Journal of Microbiology (2014) Vol. 52, No. 6, pp. 515–520
DOI 10.1007/s12275-014-4012-5

Superinfection Exclusion Reveals Heteroimmunity between *Pseudomonas aeruginosa* Temperate Phages

In-Young Chung, Hee-Won Bae, Hye-Jung Jang, Bi-o Kim, and You-Hee Cho*

Department of Pharmacy, College of Pharmacy, CHA University, Gyeonggi-do 463-840, Republic of Korea

(Received Jan 7, 2014 / Revised Feb 10, 2014 / Accepted Feb 10, 2014)

Temperate siphophages (MP29, MP42, and MP48) were isolated from the culture supernatant of clinical *Pseudomonas aeruginosa* isolates. The complete nucleotide sequences and annotation of the phage genomes revealed the overall synteny to the known temperate *P. aeruginosa* phages such as MP22, D3112, and DMS3. Genome-level sequence analysis showed the conservation of both ends of the linear genome and the divergence at the previously identified dissimilarity regions (R1 to R9). Protein sequence alignment of the c repressor (ORF1) of each phage enabled us to divide the six phages into two groups: D3112 group (D3112, MP29, MP42, and MP48) and MP22 group (MP22 and DMS3). Superinfection exclusion was observed between the phages belonging to the same group, which was mediated by the specific interaction between the c repressor and the cognate operator. Based on these, we suggest that the temperate siphophages prevalent in the clinical strains of *P. aeruginosa* represent at least two distinct heteroimmunity groups.

Keywords: *Pseudomonas aeruginosa*, temperate phage, *Siphoviridae*, genome, superinfection, c repressor

Introduction

Bacteriophages or phages are the viruses that infect bacteria, play a major role in bacterial physiology and ecology through their global abundance and transducing ability (Fuhrman, 1999; Chibani-Chennoufi *et al.*, 2004; Clokie *et al.*, 2011). The most commonly found phages belong to the order of *Caudovirales*, the tailed double-stranded DNA phages that outnumber the bacterial cells by 10-fold in environmental samples (Brussow and Hendrix, 2002), and are believed to be responsible for the majority of genome diversification and the subsequent evolution of bacteria (Weinbauer and Rassoulzadegan, 2004; Gomez and Buckling, 2011). This phage-mediated bacterial genome diversification is due to phage-derived changes in genome structure (rearrangement and insertion) and/or genome content (transmission of new genes), which are usually managed by temperate phages that have the ability to integrate into the host chromosome and replicate vertically with the host, which is called lysogeny. Temperate phages are well known to alter host bacterial physiology by facilitating the transfer of virulence factors such as superantigens, extracellular toxins, and effector proteins that modulate host-cell invasion and host-cell adhesion, which is called lysogenic conversion (Brussow *et al.*, 2004). Due to the significance of the temperate phage-mediated lysogenic conversion and genome evolution in bacterial survival and virulence traits, much attention has been paid to the comprehensive studies on the genome and the life cycle of temperate phages that can infect the major pathogenic bacteria.

Among the major human bacterial pathogens, *Pseudomonas aeruginosa,* one of ESKAPE pathogens that are highly refractile to the currently available antibiotics regimen (Rice, 2008), can cause life-threatening infections in the patients who undergo immunosuppressive therapies or suffer from traumatic skin damage, HIV infections, and cystic fibrosis (Kielhofner *et al.*, 1992; Lyczak *et al.*, 2000; Murray *et al.*, 2007). By virtue of its highly adaptable nature with extreme metabolic capabilities, *P. aeruginosa* is ubiquitously found in soil and water reservoirs as well as on almost all moist environments of fruits, vegetables, and clinical equipments and devices (Alonso *et al.*, 1999; Frimmersdorf *et al.*, 2010). It is able to colonize various natural and host environments and persist under nutrient-poor and hostile conditions (Favero *et al.*, 1971). In part to understand such versatility to enhance the ecological fitness, which may involve phage-mediated horizontal gene transfer, more systematic approaches to uncover temperate phage-mediated genome diversification and evolution in *P. aeruginosa* needs to be performed, based on the growing information of the phage diversity.

