

Comparison of 26 Sphingomonad Genomes Reveals Diverse Environmental Adaptations and Biodegradative Capabilities

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Sphingomonads comprise a physiologically versatile group within the *Alphaproteobacteria* that includes strains of interest for biotechnology, human health, and environmental nutrient cycling. In this study, we compared 26 sphingomonad genome sequences to gain insight into their ecology, metabolic versatility, and environmental adaptations. Our multilocus phylogenetic and average amino acid identity (AAI) analyses confirm that *Sphingomonas*, *Sphingobium*, *Sphingopyxis*, and *Novosphingobium* are well-resolved monophyletic groups with the exception of *Sphingomonas* sp. strain SKA58, which we propose belongs to the genus *Sphingobium*. Our pan-genomic analysis of sphingomonads reveals numerous species-specific open reading frames (ORFs) but few signatures of genus-specific cores. The organization and coding potential of the sphingomonad genomes appear to be highly variable, and plasmid-mediated gene transfer and chromosome-plasmid recombination, together with prophage- and transposon-mediated rearrangements, appear to play prominent roles in the genome evolution of this group. We find that many of the sphingomonad genomes encode numerous oxygenases and glycoside hydrolases, which are likely responsible for their ability to degrade various recalcitrant aromatic compounds and polysaccharides, respectively. Many of these enzymes are encoded on megaplasmids, suggesting that they may be readily transferred between species. We also identified enzymes putatively used for the catabolism of sulfonate and nitroaromatic compounds in many of the genomes, suggesting that plant-based compounds or chemical contaminants may be sources of nitrogen and sulfur. Many of these sphingomonads appear to be adapted to oligotrophic environments, but several contain genomic features indicative of host associations. Our work provides a basis for understanding the ecological strategies employed by sphingomonads and their role in environmental nutrient cycling.

Bacteria belonging to the genera *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis*, collectively referred to as sphingomonads, comprise an assortment of physiologically diverse bacteria within the phylum *Alphaproteobacteria* (1). Members of this group are broadly distributed in nature and have been isolated from a variety of environments, including human-associated niches (2), chemically contaminated water and sediment (3), activated sludge from wastewater treatment plants (4), seawater (5), and stromatolites (6). Many sphingomonads have been demonstrated to play an important role in nutrient cycling, especially in oligotrophic environments (5). Others reside in various plant- and animal-associated ecosystems and can even be responsible for nosocomial infections in humans (2).

A salient characteristic of many sphingomonads is their ability to degrade a variety of recalcitrant aromatic compounds and polysaccharides (1). A number of sphingomonads have been isolated from environments heavily contaminated with pesticides, herbicides, and other xenobiotics, and some have been shown to use these compounds as a sole carbon source (7, 8). Some sphingomonads have been shown to degrade dioxins (9), diphenyl ethers (7, 10), polyethylene glycol (11), dibenzofurans (12, 13), and many other aromatic and chloroaromatic compounds (8, 14). Moreover, many strains are known to metabolize lignin (15), an aromatic polymer abundant in plant cell walls. Taken together with the ability of many sphingomonads to deconstruct polysaccharides (16, 17), it is clear that these bacteria play important roles in the degradation of diverse recalcitrant compounds in the environment.

To provide insight into the ecology of sphingomonads, we

compared the genomes of 26 bacteria belonging to the genera *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis*. Additionally, we sequenced and analyzed draft genomes of *Sphingomonas* sp. strain Mn802 and *Sphingomonas* sp. strain PR090111-T3T-6A, two isolates associated with termites. The primary objectives of this study were to use genomic tools to shed light on the ecological strategies utilized by these bacteria and to broaden our knowledge of the evolutionary processes affecting their genomes. We compared the architecture of all sphingomonad genomes and, through analysis of the sphingomonad pan-genome, identified shared and variably distributed genes. Using targeted comparative analyses of specific genes involved in nutrient uptake and metabolism, our work provides hypotheses regarding the genomic content necessary for sphingomonads to thrive in a diversity of environments. Given the role many sphingomonads have been postulated to play in the cycling of recalcitrant carbon compounds in the environment, we also compared the complement of genes involved in the degradation of polysaccharides and aromatic compounds.

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MATERIALS AND METHODS

Termite collection and bacterial isolations. Termite colonies were sampled from natural populations in South Africa and Puerto Rico (see Table S1 in the supplemental material). *Sphingomonas* sp. strain Mn802 was isolated from a worker in a nest of the fungus-growing termite *Macrotermes natalensis* in 2008, and strain PR090111-T3T-6A was isolated from nest material from a *Nasutitermes* sp. in 2009. For strain Mn802, an underground fungus-growing termite colony of *Macrotermes natalensis* was excavated in South Africa and workers were collected using sterile forceps. Workers were kept together with fungus material in alcohol-wiped plastic containers until they were processed at the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa. In the laboratory, major workers were ground in 0.5 ml water and a homogenate was plated on 1% carboxymethyl cellulose (CMC) medium (10 g CMC and 20 g agar/liter), and a *Sphingomonas* isolate was obtained through serial transfer. For strain PR090111-T3T-6A, a colony of a *Nasutitermes* sp. was sampled from a termitarium in Bosque Estatal Maricao, Puerto Rico, in 2009. A homogenate of nest material in sterile water was plated on chitin medium (4 g chitin, 0.7 g K_2HPO_4 , 0.3 g KH_2PO_4 , 0.5 g $MgSO_4 \cdot 5H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnSO_4$, 0.0001 g $MnCl_2$, 1 liter water) (18), and colonies on the plates were isolated by serial transfer.

