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Dynamic Aspects of the Structured Cell Population in a Swarming Colony of *Proteus mirabilis*

TOHEY MATSUYAMA,[1]* YUKO TAKAGI,[2] YOJI NAKAGAWA,[2] HIROTO ITOH,[3] JUNICHI WAKITA,[3] AND MITSUGU MATSUSHITA[3]

Department of Bacteriology, Niigata University School of Medicine, Niigata 951-8510,[1] Faculty of Agriculture, Niigata University, Niigata 950-2181,[2] and Department of Physics, Chuo University, Tokyo 112-8551,[3] Japan

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Proteus mirabilis **forms a concentric-ring colony by undergoing periodic swarming. A colony in the process of such synchronized expansion was examined for its internal population structure. In alternating phases, i.e., swarming (active migration) and consolidation (growth without colony perimeter expansion), phase-specific distribution of cells differing in length, in situ mobility, and migration ability on an agar medium were recognized. In the consolidation phase, the distribution of mobile cells was restricted to the inner part of a new ring and a previous terrace. Cells composing the outer part of the ring were immobile in spite of their ordinary swimming ability in a viscous solution. A sectorial cell population having such an internal structure was replica printed on fresh agar medium. After printing, a transplant which was in the swarming phase continued its ongoing swarming while a transplanted consolidation front continued its scheduled consolidation. This shows that cessation of migration during the consolidation phase was not due to substances present in the underlying agar medium. The ongoing swarming schedule was modifiable by separative cutting of the swarming front or disruption of the ring pattern by random mixing of the pattern-forming cell population. The structured cell population seemed to play a role in characteristic colony growth. However, separation of a narrow consolidation front from a backward area did not induce disturbance in the ongoing swarming schedule. Thus, cells at the frontal part of consolidation area were independent of the internal cell population and destined to exert consolidation and swarming with the ongoing ordinary schedule.**

There are various kinds of surfaces in nature. Most of these surfaces have fractal properties and are able to provide an enormous amount of places for microbial habitation (17). For efficient and flexible colonization on these surfaces, microbes seem to have evolved special strategies in each special situation, e.g., the flagellar and twitching motility of *Pseudomonas aeruginosa* in biofilm formation (21). Surface translocation by flagellated bacteria may be one of these examples. Many species of bacteria have been reported to have such a surface swarming ability (2, 4, 13). Among these, *Proteus mirabilis* is well known for forming a giant colony with a concentric-ring pattern and cellular dimorphism (differentiation to extremely elongated and hyperflagellated swarmer cells).

The growth process of a *P. mirabilis* colony is composed of cyclic repetition of a swarming (expansion) phase and a consolidation (no-expansion) phase. During the consolidation phase, swarmer cells dedifferentiate into vegetative cells for intensive cell multiplication. These vegetative cells will become the source of future swarmer cells after differentiation (2). Since many types of mutants which show abnormal patterns of colony growth have been isolated (3, 5, 29), it is evident that *P. mirabilis* has a special genetic program for such characteristic swarming. Although mutational analyses clarifying responsible factors for each process are in progress (6, 9), we intend to show the dynamic population structure developing in a *P. mirabilis* swarming colony. Reflecting a century-old history of studies on the swarming phenomenon of *P. mirabilis*, there are a large number of manuscripts on this subject. However, information on the dynamic features of the bacterial population is scanty and obscure. Some of the previous experimental data (16) have not been reproducible by our experiments, as already pointed out by others (30). Thus, we examined the phenotypic profiles of *P. mirabilis* swarming by our own analytical methods. This paper focuses on specific factors of the cyclic swarming behavior of the bacteria. There are at least two models which recently have been proposed to explain periodic colony growth, those of Mimura et al. (M. Mimura, H. Sakaguchi, and M. Matsushita, submitted for publication) and Esipov and Shapiro (11). Later we briefly discuss the validity of these models.

It is quite difficult for a single bacterium to translocate on a surface, due to physicochemical factors such as interfacial and frictional inhibitory forces (18). Special organization by a structured cell population seems to enable efficient swarming by some species of bacteria. In examination of a *Bacillus subtilis* swarming colony, which is composed of dendritic branches radially extending from a point-inoculated site (20), we found a special internal structure in an actively extending branch (19, 28). That is, the branch was encapsulated with immobile cells, and these cells at the branch tip were pressed out to extending side by actively swirling cell clusters positioned just behind the tip. Therefore, branch growth was not a result of active locomotion of the outermost front cells but instead was the result of passive translocation of the outermost front cells by the pressure generated by the actively swirling cell population located slightly in the interior. The restricted presence of such a pressure-generating region in a branch front area seems to lead to the unidirectional growth of the branch without thickening or fusion of the branches.

In the swarming of *P. mirabilis*, does the translocating cell population expand only outward? For translocation to an unoccupied surface area, *P. mirabilis* is known to exert collective behavior by mutual sliding of elongated cells. However, migration by making such a cooperative cell mass does not progress

* Corresponding author. Mailing address: Department of Bacteriology, Niigata University School of Medicine, Niigata 951-8510, Japan. Phone: 81 25 227 2111. Fax: 81 25 227 0762. E-mail: tohey@med.niigata-u.ac.jp.

continuously. Outward migration undergoes temporary cessation at regular intervals. For the next migration to occur, a consolidation phase must intervene. In addition, the presence of successive internal waves toward a colony front has been visualized by time-lapse video recording of colony growth (14, 22). As indicated by the shadowing effect of a trench on periodic swarming progression (24), a dynamic organization or intercellular communication in a colonial cell population seems to be occurring for the swarming/consolidation periodic program. In this study, the site- and phase-specific distribution of cells differing in morphology and mobile activity was shown and examined for its relationship to programmed swarming.

