level of background labeling (Fig. 10B). These results demonstrate that the isolation procedure yielded fibrils that are structurally intact and that those fibrils apparently maintained their biochemical integrity (as evidenced by the binding of MAb 2105).

DISCUSSION

On the basis of chemical and genetic evidence, the extracellular fibrils of *M. xanthus* have been shown to be the mediators of cell-cell cohesion and as such they are required for S (group) motility (2). Our observation of cells of *M. xanthus* by LV-SEM have revealed that fibril formation is regulated by cell density and contact with the substratum; under the appropriate conditions, a matrix is formed around the cells consisting of lateral, branching fibrils measuring 30 nm in diameter. The size, morphology, and absence of these fibrils on *dsp* mutants have indicated to us that they are the same as those reported by Arnold and Shimkets (1).

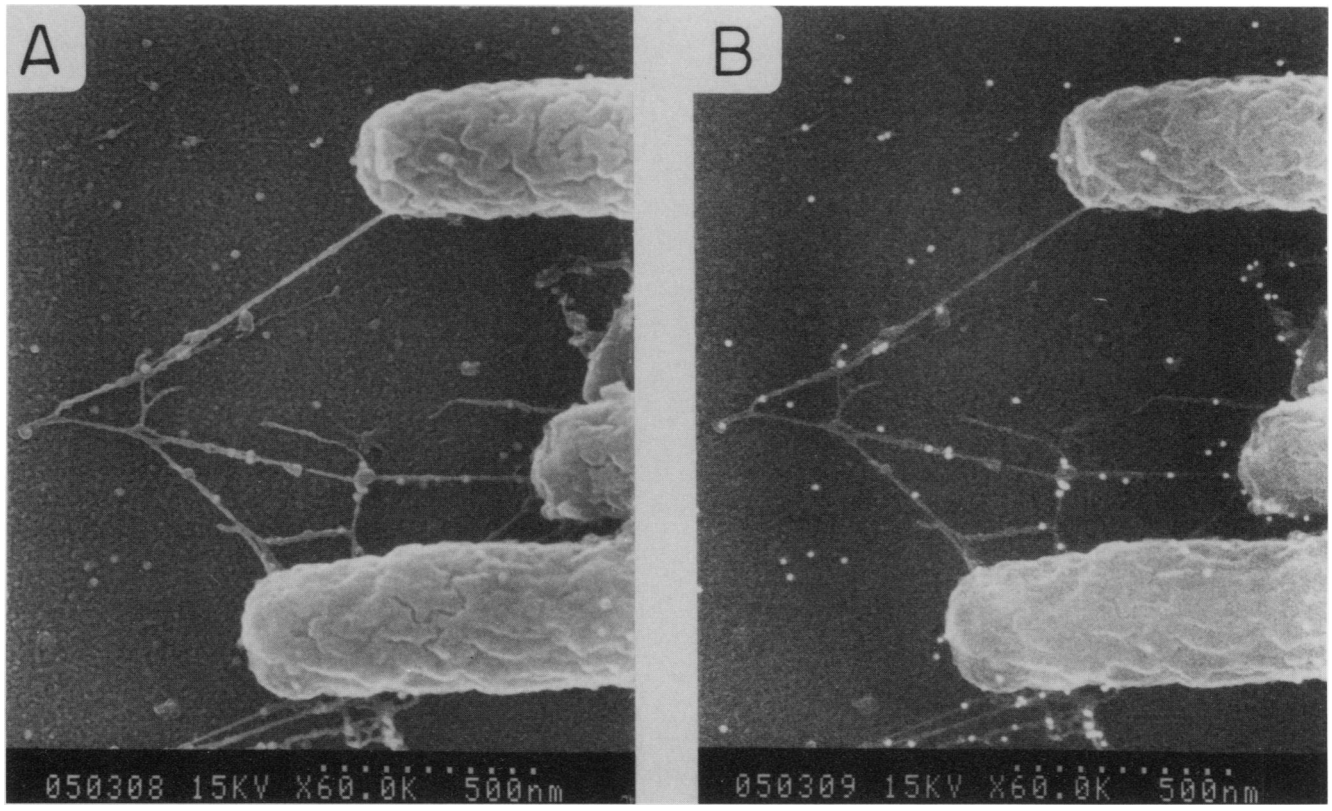

FIG. 7. Immunoelectron localization of FA-1. Cells were probed with MAb 2105 and a 15-nm gold conjugate (protein A). (A) Secondary electron image; (B) backscatter electron image. The bright spots in the backscatter image are the gold particles. Note the fibril fragments visible on the substrate in the secondary electron image. These micrographs show slightly less surface detail than others because they are taken from images formed at an accelerating voltage of 15 kV rather than the 1.5 to 4.0 kV normally used.