Genome sequencing. DNA was extracted from two pure cultures of *Sphingomonas* isolates grown in liquid Luria-Bertani (LB) media using the Qiagen DNeasy blood and tissue kit (Qiagen, CA). Shotgun sequencing libraries were constructed at the DOE Joint Genome Institute (JGI), and all aspects related to library construction and sequencing are available at <http://www.jgi.doe.gov/>. Whole-genome sequencing was performed for both isolates using a single picotiter plate on a 454 FLX Titanium pyrosequencer (19) and *de novo* assembled using Newbler, version 2.3. The draft genome of strain Mn802 consists of 25 contigs comprising 3.19 Mb, while the genome of strain PR090111-T3T-6A consists of 26 contigs comprising 3.91 Mb.

Genome annotations. Proteins from all draft and complete genomes were predicted *de novo* using Prodigal (20). Protein clusters were constructed through comparison with previously constructed clusters available on the eggNOG Database, version 3.0 (21), using HMMER (22). To do this, all bacterial cluster alignments (bactNOGs) available from eggNOG were downloaded on 28 September 2011, and HMMER3 was used to construct hidden-Markov model profiles for each cluster. For the clusters that lacked alignments on the eggNOG file transfer protocol (FTP), alignments were generated using MUSCLE (23). All sphingomonad proteins predicted were compared to these clusters using hmmscan from HMMer3, with a minimum E value of $<1e-5$. Proteins were designated as belonging to the same cluster as their top HMMer model hit. Raw data regarding cluster membership can be found in Data Set S1 in the supplemental material.

To identify distinguishing genomic features in the three sphingomonad genera for which multiple genomes are available (*Sphingomonas*, *Sphingobium*, and *Novosphingobium*), we performed an enrichment analysis of the protein clusters represented in these genera. To do this, we used Fisher's exact test ($P < 0.01$) to identify protein clusters that were overrepresented in one of these genera compared to the other two genera combined (raw data available in Data Set S1 in the supplemental material). For our analysis of highly variable coding potential, protein clusters with highly variable membership across the sphingomonads were identified by calculating the standard deviation of cluster membership across all genomes. The annotations of representative proteins belonging to the clusters with the highest variability (defined here as having a standard deviation of >1.5) were analyzed using the same tools as implemented for whole-genome annotations (see below). Visualization of Venn diagrams was performed using the tool Venny (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>).

Proteins predicted from the sphingomonad genomes were annotated using the KEGG KAAS server (24) and by comparison with the Clusters of Orthologous Groups (COG) database (25) using RPSBLAST (26) (E value $< 1e-5$). To identify transposons, we found all proteins with hits to one

or more transposon-associated COGs (full list and details in Data Set S2 in the supplemental material). Prophage and phage-like proteins on the chromosomes of the complete sphingomonad genomes were identified using *PhiSpy* (27) with the associated GenBank files available in the GenBank database (<http://www.ncbi.nlm.nih.gov/GenBank/index.html>) as the input. Oxygenases were determined by identifying all hits to mono- and dioxygenases in the KEGG Orthology (28) database (full KEGG annotations are available in Data Set S3 in the supplemental material). For carbohydrate-active enzymes (CAZymes) (29), predictions were made as previously described (30) (full annotations for oxygenases and CAZymes are available in Data Set S2). To identify transposons, we found proteins with homology to 27 COG profiles associated with these elements (details and full annotations in Data Set S2).

Phylogenetic analyses. A multilocus phylogenetic tree was constructed by identifying orthologous protein-coding genes between genomes. This was done by identifying all universally conserved eggNOG clusters and, for each genome, retaining only the protein having the highest HMMER hit to the Markov model for that cluster. This resulted in 268 universally conserved protein-coding genes for each genome. Protein sequences for each conserved cluster were aligned using the E-INS-i algorithm implemented in MAFFT, version 6 (31), and then converted to nucleotide alignments. These were concatenated and used to generate a phylogeny using RAxML (32). The concatenated alignment was partitioned by gene, and GTRGAMMA model parameters were estimated for each partition of the alignment separately. The phylogeny was generated with 100 bootstrap iterations.

For the average amino acid identity (AAI) analyses, we used a strategy similar to one previously described (33). AAI values were calculated for each pair of genomes using the reciprocal best BLASTP hits of their predicted proteins (parameters: X 150, $-q -1$, $-F F$, $-e 1e-5$). The AAI values were then converted into distances by subtracting them from 100. The dendrogram was created by using the resulting pairwise distances as input for the Neighbor package in Phylip (34). Both the multilocus phylogeny and AAI dendrogram were visualized using the interactive Tree of Life (iTOL) (35). The pairwise synteny plots were constructed using the same reciprocal best BLASTP hits as used for the AAI analysis and plotting them using R (36).

Nucleotide sequence accession numbers. The raw 454 sequences for *Sphingomonas* sp. strain Mn802 and strain PR090111-T3T-6A are available from the DDBJ Sequence Read Archive (SRA) under accession numbers SRA037852 and SRA037853, respectively, and the assemblies used in this study have been deposited in DDBJ/EMBL/GenBank under the accession numbers AORY0000000 and AORL0000000, respectively.

RESULTS AND DISCUSSION

Phylogenetic analyses of the sphingomonads. We analyzed the phylogenetic relationships of the sphingomonad genomes using both multilocus sequence analysis (MLSA) and AAI. For the MLSA, we used a concatenated alignment of 268 orthologous genes found to be universally conserved across all genomes. The resulting phylogeny revealed distinct clustering of the four genera, consistent with biochemical and 16S-based phylogenetic analyses distinguishing these groups (37) (Fig. 1). Maximum-likelihood bootstrap support was 100% for all nodes, and the topology of this tree was identical to a dendrogram created by clustering pairwise AAI analysis (Fig. 1), suggesting well-supported phylogenetic relationships. The pairwise AAI values ranged from 55.4% (between *Sphingomonas wittichii* RW1 and *Novosphingobium* sp. strain AP12) and 99.5% (between *Sphingomonas wittichii* RW1 and DP58) (all available in Data Set S4 in the supplemental material). Despite its classification in the genus *Sphingomonas*, both our MLSA and AAI analysis revealed that strain SKA58 clusters within the genus *Sphingobium* in the same clade as *Sphingobium yanoikuyae* XLDN2-5 and strain AP49 (Fig. 1), suggesting that this bacterium should be classified within this genus.

