Draft Genome Sequence of *Tokyovirus*, a Member of the Family *Marseilleviridae* Isolated from the Arakawa River of Tokyo, Japan

Masaharu Takemura

Laboratory of Biology, Department of Liberal Arts, Faculty of Science, Tokyo University of Science, Shinjuku, Tokyo, Japan

Members of the *Marseilleviridae* family are large DNA viruses with icosahedral particles that infect *Acanthamoeba* cells. This report presents a new *Marseilleviridae* family member discovered in a water/soil sample from a river in Tokyo, named *Tokyovirus*, with genome size of 370 to 380 kb.

The *Marseilleviridae* family is a group of giant viruses with smaller particles of 200-nm diameter and with a genome size of 300 to 400 kb (1–9). This article reports the draft genome of a new virus belonging to the family *Marseilleviridae*, isolated from the coast of the Arakawa River, located in Tokyo, Japan. It was named *Tokyovirus* according to the rules of nomenclature for *Marseilleviridae*.

Water/soil samples were collected from a bank of the Arakawa River. After mud was removed by filtration (filter paper 43; Whatman PLC), samples were further filtered (0.8-μm pore size, Millex-AA; Millipore). Filtered samples were concentrated by polyethylene glycol precipitation overnight at 4°C, followed by centrifugation at 1,500 × g for 30 min at 4°C (10). After the supernatant was removed, the pellet was resuspended in 4 ml of peptone yeast extract-glucose (PYG) broth and then filtered again (Millex-AA; Millipore). Then, 4 ml of fresh PYG and a 1-ml suspension of amoeba cells were added to this viral solution, which was divided and cultured on 56 wells in a 96-well culture plate at 26°C. After 10 days, I found that amoeba cells in only 1 well out of 56 wells had delayed proliferation and had been almost round. The supernatant of the culture in this single well was inoculated to fresh amoeba cells of 3 wells in a 96-well culture plate. Almost all cells were rounded. The supernatant was inoculated to fresh amoeba cells in a 25 cm² culture flask. After 2 days, rounded amoeba cells were harvested. Then, the supernatant was stored at 4°C as an isolated virus solution. After virus cloning (11), genomic DNA of *Tokyovirus* (1.1 μg) was prepared from PYG culture media including viral particles using NucleoSpin Tissue (Macherey-Nagel GmbH and Co. KG) according to the manufacturer’s protocol. A DNA library for sequencing was prepared using the TrueSeq Nano DNA LT library prep kit (Illumina, Inc.), and sequencing was performed on a HiSeq 2500 platform (Illumina, Inc.). Edena software was used to assemble 1,000,000 reads into 68 contigs with an average length of 5,481 nucleotides (nt) and a maximum contig length of 360,777 nt. The total length of the 68 contigs was 372,707 nt. Prediction of the coding region of the *Tokyovirus* genome was conducted using CRITICA version 1.05b and Glimmer 2 version 2.10. Prediction of tRNA was conducted using tRNAscan-SE version 1.23, according to the manufacturer’s protocols. The prediction of gene function was conducted using NCBI BLASTp in the NCBI NR and NCBI COG databases.

Genome analysis showed that *Tokyovirus* has a 370- to 380-kb genome. The total length of the 68 contigs was 372,707 nt, which is approximately equal to the genome sizes of other *Marseilleviridae* members (1, 2, 4, 7). This draft sequence is predicted to have 487 coding sequences (CDSs) and 2 tRNA genes (one of them is a pseudogene). Genome comparison revealed that *Tokyovirus* closely resembles *Marseillevirus* and *Melbournevirus*. *Tokyovirus* has 47 CDSs, which are predicted to be highly similar (>90% identical) with *Marseillevirus* homologs.

**Nucleotide sequence accession number.** The draft genomic sequence of *Tokyovirus* has been deposited in DDBJ/ENA/GenBank under the accession number AP017398.

