**Biofilm and surface microbiology バイオフィルム・界面**

**PM-01**
Dispersal of *Pseudomonas aeruginosa* biofilms at oxygen levels change

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酸素条件下の変化による細菌バイオフィルムの脱離
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Key word: *Biofilm, Pseudomonas aeruginosa, oxygen*

Bacteria have an ability to migrate to better habitat, which is called chemotaxis. The mechanism was mostly studied in the form of planktonic. However, in natural environment, a lot of bacteria are present in the form of biofilms, which consist of sessile bacteria embedded within a hydrated extracellular matrix. In recent studies, it is reported that the biofilms also have an ability to migrate called dispersion in which the mechanism is different from chemotaxis. *Pseudomonas aeruginosa* is well-known for forming biofilms and ability to live both aerobic and anaerobic. In this study, we newly found that oxygen levels change induces significant *P. aeruginosa* biofilms dispersion. Therefore, we aimed to investigate the mechanism of dispersion. By using crystal violet staining method and confocal laser microscopy, we demonstrated that extracellular DNA, which is one of the biofilms components, decreased with dispersion. Moreover, the supernatant extracted during dispersal phase induced more disassembly of *P. aeruginosa* biofilms. These results suggest that when the oxygen levels are low, they secrete disassembly factor, which includes nucleases, and disperse in order to migrate to better habitat.

**PM-02**
Analysis of the mechanism of *Pseudomonas aeruginosa* biofilm formation under anaerobic conditions

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嫌気脱窒環境下における細菌のBiofilm形成メカニズムの解析
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気液界面と生命環境。

Key word: *Biofilm, anaerobic, Pseudomonas aeruginosa, EPS*

Microorganisms do not live as pure cultures of dispersed single cells but instead accumulate at interfaces to form polymicrobial aggregates such as biofilms. Biofilms are comprised of microorganisms enmeshed in extracellular polymeric substances (EPS), which protect organisms from antibiotics and ultraviolet radiation. Ecologically, competition and cooperation in the confined space of the EPS matrix lead to a constant adaptation of the population. Biofilm formation has been intensively studied in *Pseudomonas aeruginosa*. However, most of the studies of biofilm formation have been performed under aerobic conditions. *P. aeruginosa* can use nitric oxides as alternative electron acceptors to produce energy when oxygen is depleted. Recently, we showed that under anaerobic conditions, cell shape and biofilm structure are different from aerobic conditions. These changes led us to question how the biofilm development process differs from aerobic conditions. In this study, it was observed that the lifecycle of biofilm under anaerobic conditions was the same as aerobic conditions. However, EPS production was observed at the bottom layer of anaerobic biofilms which localization was not observed in aerobic biofilms. These results indicate that the role of EPS under anaerobic conditions may differ from aerobic conditions.

**PM-03**
*Cbb3*-type cytochrome *c* oxidase in aerobic respiration regulates anaerobic dentifying growth and biofilm-like aggregate formation of *Pseudomonas aeruginosa*

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好気呼吸において機能する*Cbb3*型シトクロム *c* オキシダーゼが嫌気脱窒環境下における細菌の増殖及びバイオフィルム様凝集体形成を制御する
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Key word: *Respiration, Electron flow, Pseudomonas aeruginosa, Biofilm, Extracellular DNA*

Major ubiquitous bacterium *Pseudomonas aeruginosa* possesses a highly branched respiratory electron transport chain terminated by aerobic five terminal oxidases. Still, it is little known about the physiological functions. In this study, we focused on *Cbb3*-type cytochrome *c* oxidase. In a variety of bacteria, *Cbb3* oxidase is known as a crucial enzyme in oxygen respiration, while it has been indicated that *Cbb3* oxidase might also function under anaerobic environments. Therefore, we explored the roles of *Cbb3* oxidase in life cycles of *P. aeruginosa* under anaerobic conditions.

When double deletion mutant of *Cbb3* oxidases of *P. aeruginosa* and wild type were cultured under anaerobic dentifying conditions, the *Cbb3* mutant reduced N-oxides such as NO₂, NO, N₂O and grew better than wild type. Interestingly, wild type formed many aggregates and *Cbb3* mutant did not. Staining of the aggregates with SYTO6 and propidium iodide suggested that the aggregate is a complex of live cells, dead cells and extracellular DNA, previously shown to be a component of biofilm matrix. Compared with the culture supernatant of *Cbb3* mutant, wild type contained a large amount of extracellular DNA.

These findings suggest that aerobic terminal oxidase *Cbb3* of *P. aeruginosa* possesses unique physiological functions by which positively inhibit anaerobic growth and promote biofilm-like aggregate formation.

**PM-04**
Growth, mutation frequency and biofilm formation of *Escherichia coli* cells exposed to imidazolium ionic liquids

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イミダゾリウム系イオン液体が大腸菌の生育、変異率及びバイオフィルム形成に及ぼす影響
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気液界面と生命環境。

Key word: *Ionic liquid, Imidazolium, Biofilm, Escherichia coli*

Ionic liquids, salts in the liquid state at room temperature, are the new class of solvents required for various industrial applications. Many ionic liquids have been synthesized and extensively studied for their applications. We analyzed the growth, mutation frequency and biofilm formation of *Escherichia coli* cells exposed to low concentrations of imidazolium ionic liquids such as 1-ethyl-3-methylimidazolium tetrafluoroborate (EMImBF₄). Growth rate of *E. coli* cells incubated with 0.001% EMImBF₄ was almost the same as that of cells incubated without EMImBF₄. SEM analysis showed that the shape of *E. coli* cells exposed to 0.001% EMImBF₄ was almost the same as that of control cells. There was no significant difference in rate of mutation to rifampicin resistance between EMImBF₄-exposed cells. These results indicated that *E. coli* cells grew normally in the presence of 0.001% EMImBF₄. Amounts of biofilms formed by *E. coli* cells in the presence or absence of 0.001% EMImBF₄ were analyzed by SEM. Experimental data showed that *E. coli* cells formed less biofilms when incubated with the agent compared to cells incubated without the agent. Our experimental results indicated that EMImBF₄ affected biofilm formation even at low concentrations harmless for bacterial growth and mutagenesis. Investigation of the effects of other ionic liquids on biofilm formation is in progress.