Supplementary Information for Aylward *et al*., "**Comparison of 26 Sphingomonad Genomes Reveals Diverse Environmental Adaptations and Biodegradative Capabilities**"

**Supplementary Text**

**Metabolic Features of the sphingomonads**

We examined particular gene sets encoded in the sphingomonad genomes that are markers for specific metabolic capabilities. For example, serine palmitoyltransferase (*spt*) homologs were identified by comparing all of the sphingomonad proteins to the swit_3900 protein (accession YP_001264383) encoded in *Sphingomonas wittichii* RW1 using BLASTP (1) (e-value < 1e-100). All of the sphingomonad genomes contained a homolog of this enzyme, which has been characterized in *Sphingomonas wittichii* RW1 and thought to be critical for sphingolipid biosynthesis (2). Moreover, few genes associated with lipopolysaccharide biosynthesis could be identified in all genomes, consistent with biochemical characterizations of their lipid membranes (full KEGG annotations can be found in Dataset S3).

Inorganic phosphate appears to be acquired in all of the sphingomonads through use of a high-affinity phosphate pump (*pstABCS* genes, Table 3). Genes associated with the uptake of phosphonates were identified in three of the sphingomonads (*Novosphingobium aromaticivorans* DSM 12444, *Sphingobium yanoikuyae* XLDN2-5, and *Sphingomonas* sp. strain ATCC 31555), indicating that these compounds could be used to supplement phosphate requirements.

*Novosphingobium nitrogenifigens* Y88$^T$ is the only sphingomonad found to have the genes required for nitrogen fixation, consistent with previous experimental data and its isolation from nitrogen-poor paper mill wastewater (3, 4). In the other strains nitrogen appears to be acquired through the assimilatory reduction of nitrate, as all of the sphingomonads genomes encode homologs of the *nas* system for nitrate/nitrite reduction and import (Table 3). Interestingly, both *Sphingomonas wittichii* strains RW1 and DP58 encode homologs of *nirK* and *norB*, enzymes involved in the dissimilatory conversion of nitrite to nitrous oxide, the first step of denitrification. Although sphingomonads are not typically considered to be denitrifiers, the aerobic dissimilatory reduction of nitrate has been suggested to be relatively common in bacteria (5, 6). The benefits of this strategy are unclear, but it may provide an advantage in environments where oxygen levels are variable (5).