The need to further our understanding of *P. aeruginosa* temperate phages has prompted us to isolate and characterize new temperate phages that can infect *P. aeruginosa* strains. Here, we report the isolation, genome sequencing, and characterization of three temperate phages, MP29, MP42, and MP48 from the culture supernatants of clinical *P. aeruginosa* isolates. They were able to form discernible plaques on *P. aeruginosa* strains PA14 and PAO1, highly virulent clinical isolates that have been widely used in *P. aeruginosa* researches. They showed overall synteny to the known temperate phages such as MP22, D3112, and DMS3 and can be classified into two heteroimmunity groups based on the superinfection exclusion directed by the phage c repressor.

*For correspondence. E-mail: youhee@cha.ac.kr; Tel.: +82-31-8017-9463; Fax: +82-31-8017-9420

Materials and Methods

Bacterial strains and culture conditions

P. aeruginosa strains (Heo *et al.*, 2007; Chung *et al.*, 2012) were grown at 37°C using Luria-Bertani (LB) broth or on 2% Bacto-agar (Difco, USA) LB or Cetrimide-agar (Fluka, USA) plates as previously described elsewhere (Heo *et al.*, 2007). Stationary phase cultures were inoculated into fresh LB broth with an inoculum size of 1.6×10^7 CFU/ml, and then grown and used for experiments.

Preparation of phage lysates and PA14 lysogens

Filtrates of the culture supernatants from the phage lysogens were used as the phage lysates. Briefly, PAS429, PMM22, PMM29, PMM42, PMM45, and PA14LysNeg6 (Wang *et al.*, 2004; Heo *et al.*, 2007; Zegans *et al.*, 2009) were grown in LB for more than 18 h. After pelleting bacterial cells, the phage particles were precipitated from culture supernatant with 10% PEG and 1 M NaCl, and dissolved in 5 ml phage buffer [10 mM $MgSO_4$, 10 mM Tris; pH 7.6, 1 mM EDTA]. PA14-derived lysogens for each phage were obtained as described previously (Heo *et al.*, 2007). Stationary-phase PA14 bacteria were used to inoculate fresh LB that contained phage lysates. After 18 h incubation, the surviving bacteria were plated on LB for single colony isolation. Each purified colony was subjected to PCR using phage-specific primers to obtain each lysogen.

Transmission electron microscopy

Phage particles were prepared after ultracentrifugation of phage lysates at 450,000×g for 4 h, resuspended in phage buffer for transmission electron microscopy (TEM) to determine the virion morphology. Formvar-coated TEM grids were subjected to hydrophilic treatment for 10 min and floated with 20 ml of phage samples containing ~10^4 PFU immediately followed by negative staining for 5 sec using 20 ml of 2% uranyl acetate (pH 4.0). The grids were air-dried for 30 min and viewed using a transmission electron microscope (JEM 1010 EM; JEOL Ltd, Japan) at 120–500 K magnification.

Sequence analysis

The whole-genome sequences of phages have been deposited under accession numbers EU272036 (MP29), JQ762257 (MP42), and KF475786 (MP48) (Chung and Cho, 2012). The general sequence manipulation was done using Vector NTI suite (Lu and Moriyama, 2004). Multiple sequence alignments were generated with CLUSTAL W (Thompson *et al.*, 1994; http://www.ebi.ac.uk/clustalw). Protein motifs were scanned at the PROSITE server (http://www.expasy.org/prosite), and DNA motifs were investigated manually. Genome alignment was performed using the MAUVE program under the default setting (Darling *et al.*, 2004; http://gel.ahabs.wisc.edu/mauve). The phylogenomic tree has been created based on the phylogenetic analysis using the MEGA5 program (Tamura *et al.*, 2011).

RNA isolation and S1 nuclease protection analysis

RNA isolation and S1 protection analysis were performed as described elsewhere (Heo *et al.*, 2010). Briefly, PA14 bacteria lysogenized with MP22 was grown until the late-logarithmic growth phase (OD_{600}=0.7) in LB medium and the total RNA was isolated by using Qiagen RNeasy kit (Qiagen, Germany). The oligonucleotide primer pairs, MP22-F2 (AGG TTC GGT TGC GGA ACT TG) and MP22-R2 (TAT TTG ATC CAC TCC CAG CGG) were used for the S1 nuclease protection. PCR-generated probes were labeled with [γ-^{32}P]dATP by T4 polynucleotide kinase and then used for high resolution S1 nuclease protection analysis using 50 μg of the RNA sample. The unlabeled MP22-R2 primer was used to generate the nucleotide sequence ladder using Sequenase Version 2.0 DNA Sequencing Kit (USB, USA) with [α-^{32}P] dATP.

