



# Exopolysaccharides Play a Role in the Swarming of the Benthic Bacterium *Pseudoalteromonas* sp. SM9913

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Most marine bacteria secrete exopolysaccharide (EPS), which is important for bacterial survival in the marine environment. However, it is still unclear whether the self-secreted EPS is involved in marine bacterial motility. Here we studied the role of EPS in the lateral flagella-driven swarming motility of benthic bacterium *Pseudoalteromonas* sp. SM9913 (SM9913) by a comparison of wild SM9913 and  $\Delta epsT$ , an EPS synthesis defective mutant. Reduction of EPS production in  $\Delta epsT$  did not affect the growth rate or the swimming motility, but significantly decreased the swarming motility on a swarming plate, suggesting that the EPS may play a role in SM9913 swarming. However, the expression and assembly of lateral flagella in  $\Delta epsT$  were not affected. Instead,  $\Delta epsT$  had a different swarming behavior from wild SM9913. The swarming of  $\Delta epsT$  did not have an obvious rapid swarming period, and its rate became much lower than that of wild SM9913 after 35 h incubation. An addition of surfactin or SM9913 EPS on the surface of the swarming plate could rescue the swarming level. These results indicate that the self-secreted EPS is required for the swarming of SM9913. This study widens our understanding of the function of the EPS of benthic bacteria.

**Keywords:** exopolysaccharide, swarming, marine sediment, benthic bacteria, flagella

## INTRODUCTION

Bacterial exopolysaccharide (EPS) is mostly generated intracellularly and then exported to the extracellular environment (Ates, 2015). Most marine bacteria are surrounded by self-secreted EPS, which is believed to be of great importance to the survival of marine bacteria in various oceanic environments (Nichols et al., 2005; Poli et al., 2010). Marine bacterial EPS can act as a cryoprotectant to help bacteria adapt to the extreme low temperature in sea ice (Nichols et al., 2005; Carrion et al., 2015), aid bacteria in absorbing nutrient elements (Decho and Lopez, 1993; Guezennec, 2002; Nichols et al., 2005), and assist bacteria in attaching to organic particles and other surfaces (Fletcher and Floodgate, 1973; Paerl, 1975; Holmstrom and Kjelleberg, 1999). However, it is still unknown whether self-secreted EPS is involved in the motility of marine bacteria.

Bacteria have several motile patterns. Swarming is a kind of rapid surface translocation powered by flagella, and is a typical pattern of surface-associated motile lifestyle (Henrichsen, 1972; Kearns, 2010). Some bacteria have been reported to have swarming motility, such as strains in *Proteus*

(Mobley and Belas, 1995), *Vibrio* (McCarter and Silverman, 1990), *Bacillus* (Kearns and Losick, 2003), *Pseudomonas* (Kohler et al., 2000), *Escherichia* (Harshey, 1994), *Salmonella* (Harshey, 1994), *Serratia* (Eberl et al., 1999), *Aeromonas* (Kirov et al., 2002), *Azospirillum* (Hall and Krieg, 1983), *Clostridium* (Hernandez and Rodriguez, 1993), *Rhodospirillum* (Ragatz et al., 1995), and *Yersinia* (Young et al., 1999). The roles of EPS in the swarming of several non-marine bacteria have been studied, but the results are different, even controversial, in different strains. In *Sinorhizobium meliloti* RMB7201, fast-swarming mutants were found to synthesize less EPS and all EPS-I overproducing mutants had swarming defects in different degrees (Wei and Bauer, 1999). A mutant of *Pseudomonas aeruginosa* with increased swarming motility was also found to have defect in EPS production (Merritt et al., 2007). In contrast, the EPS defective mutants of *Bacillus subtilis* could not swarm (Nagorska et al., 2010). The EPS II defective strain of *S. meliloti* Rm8530 was also found to be unable to swarm (Gao et al., 2012). Therefore, it is necessary to further examine the effect of self-secreted EPS on bacterial swarming motility. The role of self-secreted EPS in the swarming motility of marine bacteria has not been reported. Determining the relationship of the EPS and the swarming motility of marine bacteria would broaden our understanding of marine bacteria.

*Pseudoalteromonas* sp. SM9913 (hereafter called SM9913) is a marine bacterium isolated from the deep-sea sediment of Okinawa Trough and noted for its good extracellular protease-producing ability (Chen et al., 2003; Zhao et al., 2008; Yan et al., 2009). Due to its good extracellular protease-producing ability, SM9913 may play a role in organic nitrogen degradation in deep-sea sediment (Zhao et al., 2008). SM9913 also secretes a large amount of EPS (Qin et al., 2007). The EPS of SM9913 has been shown to have several ecological roles, such as stabilizing the protease secreted by the same strain and binding metal ions, which may be important for the survival of SM9913 in the extreme sedimentary environment (Qin et al., 2007). It was also found that the EPS of SM9913 played a role in the attachment of SM9913 cells to clay particles (Qin et al., 2007; Mi et al., 2015). Recently, SM9913 was found to have lateral flagella propelled swarming motility, which is helpful for the strain to survive in the deep-sea sediment (Mi et al., 2015). However, it is still unknown whether the EPS of SM9913 has an effect on its swarming motility.

