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GENOME SEQUENCES

Sphingopyxis Species Isolated from Sand Filter Biofilm at an Australian Drinking Water Treatment Works

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ABSTRACT Three strains isolated by geosmin enrichment from a sand filter in an Australian drinking water treatment works were genome sequenced to identify their taxonomic placement, and a bench-scale batch experiment confirmed their geosmin-degrading capability. Using the average nucleotide identity based on the MUMmer algorithm (ANIm), pairwise digital DNA-DNA hybridization (dDDH), and phylogenomic analyses, the strains were identified as *Sphingopyxis* species.

Proposed in 2001 (1), the genus *Sphingopyxis* currently comprises 21 validly published species (2), belonging to the family *Sphingomonadaceae* of the class *Alphaproteobacteria*. *Sphingopyxis* strains have been isolated from diverse natural environments, including volcanic rock (3), freshwater (4), soils (5), seawater (6), and some contaminated sites (7–9). *Sphingopyxis* species can utilize a broad range of carbon sources (1), including aromatic compounds such as tetralin (10) and microcystins (11). *Sphingopyxis* species have also been found to biotransform heavy metals and biodegrade polyethers, antibacterials, and geosmin (1, 12).

Sphingopyxis strains Geo24, Geo25, and Geo48 were originally isolated in the lab by geosmin enrichment from a sand filter from an Australian drinking water treatment works (13, 14). Geo24 and Geo25 were identified as part of a bacterial consortium able to biodegrade geosmin as the sole carbon source (13), and Geo48 was later identified as an isolate capable of geosmin biodegradation (14). The isolates were stored long term at −80°C, shipped on charcoal transport swabs, streaked onto tryptic soy agar (TSA), and grown for 48 h at 30°C. All strains were streaked three times to ensure the purity of individual colonies.

For genome sequencing, the strains were grown in 5 mL tryptic soy broth (TSB) at 30°C for 48 h, and total DNA was extracted using the FastDNA spin kit for soil (MP Biomedicals). Sequencing was performed on a NovaSeq 6000 SP instrument using a NEBNext Ultra II DNA library prep kit. Between 7 and 9 million (150-bp) read pairs were generated for each genome. The read quality was checked using FastQC v0.11.9, trimming and adapter removal was performed using Fastp v0.20.1, and the genomes were assembled using Unicycler v0.4.7. The genome sizes and other metrics are as follows: Geo24 has a size of 3.86 Mbp, 23 contigs, an N_{50} value of 665,169 bp, and a GC content of 65.3%; Geo25 comprises 3.87 Mbp, 23 contigs, an N_{50} value of 672,543 bp, and a GC content of 65.3%; and Geo48 has a size of 3.96 Mbp, 25 contigs, an N_{50} value of 978,883 bp, and a GC content of 65.2%.

Phylogenetic analysis of *Sphingopyxis* strains Geo24, Geo25, and Geo48 was performed against all available *Sphingopyxis* type strain genomes, downloaded using the NCBI genome download tool v0.2.10 and annotated using Prokka v1.14.6. The average nucleotide identity

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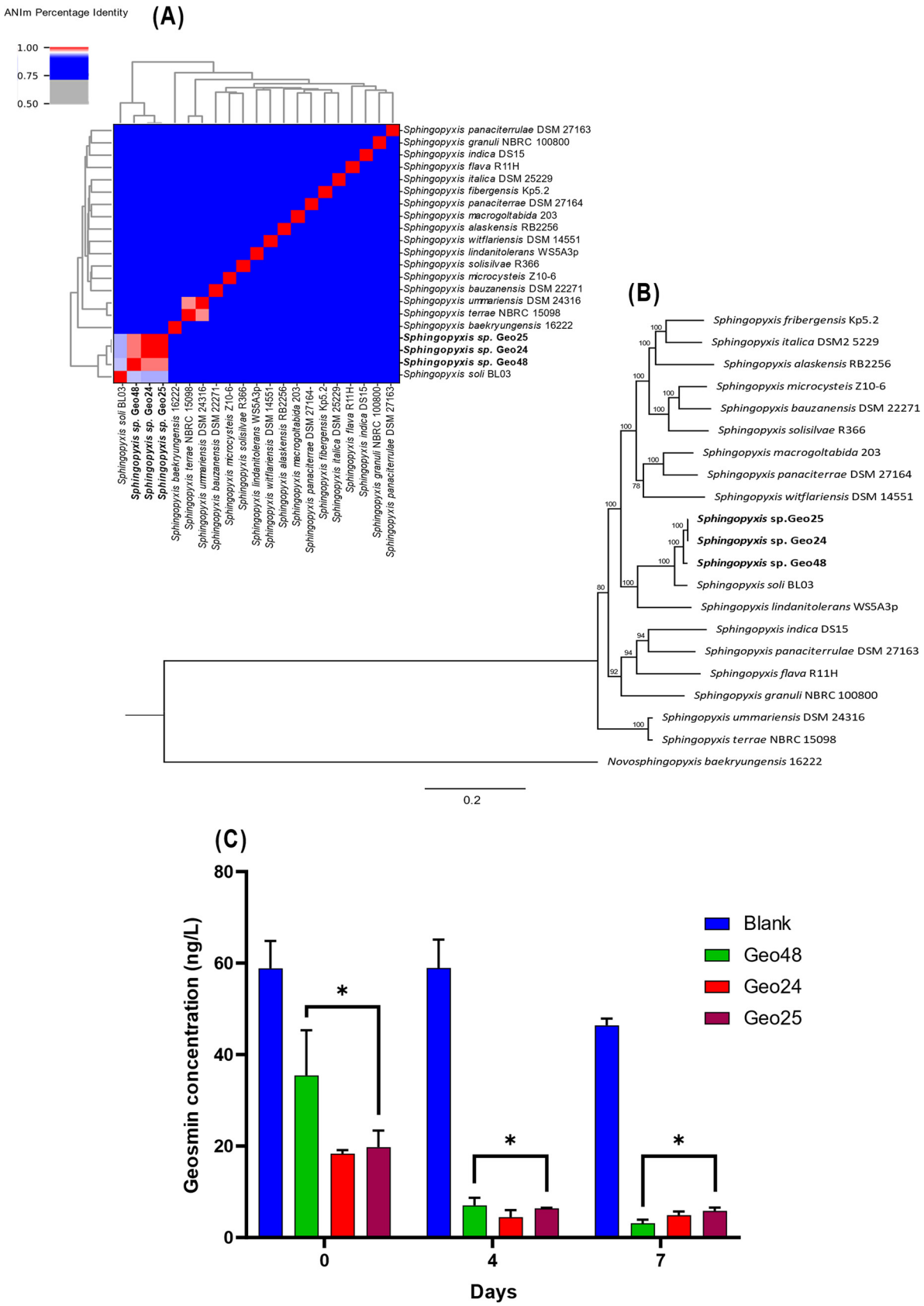

