

Genome Sequence of *Novosphingobium* sp. Strain Rr 2-17, a Nopaline Crown Gall-Associated Bacterium Isolated from *Vitis vinifera* L. Grapevine

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***Novosphingobium* sp. strain Rr 2-17 is an *N*-acyl homoserine lactone (AHL)-producing bacterium isolated from the crown gall tumor of a grapevine. To our knowledge, this is the first draft genome announcement of a plant-associated strain from the genus *Novosphingobium*.**

Crown gall tumor disease is characterized by uncontrolled tissue proliferation at the site of bacterial infection. In the field, crown gall diseases are of common occurrence on dicotyledonous plants such as grapevine, stone fruits, nut trees, pomes, woody ornamentals, and fruit canes and are rarely found associated with annual row and field crops (2, 5). Crown gall disease has a significant negative impact on agriculture, as it has been shown to severely decrease the yields of various crops such as grape berries that are important for the production of juice, jam, raisin, and wine (1). Trends indicate that there is an increased demand for grape production from areas that are deemed traditional for the growing and processing of grape in addition to the nontraditional regions. Therefore, the development of novel protection or enhancement strategies for grape production against phytopathogens is of interest, given the economic importance of the plant (1, 5).

This sequenced strain was isolated from a Hungarian Reisling grapevine nopaline-type tumor and has been previously identified via 16S rRNA gene sequencing as a strain of the genus *Novosphingobium*. Few studies have characterized non-*Agrobacterium* species in association with this proliferative disease on grapevines. *Novosphingobium* sp. strain Rr 2-17 was shown to produce *N*-acyl homoserine lactones (AHLs) that can activate the TraR receptor protein implicated in the quorum-sensing regulation of pTi replication and conjugation in plant tumors and thus potentially influence virulence in *Agrobacterium tumefaciens* and *Agrobacterium vitis*, the causal agent of crown gall disease for dicotyledonous and grapevine plants, respectively (3, 6). In this study, the genome sequence of *Novosphingobium* sp. Rr 2-17 was determined to provide novel insight into the molecular principles of non-*Agrobacterium* strains that compete and persist in the crown gall tumor environment.

The whole-genome sequencing of *Novosphingobium* sp. Rr 2-17 was performed using an Illumina Genome Analyzer IIx sequencer (100-bp paired-end reads). The paired-end reads were trimmed and assembled *de novo* using CLC Genomics Workbench 4.8 (CLC Bio, Denmark). Prodigal 2.50, tRNAscan-SE-1.3, and RNAmmer 1.2 (7–9) were used to predict open reading frames (ORFs), tRNAs, and rRNAs, respectively. Subsequent genome annotation was performed using Blast2GO 2.5.0 (4). The draft genome sequence of *Novosphingobium* Rr 2-17 consists of 4,539,029 bp (148× coverage) contained in 166 contigs with an average GC content of 62.71%. The N50 contig was approximately

130 kb, and the largest contig assembled was approximately 318 kb. A total of 4,307 ORFs, 47 tRNAs, and 3 rRNAs were identified in the draft genome.

Novosphingobium sp. Rr 2-17 possesses *nif* genes involved in the nitrogen fixation process (10) at contig 28, which may stimulate a higher production of opines in crown gall tumor cells by increasing the bioavailability of nitrogen in the form of ammonia. In addition, it also contains the complete tricarboxylic acid (TCA) cycle, thus enabling the utilization of α -ketoglutarate, an intermediate in the catabolism of nopaline (11), as an energy source for its survival and persistence in the crown gall tumor environment.

Nucleotide sequence accession numbers. The results of this Whole Genome Shotgun project have been deposited at DDBJ/EMBL/GenBank under accession number [AKFJ000000000](https://www.ncbi.nlm.nih.gov/nuccore/AKFJ000000000). The version described in this paper is the first version, AKFJ010000000.

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REFERENCES

1. Bisztray GD, et al. 2012. Grapevine pathogens spreading with propagating plant stock: detection and methods for elimination, p 1–88. In Szabo PV, Shojania J (ed), *Grapevines: varieties, cultivation and management*. Nova Science Publishers, Inc., Hauppauge, NY.
2. Burr TJ, Otten L. 1999. Crown gall of grape: biology and disease management. *Annu. Rev. Phytopathol.* 37:53–80.
3. Cha C, Gao P, Chen YC, Shaw PD, Farrand SK. 1998. Production of acyl-homoserine lactone quorum-sensing signals by Gram-negative plant-associated bacteria. *Mol. Plant Microbe Interact.* 11:1119–1129.
4. Conesa A, et al. 2005. Blast2GO: a universal tool for annotation, visual-

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- ization and analysis in functional genomics research. *Bioinformatics* 21:3674–3676.
5. Escobar MA, Dandekar AM. 2003. *Agrobacterium tumefaciens* as an agent of disease. *Trends Plant Sci.* 8:380–386.
 6. Gan HM, Buckley L, Szegedi E, Hudson AO, Savka MA. 2009. Identification of an *rsh* gene from a *Novosphingobium* sp. necessary for quorum-sensing signal accumulation. *J. Bacteriol.* 191:2551–2560.
 7. Hyatt D, et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119.doi:10.1186/1471-2105-11-119.
 8. Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108.
 9. Lowe TM, Eddy SR. 1997. tRNA-scan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.
 10. Raymond J, Siefert JL, Staples CR, Blankenship RE. 2004. The natural history of nitrogen fixation. *Mol. Biol. Evol.* 21:541–554.
 11. Zanker H, et al. 1994. Octopine and nopaline oxidases from Ti plasmids of *Agrobacterium tumefaciens*: molecular analysis, relationship, and functional characterization. *J. Bacteriol.* 176:4511–4517.