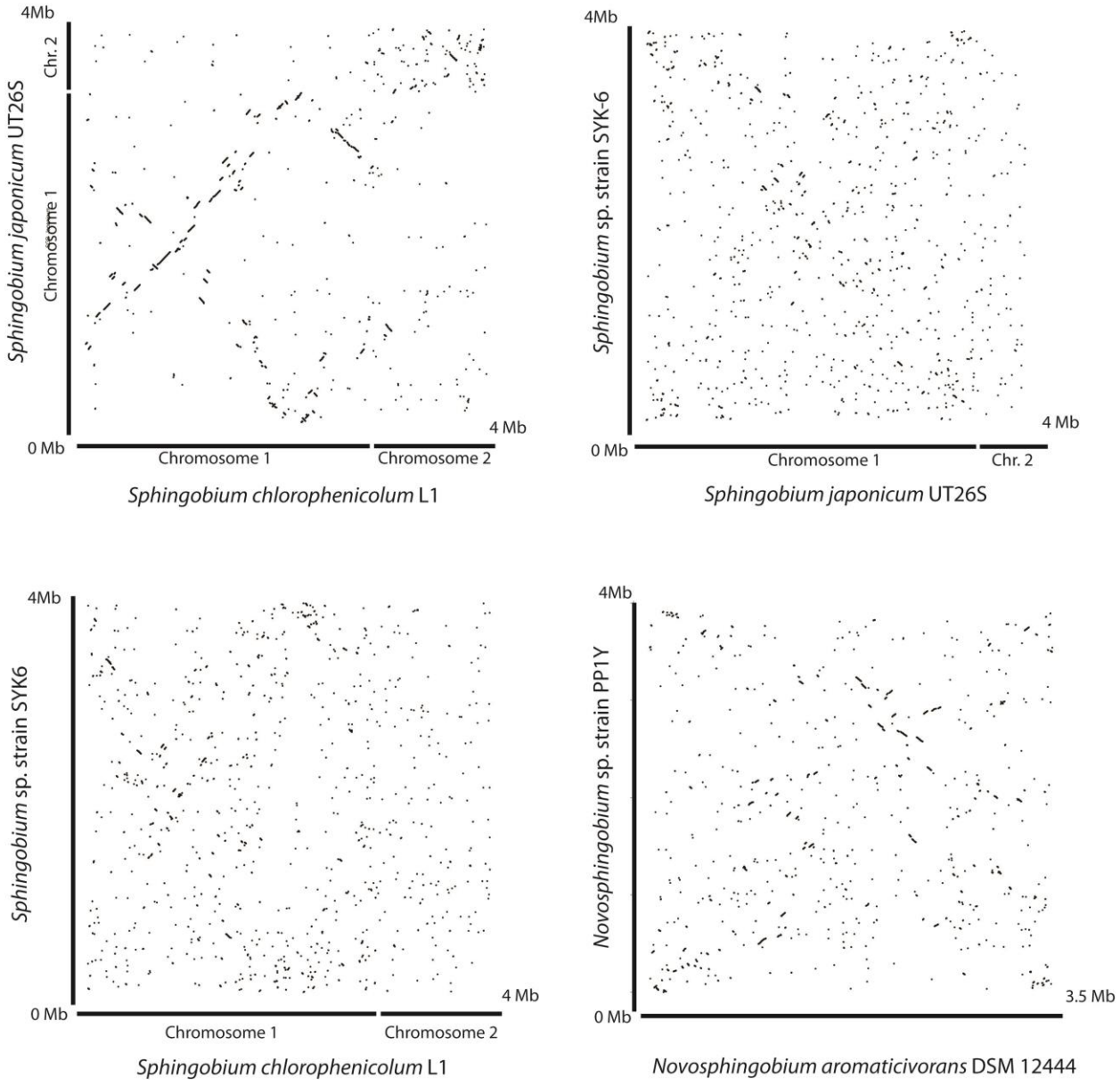


Figure S1. Pairwise synteny plots for the three complete *Sphingobium* species and two *Novosphingobium* genomes for which some degree of synteny could be detected. The *Sphingobium* species *japonicum* UT26S, *chlorophenolicum* L-1, and strain SYK-6, are shown, as are the *Novosphingobium* species strain PP1Y and *aromaticivorans* DSM 12444.