MATERIALS AND METHODS

Strain and culture conditions. *P. mirabilis* ATCC 29906 was purchased from American Type Culture Collection, Rockville, Md. This strain developed a representative *P. mirabilis* colony by forming clear concentric rings with microcolonies at terraces and was used throughout in our experiments. Nutrient agar medium containing 0.3% meat extract, 1.0% peptone, 0.5% NaCl, and 1.8% Agar Eiken (Eiken Chemical Co., Ltd., Tokyo) was solidified in a 8.5-cm-diameter petri dish (16 ml/dish). The plates were partly opened and dried at 37°C for 4 h. For swarming-colony development, bacterial cells were grown to the stationary phase in nutrient broth and then washed with PA buffer (14). A 3-μl volume of bacterial suspension (~10^9 CFU/ml of PA buffer) was point inoculated at the center of the plate, and the plate was incubated at 37°C. Under the above conditions, the periods of swarming and consolidation were found to be 2.5 ± 0.2 h and 2.6 ± 0.1 h (n = 8), respectively. Macroscopic colony growth processes were examined by time-lapse video recording, as described previously (28).

Measurement of bacterial size and swimming velocity. Bacteria taken from a specified point of a swarming colony were simply stained with a 0.3% crystal violet solution, and microscopic figures in a monitor as seen through a microscope (Olympus BH2) and a charge-coupled device camera (ICD 740; Ikegami, Tokyo, Japan) were examined for their size with a video micrometer (VM30; Olympus, Tokyo, Japan). To measure the swimming velocity, bacteria taken from a swarming colony with a prewetted toothpick were suspended in 5 μl of chemotaxis medium (1) containing 2% polyvinylpyrrolidone 360 (PVP) on a glass slide. In the previous experiment, 2 to 5% PVP solution (fluidity, 0.164 to 0.0294 cP^{-1}) was shown to be appropriate for efficient swimming (27). The suspension was sealed under a coverglass and examined with a phase-contrast microscope, and swimming bacteria were video recorded. From cell sampling to video recording, each procedure was completed within 5 min, because the swimming velocity gradually decreased over a longer observation time in sealed buffer. The swimming velocity of each cell was measured by using an Olympus video micrometer. The researchers in charge of measurement were not informed of the source of each sample.

Examination of the in situ mobile activity of cells in a colony. A plastic dish with a giant colony was directly placed on a stage of a microscope equipped with a dry 40× phase lens and examined for translocating cell distribution in a colony. Observation was carried out within a short time (less than 5 min) in a room kept at 32 to 34°C. Since it was impossible to obtain quantitative data concerning in situ cell mobility, microscopically confirmed mobile activity was recorded as blurred images of translocating cells (1-s exposure). More than 10 plates were examined at each stage (e.g., the beginning stage of the swarming phase) of colony growth.

Replica printing of the structured cell population. A sectorial or a trapezial part of a giant colony with underlying agar medium was excised with a sterile surgical knife and placed on the flat face of a lead cylinder (diameter, 40 mm). The cell population on the agar block was transferred to fresh agar medium, which was pressed lightly onto the cell population and immediately detached. The replica-printed plate was incubated at 37°C and examined for swarming activity in parallel with the original plate (a block-removed plate) at intervals. The migrating cell population, which was mostly a monolayer, could be observed and photographed under oblique illumination.

Statistics. Differences between means were compared by Student's t test. Unless otherwise indicated, variations around means were 1 standard deviation.

RESULTS

Cell lengths at characteristic sites of a swarming colony. Macroscopically, a giant colony of *P. mirabilis* has a characteristic pattern composed of several concentric rings. These rings are discernible by the presence of a terrace zone with microcolonies and a low-cell-density zone intervening between the inner and outer terrace zones (Fig. 1A). Cells forming a giant colony which is in the swarming phase were taken from each characteristic ring site and examined for their length. As shown in Fig. 1B, at the extending thin front area, elongated cells (longer than 10 μm) were predominant. At the outermost (latest) terrace zone, 60% of cells were less than 2 μm long and the rest were 2 to 10 μm long. Cells taken from the inner terrace and low-cell-density zones showed no remarkable differences in cell length and were mostly composed of short cells (less than 2 μm long).

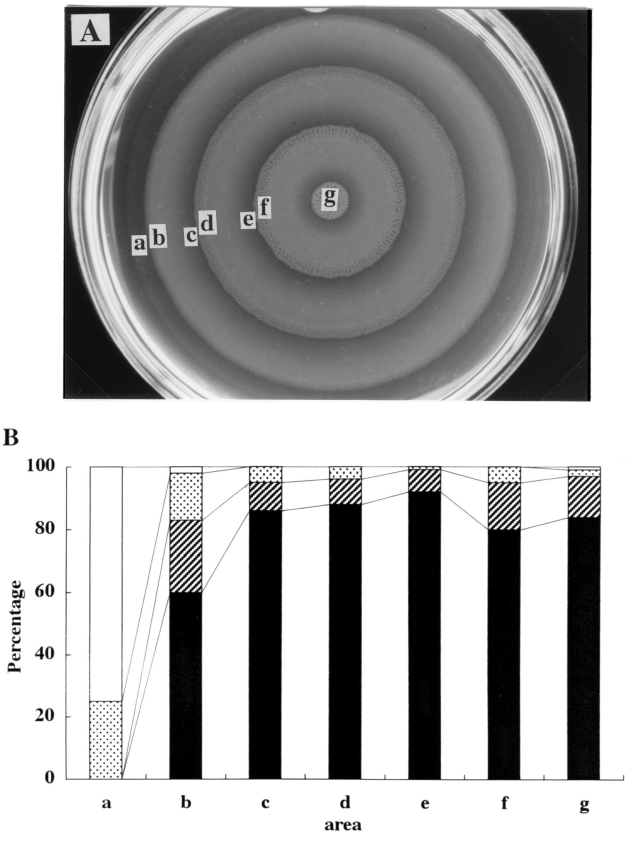

FIG. 1. Length of cells making up distinctive areas of a *P. mirabilis* colony. (A) Cells taken from the designated areas (marked a, b, c, d, e, f, and g) in an actively swarming colony were examined for cell length (more than 100 cells per area). Cell length was graded into four classes. (B) The percentages of cells shorter than 2 μm (black bars), 2 to 5 μm (hatched bars), 5 to 10 μm (dotted bars), and longer than 10 μm (white bars) at each area are shown.