During bacterial EPS biosynthesis, a majority of EPS share an initiation step requiring a UDP sugar and the undecaprenyl-phosphate (Und-P) lipid carrier (Whitfield, 2006). In this step, UDP-glucose lipid carrier transferase transfers a UDP sugar to a repeating unit attached to glycosyl carrier lipid and catalyzes the formation of a phosphoanhydride bond between them. Then the repeating units are polymerized, and the synthesized polysaccharide is secreted across membranes and cell wall (Ates, 2015). Therefore, UDP-glucose lipid carrier transferase is essential for bacterial EPS biosynthesis. In the genome of SM9913, a EPS gene cluster is predicted to contain 21 genes (from gene PSM\_A1265 to PSM\_A1287) and the gene *epsT* (PSM\_A1282) encodes the initial UDP-glucose lipid

carrier transferase (Qin et al., 2011). We have deleted the gene *epsT* from SM9913 and an EPS synthesis defective mutant  $\Delta epsT$  was obtained. The EPS production of  $\Delta epsT$  was only 27% of that of wild SM9913 (Yu et al., 2014). The reduction of EPS production severely decreased the particle-attached ability of  $\Delta epsT$ , suggesting that the EPS secreted by SM9913 is important in the attachment of SM9913 to sedimentary particles (Mi et al., 2015). In this study, wild SM9913 and  $\Delta epsT$  were compared to investigate whether the EPS of SM9913 plays a role in the swarming motility of the strain. Our results indicate that the reduction of EPS production in  $\Delta epsT$  significantly decreased the swarming motility on a swarming plate. Further analysis showed that the self-secreted EPS may facilitate SM9913 swarming by increasing the wetness of colony brim and reduce the surface tension.

## MATERIALS AND METHODS

### Strains and Culture

SM9913 was previously isolated from the deep-sea sediment of Okinawa Trough (Chen et al., 2003).  $\Delta epsT$ , an EPS biosynthesis defective mutant of SM9913, was previously constructed by deleting the *epsT* gene encoding the initial UDP-glucose lipid carrier transferase in the EPS gene cluster (Yu et al., 2014). The complement strain  $\Delta epsT/pEVepsT$  was previously constructed by introducing a multicopy plasmid carrying the *epsT* gene into  $\Delta epsT$ , and the control strain  $\Delta epsT/pEV$  was previously constructed by introducing the empty multicopy plasmid into  $\Delta epsT$  (Mi et al., 2015). These bacterial strains were grown in marine LB (Luria-Bertani) medium at 15°C as previously reported (Qin et al., 2011; Yu et al., 2014). The artificial sea water was prepared as described previously (Mi et al., 2015). To construct the growth curves of SM9913 and  $\Delta epsT$ , strains were cultured in marine LB at 15°C, and the optical density at 600 nm was measured with the time interval of 1 h using the Bioscreen C MBR microbial growth monitoring instrument (Oy Growth Curves Ab, Finland).

### Swimming and Swarming Assays

The swimming and swarming motilities of SM9913 and  $\Delta epsT$  were assayed as previously described (Mi et al., 2015). For swarming assay, 20 ml of marine LB medium solidified by 0.5% agar (Bacto™ agar, USA) were poured into each petri dish (Biologix, US) and the plates were dried under vertical flow for 50 min. For the complemented strains, chloramphenicol was added to a final concentration of 20 µg/ml. To evaluate swarming motility, overnight cultures were adjusted to the same optical density of OD<sub>600</sub> 1.5 and 5 µl of the cultures were spotted on each plate. The plates were incubated at 15°C for 5 days and motility was measured by examining the migration of bacteria through the agar from the center toward the periphery of the plate. For the swimming assay, 20 ml of marine LB medium solidified by 0.3% agar (Bacto™ agar, USA) were poured into each petri dish (Biologix, US) and the plates were dried under

vertical flow for 50 min. Overnight cultures were adjusted to the same optical density of OD<sub>600</sub> 1.5 and 5 µl of the cultures were spotted on each plate. The plates were incubated at 15°C for 3 days.

## Comparison of the Expression Levels of Genes in the EPS Gene Cluster between the Swarming and Swimming Cells of SM9913

RNA sequencing of the swarming and swimming cells of SM9913 was previously performed (Mi et al., 2015). The swimming cells for RNA sequencing were prepared by growing SM9913 in liquid marine LB to the optical density at 600 nm (OD<sub>600</sub>) of approximately 1.0. The swarming cells for RNA sequencing were taken from the edge of swarming colonies with a sterile cotton swab (Mi et al., 2015). In this study, based on the RNA sequencing data, the expression levels of genes (from PSM\_A1265 to PSM\_A1287, among which PSM\_A1277 and PSM\_A1278 were pseudogenes) in the EPS gene cluster were calculated using the RPKM method (Mortazavi et al., 2008) and compared between the swarming and swimming cells. The comparison was indicated as relative expression level = log<sub>2</sub>(swarming/swimming). A gene was regarded as differentially expressed when its log<sub>2</sub>(swarming/swimming) ≥ 1 and when its *P* ≤ 0.001.

