論文 / 著書情報
Article / Book Information

題目(和文)	ファージΦMR003の宿主認識機構と黄色ブドウ球菌制御への応用
Title(English)	Host-recognition mechanism of phage ϕMR003 and its application to controlling of Staphylococcus aureus
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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	Life Science and Technology	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	（Engineering）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

The doctoral thesis is titled "Host-recognition mechanism of phage ϕMR003 and its application to controlling of *Staphylococcus aureus*", and has six chapters including conclusion and perspectives.

Chapter 1, General Introduction. *Staphylococcus aureus* is one of the serious human pathogens and the use of antibiotics as a standard treatment has led to the emergence of antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is a multi-drug resistant *S. aureus* wide-spreading in both healthcare and community settings worldwide. In order to control this pathogen, the bacteriophages have been proposed as an alternative therapeutic agent to the antibiotics. The broad host range phage is an important criterion for the phage therapy candidate. However, the number of the broad host range phage against MRSA is limited. Moreover, host-recognition mechanism of the phage candidate is important to deepen understanding its potential for the success of the phage therapy.

Chapter 2, Evolution of *S. aureus* in the presence of the antibiotic. The acquisition of antibiotic-resistant in *S. aureus* is not only through horizontal gene transfer but also through spontaneous mutation in its chromosome. Through stepwise repeated batch culturing of *S. aureus* with increasing concentration of oxacillin for 17 rounds, the sensitive *S. aureus* strain developed into the resistant strain through acquiring the spontaneous mutation in genes associated to cell wall synthesis that is the main target of the oxacillin.

Chapter 3, Isolation and characterization of MRSA infectious phage. The rapid resistant development of *S. aureus* to antibiotic has limit treatment option for *S. aureus* infection. Therefore, the therapeutic agent such as phage is a suitable strategy to combat MRSA. Phage which is strictly lytic and possesses a broad host range is an ideal phage therapy candidate. In this chapter, ϕMR003 was isolated from a municipal wastewater treatment plant in Tokyo. ϕMR003 infected 97% of MRSA strains that originated from humans (i.e. 101 out of 104 MRSA strains of human origin). In contrast, ϕSA012 and ϕSA039 (previously isolated phages that have broad host range against bovine mastitis *S. aureus*) infected 73% and 57% of human

origin MRSA, respectively. Based on the whole-genome analysis and comparison to the phage database, ϕMR003 belongs to the genus *Silviavirus* which has not been well-studied extensively.

Chapter 4, Host-recognition mechanism of phage ϕMR003. The adsorption assay between ϕMR003 and wall teichoic acid (WTA)-deficient RN4220 strains was conducted. The disruption of *tar*O gene (encoding transferase responsible for the initiation of the WTA synthesis) in RN4220 resulted in the significant reduction of adsorbed ϕMR003 onto the host cell. Moreover, the modification of either β-GlcNAc and α-GlcNAc residues (transferred by TarS and TarM transferase) on the WTA did not affect the adsorption of ϕMR003. Therefore, ϕMR003 recognizes and binds to WTA of the *S. aureus* during infection. In silico comparisons of the genomes of ϕMR003 and ϕSA012 revealed that ORF117 and ORF119 of ϕMR003 are homologs to the receptor binding proteins (RBPs) ORF103 and ORF105 of ϕSA012, with amino acid similarities of 75% and 72%, respectively. The differences in tail and baseplate proteins of both phages were detected which are key contributing factors to the different host specificities of ϕMR003 and ϕSA012.

Chapter 5. Synergistic effects of ϕMR003 and antibiotic on control of MRSA. The drawback of using a single antibiotic or phage is the selection of the resistant strains. In order to overcome this problem, combination treatment is suggested to be more effective than the single treatment. When ϕMR003 was combined with oxacillin by either adding both agents simultaneously or sequentially, the significant reduction of the viable cell compared to the single treatment was observed. In the single phage or oxacillin treatment, the emergence of resistant strain was observed but not in the combined treatment.

Chapter 6, Conclusion and perspectives. *S. aureus* can easily develop resistant to antibiotic making it difficult to treat in clinical setting. ϕMR003 possesses a broad host range against MRSA strains of human origin. ϕMR003 requires backbone of the WTA for adsorption onto *S. aureus* host cell surface. ORF117 and ORF119 are RBPs of ϕMR003. The synergistic effects were observed in the combination of ϕMR003 and oxacillin which give a prospect application in the clinical practice. As the future study in vivo of infectivity of ϕMR003 on controlling of MRSA is essential to better understand the potential of ϕMR003 as a phage therapy candidate.