

Note

## Flagellum-independent Trail Formation of *Escherichia coli* on Semi-solid Agar

Hajime FUKUOKA,<sup>1,2,†</sup> Michio HOMMA,<sup>3</sup> and Shigeyuki ICHIHARA<sup>1,2</sup>

<sup>1</sup>The Agricultural High-Tech Research Center, Meijo University, Tenpaku-Ku, Nagoya 468-8502, Japan

<sup>2</sup>Laboratory of Microbiology, School of Agriculture, Meijo University, Tenpaku-Ku, Nagoya 468-8502, Japan

<sup>3</sup>Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-Ku, Nagoya 464-8602, Japan

Received November 25, 2002; Accepted April 25, 2003

***Escherichia coli* can form linear trails and move in a flagellum-independent manner on semisolid agar containing carbon sources. Trail formation seemed to correlate with the growth speed and/or carbon metabolism. Cell morphology in linear trails changed into larger cell sizes. We speculate that the flagellum-independent trail formation is a new mechanism for migration of *E. coli* cells.**

**Key words:** *Escherichia coli*; cell morphology; flagella; migration; growth

Bacteria can sense contact to their growth surface and adapt to their environment by changing their growth mode. Several bacteria can translocate on the their growth surface in an inherent manner. Surface translocation of bacteria is relevant to symbiosis and pathogenicity to their host.<sup>1–5</sup> Translocation is also important for finding nutrients or colonization sites within the host and for avoiding toxic substrates.

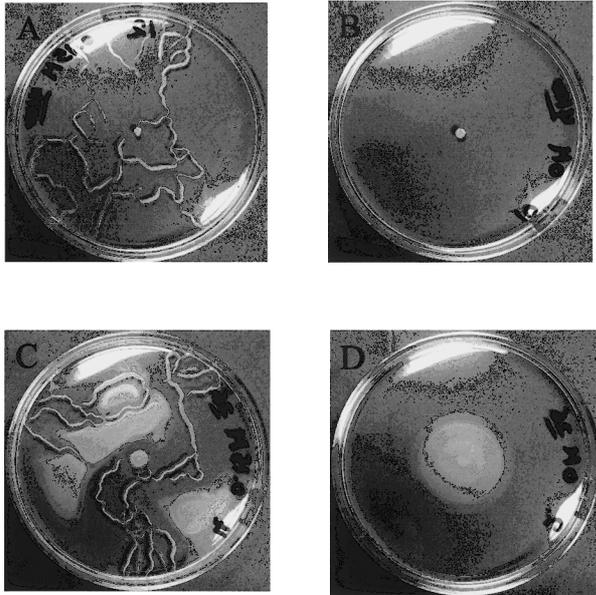
Several different types of bacterial surface translocation have been observed and classified into the following six types: (i) swimming, (ii) swarming, (iii) gliding, (iv) twitching, (v) sliding and (vi) darting.<sup>6</sup> Each of these modes of surface translocation requires specific structures such as flagella, pili, surfactants, slime, or capsules. Flagella, which contribute to swimming and swarming ability, are the best characterized locomotive organelles. On the other hand, there are many cases of flagellum-independent surface translocation. *Serratia marcescens* secretes an extracellular surfactant, called serrawettin, which enables cells to spread on a semisolid agar plate by reducing the friction between the cell and the semisolid agar surface.<sup>7,8</sup> *Mycobacterium smegmatis*, a non-flagellated Gram-positive bacterium, can also spread on semisolid agar plates.<sup>9,10</sup> Spreading of *M. smegmatis* requires glycopeptidolipid (GPL), which exists in the outermost layer of the cell wall.

In *Escherichia coli*, surface translocation has been described during cell differentiation, when the cells are hyperflagellated and elongated, similar to swarming cells.<sup>11</sup> Flagellum-independent surface translocation of *E. coli* has been reported to be an active process.<sup>12</sup> In this regard, we identified flagellum-independent surface translocation in non-flagellated strains of *E. coli*, which form linear trails of cells on soft agar containing either glucose or other carbon sources which *E. coli* can metabolize.

