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Diversity of Bacteriophages Infecting *Xanthomonas oryzae* pv. *oryzae* in Paddy Fields and Its Potential to Control Bacterial Leaf Blight of Rice

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Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a very serious disease in rice-growing regions of the world. In spite of their economic importance, there are no effective ways of protecting rice plants from this disease. Bacteriophages infecting Xoo affect the population dynamics of the pathogen and consequently the occurrence of the disease. In this study, we investigated the diversity, host range, and infectivity of Xoo phages, and their use as a bicontrol agent on BLB was tested. Among the 34 phages that were isolated from floodwater in paddy fields, 29 belonged to the *Myoviridae* family, which suggests that the dominant phage in the ecosystem was *Myoviridae*. The isolated phages were classified into two groups based on plaque size produced on the lawn of Xoo. In general, there was a negative relationship between plaque size and host range, and interestingly the phages having a narrow host range had low efficiency of infectivity. The deduced protein sequence analysis of *htf* genes indicated that the gene was not a determinant of host specificity. Although the difference in host range and infectivity depending on morphotype needs to be addressed, the results revealed deeper understanding of the interaction between the phages and Xoo strains in floodwater and damp soil environments. The phage mixtures reduced the occurrence of BLB when they were treated with skim milk. The results indicate that the Xoo phages could be used as an alternative control method to increase the control efficacy and reduce the use of agrochemicals.

Keywords: Bacteriophage, bacterial leaf blight, diversity, *Xanthomonas oryzae*

Introduction

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is one of the most important bacterial diseases of rice worldwide. Although agrochemicals and resistance cultivars help to reduce the incidence of the disease, the control efficacy was not satisfactory. The poor disease control efficacy, development of fungicide-resistant pathogens, and public concerns on health have spurred research on alternative methods for the disease management.

Although phages have long been proposed as plant disease control agents, they have not received much attention as an alternative disease control strategy because of specificity on bacterial strains, variable bacterial sensitivity, rapid development of bacterial resistance to the phages, and inactivation of phages by ultraviolet light [25, 31]. However, phage-assisted biocontrol has again attracted attention to decrease the use of agrochemicals and to guard against the emergence of antibiotic-resistant bacteria [11, 24].

A number of phages infecting Xoo have been described, and many of them have been characterized and used for phage typing [18, 22]. Wakimoto [32] previously classified phages infecting Xoo into two types, OP1 and OP2, based on their morphological and serological properties. Kuo *et al.* [20] described morphologically distinct types of Xoo

phages, Xp10, Xp20, and Xp12, in Taiwan. The genomes of Xp10, OP1, and OP2, which have double-stranded 44,373, 43,785, and 46,643 bp DNA, respectively, have been sequenced and analyzed [12, 13, 34]. Recently, a Xoo phage named Xop411 was found to be nearly identical to both OP1 and Xp10 in organization, genome size, morphology, and even genome sequences, but entirely different from OP2 [21].

Phages have a very heterogeneous structure and morphotype, such as tailed, polyhedral, filamentous, and pleomorphic. Ackermann [3] classified phages into 1 order and 13 families upon electron microscopic examination of more than 5,100 phages. Tailed phages occupied the largest and most wide-spread group of bacterial phages. Phages infecting *Xanthomonas* were mostly tailed phages that belong to the *Myoviridae*, *Siphoviridae*, and *Podoviridae* families [2, 3]. Most previously reported Xoo phages, OP1, OP2, XP12, XP10, and Xo411, belong to the *Siphoviridae* [2, 12, 13]. In spite of its rare occurrence, filamentous Xoo phage Xf, which belongs to the *Inoviridae* family, has also been reported [19].

Generally, phages that can infect one species of bacteria are unable to infect other species of bacteria, possibly due to the differences in receptors or intracellular phage production [28]. The specificity would be a disadvantage to use as a biological agent in which a single bacteriophage may not be effective against all the isolates of a particular species. The host range (h-) mutant produced in the laboratory exhibits a wider intraspecific range and maintains specificity toward the wild-type bacteria [14]. The disease incidence of BLB in geranium was reduced by 50% by treating with an h-mutant phage mixture at 5×10^8 plaque-forming unit (PFU)/ml daily [9]. Inoue *et al*. [13] reported that the homolog of the tail fiber (*htf*) gene is possibly involved in host specificity. Although the host specificities of phages with their representative hosts have been widely researched [10], the relationships between hosts and their phages are still limited from an ecological viewpoint, especially in floodwater and terrestrial environments.