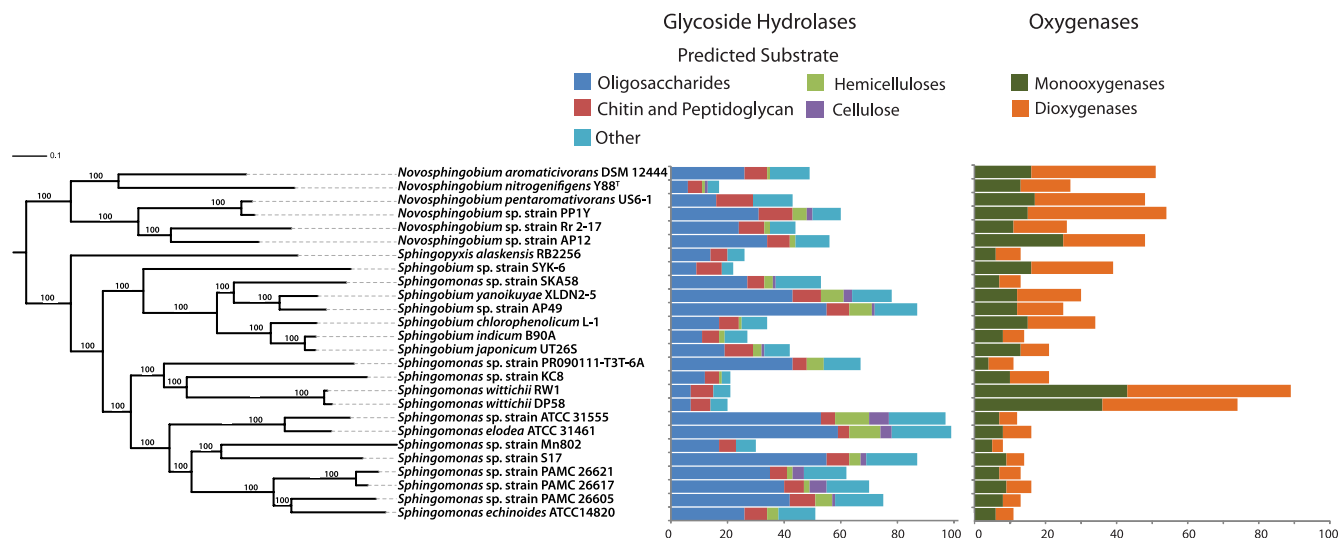


FIG 1 Maximum-likelihood MLSA of 26 sphingomonad genomes constructed from concatenated nucleotide sequences of 268 universally conserved genes, together with the distribution of glycoside hydrolases (GHs) and mono- and dioxygenases identified in the sphingomonad genomes. The topology of this tree is identical to the AAI dendrogram created from the proteins encoded in these genomes. Bootstrap support for the MLSA is given at each node, and the bar indicates the number of nucleotide substitutions per site. GHs were divided into categories based on their predicted substrate as follows: oligosaccharides, GH1, GH2, GH3, GH13, GH15, GH28, GH29, GH31, GH35, GH36, GH39, GH42, GH43, and GH92; hemicelluloses, GH8, GH10, GH26, GH51, GH53, and GH105; chitin and peptidoglycan, GH18, GH20, GH23, GH24, GH25, and GH73; cellulose, GH5, GH6, GH9, and GH44; other, all other GH families.

General genomic organization and selfish genetic elements.

Statistics for the 26 genomes analyzed in this study can be found in Table 1. Considerable variation in the organization of the seven complete genomes was observed (Table 2). For example, *Sphingobium chlorophenolicum* L-1 and *Sphingobium japonicum* UT26S contain two chromosomes, while all other complete genomes contain only one. Synteny analysis revealed only a small degree of conserved gene order between the complete genomes (see Fig. S1 in the supplemental material). As noted previously (38), a small degree of shared gene order is present between *Sphingobium japonicum* UT26S and *Sphingobium chlorophenolicum* L-1. These genomes appear to have undergone multiple chromosomal rearrangements since their divergence, and neither shares identifiable synteny with strain SYK-6. Also, only a small degree of synteny was observed between the chromosomes of *Novosphingobium aromaticivorans* DSM 12444 and *Novosphingobium* sp. strain PP1Y (see Fig. S1).

All of the complete genomes, except that of *Sphingopyxis alaskensis* RB2256, contained megaplasmids (plasmids of >100 kb [39]), consistent with previous suggestions that these replicons are common in sphingomonads (40). Although some megaplasmids were comparable in size to the secondary chromosomes of *Sphingobium chlorophenolicum* L-1 and *Sphingobium japonicum* UT26S, these replicons do not encode 16S rRNA operons or translational machinery characteristic of chromosomes (see the Translation category in Fig. S2 in the supplemental material). Furthermore, the metabolic potential encoded on the different replicons is markedly different, with the vast majority of proteins involved in essential processes such as amino acid and coenzyme metabolism encoded on the chromosomes, while hypothetical proteins are more commonly encoded on megaplasmids and plasmids (see Fig. S2).

We identified type IV secretion system components encoded in the majority of the genomes (see Table S2 in the supplemental material), raising the possibility for natural transformation or

plasmid-mediated gene transfer as mechanisms for gene exchange. Components of the type IV apparatus are encoded on the megaplasmid of each megaplasmid-containing bacterium except *Novosphingobium aromaticivorans* DSM 12444, suggesting that these replicons may encode their own mechanisms for transfer. Experiments demonstrating the transfer of the 184.5-kb megaplasmid pNL1 of *Novosphingobium aromaticivorans* DSM 12444 to other sphingomonads have previously been reported (40), and these experiments also showed rapid recombination between the transferred plasmid and the chromosome of the new host. Moreover, ~120 open reading frames (ORFs) with shared synteny and >98% amino acid identity are found on plasmid pSLGB of *Sphingobium* sp. strain SYK-6, the main chromosome of *Sphingobium japonicum* UT26S, and plasmid pSPHCH01 of *Sphingobium chlorophenolicum* L-1 (previously reported in references 38 and 41), suggesting recent transfer events. The transfer of essential genes to plasmids has been postulated to give rise to secondary chromosomes in other bacteria (42), providing a potential explanation for the multichromosomal arrangement in *Sphingobium chlorophenolicum* L-1 and *Sphingobium japonicum* UT26S.

