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Complete genomes of two *Variovorax* endophytes isolated from surface-sterilized alfalfa nodules

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ABSTRACT *Variovorax* species catabolize a wide range of natural and industrial products and have been shown to be integral rhizosphere inhabitants. Here, we report the complete genomes of *V. paradoxus* 2u118 and *V.* sp. SPNA7, which were isolated from alfalfa root nodules and possess plant growth-promoting properties.

KEYWORDS endophytes, plant growth-promotion, alfalfa nodule, bioremediation, *Variovorax*

The genus *Variovorax* is known to metabolize a wide range of substrates, including pesticides (1, 2), acyl homoserine lactones (3), and acrylamide (4). *Variovorax* species have the potential as plant growth-promoting bacteria via several strategies including lowering plant ethylene levels (5) and remediating metal-contaminated soil (6), and have been identified as keystone species for maintaining root growth in *Arabidopsis* (7). In a study of alfalfa nodule-associated bacteria, two *Variovorax* isolates were collected from an alfalfa field at CalPoly Pomona (34.045075, -117.812530). Surface-sterilized nodules were crushed with a mortar and pestle, serial dilutions were plated on LB agar, and single colonies were picked after incubation at 30°C for 1 week and streaked to obtain pure cultures. DNA was extracted using a Quick-DNA HMW Magbead Kit (Zymo Research) per the manufacturer's instructions, and fragmented using Covaris gTubes following instructions from the manufacturer (4 passes at 7,000 rpm through the gTube orifice). The average size of the sheared gDNA was checked at the TapeStation 4200 (Agilent). Multiplexed microbial libraries were prepared using the PacBio SMRTbell prep kit 3.0 together with the SMRTbell barcoded adapters 3.0 according to the PacBio protocol. Final whole genome libraries were not size-selected but simply purified via a standard procedure using 1× SMRTbell clean-up beads. DNA sequencing was performed using the PacBio Sequel IIe platform. Demultiplexing and adapter trimming were done using Lima v2.9.0 (https://github.com/pacificbiosciences/barcoding). All reads were then targeted for genome assembly by Canu v2.2 (8) and the assembled genomes were further refined by Circlator v1.5.5 (9) to identify circular contigs, remove redundant non-circular contigs, and rotate circular contigs to start with *dnaA*. This resulted in circular genomes and plasmids (Table 1). A completeness check was performed by CheckM v1.0.18 (10) and the N50 value was determined by Assembly stats ver1.01 (https://github.com/sanger-pathogens/assembly-stats). High-quality reads, completeness, and N50 quality values for each strain were as follows: *V.* sp. SPNA7: 38,196, 100%, and 5,887,536 bp; *V. paradoxus* 2u118: 23,568, 100%, and 5,622,806 bp. Genome ORF calling and annotation were performed by NCBI's PGAP v6.6 (11) and the IMG Annotation Pipeline v.5.1.17 (12). All software tools used default parameters that were stated in each tool's manual.

Properties of the finished genomes of each *Variovorax* strain are summarized in Table 1. All 16S sequences had greater than 99.45% similarity to the published 16S sequences of *V. paradoxus* NBRC 15149^T (13, 14). ANI values against *V. paradoxus* NBRC 15149^T were 93.94% and 95.27% for *V.* sp. SPNA7 and *V. paradoxus* 2u118, suggesting that SPNA7

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TABLE 1 *V.* sp. SPNA7 and *V. paradoxus* 2u118 genome information

Strain	Contig	Topology	Size (bp)	GC%	Coverage	Protein coding	# 16S	# tRNA
SPNA7	#1	Circular	5,887,536	67.5	39.0×	5,459	2	46
	#2	Circular	1,192,185	67.5	39.0×	1,112	0	10
	Total	n/a[a]	7,079,721	67.5	39.0×	6,571	2	56
2u118	#1	Circular	5,622,806	67.5	37.0×	5,238	2	46
	#2	Circular	1,298,350	67	37.0×	1,241	0	0
	Total	n/a	6,921,156	67.5	37.0×	6,479	2	46

[a]n/a: not available.

could potentially represent a new species. ANI was calculated using contigs and the Ezbiocloud ANI Calculator (15).

Both genomes are enriched in genes related to heavy metal resistance (MerR family copper efflux transcriptional regulators, copper-responsive two-component system CusR/CusS, arsenate reductase, etc.) and xenobiotics degradation (including genes related to degradation of chloroalkanes, dioxins, styrene, toluene, xylene, etc.). Xenobiotics degradation/metabolism accounts for 3.73%–3.79% of genes assigned to KEGG categories. Additionally, both genomes encode the enzyme ACC deaminase (*acdS*), genes for indole-3-acetic acid (IAA) biosynthesis, and acetoin and trehalose biosynthesis, all of which may contribute to plant growth promotion.

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DATA AVAILABILITY STATEMENT

The complete genome sequences of *V. paradoxus* 2u118 and *V.* sp. SPNA7 have been deposited in IMG/M under the taxon IDs 8045543215 and 8045536498, respectively. The assembled genomes are listed under the GenBank accession numbers CP138515 and

CP138516 for 2u118, and CP138513 and CP138514 for SPNA7. The raw sequencing reads have been deposited under the NCBI BioProject numbers PRJNA1026576 for 2u118 and PRJNA1026575 for SPNA7.

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