## Real-Time Quantitative PCR

The expression of four genes involved in the biosynthesis of lateral flagella in the lateral flagella gene cluster in wild SM9913 and *ΔepsT* was examined by real-time quantitative PCR. The total RNA was extracted from SM9913 and *ΔepsT* as described previously (Mi et al., 2015). The cells for RNA extraction were harvested by sterile cotton swabs from the edge of swarming colonies after 72 h incubation. Reverse transcription was performed using the PrimeScript™ RT reagent kit with gDNA Eraser (perfect Real Time; TaKaRa, Japan). The obtained cDNA and the SYBR® Premix Ex Taq™ (Tli RNaseH plus; TaKaRa, Japan) were used in preparing the quantitative PCR reaction system and real-time quantitative PCR was performed on LightCycler 480® (Roche, Switzerland). Fold change of the expression level of target genes was

calculated by the software in LightCycler 480® with *rpoD* as the reference gene. The extraction of total RNA and the subsequent quantitative PCR were repeated three times with three replicates. Primers used for PCR are listed in Table 1.

## Atomic Force Microscopy

Lateral flagella of SM9913 and *ΔepsT* were observed by using an atomic force microscope (AFM) as previously reported (Mi et al., 2015). Briefly, cells were taken from the edge of a swarming colony and suspended in distilled water. The obtained cell suspension was imaged using an AFM (Bruker AXS, Germany).

## Quantification of Swarming Behavior

To measure the diameter of each colony, a line was drawn on the bottom side of the petri dish across the center of each colony and the diameter was measured along the drawn line. Data of diameters were recorded and plotted against time. The obtained plots were fitted using growth equation for SM9913 and *ΔepsT*. Rate of colony expansion was calculated by differentiating diameter with respect to time using the fitted curves. Line fitting and curve differentiating were performed using the software OriginPro 8. The experiment was repeated three times with at least three replicates. *P*-value was calculated from *t*-test.

## Preparation of EPS from SM9913

The EPS of SM9913 was prepared with the method of Liu et al. (2013). Briefly, marine LB medium with 2% inoculum was incubated at 15°C, 200 rpm for 5 days. After fermentation, EPS in the supernatant of the culture was precipitated with chilled absolute ethanol. The precipitate was dissolved in distilled water. Proteins in the EPS solution were removed by the sevag method (Staub, 1965), and small molecular carbohydrates were removed by dialyzing the EPS solution against distilled water. The polysaccharide content of the EPS solution was determined using the phenol-sulfuric acid method.

TABLE 1 | Primers used in this study.

Primer	Sequence (5'-3')	Target gene
<i>rpoDF</i>	CGCATATTATTGACTGGTTAGGTG	<i>rpoD</i>
<i>rpoDR</i>	CAAGGGTTGAGGGTTCATAGC	
RT0917F	CTCGTTCTGTGTCGGTGGTG	PSM_A0917
RT0917R	TTTATCGGTTTCTTTGCCTACTGG	
RT0915F	GGCATCGCACCCAAACAG	PSM_A0915
RT0915R	ATCAATATCAATACCGCATTACAG	
RT0906F	ATTACGAGCCAGATCAGTTCAG	PSM_A0906
RT0906R	CCACACCACCCACGCTAATG	
RT0897F	GGCTGGTCTACAAATCGCTTCAC	PSM_A0897
RT0897R	TCATTTTCATCAA'ACGCACCTTCAG	