When the CSH26 (*ara thi*Δ(*lac-pro*))<sup>13</sup> flagellated strain was inoculated onto ×0.5LB (-NaCl) soft agar plates (0.3% agar) and incubated at 37°C, CSH26 formed a halo in the soft agar (Fig. 1D). This translocation phenotype is based on the ability of cells to swim by making use of flagella.<sup>6</sup> The strain MC4100 (*relA* Δ(*argF-lac*)U169 *deoC araD rpsL thiA ptsF flhD tonA*),<sup>14</sup> on the other hand, did not form a halo (Fig. 1B), because this strain is defective in the *flhD* gene, the master gene for flagellar gene expression. However, we did observe that MC4100 cells formed some white trails and spread on soft agar containing 150 mM glucose, despite the absence of flagella (Fig. 1A). Similar trails were observed in CSH26 cells on soft agar containing 150 mM glucose (Fig. 1C). Although CSH26 is a flagellated strain, flagella synthesis would be repressed by high concentrations of glucose. These results suggest that trail formation is a flagellum-independent mode of surface translocation.

Initially, the MC4100 and CSH26 strains grew at the inoculation position on the surface of the plate. After 3 hours, we could observe trails that began to extend from the original point and spread over the entire plate after 24 hours. The trails are probably formed by the grown cells. The MC4100 strain did not form trails in the absence of glucose, even after 48 hours. The trails extended on the soft agar plate, appearing to sink into grooves. In the early stage of

† To whom correspondence should be addressed. Tel: +81-52-789-2991; Fax: +81-52-789-3001; E-mail: 4hajime@bunshi3.bio.nagoya-u.ac.jp; Present address: Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-Ku, Nagoya 464-8602, Japan



**Fig. 1.** *E. coli* Trail Formation on Soft Agar.

*E. coli* cultures (1  $\mu$ l) were inoculated onto 0.3% soft agar plates in the presence (A, C) or absence (B, D) of 150 mM glucose and incubated at 37°C for 24 hours (A, B, C) or 8 hours (D). A, B; MC4100, the flagella-defective mutant (the *flhD* mutant). C, D; CSH26, the flagellated strain.

incubation, a transparent region appeared around the trails, but not around colonies that formed without trail formation (data not shown).

To confirm whether trail formation is due to a spontaneous mutation, cells were picked up from the trail and spotted on a soft agar plate in the presence or absence of glucose. A new trail formed on plates containing glucose, but not on plates lacking glucose (data not shown). This indicates that trail formation is not the result of a mutation, but is rather a response to environmental stimuli.

We investigated whether trail formation is strain-specific or a common phenotype among *E. coli*. We used JM103<sup>15</sup> or YK410<sup>16</sup> as well as derivative strains defective in one of the flagellar genes. JM103 formed trails on soft agar plates containing glucose. On the other hand, the YK strains, YK410 (WT), YK4130 (*flhC*), YK4131 (*flhD*), and YK4136 (*flhC*), did not form trails (data not shown).

Various environmental factors such as viscosity, growth temperature, osmolarity, and nutrients affect bacterial phenotype. We studied whether trails formed under various growth conditions. First, we investigated the agar concentration of the plate. A culture of the MC4100 strain was spotted onto 0.3%–0.8% agar containing 150 mM glucose, and incubated at 37°C. Trail formation was observed at 0.3% agar, but not when agar was higher than 0.4%. In 0.4% agar in the absence of glucose, the CSH26 strain was able to swarm, by flagellum-dependent translocation, although the swarm size was smaller