Gel mobility shift assay

Gel mobility shift assay was performed as described previously (Heo *et al.*, 2010). Briefly, DNA fragments of the phage operator regions were generated by PCR with primer pairs, MP22-F2 and MP22-R2 for MP22 probe and D3112-F2 (CGT GAA AGT CGG AAC AGC TGA C) and D3112-R2 (TAC TTG ATC CAC TCC CAG CGC) and for D3112 probe, respectively. PCR products were then end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Ten femtomole of the labeled probe was incubated with crude extract of *P. aeruginosa* bacteria expressing c repressor of either MP22 or D3112 (Heo *et al.*, 2007) in 20 μl of binding buffer [2 mM Tris-HCl; pH 7.8, 0.1 mM EDTA, 0.2 mM DTT, 4 mM KCl, 0.5 mM $MgCl_2$, 10 ng/ml BSA, and 10% glycerol] that contains 1 μg of poly(dI-dC) for 10 min at 25°C. The DNA-protein mixture was resolved on a 5% native PAGE in 0.5× Tris-borate-EDTA buffer, and then analyzed by a phosphorimage analyzer (Fuji, Japan).

Results and Discussion

Identification and isolation of *P. aeruginosa* temperate phages

During the course of profiling the susceptibility of *P. aeruginosa* isolates to various phages, the stationary growth phase culture supernatants of clinical and environmental isolates were tested for the presence of plaque-forming activity on *P. aeruginosa* strain PAO1 without functional CRISPR/Cas system (Cady *et al.*, 2012), and any observed plaques were

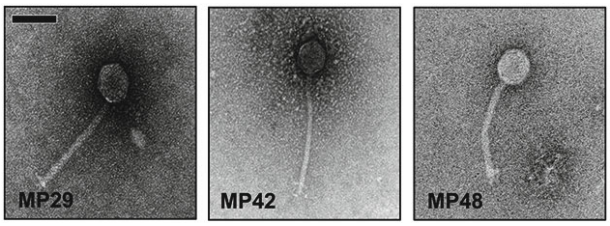

Fig. 1. Electron micrograph of phages MP29, MP42, and MP48. Transmission electron micrographs of MP29, MP42, and MP48 negatively stained with uranylacetate revealing its virion structure and flexible tail.

