Complete Genome Sequences of 10 *Xanthomonas oryzae* pv. oryzae Bacteriophages

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ABSTRACT *Xanthomonas oryzae* pv. oryzae is the causative agent of bacterial leaf blight of rice. The application of bacteriophages may provide an effective tool against this bacterium. Here, we report the complete genome sequences of 10 newly isolated OP2-like *X. oryzae* pv. oryzae bacteriophages.

Bacterial leaf blight (BLB) of rice is a devastating disease causing severe economic losses, especially in Asia and western Africa (1). The etiologic agent of this infection is the Gram-negative bacterium *Xanthomonas oryzae* pv. oryzae (2). Due to the low efficacy of current BLB treatment tools, the emergence of resistance in *X. oryzae* pv. oryzae against applied agents, and public health concerns, an efficient, flexible, and environmentally sound approach is needed for controlling BLB.

The application of bacteriophages provides an alternative option for defense against plant-pathogenic bacteria, including *X. oryzae* pv. oryzae (3). Phages against *X. oryzae* pv. oryzae have been isolated extensively (4–7). Based on their morphological and serological features, Wakimoto (8) classified *X. oryzae* pv. oryzae phages into two major groups, OP1 and OP2. Kuo et al. isolated and characterized a morphologically distinct type of *Caudovirales* (Xp20) and a filamentous phage (Xf) (4). Recently, five new OP2-like bacteriophages were isolated and characterized, but their complete genome sequences have not been determined until now (9). The complete genomes of OP1, OP2, Xp10, and Xop411 phages have been determined (10–13); however, no complete genome sequences of other OP2-like bacteriophages have been determined until now.

For bacteriophage isolation, infected leaves originating from the Mekong Delta, Vietnam, were collected in the summer of 2014, and infected leaves, paddy water, and soil were collected from the Philippines in the summer of 2016. Either 10-g soil samples were suspended in 50 ml PSA medium (14) or 25-ml filtered water samples were mixed with 25 ml PSA medium and shaken at 160 rpm at 28°C for 24 h. Cultures were sieved through sterilized gauze and centrifuged at 2,600 × *g* for 30 min at 4°C. Supernatants were filtered using 0.2-μm syringe filters. Twenty milliliters of filtrate was supplemented with 20 ml of PSA medium, 40 μl of 1 mM MgCl$_2$, 40 μl of 1 mM CaCl$_2$ and 250 μl of overnight cultures of *X. oryzae* pv. oryzae strains LMG 641 and LMG 796 (10^8 CFU ml^{-1}). The phage lysates were filtered using 0.2-μm syringe filters and stored at 4°C until further study. The presence of lytic phages was tested by spotting 10 μl filtrate onto an *X. oryzae* pv. oryzae bacterial lawn grown on PSA medium and incubating further at

TABLE 1 Genome data of *X. oryzae* pv. oryzae bacteriophages

Bacteriophage	Genome length (bp)	Terminal repeat length (bp)	G+C mol%	GenBank accession no.	SRA accession no.
XPP1	46,195	0	62.4	MG944227	SAMN11254550
XPP2	46,204	276	61.0	MG944228	SAMN11254551
XPP3	46,201	3,411	62.2	MG944229	SAMN11254552
XPP4	46,200	1,197	62.1	MG944230	SAMN11254553
XPP6	46,204	77	61.0	MG944231	SAMN11254554
XPP8	46,184	0	62.2	MG944232	SAMN11254555
XPP9	46,201	2,485	61.0	MG944233	SAMN11254556
XPV1	46,503	0	60.0	MG944234	SAMN11254557
XPV2	45,969	0	64.3	MG944235	SAMN11254558
XPV3	47,046	0	60.0	MG944236	SAMN11254559
OP2	46,643	71	60.9	AP008986[a]	

[a] Sequencing of the OP2 genome was performed by Inoue et al. (10).