In this article we also demonstrate the utility of the ultra-high-resolution technique of LV-SEM for the study of cell-cell interactions. With this technique, it has been possible to examine the surface of the cells of *M. xanthus* with a far higher degree of resolution than has previously been possible with conventional SEM or transmission electron microscopy. In addition, our observations, along with improved analytical techniques (i.e., the incorporation of critical-point drying of samples [5]), lend support to the view that the extracellular polysaccharides (glycocalyces) of many microorganisms are organized as fibrils (8).

Cells in a swarm differentially regulate the expression of fibrils. Those cells moving as individual cells (presumably the adventurously motile cells) do not express fibrillar connections, whereas a majority of the cells moving in groups (presumably the socially motile cells) form intercellular connections. Further experiments demonstrated that cell density, rather than simple cell contact with the substratum, is required for fibril expression. These findings suggest the existence of a mechanism for the regulation of fibril expression (biosynthesis) that functions within the overall system governing S and A motility behaviors in *M. xanthus*.

FA-1, recognized by MAb 2105, was identified as a series of protein antigens localized exclusively on the extracellular fibrils of *M. xanthus*. (Our laboratory has also used immunogold LV-SEM to localize a developmentally expressed fibrillar antigen, dFA-1 [7], and a cell surface antigen, CSA 302 [6].) The recognition of several bands on a Western blot by a single monoclonal antibody can by accounted for in one of two ways: either MAb 2105 recognizes different forms of a single gene product (produced either through posttranslational modification or degradation) or the antibody recognizes a common epitope (either protein or carbohydrate) on several different proteins. Until the nature of the different immunoreactive bands is resolved, the designation FA-1 will be retained for all of them. The amount of MAb 2105 bound by the cohesion-deficient *dsp* mutants is consistent with the observation that these mutants produce a small amount of what appears to be aberrantly formed fibrillar material. Attempts to label this material with MAb 2105 for immunogold analysis have been unsuccessful.

The observation of cells of *M. xanthus* by LV-SEM revealed that, under the appropriate conditions of cell density and contact with the substratum, a matrix is formed around the cells consisting of lateral, branching fibrils measuring 30 nm in diameter. The fibrils actually appear to be of different diameters, but the diameters of the fibrils at their insertion points at the cell surface are consistent. This might suggest that the fibrils are capable of cohering to each other in a longitudinal fashion. The size, morphology, and absence of these fibrils on *dsp* mutants indicated that they are the same as those reported by Arnold and Shimkets (1).

By using FA-1 as a fibril-specific marker, a technique for the isolation of the 30-nm fibrils was developed. Observation of the isolated fibrils by using immunogold LV-SEM indicates that it is indeed composed of the 30-nm fibrils that mediate cohesion (1) and make up at least part of the extracellular matrix. The isolated fibrils of *M. xanthus* bind

