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Escherichia coli K-12 Cell-Cell Interactions Seen by Time-Lapse Video

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The high degree of organization in mature bacterial colonies suggests specific interactions between the cells during colony development. We have used time-lapse video microscopy to find evidence for cell-cell interactions. In its initial stages, *Escherichia coli* K-12 colony morphogenesis displayed control of the geometry of cell growth and involved intimate side-by-side associations. When microcolonies developed from isolated single bacteria, a directed process of elongation and division resulted in the appearance of a symmetrical four-cell array. When growth began with separate but nearby bacteria, the daughters of different cells elongated towards each other and also lined up side by side. Interactions between microcolonies containing several hundred or more bacteria were visible several hours later. Control of cell morphogenesis at later stages of microcolony development was strain specific. These results show that *E. coli* K-12 cells respond to each other and adjust their cellular morphogenesis to form multicellular groups as they proliferate on agar.

Bacteria have the ability to develop into complex but organized multicellular communities. The fruiting bodies of the myxobacteria are perhaps the most spectacular examples of multicellular structures among procaryotes (20), but many observers have also reported organized growth of diverse bacteria on agar and on various substrates in nature (1, 16, 25, 28). Colony organization in a petri dish is a laboratory expression of systems that regulate multicellular growth and morphogenesis on surfaces. Thus, studying how bacteria organize themselves on agar is relevant to understanding natural situations involving surface attachment, such as pathogenesis.

Colony organization can be visualized in several ways. One convenient method is to use *lacZ* fusions and XGal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) indicator agar to display patterns of differential gene expression in mature colonies (21, 22). This method makes it possible to investigate the hereditary stability of colony patterns and the influence of physiological factors, such as medium composition, inoculation density, and colony size. In XGal-stained colonies, it was observed that patterns of differential gene expression were often independent of inoculum size, the rate of colony expansion, and the extent of colony spread (Fig. 1). The scale independence of colony patterns was one piece of evidence suggesting a regulative component to pattern formation during development which might involve cell-cell interactions.

A second way of looking at colony organization is with the scanning electron microscope (SEM). SEM examination of *Pseudomonas putida* and *Escherichia coli* colonies revealed characteristics which are usually associated with complex organisms: very large numbers of cells differentiated into many morphological types, locally specific multicellular arrays, extracellular matrix materials, and overall integration of microscopic features into recognizable hereditary patterns visible in the whole colony (23, 24). SEM analysis of *E. coli* colonies at various times after inoculation indicated that colony development is a dynamic process, involving the formation and rearrangement of specific multicellular groupings over time (24).

At what levels are regulatory processes involved as bacterial colonies develop their specific patterns? Some preliminary genetic results suggested that cell elongation and cell division constituted important targets of morphogenetic control during colony development. *E. coli* K-12 mutants with altered colony morphologies regularly arose in sectors on colonies after several days of incubation, and these mutants often displayed abnormalities in cell division and elongation (unpublished observations). In order to ask whether cell division control participated in colony development by our standard laboratory strains, we undertook to examine the initial cell divisions in *E. coli* microcolonies by time-lapse video recording. Our results showed that microcolony development from isolated bacteria was highly regular through the first three divisions, involved directed cell elongation from a single pole of each daughter cell, and proceeded through a symmetrical four-cell array. When denser inoculations placed independent cells close to each other at the start of growth, different but related patterns of cell elongation were observed. Neighboring bacteria were seen to grow preferentially towards each other and align themselves in a manner similar to that of the daughters of a single cell. Thus, specific cell elongation guided by cell-cell responsiveness appeared to be an important component of morphogenetic control during early microcolony development.

MATERIALS AND METHODS

Bacterial strains. M7124 is an F⁻ *thi* Δ(*argF-lac*)*U169* descendant of Hfr3000 (2). MS398 is a bacteriophage λ-resistant mutant of M7124. MS2168 is an Sm^r recombinant between Hfr Hayes and an M7124 derivative. MS1891 and MS1888 were isolated by infecting strain M7124 with λ*plac*Mu15 (5) and selecting Km^r transductants which expressed different XGal staining patterns. λ*plac*Mu15 inserted into the M7124 genome by means of phage Mu termini when complemented with Mu A and B functions but became genetically stable in MS1888 and MS1891 when the comple-

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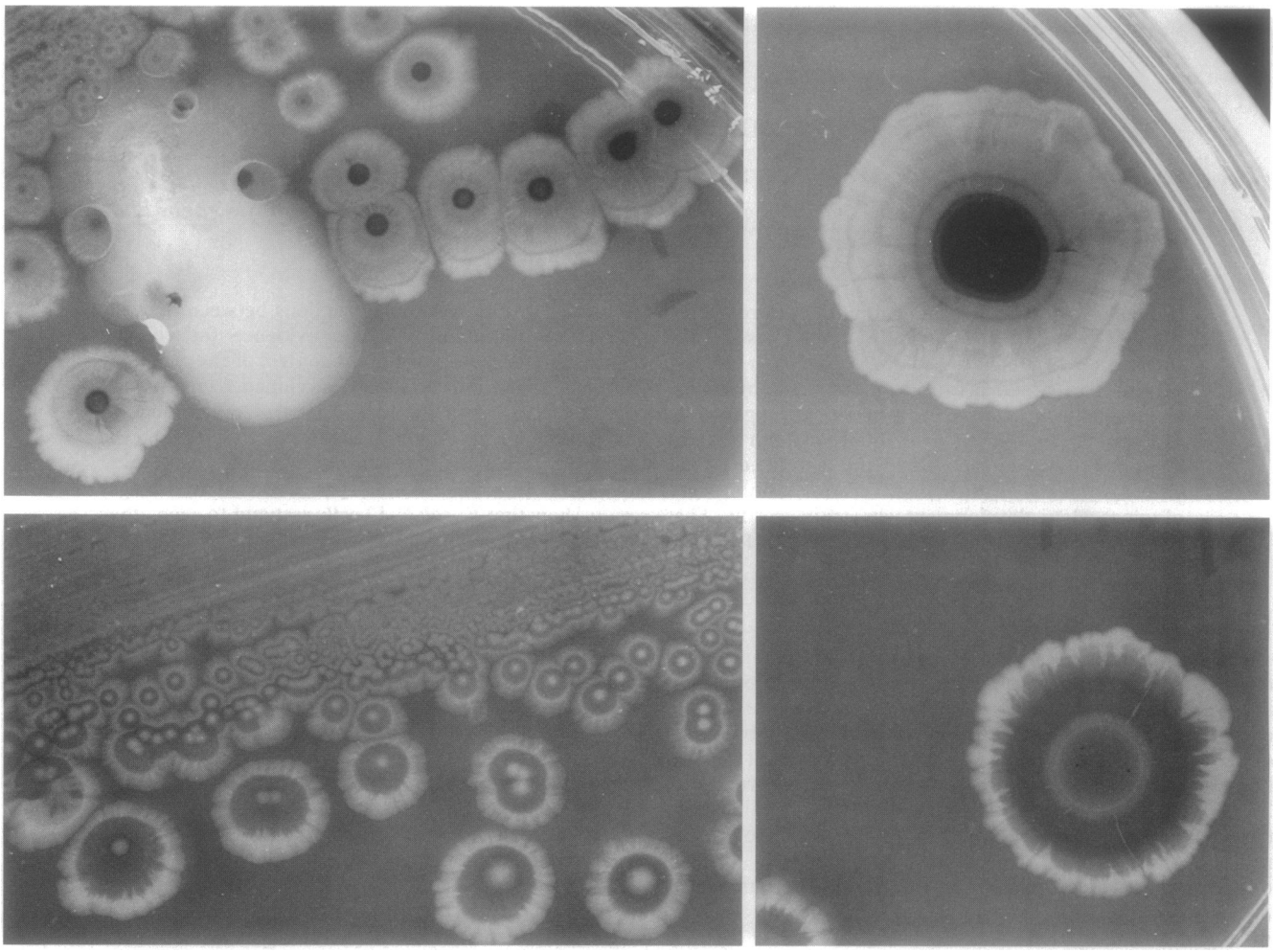