Other selfish genetic elements, such as prophage and transposons, were also prevalent features of the sphingomonads. The program *PhiSpy* (27) identified several prophage encoded on the chromosomes (Table 2), with the highest number predicted from *Novosphingobium aromaticivorans* DSM 12444: 5 putative prophage are predicted to contribute 9.3% of all proteins encoded by this bacterium. *Novosphingobium* sp. strain PP1Y encoded 3 prophage contributing 113 protein-coding genes to its chromosome. One or 2 putative prophage were also identified in all chromosomes of the complete genomes, with the exception of the smaller chromosome of *Sphingobium japonicum* UT26S (Table 2). COG-based annotation also identified a highly variable number of transposons in the sphingomonads, with between 2 and 65 of these elements encoded in the complete and draft

TABLE 1 Statistics and isolation information for the 26 sphingomonad genomes

Organism	Genome size (Mbp)	Sequencing status	No. of chromosomes (plasmids)	No. of 16S operons	Isolation source	Reference
<i>Novosphingobium</i>						
<i>Novosphingobium aromaticivorans</i> DSM 12444	4.2	Complete	1 (2)	3	Subsurface sediment	55
<i>Novosphingobium nitrogenifigens</i> Y88 ^T	4.2	Draft		1	Paper mill wastewater	56
<i>Novosphingobium pentaromativorans</i> US6-1	5.3	Draft		1	Ulsan Bay sediment	57
<i>Novosphingobium</i> sp. strain AP12	5.6	Draft		1	Cottonwood rhizosphere	58
<i>Novosphingobium</i> sp. strain PPIY	5.3	Complete	1 (3)	3	Seawater	59
<i>Novosphingobium</i> sp. strain Rr 2-17	4.5	Draft		1	Grapevine crown gall	60
<i>Sphingopyxis</i>						
<i>Sphingopyxis alaskensis</i> RB2256	3.4	Complete	1 (1)	1	Seawater	46
<i>Sphingobium</i>						
<i>Sphingobium chlorophenicum</i> L-1	4.6	Complete	2 (1)	3	Soil	38
<i>Sphingobium indicum</i> B90A	4.1	Draft		2	Sugarcane rhizosphere	61
<i>Sphingobium japonicum</i> UT26S	4.4	Complete	2 (3)	3	Contaminated soil	49
<i>Sphingobium</i> sp. strain AP49	4.5	Draft		1	Cottonwood rhizosphere	58
<i>Sphingobium</i> sp. strain SYK-6	4.3	Complete	1 (1)	2	Pulp mill wastewater	41
<i>Sphingobium yanoikuyae</i> XLDN2-5	5.4	Draft		1	Contaminated soil	62
<i>Sphingomonas</i>						
<i>Sphingomonas echinoides</i> ATCC 14820	4.2	Draft		1	Plate contaminant	63
<i>Sphingomonas elodea</i> ATCC 31461	4.1	Draft		1	Plant (<i>Elodea</i>) tissue	64
<i>Sphingomonas</i> sp. strain KC8	4.1	Draft		1	Activated sludge	4
<i>Sphingomonas</i> sp. strain Mn802	3.2	Draft		1	Termite	This study
<i>Sphingomonas</i> sp. strain PR090111-T3T-6A	3.9	Draft		1	Termite	This study
<i>Sphingomonas</i> sp. strain S17	4.3	Draft		1	Hypersaline lake	6
<i>Sphingomonas</i> sp. strain SKA58	3.9	Draft		4	Seawater	NA
<i>Sphingomonas</i> sp. strain ATCC 31555	4.0	Draft		1	Pond water	65
<i>Sphingomonas</i> sp. strain PAMC 26605	4.7	Draft		1	Arctic lichen	66
<i>Sphingomonas</i> sp. strain PAMC 26617	4.7	Draft		1	Arctic lichen	67
<i>Sphingomonas</i> sp. strain PAMC 26621	4.8	Draft		2	Arctic lichen	68
<i>Sphingomonas wittichii</i> DP58	5.6	Draft		1	Pimiento rhizosphere	69
<i>Sphingomonas wittichii</i> RW1	5.9	Complete	1 (2)	2	Elbe River	3

genomes (Table 2; see also Data Set S2 in the supplemental material). The largest numbers were found in *Sphingomonas* sp. strain PAMC 26621, *Novosphingobium* sp. strain Rr 2-17, and *Sphingobium yanoikuyae* XLDN2-5 (63 to 65 each), while the fewest were identified in the two termite-associated *Sphingomonas* strains (Mn802 and PR090111-T3T-6A).

Pan-genomics of the sphingomonads. To investigate the pan-genome of the sphingomonads, we used Prodigal (20) to identify a total of 110,698 proteins. Comparison of proteins encoded in the sphingomonad genomes to the eggNOG Database (21), which contains previously generated protein clusters from bacterial genomes, revealed a total of 23,087 protein clusters, including 268 that were universally conserved between all genomes and 492 that were conserved between the complete genomes. These genes consisted primarily of ribosomal proteins and proteins involved in replication, DNA repair, amino acid metabolism, transcription, and other basic metabolic functions (see Fig. S3 in the supplemental material).

