Dependence of swarming in *Escherichia coli* K-12 on spermidine and the spermidine importer

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Abstract

In a previous work, it was observed that the swarming of polyamine-deficient *Proteus mirabilis* (*speB::sm*) was severely inhibited on Luria–Bertani (LB) swarming plates (LBSw) (LB, 0.5% glucose, 0.5% agar), and it was clarified that extracellular putrescine was important as a signaling molecule for the induction of swarming in *P. mirabilis*. However, a polyamine-deficient strain (delta-*speAB* delta-*speC*) of *Escherichia coli* swarmed as well as the parental strain on LBSw plates. We report that the swarming phenotype of a polyamine-deficient *E. coli* strain is dependent on spermidine and PotABCD, a spermidine importer.

Introduction

Polyamines (putrescine, spermidine, and spermine) are aliphatic amines that are widely distributed in bacteria, plants, and animals (Tabor & Tabor, 1984). They play important roles in cell proliferation, tissue growth, and differentiation (Tabor & Tabor, 1984). In bacteria, polyamines bind to DNA to stabilize its structure (Tabor & Tabor, 1985), and they modulate the translation of several genes by binding to their mRNA (Igarashi & Kashiwagi, 2006). Polyamines are synthesized and exported by bacteria, and they are present in the environment where bacteria live. For example, in the intestinal tract of animals, the concentration of polyamines originating from intestinal bacteria can reach micromolar levels (Noack et al., 1998). During the growth of *Escherichia coli* in broth, putrescine is exported by *E. coli* in a manner proportional to cell density (Schiller et al., 2000).

*Escherichia coli* synthesizes two polyamines, putrescine and spermidine. There are two pathways by which putrescine is synthesized. One is the ornithine decarboxylation pathway catalyzed by SpeC, and the other is the pathway in which putrescine is synthesized from arginine by a two-step reaction: decarboxylation of arginine to yield agmatine, which is catalyzed by SpeA, and hydrolysis of agmatine to produce putrescine and urea, which is catalyzed by SpeB. Spermidine is synthesized from putrescine by SpeE by the addition of the aminopropyl moiety derived from decarboxylated S-adenosylmethionine. The decarboxylation of S-adenosylmethionine is catalyzed by SpeD.

The transport systems for polyamines have been well studied in *E. coli*. PotABCD (Pistocchi et al., 1993) is a spermidine importer that imports putrescine too, and it was previously reported (Higashi et al., 2008) that MdtJI exports spermidine. As described above, *E. coli* has both import and export systems for polyamines; therefore, it is thought that *E. coli* exports or imports polyamines according to need by altering the expression of the genes encoding the polyamine transporters. However, the function of extracellular polyamines is not well known.

Swarming is a bacterial process that allows cells to move in a coordinated manner and expand the population to new locations. The process of swarming is distinct from swimming in that swimming is a multicellular process that occurs on solid surfaces and requires the differentiation of vegetative cells into a specialized cell type called a swarmer cell (Rather, 2005). In a previous work, it was demonstrated that...
in *Proteus mirabilis*, a gram-negative bacterium and a common urinary tract pathogen in humans (Mobley et al., 1996; Rozalski et al., 1997), putrescine was used as an inducer of differentiation into swarm cells (Sturgill & Rather, 2004; Rather, 2005). In the study (Sturgill & Rather, 2004), it was observed that putrescine rescued the induction of swarming in a putrescine-deficient mutant of *P. mirabilis* (*speB::sm*), but spermidine did not. Because polyamines are highly hydrophilic, the import of polyamines through the hydrophobic membrane into the cell requires transporters; however, studies focusing on polyamine transporters as importers of the signaling molecule still need to be conducted. In this study, we report that in *E. coli*, spermidine as well as putrescine is important for swarming of the polyamine-deficient mutant (*ΔspeAB::FRT ΔspeC::FRT*), and that the induction of swarming is dependent on the spermidine importer PotABCD.