Changing size of cells taken from two designated zones. A zone 2 mm outside the latest terrace and a zone 2 mm inside the consolidation front were designated as fixed points for examination of cell size change in the ongoing swarming-consolidation cycle (zones y and z in Fig. 2A). Generally, cells present at the fixed zone changed gradually from elongated swarmer cells to short vegetative cells during a single cycle of swarming and consolidation (Figs. 2B and C). At the initial time of swarming, a small compositional increase in the number of swarmer cells (5 to 10 μm long) was recognized at the z zone.

Swimming activity of cells at the consolidation front. To learn the ordinary swimming velocity of *P. mirabilis* growing on agar medium, cells from the center of the colony, a consolidation band, and a swarming band were suspended in 2% PVP and each cell was examined for cell length and swimming velocity. As shown in Fig. 3, unlike the short cells from the colony center, cells from the consolidation and swarming bands demonstrated similar swimming velocities (20 to 35 μm/s).

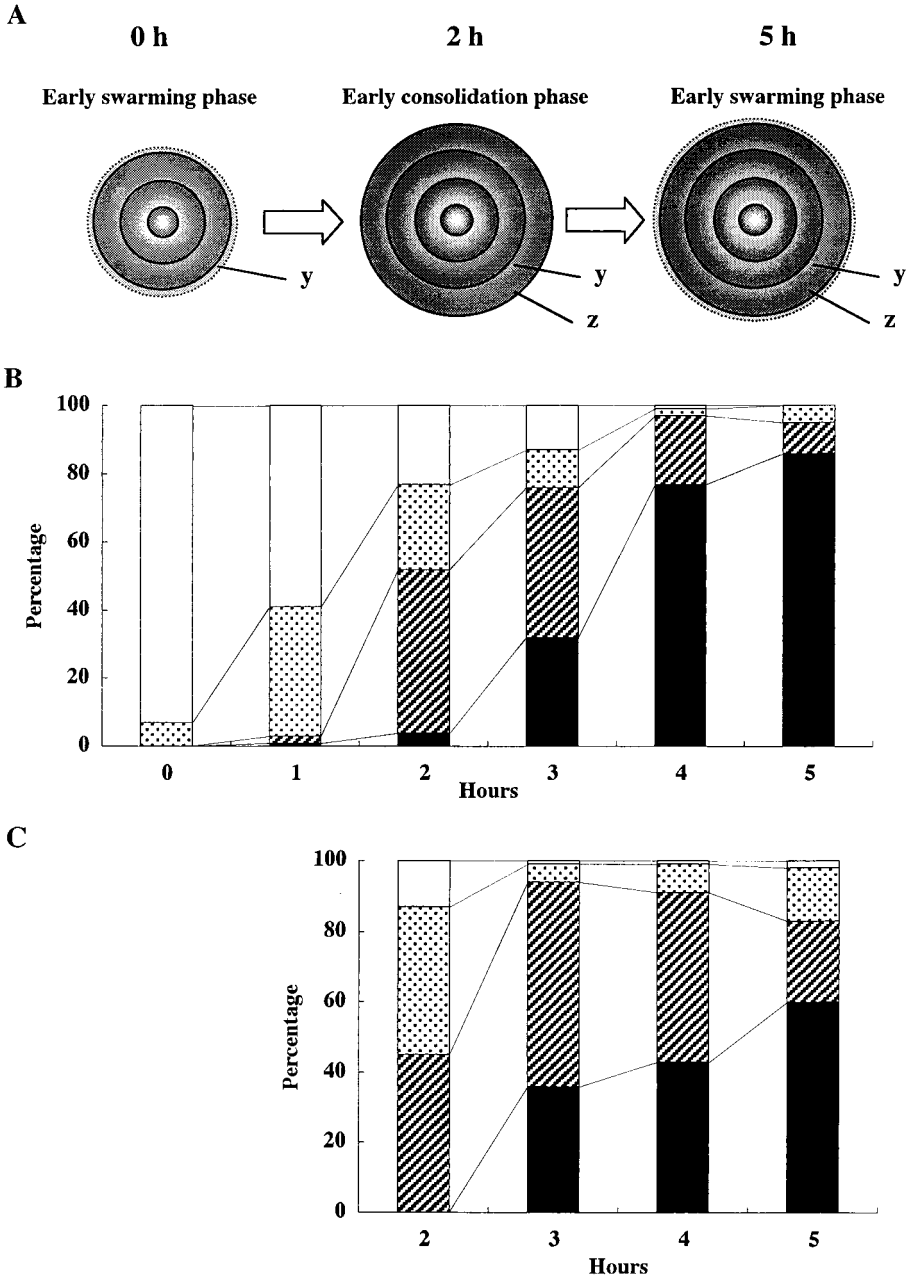

FIG. 2. Temporal change in the length of cells at the fixed zone of a swarming colony. (A) When a migration front passed the y point, cell length measurement (more than 100 cells) of the y zone started (0 h time). After the migration front passed the z zone and consolidation started (2 h time), cell length measurement of the z zone started. Just after the next swarming period started, the last measurement (5-h time) was undertaken. (B) Temporal cell length changes at the y zone. (C) Temporal cell length changes at the z zone. The percentages of cells shorter than 2 μm (black bars), 2 to 5 μm (hatched bars), 5 to 10 μm (dotted bars), and longer than 10 μm (white bars) at each time point are shown.

When the swarming phase ended, the front area became full of nontranslocating elongated cells, as shown in the following paragraph. These cells were examined for swimming activity in a viscous solution. As shown in Fig. 4, cells at the consolidation front gradually shortened but maintained ordinary swimming activity. Their rotating bundled flagella were visible in 5% PVP solution under a phase-contrast microscope (Fig. 5). Thus, nontranslocating cells at the consolidation front were shown to be neither energetically exhausted nor anatomically or functionally defective in swimming activity.