Although numerous genus-specific protein clusters were identified in our analyses (Fig. 2A), the majority of these were represented only in one or a few genomes within the genera (Fig. 2B), indicating that they do not represent features that consistently distinguish the sphingomonad genera. Note that because the genome of *Sphingopyxis alaskensis* RB2256 is the only representative

of its genus, it was not possible to ascertain whether the protein clusters unique to this bacterium are representative of other *Sphingopyxis* isolates. To investigate consistent differences in coding potential between sphingomonad genera for which multiple genomes are available, we identified protein clusters that were overrepresented in any of the genera *Sphingomonas*, *Sphingobium*, and *Novosphingobium* compared to the other two combined (Fisher's exact test, $P < 0.01$). Using this approach, we identified 87 protein clusters overrepresented in *Sphingomonas*, 163 in *Sphingobium*, and 145 in *Novosphingobium* (details are in Data Set S1 in the supplemental material). Interestingly, most of these clusters have no COG annotation, have a general or unknown functional prediction, or are associated with DNA processing (see Fig. S4 in the supplemental material), suggesting that they encode selfish genetic elements rather than conserved metabolic processes.

Consistent with intergenus findings, the majority of protein clusters identified as highly variable are predicted to encode transposases, phage-related proteins, or proteins that could not be annotated (Fig. 3). Some of these variably distributed proteins encode glycoside hydrolases (GHs) and oxygenases, suggesting that sphingomonad genomes may be particularly affected by adaptations related to changes in biodegradative capabilities. Clustering analysis of the variable protein clusters revealed that many were represented in specific genera or species, suggesting that shared

TABLE 2 Size and number of encoded selfish genetic elements and biodegradative enzymes in the 7 complete sphingomonad genomes

Organism	Size (kb)	No. of:					
		Putative prophage	Phage-like proteins (% of total proteins)	Transposons	Monooxygenases	Dioxygenases	CAZymes
<i>Novosphingobium aromaticivorans</i> DSM 12444							
Chromosome	3,561.6	5	309 (9.3)	16	13	19	58
Plasmid pNL1	184.5	1		1	1	13	1
Plasmid pNL2	487.3				2	3	8
<i>Novosphingobium</i> sp. strain PP1Y							
Chromosome	3,911.5	3	113 (3.3)	24	10	33	36
Plasmid Lpl	192.1	1		5			2
Plasmid Spl	48.7			1			1
Plasmid Mpl	1,161.6			17	5	6	41
<i>Sphingobium chlorophenicum</i> L-1							
Chromosome 1	3,080.8	1	107 (3.8)	15	9	4	30
Chromosome 2	1,368.7	1	74 (6.7)	8	6	15	17
Plasmid pSPHCH01	123.7			2			1
<i>Sphingobium japonicum</i> UT26S							
Chromosome 1	3,514.8	2	95 (2.7)	37	9	6	28
Chromosome 2	681.9			7	2	2	22
Plasmid pUT1	31.8			5			
Plasmid pUT2	5.4						
Plasmid pPCHQ1	191.0	2		6	2		2
<i>Sphingobium</i> sp. strain SYK-6							
Chromosome	4,199.3	1	88 (2.3)	29	16	23	25
Plasmid pSLPG	148.8			6			1
<i>Sphingomonas wittichii</i> RW1							
Chromosome	5,382.26	2	183 (3.8)	36	42	38	33
Plasmid pSWIT01	310.2	2		9		2	1
Plasmid pSWIT02	222.8	1		31	1	6	2
<i>Sphingopyxis alaskensis</i> RB2256							
Chromosome	3,345.2	1	62 (2.0)	36	9	7	33
F plasmid	28.5						

ancestry may be responsible for their distribution (Fig. 3). *Sphingomonas wittichii* strains RW1 and DP58, for example, both encoded multiple proteins belonging to clusters annotated as outer membrane receptors, dehydrogenases, and transposases (Fig. 3). Overall, however, our pan-genomic analysis revealed no large-scale differences in coding potential that distinguish the four sphingomonad genera.

Aromatic compound and polysaccharide degradation. To investigate the capacity of sphingomonads to degrade aromatic compounds, we analyzed the encoded mono- and dioxygenases, enzymes that catalyze the ring cleavage step critical to aromatic compound degradation (43). The distribution of mono- and dioxygenases varied by an order of magnitude across the sphingomonad phylogeny, with between 4 and 43 monooxygenases and 3 and 46 dioxygenases encoded (Fig. 1; see also Table S3 in the supplemental material). Both *Sphingomonas wittichii* RW1 and DP58 encoded the largest complement of oxygenases, although *Sphingobium chlorophenicum* L-1, *Sphingobium* sp. strain SYK-6, and all *Novosphingobium* species except for *Novosphingobium nitrogenifigens* Y88¹ also encoded a large complement of these enzymes (see Table S3). The most abundant monooxygen-

ase families identified included nitronate monooxygenase (NMO, K000459), vanillate monooxygenase (VanA, K03862), and alkanesulfonate monooxygenase (SsuD, K04091), while the most abundant dioxygenase families included taurine dioxygenase (TauD, K03119) and 4-hydroxyphenylpyruvate dioxygenase (HppD, K00457) (Table 3). Both *Sphingomonas wittichii* RW1 and DP58 contained the largest number of predicted vanillate monooxygenases (24 and 19, respectively) as well as multiple dioxin dioxygenases (HcaA homologs, K05708), with genes for the latter being generally absent from the other sphingomonad genomes (see Data Set S2 in the supplemental material).