28°C. All isolates were purified by three successive single-plaque isolation methods using the classical drop-on-lawn technique (15).

Phage nucleic acid was isolated by using the High Pure viral nucleic acid kit (Roche Diagnostics GmbH, Germany), according to the manufacturer's instructions. Genomic sequences of the *X. oryzae* pv. oryzae phages of this study were determined with MiSeq (Illumina, Inc., USA) next-generation sequencing (NGS) equipment, using Nextera XT kit (Illumina Inc.) for paired-end library preparation and Illumina V2 sequencing kit (Illumina Inc.), according to the guidelines of the manufacturer, resulting in 2,649,822 (250-bp-long) reads. The mean coverages were between 409× (XPP8) and 10,461× (XPP2).

The next-generation reads were analyzed for quality using the FastQC program (Babraham Bioinformatics, version 0.11.5), with default parameters. Low-quality bases and reads were trimmed and/or removed using the Trim Galore! (Babraham Bioinformatics, version 0.4.4 with paired mode) and Trimmomatic (version 0.36 with paired mode and using the CROP:150 MINLEN:150 parameters) programs (16). The quality-filtered reads were assembled using the MyPro software package (17). In the assembly processes, we used the Assembly.py and Integrate.py python scripts for all samples.

Genome annotation was performed using the RAST server (18), with manual curation. Each hypothetical or conserved protein-encoding gene was subjected to a search using NCBI blastx against the nonredundant protein (nr) database (19). Results were accepted when the E value was lower than e−10 and the coverage was higher than 75% as a cutoff for notable similarity.

The complete genomes of all 10 *X. oryzae* pv. oryzae phages were assembled. Table 1 contains the sequence lengths and G+C mol% of the newly isolated 10 phages and of the reference OP2. The G+C mol% contents of the newly isolated phages were in the range of 60.0 to 62.4 mol%, similar to that of OP2, with the exception of XPV2, for which it was higher (64.3 mol%; Table 1). The presence of 77- to 3,411-bp direct terminal repeats was detected (with a self dot plot, using the Geneious 8.0.5 software) in 6 newly isolated phages (Table 1). The complete genome nucleotide sequences of the phages were compared by pairwise alignments using the Geneious 8.0.5 software. Phage genome circularity was analyzed *in silico*. SAMtools/bcftools (20) was used to map the (raw) reads against the complete genomic sequence of the phage from whose genome sequence the reads originated. The positions of the mate-paired reads were investigated with IGV 2.5.2 (21). The circularity of the genomes was determined by observing mate-paired reads where the distance between the two reads spanned the whole genome. All of the investigated genomes were determined to be circular.

Genome sequencing of the newly isolated phages proved that these are OP2-like, with whole-genome nucleotide sequence similarities of 90.7 to 91.6% compared to OP2. XPV phages isolated from the Mekong Delta (Vietnam) had sequence identities of 93.2 to 96.3% compared to each other and identities of 93.0 to 94.7% compared to XPP phages isolated from the Philippines. Differences between the complete genome nucleotide sequences of the XPP phages were limited.

To the best of our knowledge, this work was the first in which the complete genomes of OP2-like *X. oryzae* pv. oryzae phages were determined.

