Figure S2. Evolutionary relationships of OTUs derived from the OB and the IO biofilm cultures with the genera *Hyphomicrobium* and *Methylophaga*

An unrooted phylogenetic tree demonstrating the evolutionary relationship of 16S rRNA gene sequences (V6-V7-V8 regions) is illustrated. The evolutionary history was inferred using the Minimum Evolution method [1]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1.7). The ME tree was searched using the Close;Neighbor;Interchange (CNI) algorithm [4] at a search level of 1. The Neighbor;joining algorithm [5] was used to generate the initial tree. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 409 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [6]. Beside OTU names are the number of reads derived from the OB and the IO biofilm cultures, respectively, and the percentage of identity with *H. nitrativorans* NL23 16S sequence (Panel A) or with *M. nitratireducenticrescens* JAM1 16S sequence (Panel B). Beside the species names is the GenBank accession number.

Panel A: *Hyphomicrobium*
The optimal tree with the sum of branch length = 1.70056439 is shown. The analysis involved 46 nucleotide sequences.

Panel B: *Methylophaga*
The optimal tree with the sum of branch length = 1.56790826 is shown. The analysis involved 54 nucleotide sequences.

1. Rzhetsky A. and Nei M. (**1992**). A simple method for estimating and testing minimum evolution trees. *Molecular Biology and Evolution* **9**:945-967.
2. Felsenstein J. (**1985**). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**:783-791.
3. Tamura K., Nei M., and Kumar S. (**2004**). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) **101**:11030-11035.
4. Nei M. and Kumar S. (**2000**). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
5. Saitou N. and Nei M. (**1987**). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**:406-425.
6. Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (**2013**). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*30: 2725-2729.

A

OTU375 (21 – 0); 90.2%
78
26   OTU029 (1501 – 1); 92.2%   } Cluster 3
      OTU274 (15 – 50); 88.8%

      H. hollandicum^T IFAM KB;677; Y14303
78   H. sulfonivorans CT; AY468372
55   OTU713 (1288 – 2); 95.6%
35   OTU285 (64 – 0); 94.6%
4   OTU123 (2481 – 217); 97.3%
11   44   OTU357 (221 – 17); 95.6%
            OTU848 (383 – 126); 96.3%
31   54   OTU106 (195 – 20); 96.8%   Cluster 1
            27   OTU147 (70 – 21); 95.1%   H. nitrativorans
      11   6   OTU329 (39 – 0); 93.7%
            9   OTU706 (4993 – 819); 97.5%
                  OTU257 (22538 – 4232); 97.3%
            16   OTU214 (7237 – 1366); 97.5%
46   7   OTU004 (107700 – 19419); 100%
            90   H. nitrativorans^T NL23; JX131369
            39   H. zavarzinii^T; ZV;622 Y14305.1; 99.2% 3 subst

            OTU980 (23 – 0); 95.8%
      67   OTU353 (17 – 0); 91.2%
0   44   OTU102 (202 – 0); 91.7%
      15   OTU010 (5273 – 29); 94.1%
            Pedomicrobium australicum^T IFAM ST1306; X97693   Cluster 2
      2   H. vulgare
            OTU212 (5264 – 501); 97.0%
0   59   H. aestuarii DSM 1564; Y14304
      99   H. vulgare^T JCM 6889; AB543807

22   Filomicrobium insigne^T SLG5B;19; EF117253
            H. denitrificans^T DSM 1869; Y14308
      100   H. facile^T IFAM H-526; Y14309
      53   50   H. methylovorum^T DSM 5458; Y14307
            55   Hyphomicrobium sp. MC1; FN667863
            98   H. chloromethanicum^T CM2; AF198623

            Sphingomonas mali^T IFO 10550;T; Y09638
2   Aquabacter spiritensis^T DSM9035; FR733686
6   Nitratireductor aquibiodomus^T NL21; AF534573
24   Devosia riboflavina^T DSM 7230; AJ549086
      48   Pelagibacterium luteolum^T 1_C16_27; EF540455
      81   Cucumibacter marinus^T CL;GR60; EF211830
      97   Maritalea mobilis^T E6; EU255260
100
            Rhodomicrobium vannielii^T DSM 162; FN666247
54   Methylorhabdus multivorans^T DM13; AF004845
77   Prosthecomicrobium hirschii^T 16; HM037994