**Materials and methods**

**Media and culture conditions**

The Luria–Bertani (LB) (BD, Franklin Lakes, NJ) swarming plates (LBSw) used in this study comprise LB supplemented with 0.5% glucose and 0.5% agar (Eiken, Tokyo, Japan). The M9 (Miller, 1992) swarming plates (M9Sw) contained M9 supplemented with 0.5% glucose, instead of the 0.2% glucose described in Miller (1992), and 0.5% agar (Eiken). The water content of the plates was equalized in each experiment by incubation of the autoclaved medium in a waterbath of 50 °C for 30 min; the medium was then poured into a Petri dish (Ø = 10 cm) and dried with the lid open on a clean bench for 30 min. The strains were precultured in 5 mL LB in a tube (Ø = 18 mm) at 37 °C overnight. In the experiments using LBSw, the overnight culture was diluted to an A600 nm of 0.4 in LB, and in the experiments using M9Sw, the overnight culture was washed once with M9 buffer (M9 medium without glucose) and diluted to an A600 nm of 0.4 in M9 medium. Then, 3 μL of the diluted culture was dropped onto the center of the plate. The plate was sealed with a paraffin film and incubated at 37 °C. The compositions of the LBSw liquid medium (LBSwL) and M9Sw liquid medium (M9SwL) are LB+0.5% glucose and M9+0.5% glucose, respectively.

**Strain and plasmid construction**

The 4130-bp fragments, including the entire region of *potABCD*, the 58-bp region downstream of *potABCD*, and the 232-bp region upstream of *potABCD* was amplified by PCR. KOD-plus-DNA polymerase (Toyobo, Osaka, Japan), the genome of SH639 (Table 1) that was used as a template, and the primers ‘potABCD up SacI’ and ‘potABCD down SmaI’ were used for the PCR, following the manufacturer’s instructions. The primers were designed so as to add Sacl and Sma1 restriction sites to the 5'-end of the amplified region. The amplified fragment was ligated to pMW119 digested with Sacl and SmaI and blunt ended with a DNA blunting kit (Takara, Otsu, Japan). The obtained fragment was cleaved by HpaI, and this plasmid was designated pBelobac11.

Table 1. Strains, plasmids, or oligonucleotides used in this study

<table>
<thead>
<tr>
<th>Strains</th>
<th>Characteristics or sequences</th>
<th>Sources or references</th>
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<tbody>
<tr>
<td>SH639</td>
<td>F′ Δagg-t</td>
<td>Suzuki et al. (1987, 2005)</td>
</tr>
<tr>
<td>SK479</td>
<td>SH639 but ΔspeAB::FRT ΔspeC::FRT</td>
<td>This study</td>
</tr>
<tr>
<td>YT16</td>
<td>SH639 but ΔspeAB::FRT ΔspeC::FRT</td>
<td>This study</td>
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<tr>
<td>Plasmids</td>
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<tr>
<td>pBelobac11</td>
<td>Mini-F replicon cat⁺</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>pBelobac11- potABCD⁺ B⁺ C⁺ D⁺</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMW119</td>
<td>pSC101 replicon bla⁺ rep⁺ lacZ⁺</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>pMW119- potABCD⁺ B⁺ C⁺ D⁺</td>
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**Results**

The swarming motility of the putrescine-deficient strain of *E. coli* is similar to that of the parental strain

As described previously, on the LBSw, the swarming ability of the *speB::sm* mutant of *P. mirabilis* was inhibited compared with that of the wild-type strain (Sturgill & Rather, 2004). However, the putrescine-deficient *E. coli* mutant YT16 (*ΔspeAB::FRT ΔspeC::FRT*) containing pBelobac11 swarmed as well as strain SH639 (*speA⁺ B⁺ speC⁺*) containing pBelobac11 did (Fig. 1).
The spermidine importer PotABCD is important for the swarming of the putrescine-deficient strain on the LBSw