Although a number of phages infecting Xoo have been described and characterized [18, 22], few trials have been carried out in paddy field systems for controlling the pathogens using the phages. In this study, we isolated Xoo phages from floodwater of paddy fields and determined the host specificities for each phage isolate using Xoo strains collected from the same or nearby fields. Each phage isolate was examined by electron microscopy and classified into phage families based on its morphotype as described by Ackermann [1, 3]. Genetic diversity was also analyzed and compared with phenotypic characteristics. Moreover, in spite of severe economic losses, the low control effect of the disease encouraged us to test the phages as a biocontrol agent.

Materials and Methods

Phage Isolation and Purification

Xoo strains, KACC 10332, 10312, and 10860, were used as hosts for the isolation of phages, and peptone sucrose (PS) medium was generally used in this study [20]. Floodwater samples collected from paddy fields were agitated thoroughly with a few drops of chloroform (ca. 2% (v/v)) and centrifuged at 10,000 ×*g* for 10 min. The supernatant was subjected to filtration (0.2 μm) and stored at 4°C. The supernatant was plated onto three lawns, each seeded with one of the isolation hosts in soft agar (0.7% agar). The soft agar together with phages within the clearing zones was picked and soaked in PS broth medium (PSB). After appropriate dilution with PSB, the suspensions were plated with the seed bacteria for plaque formation. At least two more successive single-plaque isolations were performed to obtain pure cultures. Generally a suspension (ca. 2×10^8 CFU/ml) of Xoo strain at 28°C was inoculated at a multiplicity of infection of 0.1 and aerated continuously.

Plaque Assay, Host Range Determination, and Efficiency of Plating

Fifty-nine strains of bacteria were tested for host specificity. Among them, 47 strains of Xoo were isolated at the same time with a phage sample collection at various rice growing regions. Other bacterial strains such as *Bacillus vallismortis*, *Burkholderia gladioli*, *B. glumae*, *Pseudomonas fluorescens*, *P. syringae* pv. *tomato*, *Xanthomonas axonopodis* pv. *phaseoli*, *X. a.* pv. *glycines*, *X. a.* pv. *citrumello* E, *X. a.* pv. *citri*, and *X. a.* pv. *aurantifolli* B and C were also tested.

Phage stock was diluted to 10^5 PFU/ml. Then, 30 μl of the phage suspension and 100 μl of the cells from an overnight culture of Xoo (10^8 CFU/ml) were mixed with 3 ml of the molten soft PSA and poured onto the surface of a regular PSA plate. The plaques were differentiated according to the degrees of clarity and sizes after incubation at 28°C for 18 h. The host range of the phage was determined by spotting 5 μl of the phage preparation (ca. 1×10^8 PFU/ml) on lawn cultures of the strains to be tested. To determine the relative efficiency of plating, Xoo strains of three different races (KACC 10332, 10312, and 10860) and phages of different plaque types (P4L, P43M, P23M1, P37L, P37M, P37M1) were used. The number of plaques was counted after incubating the plates overnight.

Transmission Electron Microscopy

One drop of high-titer phage suspension was placed onto a 300-mesh nickel grid coated with formvar, and the phages on the grid were negatively stained with 2% uranyl acetate or 3% sodium

phosphotungstate. Each sample was examined using a LEO 912AB (Carl Zeiss, Germany) transmission electron microscope (TEM) with an accelerating voltage of 80 kV.

