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Complete Genome Sequences of *Edwardsiella tarda*-Lytic Bacteriophages KF-1 and IW-1

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We report the complete genome sequences of two *Edwardsiella tarda*-lytic bacteriophages isolated from flounder kidney (KF-1) and seawater (IW-1). These newly sequenced phage genomes provide a novel resource for future studies on phage-host interaction mechanisms and various applications of the phages for control of edwardsiellosis in aquaculture.

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Edwardsiella tarda, a Gram-negative bacterium, has been isolated from a wide variety of animals, including humans. Particularly, *E. tarda* has long been known as a pathogen of various diseases causing severe economic losses in cultured fish species such as channel catfish (*Ictalurus punctatus*), Japanese eel (*Anguilla japonica*), and Japanese flounder (*Paralichthys olivaceus*) (reviewed in reference 1). No antimicrobial agent against *E. tarda* infections of fish is currently licensed in Japan. Although various types of experimental *E. tarda* vaccines have been reported, the resistance of *E. tarda* to phagocyte-mediated killing (2) has made it difficult to develop an effective vaccine (reviewed in references 1 and 3).

A number of bacteriophages (phages) that infect *E. tarda* have been isolated from flounder tissues and seawater samples from fish farms (4). The potential use of *E. tarda* phages as therapeutic agents against edwardsiellosis and as indicators of the presence of *E. tarda* in culture environments has been suggested (5). Genomic information regarding *E. tarda* phages is important for understanding phage-host interactions as well as for applications of the phages for the control of disease. In the present study, we determined the complete genome sequences of phages isolated from Japanese flounder kidney (KF-1) and seawater (IW-1).

Electron microscopic observations of both KF-1 and IW-1 indicated that each phage was a member of the family *Podoviridae* (6). Whole-genome shotgun sequencing of KF-1 and IW-1 was performed by using Roche 454 GS-FLX titanium pyrosequencing. *De novo* assembly of sequence reads was performed by using a 454 Newbler 2.5.3, and open reading frames (ORFs) were predicted by using GeneMarkS (7) and Glimmer3 (8). The predicted ORFs were annotated by using BLASTP (9) against the viral sequence database (E value threshold of $1E^{-3}$) and InterProScan (10). The potential presence of tRNAs was scanned by using the tRNAscan-SE 1.21 (11).

The sizes of the two entire genomes were 41,549 bp for KF-1 and 41,684 bp for IW-1. These sequences showed approximately 99% identity at the nucleotide level, with a GC content of 48.3%. Forty-eight ORFs were predicted for both genomes. Of these 48 ORFs, only 12 ORFs (25.0%) matched with at least one entry in the viral sequence database. Furthermore, a high level of sequence divergence was observed between these 12 annotated ORFs and the other phage proteins (range from 26 to 55% identities). A preliminary maximum-parsimony phylogenetic analysis based on large terminase subunit genes showed that KF-1 and IW-1 are closely related to the family *Podoviridae*. Thus, this preliminary phylogenetic analysis coincides with our morphological observations by electron microscopy. These newly sequenced *E. tarda* phage genomes and existing *E. tarda* genome sequences (12, 13) will provide the genetic resources for future studies on phages and edwardsiellosis.

Nucleotide sequence accession numbers. The complete genome sequences of the two *E. tarda* phage isolates were submitted to DDBJ under accession numbers AB757800 (KF-1) and AB757801 (IW-1).

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REFERENCES

1. **Evans JJ, Klesius PH, Plumb JA, Shoemaker CA, Han CH.** 2011. *Edwardsiella* septicaemias, p 512–569. *In* Woo PTK, Bruno DW, Han CH (ed), Fish diseases and disorders: viral, bacterial and fungal infections, 2nd ed, vol 3. CAB International, London, United Kingdom.
2. **Srinivasa Rao PS, Lim TM, Leung KY.** 2001. Opsonized virulent *Edwardsiella tarda* strains are able to adhere to and survive and replicate within fish phagocytes but fail to stimulate reactive oxygen intermediates. Infect. Immun. **69**:5689–5697.
3. **Mohanty BR, Sahoo PK.** 2007. Edwardsiellosis in fish: a brief review. J. Biosci. **32**:1331–1344.

4. **Matsuoka M, Nakai T.** 2004. Seasonal appearance of *Edwardsiella tarda* and its bacterial in the culture farm of Japanese flounder. Fish Pathol. **39**:145–152.
5. **Nakai T.** 2010. Application of bacteriophages for control of infectious diseases in aquaculture, p 257–272. *In* Sabour PM, Griffiths MW (ed), Bacteriophages in the control of food- and waterborne pathogens. ASM Press, Washington, DC.
6. **Kawato Y, Nakai T.** 2012. Infiltration of bacteriophages from intestinal tract to circulatory system in goldfish. Fish Pathol. **47**:1–6.
7. **Besemer J, Lomsadze A, Borodovsky M.** 2001. Genemarks: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. **15**:2607–2618.
8. **Delcher AL, Bratke KA, Powers EC, Salzberg SL.** 2007. Identifying bacterial genes and endosymbiont DNA with glimmer. Bioinformatics **23**:673–679.
9. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
10. **Zdobnov EM, Apweiler R.** 2001. InterProScan: an integration platform for the signature-recognition methods in InterPro. Bioinformatics **17**: 847–848.
11. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**: 955–964.
12. **Wang Q, Yang M, Xiao J, Wu H, Wang X, Lv Y, Xu L, Zheng H, Wang S, Zhao G, Liu Q, Zhang Y.** 2009. Genome sequence of the versatile fish pathogen *Edwardsiella tarda* provides insights into its adaptation to broad host ranges and intracellular niches. PLoS One **29**:e7646.
13. **Yang M, Lv Y, Xiao J, Wu H, Zheng H, Liu Q, Zhang Y, Wang Q.** 2012. Edwardsiella comparative phylogenomics reveal the new intra/inter-species taxonomic relationships, virulence evolution and niche adaptation mechanisms. PLoS One **7**:e36987.