| | |
|---|---|
| 氏　　名 | NGUYEN THI LE THUY |
| 学位の種類 | 博士（　人間生物学　） |
| 学位記番号 | 博甲第　8346　号 |
| 学位授与年月 | 平成　29 年　7 月　25 日 |
| 学位授与の要件 | 学位規則　第４条第１項該当（昭和２８年４月１日文部省令第９号） |
| 審査組織 | グローバル教育院 |
| 学位論文題目 | Study on Horizontal Transfer of Drug Resistance in *Staphylococcus aureus*<br>(黄色ブドウ球菌における薬剤耐性の水平伝達の研究) |

| | （職名） | （学位） | （氏名） |
|---|---|---|---|
| 主　査 | 筑波大学教授 | 医学博士 | 柳沢　正史 |
| 副　査 | 筑波大学教授（連携大学院） | 医学博士 | 狩野　繁之 |
| 副　査 | 筑波大学教授（連携大学院） | 獣医学博士 | 宮田　桂司 |
| 副　査 | 筑波大学教授 | 博士（人間・環境学） | 森川　一也 |

## 論文の要旨
## Abstract of thesis


**Purpose**

*Staphylococcus aureus* is the leading cause of nosocomial infections in hospitals and healthcare settings. Its ability to rapidly acquire the resistance to antimicrobial compounds by horizontal gene transfer (HGT) has been the global concern. There are three major mechanisms responsible for HGT in bacteria: conjugation, transduction and transformation. Conjugation is responsible for the transfer of DNA from one living cell to another and is mediated by special structures including conjugative pili. Transduction is mediated by bacterial virus (bacteriophage), and is considered as the major route to acquire new DNA in *S. aureus*. Transformation is through the direct uptake of free DNA by the cells that developed the ‘competence’ state. Although transduction and conjugation have been studies for decades, natural transformation was only recently described in *S. aureus*, and little is known about its regulatory mechanisms. The finding of the natural transformation also raised the necessary to re-evaluate the evolutionary ability of *S. aureus*. In this context, this study aimed to clarify some aspects of the HGT in *S. aureus*. First, the applicant aimed to specify the major transmission modes of the

linezolid resistance gene, *cfr* (chloramphenicol/florfenicol resistance), from *Staphylococcus epidermidis* strains isolated in Spanish hospitals to *S. aureus* strains isolated in Japan in which *cfr* has not been detected. Second, the applicant explored for the factor(s) that affect the natural transformation, focusing on a series of antibiotics.

**Results**

***Dissemination of linezolid resistance through HGT***

To investigate the conjugative transmission of *cfr* gene, 16 clinical MRSA (Methicillin-resistant *S. aureus*) isolated in Japan and two *S. epidermidis* strains (SE45 and SE50) from Spanish hospital were used as recipients and donors respectively. The pSCSF7-like plasmid carrying *cfr* gene responsible for the resistance was transferred from the clinical *S. epidermidis* isolates to a series of MRSA strains by filter mating method. The transmission efficiencies was different depend on the donor as well as on the recipient.

The applicant further tested whether the plasmid would be able to disseminate among *S. aureus* strains. Although the conjugation was previously believed as the only mechanism of the *cfr* dissemination, the results demonstrated that transduction could be considered as an alternative pathway for the *cfr* transmission among MRSA strains. Importantly, the whole plasmid was transferred in these transmissions. The natural transformation of *cfr* was undetectable, suggesting its low relevance in the *cfr* transmission, although there might be unknown conditions more suitable for detecting natural transformation.

***Antibiotics modulate transformation***

In *S. aureus*, the natural competence is regulated by a sigma factor – SigH, which is tightly controlled to be expressed in a minor subpopulation. In the competence-inducing medium, transformation frequency in the wild type strain is hardly detectable ($<10^{-11}$) and is low even if SigH was artificially overexpressed (~ $10^{-9}$). Taking into account that *S. aureus* has a prominent ability to swiftly acquire resistance, the applicant considered that there could be the favorable conditions to develop competence for transformation, and investigated the effect of antibiotics on the transformation efficiency in SigH-expressing strain (N315ex w/oφ h). In this strain, the prophage was eliminated to exclude the possibility of phage-dependent “pseudo-competence” DNA transfer, which is distinct from the natural competence. SigH is expressed by a plasmid, pRIT-sigH. Strikingly, the applicant found that cell wall-affecting antibiotics such as bacitracin, fosfomycin and vancomycin, but not β-lactams, increased the transformation frequency. Mitomycin C suppressed transformation in a concentration-dependent manner. Ciprofloxacin, norfloxacin and streptomycin had no significant effect. Further investigations on the external physical damages to the cell wall (by silica beads or lysostaphin, an enzyme that cleaves *S. aureus* cell walls) did not facilitate the transformation. The results suggested that effects of cell wall-affecting antibiotics on transformation might involve certain complex cellular activity.

**Discussion**

The results obtained in this study showed that the *cfr*-carrying pSCFS7-like vectors could be efficiently transferred to clinical MRSA in Japan, the country in which this gene has not been detected. Thus, the result emphasizes the importance of the surveillance programs aiming at the early detection of the *cfr* gene in both *S. aureus* and the non-pathogenic bacteria such as coagulase-negative staphylococci including *S. epidermidis*, since they are considered to be the genetic reservoir available for *S. aureus*.

Regarding the natural transformation, complex mechanisms participate in the control of the competence development in species-specific manners, which have been well studied in model bacteria such as *Streptococcus* and *Bacillus*. In *S. aureus*, it was recently found that SigH plays a key role in competence development, but many aspects of the regulation in competence development and the following transformation are not known. As part of the efforts to gain insight into the regulation after SigH expression, the present study firstly showed the effects of cell wall-affecting reagents on transformation. The positive effects of cell wall-targeting antibiotics were not simply caused by damaging the cell wall integrity. Therefore, the involvement or certain cellular response(s) was predicted in the regulation of competence and transformation.

The long-term goal in the studies of antibiotics resistance and their dissemination is to conquer the problem of antimicrobial resistance. To prevent and stop the dissemination of resistance, it is necessary to take it into account not only the dissemination among human society, but also the livestock and the environment, under the concept of “One health”. The present study provided the experimental evidence on the HGT mechanism regarding the *cfr* transmission, strongly supporting the idea that CNS species serves as the genetic pool of the antibiotics resistance. The finding of the effects of antibiotics on the natural transformation has a potential to be applied for the control of *S. aureus* evolution, but of course it requires further extensive studies.

## 審査の要旨
## Abstract of assessment result

【批評 Review】

The applicant provided experimental evidence that the conjugation-like system and the phage-mediated transduction are the potential transfer mechanisms of the linezolid resistance gene among staphylococcal species. Considering the high evolutionary potential of *Staphylococcus aureus*, the finding is relevant to predict the further dissemination of the linezolid resistance gene, which has not been detected in our country. The dissertation also describes the effects of antibiotics on another transfer mechanism, natural genetic transformation, which was recently demonstrated by her group. Although the transformation assay was carried out using the competent cells that artificially overexpress the transformation regulator, the finding that some antibiotics could suppress or facilitate the natural transformation implicated the potential effects of prescription on the *S. aureus* evolution. Further studies on horizontal gene transfer, including the mechanisms how antibiotics affect the natural transformation, would reveal the uncanny evolutionary ability of this important human pathogen.

【最終試験の結果 Result】
The final examination committee conducted a meeting as a final examination on 8 May, 2017. 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】
The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.