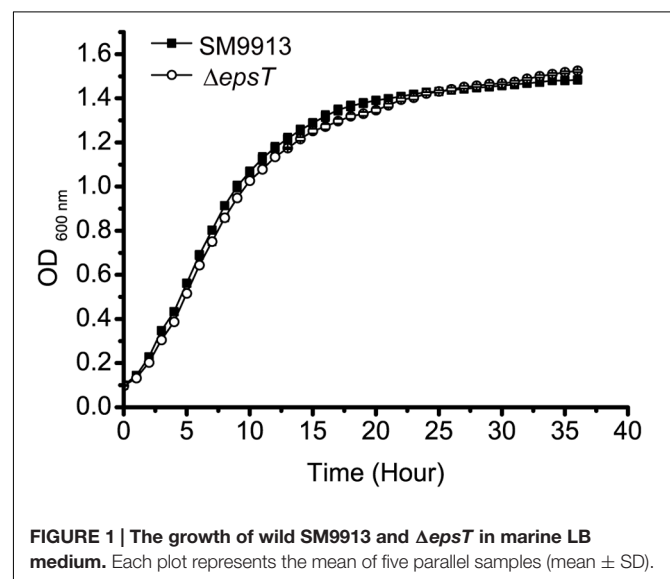
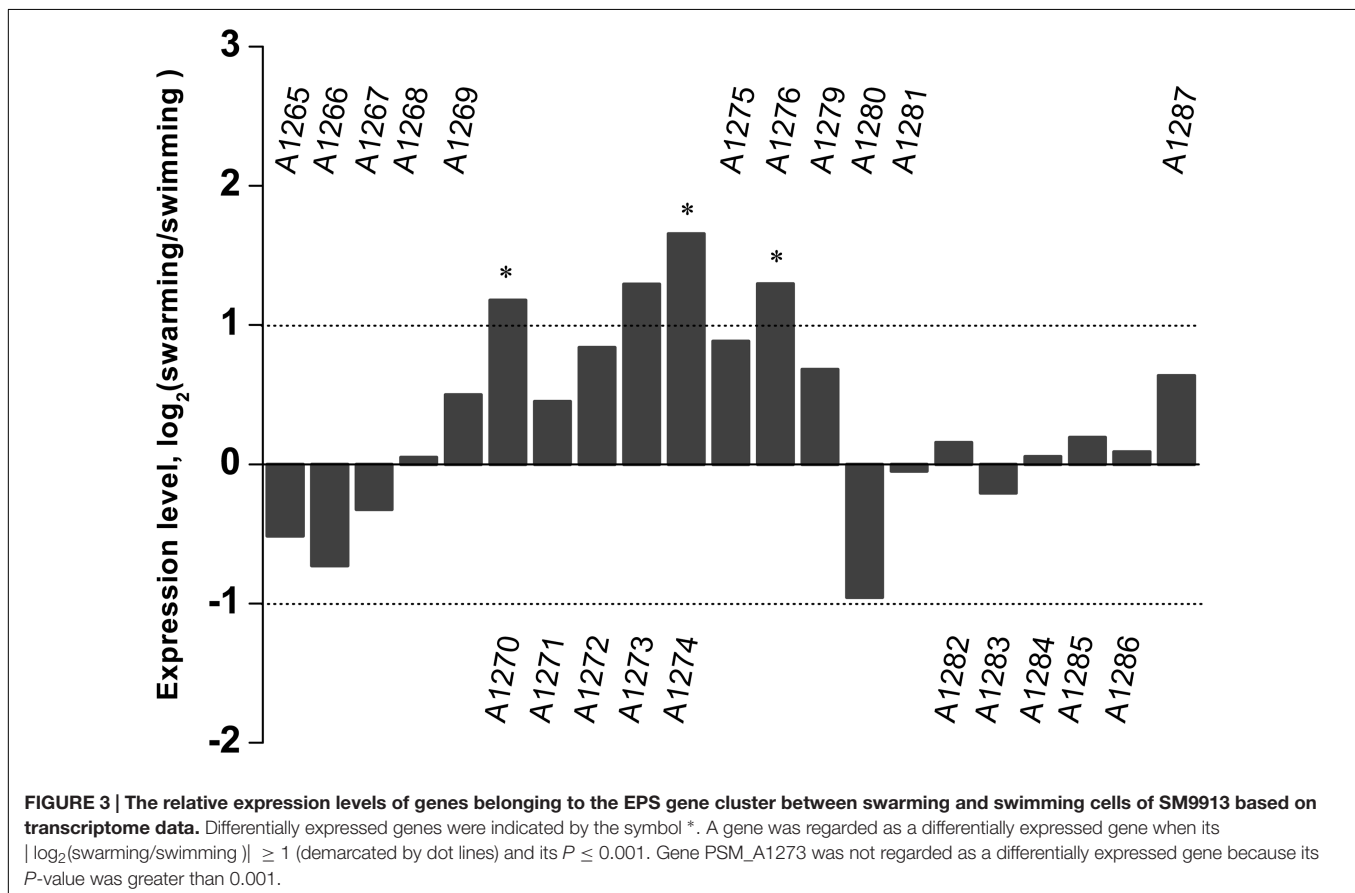
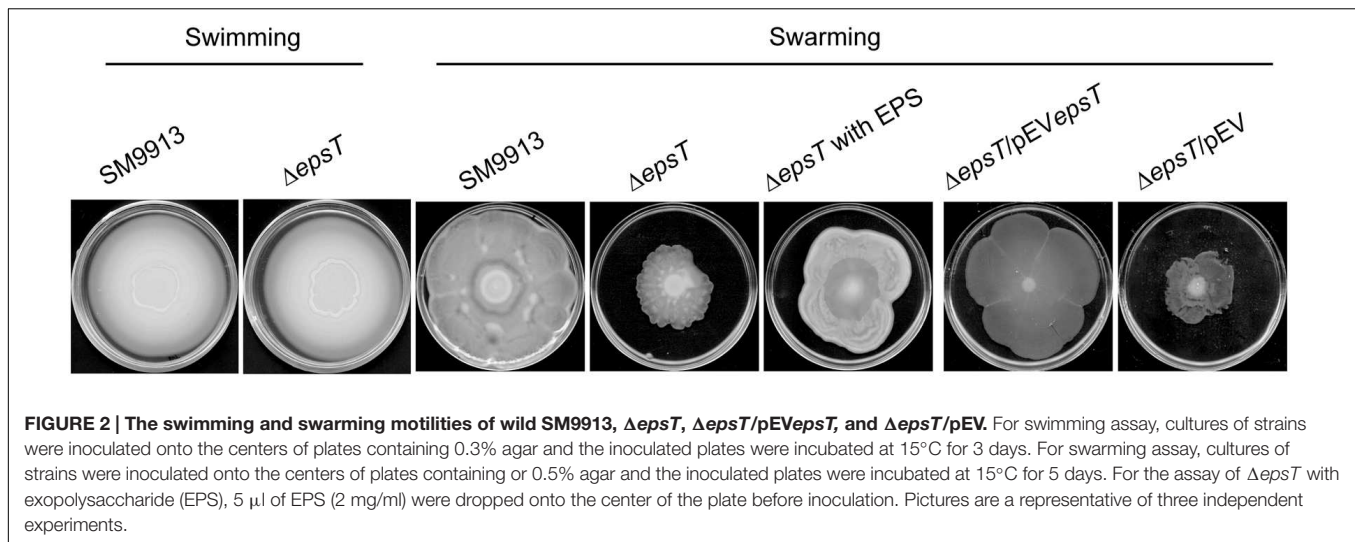


