Cyclic-di-GMP and type III secretion system are required for corneal infection by *Pseudomonas aeruginosa* and modulate host immunity

Joey Kuok Hoong Yam[1], Yang Liang[1,2], Michael Givskov[1,3]

[1]Singapore Centre for Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University. 60 Nanyang Drive, SBS-01N-27, Singapore 637551

[2]School of Biological Sciences, Nanyang Technological University, Singapore 637551

[3]Costerton Biofilm Center, Department of International Health, Immunology and Microbiology, University of Copenhagen, 2200 København N, Denmark

Email: joeyyam@ntu.edu.sg

KEY WORDS: *Pseudomonas aeruginosa*, *in vivo* biofilms, bacterial keratitis, transcriptomics, cyclic-di-GMP, type III secretion system.

Biofilms are extremely tolerant toward antimicrobial treatment and host immune clearance due to their distinct physiology and protection by extracellular polymeric substances [1]. Bis-(3´-5´)-cyclic dimeric guanosine monophosphate (cyclic-di-GMP) is an essential messenger that regulates biofilm formation by a wide range of bacteria [2]. However, there is a lack of physiological characterization of biofilms *in vivo* as well as the roles of cyclic-di-GMP signaling in mediating host-biofilm interactions. Here, we employed dual RNA-Seq to characterize the host and pathogen transcriptomes during *Pseudomonas aeruginosa* infection using a mouse keratitis model [3]. *In vivo P. aeruginosa* biofilms maintained a distinct physiology compared with *in vitro P. aeruginosa* biofilms, with enhanced virulence and iron uptake capacity. Intracellular cyclic-di-GMP was increased in *P. aeruginosa* cells *in vivo*, potentially due to down-regulation of the expression of several phosphodiesterases (e.g., DipA, NbdA). Increased intracellular cyclic-di-GMP levels and activation of the type III secretion system were found to be required for long-term ocular colonization of *P. aeruginosa* and modulation of the host innate immunity.

References

1. Flemming, H.-C., et al., *Biofilms: an emergent form of bacterial life.* Nature Reviews Microbiology, 2016. **14**(9): p. 563-575.
2. Hengge, R., *Principles of c-di-GMP signalling in bacteria.* Nature Reviews Microbiology, 2009. **7**(4): p. 263-273.
3. Saraswathi, P. and R.W. Beuerman, *Corneal Biofilms: From planktonic to microcolony formation in an experimental keratitis infection with Pseudomonas aeruginosa.* The ocular surface, 2015.