Mucoid Variants Alter the Intercellular Interaction of *Pseudomonas aeruginosa*

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Chapter 1 Introduction

Most microbes exist in environment not as an individual cell, but as microbial communities. It is known that many bacteria doing cell-to-cell communication with each other using signaling molecules (Bassler, 2002). Bacteria release certain kind of signaling molecule and receive it by a specific receptor to do cell-to-cell communication (Bassler, 2002). Quorum sensing (QS) is a kind of cell-to-cell communication. In QS, gene expressions are regulated when the signaling molecules reach a threshold concentration (Bassler, 2002). Various bacterial activities are regulated by QS such as biofilms formation, motility and denitrification (Davies *et al*., 1998; Boles *et al*., 2005; Toyofuku *et al*., 2007). During this process, it is important for the signaling molecules to reach the cells and to be received by its cognate receptor. However, it is reported that there are various kind of extracellular matrix such as extracellular DNA, protein, and polysaccharide exist outside of bacteria (Flemming and Wingender, 2010). And certain barriers may prevent the signals from reaching the cells, while the influence of extracellular matrix on cell-to-cell communication has scarcely been studied.

In this study, we examined the influence of extracellular matrix overproducer on the signaling molecules response of the bacterial community. *Pseudomonas aeruginosa* mucoid variant which overproduce extracellular polysaccharide (EPS) alginate was used in this study. Mucoid variant is frequently isolated from the sputum of heredity disease cystic fibrosis (CF) patients and coexist with QS positive WT strain in CF lung (Govan and Deretic, 1996). *P.aeruginosa* produces two kinds of AHL signal: N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) and N-butyryl-L-homoserine lactone (C4-HSL) (Jimenez *et al*., 2012). In addition to the AHLs, *P. aeruginosa* produces a quinolone signal: 2-heptyl-3-hydroxy-4-quinolone, also called *Pseudomonas* quinolone signal (PQS) (Jimenez *et al*., 2012). The immediate precursor of PQS 2-heptyl-4-quinolone (HHQ), is also reported to act as signaling molecule (Jimenez *et al*., 2012). In this study, the effect of alginate on the signaling response to each signals were examined.

Chapter 2 Results

Since it is reported that the signaling molecules auto-positive regulate the transcription of its own synthesis gene, the synthesis gene promoter fused EGFP reporter plasmids were used to examine the signaling response in this study. In order to avoid the self-signaling, the signal production defected strains were generated and were transformed the reporter plasmids. First, the response to quinolone signals was examined. As the result, the mucoid variant almost lost the response to PQS and HHQ compared to WT and it is indicated that the overproduction of alginate in mucoid variant interfering the response to PQS and HHQ. PQS system regulate various virulence production in *P.aeruginosa*. Therefore, we further confirm the interfering of alginate overproduction on quinolone signals response by measure the pyocyanin production that induced by quinolone signals. Since *P.aeruginosa* also produce 2 kinds of AHL signal, then we examine the effect of alginate on the response to AHL signal. The signaling molecule synthesis genes of each strain were deleted and then we added each kind of signal into the medium to examine their response to each kind of signal. Because the expression of signaling molecule production genes are upregulated by their own products, we measured their promoter activity with reporter vectors to assess the activity. It is indicated that alginate production have no effect to *N*-acyl-L-homoserine lactones (AHLs).

Mucoid strain is well known to derive non-mucoid suppressed revertants. The suppressed revertants usually exhibit the same phenotype to WT strain. Therefore we isolated an isogenic non-mucoid suppressed revertant from mucoid variant that lose alginate overproduction phenotype and examine its quinolone signals response. It is indicated that non-mucoid suppressed revertants restored the quinolone signals response.

Also, the co-cultures of mucoid variants and the WT were perform to see the effect of alginate production on the alginate non-producing neighbor cells and the effect of the elements released from WT on the signaling response of mucoid variant. It is indicated that the inhibition of quinolone signaling by alginate was limited to the alginate overproducer and had no effect on the alginate non-producing neighbor cells and the mucoid variant did not recover the response to quinolone signal in the co-culture with WT.

Chapter 3 Discussion

This study found that the alginate production alter the signaling response. In previous study, it is indicated that mucoid variant decrease the production of PQS, C4-HSL, and 3-oxo-C12-HSL (Ryall *et al*., 2014). This study further indicated that the alginate production only interfere the response to quinolone signals, but had no effect upon AHL response. Therefore, mucoid variant lose the response to the exogenous quinolone signals but remain the response to AHLs. It is reported that the 3-oxo-C12-HSL receptor defected strains have been isolated from CF sputum where mucoid variant is well isolated (Ciofu *et al*., 2010; Hoffman *et al*., 2009). Besides, it is reported that the defect of PQS response would contribute to the fitness advantage to WT (Wilder *et al*., 2011). Therefore, the PQS blindness caused by alginate may contribute to the fitness advantage in CF. It has been considered that the cells in the microbial communities respond to signaling molecules synchronously and do cell-cell communication. Since cell-to-cell communication regulate various behavior of *P.aeruginosa*, it is considered that this change of the signaling response of mucoid variant may result in different downstream behaviors when it emerge in a signaling-positive WT community. The emergence of such QS un-synchronal strain may contribute to the survival of the community when it faces various environmental changes. The present study adds a new function of alginate in cell-to-cell communication that may have a great impact on bacterial communities.

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