FIGURE 1 | The growth of wild SM9913 and *ΔepsT* in marine LB medium. Each plot represents the mean of five parallel samples (mean ± SD).



## Rescue of the Swarming Defect of $\Delta epsT$ with Surfactin and the EPS from Wild SM9913

Surfactin from *B. subtilis* (Sigma, USA) was dissolved in 20 mM NaOH. Five microliters of surfactin (2 mg/ml) or the EPS extracted from wild SM9913 (2 mg/ml) were spotted onto

swarming plates and air dried. Then, 5  $\mu$ l of the overnight culture of  $\Delta epsT$  or wild SM9913 was spotted on the location of surfactin or EPS. The inoculated plates were incubated at 15°C for 5 days. The measurement of the diameter against time was performed as described above. The experiment was repeated three times with at least three replicates.  $P$ -value was calculated from  $t$ -test.

## RESULTS

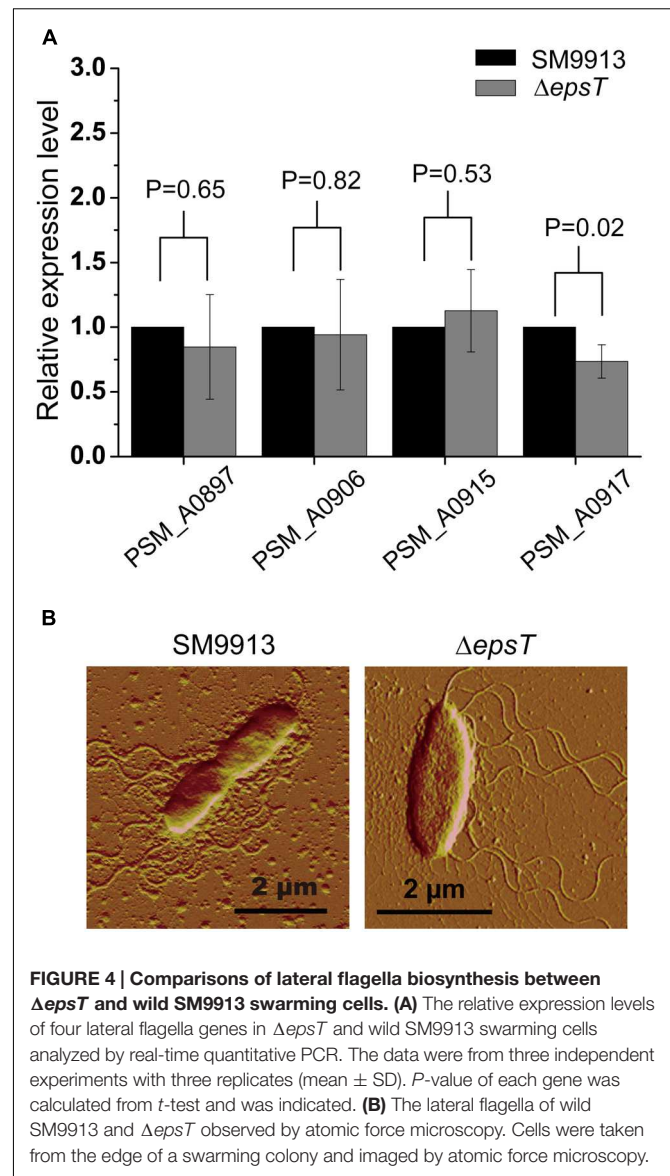
### Reduction of EPS Production Caused a Decrease in the Swarming Motility of SM9913

$\Delta epsT$  is an EPS biosynthesis defective mutant of SM9913 with the *epsT* gene being knocked out and 73% reduction of EPS production (Yu et al., 2014). To investigate whether the reduction of EPS production affects the growth of SM9913, the growth of wild SM9913 and  $\Delta epsT$  was compared. As shown in **Figure 1**,  $\Delta epsT$  displayed a similar growth rate as wild SM9913, indicating that the reduction of EPS production does not affect the growth of SM9913.