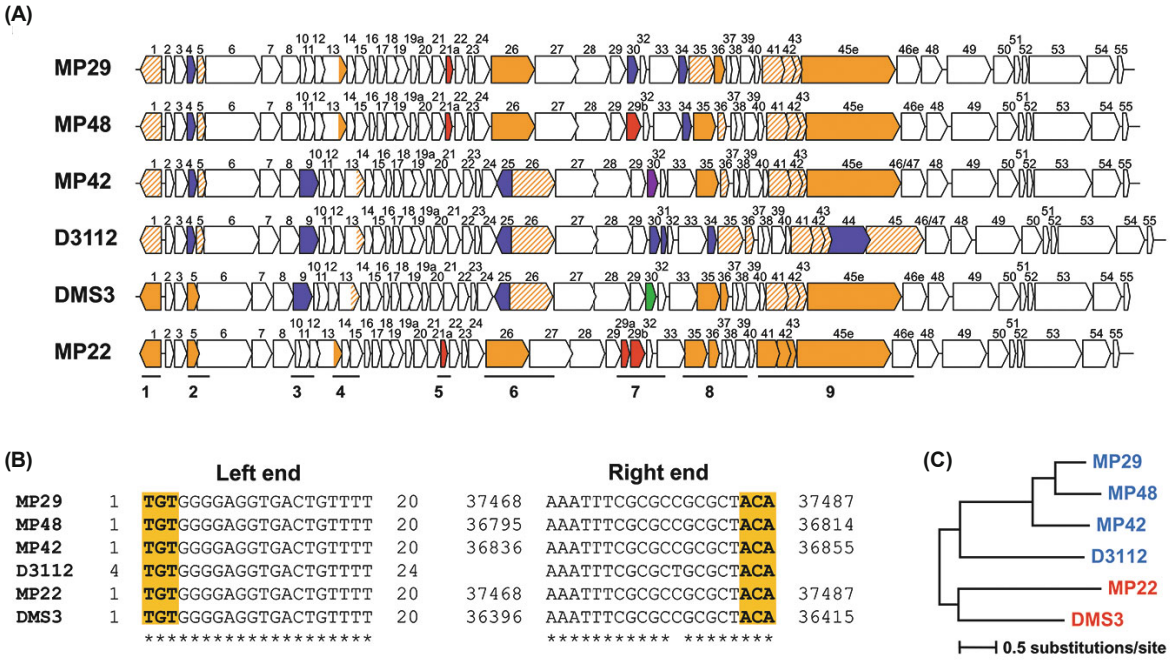

Fig. 2. Comparison of the phage genomes. (A) The putative coding regions of MP29, MP42, MP48 are schematically drawn approximately to scale in parallel with those of D3112, MP22, and DMS3. The ORFs are shown as boxes and gene numbers. The relative locations of orthologous genes, shown by empty boxes, reveal a high degree of synteny (Heo *et al.*, 2007). The ORF color schemes are as follows; white, present in all phages; blue and red present in a set of phages; orange and hatched orange represent relatively low similarity between the genes. All the genes are transcribed rightward, except for the c repressors and the ORF25 (MP42 and D3112 only). (B) The conserved genome end sequences are compared; with the numbers designate the corresponding position, except for the right end sequence of D3112 that has been absent in the GenBank. (C) Phylogenetic tree inferred from the genome sequences. The neighbor-joining tree was obtained using the MEGA5 program with an output from a ClustalW alignment.

subsequently purified. Among them, three phage lysates (MP29, MP42, and MP48), most likely temperate, could form discernible plaques on *P. aeruginosa* strain PA14 with functional CRISPR/Cas system and then subjected to further characterization.

Morphology and receptor of the temperate phages: an MP22-like phage

We determined the morphology of the phage particles by transmission electron microscopy. All have a flexible tail of ~150 nm in length and an icosahedral head of ~50 nm in diameter (Fig. 1). This morphology is similar to the virion structure of the previously identified MP22 of the *Siphoviridae* family, which have characteristic isometric heads and long, flexible, and noncontractile tails. Furthermore, because all three phages were unable to form plaques on *pilA* mutant bacteria in any conditions observed, the *P. aeruginosa* type IV pili (TFP) are the primary receptors for these phages like MP22 and D3112 (data not shown).

Alignment of the temperate phage genome ends

Sequencing of the leftmost and the rightmost ends of the temperate phages revealed heterogeneous *P. aeruginosa* sequences due to their capability to integrate into different locations on the *P. aeruginosa* genome (Heo *et al.*, 2007). Based on the experimental determination of both ends of the phage MP22, which are 5′-TGT-3′, we trimmed the heterogeneous sequences and found that all the phages have 5′-TGT-3′ at both ends (Fig. 2B). While sequence variations as well as imprecise excision could occur during prophage excision (Darzins and Casadaban, 1989; Wang *et al.*, 2004), this approach seems valid to determine the genome ends of the related phages which are sporadically found in the clinical and environmental isolates of *P. aeruginosa*.

Genome and coding region comparisons between temperate phages

The BLASTP search results showed that a majority of the phage genes are similar to genes in MP22 and D3112 with overall synteny. The ORFs of the phages are assigned with the number chosen to correspond to the orthologous ORF of phage MP22 (Fig. 2A).

Comparison of the genome sequences of the three phages and MP22, D3112, and DMS3 led us to suggest that the significant sequence divergence were observed only in the nine dissimilarity regions (R1 to R9) that has been identified by the comparison between MP22 and D3112 (Heo *et al.*, 2007). Among them, the 7th region, R7 is noteworthy, in that all the six phages have unique ORFs between two conserved genes (ORF29 and 32). This region has been shown to contain new functional proteins to antagonize the host CRISPR/Cas system (Bondy-Denomy *et al.*, 2013). All the phages, except for DMS3, contain the anti-CRISPR gene, which is almost identical to the anti-CRISPR genes previously identified that include MP29-ORF30 and D3112-ORF30: ORF29b of MP22 and MP48 is similar to JBD35-ORF35

