Effect of *M. xanthus* Exopolysaccharides on Twitching Motility of *P. aeruginosa*

Huining Lu[1*]

[1] Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Tianjin, 300350, China

[*]Corresponding author: tjuluhuining@163.com

Keywords: *M. xanthus*; *aeruginosa*; sl; EPS; witching motility

Abstract: Exopolysaccharides (EPS) play important roles in bacterial surface motilities for a variety of bacterial species. However, the role of EPS in inter-species interactions remains unclear. In this study, by employing high-throughput bacterial tracking techniques, we investigated the effect of EPS of *M. xanthus* (*Mx*EPS) on the twitching movement of *P. aeruginosa* cells. Our results showed *Mx*EPS exhibits 'glue' effect, and Psl plays significant role in *Mx*EPS-mediated cell-surface interactions. Our study shed light on our further understanding of the role of EPS in the species-specific interactions when multiple species coexist.

1 Introduction

Twitching motility is a pili-driven form of bacterial translocation on surfaces [1]. It plays important roles in a variety of bacterial activities such as surface sensing, host colonization, biofilm, which have been observed in many bacterial species, and *P. aeruginosa* and *M. xanthus* are two model bacterial species [2-3].

P. aeruginosa is a gram-negative opportunistic pathogen, perform different types of twitching mode. A "slingshot" motion has been observed when cells release one attached pilus during crawling[4]. *M. xanthus* is another widely-studied gram-negative soil bacterium and has two independent surface motility systems, which play key roles in a variety of activities, including vegetative swarming, fruiting body formation and predation [5].

EPS secreted by cells play an important role in the regulation of twitching motility [6-7]. Psl has shown to guide the bacterial surface movement pattern through a Psl-based rich-get-richer mechanism. *Mx*EPS deposited on soft surfaces could be sculpted into microchannels structures which guide cell movement [8]. Purified *Mx*EPS materials can be shared among closely related strains, however, the role of EPS in inter-species remains unclear. Given the similarity in composed ingredients between *Mx*EPS and Psl, it is intriguing whether and how the *Mx*EPS affect the surface motility of *P. aeruginosa*?

In this work, we studied the effect of *Mx*EPS on the twitching movement of *P. aeruginosa* using high-throughput cell-tracking techniques. We modified glass surfaces with *Mx*EPS and Psl, and characterized surface movement of *P. aeruginosa* cells on these surfaces. Our study shed light on our further understanding of the role of EPS in the species-specific interactions when multiple species coexist.

  DOI: 10.25236/icme.2019.023

2 Materials and Methods

2.1 Bacteria Strains and Culture Conditions

Wild-type *P. aeruginosa* strain and its isogenic mutants △*pslBCD*、△P_{psl}/P_{BAD}-*psl* strain, wild-type *M. xanthus* DK1622 were used in this study.

Strains *P. aeruginosa* were grown on LB agar plates at 37℃ for 12 h. An inoculum was prepared by growing strains in test tubes containing FAB medium [2 g L^{-1} $(NH_4)_2SO_4$, 9 g L^{-1} $Na_2HPO_4 \cdot 7H_2O$, 3 g L^{-1} KH_2PO_4, 3 g L^{-1} NaCl, 93 mg $L^{-1}MgCl_2$, 14 mg $L^{-1}CaCl_2 \cdot 2H_2O$, 1 ml L^{-1} trace metals solution (200 mg L^{-1} $CaSO_4 \cdot 2H_2O$, 200 mg L^{-1} $FeSO_4 \cdot 7H_2O$, 20 mg L^{-1} $MnSO_4 \cdot H_2O$, 20 mg L^{-1} $CuSO_4 \cdot 5H_2O$, 20 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 10 mg $L^{-1}CoSO_4 \cdot 7H_2O$, 10 mg $L^{-1}Na_2MoO_4 \cdot 2H_2O$, 5 mg L^{-1} H_3BO_3),pH 6.9] with 30 mM glutamate, shaking at 220 rpm and 37°C for about 10 h. Cultures were diluted to an OD_{600} of 0.01.