SM9913 has both swimming and swarming motilities (Mi et al., 2015). To study the role of the EPS in the motilities of SM9913, the swimming and swarming motilities of wild SM9913 and  $\Delta epsT$  were compared. On the swimming plates, wild SM9913 and  $\Delta epsT$  displayed similar swimming motility (**Figure 2**), indicating that the reduction of EPS had no effect on the swimming motility of SM9913. In contrast, the swarming colony of  $\Delta epsT$  was much smaller than that of wild SM9913 after 5-day incubation (**Figure 2**), indicating a lower swarming motility of  $\Delta epsT$  than wild SM9913. In addition, the swarming motility of the complement strain  $\Delta epsT/pEVepsT$  could be rescued to the level of wild SM9913 by re-introducing the gene *epsT* into  $\Delta epsT$ , but the swarming of the control strain  $\Delta epsT/pEV$  was not rescued (**Figure 2**). Moreover, when the EPS extracted from SM9913 was spotted in advance on the location where  $\Delta epsT$  was inoculated on the swarming plate, the swarming defect of  $\Delta epsT$  could be rescued to a large extent (**Figure 2**). EPS is a polymer and is difficult to diffuse in the agar on the swarming plate, which may be the main reason why the swarming defect of  $\Delta epsT$  was not completely rescued by the EPS from SM9913. Altogether, these results showed that the reduction of EPS production in  $\Delta epsT$  affected its swarming, which suggests that the self-secreted EPS of SM9913 may play a role in its swarming.

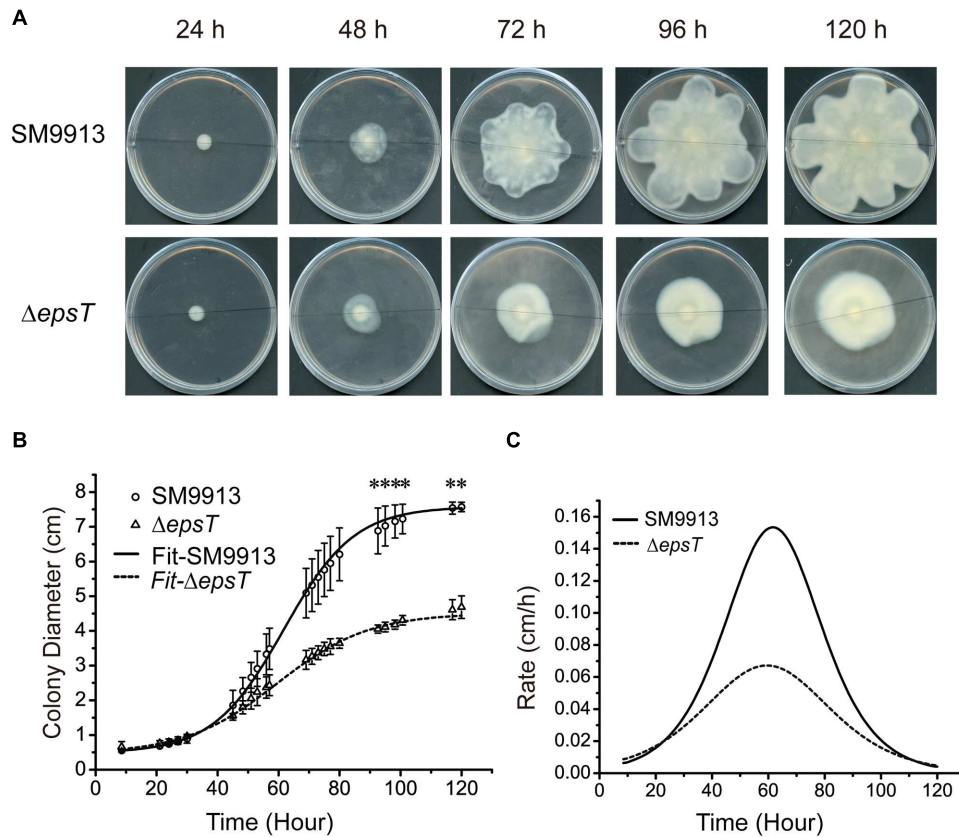
### EPS Production in SM9913 Was Not Coupled with Swarming

Transcriptome sequencing of the swarming and swimming cells of SM9913 were previously performed (Mi et al., 2015). To analyze whether the production of EPS in SM9913 is coupled with swarming, the expression levels of genes in the EPS gene cluster were compared between the swarming and swimming cells according to the transcriptome data. The results showed that, while 3 of the 21 genes in the EPS gene cluster were differentially expressed slightly, the other 18 genes (including the deleted gene *epsT*) did not show differential expression between the swarming and swimming cells (**Figure 3**). This suggests that the expression of genes in the EPS gene cluster is not coupled with the regulation of swarming, and that the swarming of SM9913 does not induce EPS production.