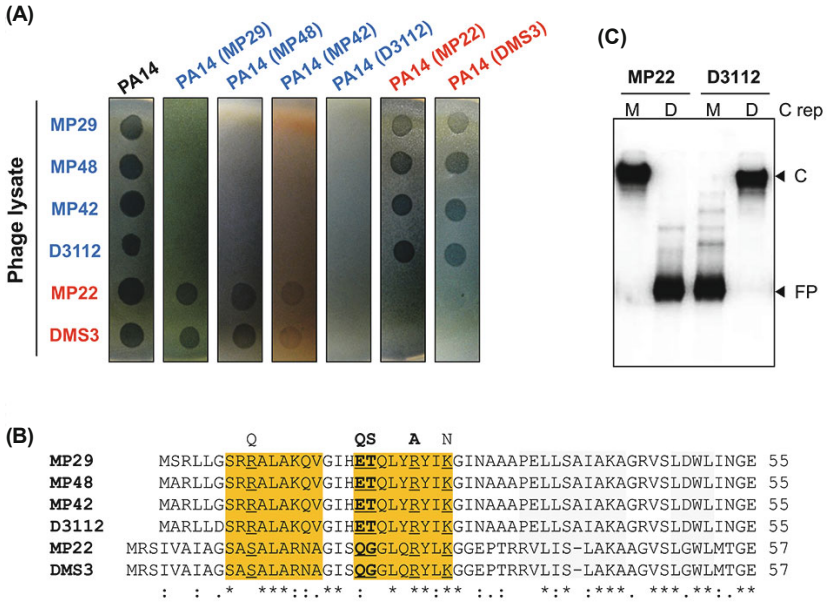

Fig. 3. Superinfection between phages and c repressor specificity. (A) Plaque formation by the phage lysates from each PA14 lysogen (indicated at the left) that were spotted onto lawns of PA14 lysogens as indicated in the parentheses. The zone of lysis on the plate indicates that the lysate was able to infect the cells. (B) Alignment of the N-terminal regions of the c repressor proteins with ClustalW. Conservation of identical residues is designated by asterisks. Colons and dots indicate the conservation of similar residues based on the Gonnet PAM250 matrix similarity scores: colon, strong similarity; dots, weak similarity (Thompson *et al.*, 1994). The helix-turn-helix motifs are designated, which are included in the phage repressor motif (PS50943) in the PROSITE database. The putative DNA binding helix-turn-helix motif is represented with. Critical residues in DNA interaction are underlined and bold-faced in analogy to lcI repressor above (Pabo and Sauer, 1992): Q, S, and A residues in the second helix required for sequence-specific DNA binding and the other two residues (Q and N) interacting with the DNA phosphate backbone are shown. (C) Gel mobility shift assay using the crude extract from cells expressing c repressors, M (from MP22) and D (from D3112) and the labeled operator fragments from MP22 and D3112. Binding complex (C) and free probe (FP) are designated.

and JBN88a-ORF33, whereas ORF30 of MP42 is similar to JBD30-ORF35. DMS3 has one ORF (ORF30) in the region, which is not related with anti-CRISPR function. Since anti-CRISPR containing phages, except MP22, were unable to inhibit bacterial swarming motility (Chung *et al.*, 2012) which is shown to depend on the non-identity match between the part of phage gene (ORF42) and one of the spacers in the host CRISPR region (Cady and O'Toole, 2011). The anti-CRISPR function may be generally incompatible with swarming motility inhibition. In case of MP22, another swarming inhibitor but with anti-CRISPR gene (ORF29b), it should be noted that an additional ORF (ORF29a) is present immediately upstream of ORF29b, which is unique to MP22 (Fig. 2A). The presence of an upstream ORF for the anti-CRISPR gene may affect its expression and/or function. This heterogeneity in the R7 region of these phages may be associated with the modulation of the bacterial immunity function of the CRISPR system to affect the bacterial group motility, which will be a future avenue to further understanding of the physiological function and evolution of the CRISPR/Cas system in *P. aeruginosa*.

The degree of the synteny between the six phages was further investigated by creating the phylogenomic tree as shown in Fig. 2C. As a result, they are divided into two distinct groups: MP29 and MP48 are very close and comprise one group with MP42 and D3112, whereas MP22 and DMS3 belong to the other group. We previously observed that the c repressors from D3112 and MP22, which belong to the different groups, are unable to exclude the infection of the non-cognate phages, which indicated the heteroimmunity between both phages (Heo *et al.*, 2007). We, therefore, needed to investigate the presence of additional heteroimmunity group(s) in these phages.