Inside distribution of actively translocating cells. The interior of a giant colony was surveyed for translocating cells by using a phase-contrast microscope equipped with a dry objective lens. At the initial stage of the consolidation phase, marginal-front cells were actively translocating (blurred images in Fig. 6A). However, cells just behind the front were mostly immobile (Fig. 6A and B). Therefore, the supply of mobile cells to the expanding front was ceasing. Consequently, in contrast to the smoothly expanding margin in the middle phase of swarming, the contour at the colony margin at this stage was

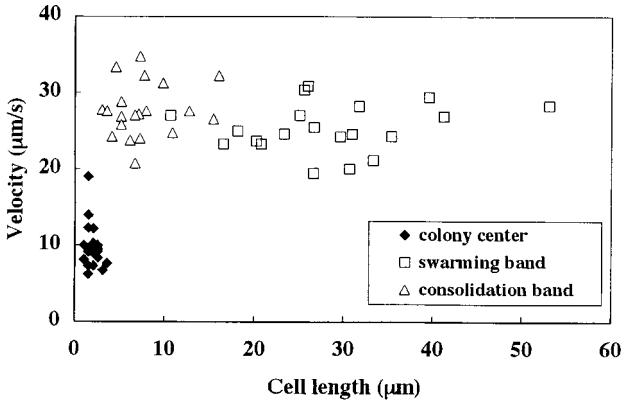

FIG. 3. Swimming activity of cells from representative areas (the center of the colony, swarming band, and consolidation band) of a swarming colony. The cell length and swimming velocity (in 2% PVP solution) of each cell are shown.

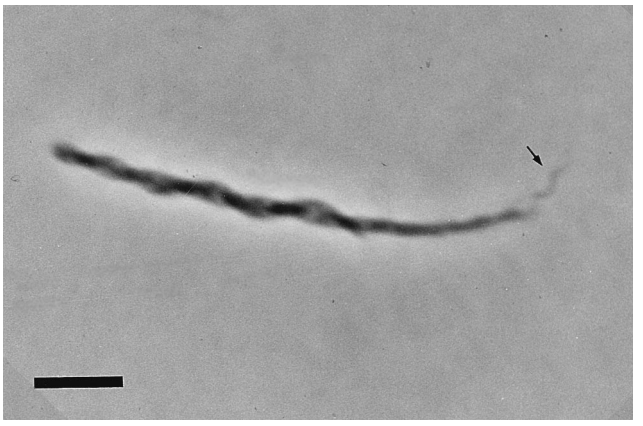

FIG. 5. Swimming swarmer cell with a bundle of rotating flagella. Cells taken from the consolidation area were suspended in 5% PVP. Swarmer cells were swimming straight and independently without making an interactive cell group. Some cells made a folded helix form, as shown here. To visualize flagella in a photograph, a cell with slowly rotating flagella (arrowhead) was photographed with Fuji PROVIA (ASA 1600) film. Bar, 5 μm.

becoming rough due to a shortage of space-filling bacteria. During the early consolidation phase, cells which were distributed from the front to a middle zone of the ring showed no mobility (Fig. 6C). However, cells at the inner half of the ring (Fig. 6D) and the previous terrace (Fig. 6E) were exerting chaotic translocation. It is noteworthy that the terrace area was full of nontranslocating vegetative cells in the previous consolidation phase. Therefore, a swarming colony of *P. mirabilis* seems to have a defined time schedule for reappearance of mobile cells in the most recent terrace.

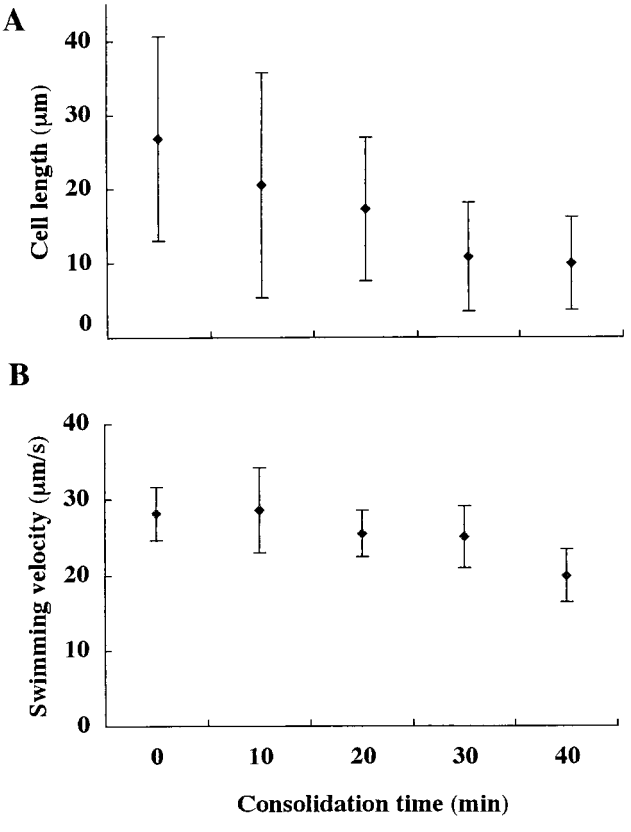

FIG. 4. Swimming activity of cells entering into the consolidation phase. The length (A) and swimming velocity (B) of cells taken at the indicated time from the front area, which was full of nontranslocating cells, are shown. Cells were suspended in 2% PVP solution. Vertical error bars denote SD (n = 20).

In the late phase of consolidation, cells in the inner-half low-cell-density zone and in the previous terrace area ceased moving gradually and never resumed mobile activity. The outer half of the ring changed to a new terrace by cell multiplication. Cells at the new terrace showed no in situ mobility. Thus, mobile cells were disappearing from the whole colony at this end stage. The reappearance of actively translocating cells, however, took place suddenly. Rafts of translocating cells spurred synchronously around the outermost end of the newly formed terrace (Fig. 6F). Since the terrace appeared as a composed cell mass at that time, translocating cells seemed to appear from underneath the terrace facing the agar medium. Thereafter, the new terrace gradually became a mass of mobile cells. From the mass of mobile cells at the newly formed terrace, successive internal waves (14, 22) moving to the swarming front (only in an outward direction, not in an inward direction) were observed as the advancing edges of increasingly thicker populations of swarmer cells by macroscopic time-lapse video recording. Such internal waves disappeared when the consolidation phase began (data not shown).