The abundance of *tauD* and *ssuD* homologs in many of the genomes suggests that sulfonated compounds may be sources of both carbon and sulfur for many of the sphingomonads. Bacteria have been shown to use a variety of organosulfur compounds as carbon and sulfur sources, and these compounds are abundant in soil and aquatic environments, as well as in environmental pollutants and fossil fuels (44). *Novosphingobium* sp. strain AP12, isolated from the rhizosphere of the cottonwood tree (*Populus deltoides*), for example, contained a particularly large amount of these genes (16 *ssuD* homologs and 17 *tauD* homologs). Ho-

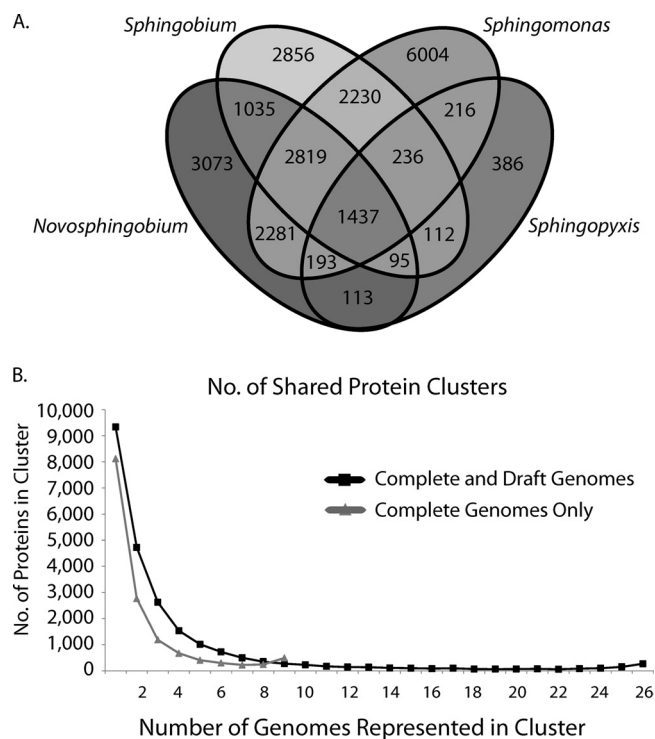


FIG 2 (A) Venn diagram showing the number of shared and unique protein clusters identified in the 4 sphingomonad genera. (B) Distribution of protein clusters identified in the complete and draft sphingomonad genomes. The x axis represents the number of genomes encoding a member of a protein cluster, and the y axis represents the total number of clusters having that many genomes represented.

mologs to the sulfonate transport apparatus (*ssuABC*) were also identified in strain AP12 as well as all of the soil- or rhizosphere-associated *Sphingobium* species (*S. chlorophenolicum* L-1, *S. japonicum* UT26S, strain AP49, and *S. yanoikuyae* XLDN2-5) (see Table S2 in the supplemental material). Interestingly, *S. japonicum* UT26S contained all *ssuABC* homologs but no *ssuD* dioxygenase, suggesting that it may degrade sulfur-containing compounds using alternative enzymes (Table 3; see also Table S2 and Data Set S3 in the supplemental material).

In contrast to *ssuD* and *tauD*, the presence of nitronate monooxygenase (NMO, K00457) gene homologs in the sphingomonad genomes was more consistent, with all genomes coding for between 2 and 7 copies. The largest complement of these enzymes was found in *Sphingobium chlorophenolicum* L-1 and *Sphingobium japonicum* UT26S. NMO preferentially acts on nitroalkanes, raising the possibility that these compounds may be naturally occurring sources of both nitrogen and carbon to some sphingomonads.

Many of the sphingomonads contained a diversity of GHs, suggesting adaptations to degrade plant polysaccharides. *Sphingomonas elodea* ATCC 31461 and *Sphingomonas* sp. strain ATCC 31555 contained the largest complement of these enzymes (99 and 97, respectively), including cellulases and hemicellulases (Fig. 1). The genomes with the most predicted GHs also encoded representatives from the cellulase families GH5, GH6, and GH9 (Table 3), in support of their ability to hydrolyze cellulose. The genomes of these also encode a diversity of other GHs, carbohydrate esterases,

and polysaccharide lyases that likely target pectins, xylans, mannans, and other plant cell wall polysaccharides (see Table S3 in the supplemental material). The isolates with the largest complement of hemicellulases and cellulases, *Sphingomonas elodea* ATCC 31461, *Sphingomonas* sp. ATCC 31555, *Sphingobium yanoikuyae* XLDN2-5, and *Sphingobium* sp. strain AP49, were isolated from environments rich in plant biomass, such as plant tissue, soil, and pond water, suggesting sphingomonad participation in the degradation of environmental plant biomass.

Among the sphingomonads with the fewest GHs (20 to 22 each) were strains encoding a large number of oxygenases (e.g., *Sphingomonas wittichii* strains RW1 and DP58 and *Sphingobium* sp. strain SYK-6 [Fig. 1; see also Table S3 in the supplemental material]), suggesting specialization in the degradation of aromatic compounds and an absence of the capacity to deconstruct recalcitrant polysaccharides. However, although *Novosphingobium nitrogenifigens* Y88^T encoded only 17 GHs, it also lacked a substantial coding potential for oxygenases compared to other *Novosphingobium* strains (Fig. 1; see also Table S3), suggesting that the degradation of a wide diversity of recalcitrant carbon compounds may be less important for this strain than for other sphingomonads.

Analysis of the seven complete sphingomonad genomes revealed that many oxygenases and GHs are encoded on plasmids or megaplasmids (Table 2). In *Novosphingobium* sp. strain PP1Y, more GHs were encoded on the megaplasmid than the chromosome (41 versus 36), and the plasmids of *Sphingomonas wittichii* RW1 and *Novosphingobium aromaticivorans* DSM 12444 contain several dioxygenases. Moreover, the smaller of the two chromosomes in *Sphingobium chlorophenolicum* L-1 has been shown to encode the genes necessary to degrade the aromatic pesticide pentachlorophenol, and evidence suggests that three genes involved have arisen through multiple horizontal gene transfer events (38). Together with the high incidence of chromosomal rearrangements and plasmid-mediated gene transfers observed, this is consistent with the assertion that plasmids and other selfish genetic elements are likely responsible for frequent exchange of biodegradative capabilities in sphingomonads (14).