FIG. 1. Colonies of *lacZ* fusion strains MS1891 (top) and MS1888 (bottom) on β-galactosidase indicator agar. Each row shows colonies produced by a particular *E. coli* K-12 strain which had no *lac* operon but expressed a hybrid β-galactosidase activity under the control of unknown transcription and translation regulatory elements in the bacterial chromosome. The medium contained the chromogenic substrate XGal, which liberated an insoluble blue dye when hydrolyzed by β-galactosidase. Thus, the darker regions on the colonies indicated areas of greater enzyme activity. Note how the staining patterns had both concentric and sectorial (radially oriented, wedge-shaped) elements. The sectorial elements reflected the appearance of differentiated cell clones descended from a common ancestor. The concentric elements reflected the appearance of differentiated populations which did not share common ancestry but which were formed at similar times during colony development. The colonies on the left arose out of inoculations in which the individual bacteria were separated by streaking over the agar surface with a sterile glass rod. The colonies on the right formed from 1-μl spots containing about 10^5 bacteria of the same cultures placed directly on the agar surface without disturbance. The spot colonies were larger for two reasons: (i) they began growth from the perimeter of the inoculation zone (visible as a distinctly staining disk at the colony center), and (ii) the growing cell populations spread more rapidly over the agar substrate. Note that the centers of the spot colonies grew upwards from a disk layer of cells in the inoculated zone and were not comparable with the central zones of the streak colonies, which were formed by outward expansion from a microscopic point. These colonies were photographed after 6 days of incubation at 37°C and are magnified ×4.

menting phage DNA encoding these functions segregated out of the transductant bacteria. λp*lac*Mu15 was designed so that insertions in the proper reading frame would direct the synthesis of a hybrid β-galactosidase protein.

Time-lapse video recording. To study individual cells, a Zeiss GFL microscope with a 6-V incandescent light source was used to visualize the bacteria on slides through an oil immersion, 100×, 1.3 NA phase lens. A Panasonic WV-1550 black-and-white camera was mounted on the photo tube, and images were recorded on a Panasonic NV-8050 time-lapse videocassette recorder (VCR) with a Panasonic WJ-810 time and date generator between the camera and the VCR. Recordings were made in the 240-h-per-tape mode. The slides were warmed by a Sage model 279 air curtain. It was not possible to measure the exact temperature of the slide, and control was accomplished empirically by moving the air curtain far enough from the microscope so that development proceeded without visible cell death or abnormal filamentation, which did occur if the air curtain was placed too close. Slides were prepared by warming them on a 55°C heating block, adding 0.2 to 0.3 ml of molten 1.5% agar, allowing the agar to puddle in the molten state, and then removing the slide to cool on the lab bench. This procedure reliably gave us a good flat surface for viewing bacteria. In early experiments, the agar contained only phosphate buffer, but for later experiments (including those in Fig. 2, 6, and 7), the agar contained tryptone (1%), yeast extract (0.5%), and NaCl (0.5%) (TYE agar). No difference in cell division

patterns was noted when recordings made on the two types of agar were reviewed.

To initiate microcolony development, saturated cultures of *E. coli* K-12 were diluted in TYE broth, and about 20 μl of the diluted cell suspension was placed on a thin agar layer on a microscope slide. After excess liquid was blotted, a cover slip was placed on the inoculated agar, and the slide was incubated under an air curtain on the microscope stage.

To study expanding microcolonies on agar petri dishes, the same microscope was used with a 20×, 0.25 NA long-working-distance objective. The bacteria were visualized through the plastic lid of the dish by using bright-field illumination with a defocused condenser. The lid was sealed to the dish with freezer tape all around its perimeter, except for a small opening to allow gas exchange. This was done to prevent excessive drying of the agar under the air curtain. To bring the agar surface close enough to the lens, it was necessary to fill the bottom of the petri dish completely with agar medium.

Petri dish microphotography. Microcolonies on agar plates were photographed with a 50×, 0.85 NA Epiplan objective (i.e., not cover slip corrected) on a Zeiss Axiophot microscope. The plate was illuminated with transmitted light through a bright-field condenser and viewed through Nomarski optics polarizing filters between the objective and the ocular/camera. By altering the plane of focus, the bacteria appeared either dark or light, and at appropriate focal planes, pseudo-Nomarski images could be obtained. It was necessary to prewarm the objective and maintain an air curtain over the objective and plate during observation to prevent condensation at such a close working distance.

Digital analysis of time-lapse sequences. Photographs of frames from the time-lapse sequences were viewed with a television camera and digitized into MacPaint documents by a Koala MacVision digitizer unit attached to a Macintosh Plus computer. For measurements of the positions of the cell poles, the MacPaint documents were converted into SuperPaint documents, and the coordinates of the cursor when it was positioned over the end of each cell were noted with rulers calibrated in screen dots. To normalize these individual measurements for frame-to-frame comparison, the position of a marker on the video image in each frame was also determined. The coordinates of the marker were then subtracted from each measurement to give the normalized values used in preparing the graphs in Fig. 3.

RESULTS

Time-lapse recording of initial cell divisions on slides. Individual cells from dilutions of saturated *E. coli* K-12 cultures were visualized on a thin agar layer on a microscope slide with oil immersion phase contrast optics, and video recordings were made of the growing bacteria for several hours. Figure 2 presents a selection of frames from three sequences illustrating growth to the four-cell stage. Following the first division, cell elongation became asymmetric, so that the two daughter cells elongated preferentially towards each other, displacing their inside poles and continuing to elongate alongside each other until the four poles were aligned. Septation and division occurred during the elongation process so that a characteristic four-celled structure resulted after somewhat less than 2 h of incubation.

Six of seven recordings showed the same initial cell division pattern. In the seventh recording, one of the first two daughter cells stopped growing due to overheating (see reference 13). In three of the seven cases in which the bacteria developed from one cell, viewing of the recordings at high speed (120 times the rate of recording) revealed a sudden movement of the initial two daughter cells towards each other several minutes after the first division. At later stages of microcolony development, other abrupt movements could be observed bringing cells into parallel alignment. The possible physical basis of these movements will be discussed below.

By graphing the positions of the ends of the first two daughter cells over time, it was possible to confirm where elongation occurred and discern the sudden displacement of one daughter cell towards the other when it occurred (Fig. 3). The positions of the outside poles (represented by solid diamonds and open triangles) did not change as much as the positions of the inside poles (represented by solid squares and open diamonds). Note that the outside poles were virtually stationary during an interval of significant elongation from 11 h 14 min (11:14) to 11:40. It can also be seen that the rate of elongation was not constant but varied over time. The arrow on the graph indicates the time when the right-hand cell moved abruptly towards the left-hand cell; this movement can be seen by the simultaneous and equal displacement of both cell poles to the left. The sudden change in relative positions of these two daughter cells is also illustrated by two frames of the video sequence separated in recording time by an interval of only 32 s.

