SHORT COMMUNICATION

The roles of biofilm matrix polysaccharide Psl in mucoid Pseudomonas aeruginosa biofilms

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Abstract
The opportunistic pathogen Pseudomonas aeruginosa causes life-threatening, persistent infections in patients with cystic fibrosis (CF). Persistence is attributed to the ability of these bacteria to form structured communities (biofilms). Biofilms rely on an extracellular polymeric substances matrix to maintain structure. Psl exopolysaccharide is a key matrix component of nonmucoid biofilms, yet the role of Psl in mucoid biofilms is unknown. In this report, using a variety of mutants in a mucoid P. aeruginosa background, we found that deletion of Psl-encoding genes dramatically decreased their biofilm formation ability, indicating that Psl is also a critical matrix component of mucoid biofilms. Our data also suggest that the overproduction of alginate leads to mucoid biofilms, which occupy more space, whereas Psl-dependent biofilms are densely packed. These data suggest that Psl polysaccharide may have significant contributions in biofilm persistence in patients with CF and may be helpful for designing therapies for P. aeruginosa CF infection.

Pseudomonas aeruginosa is an important opportunistic human pathogen that can cause life-threatening persistent infections in patients with cystic fibrosis (CF) and individuals with a compromised immune system. Persistence is due, in part, to the ability of this organism to form biofilms. Pseudomonas aeruginosa cells often become mucoid once they have colonized the CF lung. Mucoidy is caused by the overproduction of alginate that provides an advantage for P. aeruginosa in the airway of patients with CF (Ramsey & Wozniak, 2005). Psl is an essential matrix component that is required for nonmucoid P. aeruginosa to initiate and maintain biofilms (Ma et al., 2009), but it is not clear yet whether this will hold true in biofilms composed of mucoid P. aeruginosa.

To investigate whether Psl has any contribution to mucoid biofilm formation, we constructed a Δpsl strain, FRD3001, in an FRD1 background using the same deletion strategy for the PAO1-derived Δpsl strain WFPA800 (Ma et al., 2006). FRD1 is a mucoid P. aeruginosa isolate derived from a patient with CF (Ohman & Chakrabarty, 1981), and PAO1 is a nonmucoid wild-type strain. To determine the effect of psl deletion, the biofilms of FRD1-derived strains were grown in Jensen’s chemically defined media at room temperature in three-channel flow chambers (Stovall Life Science, INC) for 2 days. The biofilms were stained by membrane stain FM4-64 (1 μM final concentration, Molecular Probes, Invitrogen), imaged by Zeiss LSM510, and quantified by COMSTAT software as described previously (Ma et al., 2006). Similar to the Δpsl strain WFPA800, the mucoid Δpsl strain FRD3001 was unable to form a biofilm under flow conditions (Fig. 1, red panel). In contrast, biofilms formed by the parental FRD1 strain had an average biofilm thickness of 25 μm and 61-fold more biomass than that of FRD3001 (Fig. 1). These results indicate that Psl is also essential for the biofilm formation of mucoid P. aeruginosa.

The biofilm microcolonies of FRD1 were similar to those of the PAO1-derived mucoid strain PDO300 (Fig. S1B), yet different in architecture from microcolonies not expressing alginate, which were densely packed (Fig. 1 FRD870 biofilm and Fig. S1A). Consistent with previous reports (Purevdorj-Gage et al., 2005), the microcolonies
of mucoid strains had fewer bacteria but expanded more in depth, and the bacterial cells tended to form chains within and between microcolonies (Fig. 1 red panel and Fig. S1B). This may be due to the combined effects of Psl and alginate. Interestingly, the FRD1-derived \( \Delta \text{algD} \) strain FRD870, which was not able to produce alginate yet still expressed Psl, formed a densely packed biofilm (Fig. 1) with half the thickness of the FRD1 biofilm yet 12-fold more biomass. These data suggest that the overproduction of alginate leads to mucoid biofilms, which occupy more space, whereas Psl-dependent biofilms are densely packed.

To detect Psl in the biofilms of FRD-derived strains, we constructed Psl-inducible strains and utilized Psl-specific lectin HHA staining (FITC-HHA 100 \( \mu \)g mL\(^{-1}\), EY lab, INC) as previously described (Ma et al., 2007, 2009). Lectin staining results showed that FRD1 could produce some Psl polysaccharide (Fig. 1 green panel). Psl staining signal can be increased in Psl-inducible nonmucoid strain FRD2800 (Fig. 1, green panel) and mucoid FRD1 \( \text{P}_{\text{BAD}}\text{-psl} \) strain (data not shown). In contrast, no staining signal was detected in the \( \Delta \text{psl} \) strain FRD3001 (Fig. 1, green panel). This suggested that the Psl polysaccharide from mucoid FRD1 and nonmucoid PAO1 might have similar chemical structure detected by the lectin. Psl in FRD1 was associated with the bacterial surface as we observed in nonmucoid PAO1 (Fig. 1, merged panel), suggesting that the production of alginate did not affect the localization of Psl.