Unlike in the case of P. mirabilis, in E. coli, the ability to synthesize putrescine was not involved in the swarming ability on LBSw. This result suggested that spermidine present in the medium induced the swarming of the putrescine-deficient strain YT16 containing pBelobac11 because the LB medium contains c. 100 μM of spermidine. Therefore, to examine the role of the spermidine importer encoded by potABCD, we constructed a potABCD deletion mutant in the speABC mutant background (Fig. 2). As shown in Fig. 2a, both the strain YT16 (ΔspeAB ΔspeC potAΔ B ΔC ΔDΔ) containing pBelobac11 and the strain SK479 (ΔspeAB ΔspeC ΔpotABCD) complemented with pBelobac11-potAΔ B ΔC ΔDΔ swarmed well on LBSw plate. In contrast, the swarming of SK479 (ΔspeAB ΔspeC ΔpotABCD) containing pBelobac11 was severely inhibited (Fig. 2a). We determined the influence of cell growth on the extent of swarming by growing three strains in LBSwL (Fig. 2b) and measuring the A_{600 nm} values. Growth of SK479 containing pBelobac11 was partially inhibited (Fig. 2b) and its swarming activity was severely inhibited, compared with YT16 containing pBelobac11 and SK479 containing pBelobac11-potAΔ B ΔC ΔDΔ+, which showed good swarming. This result suggested that although the growth inhibition partially inhibited the swarming activity, other factors also existed.

PotABCD and spermidine are essential for swarming on M9Sw minimal medium

To clearly demonstrate that spermidine and PotABCD are important for the swarming of E. coli, we used M9Sw minimal medium. YT16 containing pBelobac11, SK479 containing pBelobac11, and SK479 containing pBelobac11-potAΔ B ΔC ΔDΔ did not show swarming activity on M9Sw minimal medium. However, YT16 containing pBelobac11 (carrying potAΔ B ΔC ΔDΔ in the genome) and SK479 containing pBelobac11-potAΔ B ΔC ΔDΔ showed good swarming on M9Sw supplemented with 1 μM spermidine. In contrast, SK479 (ΔpotABCD) containing pBelobac11 did not show swarming activity on the same plates (Fig. 3a). In order to determine the influence of growth on the extent of swarming, three strains were grown in M9SwL supplemented with or without 1 μM spermidine, and the A_{600 nm} values were measured (Fig. 3b). In M9SwL supplemented with 1 μM spermidine, the growth of YT16 containing pBelobac11 and SK479 containing pBelobac11-potAΔ B ΔC ΔDΔ+, which swarmed well on the semisolid medium, was better than the growth of SK479 containing pBelobac11. However, the inhibition of swarming was not mainly due to the growth inhibition, because in M9SwL supplemented with 1 μM spermidine, SK479 containing pBelobac11, which did not swarm, showed 80% of the growth observed in YT16.
containing pBelobac11 and SK479 containing pBelobac11-potA\(^\Delta\)B\(^\Delta\)C\(^\Delta\)D\(^\Delta\), which swarmed well. In addition, SK479 containing pBelobac11 showed the best growth among the three strains in M9SwL and the growth of SK479 containing pBelobac11 in M9SwL (closed triangles) was comparable to that of YT16 containing pBelobac11 (open squares) and SK479 containing pBelobac11-potA\(^\Delta\)B\(^\Delta\)C\(^\Delta\)D\(^\Delta\) (open diamonds) in M9SwL supplemented with 1 μM spermidine at 45 h after inoculation. Therefore, it is thought that growth is not related directly to the swarming activity, although the growth speed in the initial growth phase may partially affect the activity of swarming.