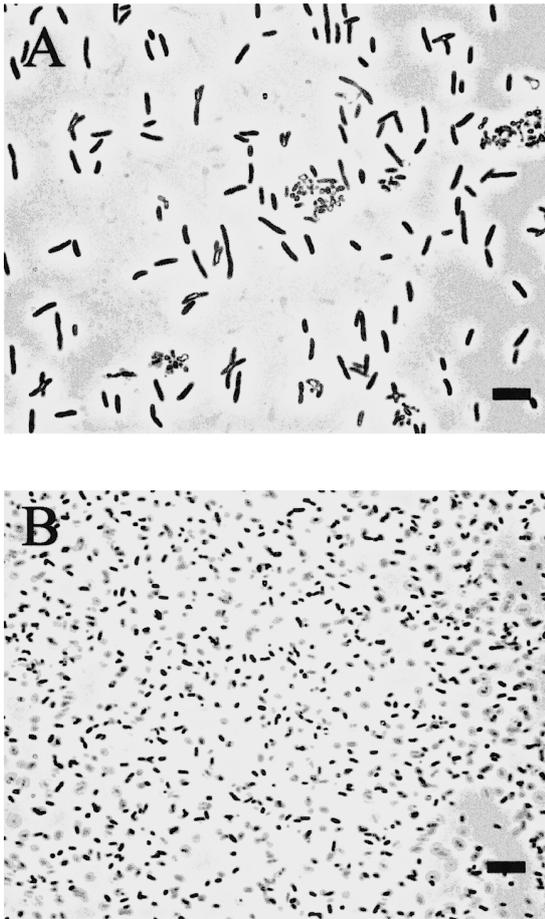
than in 0.3% agar. This result suggests that the hardness of the plate is critical for trail formation and flagellum-dependent translocation. Second, the MC4100 strain was incubated at 25°C, 30°C, and 37°C on 0.3% soft agar containing 150 mM glucose. Compared to incubation at 37°C, branching and trail length were reduced at 30°C. At 25°C, trail formation was not observed even with a longer incubation time (72 hours). This result suggests that the trail formation may correlate with growth speed, because 37°C is the optimal growth temperature for *E. coli*. Third, the glucose concentration of the soft agar plates was changed. The MC4100 strain was incubated at 37°C on 0.3% soft agar containing 150 mM, 15 mM, 5 mM, and 1.5 mM glucose. Trail formation was observed on agar containing at least 5 mM glucose, but not on agar containing 1.5 mM glucose. This indicates that the lower threshold glucose concentration for trail formation was between 5 mM and 1.5 mM. Trails were also noted to be straighter and shorter at lower glucose concentrations.

We also tested the involvement of cAMP and the cAMP receptor protein (CRP) on the trail formation, using the TB100 (WT), TB102 (*crp*), and TB105 (*cyd*) strains (provided by Dr. H. Aiba).<sup>17</sup> Although trail shape appeared to be different from MC4100, trail formation was observed in all the strains on plates containing glucose (data not shown). Thus, cAMP and cAMP receptor protein are not likely to be required for trail formation.

We investigated whether trail formation was induced by carbon sources other than glucose. Galactose, maltose, trehalose, pyruvate, or glycerol induced trail formation while fructose, lactose, sucrose, and cellobiose did not. *E. coli* cannot metabolize sucrose and cellobiose. In addition, the MC4100 strain cannot metabolize lactose, due to a mutation in the *lac* gene. When MC4100 was grown in a medium containing fructose as the sole carbon source, the growth rate was very slow compared to growth in media supplemented with glucose at the same concentration. These results suggest that trail formation correlates with the growth speed and/or metabolic activity of *E. coli*.

*E. coli* produces acetic acid when grown in medium containing high concentrations of glucose.<sup>18</sup> The pH of the medium around the cells was measured with pH test papers. In almost all media, pH was reduced to 4–5 from the original pH of 7 around the trails except in the case of pyruvate (pH 9). On the other hand, the pH of the medium around the colony that could not form trails was 7. Therefore, low pH appears to not be directly correlated with trail formation.