Swirling stream patterns generated by grouping cells were predominant, as shown in Fig. 6D. Thus, inside a swarming colony, the cells were not translocating independently. Similar to cells at the swarming front, inner cells were exerting collective behavior by forming transient groups in a crowded cell population.

Modification of the ongoing swarming time schedule. The time schedule of *P. mirabilis* swarming-consolidation has been reported to be quite robust (22). To examine the background of such bacterial temporal behavior, we looked for effective ways to modify the bacterial schedule. Two methods were found to be effective in modification of the ongoing swarming schedule. First, separation of the migration front from the hind area of the swarming colony by cutting with a knife was effective for premature cessation of the swarming phase (Fig. 7). Second, random mixing of a colonial cell population to disrupt the characteristic cell distribution resulted in continuous swarming by canceling the scheduled consolidation period (Fig. 7). Microscopically cells in the mixed area were densely and uniformly distributed, and from the outermost margin of

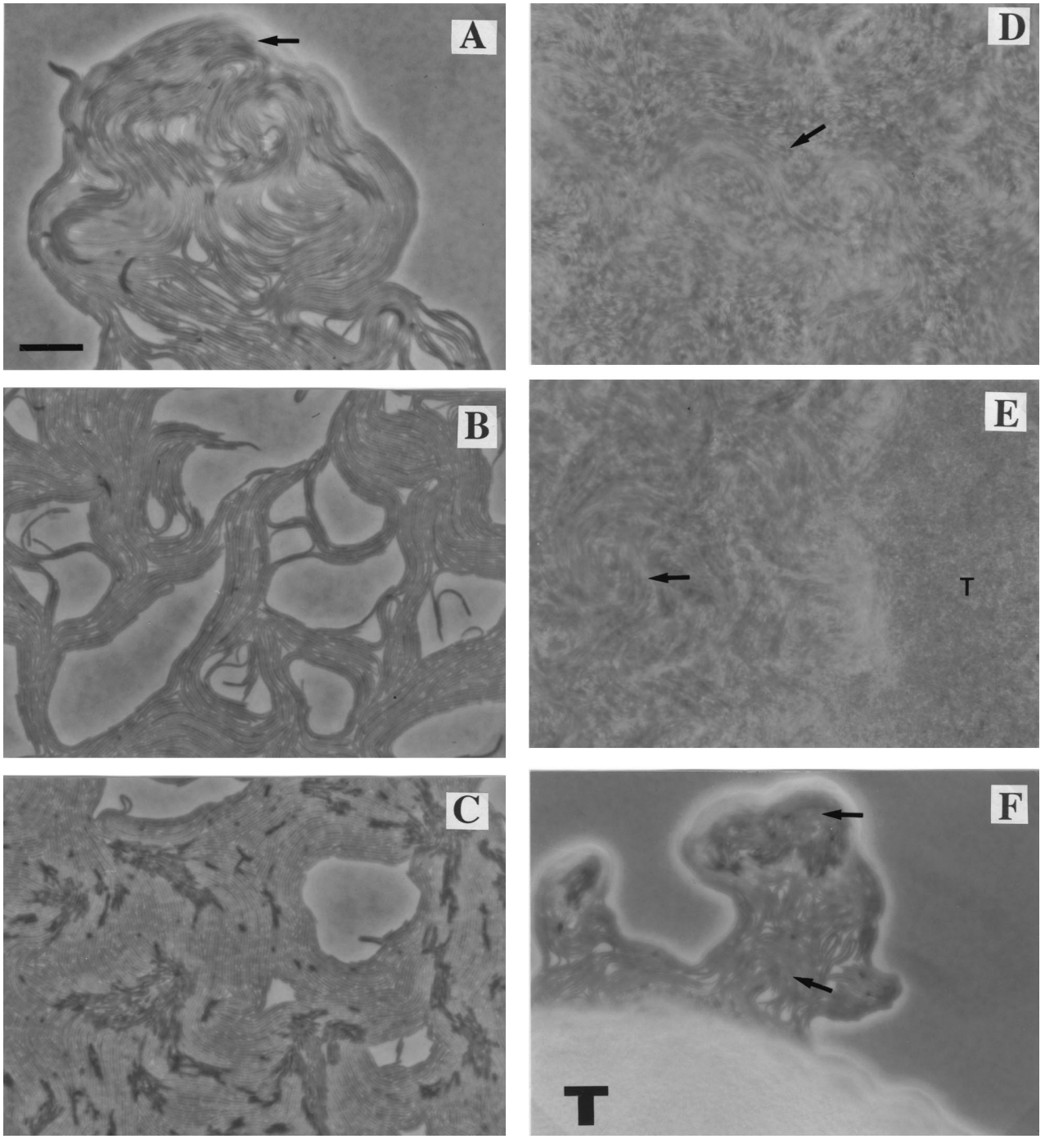

FIG. 6. In situ mobile activities of cells at the front of and inside a *P. mirabilis* colony. A front area (A) and an area just behind the front (B) at the beginning of consolidation, a 1-mm inside front area (C), a backward area near to the latest terrace (D), and the latest terrace area (E) at the early consolidation phase, and a margin of a newly formed terrace (F) at the beginning of swarming were directly examined under a phase-contrast microscope. All micrographs were taken at a 1-s exposure to discriminate moving cells (blurred images as partly indicated by arrows) from nonmoving cells. T, silent cell mass forming a terrace. Bar, 20 μm.

the mixed area, elongated cells were swarming beyond the expected consolidation line. In this experiment, the timing of cell mixing was important. Mixing just before the end of the swarming phase was most effective. Thus, the time schedule of *P. mirabilis* swarming behavior was modifiable by timely disruption of the structured cell population of the colony.