Strain *M. xanthus* were grown on CYE agar (10 g L^{-1} casitone, 5 g L^{-1} yeast extract, 0.986 g L^{-1} $MgSO_4 \cdot 7H_2O$, 2.093 g L^{-1} MOPS) plates at 30℃ for 2~3 d. An inoculum was prepared by growing strains in flask containing CYE (pH 7.6) medium shaking at 200 rpm and 30℃ for 12 h. The Cultures were diluted to an OD_{600} of 0.05.

2.2 Flow Cell Preparation and Data Analysis

Flow cell has six identical rectangle channels which are connected to a syringe through a filter using silicon tube. *M. xanthus* culture was injected for 8 h. Then, WGA-555 was injected to stain *Mx*EPS, SDS (2 %) was injected to lyse the cells, *P. aeruginosa* (WT、△*pslBCD*) was injected to start image recording. Similarly, △P_{psl}/P_{BAD}-*psl* culture was injected for 6 h. Next, HHA-FITC was injected to stain Psl, meropenem solution (1 %) and SDS (2 %) were used to lyse the cells. *P. aeruginosa* (WT、△*pslBCD*) was injected to start image recording.

Images were captured using a NEO-5.5-CL3 camera on a Leica DMi8 microscope. Bright-field images were recorded every 3 s. The trajectories of *P. aeruginosa* cells were analyzed by MATLAB and IDL software.

3 Results and Discussion

3.1 The Preparation and Characterization of *Mx*EPS-modified and Psl-modified Glass Surfaces

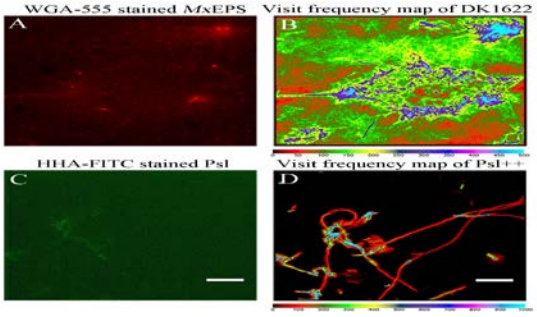

Fig 1. (A) Florescent image of the glass surface showing WGA-555 stained *Mx*EPS left on the glass surface. (B) A visit frequency map of DK1622 after incubation on a glass surface. (C) Fluorescent image of the glass surface showing HHA-FITC stained Psl left on the glass surface. (D) A visit frequency map of Psl++ after incubation on a glass surface. Scale bars are 10 μm.

We prepared a *Mx*EPS-modified glass surface by incubating DK1622 cells on a bare glass surface to. As the effect of fluorescent molecules attached to functionality of *Mx*EPS is not clear, we reconstructed a visit frequency map of DK1622 from their trajectories as a measure for the distribution of *Mx*EPS(Fig 1.A-B). Similarly, we prepare a Psl-modified glass surface and used the distribution of bacterial visits as a measure for the distribution of Psl(Fig 1.C-D). One caveat here is that the quantitative relationship between bacterial visits and fluorescence intensities is unknown, so we just categorize the surface into two types: surfaces with and without bacterial visits, then study how bacteria behave differently on them.

