論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Analysis of phage resistance mechanism of Staphylococcus aureus SA003 which causes bovine mastitis against phages ϕSA012 and ϕSA039
著者(和文)	アア ハエルマン アザム
Author(English)	Aa Haeruman Azam
出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第11103号, 授与年月日:2019年3月26日, 学位の種別:課程博士, 審査員:丹治 保典,山本 直之,廣田 順二,小倉 俊一郎,堤 浩
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第11103号, Conferred date:2019/3/26, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨
THESIS SUMMARY

専攻： Department of	Bioengineering 専攻	申請学位（専攻分）： Academic Degree Requested	博士 Doctor of Engineering
学生氏名： Student's Name	Aa Haeruman Azam	指導教員（主）： Academic Supervisor(main)	丹治保典
		指導教員（副）： Academic Supervisor(sub)	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words）

The doctoral thesis is titled "Analysis of phage resistance mechanism of *Staphylococcus aureus* SA003 which causes bovine mastitis against phages ϕSA012 and ϕSA039", and consists of six chapters including conclusion and prospective.

Chapter 1, General Introduction. *Staphylococcus aureus* is one of the most frequent agents of bovine mastitis. The use of antibiotic as a standard treatment has led to the emergence of antibiotic-resistant strains. One alternative strategy to control *S. aureus* is to exploit lytic phages as an agent to kill the bacteria. Wall teichoic acid (WTA) is famous as a phage receptor in *S. aureus*. Staphylococcal phages utilize the backbone of WTA and/or *N*-acetyl glucosamine (GlcNAc) residues consist of α-GlcNac and β-GlcNac which transfer by TarM and TarS, respectively. In the evolution of WTA, the backbone is preserved in all *S. aureus* strains while one of the GlcNac residues is often missing (e.g. RN4220 has both GlcNAc residues but SA003 naturally lacks of α-GlcNAc). Staphylococcal Twort-like phages (including ϕSA012 and ϕSA039) are well known to utilize the backbone of WTA hence expected to have wide host range. ϕSA012 and ϕSA039 is similar in genomic level yet showing different host preference. The detail host recognition mechanism of those phages is unknown to date.

Chapter 2, Whole genome sequencing of phage-resistant SA003. The whole genome of phage resistance SA003 co-cultured with ϕSA012 was analyzed in this chapter. The study showed that most of mutated genes product were linked to the cell wall synthesis including WTA. One mutated gene encodes RNA polymerase alpha subunit which may contribute in the inhibition of post-adsorption of the phage.

Chapter 3, mutated host-genes are linked to inhibition of phage-adsorption and post-adsorption. The complementation study was conducted to confirm the role of mutated genes in phage resistance. The products of six genes were linked to phage adsorption efficiency while two genes were linked to post-adsorption. Mutation in two WTA-related gene products (TagO which transfers WTA linker and TarS which transfers β-GlcNAc residue into WTA) were manifested by the decrease of WTA production. While mutation in other cell wall-related genes were manifested by sliminess suggestive capsular polysaccharide and total sugar of whole cell.

Chapter 4, Phages ϕSA012 and ϕSA039 use different receptor in *S. aureus* cell. I used the phage-resistant SA003 and various deletion mutants of *S. aureus* to compare the host recognition mechanism of ϕSA012 and ϕSA039. Even though the phages belong to Twort-like group, ϕSA039 exhibited different host recognition mechanism compare to current well known phages. ϕSA039 utilized β-GlcNAc residue and the backbone of WTA in which β-GlcNAc residue was used as the main receptor. Twort-like phages are well known to use backbone of WTA as their receptor; therefore, further investigation of β-GlcNAc-dependent adsorption of ϕSA039 was necessary.

Chapter 5, Mutant ϕSA039 with point mutation in ORF100 is able to infect Tar-null *S. aureus*. I generated spontaneous mutants of ϕSA039 which was able to infect TarS-null *S. aureus* (lacks β-GlcNAc residue in WTA). I found that mutation in ORF100 of ϕSA039 was the key factor for this infectivity. However, the mutation in ORF100 caused inability of the phage to infect RN4220 (which has α-GlcNAc residue in the WTA). By deleting TarM gene in RN4220, the susceptibility against ORF100-mutated mutant phage was restored. Additional mutation in ORF102 in ORF100-mutated phage could restore the infectivity of the phage toward RN4220. Therefore, I concluded that mutation in ORF100 enabled ϕSA039 to infect TarS-null *S. aureus* and additional mutation in ORF102 restored the infectivity of ORF100-mutated mutants of ϕSA039 toward *S. aureus* harboring α-GlcNAc residue in the

WTA. ORF100 and ORF102 are likely ideal “host-spots” for artificial modification of the phage for future application.

Chapter 6, Conclusion and perspective. This study revealed that *S. aureus* SA003 developed phage resistance mechanism through inhibition of phage adsorption and post-adsorption. Staphylococcal phages utilize WTA component as their receptors hence make us easy to categorize them in the certain group (e.g. *Myoviridae* group is well known to use backbone of WTA). However, ϕSA039 (a member of *Myoviridae* Twort-like phages) utilizes not only the backbone but also β-GlcNAc residue as its receptors, therefore, generalizing the Twort-like phages by observing certain representative species may lead to misunderstanding. The glycosylation of WTA is varied in *S. aureus* and may determine their susceptibility toward phages. Thus, more detail study on host-recognition mechanism of staphylococcal phage using more species of phages is important before applying phage therapy. Noteworthy, I identified that ϕSA039 was infective toward other *Staphylococcus* species which have different WTA structure compared with common *S. aureus* lineage. The phage may able to utilize other glycoepitope in other type of WTA backbone which make the phage to has broad host range of *Staphylococcus* bacteria.