Replica-printing of a structured cell population. To confirm the role of the structured cell population in the scheduled swarming, a sectorial part of a swarming colony was excised and immediately replica-printed onto fresh agar medium. The printed cell population demonstrated swarming behavior depending on the time of excision. That is, replica-printing of a

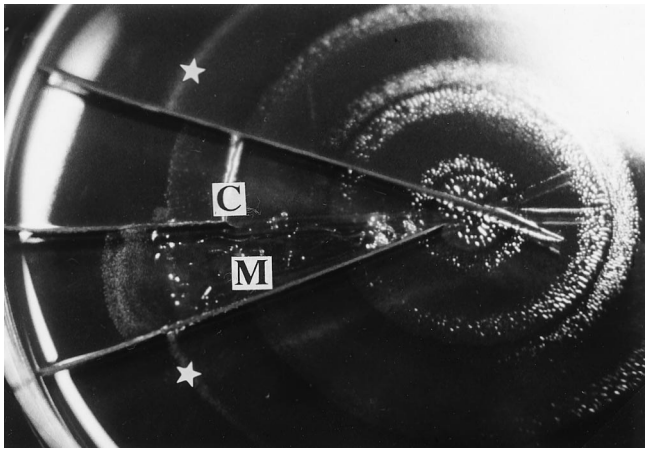

FIG. 7. Modification of the swarming schedule by disorganization of the structured cell population. Upper sector, early ending of swarming phase after separation (C indicates the cut line) from the latest terrace. Lower sector, extended migration canceling the scheduled consolidation period after random mixing (marked with M) of the sectorial cell population. The stars indicate the growing edge of part of the untreated colony. The photograph was taken 200 min after cutting and 60 min after mixing.

sectorial cell population in the swarming phase resulted in successive migration from the front area (Fig. 8A), consistent with previous reports (26, 30) which stated that swarmer cells taken from the swarming zone demonstrated migration activity immediately after transfer onto agar medium. Migration from the lateral margin of the printed cell population demonstrated that translocating cells are able to migrate in all directions. In contrast, the cell population at the consolidation front remained in the consolidation state (Fig. 8B) and started migration from the front margin with a delay (17.8 ± 8.6 min; n = 6) compared to the starting time of migration of a mother colony. It is noteworthy that cell migration was observed at first from the lateral cut line of the previous terrace area (Fig. 8B) ahead of swarming from the consolidation front. This finding is consistent with the presence of in situ mobile cells in the most recent terrace area (Fig. 6D and E). Since swarming from all cut edges of the printed cell population began later (2 to 5 h after printing), swarming from the printed original front was examined only following two migration starting times. The initial delay was not adjusted and carried over to the next cycle. Thus, the swarming schedule was shown to be transferable by replica-printing of the structured cell population.

These results of replica-printing indicated that nutrient depletion, waste product accumulation, or signal substance diffusion in an agar substrate is not a determinative factor in the achievement of periodic swarming.

When the second consolidation phase began (before terrace formation), a newly formed ring band was separated from the most recent terrace by using a knife, excised as a trapezoidal block (the shorter edge is the inner side), and replica-printed onto fresh agar medium. Cell migration started immediately around the shorter edge of a transprint, i.e., without the second consolidation which was on the schedule of the colony, whereas swarming from the printed outer edge started at the end of the second consolidation period. Since a concentric-ring pattern formed from the shorter edge was kept clear, migration that started later from the longer edge did not seem to progress inward and disturb the preformed pattern. It is noteworthy that characteristic microcolonies which may be the result of nonuniform focal multiplication of the bacteria in the outer part of the ring appeared at each terrace and not at the shorter edge of the printed cell population (Fig. 9). Thus, in a newly formed ring band, the roles of the cells have already been determined. Cells in the front area will eventually multiply to generate microcolonies later, and cells in the rear area will continue to migrate for a while.

Effects of azimuthal and radial cutting on a colony. In Fig. 9, swarming from the replica-printed consolidation front progressed on a regular time schedule. This was further confirmed by replica-printing of a thin cell film at the narrow front area (2 by 8 mm) of the ring onto fresh agar medium (data not shown). To initiate the next scheduled swarming, cells localized only at the consolidation front seemed to be sufficient. Then a thin cell film at the narrow front area (2 mm wide) was simply separated from the whole cell population by cutting through the cell

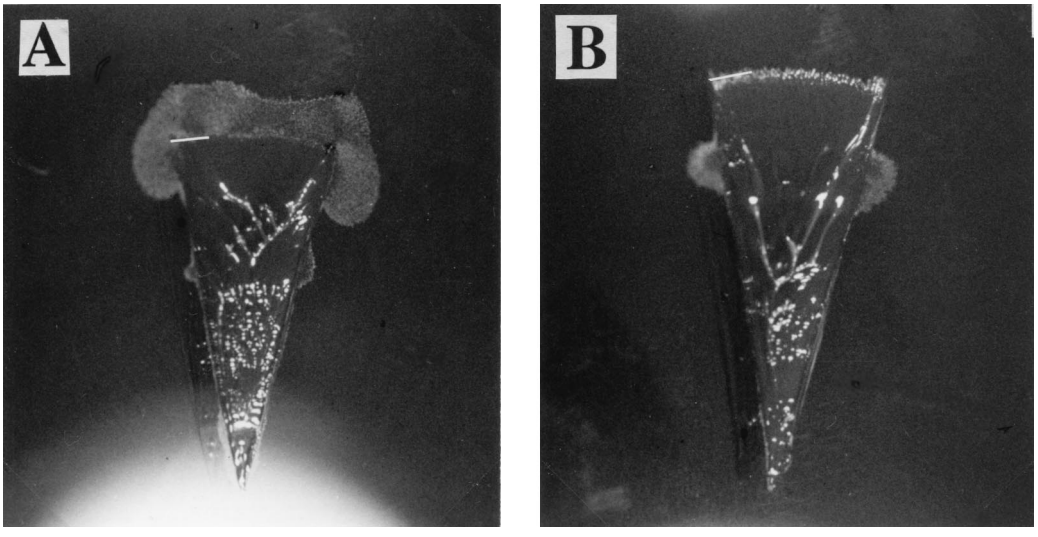

FIG. 8. Behavior of a replica-printed cell population. The sectorial part of a colony at the early swarming phase (A) and the early consolidation phase (B) was replica-printed onto fresh agar medium. A thin cell population is migrating from the printed swarming front, lateral cut ends near the front, and lateral cut ends near the latest terrace. There is no cell migration from the consolidation front at this stage. Photographs were taken 60 min after replica-printing. Short lines indicate the original front of the printed cell population.