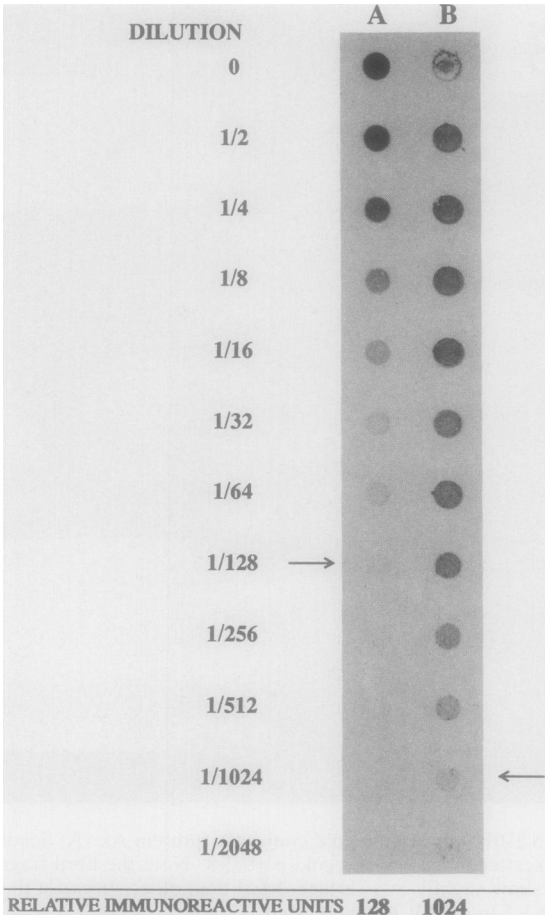

FIG. 8. Enrichment of FA-1 in isolated fibrils. Endpoint dilution dot blots of whole cells (column A) and isolated fibrils (column B) from *M. xanthus* were done. Each spot is a twofold dilution of the previous spot. The final dilution displaying immunoreactivity is indicated with an arrow. The inverse of the dilution factor for the last immunoreactive spot gave the number of relative immunoreactive units in the sample. Each of the initial spots (labeled 0) contained 25 mg of protein. Blots were probed with MAb 2105.

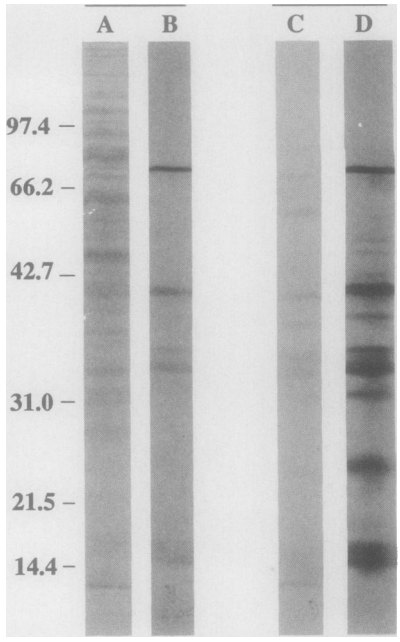

FIG. 9. Western blots probed with MAb 2105 and SDS-PAGE stained with Ponceau S (for total protein) of whole cells (lanes A and B) and isolated fibrils (lanes C and D). Each lane contained 25 mg of protein. Lanes A and C were stained with Ponceau S; lanes B and D were probed with MAb 2105.

Congo red and remove the dye from solution, suggesting that they constitute what Arnold and Shimkets referred to as the Congo red receptor (1). On the basis of an analogy to the extracellular fibrils of other bacteria that bind Congo red, it is likely that the fibrils of *M. xanthus* contain substantial amounts of polysaccharides. Indeed, extracellular fibrils composed of polysaccharides are the apparent mediators of cohesion in several other prokaryotes, including *Agrobacterium tumefaciens* (21), *Zooglea ramigera* (12), and *Rhizobium leguminosum* (30), among others (27).

Sutherland and Thomson isolated exopolysaccharide from several genera of myxobacteria as an acetone-precipitable fraction released from intact cells by shear forces (33). Exopolysaccharide from several species of the genus *Myxococcus* constitutes 5 to 10% of the dry weight of the starting material and is predominantly composed of carbohydrates (neutral and amino sugars) (32). While it seems likely that the 30-nm extracellular fibrils that have been isolated from *M. xanthus* comprise at least part of the extracellular matrix, complete compositional analysis has yet to be done. The possible existence of amorphous polysaccharides (not detected by LV-SEM) must also be explained. A more complete structural and compositional analysis of the isolated fibrils will allow more detailed conclusions to be drawn about their precise function in *M. xanthus*.

By using the ultra-high-resolution technique of LV-SEM, new insights have been gained with regard to the cell-cell interactions of *M. xanthus*. We have demonstrated that the production of extracellular matrix fibrils is differentially regulated between cells expressing A and S motility behaviors. That the expression of extracellular fibrils is dependent on cell density indicates the existence of a mechanism for the perception of cell density and the regulation of fibril production, a system which seems to operate within the overall system governing A and S behaviors in *M. xanthus*. By isolating the extracellular fibrils and purifying the proteins uniquely associated with them, we hope to gain a better understanding of their structure and function. It is possible that the extracellular fibrils are not only the mediators of cell-cell cohesion but also function as an integral part of a contact-mediated signaling system used to sense and regulate cell density in *M. xanthus*.