Initial cell divisions on the surface of agar petri dishes. One major question about the biological significance of these results arose from the need to use agar slide cultures under a cover slip in order to achieve adequate resolution for the time-lapse recording. Could the cover slip have constrained the geometry of cell divisions in such a way as to create artifactually the specific pattern we observed? In order to address this question, cultures were diluted and plated on standard petri dishes containing agar substrate, incubated at 37°C, and examined at intervals to see what kind of microcolonies formed under these less-constrained conditions. Fresh cultures of strain MS2168 showed reasonably uniform staging of microcolony development on petri dishes, and it was easy to view fields after 90 min of incubation, at which time the large majority of the microcolonies consisted of figures clearly related to the four-cell arrays observed on slides (Fig. 4).

Interactions between bacteria descended from different ancestors. Donachie and Begg (8) reported previously the same division pattern as we observed leading to the four-cell array with *E. coli* 15T$^-$ on minimal agar. They attributed this pattern to an automatic "rule" that elongation under these conditions proceeded only from the pole which had previously participated in a septation. While this rule would account for the results we obtained with well-isolated bacteria, it was not consistent with observations with higher-density inoculations, in which nearby bacteria frequently attracted each other and altered their normal patterns of elongation and division.

In dozens of lower-magnification recordings, we invariably saw microcolonies fuse to form larger structures which had the same multicellular patterns as the progeny of single bacteria. In order to find out whether directed interactions between neighboring cells played a role in these fusions, we did nine high-magnification time-lapse recordings to follow the progeny of neighboring bacteria. In two instances, it was not possible to distinguish whether the two microcolonies actively joined together or simply expanded into each other as they spread over the substrate. In the remaining seven cases, however, our recordings of growth from two or more

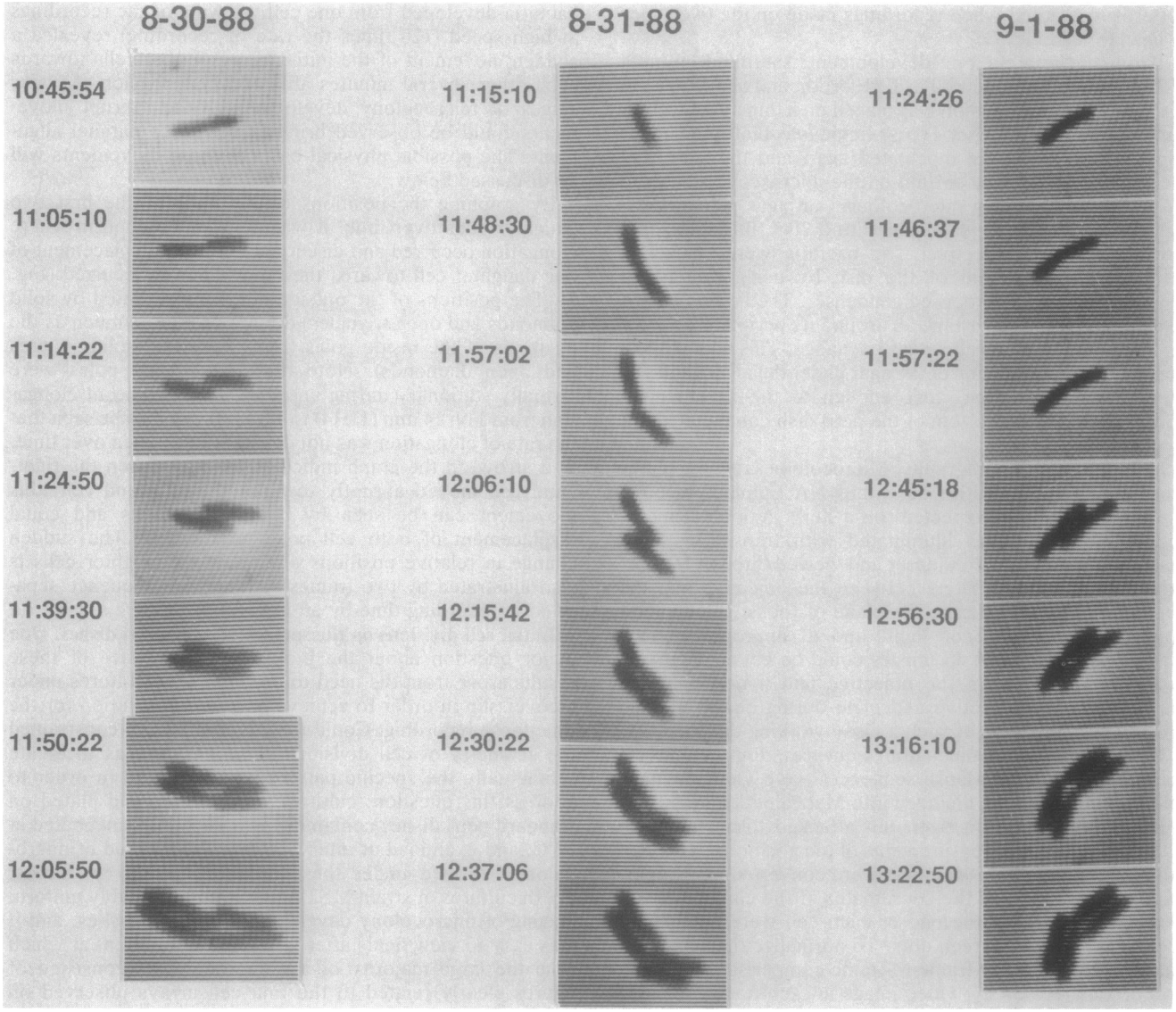

FIG. 2. Initial divisions of three *E. coli* K-12 cells leading to microcolony formation on agar-coated slides. Selected video frames were photographed off the monitor and are arranged here in sequential order from top to bottom to show the development from a single cell to a characteristic four-cell array. In the last frame of each sequence, subsequent developmental events can be seen to begin with internal cell elongation and consequent displacement of the inside ends of the bacteria. The numerals at the left of each panel give the time of the video frame (hours:minutes:seconds). The 8-30-88 sequence shows strain MS398, and the 8-31-88 and 9-1-88 sequences show strain MS2168. The exact magnifications of these figures were not determined, but they are on the order of ×2,000.

nearby single cells gave clear evidence of specific interactions between bacteria from different clones. As a "specific interaction," we scored directed and/or accelerated elongation of a cell in one clone towards another clone or preferential side-by-side alignment of cells from two different clones. Figure 5, for example, shows a sequence in which the daughter of one MS398 cell elongated at its outside pole and curved towards the daughter of another cell. These unusual growth events (elongation away from the sibling and cell curvature at this stage) produced an aligned cell pair involving the progeny of two different bacteria. In a similar way, Fig. 6 shows a sequence involving three nearby MS398 cells, labeled A, B, and C. In the first four frames, the interaction between daughter cells from B and C could be seen to give the same result as in Fig. 5, except that neither cell appeared to curve. Note that after contact, the lower daughter of cell B elongated at its outside pole along the upper daughter of cell C. In the next four frames, the progeny of A showed a striking example of differential cell elongation. At 15:03 (135 min after inoculation), cell A1 had divided twice to give four progeny, but cell A2 had only elongated. In the next 12 min, A2 divided, and one of its daughters preferentially elongated towards the merged progeny of B and C, reaching them in another 6 min. The final frame, taken 217 min after inoculation, illustrates how the progeny of these three independent cells were unified into a single microcolony which would have been difficult to distinguish from a single clone of cells.

