

The following supplement accompanies the article

Isolates as models to study bacterial ecophysiology and biogeochemistry

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Supplementary Materials & Methods

The bacteria characterized in this study were collected from sites at three different sea areas; the Northern Baltic Sea (63°30'N, 19°48'E), Northwest Mediterranean Sea (43°41'N, 7°19'E) and Southern California Bight (32°53'N, 117°15'W). Seawater was spread onto Zobell agar plates or marine agar plates (DIFCO) and incubated at *in situ* temperature. Colonies were picked and plate-purified before being frozen in liquid medium with 20% glycerol. The collection represents aerobic heterotrophic bacteria from pelagic waters. Bacteria were grown in media according to their physiological needs of salinity. Isolates from the Baltic Sea were grown on Zobell media (ZoBELL, 1941) (800 ml filtered seawater from the Baltic, 200 ml Milli-Q water, 5g Bacto-peptone, 1g Bacto-yeast extract). Isolates from the Mediterranean Sea and the Southern California Bight were grown on marine agar or marine broth (DIFCO laboratories). The optimal temperature for growth was determined by growing each isolate in 4ml of appropriate media at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50° C with gentle shaking. Growth was measured by an increase in absorbance at 550nm.

Statistical analyses

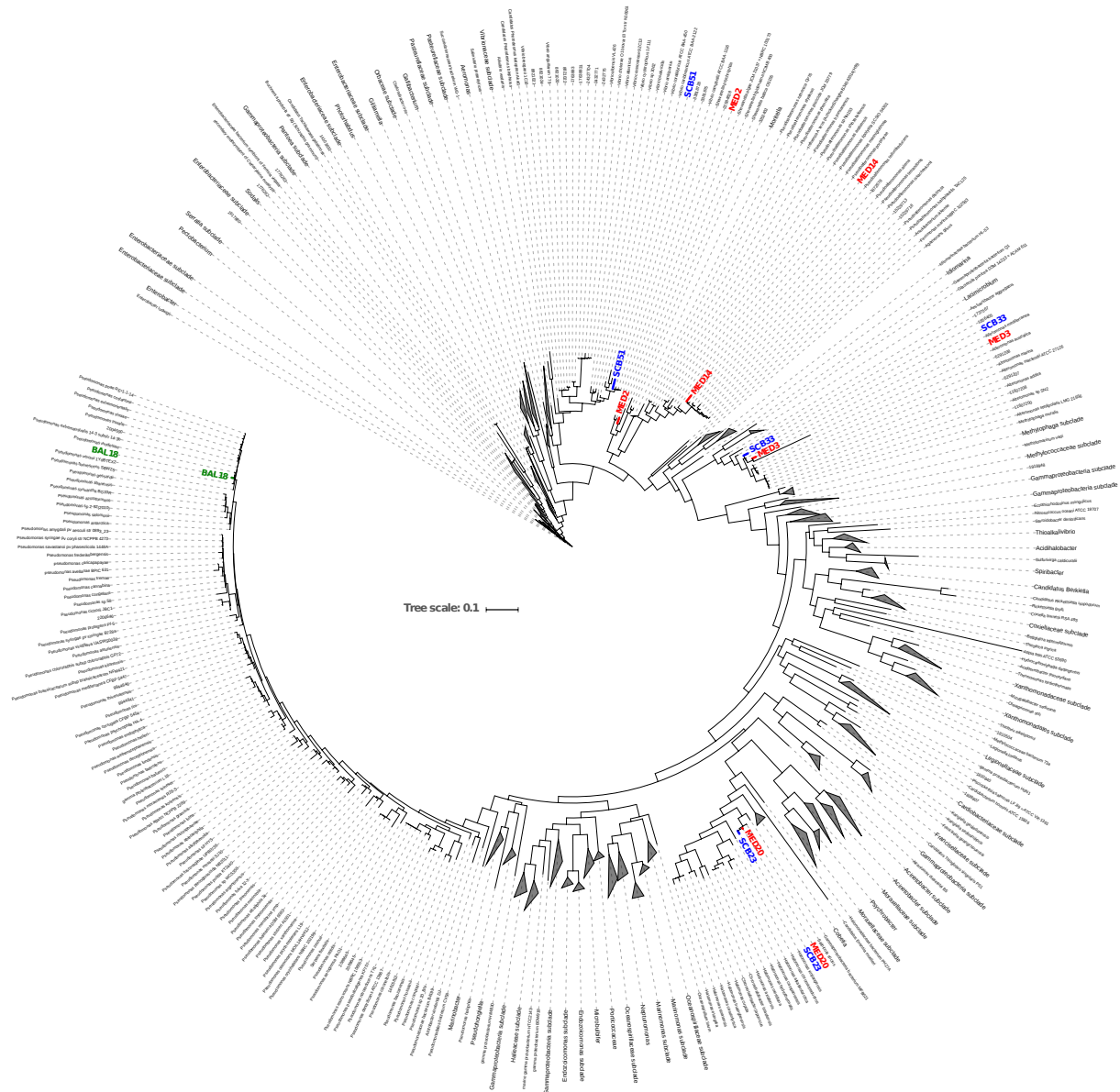
The influence of temperature, geographical origin and taxonomic affiliation on growth rates was assessed by a two-way analysis of variance (ANOVA) in R (<http://www.r-project.org/>) and the “car” package. Subsequently, a Tukey honest significant difference (HSD) test was performed to determine which groups are significantly different using the “agricolae” package. Plots were created in R using the “ggplot2” package Wickham H. ggplot2: Elegant Graphics for Data Analysis. New York: Springer; 2016.