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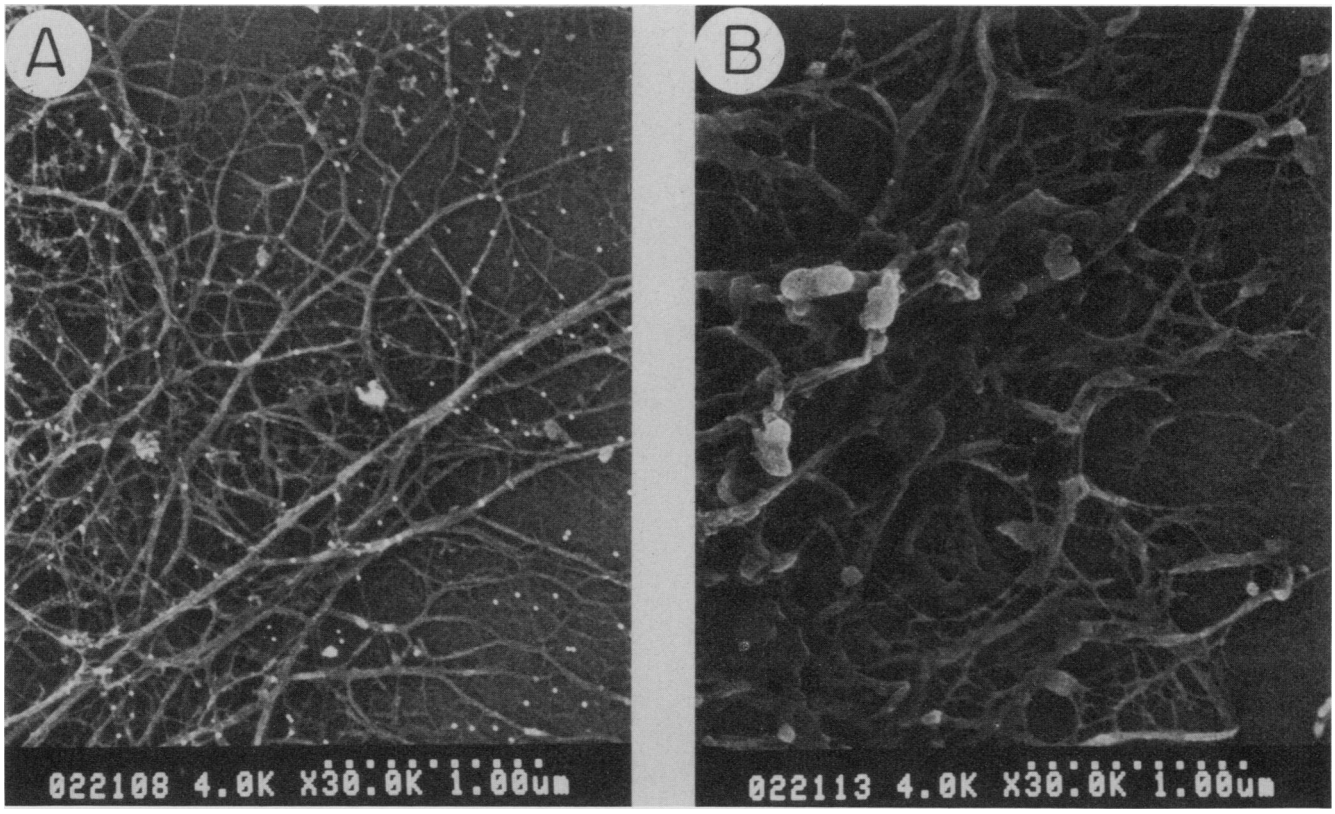

FIG. 10. Immunogold labeling of FA-1 on isolated fibrils. (A) Fibrils labeled with MAb 2105; (B) fibrils labeled with conjugate only as a control for background binding. Both micrographs are from backscatter images taken at an accelerating voltage of 4.0 kV.

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