Heteroimmunity between temperate phages

We investigated the superinfection exclusion phenotypes between the lysogens for each phage. To avoid the potential host-specific restriction, we first created the lysogens for one of the six phages which are derived from a single *P. aeruginosa* strain (PA14) and the six PA14 lysogens were verified for their ability to produce phage lysates. As shown in Fig. 3A, two distinct heteroimmunity groups were observed: MP22 and DMS3 could infect the lysogens for MP29, MP48, and MP42, but could not infect MP22 and DMS3, indicating that both MP22 and DMS3 belong to the same heteroimmunity group. Likewise, MP29, MP48, MP42, and D3112 could infect the lysogens for MP22 or DMS3, but not infect the lysogens for MP29, MP48, MP42, or D3112, indicating that those belong to another heteroimmunity group. This result suggests that, despite high similarity in their genomic and morphological characteristics, the six phages are in two distinct heteroimmunity groups.

It is already known that PA14(D3112) bacteria (lysogenized with D3112 phage) did not allow superinfection by MP22, unlike PA14 cells expressing the c repressor from D3112 did, while PA14(MP22) bacteria (lysogenized with MP22 phage) allowed superinfection as PA14 cells expressing the c repressor from MP22 (Heo *et al.*, 2007). DMS3 like MP22 also failed to superinfect PA14(D3112) (Fig. 3A), suggesting that the superinfection exclusion phenotype by PA14(D3112) may not involve c repressor. We also verified that the phenotype was observed at 42°C, a restrictive temperature for the temperature-sensitive c repressor from D3112 and that PA14(D3112) could exclude superinfection by phylogenetically unrelated phages such as MPK7 (a podophage) and PP7 (a leviphage), both of which require TFP (data not shown).

We made a tentative conclusion that this superinfection exclusion by D3112 lysogenization is most likely attributed to the twitching motility inhibition by D3112 lysogenization, since twitching motility is critical for the phage infection that requires TFP as the phage receptor (Chung *et al.*, 2012). The molecular mechanism by which this superinfection exclusion takes place toward TFP-requiring phages warrants further investigation.

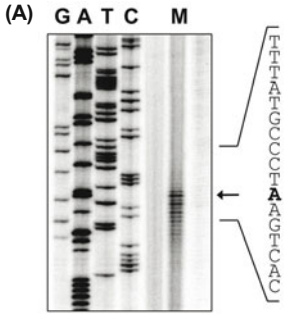

Fig. 4. Sequences of the early promoter region of the phages. (A) High-resolution S1 nuclease mapping of the 5′ end of the major promoter (P_{R1})-derived transcript. RNA isolated from PA14(MP22) were subjected to S1 mapping with the 293-bp probe generated by PCR (lane M). The same downstream primer (MP22-R2) was used to generate the DNA sequencing ladders (lanes G, A, T and C). The transcription start site of P_{R1} is indicated by an arrow from the A residue (bold) on the sense sequence. (B) The transcription start sites for P_{R1} promoter (A) and P_L promoter for c repressor that was identified elsewhere (Salmon *et al.*, 2000) are marked by bent arrows. The putative promoter element hexamers are designated by boxes and underlines. Potential operators (O_1) for c repressor binding with weak inverted repeat sequences (TG-N_{15}-CA for D3112 and AG-N_{15}-CT for MP22) are shown. Palindromic residues are marked in bold and underlined for each sequence.

The c repressor and the cognate operator divergence is associated with the heteroimmunity

Diversity of ORF1 encoding phage c repressor accounts for the divergence of the R1 region (Fig. 2A). The R1 divergence is primary due to the N-terminal 60 amino acid residues of the c repressor that include the proposed helix-turn-helix DNA binding motif, with the key difference especially at the second recognition helix (Heo *et al.*, 2007). Multiple alignment of c repressors of the six phages revealed that each heteroimmunity group represent identical N-terminal amino acid sequences within the group, except for the 6th amino acid for D3112 (aspartate instead of glycine for the others) (Fig. 3B). To examine whether the divergence in the c repressor might be involved in specific DNA recognition, gel mobility shift assay was performed using c repressor proteins and potential operator regions. The intergenic regions between ORF1 and ORF2 from D3112 and MP22 were amplified (273 and 293 bp, respectively) and used for the potential operator regions. As shown in Fig. 3C, c repressor from MP22 could bind to the operator of MP22, but not to the operator of D3112, whereas c repressor from D3112 could bind only to its own operator. Since the c repressor normally functions to repress all transcription units (Krylov *et al.*, 1985), the specific binding to the cognate operator regions may explain the c repressor-mediated heteroimmunity in these phages.