3.2 The Effect of *Mx*EPS on the Twitching Motility of *P. aeruginosa*

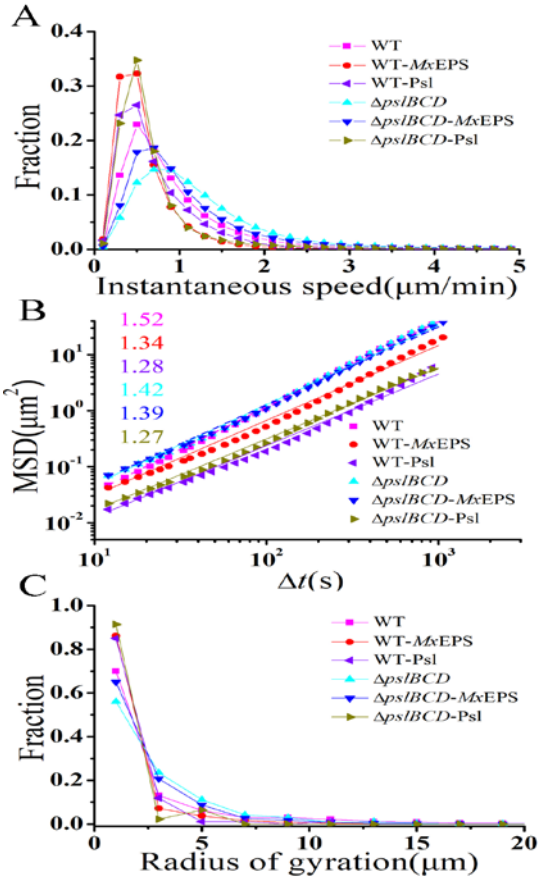

Fig 2. The effect of *Mx*EPS and exogenous Psl on the twitching motility of *P. aeruginosa*. (A) Instantaneous speed distributions, (B) Mean square displacements and (C) Radius of gyration (R_{gyr}) for WT cells and △*pslBCD* cells on bare, *Mx*EPS-modified and Psl-modified glass surfaces. The results are obtained from three repeats.

To investigate the effect of *Mx*EPS on twitching motility of *P. aeruginosa*, we first characterized single-cell movement of *P. aeruginosa* WT. On *Mx*EPS-modified glass surfaces, instantaneous speed and R_{gyr} distribution has a narrower distribution, MSD slope was smaller than on bare glass surfaces (Fig 2). The results indicated that *Mx*EPS confined the motion of *P. aeruginosa* cells,which is consistent with the known “glue” function of EPS.

Considering that Psl plays an important role in cell-surface interactions, we also repeated measurements using △pslBCD strain. A similar confined trend is observed, however, the differences between two surfaces are not as strong as those in WT cells, which implies a role of Psl in *Mx*EPS-mediated cell-surface interactions.

3.3 The Effect of Exogenous Psl on the Twitching Motility of *P. aeruginosa*

To further understand the role of Psl in *Mx*EPS-mediated cell-surface interactions, we characterized single-cell movement of *P. aeruginosa* on Psl-modified glass surfaces.

Similarly, on Psl-modified glass surfaces, both WT and △*pslBCD* cells have a narrower instantaneous speed and R_{gyr} distribution, smaller MSD slope than on bare glass surfaces(Fig 2), which represents the 'glue' effect of Psl. Moreover, MSD slope of △*pslBCD* cells show a very similar surface behavior as WT cells. This indicates that exogenous Psl could complement (at least partially) the Psl deficiency of △*pslBCD*.

3.4 The Comparation of Influence of *Mx*EPS and Exogenous Psl on the Twitching Motility of *P. aeruginosa*

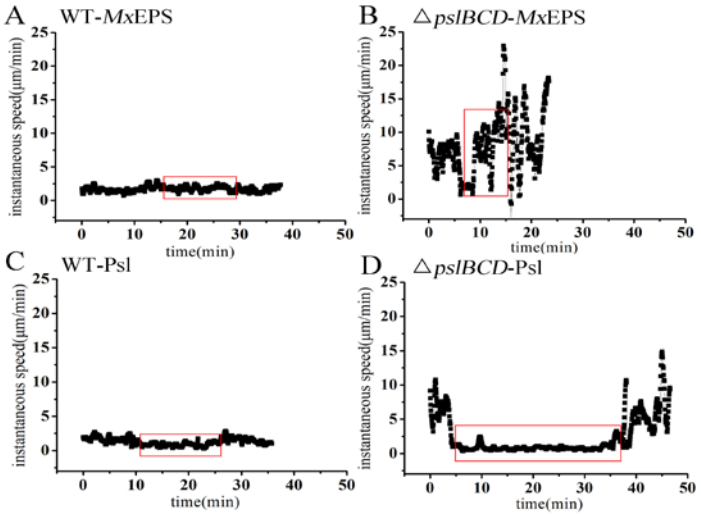

Fig 3. (A-B) Instantaneous speed variation of a typical WT cell and △*pslBCD* cell passing *Mx*EPS-rich and *Mx*EPS-none regions respectively. (C-D) Instantaneous speed variation of a typical WT cell and △*pslBCD* cell passing on Psl-rich and Psl-none regions respectively. The red square stand for the *Mx*EPS and Psl regions.