Sequencing and Phylogenetic Analysis of *htf* Genes

Phage DNA was isolated as previously reported [10] and used as template for the amplification of *htf* genes. The amplification reaction was performed in a 50 µl solution containing 50 ng of template DNA, 10 pmol of both primers, 0.2 mM of each dNTP with 1.5 mM $MgCl_2$, and 1 U of E-Taq DNA polymerase (SolGent Ltd., Korea). Each reaction mixture was run for 30 cycles with the following temperature profiles: denaturation at 95°C for 45 sec, annealing at 50°C for 45 sec, and extension at 72°C for 1 min, and 30 sec plus an initial step of 95°C for 15 min and a final step of 72°C for 5 min. Primers HtfF (5′-ATGGCAATTGTGAACATC GACTT-3′) and HtfR1 (5′-TTATAGCTCCGCCGACATAATAT-3′), which were designed from the sequence of OP1 phage, were used [13]. The PCR products were purified and cloned to pGEM-T easy vector (Promega, USA). The cloned plasmid was sequenced, and deduced protein sequences of *htf* genes were analyzed with OP1 using MEGA5 software [29]. The phylogenetic tree was constructed using the UPGMA (unweighted pair group method with arithmetic mean) method.

Phage Production

For the mass production of phages, a suspension (ca. 2 × 10^8 cells/ml) of Xoo strains, KACC 10332, 10312, and 10860, was inoculated at a multiplicity of infection of 0.1 and aerated continuously overnight at 28°C. When lysis became evident, a few drops of chloroform (ca. 2% (v/v)) were added and cell debris was removed from the lysate by centrifugation at 10,000 ×*g* for 10 min. In general, the burst size was 45 per infected cell and the latent period was ca. 140 min. The resultant phage stock was stored at 4°C until used. The enriched phages were subjected to serial dilution with PSB. Then, 30 µl of a phage suspension and 100 µl of the cells from an overnight culture of Xoo were mixed with 3 ml of the molten soft PSA and poured on the surface of a regular PSA plate. The number of the plaques was estimated after incubating the plates overnight.

Field Trial of the Phage as an Agent for Biocontrol of Xoo

The field experiment was conducted with the rice variety Nampyoung, at Kimje in Jeonbuk Province for 2 consecutive years. Phages P4L, P43M, and P23M1 having different host ranges were selected and mass produced as described above. The selected phage isolates were subjected to produce h-mutants as reported by Jackson [14] and Flaherty *et al*. [9] with minor modifications. Briefly, each selected phage was mixed with its respective Xoo strain. After culturing 2 to 3 days, the resistant colonies were obtained and resistance to the wild-type phages was confirmed by using the soft agar method. The resistant Xoo isolates were combined with wild-type phages and poured onto the plates. Subsequently, h-mutants that infected not only resistant but also wild-type Xoo strains were selected.

To produce h-mutant, KACC 10860 strain, which is a K3 race, was used. The phages were diluted in skim milk (0.75 g/l) at concentrations of 5 × 10^8 PFU/ml before treatment. Phage mixture (PM; P4L, P43M, and P23M1), h-mutant phage mixture (PMh; h-mutants of P4L + P43M + P23M1), and skim milk were applied for the control of BLB. Tecloftalam wettable powder, which is recommended for the control of BLB, was applied (1 g/l) as and agrochemical control. Three replications were implemented for each treatment, and the plots were arranged in a randomized complete block design. Each plot consisted of 20 m^2. Each treatment (2 L per plot) was sprayed with a compression sprayer. All treatments were applied three times, in about 10 days interval after sunset, and disease occurrence was surveyed 10 days after treatment. Disease severity was assessed by estimating the diseased leaf area. The experiment was maintained with conventional fertilization, irrigation, and weed and insect control.

Statistical Analysis

Statistical analysis was carried out with SAS software (Statistical Analysis System 9.2; USA) using Tukey's test. Means were compared by the least significant difference (LSD) method at $P < 0.05$.

Results

Characterization of Xoo Phages

Seventy-five floodwater samples, which were collected from paddy fields in various regions of Korea, were tested for the presence of Xoo phages. Three host strains were used for the initial isolation to overcome no recovery because of potential host-specificity. We recovered 34 phages from the samples and differentiated the obtained phages on the basis of plaque diameter (Table 1). Eight phages formed more than 4-mm-diameter plaques on the lawns of host strain KACC 10860, and most of the other phages formed 2–4 mm plaques.

