



Draft Genome Sequence of *Pseudomonas syringae* RAYR-BL, a Strain Isolated from Natural Accessions of *Arabidopsis thaliana* Plants

Isabel Fuenzalida-Valdivia,^{a,c} Maria Victoria Gangas,^{a,c} Diego Zavala,^{a,b,c} Ariel Herrera-Vásquez,^{a,c} Fabrice Roux,^e Claudio Meneses,^{a,d}  Francisca Blanco-Herrera^{a,b,c}

^aCentro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile

^bCenter of Applied Ecology and Sustainability (CAPES), Santiago, Chile

^cANID-Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio), Santiago, Chile

^dFONDAP Center for Genome Regulation, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile

^eLaboratoire des Interactions Plantes-Microbes-Environnement, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, CNRS, Université de Toulouse, Castanet-Tolosan, France

ABSTRACT Here, we report the genome sequence of the *P. syringae* strain RAYR-BL, isolated from natural accessions of *Arabidopsis* plants. The draft genome sequence consists of 5.85 Mbp assembled in 110 contigs. The study of *P. syringae* RAYR-BL is a valuable tool to investigate molecular features of plant-pathogen interaction under environmental conditions.

Pseudomonas syringae is a Gram-negative bacterium able to colonize plants and produce disease in the most economically important crop species (1). More than 60 pathovars have been recognized in the species (2), each with genetic characteristics improving their fitness to colonize particular plant species, even when potentially affecting different hosts (3). In 1991, Whalen et al. reported that *P. syringae* pv. tomato isolated from tomato plants can also infect the model plant *Arabidopsis thaliana* (4, 5). The wide range of genetic and technical tools offered by *Arabidopsis* and its capacity to host *P. syringae* pv. tomato as a pathogen result in a high-impact model system to dissect the physiological and molecular bases of the plant defense response (6, 7) and the mechanisms of bacterial pathogenicity.

In 2018, Bartoli et al. isolated bacterial microorganisms from natural *Arabidopsis* accessions located in the south of France, identifying a new strain of *Pseudomonas*, *P. syringae* RAYR-BL, able to produce severe disease symptoms on different natural *Arabidopsis* accessions (8). This strain also infects *Arabidopsis* Col-0 plants, increasing the bacterial leaf population and inducing disease-associated phenotypic changes (Fig. 1). The study of the genome of the *P. syringae* RAYR-BL strain found in *Arabidopsis* plants will allow us to determine the pathogenicity mechanisms of species that naturally coexist and interact in the environment.

To study the genetic features of the *P. syringae* RAYR-BL strain, the bacteria were sent on semisolid medium from INRA-France from Fabrice Roux's laboratory. The bacteria were expanded on liquid King's B medium and stored at -80°C (15% glycerol). For DNA extraction, the stored bacteria were plated on King's B medium, and then a single colony was grown on King's B medium for 24 h at 28°C under shaking conditions. Genomic DNA isolation was performed using a Qiagen DNeasy blood and tissue kit. Then, 100 ng of the isolated DNA was used in a TruSeq DNA sample preparation kit (Illumina, Inc., USA). Whole-genome sequencing of *P. syringae* RAYR-BL was performed on a MiSeq platform at the Plant Biotechnology Center of Universidad Andres Bello, Chile. We obtained 2,723,120 paired-end reads of 300-bp length (2×300 bp). Default

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Address correspondence to Francisca Blanco-Herrera, mblanco@unab.cl.

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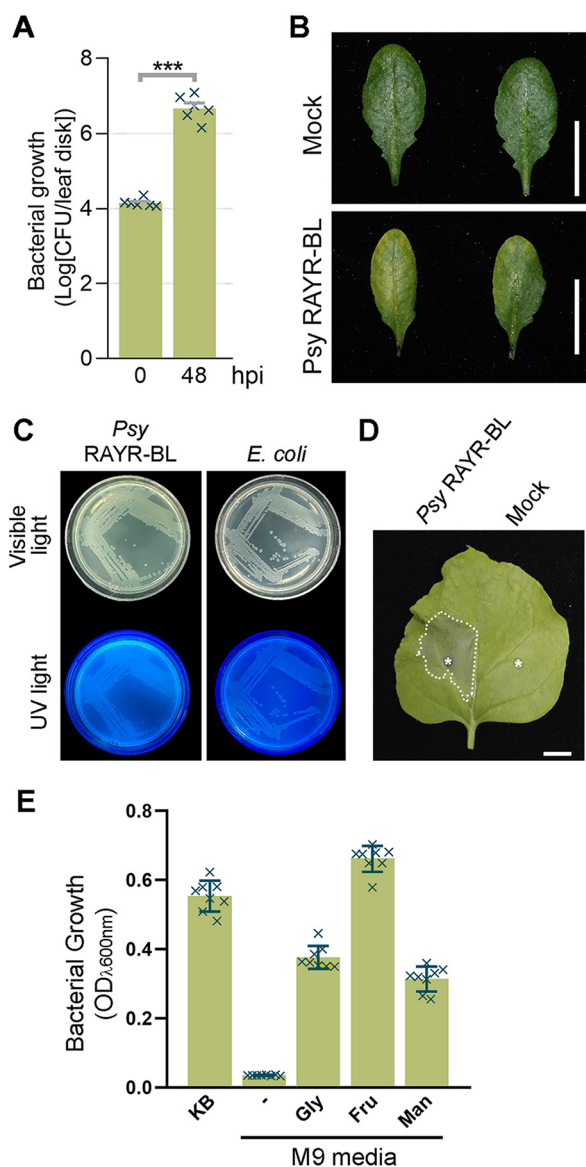