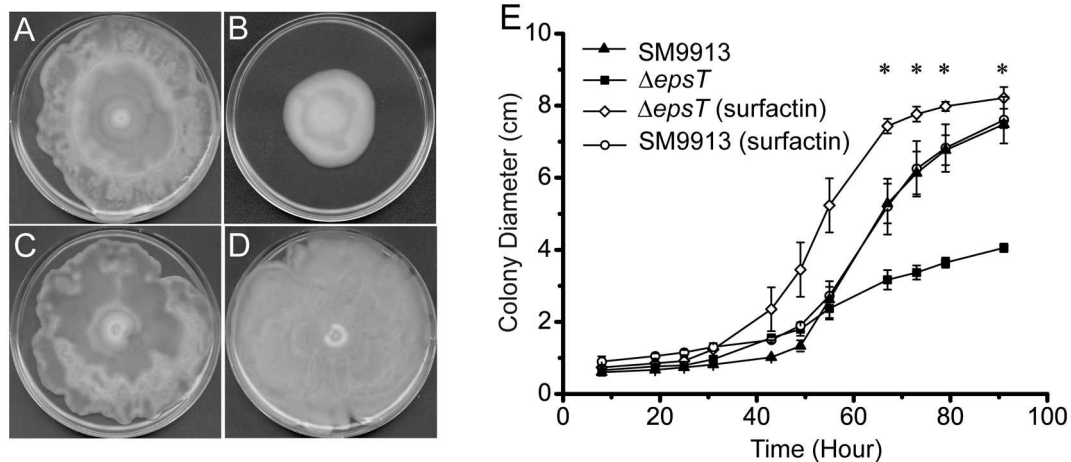


### Deletion of Gene *epsT* Did Not Affect the Biosynthesis of Lateral Flagella in SM9913

SM9913 uses lateral flagella to power its swarming motility (Mi et al., 2015). To investigate whether the deletion of gene *epsT* affected the biosynthesis of lateral flagella in SM9913, the expression of four genes involved in the biosynthesis of lateral flagella in the lateral flagella gene cluster in wild SM9913 and  $\Delta epsT$  was examined by real-time quantitative PCR. The result showed that the expression of these four genes displayed no significant difference between wild SM9913 and  $\Delta epsT$  (**Figure 4A**), which indicated that the deletion of gene *epsT* did not affect the biosynthesis of lateral flagella in SM9913. An AFM observation of their lateral flagella also supported this. The lateral flagella of  $\Delta epsT$  were clearly viewed under AFM (**Figure 4B**). These results indicate that



**FIGURE 5 | Analyses of the swarming modes of wild SM9913 and  $\Delta epsT$ .** (A) Swarming colonies of SM9913 and  $\Delta epsT$  at different incubation time. Pictures are a representative of three independent experiments. (B) Colony expansion curves of wild SM9913 and  $\Delta epsT$  fitted with growth equation based on measured colony diameters (scatters). Data were from three independent experiments with at least three replicates (mean  $\pm$  SD).  $P$ -value was calculated from  $t$ -test and the symbol \* indicates that the difference in colony diameter between wild SM9913 and  $\Delta epsT$  was significant ( $P < 0.001$ ). (C) Colony expansion rates of wild SM9913 and  $\Delta epsT$ . Curves were obtained by differentiating diameter with respect to time using fitted curves.



**FIGURE 6 | The rescue of the swarming of  $\Delta epsT$  with surfactin.** (A) Wild SM9913 on a swarming plate without surfactin. (B)  $\Delta epsT$  on a swarming plate without surfactin. (C) Wild SM9913 on a swarming plate with surfactin. (D)  $\Delta epsT$  on a swarming plate with surfactin. Five microliters of surfactin (2 mg/ml) were dropped onto the center of the plate before inoculation. (E) Colony diameters of wild SM9913 in (A) and (C) and  $\Delta epsT$  in (B) and (D). Data were from three independent experiments with at least three replicates (mean  $\pm$  SD).  $P$ -value was calculated from  $t$ -test and the symbol \* indicates that the difference in colony diameter between  $\Delta epsT$  with or without surfactin was significant ( $P < 0.001$ ). Pictures are a representative of three independent experiments.

the expression and assembly of lateral flagella in  $\Delta epsT$  are not affected and that the decrease of the swarming motility of  $\Delta epsT$  is not due to the lack of lateral flagella. Alternatively, EPS reduction in  $\Delta epsT$  may affect the function of lateral flagella.

## EPS Reduction in $\Delta epsT$ Caused Changes in the Swarming Behavior

To analyze the possible change in the swarming behavior of  $\Delta epsT$  caused by EPS reduction, we quantified the swarming of wild SM9913 and  $\Delta epsT$  by recording the diameters of swarming colonies with incubation time and calculated their expansion rates. The result showed that the diameter of the swarming colony of  $\Delta epsT$  was smaller than that of SM9913 after 120-h incubation (Figure 5A). Statistical analysis indicated that the difference in colony diameter between wild SM9913 and  $\Delta epsT$  was significant ( $P < 0.001$ ). Moreover, it can be seen from Figures 5A,B that the colony expansion modes between wild SM9913 and  $\Delta epsT$  were also different. During the first 30 h of incubation, diameters of wild SM9913 and  $\Delta epsT$  colonies increased very slowly (Figure 5B), showing an obvious swarming lag. After the lag period, the colony of wild SM9913 expanded quickly. The colony expansion rate increased with time, reached the maximum (0.16 cm/h) at the 65th hour, and then declined (Figure 5C). In contrast, the colony of  $\Delta epsT$  did not show an obvious rapid expansion period. Its expansion rate increased almost constantly from the beginning, reached a much lower maximum (approximately 0.06 cm/h) at the 60th hour, and then declined (Figure 5C). Thus,  $\Delta epsT$  finally formed a much smaller colony compared to the wild strain (Figure 5A). These results indicated that EPS reduction led to a change in the swarming behavior of SM9913, which caused the decrease of its swarming motility on the swarming plate.