When bacteria swarm on soft agar, cells have been observed to develop excessive numbers of flagella and to elongate their cell bodies.<sup>12</sup> We investigated the morphology of cells that form trails (Fig. 2). Cells



**Fig. 2.** Microscopic Analysis of MC4100 Cells in Trails.

Cells from the plates in Fig. 1-A and Fig. 1-B, from trails in 0.3% soft agar plates containing 150 mM glucose (A), and from colonies in 0.3% soft agar plates containing no glucose (B), respectively, were picked up by pipette and observed under a microscope (OLYMPUS BX60). Photographs were taken with a digital camera (OLYMPUS DP10). Bar, 10  $\mu$ m.

forming trails (on agar containing 150 mM glucose) appeared larger than cells forming colonies (on agar containing no glucose): about 10  $\mu$ m long and 2  $\mu$ m wide (maximal size) for trails and about 2  $\mu$ m long and 1  $\mu$ m wide for colonies. We observed that cells were enlarged anywhere in trails and in the original inoculation point. Cells grown in liquid media with or without 150 mM glucose have the same morphology. There is a possibility that a change in cell morphology contributes to trail formation. Little is known about the mechanism and regulation of this putative morphological transformation.

The *E. coli* MC4100, non-flagellated strain formed trails on soft agar containing glucose. The trail formation is probably the movement of whole cells and we do not know about the movement of individual cells. In other words, the trail formation may be the expansion of the cell population by cell division. Trail formation seemed to correlate with the growth speed in MC4100 because it was greatest at 37°C, the

optimal growth temperature for *E. coli* and, in addition, trail formation was only observed on agar containing carbon sources that can be metabolized by *E. coli*. Flagellum-independent migration of *E. coli* has been reported, but the described migration profiles are very different from one another.<sup>12)</sup> Currently, we do not know whether these phenotypes are due to a similar mode of migration. Many flagella-independent migration phenomena have been observed in various species. In flagellum-independent surface translocation, secreted surfactants or slime materials are thought to be essential for reducing friction between the attached surface and the cell surface.<sup>8,19)</sup> We observed a transparent region around the trails at an early stage of incubation. From this result, there is a possibility that *E. coli* also secretes materials that might assist in trail formation.

In flagellum-dependent translocation of *Proteus mirabilis* and *Salmonella typhimurium*, mutants defective in the biosynthesis of polysaccharide (*Proteus*) or lipopolysaccharide (*Salmonella*) cannot swarm.<sup>19-21)</sup> Similarly, lipopolysaccharide has been shown to be important for flagella-independent surface translocation of *Vibrio cholerae*,<sup>12)</sup> and glycopeptide lipids (GPL), a component of mycobacteria cell walls, are required for sliding.<sup>9,10)</sup> The biosynthesis of lipopolysaccharide is known to be affected by environmental signals.<sup>22)</sup> Thus, the structure and/or the components of bacterial cell walls may be essential for surface translocation in both flagellum-dependent and independent translocation.

During trail formation, changes in cell morphology were observed. These phenotypes might be induced by carbon catabolism on the soft agar. We have shown that *E. coli* can migrate by trail formation in an environment without the locomotive organelle, the flagella. This translocation ability can be expressed in the host and extended to a new, nutrient-rich environment. To further elucidate the mechanism of trail formation or flagellum independent migration, we are presently trying to isolate transposon mutants defective in trail formation.

## Acknowledgments

We thank Dr. Kutsukake for providing the strain YK410, and Dr. Aiba for providing for the starin TB100, TB102, and TB105. This study was supported by the Agricultural High-Tech Research Center, Meijo University, under the "Environmental Control through the Functions of Microorganisms" project.

## References

- 1) Watnick, P., and Kolter, R., Biofilm, city of microbes. *J. Bacteriol.*, **182**, 2675-2679 (2000).
- 2) Allison, C., Lai, H. C., and Hughes, C., Co-ordinate expression of virulence genes during swarm-cell

