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Involvement of a respiratory terminal oxidase in *Pseduomonas aeruginosa* biofilm formation

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Keywords: *Pseudomonas aeruginosa*, biofilm formation, terminal oxidase

【Objective】 Biofilm is a microorganisms' habitat and it sometimes cause adverse effect against humans. For regulating the biofilm formation, how microorganisms gain energy and grow in biofilms should be investigated. Interestingly, *Pseudomonas aeruginosa*, a model used in this reserch, is suggested to possess five terminal oxidases to achieve energy aerobically. Here, we focused on the function of two homologous terminal oxidase, named cbb_3-1 oxidase and cbb_3-2 oxidase, respectively, in biofilm formation.

【Materials and Methods】 *Pseudomonas aeruginosa* Wild type (WT) strain, cbb_3-1oxidase mutant strain(N1), cbb_3-2 oxidase mutant strain(N2), and cbb_3-1,2 oxidase double mutant strain(N12) were inoculated to 96 well microtiter plate for biofilm formation assay. Planctonic growth was analyzed by using hungate tube. All experiments were done in denitrifying conditions.

【Results and Discussion】 Biofilm formation did not change between WT strain, strain N1and N2, but significantly decreased in strain N12. However, planctonic growth assay showed that growth of strain N12 exceeded that of WT strain. These results suggest that these cbb_3 type oxidases are greatly related in biofilm formation in denitrifying conditions. We are now investigating the effect of the terminal oxidases on adhesion to a solid surface, and on motility.

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The contribution of *rpo*S to formation of *Escherichia coli* biofilms

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Key words: biofilms, gene expression, *rpoS*, *Escherichia coli*

Introduction. The creation of starved, stationary phase-like zones in biofilms seems to be an important factor for biofilm formation. Our previous study revealed that *Escherichia coli* cannot form mature biofilms without *rpoS* gene, and the gene expression patterns of wild type biofilms were different from that of *rpoS* mutant biofilms. In this study, global gene expression changes and localized gene expression of the *rpoS* during biofilm formation were analyzed. **Materials and Methods.** Gene expression analysis was performed with biofilm cells harvested at the three time points during biofilm formation process. For the observation of *rpoS* gene expression during biofilm formation, the green fluorescent protein (GFP) was inserted into the downstream of the *rpoS* gene. The biofilm formation experiments were performed in the flow system, and the *rpoS* gene expression was observed. **Results and Discussion.** The gene expression analysis revealed that the expression patterns of maturaion process were different from that of attachment and colony formation processes. Genes involved in the energy metabolism and motility were induced at attachment process, and genes involved in the stress response were induced at maturation process. The observation of *rpoS* gene expression revealed that the *rpoS* gene was expressed in most of the cells at attachment and colony formation processes but expressed only in the cells outside of the biofilms at maturation process, which suggested that the region of the *rpoS* gene expression moved as the thickness of biofilm increased.

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