We visually observed the relative change of instantaneous speed variation when cells pass through EPS and Psl regions respectively. On *Mx*EPS-modified glass surfaces, both WT and △*pslBCD* cells didn't show apparent change when entered or left *Mx*EPS-rich regions (Fig 3.A-B). On Psl-modified glass surfaces, the average speed of a WT cell was 1.7±0.4 μm/min before entered a Psl-rich region, then decreased to 0.9±0.3 μm/min when was in Psl-rich region, and increased to 1.5±0.4 μm/min after left the Psl-rich region(Fig 3.C). A similar but more significant change in the instantaneous speed was observed for △*pslBCD* cell when entered and left Psl-rich regions from Psl-none regions. The average speed of the △*pslBCD* cell was 3.1±3.0 μm/min before entered a Psl-rich region, 0.77±0.23 μm/min when was in Psl region and 5.34±2.95 μm/min after left Psl region(Fig 3.D). △*pslBCD* cells showed a distinct difference between Psl-rich regions and Psl-none regions. These results clearly demonstrate the different effects of *Mx*EPS and Psl on the surface movement of cells.

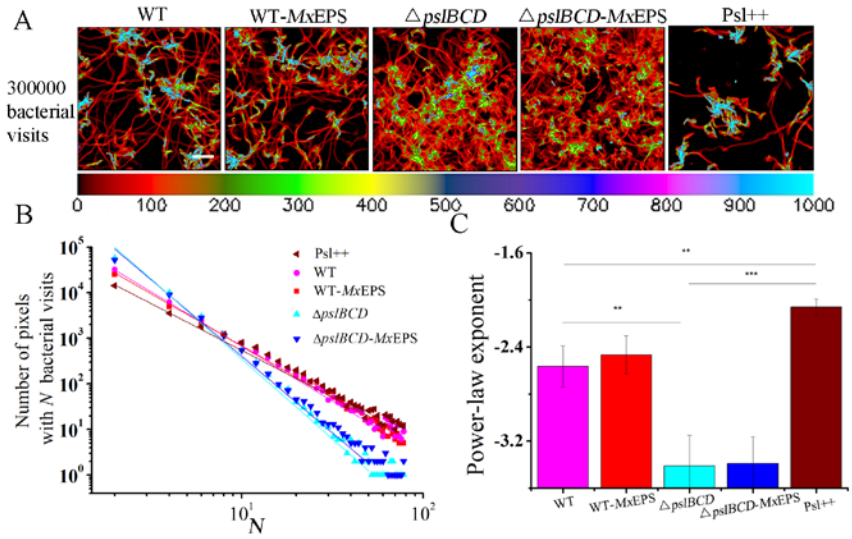

Fig 4. Characterization of collective surface motion. (A) Visit frequency maps at a total of 300,000 visits for WT and △*pslBCD* cells on bare and *Mx*EPS-modified glass surfaces, and Psl++ cells on bare glass surfaces. (B) The visit frequency distributions. (C) Average power-law exponents. Statistical significance was measured using one-way ANOVA. **, P <0.05; ***, P < 0.01. Scale bar is 10 μm.