As shown in Fig. 3B, one of the most striking dissimilarities between the c repressors from both heteroimmunity groups lies within the second helix of the helix-turn-helix DNA binding motif (ET for D3112, MP29, MP42, and MP48 vs QG for MP22 and DMS3). The corresponding residues for lcI repressor (QS) are involved in direct contact with the base pairs in the operator region (Pabo and Sauer, 1992).

To elucidate the difference in the molecular interaction between the c repressor and the operator, the operator sequence needed to be identified. Because the early leftward promoter (P_L) that drives ORF1 transcription has been already identified (Salmon *et al.*, 2000) and the potential operator would be positively acting on the P_L promoter and negatively acting on the earliest promoter (P_{R1}), we first performed S1 nuclease mapping to determine the transcription start site of the P_{R1} promoter of MP22 using the RNA samples extracted from the PA14(MP22) lysogens. As shown in Fig. 4, the transcription start site was determined as the A1004 on the MP22 genome (corresponding to the A993 on the MP29 genome), enabling us to purport the promoter hexamers (i.e. -35 and -10 boxes) in the six phages. The proposed P_{R1} promoter are overlapped with the P_L promoter and we found a weak inverted repeat sequences of 19 bp (designated by O_1 in Fig. 4B) whose binding center (T) was localized within the -10 box of the P_{R1} promoter (i.e. +10 position), but proximally located upstream of the -35 box of the P_L promoter (i.e. +43 position). Although the function of the operator O_1 in regulation of both promoters awaits experimental verification, we tentatively concluded that this operator may be able to positively and negatively regulate the P_L and the P_{R1} promoters, respectively, based on the configuration within the intergenic region of the two early promoters. This tentative conclusion would be also substantiated by the slight difference between the two heteroimmunity groups in the operator sequences. The D3112-group phages, which contain ET residues at the second helix of the c repressor, display AG-N_{15}-CT, while MP22 and DMS3, both of which contain QG residues at the second helix, have TG-N_{15}-CA. The actual involvement of this amino acid and nucleotide residues in specific interaction between cognate repressor-operator pairs will be geared by creating the mutations of these residues as well as the hybrid repressors and/or operators to reveal the recognition code governing the heteroimmunity of these siphophages sporadically carried by *P. aeruginosa* strain. Further molecular biological studies focusing on the dissimilarity regions will be useful to facilitate the elucidation of biogenesis, function and evolution of these related phages.

Acknowledgments

This work was supported by the grants from the National Research Foundation (NRF) of Korea (2011-0015953).

References

Alonso, A., Rojo, F., and Martinez, J.L. 1999. Environmental and clinical isolates of *Pseudomonas aeruginosa* show pathogenic and biodegradative properties irrespective of their origin. *Environ. Microbiol.* **1**, 421–430.

Bondy-Denomy, J., Pawluk, A., Maxwell, K.L., and Davidson, A.R. 2013. Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system. *Nature* **493**, 429–432.

Brussow, H., Canchaya, C., and Hardt, W.D. 2004. Phages and the evolution of bacterial pathogens: From genomic rearrangements to lysogenic conversion. *Microbiol. Mol. Biol. Rev.* **68**, 560–602.

Brussow, H. and Hendrix, R.W. 2002. Phage genomics: Small is beautiful. *Cell* **108**, 13–16.

Cady, K.C., Bondy-Denomy, J., Heussler, G.E., Davidson, A.R., and O'Toole, G.A. 2012. The CRISPR/Cas adaptive immune system of *Pseudomonas aeruginosa* mediates resistance to naturally occurring and engineered phages. *J. Bacteriol.* **194**, 5728–5738.

Cady, K.C. and O'Toole, G.A. 2011. Non-identity-mediated CRISPR-bacteriophage interaction mediated via the Csy and Cas3 proteins. *J. Bacteriol.* **193**, 3433–3445.

Chibani-Chennoufi, S., Bruttin, A., Dillmann, M.L., and Brussow, H. 2004. Phage-host interaction: An ecological perspective. *J. Bacteriol.* **186**, 3677–3686.

Chung, I.Y. and Cho, Y.H. 2012. Complete genome sequences of two *Pseudomonas aeruginosa* temperate phages, MP29 and MP42, which lack the phage-host CRISPR interaction. *J. Virol.* **86**, 8336.

Chung, I.Y., Sim, N., and Cho, Y.H. 2012. Antibacterial efficacy of temperate phage-mediated inhibition of bacterial group motilities. *Antimicrob. Agents Chemother.* **56**, 5612–5617.