Adaptations to the environment. All of the sphingomonad genomes contained between 1 and 4 copies of the 16S rRNA gene, with the majority containing one or two copies (Table 1). Previous work has shown that low 16S rRNA copy number is a characteristic commonly associated with bacteria that inhabit a specialized niche and do not rapidly respond to changing environmental conditions (45). Together with the specific sets of oxygenases and GHs encoded by most of the sphingomonads, it seems likely that many of these bacteria are specialized heterotrophs capable of utilizing components of refractory detritus or xenobiotics as carbon, sulfur, and phosphorus sources. This has previously been suggested for *Sphingopyxis alaskensis* RB2256 (46–48), *Sphingobium japonicum* UT26S (49), and sphingomonads residing in subsurface sediment, such as *Novosphingobium aromaticivorans* DSM 12444 (50). This is likely a successful ecological strategy in oligotrophic environments, where few alternative nutrient sources would be available and where other microbes would likely not possess the enzymatic machinery necessary to compete for their use. Thus, rather than remaining dormant for long periods and doubling rapidly when environmental conditions become favorable, sphingomonads may be adapted

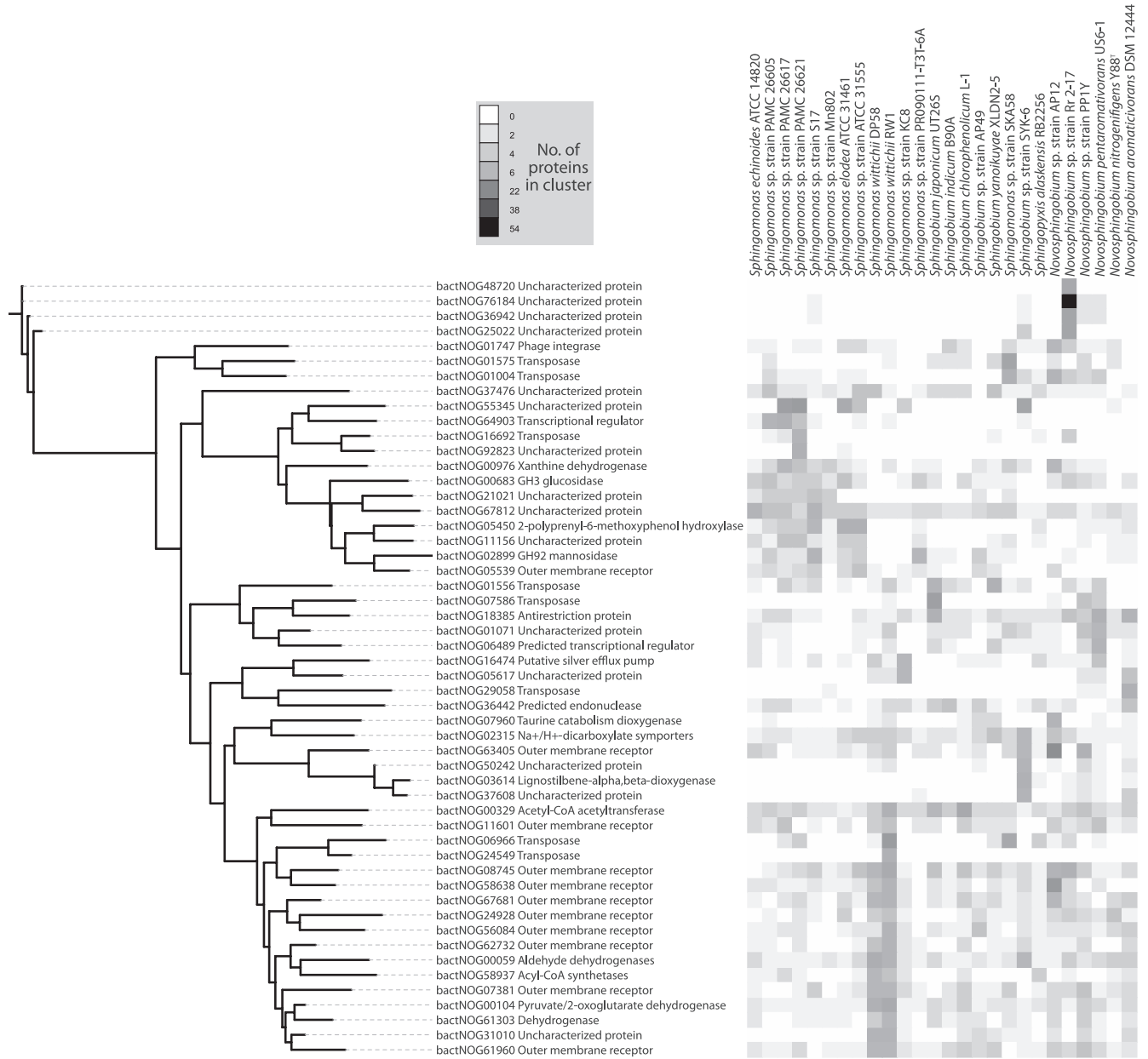


FIG 3 Heat map representing the sphingomonad protein clusters with the most variable distributions across the 26 genomes. The dendrogram was constructed using the pairwise Pearson's *r* correlation calculated for the protein cluster membership distributions (raw values in Data Set S1 in the supplemental material).

to specialize in the utilization of particular recalcitrant compounds in oligotrophic conditions.

Adaptation to oligotrophic conditions has already been shown for the marine bacterium *Sphingopyxis alaskensis* RB2256, an important contributor to nutrient cycling in marine ecosystems (46, 47, 51). This ultramicrobacterium, which maintains an extremely small cell volume (<0.08 μm^3), has adapted to persist in seawater containing extremely low concentrations of amino acids and other nutrients (5, 47). Previous analyses have suggested that this genome has not undergone the same degree of genomic reduction as has been observed in other marine ultramicrobacteria (47, 52). This species has the second-smallest genome of all of the sphingomonads analyzed in this study (smallest among the complete

genomes) and is the only complete sphingomonad genome lacking a megaplasmid, suggesting that it may in fact have undergone a considerable amount of genomic streamlining compared to other sphingomonads.