## The Rescue of $\Delta epsT$ Swarming by Extracellularly Added Biosurfactant Surfactin

Surfactin is a biosurfactant from *B. subtilis*. We investigated whether surfactin could rescue the swarming of  $\Delta epsT$ . As shown in Figure 6, the colony diameter of  $\Delta epsT$  on the plate with surfactin was significantly larger than that of this mutant without surfactin after 91 h incubation ( $P < 0.001$ ) and similar to that of wild SM9913 (Figures 6A,B,D,E), indicating that the addition of surfactin could rescue the swarming of  $\Delta epsT$ . However, surfactin added on the plate had no effect on the swarming of wild SM9913 (Figures 6A,C).

## DISCUSSION

The results in this study indicated that the EPS of SM9913 had a positive effect on its swarming motility, which is consistent with the role of EPS on the swarming motilities of *B. subtilis* (Nagorska et al., 2010) and *S. meliloti* Rm8530 (Gao et al., 2012). Bacterial

swarming motility is driven by rotating flagella and coupled to the production of a mucoid layer that facilitates the movement (Verstraeten et al., 2008; Kearns, 2010). The mucoid layer serves as wetting agents to extract water from the surroundings and also as surfactants to reduce tension between the substrate and the bacterial cells at the swarming front (Verstraeten et al., 2008; Kearns, 2010). The swarming of SM9913 is driven by its lateral flagella (Mi et al., 2015). In its mutant  $\Delta epsT$ , the expression and assembly of lateral flagella was not altered (Figure 4), but the EPS production was severely reduced (Yu et al., 2014), which would affect the mucoid layer. This is likely the main cause leading to the swarming defect of  $\Delta epsT$ . In the meantime, the possibility that the loss of the *espT* gene in  $\Delta epsT$  has other effects on the swarming behavior that is independent of EPS production can not be excluded completely, which needs further study.

Reports on the mechanistic benefit of EPS during bacterial swarming are quite limited. It was suggested that during *Escherichia coli* swarming, lipopolysaccharides (LPS) likely functioned as osmolytes facilitating water extraction from the agar, which then advantaged the swarming motility (Ping et al., 2014). Bacterial capsular exopolysaccharides (CPS) were also suggested to facilitate the translocation over solid surfaces of differentiated cell populations by aiding colony hydration (Gygi et al., 1995; Rahman et al., 1999). Thus, although bacterial LPS and CPS have differences in their characteristics, they seem to play similar roles in bacterial swarming motility, increasing the wetness of colony brim to provide a liquid environment for flagella to function properly (Gygi et al., 1995; Rauprich et al., 1996; Toguchi et al., 2000; Chen et al., 2007). Therefore, the EPS of SM9913 may also play a similar role in its swarming motility, increasing the wetness of colony brim to provide a liquid environment for flagella movement, and thereby facilitating the swarming of SM9913.

Some species, such as *B. subtilis*, secrete both surfactin as a surfactant and EPS as a wetting agent to facilitate its swarming motility (Nagorska et al., 2010). SM9913 secretes a large amount of EPS (Qin et al., 2007), but does not secrete surface-active agent (data not shown). Surfactin is a kind of amphiphathic molecules. Surfactin can improve the wettability of the substrate, and subsequently reduce surface tension, which permit bacteria spreading over surfaces (Julkowska et al., 2004; Ke et al., 2015). It is reported that surfactin was able to rescue the swarming of LPS biosynthesis defective mutant in *Salmonella enterica* (Toguchi et al., 2000). Similar to the effect of surfactin on *S. enterica* (Toguchi et al., 2000), our results showed that surfactin could completely compensate the swarming defect of  $\Delta epsT$  by improving the colony expanding rate, but had little effect on the swarming motility of wild SM9913 (Figure 6). This implies that the EPS of SM9913 may be also helpful in reducing the surface tension in SM9913 swarming.

In summary, our results in this study show that the self-secreted EPS of benthic strain SM9913 plays a positive role in its swarming motility. The EPS may facilitate SM9913 swarming by increasing the wetness of colony brim and reducing the surface tension. This study sheds more light on the function of the EPS of benthic bacteria.

## AUTHOR CONTRIBUTIONS

AL, Z-HM, and X-LC designed, conducted and wrote the paper. X-YZ, YY, and H-NS conducted the experiments. B-BX, B-CZ, and Y-ZZ analyzed the data. Q-LQ instructed the study and revised the paper.

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