Burkholderia graminis^T C4D1M; U96939 (Betaproteobacteria)

Pseudomonas fluorescens^T IAM 12022; D84013   } Gammaproteobacteria
100   Pseudomonas putida^T IAM 1236; D84020

0.02

Hyphomicrobium

Alphaproteobacteria;Rhizobia; Hyphomicrobiaceae

B

- 98 — *Methylophaga* sp. Pbvc023; KT921330
- 56 — *Methylophaga* sp. SM14; KF790925
- 18 — *M. marina*ᵀ DSM 5989; X95459
- OTU282 (23 – 1); 95.3%
- 17 — *M. muralis*ᵀ Kr3; AY694421
- OTU634 (110 – 1); 96.3%
- 1
- 88 — OTU076 (210 – 0); 93.9%
- OTU068 (1 – 283); 94.9%
- 63 — *M. nitratireducenticrescens*ᵀ JAM1; CP003390
- OTU005 (10486 – 120034); 100%
- 36 — OTU826 (1055 – 11840); 99.2%
- 41 — OTU475 (9 – 52); 97.0%
- 33 — OTU273 (3 – 219); 97.3%
- 22 — 29 — OTU258 (139 – 1817); 97.0%
- OTU313 (9 – 212); 96.6%
- 38 — OTU538 (0 – 20); 95.4%
- 17 — OTU593 (0 – 35); 94.9%
- 10 — OTU299 (0 – 130); 95.1%
- 42 — OTU128 (12 – 211); 96.1%
- 96 — OTU408 (0 – 55); 93.7%

Cluster 1
M. nitratireducenticrescens

- OTU645 (19 – 78); 97.5%;
- 96 — OTU101 (2 – 1069); 93.2%
- 11 — 53 — OTU181 (52 – 85); 93.6%
- 50 — OTU247 (0 – 38); 93.6%
- 100 — OTU938 (0 – 24); 93.6%
- OTU085 (0 – 277); 92.9%

Cluster 2

- 5 — 2
- 98 — bacterium DS-4; JN003578
- *Methylophaga* sp. LMN; HQ268014
- bacterium SH3-2; JQ269254
- 3 — 19 — 95 — *Methylophaga* sp. R1; KT461669
- 58 — *M. thiooxydans*ᵀ DMS010; DQ660915
- 0 — 47 — 31 — *M. sulfidovorans*ᵀ RB-1 94-792; X95461
- bacterium SH6-1; JQ269264
- 47 — *Methylophaga* sp. AQMP1; JF794990
- 73 — *M. natronica* Bur2; AY128533
- 56 — *M. aminisulfidivorans*ᵀ MP; DQ463161
- 68 — *M. thalassica*ᵀ DSM5690; X95460
- *Methylophaga* sp. V4-ME-29; AJ244761
- 0 — *M. alcalica*ᵀ M39; AF384373
- 24 — *M. frappieri*ᵀ JAM7; CP003380
- 15 — *M. lonarensis*ᵀ MPL; JF330773
- 67 — *Methylophaga* sp. RS-MM3; KF371656
- OTU159 (115 – 4); 93.4%
- 49 — OTU206 (0 – 61); 94.1%

Cluster 3

Methylophaga

- 63 — *Enterobacter kobei*ᵀ CIP 105566; AJ508301
- 29 — *Cycloclasticus pugetii*ᵀ PS-1; L34955
- *Pseudomonas amygdali*ᵀ; LMG 2123T; Z76654
- 61 — *Piscirickettsia salmonis* SCO-02A; AY498635
- 47 — *Sulfurivirga caldicuralii*ᵀ MM1; AB245479
- *Thioalkalimicrobium aerophilum*ᵀ AL3; AF126548
- 62 — *Galenea microaerophila*ᵀ P2D; JQ080912
- 89 — *Thiomicrospira frisia*ᵀ JB-A2; AF013974
- 74 — 89 — *Hydrogenovibrio marinus*ᵀ JCM7688; MH-110; D86374
- 99 — *Thiomicrospira chilensis*ᵀ Ch-1; AF013975

0.02