論文の要旨
（Thesis Summary）

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論文題目(Thesis Title)

Screening, isolation and characterization of large bacteriophages for use in biocontrol of a wide-range of pathogenic bacteria

（広範囲の病原菌を対象とした生物防除に有用な大型バクテリオファージのスクリーニング、単離および特徴付け）

Introduction: Bacteriophages (phages; viruses that attack and kill bacteria) have been used for clinical applications since their first discovery at the beginning of the 20th century. The recent worldwide revival of interest in phage therapy is mainly attributed to declines in antibiotic efficacy, mainly an increase of antibiotic-resistant pathogenic bacteria in the environment, and also to accumulated knowledge about phage genomics. The most important factors in evaluating phages as effective biocontrol agents are their host ranges and ability to execute long-lasting infection. Jumbo phages are tailed phages characterized by their large genome (>200 kbp) and most of them are members of the family *Myoviridae*. Some jumbo phages were reported to have a wide host range and execute sustainable, long-lasting infection. Although such jumbo phages may be abundant in our environments with a high impact on microbial ecology, standard methods have unfortunately failed to detect them. The aim of my study is to develop a method for selective and efficient isolation of large phages such as jumbo phages (with a genome larger than 200 kbp) and phages with a genome about 200 kbp including T4-like phages. Moreover, isolated phages are to be fully characterized for their genomic, genetic, and virological features as well as their host-ranges to be utilized in biocontrol of pathogenic bacteria.

Systemic method to isolate large phages for use in biocontrol of a wide-range of pathogenic bacteria: Most of jumbo phages are thought to have a broad host range because of their reduced dependence on their host species. Despite the amazing characteristics of jumbo phages, less than 100 jumbo phages have been isolated since the first discovery of phage at the beginning of the last century. Most jumbo phages have been isolated by chance using classical methods for phage detection. I have succeeded to develop smart method for isolation of large phages with a wide host range. This method depends on three successive centrifugations for raw samples at 15,000 x g for 1 hour followed by treatment with chloroform without any filtration. Plaque assay was performed at low temperature (28°C) by using low concentration of top agar

(0.35%), and only the smallest plaques (less than 0.5 mm) were selected. By my method, I have succeeded in isolating 11 large phages, named Escherichia phage E1 ~ E11. Electron microscopic observations revealed that they are typical Myoviridae phages with a big capsid and a long contractile tail. Genome sizes of the isolated phages were determined by pulsed-field gel electrophoresis and found to be in two groups, those around 200 kbp for E1, E2, E5, E6, E7, E9 and E10 phages, and others of approximately 450 kbp for E3, E4, E8 and E11 phages. The isolated large phages had wide host ranges. For example, E9 was effective against *Shigella sonnei* SH05001, *Shigella bydii* SH00007, *Shigella flexneri* SH00006, *Salmonella enterica* serovar Enteritidis SAL01078 and *Escherichia coli* C3000 (K-12 derivative), as well as its original host *E. coli* BL21. Phage E4 also showed a characteristic host range covering three genera such as *Escherichia*, *Salmonella*, and *Serratia*. Hosts of phages are usually species-specific and even strain-specific. Therefore, these phages are very interesting for research not only in applied fields but also in basic ones.

Full genome sequence of a polyvalent bacteriophage infecting strains of *Shigella*, *Salmonella*, and *Escherichia*: From the isolated phages, we have succeeded to determine the full genome sequence of phage E9 (named EcS1). The sequence was determined using the Illumina Miseq System. The whole genome of EcS1 was found to be 175,437 bp in length with a mean G+C content of 37.8%. A total of 295 open reading frames (ORFs) were identified as structural, functional, and hypothetical genes. BLAST analyses of the EcS1 genomic sequence revealed the highest identity (79%; query cover of 73–74%) with three T4-related phages that infect *Serratia* sp. ATCC 39006 (Serratia phages CBH8, CHI14, and X20). Host range analyses revealed that EcS1 has lytic effects on three pathogenic strains of *Shigella* spp. and a pathogenic strain of *S. enterica* as well as *E. coli* strains. However, two strains of *Serratia marcescens* interestingly showed resistance to this phage. Making phylogenetic trees for phage tail fiber protein sequences revealed that EcS1 is closely related to *Enterobacteriaceae*-infecting phages. Thus, EcS1 is a novel phage that infects pathogenic strains of the family *Enterobacteriaceae*.

Conclusion: My newly developed smart method for isolation of large phages opened a way to screen and isolate most of missed large phages (especially jumbo phages) in our environments to be used for control of pathogenic bacteria especially antibiotic resistant ones. Moreover, using non-pathogenic laboratory strains (e.g. *E. coli* BL21) makes the screening feasible for research groups that face difficulties in handling pathogenic strains in lower biosafety level laboratories.