Complete Genome Sequence Data of Nonpathogenic and Nonantagonistic Strain of *Rhizobium vitis* VAR06-30 Isolated From Grapevine Rhizosphere

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Abstract

Rhizobium (*Agrobacterium*) is one genus in the family Rhizobiaceae. Most of the species are epi- or endophytic bacteria which include tumorigenic or rhizogenic pathogens, root nodule bacteria, and commensal endosymbionts. *Rhizobium vitis* strain VAR06-30 is a commensal bacterium without pathogenicity which was isolated from a rootstock of grapevine in Japan. It also does not have antagonistic activity to the pathogenic strain of *R. vitis*. Here, we show the complete genome sequence data with annotation of *R. vitis* VAR06-30 which was analyzed by sequence reads obtained from both PacBio and Illumina platforms. This genome sequence would contribute to the understanding of evolutionary lineage and characteristics of *Rhizobium* commensal bacteria.

Genome Announcement

In Japan, tumorigenic *Rhizobium radiobacter* (=tumorigenic *Agrobacterium tumefaciens*, *A. tumefaciens* biovar 1) and tumorigenic *R. vitis* (=tumorigenic *A. vitis*, *A. tumefaciens* biovar 3) were identified as pathogenic causal agents of grapevine crown gall disease (Kawaguchi et al. 2005a,b). In this report, the nomenclature for *Agrobacterium* and *Rhizobium* species follows a proposal by Young et al. (2001). *R. vitis* VAR06-30 was a nonpathogenic strain of *R. vitis* which was isolated from graft unions of grapevine nursery stock (*Vitis vinifera* L. 'Aurora Black') in a field in Okayama Prefecture, Japan during the investigation of causal pathogens of crown gall disease (Kawaguchi et al. 2005a, 2011). The genetic diversity of *R. vitis* strains isolated mainly in Japan was analyzed using three different methods (Kawaguchi et al. 2008). The first consisted of slide agglutination tests using antisera against somatic antigens. The second one was a phylogenetic analysis using repetitive sequence-based PCR fingerprints. The third one was multilocus sequence analysis using the partial nucleotide sequences of housekeeping genes *pyrG*, *recA*, and *rpoD* (Kawaguchi et al. 2008). The third method was also performed with an expanded number of strains (Kawaguchi 2011).

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Keywords

crown gall disease, commensalism, endophytes, grapevine, *Rhizobium vitis*, rhizosphere

These results suggest that *R. vitis* strains are genetically heterogeneous and separate into several genetic groups. VAR06-30 and another nonpathogenic strain form a separate clade from other pathogens as well as from nonpathogenic strains.

Agrobacterium and *Rhizobium* include nitrogen-fixing endosymbionts, commensal endophytes, and tumorigenic or rhizogenic pathogens. The whole-genome sequences of two pathogenic strains of this genus, tumorigenic *R. radiobacter* (=tumorigenic *A. tumefaciens*, *A. tumefaciens* biovar 1) strain C58 and tumorigenic *R. vitis* (=tumorigenic *A. vitis*, *A. tumefaciens* biovar 3) strain S4, have been determined (Goodner et al. 2001; Slater et al. 2009; Wood et al. 2001). The genome sequence of nonpathogenic *R. radiobacter* (=nonpathogenic *A. rhizogenes*, *A. radiobacter* biovar 2) K84 was also reported (Slater et al. 2009). It has antagonistic activity to the pathogenic bacterial strains and functions as a biocontrol agent. Recently, a large number of shotgun genome sequencing data of Rhizobiales bacteria, including *Agrobacterium* and *Rhizobium* strains, became available (Garrido-Oter et al. 2018). As for *R. vitis*, assembled genome sequences of 52 strains, including *R. vitis* S4, have been registered in the database.

In this study, we characterized the whole-genome sequence of *R. vitis* VAR06-30 with annotation by shotgun sequencing using PacBio and Illumina sequencing technologies. The genomic DNA was extracted with a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and sheared into fragments which sizes are ranging from 20 to 50 kbp with a g-tube device (Covaris Inc., Woburn, MA, U.S.A.). A SMRTbell library was set up using a Template Prep Kit v1.0 (Pacific Bioscience, Menlo Park, CA, U.S.A.) following the manufacturer's protocol. The size of the sequencing library was selected using the BluePippin system (Sage Science, Beverly, MA, U.S.A.) with a minimum fragment length cutoff of 17 kbp. The finished library was sequenced by the PacBio RSII sequencer with SMRT cell v3 and P6-C4v2 chemistry, yielding a total of 114,871 reads (2.73 Gbp). The assembly of these PacBio reads was performed by the HGAP3 assembler program with default settings. Apart from this, the genomic DNA was chopped into pieces with an average size of 600 bp by the DNA Shearing System M220 (Covaris Inc.). A paired-end library was prepared with a TruSeq DNA PCR-Free Library Prep kit (Illumina, Inc., San Diego, CA, U.S.A.) and its size was selected on an agarose gel using a ZymoClean Large Fragment DNA Recovery Kit (Zymo Research, Irvine, CA, U.S.A.). The finished library was analyzed by the Illumina HiSeq 2500 sequencer with a read length of 250 bp. Illumina read sequences comprising $3,233,859 \times 2$ raw reads were used to improve the base quality of the long-read assembly with the Pilon version 1.22 software tool. DFAST, a DNA Data Bank of Japan (DDBJ) Fast Annotation and Submission Tool, was used for annotation (Tanizawa et al. 2016).

