

Genome Sequence of the Pathogenic *Herbaspirillum seropedicae* Strain Os34, Isolated from Rice Roots

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Most *Herbaspirillum seropedicae* strains are beneficial endophytes to plants. In contrast, *H. seropedicae* strain Os34, isolated from rice roots, is pathogenic. The draft genome sequence of strain Os34 presented here allows in-depth comparative genome analyses to understand the specific mechanisms of beneficial and pathogenic *Herbaspirillum*-plant interactions.

Herbaspirillum seropedicae, the type species of the genus *Herbaspirillum*, has been widely isolated from plants, particularly from important cereals in the family *Poaceae*, such as rice, maize, sugarcane, and sorghum (1, 13). *H. seropedicae* can colonize in plants (6, 7) and promote plant growth by biological nitrogen fixation (1, 4). *H. seropedicae* is studied as a model of bacterial entry into host plants and plant growth promotion by endophytic bacteria (12).

Strain SmR1, a spontaneous mutant of strain Z78 (ATCC 35893), isolated from sorghum roots (1), is the best studied *H. seropedicae* strain (12). Its genome has been sequenced and annotated (14). It has multiple plant growth-promoting potentials, such as N₂ fixation, production of indoleacetic acids and siderophores, and reduction of plant ethylene generation via cleavage of the precursor 1-aminocyclopropane-1-carboxylate (ACC) catalyzed by ACC deaminase (12, 14).

Ten strains belonging to the genus *Herbaspirillum* were isolated from roots of rice cultivar C2 grown in a field on the Huajiachi campus of Zhejiang University, Hangzhou, China (9). Five of them, Os34, Os38, Os44, Os45, and Os49, were identified as *H. seropedicae*. Their 16S rRNA gene sequences (GenBank accession no. HQ728567, HQ728570, HQ728574, HQ728575, and HQ728579) showed the highest similarity (>99%) to *H. seropedicae* strains and the highest phylogenetic relatedness to the type strain Z67 of *H. seropedicae* among the type strains of all species in the genus *Herbaspirillum*. They contained nitrogenase and ACC deaminase and produced indoleacetic acids and siderophores, like SmR1. However, they inhibited the growth of rice seedlings and induced a hypersensitive response in tobacco leaves. Genome comparison of the five strains and strain SmR1 with the contrasting pathogenicity phenotype will aid in elucidating the subtle regulatory mechanisms of beneficial and pathogenic bacterium-plant interactions.

The genome DNA of strain Os34 was extracted from an overnight culture using a TIANamp bacteria DNA kit (Tiagen Biotech, Beijing, China), constructed into a 500-bp-insert library, and sequenced using an Illumina HiSeq 2000 sequencing system. Whole-genome sequencing resulted in 18,541,126 high-quality filtered paired-end reads with an average length of 95 bp and about 150-fold coverage. Filtered reads were assembled *in silico* with the Velvet program (15) and CLC Genomic Workbench 5.5.1 (CLC Bio, Aarhus, Denmark). Gaps between scaffolds and within scaffolds were closed by SSPACE-Basic 2.0 and GapFiller 1.0 (2, 3). A draft genome of strain Os34 was completed based on the reference genome of strain SmR1 (14) (the draft genome of strain

Os45 is discussed in our accompanying genome announcement [16]). By subsequent PCR and resequencing, 28 genome gaps were closed, and 252 contigs remained.

The draft genome sequence of strain Os34 comprises 6,152,986 bases representing more than 99.9% of the estimated genome size and has a G+C content of 61.2%. A total of 5,309 coding sequences (CDSs) were predicted using Prodigal version 2.6 with default parameters (5). The size and CDS numbers of the Os34 genome are approximately 12% greater than those of the reference SmR1 genome (5,513,887 bp and 4,735 CDSs). Putative functions of CDSs were automatically identified by using the GenDB annotation engine (11). The chromosome has 3 rRNA operons and 73 tRNAs predicted by RNAmmer and tRNAscan (8, 10). About 80.4% of its open reading frames (ORFs) have orthologs in the reference genome (BLASTP < 1e−5), whereas 1,040 ORFs were not found in the reference genome.

Like the plant growth-promoting strain SmR1, Os34 contains genes for nitrogen fixation, indoleacetic acid synthesis, siderophore production, and ACC deaminase and also contains genes for type III and type VI secretion systems and type IV pili, which are involved in delivering pathogenic effectors into the cytoplasm of eukaryotic host cells (14). The genome sequence of strain Os34 provides a genomic basis for in-depth comparative genome analyses to understand the specific mechanisms of beneficial and pathogenic *Herbaspirillum*-plant interactions.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [AMSB000000000](https://www.ncbi.nlm.nih.gov/nuccore/AMSB000000000). The version described in this paper is the first version, [AMSB010000000](https://www.ncbi.nlm.nih.gov/nuccore/AMSB010000000).

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