論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Genetic analysis of host-phage interaction of Escherichia coli O157:H7 and its specific phage, PP01
著者(和文)	星賀史也
Author(English)	Fumiya Hoshiga
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11102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,和地 正明,廣田 順二,小倉 俊一郎,八波 利恵
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第11102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	生物プロセス　専攻	申請学位（専攻分野）： Academic Degree Requested	博士　（　工学　） Doctor of
学生氏名： Student's Name	星賀　史也	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 ）

Introduction

Bacteriophages have drawn much attention due to their potential as an alternative therapy to antibiotics since their discovery. The therapeutic application of phages is referred to as phage therapy and has several advantages over the use of chemical drag. Understanding the underlying mechanisms of host-phage interaction would be beneficial to the practical application of phage therapy.

PP01 phage is a member of *Escherichia coli* O157:H7-specific phages and has the potential to be applied for treatment of *E. coli* O157:H7 infection. Prophylaxis of the food poisoning caused by *E.coli* O157:H7 is a big concern of health-care. Thus it should be important to analyze the interaction of PP01 and *E. coli* O157:H7 aiming at further increasing PP01's therapeutic potential.

This study focused on identification of genes which can make PP01 phage specific to *E. coli* O157:H7 based on comparison with T2 phage. T2 is one of the most well-characterized phage and homologous with PP01. Nonetheless, it has no infectivity to *E. coli* O157:H7 strains. Hence comparison of PP01 and T2 should be useful to identify essential genes of PP01 to infect *E. coli* O157:H7.

Experimental Results

i) Modification of long and short tail fibers of T2 phage by CRISPR/Cas9

The long and short tail fibers of PP01 and T2 are responsible for specific adsorption to their host and are encoded by gene *34*, *35*, *36*, *37*, *38* and gene *12*, respectively. In order to endow adsorption ability to *E. coli* O157:H7 equivalent to PP01 with T2, gene *37-38* and C-terminal part of gene *12* of T2 were replaced with the corresponding parts of PP01 exploiting CRISPR/Cas9.

The resulting T2 recombinant whose long tail fiber or both long and short tail fibers were swapped with one of PP01 were named $T2_{PP01g37\text{-}38}$ and $T2_{PP01g37\text{-}38\text{-}12}$. Adsorption assay of those recombinant showed $T2_{PP01g37\text{-}38}$ was able to adsorb to *E. coli* O157:H7 but less efficiently than PP01, whereas there was no significant difference in the adsorption abilities of $T2_{PP01g37\text{-}38\text{-}12}$ and PP01. However both recombinants had quite poor infectivity to *E. coli* O157:H7.

ii) Screening of further essential genes for infecting *E. coli* O157:H7

The inability of the T2 recombinants to infect *E. coli* O157:H7 despite of its decent adsorption ability implied that there should be other essential genes for infection to *E. coli* O157:H7 other than genes encoding the long and short tail fibers. In order to screen such genes, whole genome analysis of PP01 and T2 were performed. Reciprocal best hit blast revealed 73 of PP01's ORFs were quite varied from those of T2. Then essentiality of 28 of those ORFs were assessed exploiting CRISPR/Cas9, resulting in gene *motB* turned out to be the most possible candidate of the gene of interest.

iii) Modification of *motB* of PP01 and T2

In order to elucidate the importance of *motB* for infection to *E. coli* O157:H7, two recombinant phages were constructed in the same way for modification of the long and the short tail fibers: PP01ΔmotB, *motB* deletion mutant of PP01; $T2_{PP01g37\text{-}38\text{-}motB,}$ T2 recombinant whose genes *37*, *38* and *motB* were replaced with the counterpart of PP01. PP01ΔmotB showed quite poor infectivity to *E. coli* O157:H7. Meanwhile, the infectivity of $T2_{PP01g37\text{-}38\text{-}motB}$ was comparable with one of wild type PP01. Thus the results confirmed that difference of infectivity to *E. coli* O157:H7 between PP01 and T2 was accounted for their dissimilarity in genes encoding the long tail fiber and gene *motB*.

Conclusion

By whole genome analysis and genome editing using CRISPR/Cas9, it turned out that gene *37* and *38* encoding the long tail fiber and *motB* gene, whose function remains to be known, were essential for infection to *E. coli* O157:H7 and introducing those genes into T2 phage could even enable the T2 recombinant to infect *E. coli* O157:H7. On top of that, the replacement of the long tail fiber and the short tail fiber of T2 with the counterpart of PP01 endow the T2 mutant with adsorption ability comparable with PP01, which is not crucial for phage infectivity but still important parameter related to phage fitness. Thus the results could not only identify the essential gene of PP01's infectivity but develop a proof of concept of artificial host range alteration, which is also considered to be important for phage therapy.