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REFERENCES

1. Saha S, Garg R, Biswas A, Rai AB. 2015. Bacterial diseases of rice: an overview. J Pure Appl Microbiol 9:725–736.
2. Swings J, Van Den Mooter M, Vauterin L, Hoste B, Gillis M, Mew TW. 1990. Reclassification of the causal agents of bacterial blight Xanthomonas campestris pv. oryzae) and bacterial leaf streak (Xanthomonas campestris pv. oryzicola) of rice as pathovars of Xanthomonas oryzae ex Ishiyama 1922) sp. nov., nom. rev. Int J Syst Bacteriol 40:301–311. https://doi.org/10.1099/00207713-40-3-309.
3. Balogh B, Jones JB, Iriarte FB, Momol MT. 2010. Phage therapy for plant disease control. Curr Pharm Biotechnol 11:48–57. https://doi.org/10.2174/138920110790725302.
4. Kuo TT, Huang TC, Wu RY, Yang CM. 1967. Characterization of three bacteriophages of Xanthomonas oryzae. Bot Bull Acad Sinica 8:246–254.
5. Kuo TT, Huang TC, Chow TY. 1969. A filamentous bacteriophage from Xanthomonas oryzae. Virology 39:548–555. https://doi.org/10.1016/0042-6822(69)90102-0.
6. Lin NT, You BY, Huang CY, Kuo CW, Wen FS, Yang JS, Tseng YH. 1994. Characterization of two novel filamentous phages of Xanthomonas. J Gen Virol 75:2543–2547. https://doi.org/10.1099/0022-1317-75-9-2543.
7. Chae JC, Hung NB, Yu SM, Lee HK, Lee YH. 2014. Diversity of bacteriophages infecting Xanthomonas oryzae pv. oryzae in paddy fields and its potential to control bacterial leaf blight of rice. J Microbiol Biotechnol 24:740–747. https://doi.org/10.4014/jmb.1402.02013.
8. Wakimoto S. 1960. Classification of strains of Xanthomonas oryzae on the basis of their susceptibility against bacteriophages. Jpn J Phytopath 25:193–198. https://doi.org/10.3186/jjphytopath.25.193.
9. Ogunyemi SO, Chen J, Zhang M, Wang L, Masum MMI, Yan C, An Q, Li B, Chen J. 2018. Identification and characterization of five new OP2-related Myoviridae bacteriophages infecting different strains of Xanthomonas oryzae pv. oryzae. J Plant Pathol. https://doi.org/10.1007/s42161-018-0188-6.
10. Inoue Y, Matsuura T, Ohara T, Azegami K. 2006. Sequence analysis of the genome of OP2, a lytic bacteriophage of Xanthomonas oryzae pv. oryzae. J Gen Plant Pathol 72:104–110. https://doi.org/10.1007/s10327-005-0259-3.
11. Inoue Y, Matsuura T, Ohara T, Azegami K. 2006. Bacteriophage OP1, lytic for Xanthomonas oryzae pv. oryzae, changes its host range by duplication and deletion of the small domain in the deduced tail fiber gene. J Gen Plant Pathol 72:111–118. https://doi.org/10.1007/s10327-005-0252-x.
12. Lee CN, Hu RM, Chow TY, Lin JW, Chen HY, Tseng YH, Weng SF. 2007. Comparison of genomes of three Xanthomonas oryzae bacteriophages. BMC Genomics 8:442. https://doi.org/10.1186/1471-2164-8-442.
13. Yuzenkova J, Nechaev S, Berlin J, Rogulja D, Kuznedelov K, Inman R, Mushegian A, Severinov K. 2003. Genome of Xanthomonas oryzae bacteriophage Xp10: an odd T-odd phage. J Mol Biol 330:735–748. https://doi.org/10.1016/S0022-2836(03)00634-X.
14. Tsuchiya K, Mew TW, Wakimoto S. 1982. Bacteriological and pathological characteristics of wild types and induced mutants of Xanthomonas campestris pv. oryzae. Phytopathology 72:43–46. https://doi.org/10.1094/Phyto-77-43.
15. Adams MH. 1959. Bacteriophages. Interscience Publishers, New York, NY.
16. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
17. Liao YC, Lin HH, Sabharwal A, Haase EM, Scannapieco FA. 2015. MyPro: a seamless pipeline for automated prokaryotic genome assembly and annotation. J Microbiol Methods 113:72–74. https://doi.org/10.1016/j.mimet.2015.04.006.
18. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
20. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352.
21. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. 2011. Integrative genomics viewer. Nat Biotechnol 29:24–26. https://doi.org/10.1038/nbt.1754.