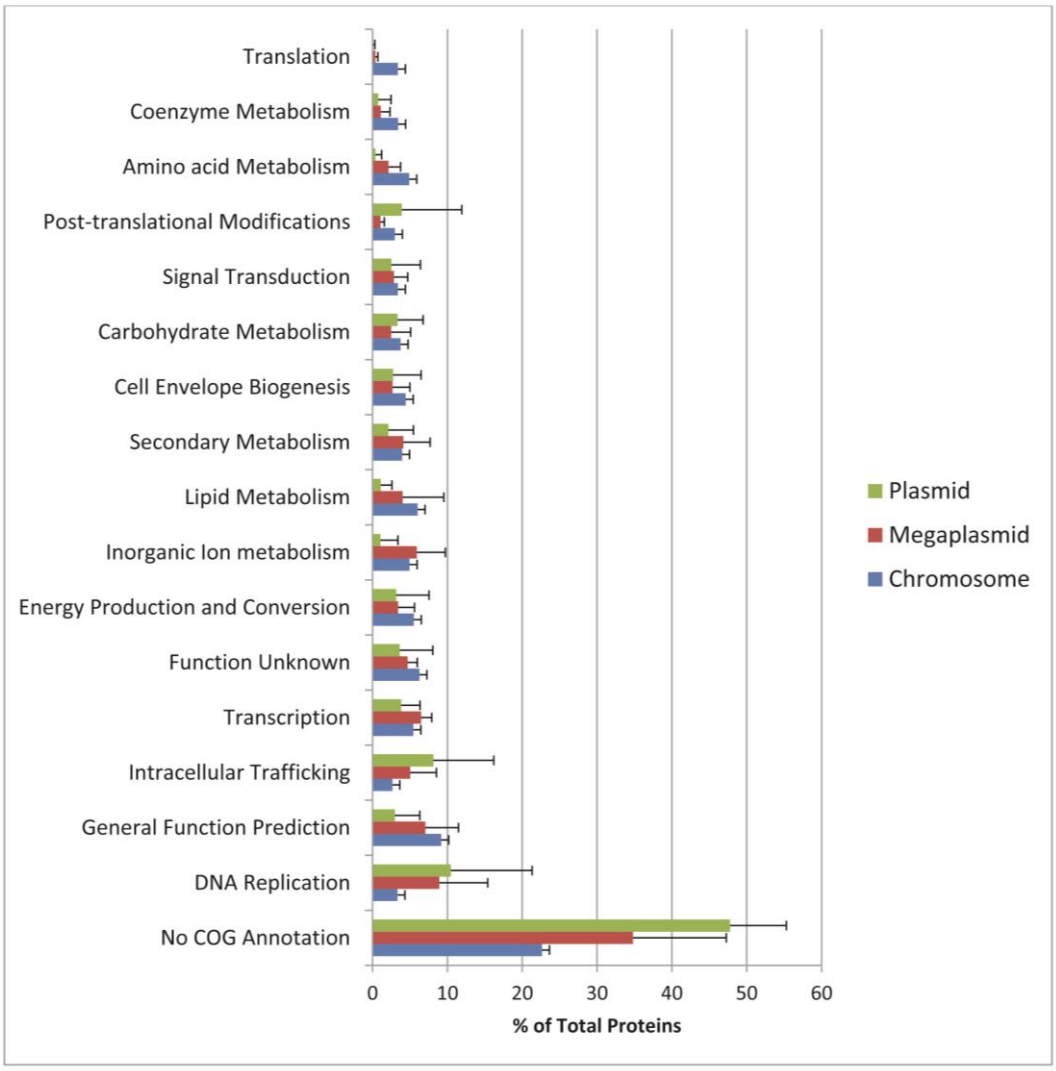


Figure S2. COG category annotations for the predicted proteins encoded on the chromosomes, megaplasmids, and plasmids of the 7 complete sphingomonad genomes analyzed. Plasmids > 100 Kb were considered megaplasmids. The secondary chromosomes of *Sphingobium japonicum* UT26S and *Sphingobium chlorophenolicum* L-1 were analyzed individually. Error bars indicate standard deviation.

