Draft Genome Sequences of Three Strains of *Pseudomonas syringae* pv. *eriobotryae*, a Pathogen Causing Canker Disease in Loquat, Isolated in Japan

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**ABSTRACT** The phytopathogenic bacterium *Pseudomonas syringae* pv. *eriobotryae* causes canker disease in loquat. Isolates from Japan are classified into three groups based on pathogenicity and pigment production. In this study, we report the draft genome sequences of three strains, one belonging to each of the three groups.

Loquat (*Eriobotrya japonica* [Thunb.] Lindl.), a subtropical evergreen fruit tree, is widely cultivated in subtropical regions. *Pseudomonas syringae* pv. *eriobotryae* causes loquat canker disease, the most serious disease in loquat cultivation (1). Isolates from Japan are classified into three groups: group A is not pathogenic to mesophyll and produces no pigment in culture medium; group B is pathogenic to mesophyll and produces no pigment; group C is not pathogenic to mesophyll and produces brown pigment (2). In loquat, a single dominant gene (*Pse-a*) controls resistance to group A (3), and a single recessive gene (*Pse-c*) controls resistance to group C (4). Here, we report the draft genome sequences of three strains isolated in Japan (AM001, BM001, and CG001), one from each group.

Three strains of *Pseudomonas syringae* pv. *eriobotryae* were isolated from a leaf from a diseased loquat tree (3, 4). The bacteria were cultured at 37°C for 16 to 18 h in King’s medium (5) with shaking. DNA was isolated using cetyltrimethylammonium bromide (CTAB)/NaCl and phenol-chloroform extractions, followed by isopropanol precipitation (6). Sequencing libraries were generated using the NEBNext Ultra DNA library prep kit for Illumina (NEB, USA). The libraries were sequenced with 150-bp paired-end reads by Novogene (Beijing, China), using a NovaSeq 6000 system (Illumina, San Diego, CA, USA). Adapter sequences and low-quality bases were trimmed using Trimmomatic v 0.39 (ILLUMINACTION:adapter_sequence:2:30 LEADING:20 TRAILING:20 SLIDINGWINDOW:5:20 MINLEN:50) (7). Assembly was performed using Unicycler v 0.4.8 (8), and gene annotation was performed using DFAST (https://dfast.nig.ac.jp) (9). To construct a phylogenetic tree, all combinations of 2,246 orthologous proteins were extracted using OrthoFinder v 2.3.12 (-og) (10), multiple alignments were performed using MAFFT v 7.471 (--maxiterate 1000 --localpair) (11) and trimmed using trimAl v 1.4 (-automated1) (12), and a maximum-likelihood tree was constructed using IQ-TREE (-sp, -bb 1000) (v 2.0.6) (13). Default parameters were used for all software tools, unless otherwise stated.

The resulting genome assemblies are shown in Table 1.

The draft genome sequences of five *Pseudomonas* strains pathogenic to loquat (CFBP2343, ICMP4316, ICMP4455, ICMP4967, and ICMP8636) are available in DDBJ/EMBL/GenBank. The source of ICMP8636 is unknown; the other four strains were isolated in the United States. We additionally used the genome sequences of 10 *Pseudomonas* strains pathogenic to the other plants (1448A, KBS0707, ATCC 11528, FTRS U6602, FTRS U6603, NCPPB3335, R15244, CFBP3840, CFBP2116, and LMG5095) to...
| Strain | BioSample accession no. | Group | No. of reads (bp) | Total sequence length (bp) | No. of contigs | Avg coverage (x) | No. of CDSs | No. of tRNAs | No. of rRNAs | G+C content (%) | Coding ratio (%) | DDBJ/GenBank accession no. | SRA accession no. |
|--------|------------------------|-------|------------------|---------------------------|---------------|-----------------|-------------|-------------|-------------|-----------------|----------------|----------------------|------------------|
| AM001  | SAMD00239674           | A     | 8,367,914        | 6,576,215                 | 226           | 184              | 5,989       | 4           | 4           | 57.6            | 84.9            | BMZW000000000.1     | DRA010831        |
| BM001  | SAMD00239675           | B     | 9,420,632        | 6,324,864                 | 140           | 202              | 5,780       | 77          | 4           | 57.6            | 85.9            | BMZX000000000.1     | DRA010830        |
| CG001  | SAMD00239676           | C     | 8,506,582        | 6,327,638                 | 227           | 194              | 5,784       | 55          | 3           | 58.0            | 85.2            | BMZY000000000.1     | DRA010832        |

*CDSs, coding DNA sequences.*
construct a phylogenetic tree (Fig. 1). In this tree, two Japanese strains (AM001 and CG001), three U.S. strains (CFBP2343, ICMP4316, and ICMP4455), and ICMP8636 formed a single clade. Because loquat was introduced into the United States from Japan or China, the three U.S. strains and ICMP8636 probably came from East Asia. Interestingly, one of the Japanese strains, BM001, was closely related to P. savastanoi pv. savastanoi NCPPB3335, the causal agent of olive knot disease. Mechanisms such as horizontal gene transfer and hybridization might have genetically separated BM001 from the other two Japanese strains. More interestingly, one of the U.S. strains, ICMP4967, was genetically distant from the other seven strains. Although further study is required, the progenitor of this strain might have been present in the United States before the introduction of loquat.

The draft genome sequences reported here will provide fundamental information for elucidating the mechanisms of infection of P. syringae pv. eriobotryae and facilitate progress toward protection against this pathogen.

**Data availability.** The draft genome sequences and corresponding read data are available in DDBJ/GenBank. The DDBJ/GenBank and SRA accession numbers for P. syringae pv. eriobotryae AM001, BM001, and CG001 are listed in Table 1.

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**REFERENCES**

1. Morita A. 1991. Effect of the inoculation of Pseudomonas syringae pv. eriobotryae at seedling stage upon its growth and fruit-productivity. Jpn J Phytopathol 57:629–633. (In Japanese.) [https://doi.org/10.3186/jjphytopath.57.629](https://doi.org/10.3186/jjphytopath.57.629).

2. Morita A. 1978. Studies on the loquat canker caused by Pseudomonas eriobotryae (Takimoto) Dowson. II: grouping of the bacterial isolates on the basis of their pigment producibility and pathogenicity. Jpn J Phytopathol 44:6–13. (In Japanese.) [https://doi.org/10.3186/jjphytopath.44.6](https://doi.org/10.3186/jjphytopath.44.6).

3. Hiehata N, Sato Y, Fukuda S, Terai O. 2002. Inheritance of resistance to loquat canker (Pseudomonas syringae pv. eriobotryae, Group A) in loquat
(Eriobotrya japonica). Jpn Soc Hort Sci 71:255–261. https://doi.org/10.2503/jjshs.71.255.

4. Hiehata N, Sato Y, Fukuda S, Tominaga Y, Terai O, Nesumi H. 2012. Inheritance of resistance to loquat canker (Group C) in progenies derived from ‘Shiromogi’ loquat. J Amer Soc Hort Sci 137:152–156. https://doi.org/10.21273/JASHS.137.3.152.

5. King EO, Ward MK, Raney DE. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med 44:301–307.

6. Shingu Y. 2003. Studies on the analysis of pathogenic genes in the bacterial grain rot of rice. PhD thesis. University of Meiji, Tokyo, Japan.

7. 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.

8. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.

9. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713.

10. Emms DM, Kelly S. 2019. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol 20:238. https://doi.org/10.1186/s13059-019-1832-y.

11. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010.

12. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25:1972–1973. https://doi.org/10.1093/bioinformatics/btp348.

13. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300.