- differentiation and population migration of *Proteus mirabilis*. *Mol. Microbiol.*, **6**, 1583–1591 (1992).
- 3) Ottemann, K. M., and Miller, J. F., Roles for motility in bacterial-host interactions. *Mol. Microbiol.*, **24**, 1109–1117 (1997).
  - 4) Rozalski, A., Sidorczyk, Z., and Kotelko, K., Potential virulence factors of *Proteus bacilli*. *Microbiol. Mol. Biol. Rev.*, **61**, 65–89 (1997).
  - 5) Walker, K. E., Moghaddame-Jafari, S., Lockett, C. V., Johnson, D., and Belas, R., ZapA, the IgA-degrading metalloprotease of *Proteus mirabilis*, is a virulence factor expressed specifically in swarmer cells. *Mol. Microbiol.*, **32**, 825–836 (1999).
  - 6) Henrichsen, J., Bacterial surface translocation: a survey and a classification. *Bacteriol. Rev.*, **36**, 478–503 (1972).
  - 7) Matsuyama, T., Bhasin, A., and Harshey, R. M., Mutational analysis of flagellum-independent surface spreading of *Serratia marcescens* 274 on a low-agar medium. *J. Bacteriol.*, **177**, 987–991 (1995).
  - 8) Matsuyama, T., Kaneda, K., Nakagawa, Y., Isa, K., Hara-Hotta, H., and Yano, I., A novel extracellular cyclic lipopeptide which promotes flagellum-dependent and -independent spreading growth of *Serratia marcescens*. *J. Bacteriol.*, **174**, 1769–1776 (1992).
  - 9) Martínez, A., Torello, S., and Kolter, R., Sliding motility in mycobacteria. *J. Bacteriol.*, **181**, 7331–7338 (1999).
  - 10) Recht, J., Martínez, A., Torello, S., and Kolter, R., Genetic analysis of sliding motility in *Mycobacterium smegmatis*. *J. Bacteriol.*, **182**, 4348–4351 (2000).
  - 11) Harshey, R. M., and Matsuyama, T., Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: Surface-induced differentiation into hyperflagellate swarmer cells. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 8631–8635 (1994).
  - 12) Brown, I. I., and Häse, C. C., Flagellum-independent surface migration of *Vibrio cholerae* and *Escherichia coli*. *J. Bacteriol.*, **183**, 3784–3790 (2001).
  - 13) Miller, V. L., and Mekalanos, J. J., A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires toxR. *J. Bacteriol.*, **170**, 2575–2583 (1988).
  - 14) Schweizer, H., and Boos, W., Transfer of the delta (argF-lac)U169 mutation between *Escherichia coli* strains by selection for a closely linked Tn10 insertion. *Mol. Gen. Genet.*, **192**, 293–294 (1983).
  - 15) Bolivar, F., and Backman, K., Plasmids of *Escherichia coli* as cloning vectors. *Methods Enzymol.*, **68**, 245–267 (1979).
  - 16) Komeda, Y., Kutsukake, K., and Iino, T., Definition of additional flagellar genes in *Escherichia coli* K12. *Genetics*, **94**, 277–290 (1980).
  - 17) Aiba, H., Hanamura, A., and Tobe, T., Semisynthetic promoters activated by cyclic AMP receptor protein of *Escherichia coli*. *Gene*, **85**, 91–97 (1989).
  - 18) Hosono, K., Kakuda, H., and Ichihara, S., Decreasing accumulation of acetate in a rich medium by *Escherichia coli* on introduction of genes on a multicopy plasmid. *Biosci. Biotechnol. Biochem.*, **59**, 256–261 (1995).
  - 19) Toguchi, A., Siano, M., Burkart, M., and Harshey, R. M., Genetics of swarming motility in *Salmonella enterica* serovar *typhimurium*: critical role for lipopolysaccharide. *J. Bacteriol.*, **182**, 6308–6321 (2000).
  - 20) Gygi, D., Rahman, M. M., Lai, H.-C., Carlson, R., Guard-Petter, J., and Hughes, C., A cell-surface polysaccharide that facilitates rapid population migration by differentiated swarm cells of *Proteus mirabilis*. *Mol. Microbiol.*, **15**, 1167–1175 (1995).
  - 21) Rahman, M. M., Guard-Petter, J., Asokan, K., Hughes, C., and Carlson, R. W., The structure of the colony migration factor from pathogenic *Proteus mirabilis*. A capsular polysaccharide that facilitates swarming. *J. Biol. Chem.*, **274**, 22993–22998 (1999).
  - 22) Ernst, R. K., Guina, T., and Miller, S. I., *Salmonella typhimurium* outer membrane remodeling: role in resistance to host innate immunity. *Microbes. Infect.*, **3**, 1327–1334 (2001).