The final assembled genome length is 5,882,175 bp, with no gaps, and its GC content is 57.5%. There were, in total, 5,408 protein-coding sequences. The genome is divided into two chromosomes (circular) and five plasmids whose sizes are 3,769,447, 1,162,882, 409,071, 309,504, 96,407, 88,752, and 46,112 bp, respectively. In all, nine ribosomal RNAs and 52 transfer RNAs are located on chromosome 1, and three ribosomal RNAs and four transfer RNAs exist on chromosome 2. The small number of virulence-related genes such as *virA*, *virB*, *virC*, *virD*, and *virG* are found in the 96,407- and 88,752-bp-length plasmids but *virE1*, *virE2*, *virB4*, *virB5*, *virB6*, *virB7*, *virB10*, *virD5*, and *virH1* were missing in the genome, indicating an insufficient ability for tumor induction.

This genome sequence could contribute to the understanding of genetic and genomic diversities of *R. vitis* as well as differences between the genome organization of commensal and pathogenic rhizobia. It could also provide opportunities to analyze the molecular basis of commensalism.

This complete whole-genome sequence has been deposited in the DDBJ database under accession numbers AP023272 (chr1), AP023273 (chr2), AP023274 (pRvVAR0630a), AP023275 (pRvVAR0630b), AP023276 (pRvVAR0630c), AP023277 (pRvVAR0630d), and AP023278 (pRvVAR0630e) (PRJDB10104 for BioProject and SAMD00233297 for BioSample).

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Literature Cited

- Garrido-Oter, R., Nakano, R. T., Dombrowski, N., Ma, K.-W., McHardy, A. C. Schulze-Lefert, P., and AgBiome Team. 2018. Modular traits of the Rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. *Cell Host Microbe* 24:155-167.e5.
- Goodner, B., Hinkle, G., Gattung, S., Miller, N., Blanchard, M., Qurollo, B., Goldman, B. S., Cao, Y., Askenazi, M., Halling, C., Mullin, L., Houmiel, K., Gordon, J., Vaudin, M., Iartchouk, O., Epp, A., Liu, F., Wollam, C., Allinger, M., Doughty, D., Scott, C., Lappas, C., Markelz, B., Flanagan, C., Crowell, C., Gurson, J., Lomo, C., Sear, C., Strub, G., Cielo, C., and Slater, S. 2001. Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science* 294:2323-2328.
- Kawaguchi, A. 2011. Genetic diversity of *Rhizobium vitis* strains in Japan based on multilocus sequence analysis of *pyrG*, *recA* and *rpoD*. *J. Gen. Plant Pathol.* 77: 299-303.
- Kawaguchi, A., Inoue, K., and Nasu, H. 2005a. Inhibition of crown gall formation by *Agrobacterium radiobacter* biovar 3 strains isolated from grapevine. *J. Gen. Plant Pathol.* 71:422-430.
- Kawaguchi, A., Sawada, H., and Ichinose, Y. 2008. Phylogenetic and serological analyses reveal genetic diversity of *Agrobacterium vitis* strains in Japan. *Plant Pathol.* 57:747-753.
- Kawaguchi, A., Sawada, H., Inoue, K., and Nasu, H. 2005b. Multiplex PCR for the identification of *Agrobacterium* biovar 3 strains. *J. Gen. Plant Pathol.* 71:54-59.
- Slater, S. C., Goldman, B. S., Goodner, B., Setubal, J. C., Farrand, S. K., Nester, E. W., Burr, T. J., Banta, L., Dickerman, A. W., Paulsen, I., Otten, L., Suen, G., Welch, R., Almeida, N. F., Arnold, F., Burton, O. T., Du, Z., Ewing, A., Godsy, E., Heisel, S., Houmiel, K. L., Jhaveri, J., Lu, J., Miller, N. M., Norton, S., Chen, Q., Phoolcharoen, W., Ohlin, V., Ondrusek, D., Pride, N., Stricklin, S. L., Sun, J., Wheeler, C., Wilson, L., Zhu, H., and Wood, D. W. 2009. Genome sequences of three *agrobacterium* biovars help elucidate the evolution of multichromosome genomes in bacteria. *J. Bacteriol.* 191:2501-2511.
- Tanizawa, Y., Fujisawa, T., Kaminuma, E., Nakamura, Y., and Arita, M. 2016. DFAST and DAGA: Web-based integrated genome annotation tools and resources. *Biosci. Microbiota Food Health* 35:173-184.
- Wood, D. W., Setubal, J. C., Kaul, R., Monks, D. E., Kitajima, J. P., Okura, V. K., Zhou, Y., Chen, L., Wood, G. E., Almeida, N. F., Jr., Woo, L., Chen, Y., Paulsen, I. T., Eisen, J. A., Karp, P. D., Bovee, D., Sr., Chapman, P., Clendenning, J., Deatherage, G., Gillet, W., Grant, C., Kutayavin, T., Levy, R., Li, M. J., McClelland, E., Palmieri, A., Raymond, C., Rouse, G., Saenphimmachak, C., Wu, Z., Romero, P., Gordon, D., Zhang, S., Yoo, H., Tao, Y., Biddle, P., Jung, M., Krespan, W., Perry, M., Gordon-Kamm, B., Liao, L., Kim, S., Hendrick, C., Zhao, Z. Y., Dolan, M., Chumley, F., Tingey, S. V., Tomb, J. F., Gordon, M. P., Olson, M. V., and Nester, E. W. 2001. The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science* 294:2317-2323.
- Young, J. M., Kuykendall, L. D., Martinez-Romero, E., Kerr, A., and Sawada, H. 2001. A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et al. 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*. *Int. J. Syst. Evol. Microbiol.* 51: 89-103.