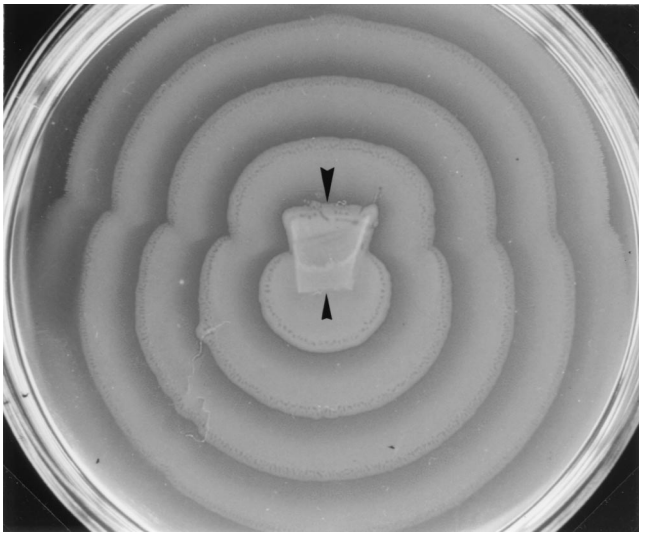

FIG. 9. Anisotropic swarming from a printed ring band area. At the early consolidation phase, a trapezoidal area with a newly formed ring band was excised and replica-printed onto fresh agar medium. Cell migration from the shorter edge (small arrowhead, inner boundary of the ring band) began immediately. Cell migration from the longer edge (large arrowhead, consolidation front of the band) began at the expected consolidation-ending time. Rings differing in phases developed without entrainment. A replica-printed agar plate was incubated for 23 h. Formation of terraces with microcolonies (lines of dark dots along the terrace edge) is recognizable, except for the shorter edge of the printed cell population.

population and underlying agar medium with a surgical knife. For this experiment, radial cutting of the agar plate was also carried out to eliminate effects from the surrounding lateral areas. After incubation, the isolated cell film grew as a terrace and became the starting site of swarming with the same periodic cycle as the control sector (Fig. 10A, a and b, Fig. 10B). In addition, it is noteworthy that swarming from smaller sectors (a and b) progressed sooner than swarming from the semicircular part (c) of the colony. Divided swarming colonies in more than 16 plates consistently showed the same tendency. Cells in these small isolated sectors seemed to migrate with a higher velocity after separation from the whole cell population but seemed to keep the same timing of onsets of swarming and consolidation phases.

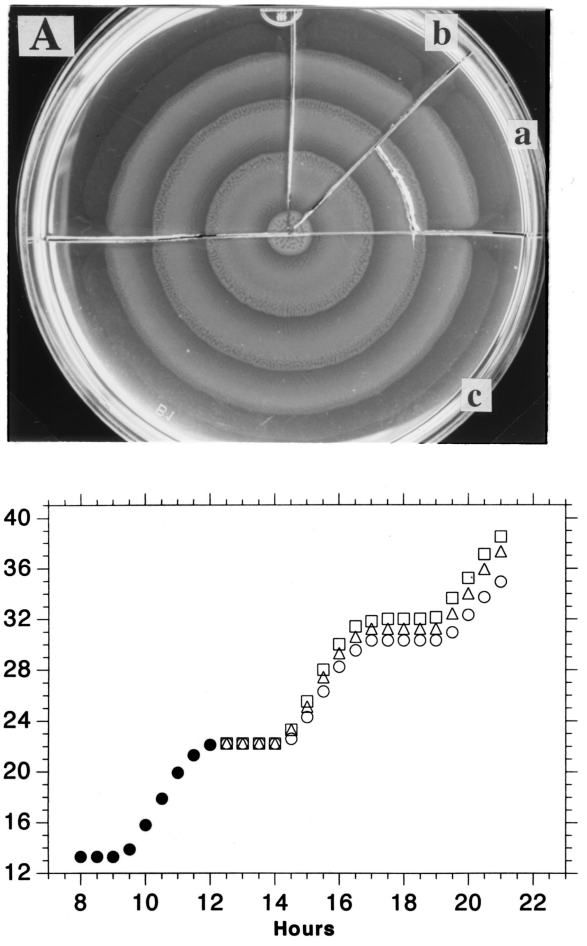

FIG. 10. Effects of azimuthal and radial cuts of a swarming colony. At the beginning stage of the second consolidation, a colony was divided into four parts by radial cuts, and then a thin front cell film on one of one-eighth sectors was separated from the rear area by an azimuthal cut. (A) A divided colony. a, sector with an azimuthal cut; b, sector without an azimuthal cut; c, semicircular part of a colony. (B) Expansion time course of the divided colony edges. Each point indicates a mean of 10 experiments. SD values were less than 3.6%. Solid circle, radius before cutting; open square, radius of a; open triangle, radius of b; open circle, radius of c. Differences in radius measurement were significant between a and b ($P < 0.05$ at 16.5 and 21.0 h postinoculation) and b and c ($P < 0.01$ at 16.5 and 21.0 h postinoculation).

DISCUSSION

Recently, the study of structured bacterial populations has focused on the unique life forms developed by intercellular cross-talking and collaboration (7, 10, 15, 23, 31). Since the cell population of *P. mirabilis* develops a concentric-ring pattern which becomes clearer during colony growth, a special arrangement may be taking place in the cell population. Therefore, precise analyses of the structured cell population, especially about the mobility of inner cells in relation to the swarming-consolidation cycle, would be helpful. Recent reports about internal waves recognized in a time-lapsed overview of a swarming colony by Rauprich et al. (22) and Itoh et al. (14) also strengthened our interest on the interior structure of a colony.