The isolated Xoo phages were divided into two morphotype groups by the method of Ackermann [3]. All of them were tailed phages, either A1 type of the *Myoviridae* family (Figs. 1A–1D) or B1 type of the *Siphoviridae* family (Figs. 1E and 1F), which represent the largest groups of phage isolates based on the observation by Ackermann [3]. The head of the *Myoviridae* phages had a hexagonal outline approximately 56.7 ± 2.0 nm in length and 60.8 ± 2.8 nm in width. The head of the *Siphoviridae* was also hexagonal with a width of 53.5 ± 1.8 nm and length of 57.0 ± 1.4 nm. The tail was approximately 85 and 145 nm in length for the *Myoviridae* and *Siphoviridae* phages, respectively (data not shown). The contracted stage was observed in some of the *Myoviridae* phages such as P68M and P70M (Fig. 1D).

Table 1. Plaque size, morphotype, and host range of the tested phages.

Phages	Plaque diameter[a] (mm)	Morpho-type[b]	No.[c]			Phages	Plaque diameter (mm)	Morpho-type	No.		
			-	+	++				-	+	++
P4L	4>	A1	14	6	27	P45M	3~4	A1	14	5	28
P4M	3~4	A1	1	1	45	P47M	3~4	A1	0	2	45
P6M	3~4	A1	0	1	46	P50M	3~4	A1	0	3	44
P6M1	2~3	A1	0	2	45	P53M	3~4	A1	0	1	46
P8L	4>	B1	11	7	29	P54M	3~4	A1	0	1	46
P14M	3~4	A1	0	1	46	P57M	3~4	A1	0	2	45
P14M1	2~3	A1	0	1	46	P58M	3~4	A1	0	2	45
P18M	3~4	A1	0	1	46	P59L	4>	B1	16	4	27
P23M1	2~3	A1	0	2	45	P60M	3~4	A1	19	1	27
P27L	4>	B1	14	4	29	P61M	3~4	A1	0	1	46
P30L	4>	B1	16	4	27	P62M	3~4	A1	0	3	44
P33M	3~4	A1	0	1	46	P66M	3~4	A1	1	0	46
P37L	4>	A1	14	4	29	P68M	3~4	A1	0	1	46
P37M	3~4	A1	0	2	45	P70M	3~4	A1	0	2	45
P37M1	2~3	A1	1	1	45	P71L	4>	A1	21	0	27
P41M	3~4	A1	0	2	45	P72M	3~4	A1	0	1	46
P43M	3~4	A1	0	2	45	P73L	4>	B1	1	19	27

[a]The diameter of plaque in PSA medium after incubation overnight.

[b]Phage morphology for phage families as described by Ackermann [3].

[c]Number of strains infected by the phages among 47 Xoo strains. According to the degrees of clarity, the spots were differentiated into three categories: ++, clearly visible plaque formation; +, small or faint plaque formation; -, no plaque.

The phages that belonged to the *Siphoviridae* family (P8L, P27L, P30L, P59L, P73L) produced more than 4 mm plaques in diameter on the lawns of host strain KACC 10860. However, the phages that belonged to the *Myoviridae* family produced relatively small-sized plaques with variable diameters ranging from 2 to 4 mm, except for P4L, P37L, and P71L.

Host Range and Efficiency of Plating

To test the host range, 59 strains of bacteria were subjected to spot tests with the isolated phages. The host range of the tested phages differed greatly according to Xoo strains. None of the tested Xoo strains were resistant to all of the tested 34 phages (Table 1). The phages that formed small-sized plaques infected most of the tested Xoo strains, except for P45M and P60M. Interestingly, the phages that formed large-sized plaques infected a lower number of Xoo strains than the phages that produced small-sized plaques, except P73L. The recently reported K3a race has similar susceptibility to each phage as that of the K3 race [27].

Several bacteria, including *B. glumae*, *P. s.* pv. *tomato*, *B. vallismorti*, *B. gladioli*, *P. fluorescence*, and various *Xanthomonas* isolates, were also subjected to spot test for the evaluation of the isolated phages that infect bacterial species other than Xoo. None of these bacteria were susceptible to any of the phages, indicating that the phages had host specificity only to Xoo.