*Pseudomonas aeruginosa* strains isolated from the CF airway often lose motility and tend to be mucoid (Tart et al., 2006). Thus, nonmotile/mucoid bacteria contribute to *P. aeruginosa* biofilm persistence in patients with CF. In this report, we provide some evidence for how nonmotile and mucoid *P. aeruginosa* can manage to form biofilms. Besides polysaccharide, many other factors affect biofilm formation of nonmucoid *P. aeruginosa*, such as bacterial motility and quorum sensing (Karatan & Watnick, 2009). Mucoid strain FRD1 is nonmotile and has lost flagellum-mediated swimming motility and type IV pili-mediated twitching motility (Tart et al., 2006 and data not shown). Thus, it appears to depend more on polysaccharide to form a biofilm as we show in this report. We have shown that mucoid nonmotile *P. aeruginosa* depends on Psl to connect bacteria together to form a biofilm, which suggests that Psl may have significant contributions in biofilm persistence in patients with CF.

Our previous data showed that Psl provided cell–cell and cell–surface interactions, allowing *P. aeruginosa* to rapidly form a biofilm (Ma et al., 2006). Rapid biofilm formation may be critical for bacteria to resist killing by antimicrobials or engulfment by phagocytic cells. In this regard, the Psl matrix may afford protection for the *P. aeruginosa* cells prior to mucoid conversion. A recent report did show that *P. aeruginosa* Psl polysaccharide reduces neutrophil phagocytosis and the oxidative response (Mishra et al., 2012). In this study, we showed that Psl also had a key role in the formation of mucoid biofilms. *Pseudomonas aeruginosa* cells often become mucoid once they have colonized the CF lung (Ramsey & Wozniak, 2005). However, the initially colonizing strains are nonmucoid and likely express Psl/Pel (Colvin et al.,

<table>
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<tr>
<th>Strains</th>
<th>Biofilm</th>
<th>Psl</th>
<th>Merge</th>
<th>DIC</th>
<th>Biofilm max thickness (µm)</th>
<th>Biofilm Biomass (µm(^3)/µm(^2))</th>
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<td><img src="image7" alt="Merge" /></td>
<td><img src="image8" alt="DIC" /></td>
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<td>0.01±0.004</td>
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<td><img src="image10" alt="Psl" /></td>
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Fig. 1. Psl is necessary for mucoid *Pseudomonas aeruginosa* biofilm formation. Shown are biofilms of FRD1, FRD3001 (Δpsl), FRD870 (ΔalgD), and FRD2800 (ΔalgD, P_{BAD}-psl), which were grown for 2 days prior to staining with HHA-FITC (green, Psl matrix) and FM4-64 (red, biofilm). The biofilm biomass and maximum thickness were quantified by COMSTAT software (47). Bar represents 1 µm for FRD3001 and 5 µm for the other strains.
The role of Psl in mucoid biofilms

et al. (2011). The viscous alginate coating is believed to play a role in resistance to antibiotics and also to reduce the effectiveness of the host immune system (Nivens et al., 2001; Høiby, 2006). Coexisting mucoid and nonmucoid phenotypes can often be isolated from the sputa of the same patient with CF, and such isolates display a hypermutable phenotype and resistance to antibiotics. Nonmucoid P. aeruginosa cells are also released from mucoid biofilms in vivo (Høiby, 2006). This suggests that both mucoid and nonmucoid P. aeruginosa contribute to persistence in the CF lung. Two questions arise from these data: Is there an advantage of having both mucoid and nonmucoid cells present in the same community? How are these two types of cells organized in a single biofilm?

Our data show that the overproduction of alginate leads to mucoid biofilms, which occupy more space, whereas Psl-dependent biofilms are more densely packed. Yang et al. (2008) have studied the P. aeruginosa populations in CF sputum during a course of intravenous antibiotic therapy. They found that immediately following treatment, the P. aeruginosa isolates that remained are predominantly nonmucoid but 2 months after antibiotic therapy, the ratio of mucoid and nonmucoid returns to a ratio similar to that before treatment (85% nonmucoid and 15% mucoid). These results suggest that nonmucoid P. aeruginosa biofilms may persist in the CF lung by shielding themselves within mucoid bacterial cells. Mucoid bacteria could potentially protect nonmucoid biofilm from antibiotics and immune-mediated killing, leading to P. aeruginosa persistence in the CF lung. Recent reports show that Psl contributes to the antibiotic resistance of P. aeruginosa biofilms (Yang et al., 2011). Therefore, even if outer-layer mucoid bacteria of a biofilm were killed by antibiotics, the inner-layer nonmucoid bacteria may still resist antibiotics because of the presence of the Psl polysaccharide. Alternatively, mucoid P. aeruginosa and nonmucoid P. aeruginosa may colonize different niches in the CF lung. Nonmucoid bacteria persist because they evolve faster than mucoid bacteria (Lee, et al. 2011) and become more resistant to antibiotics.

Acknowledgements

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. The biofilm and corresponding Psl matrix of PAO1 and PAO1-derived mucoid strain PDO300.

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