Interactions between cells from different clones to produce fused microcolonies were an ongoing feature of *E. coli* development on densely inoculated agar, and microcolony fusions help explain why smooth lawns grow from such

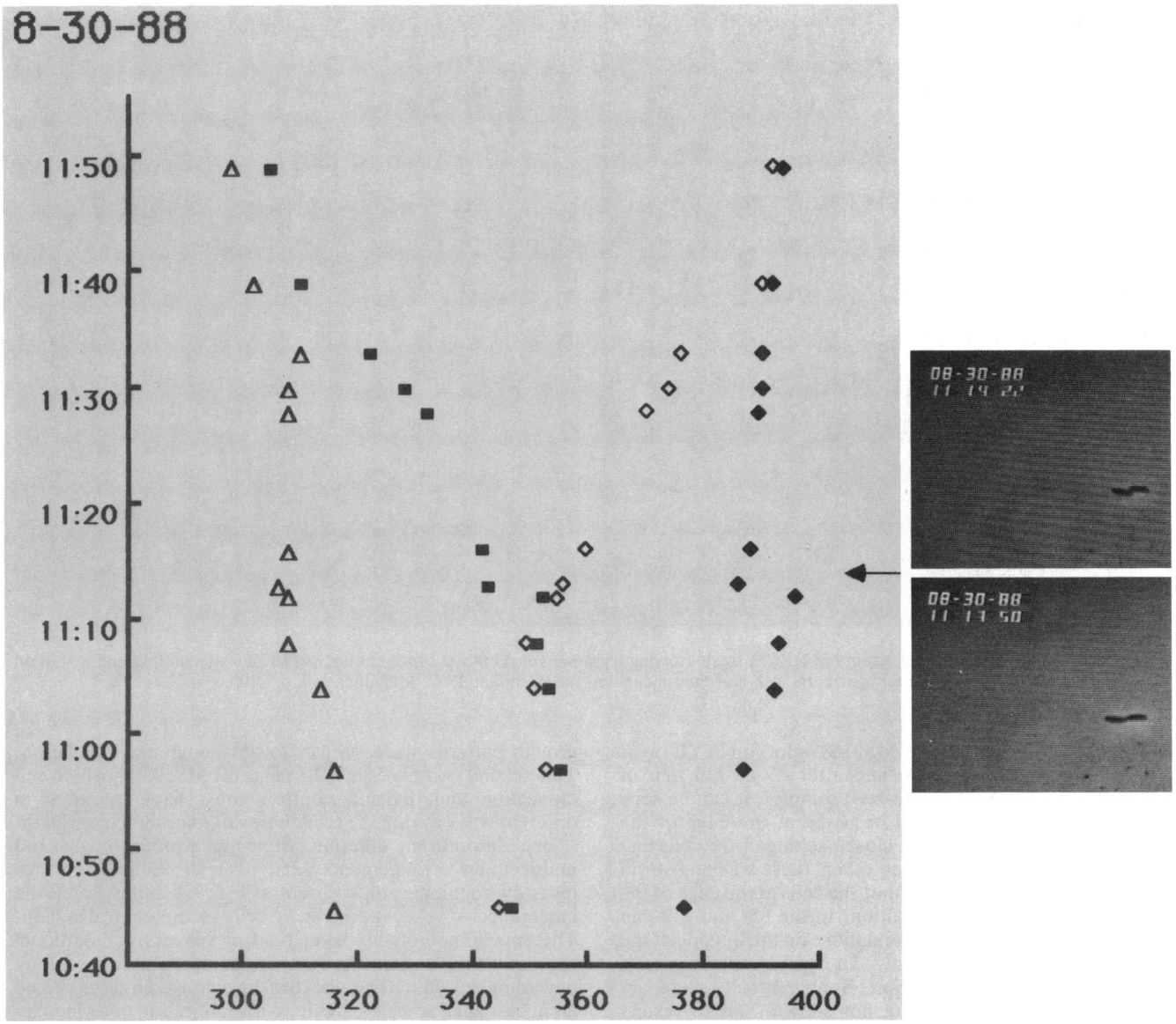

FIG. 3. Digital analysis of the movement of the cell poles in the 8-30-88 sequence. The left-hand scale gives the time of each frame analyzed. The abscissa gives the normalized x-axis coordinate of the four cell poles emerging from the first division. The poles are indicated by the following symbols: solid diamond, right pole of right cell; solid square, left pole of right cell; open triangle, left pole of left cell; open diamond, right pole of left cell. Increasing values for the coordinates indicate movement towards the right of the video screen. The arrow and video frames indicate the interval when the relative positions of the two cells changed abruptly.

inoculations. At later stages of development, the influence that nearby microcolonies had on each other's growth was seen very clearly. When microcolonies containing several hundred or more bacteria approached each other, expansion towards the neighbor accelerated so that each one stretched out towards it until they fused. Characteristic changes occurred in the structures of the expanding microcolony perimeters during these fusions. Figure 7 shows the initial encounter of two M7124 microcolonies after about 10.5 h of incubation on a minimal salts-glucose agar plate. In this case, the distortions of the microcolony borders involved many bacteria. On the actual videotapes of this and other encounters, individual bacteria could be seen to separate from the edge of one microcolony and "jump" towards the other microcolony when they were approximately 2 to 5 μm apart. It is interesting that the characteristic structure of the microcolony perimeter (here seen as concentric darker rings) began to reform at the points of fusion once the two clones had joined together.

Cellular alignments in multicellular inocula. One of the most remarkable aspects of bacterial colony patterns was their independence of colony size and mode of inoculation (Fig. 1). These observations indicated that there were probably important similarities between early events in colony morphogenesis from individual bacteria and from multicellular spots. We previously reported that the bacteria at the perimeter of a multicellular inoculum showed considerable palisading after 3.5 and 7.5 h of incubation when visualized in the SEM (24). Reexamination of what happened to multicellular spots on agar plates by light microscopy confirmed this report and showed that considerable cell-cell alignment was visible within the first 85 min of development. Figure 8