Discussion

In the previous study on the swarming of P. mirabilis (Sturgill & Rather, 2004), the swarming of the putrescine-deficient mutant (speB::sm) was significantly inhibited on the LBSw. In this study, however, the ΔspeAB ΔspeC mutant of E. coli swarmed as well as the parental strain on the LBSw (Fig. 1). Furthermore, it was clarified that the swarming of the ΔspeAB ΔspeC E. coli mutant was induced by spermidine imported by the spermidine importer PotABCD (Figs 2 and 3). In the previous study on P. mirabilis (Sturgill & Rather, 2004), putrescine was identified as an extracellular signaling molecule that induced swarming, but the importer responsible for the uptake of the signaling molecule was not identified. In this study, spermidine was identified as a molecule that induces swarming; moreover, PotABCD was identified as a spermidine importer that is indispensable for the induction of swarming. In E. coli, polyamine oxidase activity, by which putrescine can be formed from N\(^1\)-acetyl spermidine yielded by the acetylation reaction of catalyzed spermidine by SpeG, has not been detected (Ignatenko et al., 1996). Therefore, it is thought that the induction of swarming on media containing spermidine is not the consequence of the conversion of spermidine to putrescine but the direct effect of spermidine itself. In the previous study on P. mirabilis (Sturgill & Rather, 2004), the polyamine-deficient mutant (speB::sm) absorbed the extracellular putrescine exported by the speB\(^\Delta\) strain adjacent cultured on the LBSw, and it showed swarming activity. This experiment clearly showed that putrescine can be physiologically characterized as an extracellular signaling molecule that is transferred from cell to cell during the induction of swarming. However, it was impossible to show that spermidine exported by E. coli induced swarming of the polyamine-deficient mutant, because the effect of spermidine is indistinguishable from the effect of putrescine on swarming; their effects are indistinguishable because spermidine is synthesized from putrescine in E. coli cells and E. coli cells have both spermidine and putrescine. However, spermidine exported from bacteria is present in the environment; for example, in the intestinal tract, several tens of micromolar

Fig. 3. PotABCD-dependent swarming induced by spermidine on the M9Sw. (a) The effect of deletion and complementation of potABCD on swarming on the M9Sw plate supplemented with or without 1 μM spermidine. YT16 containing pBelobac11, SK479 containing pBelobac11, and SK479 containing pBelobac11-potA\(^\Delta\)B\(^\Delta\)C\(^\Delta\)D\(^\Delta\) were used. At 120 h after inoculation, the plates were photographed. (b) The growth of the three strains used in (a) in M9SwL supplemented with or without 1 μM spermidine. The overnight precultures in LB of the three strains were washed once with M9 medium. Bacteria were inoculated into 100-mL M9SwL supplemented with or without 1 μM spermidine in 500-mL Erlenmeyer flasks. The A\(600\) of the culture was adjusted to 0.001. The flasks were shaken at 150 r.p.m. at 37°C. Open squares, the growth of YT16 containing pBelobac11 in M9SwL supplemented with 1 μM spermidine; open triangles, the growth of SK479 containing pBelobac11 in M9SwL supplemented with 1 μM spermidine; open diamonds, the growth of SK479 containing pBelobac11-potA\(^\Delta\)B\(^\Delta\)C\(^\Delta\)D\(^\Delta\) in M9SwL supplemented with 1 μM spermidine; closed squares, the growth of YT16 containing pBelobac11 in M9SwL; closed triangles, the growth of SK479 containing pBelobac11 in M9SwL; closed diamonds, the growth of SK479 containing pBelobac11-potA\(^\Delta\)B\(^\Delta\)C\(^\Delta\)D\(^\Delta\) in M9SwL.

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spermidine (Matsumoto & Benno, 2007) is synthesized and exported by intestinal bacteria (Noack et al., 1998). Furthermore, the spermidine exporter of *E. coli* has been identified previously (Higashi et al., 2008). All the results obtained in this study indicate that spermidine as well as putrescine plays a role in the induction of swarming as an extracellular signaling molecule, which is transferred from cell to cell in *E. coli*.

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**References**