**ACKNOWLEDGMENTS**

I thank Yasunobu Terabayashi of Takara Bio, Inc. (Yokkaichi, Mie, Japan) for *Tokyovirus* genome analysis and Shingo Murono and Tatsuya Mikami of my laboratory for their useful discussions. I also thank Hiroyuki Ogata of Kyoto University, Hiroyuki Yamaguchi of Hokkaido University, Satoru Arai of the National Institute of Infectious Diseases of Japan, Bernard La Scola of the Faculté de Médecine, Aix-Marseille Université, and Chantal Abergel of CNRS, Aix-Marseille Université, for their useful comments and discussions.

**FUNDING INFORMATION**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**REFERENCES**

1. Boyer M, Yutin N, Pagnier J, Barrassi L, Fournous G, Espinosa L, Robert C, Azza S, Sun S, Rossmann MG, Suzan-Monti M, La Scola B, Koonin EV, Raoult D. 2009. Giant Marseillevirus highlights the role of amoebae as a melting pot in emergence of chimeric microorganisms. Proc Natl Acad Sci USA 106:21848–21853. http://dx.doi.org/10.1073/pnas.0911354106.

2. Thomas V, Bertelli C, Collyn F, Casson N, Teltens A, Goessmann A, Croxatto A, Greub G. 2011. Lausannevirus, a giant amoebal virus encod-
ing histone doublets. Environ Microbiol 13:1454–1466. http://dx.doi.org/10.1111/j.1462-2920.2011.02446.x.

3. Lagier J-C, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G, Maraninchi M, Trape J-F, Koonin EV, La Scola B, Raoult D. 2012. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 18:1185–1193. http://dx.doi.org/10.1111/1469-0691.12023.

4. Aherfi S, Pagnier I, Fournous G, Raoult D, La Scola B, Colson P. 2013. Complete genome sequence of Cannes 8 virus, a new member of the proposed family “Marseilleviridae.” Virus Genes 47:550–555. http://dx.doi.org/10.1007/s11262-013-0965-4.

5. Boughalmi M, Pagnier I, Aherfi S, Colson P, Raoult D, La Scola B. 2013. First isolation of a Marseillevirus in the Diptera syrphidae Eristalis tenax. Intervirology 56:386–394. http://dx.doi.org/10.1159/000354560.

6. Aherfi S, Boughalmi M, Pagnier I, Fournous G, La Scola B, Raoult D, Colson P. 2014. Complete genome sequence of Tunisivirus, a new member of the proposed family Marseilleviridae. Arch Virol 159:2349–2358. http://dx.doi.org/10.1007/s00705-014-2023-5.

7. Doutre G, Philippe N, Abergel C, Claverie J-M. 2014. Genome analysis of the first Marseilleviridae representative from Australia indicates that most of its genes contribute to virus fitness. J Virol 88:14340–14349. http://dx.doi.org/10.1128/JVI.02414-14.

8. Doutre G, Arfib B, Rochette P, Claverie J-M, Bonin P, Abergel C. 2015. Complete genome sequence of a new member of the Marseilleviridae recovered from the brackish submarine spring in the cassis Port-Miou Calanque, France. Genome Announc 3(6):e01148-15. http://dx.doi.org/10.1128/genomeA.01148-15.

9. Dornas FP, Assis FL, Aherfi S, Arantes T, Abrahão JS, Colson P, La Scola B. 2016. A Brazilian Marseillevirus is the founding member of a lineage in family Marseilleviridae. Viruses 8(6). http://dx.doi.org/10.3390/v8060076.

10. Philippe N, Legendre M, Doutre G, Couté Y, Poirot O, Lescot M, Arslan D, Seltzer V, Bertaux L, Bruley C, Garin J, Claverie J-M, Abergel C. 2013. Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes. Science 341:281–286. http://dx.doi.org/10.1126/science.1239181.

11. Legendre M, Lartigue A, Bertaux L, Jeudy S, Bartoli J, Lescot M, Alempic J-M, Ramus C, Bruley C, Labadie K, Shmakova L, Rivkina E, Couté Y, Abergel C, Claverie J-M. 2015. In-depth study of Mollivirus sibericum, a new 30,000-γ-old giant virus infecting Acanthamoeba. Proc Natl Acad Sci USA 112:E5327–E5335. http://dx.doi.org/10.1073/pnas.1510795112.