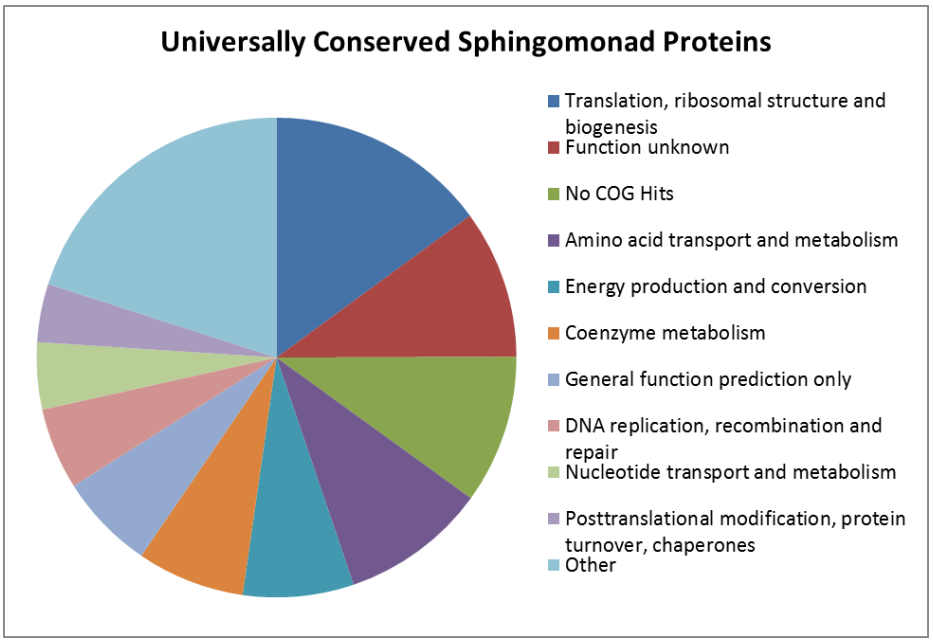


Figure S3. COG category annotation for the 268 protein clusters universally conserved in the 26 sphingomonad genomes.

