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PROKARYOTES

Genome Sequence of *Rhizobium sullae* HCNT1 Isolated from *Hedysarum coronarium* Nodules and Featuring Peculiar Denitrification Phenotypes

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ABSTRACT The genome sequence of *Rhizobium sullae* strain HCNT1, isolated from root nodules of the legume *Hedysarum coronarium* growing in wild stands in Tuscany, Italy, is described here. Unlike other *R. sullae* strains, this isolate features a truncated denitrification pathway lacking NO/N_2O reductase activity and displaying high sensitivity to nitrite under anaerobic conditions.

Rhizobium sullae (1) is a nitrogen-fixing symbiont of the legume *Hedysarum coronarium* L. (= *Sulla coronaria*, Medik), known as French honeysuckle, or sulla. The plant is still occurring both as a cropped legume and as a spontaneous weed in Mediterranean habitats, and it is particularly tolerant to drought and alkalinity. The genome sequences of two other strains of the same bacterial species have been published, including that of the type strain IS123 (2) isolated in southern Spain from its host growing in the wild, and that of strain WSM1592 (3), isolated from cropped sulla on the island of Sardinia (Italy). The present isolate, HCNT1 (=IMAP 801, =ATCC 43676) (4), was isolated from root nodules of its host collected under spontaneous conditions in the highly calcareous pliocenic clays near Volterra (Tuscany, Italy). The strain has demonstrated peculiarities in comparison to its conspecific relatives, as it is endowed with a unique denitrifying phenotype. While capable of reducing nitrite, it appears to be incapable of coupling such a reaction to energy metabolism as true denitrifiers would (5, 6). Cell growth is also inhibited under anaerobic conditions in the presence of nitrite due to nitric oxide accumulation (7). The nitrite reductase of *R. sullae* HCNT1 has also shown to be active in reducing selenite besides nitrite (8).

Whole-genome sequencing was carried out using Illumina MiSeq sequencing technology (Ramaciotti Centre for Genomics, Sydney, Australia). The Nextera XT kit (Illumina, Inc., San Diego, CA, USA) was employed for the generation of genomic libraries. The number of paired-end reads (2 × 250 bp) obtained was 3,626,956, and this accounted for 124-fold coverage of the studied genome. Up to 99.93% of these reads were assembled into 54 scaffolds, and an N_{50} as high as 373,743 bp was obtained. *R. sullae* HCNT1 has been shown to have a size of 7,298,178 bp, in which the G+C content is 59.9%. Version 10.1.1 of CLC Genomics Workbench software (Qiagen Bioinformatics, Germany) was used to perform all the above-mentioned analyses.

The Rapid Annotations using Subsystems Technology (RAST) software (9) allowed the identification of 7,441 coding sequences and 50 RNAs. Thirteen prophage and phage sequences were found as well. It is also worth mentioning that virulence, disease, and defense sequences accounted for up to 99 genes in this genome, of which

Received 7 December 2017 **Accepted** 15 December 2017 **Published** 25 January 2018

Citation de Diego-Diaz B, Treu L, Campanaro S, da Silva Duarte V, Basaglia M, Favaro L, Casella S, Squartini A. 2018. Genome sequence of *Rhizobium sullae* HCNT1 isolated from *Hedysarum coronarium* nodules and featuring peculiar denitrification phenotypes. Genome Announc 6:e01518-17. https://doi.org/10.1128/genomeA.01518-17.

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the vast majority (80 genes) are related to resistance to antibiotics and toxic compounds. Regarding the species characteristics, it is important to note that nitrogen metabolism is represented by 70 genes, including a copper-containing nitrite reductase (EC 1.7.2.1), 32 genes of which are related to denitrification. The genome sequence was used as input for the PHASTER software (10). This yielded two incomplete prophage regions.

A BLAST search of the 16S rRNA gene sequence against the NCBI nr database was carried out, and interestingly, the highest similarity (99% identity) was obtained with *Rhizobium* sp. strain WSM749 (not with *R. sullae*). This uncharacterized organism was isolated in Morocco from *Hedysarum flexuosum* (11), which is the closest relative of *H. coronarium* in terms of bacterial symbiont intercompatibility. In fact, isolates from *H. flexuosum* can nodulate *H. coronarium* but are ineffective in fixing nitrogen (12).

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number PIQN00000000. The version described in this paper is version PIQN01000000.

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