FIG 1 (A and B) *Pseudomonas syringae* RAYR-BL phenotypes. *P. syringae* RAYR-BL produces disease on the model plant *Arabidopsis thaliana* ecotype Columbia 0. Four-week-old *A. thaliana* (Col-0) plants were syringe-inoculated with *P. syringae* RAYR-BL (optical density at 600 nm [OD₆₀₀], 0.1) or 10 mM MgCl₂ as the control (mock), and they were maintained under normal growing conditions (16 h light/8 h dark cycle at 22°C, 100 mmol m⁻² s⁻¹, humidity ≈90%) until the leaves were sampled. (A) Bacterial proliferation is expressed as the mean of the log CFU/leaf disk ± standard deviation (SD) (*n* = 6). Disks (5 mm²) from inoculated leaves were cut using a punch immediately after inoculation (0 h postinfiltration [hpi]) and 48 hpi. Each disk was sampled from a different leaf from a different plant. The disks were ground on sterile MgCl₂ and then plated on solid King's B selective medium. The colonies were manually counted. Asterisks indicate statistical differences (*P* < 0.001) in an unpaired *t* test. (B) The plants inoculated with *P. syringae* RAYR-BL displayed a disease phenotype characterized by chlorosis after 72 hpi. Bar = 1 cm. (C) *P. syringae* RAYR-BL produces fluorescent molecules when it grows on King's B medium. The bacteria *P. syringae* RAYR-BL and *Escherichia coli* were cultured on King's B medium plates at 28°C. After 48 h the plates were photographed under UV and visible light. (D) *P. syringae* RAYR-BL produces disease symptoms on tobacco plants; 4-week-old plants were syringe-inoculated with *P. syringae* RAYR-BL (OD₆₀₀, 0.01) on the left side of the leaf. As a control, MgCl₂ (mock) was infiltrated on the right side. The asterisks indicate the inoculation site. The plants were maintained under normal growing conditions (16 h light/8 h dark cycle at 22°C, 100 mmol m⁻² s⁻¹, humidity ≈40%) for 48 hpi. Then the inoculated leaves were collected and photographed. The dotted line limits the leaf lesion. Bar = 1 cm. (E) The *P. syringae* RAYR-BL growth was evaluated on media supplemented with different carbon sources. The strain was incubated (28°C, 24 h, 180 rpm) on King's B (KB) medium as a rich medium, M9 medium without carbon sources (-), or M9 medium supplemented with glycerol (Gly, 40 mM), fructose (Fru, 20 mM), or mannitol (Man, 20 mM). The data represent the mean of the OD₆₀₀ ± SD (*n* = 8).

parameters were used for all software unless otherwise specified. Quality assessment of the reads was done using FastQC v0.11.7 (9). Removal of low-quality reads and adapter content was performed with Trimmomatic v0.38 (SLIDINGWINDOW:10:25 MINLEN:50) (10). The filtered reads were assembled *de novo* using SPAdes v3.11.0 (--careful -k 77, 89, 99) (11), and the draft assembly metrics were obtained with QUAST v5.1 (12). Contigs shorter than 200 bp were discarded, and those remaining were ordered against the reference *Pseudomonas syringae* DC3000 genome (13) using the Mauve Contig Mover software v2.4 (14).

The resulting draft genome sequence of *P. syringae* RAYR-BL consists of 5,853,599 bp assembled in 110 contigs, with an average G+C content of 58.98%, an N_{50} value of 193,008 bp (largest contig, 1,064,144 bp), and an L_{50} value of 8 contigs. The completeness of the draft genome assembly was assessed with a BUSCO (Benchmarking Universal Single-Copy Orthologs) v4.0.5 test (15), showing a completeness of 98.6% using the *Pseudomonadales* (OrthoDB v10) as a reference orthologue set. The genome annotation was made using Prokka v1.12 (--addgenes --gram neg --rfam --usegenus --genus *Pseudomonas*, with the *Pseudomonas* database created with all the complete *Pseudomonas* genome sequences found in the NCBI assembly database) (16), which predicted 5,188 genes, 5,053 coding sequences, 6 rRNA genes, and 57 tRNA genes. A phylogeny tree based on the citrate synthase (*cts*) sequence reveals that *P. syringae* RAYR-BL belongs to the *P. syringae* complex (8). Even when average nucleotide identity (ANI) values obtained with PyANI v0.2 (17) using ANI based on BLAST (ANIb) analysis reported that the best match corresponds to *Pseudomonas tremae* (88.29%), comparison with other *P. syringae* strains such as *P. syringae* pv. *oryzae* strain 1_6 and *P. syringae* DC3000 show similar ANIb values of 87.7% and 87.4%, respectively. To clarify the taxonomic status, further phenotypic analysis demonstrates that *P. syringae* strain RAYR-BL shares the following important features with the *P. syringae* species, unlike *P. tremae*: (i) the production of fluorescent molecules when it grows on King's B medium (Fig. 1C) (18), (ii) the induction of disease phenotype on the host plant *Nicotiana benthamiana* (Fig. 1D) (19), and (iii) the capacity to metabolize and grow on glycerol, fructose, and mannitol as carbon sources (Fig. 1E) (18).

Data availability. This whole-genome shotgun project for *P. syringae* RAYR-BL has been deposited at DDBJ/ENA/GenBank under the accession number [JAHZNS000000000](https://www.ncbi.nlm.nih.gov/assembly/JAHZNS000000000/). The version described in this paper is version [JAHZNS010000000](https://www.ncbi.nlm.nih.gov/assembly/JAHZNS010000000/). The raw sequencing reads for this project can be found under SRA accession number [SRR15318771](https://www.ncbi.nlm.nih.gov/sra/SRR15318771). The BioProject accession number is [PRJNA750763](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA750763).

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