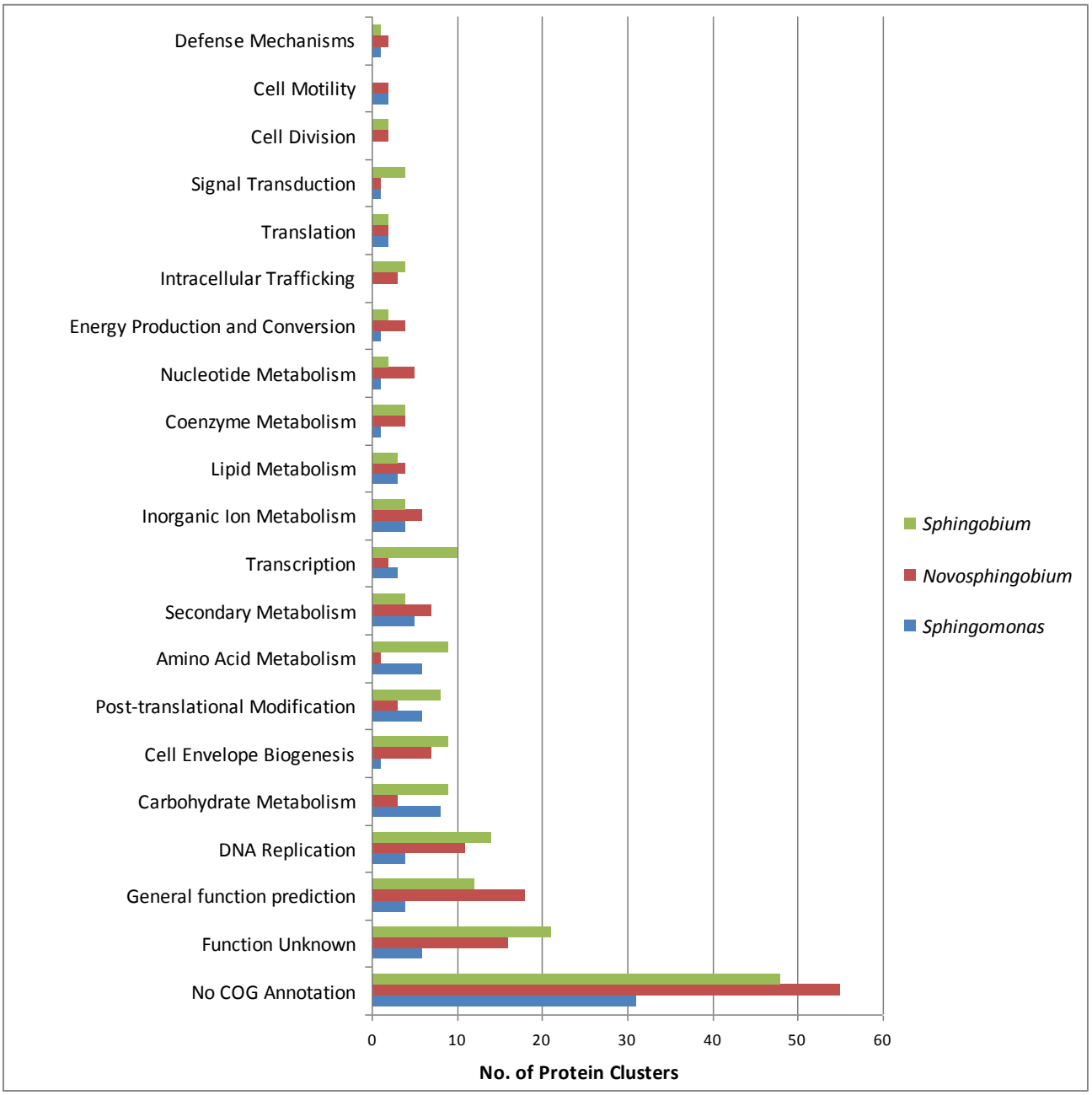


Figure S4. COG category annotations for the protein clusters found to be over-represented in the three sphingomonad genera for which multiple genomes were analyzed (*Sphingomonas*, *Sphingobium*, and *Novosphingobium*). Details regarding all clusters can be found in Dataset S1.

Table S1. Collection information for the termite-associated *Sphingomonas* isolates.

| Strain | Collection year | Termite species | Isolated from | Location |
|---|---|---|---|---|
| Mn802 | 2008 | *Macrotermes natalensis* | Worker | South Africa |
| PR090111-T3T-6A | 2009 | *Nasutitermes* sp. | Termitarium | Puerto Rico |

**Table S2**. Genes identified in the sphingomonad genomes that are signatures indicative of sphingolipid biosynthesis (*spt*), phosphonate metabolism (*phn* genes), phosphate uptake (*pst* genes), denitrification (*nirK, norB*), nitrate assimilation (*nas* genes), sulfonate metabolism (*ssu* genes), and the type IV secretion system (*vir* genes).

| Genome | *spt* | *pstABCS* | *phnCDE* | *nas* genes | *nirK, norB* | *ssuABC* | *vir* genes |
|---|---|---|---|---|---|---|---|
| ***Novosphingobium*** | | | | | | | |
| *Novosphingobium aromaticivorans* DSM 12444 | x | x | x | x | | | x |
| *Novosphingobium nitrogenifigens* DSM 19370 | x | x | | x | | | |
| *Novosphingobium pentaromativorans* US6-1 | x | x | | x | | | x |
| *Novosphingobium* sp. strain AP12 | x | x | | x | | x | |
| *Novosphingobium* sp. strain PP1Y | x | x | | x | | | x |
| *Novosphingobium* sp. strain Rr 2 17 | x | x | | x | | | x |
| ***Sphingopyxis*** | | | | | | | |
| *Sphingopyxis alaskensis* RB2256 | x | x | | x | | | x |
| ***Sphingobium*** | | | | | | | |
| *Sphingobium chlorophenolicum* L-1 | x | x | | x | | x | x |
| *Sphingobium indicum* B90A | x | x | | x | | | x |
| *Sphingobium japonicum* UT26S | x | x | | x | | x | x |
| *Sphingobium* sp. strain AP49 PMI04 | x | x | | x | | x | x |
| *Sphingobium* sp. strain SYK-6 | x | x | | x | | | x |
| *Sphingobium yanoikuyae* XLDN2 5 uid86867 | x | x | x | x | | x | x |
| ***Sphingomonas*** | | | | | | | |
| *Sphingomonas echinoides* ATCC 14820 | x | x | | | | | |
| *Sphingomonas elodea* ATCC 31461 | x | x | | x | | | |
| *Sphingomonas* sp. strain KC8 | x | x | | x | | | x |
| *Sphingomonas* sp. strain MN802 | x | x | | | | | |
| *Sphingomonas* sp. strain PR09011 | x | x | | x | | | |
| *Sphingomonas* sp. strain S17 | x | x | | x | | | x |
| *Sphingomonas* sp. strain SKA58 | x | x | | x | | | x |
| *Sphingomonas* sp. strain ATCC 31555 | x | x | x | x | | | |
| *Sphingomonas* sp. strain PAMC 26605 | x | x | | | | | x |
| *Sphingomonas* sp. strain PAMC 26617 | x | x | | | | | x |
| *Sphingomonas* sp. strain PAMC 26621 | x | x | | | | | |
| *Sphingomonas wittichii* DP58 | x | x | | x | x | | x |
| *Sphingomonas wittichii* RW1 | x | x | | x | x | | x |