We also compared the effect of *Mx*EPS and Psl on collective surface motion (Fig 4)，which cannot be detected by single-cell motility analysis alone. Cells followed a Psl-based rich-get-richer mechanism, which resulted in a distribution of bacterial visits that could be approximated by a power law with an exponent of -2.6±0.2 and -2.5±0.2 for WT cells on bare and *Mx*EPS-modified glass surfaces, -3.4±0.3-2 and -3.4±0.2 for △*pslBCD* cells on bare and *Mx*EPS-modified glass surfaces, and -2.0±0.1 for Psl++ cells on bare glass surfaces. A power law distribution with a more negative exponent indicates that the distribution is more uniform. On bare glass surfaces, the visit distribution of Psl++ cells on surface follows a power law with the least-negative exponent, indicating a more hierarchical distribution, whereas the △*pslBCD* showed a power law with the most-negative exponent, implying a more uniform distribution. However, there is not much difference between cells on bare glass surfaces and *Mx*EPS-modified glass surfaces, which implied only Psl guide the bacterial movement.

4 Conclusions

The effects of EPS of bacteria appear to have several key properties and functions, specially are a key component that influence bacteria movement and surface exploration. Previous study demonstrated that purified *Mx*EPS can be shared among closely related strains, and *P. aeruginosa* WT cells form highly structured mound-shaped cell clusters on the mucin-coated coverslip, which are more constrained on biotic surface[9]. However, the role of EPS in inter-species interactions remains unclear. Here we investigated the effect of *Mx*EPS on *P. aeruginosa* twitching movement.

Similar but different confined effects of *Mx*EPS and Psl was observed, and Psl plays significant role in *Mx*EPS-mediated *cell*-surface interactions. We also found the microcolony formation is not so related to *Mx*EPS and Psl. We propose that Psl has an important species-specific role in regulation of *P. aeruginosa* adhesion and motility, thus the mechanisms that how *P. aeruginosa* cells sense exogenous EPS need further exploration. Surface sensing in *P. aeruginosa* is a possible

factor which includes EPS and TFP-mediated cooperative participation[10]. Other sensor protein could also response to signals received in the periplasm. Our results may pave the way to control and guide the bacteria behavior by the exogenous addition of EPS, which is hopeful to further reveal the role of EPS in the species-specific interactions when multiple species coexist.

References

[1] Merz A J , So M , Sheetz M P . Pilus retraction powers bacterial twitching motility.[J]. Nature, 2000, 407(6800):98.

[2] Mattick J S. Type IV pili and twitching motility. [J]. Annual Review of Microbiology, 2002, 56(1):289-314.

[3] Lee C K, De Anda J, Baker A E, et al. Multigenerational memory and adaptive adhesion in early bacterial biofilm communities[J]. Proceedings of the National Academy of Sciences, 2018: 201720071.

[4] Zhang R, Ni L, Jin Z, et al. Bacteria slingshot more on soft surfaces[J]. Nature Communications, 2014, 5:5541.

[5] José M D, Marcos-Torres F J, Elena G B, et al. Myxobacteria: Moving, Killing, Feeding, and Surviving Together: [J]. Frontiers in Microbiology, 2016, 7(e1004474).

[6] Gelimson A, Zhao K , Lee C K , et al. Multicellular Self-Organization of P. aeruginosa, due to Interactions with Secreted Trails[J]. Physical Review Letters, 2016, 117(17):178102.

[7] Gibiansky, M. L., Hu, W., Dahmen, K. A., Shi, W., & Wong, G. C. L., Earthquake-like dynamics in myxococcus xanthus social motility. Proceedings of the National Academy of Sciences 2013, 110 (6), 2330-2335.

[8] Berleman, J. E.; Zemla, M.; Remis, J. P.; Liu, H.; Davis, A. E.; Worth, A. N.; West, Z.; Zhang, A.; Park, H.; Bosneaga, E., Exopolysaccharide microchannels direct bacterial motility and organize multicellular behavior. Isme Journal 2016, 10 (11), 2620.

[9] Ma, L.; Jackson, K. D.; Landry, R. M.; Parsek, M. R.; Wozniak, D. J., Analysis of Pseudomonas aeruginosa conditional psl variants reveals roles for the psl polysaccharide in adhesion and maintaining biofilm structure postattachment. Journal of bacteriology 2006, 188 (23), 8213-8221.

[10]O'Toole, G. A.; Wong, G. C., Sensational biofilms: surface sensing in bacteria. Current opinion in microbiology 2016, 30, 139-146.