Table 2. Relative plaque-forming efficiency of phages on *Xoo* strains.

Phages tested	Xoo race and strain			
	K1 KXO42	K2 KXO90	K3 KXO19	K1 KXO85
P4L	25[a]	0	100	0
P43M	101	116	100	98
P23M1	109	103	100	99
P37L	72	0	100	0
P37M	111	95	100	108
P37M1	73	74	100	96

[a]Efficiency of plating was compared by taking the number of plaques formed on KXO19 as 100. Data represent an average of three experiments with three replicates each.

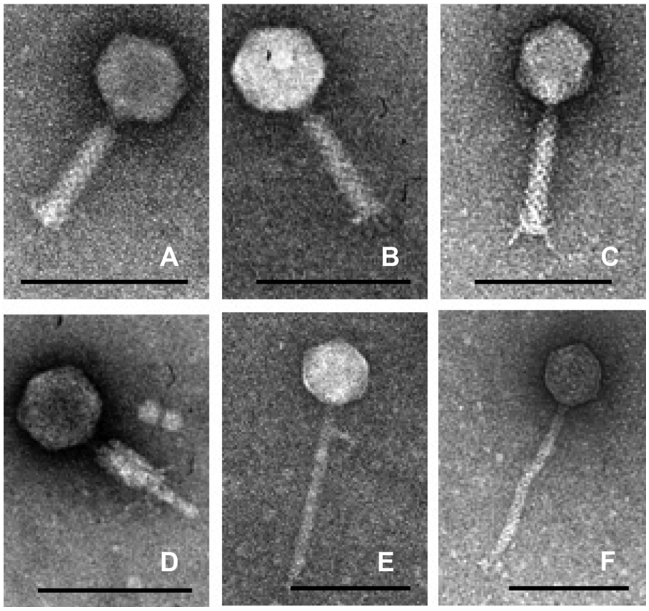

Fig. 1. Electron micrographs of phages.
Xoo phages belonging to the *Myoviridae* family in uncontracted (**A**–**C**) and contracted state (**D**) and the *Siphoviridae* family (**E**, **F**). Large (**A**) and small (**B**–**D**) plaques were formed by the phages of the *Myoviridae* family on PSA medium; however, only large plaques were formed by those of the *Siphoviridae* family. For TEM observation, a 3 µl drop of bacteriophages at a concentration of 10^{10} PFU/ml was applied to a formvar-coated grid, and then negative-stained with sodium phosphotungstate. **A**: P37L; **B**: P6M1; **C**: P50M; **D**: P68M; **E**: P30L; **F**: P59L. The bar represents 100 nm.

The relative efficiency of plating of six randomly selected phages on Xoo strain KXO19 was compared with three respective host strains (Table 2). Although the phages, P43M, P23M1, and P37M, had similar efficiencies of plating on the tested host strains, the other tested phages had different efficiencies depending on the bacterial strains. The efficiency of P4L on Xoo KXO42 strain was four times lower than on Xoo KXO19 strain. P4L and P37M could not infect Xoo KXO90 and KXO85 strains, confirming the result of the host specificity test.

htf Gene and Host Specificity

We obtained 15 PCR products from the 34 phages with the primers described above. According to the phylogenetic analysis, the deduced protein sequence of *htf* gene was differentiated into three clusters with 99% similarity (Fig. 2). The phages belonged to the *Myoviridae* and *Siphoviridae* families located together in the same group of the clusters, and the phages were not differentiated by the difference of host range.