Table S3. Total number of glycoside hydrolases, polysaccharide lyases, carbohydrate esterases, monooxygenases, and dioxygenases in the 26 sphingomonad genomes.

| Genome | Glycoside Hydrolases | Polysaccharide Lyases | Carbohydrate Esterases | Mono-oxygenases | Di-oxygenases |
|---|---|---|---|---|---|
| ***Novosphingobium*** | | | | | |
| *Novosphingobium aromaticivorans* DSM 12444 | 49 | 1 | 13 | 16 | 35 |
| *Novosphingobium nitrogenifigens* Y88$^{T}$ | 17 | | 7 | 13 | 14 |
| *Novosphingobium pentaromativorans* US6-1 | 43 | | 5 | 17 | 31 |
| *Novosphingobium* sp. strain AP12 | 56 | 1 | 16 | 25 | 23 |
| *Novosphingobium* sp. strain PP1Y | 60 | | 11 | 15 | 39 |
| *Novosphingobium* sp. strain Rr 2-17 | 44 | 1 | 16 | 11 | 15 |
| ***Sphingopyxis*** | | | | | |
| *Sphingopyxis alaskensis* RB2256 | 26 | 1 | 5 | 9 | 7 |
| ***Sphingobium*** | | | | | |
| *Sphingobium chlorophenolicum* L-1 | 34 | 2 | 11 | 15 | 19 |
| *Sphingobium indicum* B90A | 27 | 1 | 4 | 8 | 6 |
| *Sphingobium japonicum* UT26S | 42 | 1 | 5 | 13 | 8 |
| *Sphingobium* sp. strain AP49 | 87 | 5 | 14 | 12 | 13 |
| *Sphingobium* sp. strain SYK-6 | 22 | | 3 | 16 | 23 |
| *Sphingobium yanoikuyae* XLDN2-5 | 78 | 4 | 17 | 12 | 18 |
| ***Sphingomonas*** | | | | | |
| *Sphingomonas echinoides* ATCC 14820 | 51 | 1 | 16 | 6 | 5 |
| Sphingomonas elodea ATCC 31461 | 99 | 4 | 15 | 8 | 8 |
| *Sphingomonas* sp. strain KC8 | 21 | 1 | 7 | 10 | 11 |
| *Sphingomonas* sp. strain Mn802 | 30 | | 9 | 5 | 3 |
| *Sphingomonas* sp. strain PR090111-T3T-6A | 67 | 2 | 11 | 4 | 7 |
| *Sphingomonas* sp. strain S17 | 87 | | 9 | 9 | 5 |
| *Sphingomonas* sp. strain SKA58 | 53 | 2 | 6 | 7 | 6 |
| *Sphingomonas* sp. strain ATCC 31555 | 97 | 4 | 16 | 7 | 5 |
| *Sphingomonas* sp. strain PAMC 26605 | 75 | | 21 | 8 | 5 |
| *Sphingomonas* sp. strain PAMC 26617 | 70 | | 12 | 6 | 7 |
| *Sphingomonas* sp. strain PAMC 26621 | 62 | 1 | 13 | 7 | 6 |
| *Sphingomonas wittichii* DP58 | 20 | | 11 | 36 | 38 |
| *Sphingomonas wittichii* RW1 | 21 | | 12 | 43 | 46 |

## Supplementary References


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