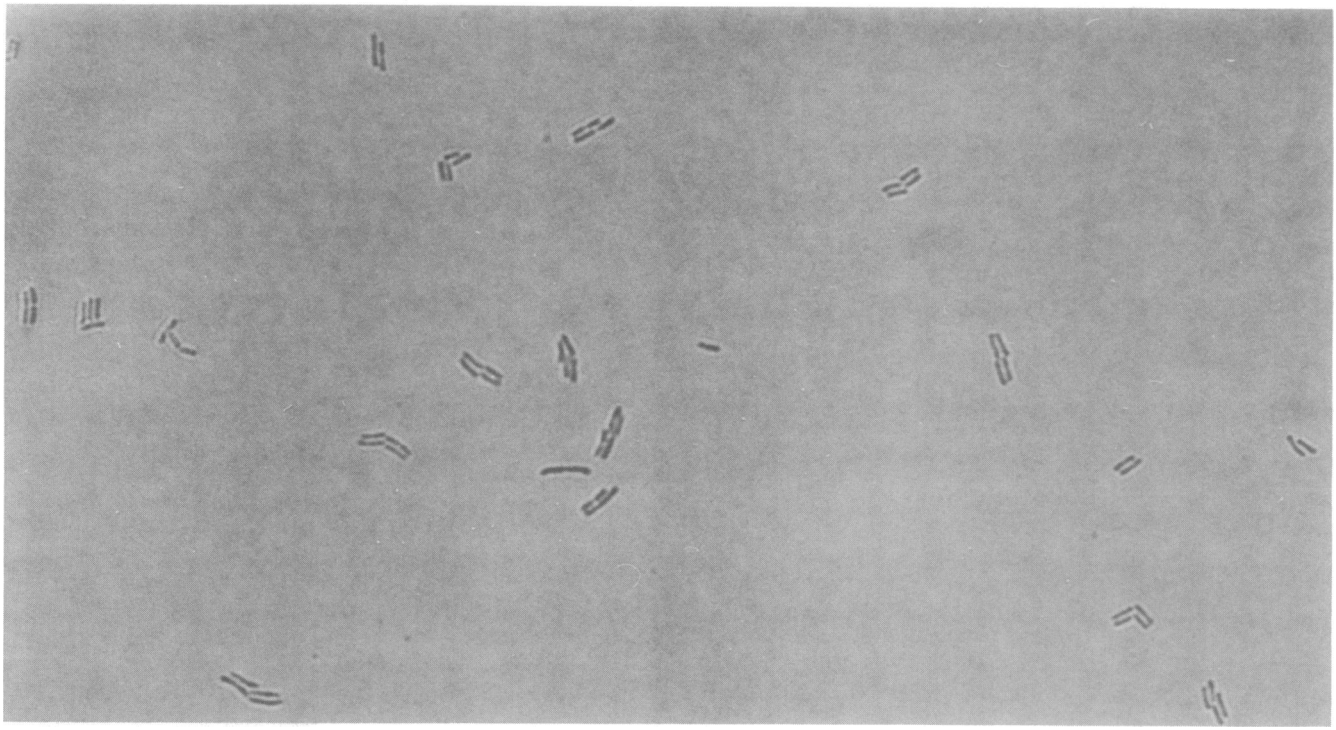

FIG. 4. Early microcolonies of strain MS2168. A fresh growing tryptone-yeast extract broth culture of MS2168 was diluted and plated on a prewarmed tryptone-yeast extract agar petri dish and then incubated for 90 min at 37°C. Magnification, ×1,100.

shows the perimeter of an MS2168 spot on TYE agar immediately after inoculation and after 85 or 110 min of incubation at 37°C. In the time-zero samples, it can be seen that the bacteria accumulated in a ring at the edge of the spot. Although there was very close packing of the cells and small groups lay parallel to each other, there was no overall pattern of alignment. In particular, the cells at the edge of the spot showed no special orientation. In the 85- and 110-min samples, the cells were longer and those on the outside of the dense bacterial ring clearly showed a considerable degree of palisading and radial orientation. Similar results were obtained with MS398 spots (data not shown). These results indicated that an active process of elongation and cell-cell alignment took place in multicellular groups, just as it did among the progeny of individual cells.

Strain specificity in microcolony development after the four-cell array. One feature of our results which merits comment was the observation that the bacteria in cultures of different *E. coli* K-12 strains produced similar four-cell arrays but then went on to develop into quite distinct microcolony types. The 8-30-88 sequence in Fig. 2 involved strain MS398, and the 8-31-88 and 9-1-88 sequences involved strain MS2168. MS398 bacteria regularly developed into microcolonies with long curving cells aligned tangentially, while MS2168 bacteria regularly developed into highly palisaded microcolonies with a large majority of very uniform shorter cells. Figure 9 illustrates these two distinct microcolony types from later times in the respective video sequences as well as from slides photographed directly through the microscope after several hours at 37°C. The importance of the finding that MS398 and MS2168 microcolonies were similar during the first 2 h of growth but very different after 8 h is twofold. First, it demonstrated genetic specificity in developmental patterns. Second, it showed that cellular growth patterns were subject to developmental regulation. This second point is especially clear for MS398, in which cell elongation and division controls must have operated in different ways during the two phases of microcolony growth.

In addition to the differences in cellular morphologies and multicellular arrangements seen near the edges, the two older microcolonies on the right of Fig. 9 also illustrated the emergence of a second layer of cells in the central region. The appearance of this layer (and of successive additional layers) was yet another regular feature of early colony development, which showed that the colony center remained an active zone of cell growth. With longer times of incubation, additional layers formed near the perimeters of older microcolonies to generate a multilayered structure of the kind shown in Fig. 7.

DISCUSSION

The present observations on growth from single *E. coli* cells fit with an extensive literature documenting various regular features in the species-specific development of young bacterial microcolonies (3, 4, 7, 11, 13, 19, 29). In addition to Donachie and Begg (8), three papers (among those known to the report by us) documented the four-celled array we have described: Graham-Smith in 1910 on *Vibrio cholerae* (11), Hoffman and Franck on *E. coli* ATCC 8677 (13), and Tanaka on *Agrobacterium tumefaciens* (26). The results presented here are thus consistent with work dating back more than three-quarters of a century, and the data extend our previous description of orderly events during the development of *E. coli* K-12 colonies (24) to the earliest cell divisions. In particular, these data showed that the start of colony development followed the same cell division pattern on a petri dish as on a slide (Fig. 4).

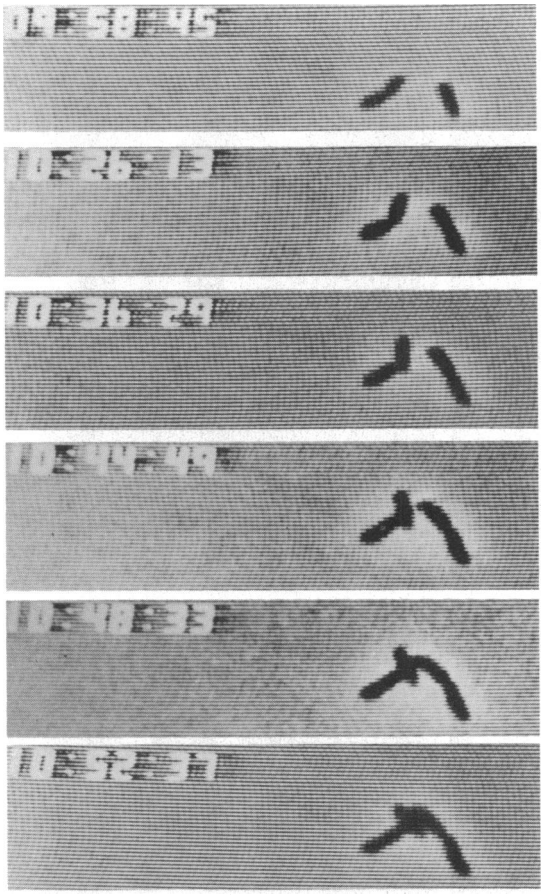

FIG. 5. Initial growth and division of two neighboring MS398 cells. Numbers in the upper left-hand corner give the time of each frame. The conditions were similar to those described for Fig. 2.