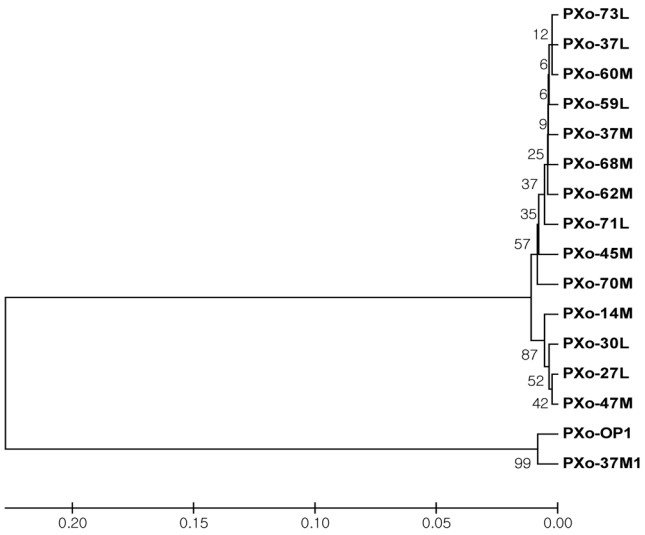

Fig. 2. Phylogenetic analysis of *htf* genes.
The deduced protein sequences of *htf* genes were analyzed with OP1 using MEGA5. The tree was inferred using the UPGMA method. The percentages of replicate trees in which the associated protein sequences clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The evolutionary distances were computed using the Poisson correction method.

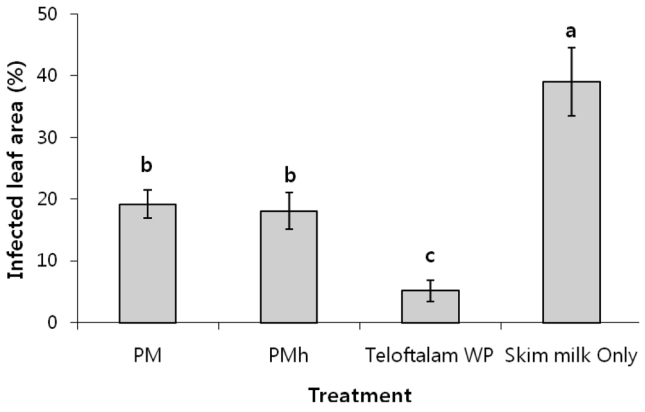

Fig. 3. Control effect of phage mixture and selected treatments on bacterial leaf blight of rice plant.
The different formulations were sprayed three times on the leaves of rice plants. Columns represent the occurrence of BLB in each treatment. The constitution of treatments was phage mixture (PM), h-mutant phage mixture (PMh), Tecloftalam WP (1 g/l), and skim milk only. The data are represented as standard deviation, and values denoted by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

Control Effect of Phages as a Biological Control Agent

The incidence of BLB on rice plants in the skim milk only treated plot was about 39.1% (Fig. 3). The application of

PM and PMh in skim milk significantly reduced the occurrence of BLB to 19.2% and 18.1%, respectively. The treatments of Tecloftalam WP provided 87.0% of control efficacy, which was better than the treatment of PM or PMh.

Discussion

Bacterial diseases can be controlled to a certain extent by agrochemicals; however, the chemicals have not always been effective and caused public concerns. To improve the control efficacy, we investigated the diversity and specificity of Xoo-infecting phages and demonstrated the use of phages as alternative measures for the management of BLB on rice plant.

Xoo-specific phages have been isolated and characterized for a long time. Moreover, the full genomes of some Xoo phages have been sequenced and annotated [12, 13, 21, 34]. However, we still need more understanding of both phage ecology and the complex phage–host interactions in various plant environments to maximize fully the use of phages as a biocontrol method [11]. In this study, 34 phages were isolated from floodwater of paddy fields in Korea and their morphotypes were characterized (Table 1). The majority of the identified phages to date possess tails and belong to the order *Caudovirales*, which encompasses over 95% of all known phages [22, 23]. Tailed phages are grouped into the *Myoviridae* family with a contractile tail, *Siphoviridae* family with a long non-contractile tail, and *Podoviridae* family with a short non-contractile tail. *Siphoviridae* is assumed to represent the most abundant phage morphotype in the environment [1, 6], and most previously reported phages infecting Xoo also belong to *Siphoviridae*. In this study, only five phages belonged to *Siphoviridae* (Figs. 1E and 1F), and the other 29 phages belonged to *Myoviridae* (Figs. 1A–1D). Although *Siphoviridae* comprises 61% of all tailed phages [3], a predominance of *Siphoviridae* among tailed viruses is not necessarily the case in marine environments [5, 16]. Although phages infecting Xoo have been studied for a long time, there has been no research on the distribution and diversity of phages in paddy fields in aquatic and damp soil environments to our knowledge. Our results indicated that *Myoviridae* were dominant tailed phages infecting Xoo in floodwater environments.

