Genome Resource of *Pseudomonas chlororaphis* L5734: A Bacterium Antagonistic to the Mulberry Gray Mold Pathogen *Botrytis cinerea*

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Keywords

biological control, fungi, genomics, microbe-genome sequencing, trees

Genome Announcement

Pseudomonas chlororaphis strain L5734 was isolated from the rhizosphere soil of mulberry (*Morus notabilis* Schneid.) in Mianyang, Sichuan Province, China, and exhibited strong antifungal activity for several fungal pathogens. Strains of *P. chlororaphis* can promote plant growth and antagonize fungi via secondary metabolites. Strain L5734 exhibited antagonistic activity against fungal pathogen *Botrytis cinerea*, which causes gray mold in *M. notabilis*. As of November 2021, 101 *P. chlororaphis* genomes have been reported in the NCBI database. Many *P. chlororaphis* strains were recognized as plant-growth-promoting rhizobacteria and have biocontrol activity because they produce many secondary metabolites such as siderophores, cyclic peptides, pyrrolnitrin, phenazine, and volatile organic compounds (Dimkpa et al. 2012; Park et al. 2011, 2012; Song et al. 2020; Zhang et al. 2019), which have inhibitory activities against plant pathogens (Hu et al. 2014; Kamou et al. 2020).

Mulberry is widely cultivated as an economic fodder for the domesticated silkworm (*Bombyx mori*) in sericulture, in addition to its delicious fruit and its uses in traditional medicine. However, the fungal pathogen *Botrytis cinerea* causes gray mold disease, which is ubiquitous and difficult to control due to its wide host range and high resistance (Mbengue et al. 2016; Williamson et al. 2007), and farming has exacerbated the damage caused by the disease (Datta 2000).

In the present study, strain L5734 was isolated from the rhizosphere of *M. notabilis*, and identified as *P. chlororaphis* by 16S ribosomal RNA (rRNA) gene sequencing analysis. Strain L5734 demonstrated a broad-spectrum antifungal activity against plant pathogens, including *B. cinerea*, *Phytophthora capsici*, and *Magnaporthe oryzae* (Supplementary Fig. S1). Here, we report the complete genome sequence data of *Pseudomonas chlororaphis* strain L5734 in order to obtain a better understanding of its genome, with a special focus

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Table 1. Genome summary statistics of *Pseudomonas chlororaphis* L5734 strain

Genome feature	Results
Genome size (bp)	6,880,469
GC content (%)	62.76
Total genes	6,242
Coding genes	5,960
Pseudogenes	193
Transfer RNA genes	69
Ribosomal RNA genes	16
Noncoding RNA genes	4
Genome coverage	186x
N ₅₀ (bp)	23,451
Protein with functional assignments	4,959 (76.92%)
Benchmarking universal single-copy orthologs	C:98.9% [S:98.8%, D:0.1%], F:0.3%, M:0.8%, n:782

on the secondary metabolite gene clusters, which suggest its potential application as bio-control agents.

Strain L5734 was cultured in 10 ml of Luria-Bertani medium at 30°C by shaking at 180 rpm overnight. Extraction of genomic DNA from L5734 was carried out as described previously (Huang et al. 2011) according to the protocol of a genomic DNA isolation kit (BioDev-Tech, Beijing, China). Whole-genome sequencing was transferred to Woosen Biotechnology (Hangzhou, China), where the analysis was performed using Oxford Nanopore Technologies (Promethlon 48), whereas the hybrid error-prone base correction was performed using the Illumina HiSeq 2500 sequencing technology (Koren et al. 2012). Thereafter, 104,789 long reads, with a mean length of 12,441 bp and N₅₀ read length of 23,451 bp and 186x coverage sequence depth (from Oxford Nanopore), and 1,933,280 paired-end Illumina reads (2 × 150 bp, from Illumina HiSeq 2500) were collected for *P. chlororaphis* L5734 assembly. The genome was assembled using raw Nanopore sequencing reads by Canu v2.0 (Koren et al. 2017) and circularized by Circlator v0.69-9 (Hunt et al. 2015). The 2 × 150 bp paired Illumina sequencing reads filtered out low-quality sequence reads (phred quality score < 30), then mapped reads to the assembled genome by bwa v0.7.17-r1188 (Li and Durbin 2009) and corrected the genome by Pilon v1.23 (Walker et al. 2014). We assessed the genome completeness with benchmarking universal single-copy orthologs (BUSCO v5.1.2) (Seppey et al. 2019) with the pseudomonadales_odb10 dataset; the completeness of the genome assembly was estimated to be 98.9%. Genome comparison between *P. chlororaphis* strain Qlu-1 (Li et al. 2020) and L5734 genomes was obtained using an average nucleotide identity calculator (Varghese et al. 2015), which revealed a 95.86% similarity. An in vitro tablet confrontation antagonistic assay (plant pathogens versus L5734) was performed as described previously (Cong et al. 2019). Inhibition rate = [(the control group colony radius – the inhibited group colony radius)/the control group colony radius] × 100%.

The *P. chlororaphis* L5734 genome was assembled into a circular contig of 6,880,469 bp, with a GC content of 62.76%. It contained 6,242 genes, including 69 transfer RNA genes, 16 rRNA genes, 4 noncoding RNA genes, 193 pseudogenes and 5,960 coding genes with NCBI Prokaryotic Genome Annotation Pipeline (Table 1).

According to clusters of orthologous group analysis, 549 genes were annotated with amino acid transport and metabolism, 514 genes were classified into general function prediction only, and the third most abundant coding genes (504 genes) were related to transcription. Furthermore, 164 genes were involved in secondary metabolite biosynthesis, transport, and catabolism, which illustrates the potential capacity of strain L5734 against *B. cinerea*.

The secondary metabolite gene clusters such as pyocyanine and bananamid produced by the strain L5734 may execute antagonistic activity against fungal pathogens, including playing a critical role in the inhibition of mulberry gray mold caused by fungal pathogen *B. cinerea*. AntiSMASH software (version 6.0.1) was used to identify the secondary metabolite gene clusters (Blin et al. 2021). Fourteen regions in the L5734 genome were predicted as possible secondary metabolite gene clusters. These results suggest that *P. chlororaphis* strain L5734 genome data could be an invaluable resource for studying its antagonistic mechanisms and application as a biocontrol agent. The secondary metabolites include

siderophore, nonribosomal peptide-synthetase, ribosomal peptide product-like, arylpolyene, redox-cofactor, phenazine, N-acetylglutaminyglutamine amide, β -lactone, and butyrolactone, in which seven pyocyanine synthetic genes (including *phzA*, *phzB*, *phzC*, *phzD*, *phzE*, *phzF*, and *phzG*) were identified with a similarity of 100% (Viswanathan and Samiyappan 2004). In addition, bananamide synthesis genes (*banA*, *banB*, *banC*, *banD*, *banE*, *banF*, *banG*, and *banH*) also exhibited 100% similarity in the genome (Omoboye et al. 2019). Both compounds have provided evidence for the antifungal activity of the L5734 strain, which will be useful for genetic engineering work and optimization of fermentation conditions in the future.

Data Availability

The complete genome sequence of strain L5734 has been deposited in GenBank under accession number CP083442 and BioProject PRJNA762430.

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