These data and our previous observations showing that each location in an *E. coli* colony had its own array of morphologically differentiated cells (24) indicated that the growth of individual bacterial cells was regulated during colony development to produce different shapes and to extend in specific directions. As seen in Fig. 2, 3, 5, and 6, individual cells did not elongate uniformly in an automatic fashion but extended one pole towards other bacteria. The biophysical bases of directed cell elongations may include a number of factors, such as discrete regions of cell envelope growth, specific zones of cell adhesion to the agar substrate, and attractive interactions between closely juxtaposed bacteria. Our results are consistent with the notion that regulation of elongation and division played a role in producing the orderly multicellular arrays seen at various times and places in developing colonies. This regulation must be subject to hereditary control, because there were dramatic differences between strains MS398 and MS2168 in cell morphogenesis at later stages of microcolony development (Fig. 9). Studies of the genetic control of cell division, cell shape, and envelope biosynthesis have uncovered a large number of loci encoding cellular morphogenesis functions, many of which can be eliminated by mutation without loss of viability (9). Each of these functions is a potential control point where the growth of individual cells could be modified in order to achieve the morphological differentiation and overall patterning that we and others have found to characterize the developing colony. The process of cell division may itself play an important role in the differentiation of *E. coli* cells during multicellular development, because macromolecules can be distributed unequally to the two daughter cells, as happens in other bacterial groups, such as *Bacillus* (17) and *Caulobacter* (10) spp.

Our results showed that neighboring cells and microcolonies responded to each other and merged into larger units (Fig. 5 to 7). The tendency of *E. coli* K-12 cells to form intimate side-by-side alignments, even when initially separated from each other, indicated that these bacteria have evolved to grow cooperatively on surfaces. If each cell were growing autonomously in competition with its neighbors, the optimum strategy for resource utilization would be to maximize the distance between bacteria, but we and others have found just the opposite growth pattern. It is probably the case, as suggested by Legroux and Magrou (16), that the organization of different cell types in colonies has a physiological significance and reflects arrangements which serve to facilitate uptake of nutrients, disposal of wastes, gas exchange, and other vital processes. This interpretation is consistent with observations that the colonies of a particular strain have different morphologies when grown on different media (22, 23) or under different conditions, such as anaerobic versus aerobic growth (R. D'Ari, personal communication). One clear example of substrate transport through many cell layers is papillation on fermentation indicator medium. Without such transport, fermentation-positive bacteria could not proliferate on the colony surface in response to a specific sugar in the agar.

We do not yet know the molecular basis of the cell-to-cell communication illustrated in Fig. 5 to 7. While diffusible metabolites and small peptides analogous to sporulation factors in *Bacillus subtilis* (12) and *Myxococcus xanthus* (14) are prime candidates for signaling molecules, there are other possibilities. We would like to emphasize the potential importance of extracellular polymers, which have been visualized in the SEM and often extend considerable distances beyond the bacterial cells onto the agar substrate (23, 24). These materials are likely to have interesting physical properties which may play a role in multicellular alignments. In this research we repeatedly observed abrupt cell movements, such as those documented in Fig. 3 and others observed later in microcolony development, when more complex arrays of many cells at different angles collapsed into parallel alignments like those seen with MS2168 in Fig. 9. These movements could have had a physical basis of elastic material connecting apparently separate bacteria. Electrostatic interactions may also have played a role in attracting and aligning cells. If so, how individual cells accumulate regions of positive and negative charge at specific places on their surfaces will be a very interesting question to resolve. Research on conjugal DNA transfer further indicates that extracellular appendages like pili, not visible in the light microscope, and surface proteins may play a critical role in intercellular signal transduction (27).

Genetic analysis of cell-cell interactions in developing *E. coli* colonies is possible because of the relationship between morphogenesis at the cellular and multicellular levels. As mentioned above, we have isolated mutants which display altered patterns of colony morphogenesis, and a number of these mutants have obvious changes in the regulation of cell elongation and division (unpublished observations). Molecular analysis of these mutants may identify new genetic loci involved in the control of cell-cell interactions. In addition, it is possible to take advantage of the large stock of available *E. coli* mutations which affect cell morphogenesis, growth

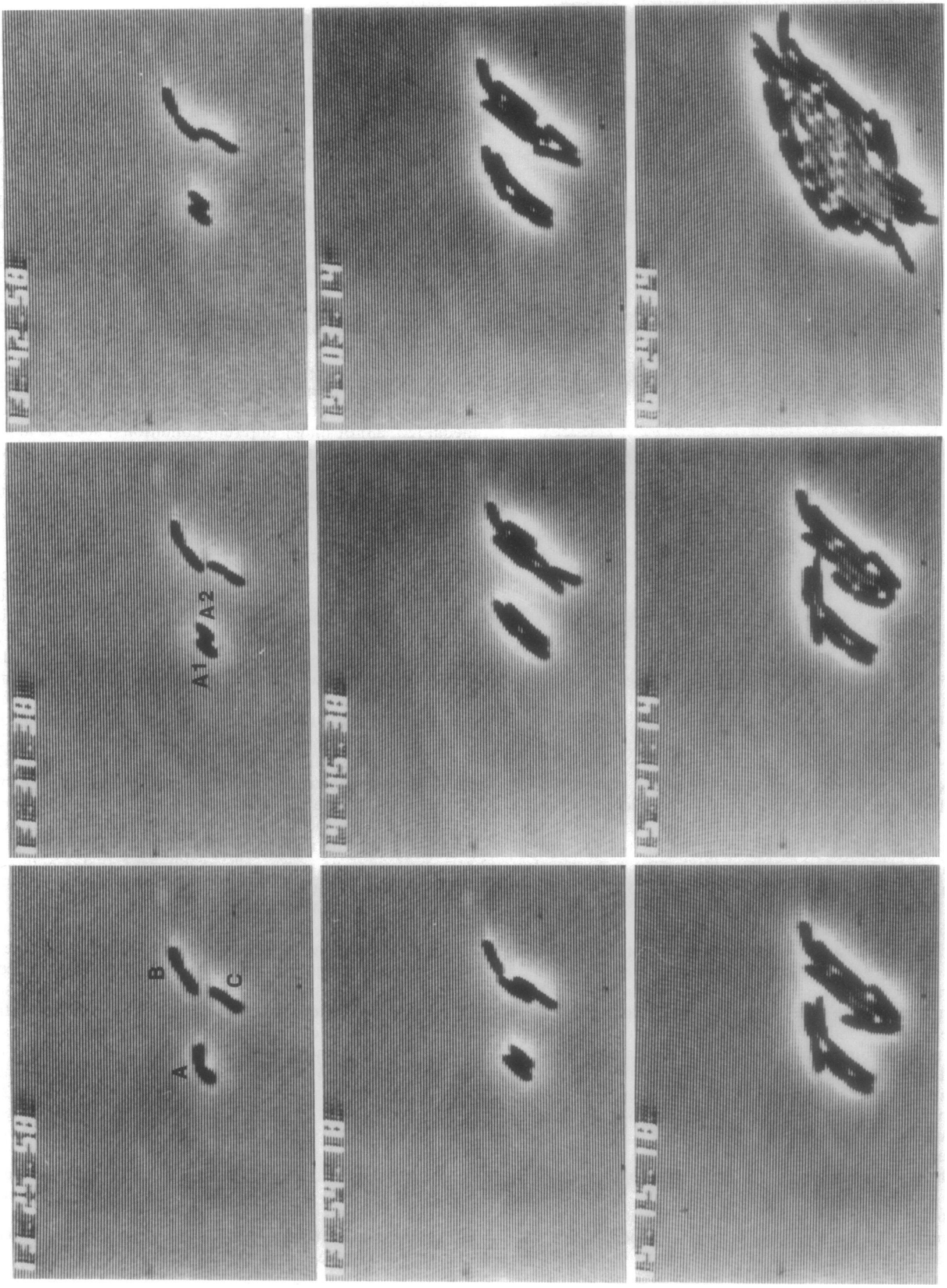

FIG. 6. Initial growth and division of three neighboring MS398 cells. The three starting cells and two daughters of cell A are labeled to facilitate the description in the text. Numbers in the upper left-hand corner give the time of each frame. The conditions were similar to those described for Fig. 2.