Although phages infecting strains of two or more species of a particular genus have been described, the results to date indicate that most of them are host species-specific and also demonstrate strain specificity [4, 8, 10]. All of the isolated phages in this experiment infected only Xoo strains. One of the interesting results was the negative relationship between plaque size and host range of the tested phages. *Siphoviridae*, which produced large plaques generally, had a narrow host range compared with those of *Myoviridae*. This result suggests that some phages specifically adapted to greatly exploit a certain range of the bacterial strains, although more investigation is needed on the relationship between host range and lysis capability. The difference in host range between *Siphoviridae* and *Myoviridae* can be an interesting subject for further research.

The coexistence of different phages for the same host in the same sample is a result of finely tuned interaction between the defense and counter-defense strategies of the phage and host, including reproductive efficiency, host range, and many other factors that we do not understand well as yet [26, 33]. Wakimoto [32] classified Xoo phages into two types, Op1 and Op2, based on their morphological and serological properties, and the phages collected from the Philippines were compared with those Op phages and classified into three types [3]. One of those types was found to be different in serological properties and host range pattern. In this study, 22 phages were able to infect Xoo strain PXO99 isolated from the Philippines, and the plaques formed in the lawn of the strain were very turbid (data not shown). This result indicated that the phages differentially adapted to the relevant bacterial strains, and thus the isolated area could affect the specificity. The results also suggested that classification of bacterial strains according to lysotype along with country-wide use of identical phages as a biological control agent needs to be investigated.

From the results of efficiency of infectivity, the average number of PFU per plate varied when an equal amount of phage was plated with different Xoo strains (Table 2). Interestingly, the phages having a narrow host range generally had low efficiency to the tested Xoo strains. The results suggested that the phages might have evolved to adapt specifically to the narrow range of the bacterial strains. In this experiment, the *htf* genes obtained from the 34 phages were analyzed in amino-acid sequence level, since the gene was reported to have a relationship with host specificity [13]. However, we could not find any relationship between *htf* gene clustering and host specificity (Fig. 2).

Phage-assisted biocontrol measures for bacterial diseases of crops were first studied in the early twentieth century [15, 30]. Civerolo and Kiel [7] reduced bacterial spot disease of peach seedlings caused by *X. oryzae* to 86% from 100% by application of phages. Kuo *et al*. [17] revealed that

phage of Xoo was widely distributed in water of paddy fields. When purified phage was applied 1, 3, and 7 days before Xoo inoculation, there were 100%, 96%, and 86% reductions of BLB, respectively. However, satisfactory results were not attained owing to the emergence of bacterial mutants resistant to the single-phage types used. To reduce the phage-resistant bacterial mutants emerging in a cropping system, a method using a mixture of different phages, including h-mutant phages, was tested [9, 14].

In this study, we tested a mixture of phages that have complementary host ranges and that can function independently in bacterial populations. Moreover, to increase the control efficacy, we produced h-mutant phages and tested the effectiveness of an h-mutant phage mixture for reducing the incidence of BLB in the field. The h-mutant phages produced in our laboratory attacked the resistant bacteria effectively and maintained specificity toward the wild-type bacteria. However, the h-mutant phages did not exhibit a wider host range (data not shown). PM and PMh in skim milk were tested for their efficacy of control of BLB. The PM and PMh provided 51.0% and 54.0% control effect, respectively, compared with the treatment of skim milk only. Although the control efficacy was not satisfactory, the results indicated that the phages could be used to reduce treatment with agrochemicals.

Overall, we demonstrated Xoo-infecting phages as an alternative measure for the management of BLB of rice. To increase the control efficiency of the phages on BLB, the interaction between bacterial pathogens and phages was studied in the area in which phages were applied. Although much research has unraveled the importance of phages in aquatic and freshwater environments, our results indicate that we need more understanding of these phages, especially regarding their relationship with their hosts.

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