Despite the apparent adaptations of many sphingomonads to oligotrophic conditions, many of these bacteria can be found in host-associated environments that are likely more nutrient rich. Many of the species analyzed in this study were isolated from environments including termites, lichen, plant tissue, or the rhizosphere (Table 1). Type IV secretion systems, implicated in host-microbe interactions in other *Alphaproteobacteria* (53), were identified in most of the sphingomonad genomes (see Table S2 in the supplemental material), raising the possibility that these sys-

TABLE 3 Selected catabolic enzymes identified in the sphingomonad genomes^a

Organism	No. of enzymes							
	Monooxygenases			Dioxygenases		Cellulase families		
	NMO (K00459)	VanA (K03862)	SsuD (K04091)	TauD (K03119)	HppD (K00457)	GH5	GH6	GH9
<i>Novosphingobium</i>								
<i>Novosphingobium aromaticivorans</i> DSM 12444	4	4	1	2	2			
<i>Novosphingobium nitrogenifigens</i> Y88 ^T	3	1	2	3		1		
<i>Novosphingobium pentaromativorans</i> US6-1	6	5	2	2	2			
<i>Novosphingobium</i> sp. strain AP12	5		16	17	1			
<i>Novosphingobium</i> sp. strain PP1Y	3	2	1	1	2		1	1
<i>Novosphingobium</i> sp. strain Rr 2-17	3		2	5	1			
<i>Sphingopyxis</i>								
<i>Sphingopyxis alaskensis</i> RB2256	6		1	1	1			
<i>Sphingobium</i>								
<i>Sphingobium chlorophenicum</i> L-1	7	1	1	6	1			
<i>Sphingobium indicum</i> B90A	5	1			1			
<i>Sphingobium japonicum</i> UT26S	7	2			1	1		
<i>Sphingobium</i> sp. strain AP49	4	1	2	4	1			
<i>Sphingobium</i> sp. strain SYK-6	2	7		7	1			
<i>Sphingobium yanoikuyae</i> XLDN2-5	4		2	4	2	2		
<i>Sphingomonas</i>								
<i>Sphingomonas echinoidea</i> ATCC 14820	2		1		1			
<i>Sphingomonas elodea</i> ATCC 31461	2	1	2	1	1	1		1
<i>Sphingomonas</i> sp. strain KC8	4	1		2	1			
<i>Sphingomonas</i> sp. strain Mn802	2		1		1			
<i>Sphingomonas</i> sp. strain PR090111-T3T-6A	3			1	1			
<i>Sphingomonas</i> sp. strain S17	3		2		1			2
<i>Sphingomonas</i> sp. strain SKA58	3	1			1			1
<i>Sphingomonas</i> sp. strain ATCC 31555	2	1			1	2		2
<i>Sphingomonas</i> sp. strain PAMC 26605	3		3	1	1			1
<i>Sphingomonas</i> sp. strain PAMC 26617	2		2	1	2	4		2
<i>Sphingomonas</i> sp. strain PAMC 26621	2		2		1	2		2
<i>Sphingomonas wittichii</i> DP58	4	19	5	8	1			
<i>Sphingomonas wittichii</i> RW1	5	24	4	7	1			

^a NMO, nitrate monoxygenase; VanA, vanillate monoxygenase; SsuD, alkanesulfonate monoxygenase; TauD, taurine dioxygenase; HppD, 4-hydroxyphenylpyruvate dioxygenase.

tems are used during host interaction, in addition to their likely role in DNA transfer. Many of these associations may, however, be short term and not involve host-microbe specificity. For example, some of the sphingomonads isolated from the rhizosphere, such as *Novosphingobium* sp. strain AP49 and *Sphingobium* sp. strain AP12, contain genes for organosulfur and nitroaromatic compound utilization, indicating that they may subsist on recalcitrant compounds or secondary metabolites produced by potential plant associates. These bacteria may therefore degrade these compounds in the soil or similar environments, without the necessity for any specific association with metazoan hosts.

Two *Sphingomonas* species sequenced as part of this study, strain Mn802 and strain PR090111-T3T-6A, were isolated from termites (see Table S1 in the supplemental material). Strain PR090111-T3T-6A encodes 80 CAZymes, suggesting that it may play a role in the degradation of the copious quantities of plant biomass ingested by its potential termite host. Strain Mn802, however, encodes relatively few CAZymes and has the smallest genome size of all the sphingomonads, suggesting that it may have reduced biodegradative capabilities and subsist on easily accessi-

ble nutrients readily available in the termite environment. Sphingomonads have previously been isolated from termites (54), indicating that members of this group may have a consistent but as-yet-uncharacterized termite association. If this is correct, our analyses suggest that termite-associated sphingomonads do not all partake in the breakdown of recalcitrant plant material but rather may perform alternative functions in the associations.

Conclusions. Consistent with previous biochemical characterizations and analyses of 16S rRNA genes (37), the genera *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis* form well-resolved phylogenetic clades at the whole-genome level, although *Sphingomonas* sp. strain SKA58 appears to belong in the genus *Sphingobium*. A large number of species-specific genes were present in the sphingomonad genomes, and few genomic features can reliably distinguish the genera. Selfish genetic elements appear to be prominent forces shaping this genome evolution, and megaplasmids, prophage, transposons, and frequent chromosomal rearrangements are prevalent features in the group. Many sphingomonads appear to successfully occupy oligotrophic niches and utilize recalcitrant compounds as sources of carbon, nitrogen, and

sulfur. Species with few oxygenases or GHs may have evolved to live in more nutrient-rich environments, including host associations, while others may have undergone genomic streamlining to adapt to extreme oligotrophic conditions. As more information on the distribution and catabolic activities of the sphingomonads emerges, insights into their ecology will greatly advance our understanding of their impact on global nutrient cycles and potential applications to biotechnology.

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