氏　　名	竹村　彩
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学位論文題目	The natural transformation in *Staphylococcus aureus* （黄色ブドウ球菌における自然形質転換）

	（職名）	（学位）	（氏名）
主　　査	筑波大学教授	博士（医学）	渋谷　彰
副　　査	筑波大学教授	博士（人間・環境学）	森川　一也
副　　査	筑波大学准教授	医学博士	竹内　薫
副　　査	筑波大学教授（グローバル教育院）	Ph.D.	Derrick Roy Robinson

論文の要旨
Abstract of thesis

Purpose

Staphylococcus aureus (*S. aureus*) is a commensal bacterium that behaves as an opportunistic pathogen. Its genome possesses various kinds of mobile genetic elements carrying pathogenic factors and antibiotic resistance genes. This thesis focuses on one of the horizontal gene transfer (HGT) systems, the natural transformation, which had not been demonstrated and had argued for a long time in *S. aureus*.

Previous study showed that alternative RNA polymerase sigma factor, sigma factor H (SigH), transcribes the operons for competence machineries required for the natural transformation. There are two distinct SigH expression mechanisms. SigH expresses via the duplication of *sigH*, generating a chimeric gene with the downstream gene. The expression frequency was 10^{-5}. Another SigH expression mechanism involves post-transcriptional regulation. It requires specific growth condition, a complete synthetic medium (CS2), suggesting that environmental factors seem to be important for the SigH expression. Using CS2 medium and the SigH over-expressing strain, we developed transformation protocol. However, the strain initially used for this assay carried a lysogenic phage. Considering the other phage dependent HGT systems and the impact to propose the natural transformation in *S. aureus* for the first time, it was necessary to demonstrate the independency from phage factors.

Mentioned above, environmental factors are thought to be required for SigH-mediated transformation. Cell wall-affecting antibiotics were also found to affect the SigH-mediated transformation. However, there is limited information for modulating elements of the transformation.

This thesis aimed to demonstrate of the natural transformation in *S. aureus*, and reveal the environmental factors modulating it.

Methods

1) The demonstration of the natural transformation in *S. aureus*

To address this issue, the applicant developed a DNA transfer system that does not contain any phage component and constructed a phage-eliminated strain with the pMAD system. She performed plasmid and genomic transformation assays with the phage-eliminated strains. Furthermore, she also performed a pseudo-transformation assay to distinguish from pseudo-transformation.

2) Environmental factors modulating the natural transformation

To address this issue, the applicant tested the effect of an autolysis inhibitor, sodium polyanethol sulfonate (SPS), on the transformation. Furthermore, she also tested the effect of physical disruption by silica beads, or lysostaphin, an enzyme that cleaves *S. aureus* cell wall to address whether the increase in transformation with SPS is simply attributed to the physically disturbed cell wall.

Result and Discussion

1) The demonstration of the natural transformation in *S. aureus*

The applicant found that SigH mediated transformation of plasmid DNA did not require the phage factors, indicating that *S. aureus* cells expressing SigH develop the genuine natural transformation. She also confirmed genomic DNA transformation without phage factors. Pseudo-transformation assay showed that the operons for competence machineries were not required for phage-mediated transformation, in contrast to SigH mediated transformation. Together, her observation demonstrated for the first time that natural genetic competence develops in a SigH-dependent manner in *S. aureus*, allowing transformation by extracellular plasmid or chromosomal DNA as well as HGT between different strains. This was first evidence for the natural transformation in *S. aureus*.

2) Environmental factors modulating the natural transformation

The applicant found that the DNA transformation efficiency varied depending on culture conditions and that cells grown in the CS2 medium exhibits enlarged morphology with disintegrated cell walls. Notably, SPS facilitated transformation in CS2 medium in a dose-dependent manner. She also found that the physical disruption of the cells did not increase the transformation efficiency in TSB medium, suggesting that the increase cannot simply be attributed to the damages in the cell wall alone. This study added new information to *S. aureus* natural transformation and supports the idea that cell wall metabolism plays an important role in the DNA incorporation process by the competence machinery expressed by SigH.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant demonstrated the natural transformation in *S. aureus* and environmental factors modulating the natural transformation. Based on this study, she proposed a unique model for staphylococcal competence regulation, which can help explain the acquisition of antibiotic resistance genes through HGT in this important pathogen.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 12 September, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded a Doctor of Philosophy in Human Biology.