Concentric rings in a colony were easily discernible by the clear contrast between high-cell-density terrace zones and neighboring translucent zones. In such cell density appearance, the translucent zone in each inner ring is similar to the outermost swarming zone. However, cells from the translucent zone were similar to cells in neighboring terraces in their cell lengths (Fig. 1). The in situ mobility of these cells depended on the timing of examination and the location in the band. At the beginning of consolidation, cessation of translocation began from cells in the inside area near to the leading edge (Fig. 6B). Cells located in the inner area of the ring (Fig. 6D) near the previous terrace maintained in situ mobile activity for a while and remained as the lowest-cell-density area (translucent zone) in each ring throughout the following colony expansion. Immediate cell migration from this inner-ring zone in replica-printing experiments (Fig. 9) also indicated the presence of mobile cells in that area. Cancellation of the scheduled consolidation shown in Fig. 7 may be the result of disruption of such a special cell orientation.

Our primary interest was the discontinuous swarming behavior of *P. mirabilis*. Why do swarmer cells residing just behind the swarming front cease their translocation at regular, synchronized intervals? When the scheduled ending of swarming nears, the leading edge of the swarming cell film becomes

thinner and decreases its expanding velocity (14). Roughening of the contour of the swarming front is a clear sign of the end of swarming. The sparse cell distribution shown in Fig. 6A and B was consistent with naked-eye observations. Initiation of migration has been reported to be a cell density-dependent process (6, 22), and threshold cell density has been postulated in the model of Esipov and Shapiro (11). If so, did lower cell density induce cessation of translocation of swarmer cells? Such lowered cell density at the consolidation front seemed to be due to a decreased cell supply from the rear area with high cell density. On the other hand, capsular polysaccharide of *P. mirabilis* is necessary for efficient coordinated migration (12, 25). Were these immobile swarmer cells temporally losing capsular polysaccharide? The actual mechanism remains to be elucidated, but several points could be clarified. These nontranslocating swarmer cells were examined for their swimming activity. Although previous studies on the swimming activity of cells in a swarming colony have indicated a decrease of swimming velocity during consolidation periods (8), the reported velocity measurement in phosphate buffer was shown to be inappropriate for elongated swarmer cells in our studies (27). In our examination, in which we used a viscous solution (containing 2 to 5% PVP), these nontranslocating swarmer cells retained ordinary swimming activity, as shown in Fig. 4. Therefore, nontranslocating cells at the consolidation front were demonstrated to have enough energy and intact cell structures for swimming. Other possible explanations are the depletion or accumulation of unknown substances related to surface translocation. Replica-printing of a sector of a consolidating colony on fresh medium had no effect on the ongoing consolidation schedule (Fig. 8B) and indicated the absence of inhibitory substances in a *P. mirabilis* agar substrate. The absence of such extracellular substances working for determination of periodical timing has also been suggested by the absence of phase entrainment when two swarming colonies (differing in phase) collided with each other (14, 22), as also shown in Fig. 9. Since mutants defective in forming the smooth circular outline of a swarming colony have been reported (3, 5), some unknown intercellular communication system seems to be working in synchronized swarming. Whether accelerated migration by the cell population on a small sector (Fig. 10) was really a result of disrupted synchronization will be examined by further experimental studies.

As shown in Fig. 7, the periodic swarming schedule was partly changed by destruction of the internal colony structure. Separation of the swarming front area from the back area resulted in earlier cessation of swarming. This result is consistent with observations of internal waves running outward from the most recent terrace during the swarming phase. That is, a successive supply of mobile cells (appearing as internal waves) sustains continuous swarming (14). In the early consolidation phase, mobile cells were also present in the back area including the previous terrace, as shown in Fig. 8. However, no internal waves running to the consolidation front were recognized by time-lapse video recording of that phase. Then, at the beginning of consolidation, the consolidation front area was separated from the inside area, which might be a possible source of swarmer cells and signal substances, by being cut with a knife. No changes in the ongoing swarming schedule or morphology of swarming front were observed. Therefore, without a supply of cells or signal substances from the inside area, cells making the consolidation front can dedifferentiate to vegetative cells, differentiate to swarmer cells, and initiate migration on their own inherent program. The sudden appearance of active swarmer cells from the base of a newly developing terrace (Fig. 6F) was the result of swarmer cell differentiation from vegetative cells at the consolidation front area.

It is very important to make simple and consistent scenarios or models for seemingly complicated phenotypic behaviors such as the periodic colony growth of *P. mirabilis*, instead of pursuing the genetic origins only. By modelling the colony growth, one can analyze the behavior quantitatively, argue which factors among many are more relevant, and predict submacroscopic growth mechanisms. The model of Mimura et al. (submitted) was proposed to explain the periodic growth of a *B. subtilis* colony and has been known among mathematical biologists for several years without its publication. Although it is based on the reaction-diffusion equations for bacterial cells and nutrients, it shows nontrivial periodic behavior. This is due to the time lag between the migration of bacteria which consume nutrients and the diffusion of nutrients toward the nutrient-depleted (cell-populated) region. In this sense, the model is of rather general use. However, the model of Mimura et al. does not seem to be appropriate for the periodic colony growth of *P. mirabilis*, because many observations indicate that the nutrient depletion is not important in this case. On the other hand, the model of Esipov and Shapiro (11) was proposed specifically for the periodic colony growth of *P. mirabilis* and assumes that initiation and cessation of swarming are dependent on cell population density. In this sense, the periodic behavior is built into this model and is rather self-evident, although the model appears very complicated. However, this model is very promising in that many of the observations in the present work, such as the one that the changes in cell population structure alter the timing of the onset of the consolidation phase (Fig. 7), are consistent with it. It would be of interest to make the model much simpler from the viewpoint of modelling.

Modification of the structured cell population of a *P. mirabilis* colony, if done at special timings and at intracolony sites, will induce some changes in periodic swarming behavior. On the other hand, replica-printing of the cell population onto fresh medium induces no remarkable changes in the ongoing time schedule. Substances diffused in an agar substrate may play limited roles in periodic behavior of *P. mirabilis*. Studies of direct cell-to-cell communication or signal substances working within a small area will lead to new insights for elucidation of collective behavior of bacteria.

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