



Draft Genome Sequence of a Terrestrial Planctomycete, *Singulisphaera* sp. Strain GP187, Isolated from Forest Soil

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ABSTRACT Here, we present the draft genome sequence of a novel species of the genus *Singulisphaera* (phylum *Planctomycetes*, family *Isosphaeraceae*) isolated from soil. *Singulisphaera* sp. strain GP187 has a relatively large mobilome and numerous novel genes that may contribute to the production of bioactive molecules.

Culture-independent analysis reveals that *Planctomycetes* is the fifth most abundant bacterial phylum in global soil samples (1), yet this phylum remains underrepresented in axenic cultures, and a large majority of these cultures are derived from aqueous environments (2). Aqueous planctomycetes are hypothesized to have evolved from terrestrial species (3).

Singulisphaera sp. strain GP187 was isolated on 3 June 2014 from the Harvard Forest, a temperate forest ecosystem in Petersham, MA (42.54°N, -72.18°W). Organic horizon soil was pretreated with 6% yeast extract plus 0.05% SDS (4), plated onto oat-meal medium, and incubated aerobically at 20°C, with colonies appearing after 8 days. GP187 was the only *Planctomycetes* strain of the hundreds of isolates from this site (5) and thus was subjected to whole-genome sequencing.

GP187 was grown aerobically on Reasoner's 2A (R2A) medium (pH 7) (6). Genomic DNA was purified using a modified cetyltrimethylammonium bromide (CTAB) procedure (7) but was not sheared or size selected. The draft genome sequence was generated at the DOE Joint Genome Institute (JGI). A PacBio SMRTbell library was constructed and sequenced on the PacBio RS platform, generating 407,937 reads (N_{50} , 3.6 kbp). The filtered raw reads (675.3 Mbp) were assembled using HGAP v2.3_p5 (protocol version, 2.3.0; method, RS HGAP Assembly.3, smrtpipe.py v1.87.139483) (8). The final draft assembly contained 5 contigs in 5 scaffolds (N_{50} , 6.278 Mbp), estimated as 99.61% complete and 5.81% contaminated using CheckM v1.0.18 (9) in KBase (10). The input read sequencing depth was 72.8×. Gene annotations were completed within the JGI's Integrated Microbial Genomes (IMG) platform (11) and KBase. Default parameters were used for all software.

The genome is 10,689,158 bp (G+C content, 63.07%) and is predicted to encode 8,388 proteins (36.6% without predicted function), 8 rRNA operons, and 110 RNA genes (24 rRNAs, 64 tRNAs). GP187 has the largest genome of cultured *Isosphaeraceae* strains and the second largest genome of cultured *Planctomycetes* strains (12).

Phylogenetically, the closest species to GP187 is the aquatic *Singulisphaera acidiphila* DSM 18658 (13). These strains share 98.84% homology for 16S rRNA genes (average as determined by searching public RNA isolates with IMG BLAST) and 86.7% whole-genome average nucleotide identity (ANI) (IMG pairwise ANI). *Isosphaeraceae* genome sequences characteristically carry large plasmids; GP187 harbors a putative plasmid of 63.8 kb (G+C content, 61.8%; a lower G+C content is typical of plasmids [14]). The subtilisin gene open reading frame spans, without gaps, the ends of contig5

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TABLE 1 Differences in select characteristics between *S. acidiphila* DSM 18658 and *S. acidiphila* sp. GP187

Strain	Genome size (Mbp)	No. (%) of:			
		Protein coding genes ^a	Genes without predicted function ^a	Giant genes without predicted function ^b	Genes without predicted function in BGCs ^c
<i>S. acidiphila</i> DSM 18658	9.76	7,576 (98.6)	2,578 (33.6)	7 (20.6)	76 (29.8)
<i>S. acidiphila</i> sp. GP187	10.69	8,388 (98.7)	3,110 (36.6)	14 (35.0)	104 (36.4)

^aPercentage of total protein coding genes (IMG).

^bPercentage of giant genes (KBase).

^cPercentage of total genes in biosynthetic gene clusters (BGCs) (IMG).

and shares 82% homology with the plasmid-encoded subtilisin gene of *S. acidiphila* DSM 18658, suggesting that contig5 is a circular plasmid.

GP187 contains 60% more mobilome-associated genes (186 [2.2% of protein-encoding genes] versus 117 [1.5%], respectively) and 78% more genomic islands (41 versus 23, respectively) than *S. acidiphila* DSM 18658, as predicted by IslandViewer4 (15). GP187 has a greater potential to synthesize specialized metabolites, given that novel genes of this species are categorized as giant genes ($\leq 5,000$ bp with Kbase RAST annotation) (16) or are located in biosynthesis gene clusters (17) more often than in *S. acidiphila* DSM 18658 (Table 1).

This genome sequence will contribute to our understanding of terrestrial species of *Planctomycetes*, a phylum abundant in soil but underrepresented in isolate genome analysis. Analysis of this genome sequence may elucidate its ecological role in terrestrial ecosystems, identify evolutionary relationships between terrestrial and aquatic *Singulisphaera* species, and contribute to the discovery of novel secondary metabolites.

Data availability. This whole-genome sequence was deposited at DDBJ/EMBL/GenBank under the accession number [NZ_FSRB00000001](https://www.ncbi.nlm.nih.gov/nuclink/NZ_FSRB00000001). The raw data were deposited in the JGI GOLD under the project number [Gp0151081](https://www.jgi.doe.gov/gold/Gp0151081) and in the Sequence Read Archive under the accession number [SRX2158412](https://www.ncbi.nlm.nih.gov/sra/SRX2158412). The JGI annotation is found at https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2695420965#.

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