Clokie, M.R., Millard, A.D., Letarov, A.V., and Heaphy, S. 2011. Phages in nature. *Bacteriophage* **1**, 31–45.

Darling, A.C., Mau, B., Blattner, F.R., and Perna, N.T. 2004. Mauve: Multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* **14**, 1394–1403.

Darzins, A. and Casadaban, M.J. 1989. *In vivo* cloning of *Pseudomonas aeruginosa* genes with mini-D3112 transposable bacteriophage. *J. Bacteriol.* **171**, 3917–3925.

Favero, M.S., Carson, L.A., Bond, W.W., and Petersen, N.J. 1971. *Pseudomonas aeruginosa*: Growth in distilled water from hospitals. *Science* **173**, 836–838.

Frimmersdorf, E., Horatzek, S., Pelnikevich, A., Wiehlmann, L., and Schomburg, D. 2010. How *Pseudomonas aeruginosa* adapts to various environments: A metabolomic approach. *Environ. Microbiol.* **12**, 1734–1747.

Fuhrman, J.A. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* **399**, 541–548.

Gomez, P. and Buckling, A. 2011. Bacteria-phage antagonistic coevolution in soil. *Science* **332**, 106–109.

Heo, Y.J., Chung, I.Y., Cho, W.J., Lee, B.Y., Kim, J.H., Choi, K.H., Lee, J.W., Hassett, D.J., and Cho, Y.H. 2010. The major catalase gene (*katA*) of *Pseudomonas aeruginosa* PA14 is under both positive and negative control of the global transactivator OxyR in response to hydrogen peroxide. *J. Bacteriol.* **192**, 381–390.

Heo, Y.J., Chung, I.Y., Choi, K.B., Lau, G.W., and Cho, Y.H. 2007. Genome sequence comparison and superinfection between two related *Pseudomonas aeruginosa* phages, D3112 and MP22. *Microbiology* **153**, 2885–2895.

Kielhofner, M., Atmar, R.L., Hamill, R.J., and Musher, D.M. 1992. Life-threatening *Pseudomonas aeruginosa* infections in patients with human immunodeficiency virus infection. *Clin. Infect. Dis.* **14**, 403–411.

Krylov, V.N., Akhverdian, V.Z., Bogush, V.G., Khrenova, E.A., and Reulets, M.A. 1985. Modular structure of the genes of phages-transposons of *Pseudomonas aeruginosa*. *Genetika* **21**, 724–734.

Lu, G. and Moriyama, E.N. 2004. Vector NTI, a balanced all-in-one sequence analysis suite. *Brief. Bioinform.* **5**, 378–388.

Lyczak, J.B., Cannon, C.L., and Pier, G.B. 2000. Establishment of *Pseudomonas aeruginosa* infection: Lessons from a versatile opportunist. *Microbes Infect.* **2**, 1051–1060.

Murray, T.S., Egan, M., and Kazmierczak, B.I. 2007. *Pseudomonas aeruginosa* chronic colonization in cystic fibrosis patients. *Curr. Opin. Pediatr.* **19**, 83–88.

Pabo, C.O. and Sauer, R.T. 1992. Transcription factors: Structural families and principles of DNA recognition. *Annu. Rev. Biochem.* **61**, 1053–1095.

Rice, L.B. 2008. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. *J. Infect. Dis.* **197**, 1079–1081.

Salmon, K.A., Freedman, O., Ritchings, B.W., and DuBow, M.S. 2000. Characterization of the lysogenic repressor (c) gene of the *Pseudomonas aeruginosa* transposable bacteriophage D3112. *Virology* **272**, 85–97.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680.

Wang, P.W., Chu, L., and Guttman, D.S. 2004. Complete sequence and evolutionary genomic analysis of the *Pseudomonas aeruginosa* transposable bacteriophage D3112. *J. Bacteriol.* **186**, 400–410.

Weinbauer, M.G. and Rassoulzadegan, F. 2004. Are viruses driving microbial diversification and diversity? *Environ. Microbiol.* **6**, 1–11.

Zegans, M.E., Wagner, J.C., Cady, K.C., Murphy, D.M., Hammond, J.H., and O'Toole, G.A. 2009. Interaction between bacteriophage DMS3 and host CRISPR region inhibits group behaviors of *Pseudomonas aeruginosa*. *J. Bacteriol.* **191**, 210–219.