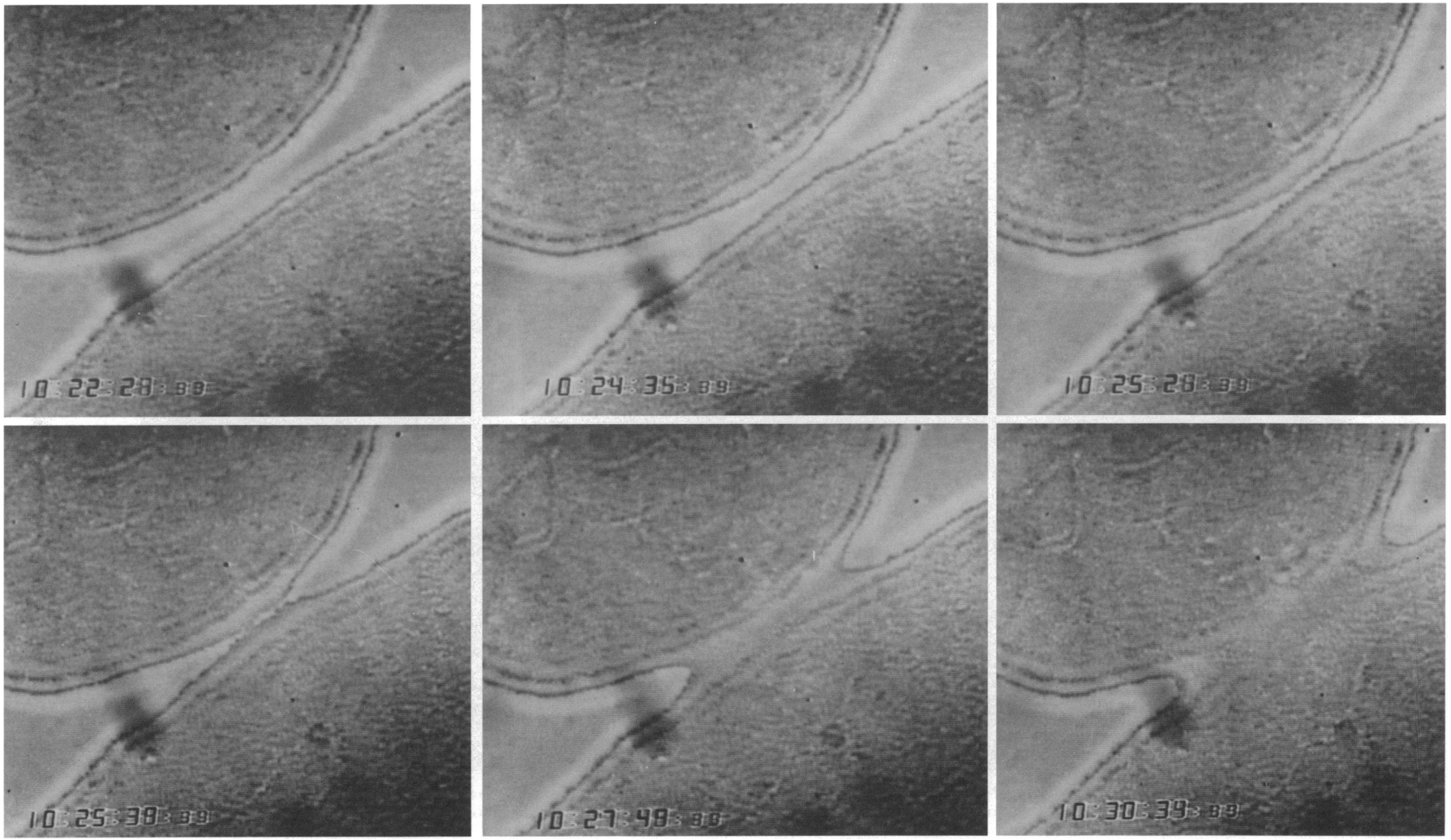

FIG. 7. Approach and merger of two microcolonies on a petri dish. The time-lapse sequence shows two M7124 microcolonies on a minimal salts-glucose agar plate almost 10.5 h after inoculation. The numbers at the lower left indicate the time elapsed since inoculation (in hours:minutes:seconds:hundredths of seconds). Each microcolony was bounded by a series of dark lines, indicating successive layers of different thicknesses at the perimeter. The zone between the outermost lines was a monolayer. As the microcolonies neared each other, this monolayer zone puckered and stretched towards its neighbor. Just before the two microcolonies came into contact (at 10:25:39 elapsed time), a few cells at the edge separated and appeared to jump from one microcolony to the other. After contact, the two microcolonies joined and began to establish the typical multilayer perimeter structure around the sites of junction. The exact magnification was not determined but is on the order of a few hundred-fold.

