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GENOME SEQUENCES

Complete Genome Sequences of Two Phylogenetically Distinct *Nitrospina* Strains Isolated from the Atlantic and Pacific Oceans

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ABSTRACT The complete genome sequences of two chemoautotrophic nitrite-oxidizing bacteria of the genus *Nitrospina* are reported. *Nitrospina gracilis* strain Nb-211 was isolated from the Atlantic Ocean, and *Nitrospina* sp. strain Nb-3 was isolated from the Pacific Ocean. We report two highly similar ~3.07-Mbp genome sequences that differ by the presence of ferric iron chelator (siderophore) biosynthesis genes.

Nitrospina species are aerobic, chemoautotrophic, nitrite-oxidizing bacteria that have so far only been found in marine habitats (1), where they play an important role in the nitrogen cycle (2). *Nitrospina gracilis* Nb-211 (ATCC 25379), first described in 1971 (3), was isolated from surface waters (13-m depth) of the Atlantic Ocean approximately 200 miles from the mouth of the Amazon River. *Nitrospina* sp. strain Nb-3 was isolated from the Pacific Ocean off the coast of Peru and has not been validly described; however, its 16S rRNA gene sequence was published in 1994 (4). Both strains belong to the *Nitrospinaceae* family within the *Nitrospinae/Nitrospinota* (Joint Genome Institute/Genome Taxonomy Database [JGI/GTDB]) phylum.

For genomic sequencing, cultures were grown in 2-L glass bottles in artificial seawater medium containing 2 mM nitrite and incubated in the dark without agitation as described previously (5). Cells were collected via centrifugation (1 h, 15,000 × *g*, 10°C), and DNA was extracted from the cell pellets using a cetyltrimethylammonium bromide (CTAB)-phenol-chloroform protocol (6). Draft genomes were generated at the DOE JGI using the Pacific Biosciences (PacBio) sequencing technology (7). Genomic DNA was sheared to 10 kb using g-TUBE columns (Covaris) and subjected to library preparation using the SMRTbell Express template prep 2.0 kit. The PacBio SMRTbell library was purified and size-selected using AMPure PB beads and sequenced on the PacBio Sequel platform, which generated 123,391 subreads (5,002.7 ± 3,337.2 bp) totaling 617,291,847 bp for strain Nb-211 and 89,053 subreads (6,935.6 ± 5,348.8 bp) totaling 617,633,853 bp for strain Nb-3. Reads of >5 kb were assembled with HGAP (v. smrtlink/8.0.0.80529, HGAP 4 [1.0]) using default settings (8). The input read coverage was 188.4× for strain Nb-211 and 189.5× for strain Nb-3. The final draft genome sequences consisted of one scaffold each, with a total size of 3,069,626 bp for strain Nb-211 and 3,075,869 bp for strain Nb-3 (Table 1). We confirmed complete circularization with the Circlator pipeline v.1.5.5 (9), which uses nucmer v.3.1 (10) to check for alignment between assembled contigs at opposite ends of the assembly, identifying a 50,007-bp alignment with 100% identity for strain Nb-211 and 50,012 bp with 99.99% identity for strain Nb-3.

Both genomes were annotated using the Integrated Microbial Genomes (IMG) Annotation Pipeline (IMGAP) v.5.0.22/3. The genome of *Nitrospina gracilis* Nb-211 contains 2,939 coding DNA sequences (CDS), and that of *Nitrospina* sp. Nb-3 contains 2,905 CDS. Both genome sequences share an average nucleotide identity (ANI) of

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TABLE 1 Genome features of *Nitrospina gracilis* Nb-211 and *Nitrospina* sp. Nb-3

Strain	GenBank accession no.	JGI taxon ID	Genome size (bp)	G+C content (%)	No. of DNA scaffolds	No. of total genes	No. of protein coding genes	No. of rRNA operons	No. of trRNA operons	CRISPR count
N. gracilis Nb-211	JAKJKD010000001	2917506613	3,069,626	57.43	1	2,939	2,879	1	50	2
Nitrospina sp. Nb-3	JAKJKC010000001	2929071401	3,075,869	56.21	1	2,939	2,846	1	49	0

85.5%, well below the intraspecies threshold of 96.5% (11). Strain Nb-3 shares 99.98% ANI with the previously published draft genome sequence of *Nitrospina gracilis* strain 3/211 (12), indicating that the latter likely derives from the culture originally designated strain Nb-3 (4). Strain Nb-3 encodes a putative iron chelator (siderophore) biosynthesis gene cluster, which is absent in strain Nb-211, potentially reflecting adaptations to differences in iron availability in the respective ocean basins the strains were isolated from. Strain Nb-211 was isolated near the Amazon River, which is a source of iron to the Atlantic Ocean (13), while strain Nb-3 was isolated from the relatively iron-deplete North Pacific (14).

Data availability. The whole-genome shotgun sequencing project of *Nitrospina gracilis* Nb-211 has been deposited at DDBJ/ENA/GenBank under BioProject number PRJNA708439 and accession number JAKJKD000000000. The whole-genome shotgun sequencing project of *Nitrospina* sp. Nb-3 has been deposited at DDBJ/ENA/GenBank under BioProject number PRJNA783628 and accession number JAKJKC000000000. The NCBI Sequence Read Archive (SRA) accession numbers for the raw reads are SRR17430281 for strain Nb-211 and SRR17430190 for strain Nb-3.

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