FIG 1 ANIm, phylogeny, and geosmin biodegradation batch experiment results for novel *Sphingopyxis* species Geo24, Geo25, and Geo48, isolated from an Australian drinking water treatment works sand filter. (A) ANIm heatmap produced using PyANI v0.2.12, depicting the average

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TABLE 1 Basic metrics and pairwise dDDH values for novel *Sphingopyxis* species and the three most closely related type strains[a]

Strain	Genome size (Mbp)	GC content (%)	Pairwise dDDH (%) with strain: Geo24	Geo25	Geo48	*Sphingopyxis soli* BL03	*Sphingopyxis lindanitolerans* WS5A3p	*Sphingopyxis macrogoltabida* 203
Sphingopyxis sp. Geo24	3.86	65.3		100.0	76.4	50.0	27.5	25.8
Sphingopyxis sp. Geo25	3.87	65.3	100.0		76.4	50.0	27.5	25.8
Sphingopyxis sp. Geo48	3.96	65.2	76.4	76.4		51.2	27.1	26.2
Sphingopyxis soli BL03	3.63	65.8	50.0	50.0	51.2		27.5	25.9
Sphingopyxis lindanitolerans WS5A3p	4.15	65.3	27.5	27.5	27.1	27.5		25.9
Sphingopyxis macrogoltabida 203	5.75	64.9	25.8	25.8	26.2	25.9	25.9	

[a] dDDH values were calculated using TYGS and according to the *Sphingopyxis* phylogenetic tree (Fig. 1A). Pairwise dDDH values of <70% indicate different species.

(ANI) was calculated using PyANI v0.2.12 (Fig. 1A), and a phylogenomic tree was constructed using OrthoFinder v2.5.4 (15, 16) and RAxML-NG v1.1 (17) (Fig. 1B). All three strains showed an ANI of <94% to the most closely related type strains and displayed phylogenomic distinction, indicating them as potentially novel species (Fig. 1B). Pairwise digital DNA-DNA hybridization (dDDH) values were determined using the Type Strain Genome Server (TYGS) with the most closely related type strains (18); the results indicated that all three *Sphingopyxis* strains represent different species (dDDH, <70%) than those previously described (Table 1).

Geosmin biodegradation capacity was confirmed in a microcosm batch experiment by analyzing the geosmin concentration over 7 days. Microcosms comprised of 10 mL bacteria, diluted to 0.1 optical density at 600 nm (OD_{600}), in basal salts medium (BSM) (19) in vials with 20 mL headspace. Geosmin losses by volatilization were controlled with microcosms of 10 mL BSM. Geosmin was added at 100 ng/L to each microcosm and measured at 0, 4, and 7 days in triplicate using solid-phase microextraction and gas chromatography mass spectrometry (GCMS) analysis. Significant removal of geosmin was observed for all inoculated microcosms compared to the control (Fig. 1C), demonstrating that all three *Sphingopyxis* strains can degrade geosmin.

Data availability. The genome sequences and raw reads have been deposited in the European Nucleotide Archive (ENA) under the project/study number PRJEB60073. The accession numbers for the genome sequences are ERS14837053, ERS14837054, and ERS14837055 for Geo24, Geo25, and Geo48, respectively.

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FIG 1 Legend (Continued)

nucleotide identity, as specified by the color bar key. Novel species are shown in bold against *Sphingopyxis* type strains. (B) Phylogenetic tree created using OrthoFinder v2.5.4 and RAxML-NG v1.1 to show the relationship between novel *Sphingopyxis* species and available type strain species, rooted with the type strain from the sister genus *Novosphingopyxis*. The maximum likelihood method was used with the LG model and G4 distribution, with bootstrap support (100 replicates) shown next to each node. Novel *Sphingopyxis* species are shown in bold. (C) Geosmin concentrations for microcosms in batch experiment comparing geosmin removal of novel *Sphingopyxis* species over 7 days. Bacteria (1 mL) grown in TSB, washed, and controlled to an OD_{600} of 1 were added to 9 mL BSM and spiked with 100 ngL^{-1} geosmin. Blank control microcosms with 10 mL BSM, spiked with 100 ngL^{-1} geosmin and with no inoculum, were run simultaneously. Geosmin concentration analysis was performed using SPME with GCMS analysis. Asterisks indicate statistical significance from the blank control at each time point, determined using the Mann-Whitney test.

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