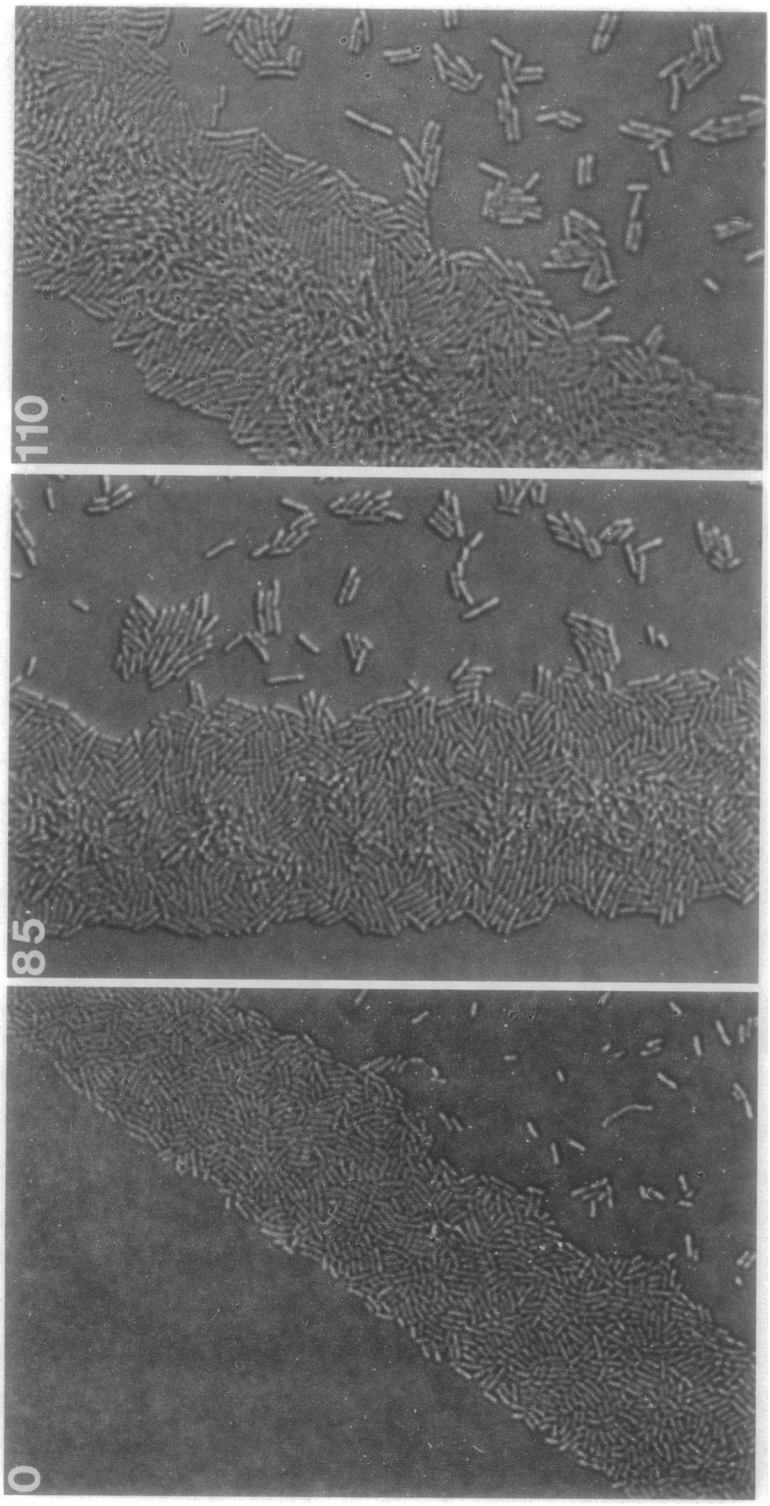

FIG. 8. Bacteria at the edge of a spot inoculum of strain MS2168 on a TYE agar plate. The left-hand panel shows the bacteria immediately after inoculation. The other two panels show the bacteria after incubation at 37°C. The numbers at the top of each panel indicate incubation time (in minutes). Magnification, ×1,000.

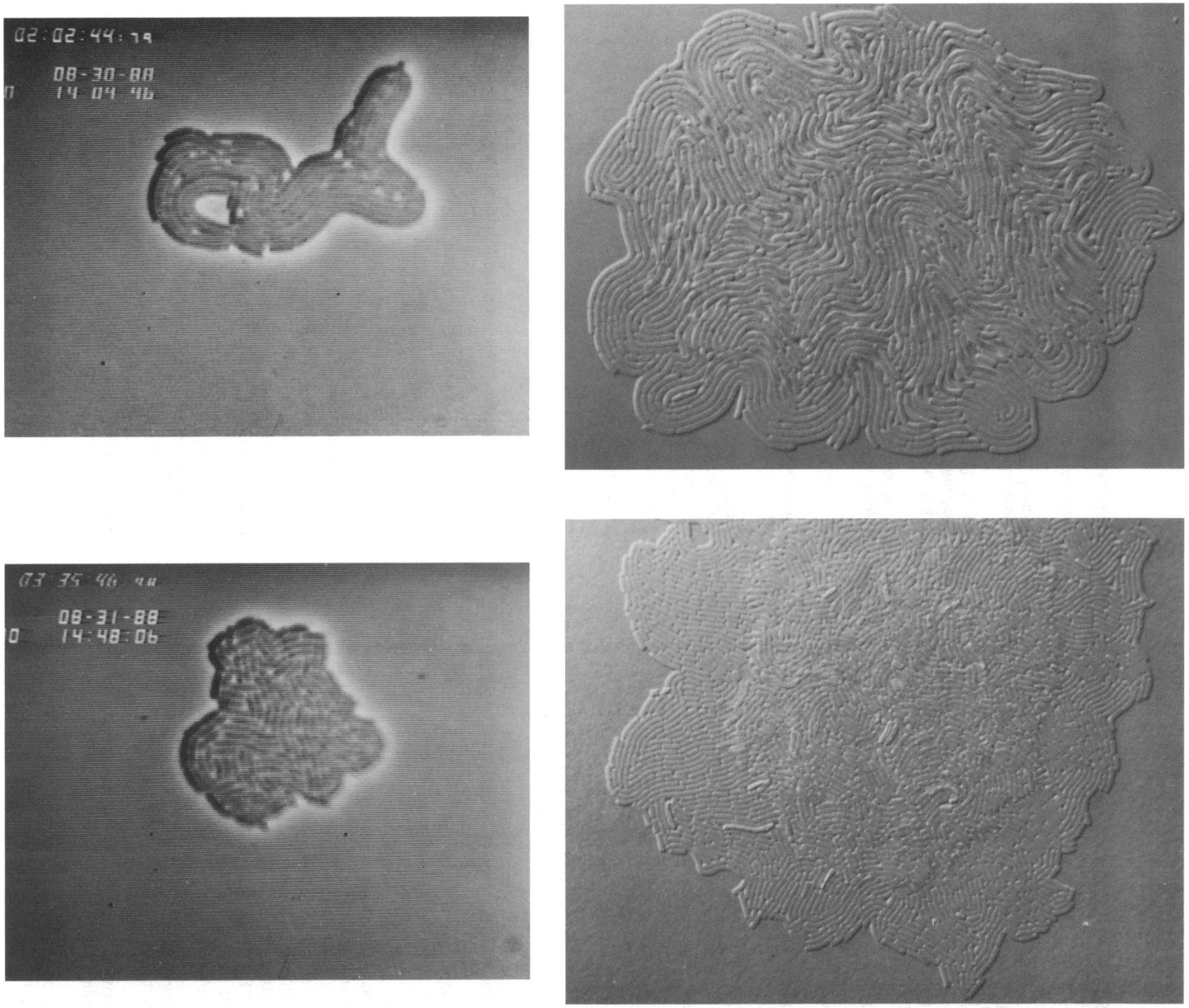

FIG. 9. Microcolonies produced after several hours by strains MS398 and MS2168. On the left are microcolonies from the 8-30-88 video sequence (MS398) and from the 8-31-88 sequence (MS2168) seen 2 h after the last frames in Fig. 1. To the right of each of these video pictures are microcolonies of the same strain incubated at 37°C on slides coated with buffered agar for 7.5 h (MS398) and 11.5 h (MS2168). These slides were photographed with Nomarski optics using a 100×, 1.3 NA oil immersion objective on a Zeiss Axiophot microscope. Magnification, ×1,170.

regulation, and signal transduction to find out whether they modify the kinds of interactions we have observed. One prediction of our view of multicellular pattern formation by bacteria is that signaling and receptor functions involved in morphogenesis will be identified.

We know that bacteria have a wide range of regulatory systems which permit them to respond to many different chemical and physical signals and adapt their metabolism and behavior accordingly. The results presented here indicate that bacteria also respond to each other as they proliferate on agar. The interactive nature of multicellular growth adds a new dimension to our analysis of physiological control in bacteria. In some cases, the functional significance of multicellular organization and the benefits it provides to the entire bacterial population are known. Examples include *Neisseria gonorrhoeae* virulence correlated with colony type (15), antibiotic-resistant *Staphylococcus aureus* biofilms (18), and predatory *M. xanthus* colonies in aqueous environments (6). It is likely that studies of pathogens, including *E. coli*, will reveal additional roles for cellular differentiation and for specific forms of multicellular aggregation in important processes such as colonization and defense against antibacterial agents.

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