Table S1 Growth rate and temperature characteristics for a number of bacterial isolates typically representing an opportunist life strategy.

Isolate name Accession # seq length	2016 similarity % Identity	2003 similarity % Identity	2016 /2003 Phylum	Motility	Temp. °C Min*, Opt, Max	Growth rate μ at Opt
MED2 AF025545440	97,7 NR_116537 <i>Shewanella corallii</i> strain fav-2-10-05 16S ribosomal RNA gene, partial sequence.	95,0 AF003549 <i>Shewanella woodyi</i> , Alboran sea	Gamma	Yes	10, 28, 34	0.82 h-1
MED3 AF025546436	96,9 NR_043977 <i>Alteromonas tagae</i> strain BCRC 17571 16S ribosomal RNA gene, partial sequence Taiwanese estuary.	98,0 Y18231; <i>Alteromonas macleodii</i> clone 1B161, Mediterranean Sea	Gamma	Yes	10, 25, 41	0.28 h-1
MED6 AF025549436	99,8 NR_025691 <i>Sulfitobacter dubius</i> strain KMM 3554 16S ribosomal RNA gene, partial sequence	99,0 AF359537; Marine bacterium SCRIPPS_101 (99%)	Alpha	Yes	ND	
MED9 AF025552435	97,9 NR_044260 <i>Salinimicrobium xinjiangense</i> strain BH206 16S ribosomal RNA gene, partial sequence	90,0 AF141552; Uncultured Cytophagales clone CRE-PA85 16S, columbia river, estuary, or adjacent coastal ocean	Bacterioidetes	No	5, 33, 41	0.44 h-1
MED10 AF025553427	94,9 NR_134725 <i>Flavirhabdus iliipiscaria</i> strain Th68 16S ribosomal RNA, partial sequence	97,0 AB015262 <i>Cytophaga</i> sp. strain JTB244, Sediments from the Japan Trench	Bacterioidetes	Yes	5, 29, 35	0.64 h-1
MED11 AF025554479	100,0 NR_025263 <i>Mesonia algae</i> strain KMM 3909 16S ribosomal RNA gene, partial sequence	99,0 AF367852; Bacterium KmC, Southern California Bight	Bacterioidetes	Yes	5, 29, 35	0.43 h-1
MED14 AF025557485	99,2 NR_025509 <i>Pseudoalteromonas agarivorans</i> strain DSM 14585 16S ribosomal RNA gene, partial sequence	99,0 AF261054; <i>Pseudoalteromonas</i> sp. SKA32, Skagerrak	Gamma	Yes	5, 20, 37	0.37 h-1
MED17 AF025560432	99,8 NR_025817 <i>Erythrobacter seohaensis</i> strain SW-135 16S ribosomal RNA gene, partial sequence	99,0 AF227259; <i>Erythrobacter citreus</i> , Mediterranean Sea	Alpha	No	ND	
MED20 AF025563475	99,2 NR_042490 <i>Salinicola salarius</i> strain M27 16S ribosomal RNA gene, partial sequence	98,0 U78786; <i>Haererehalobacter ostenderis</i>	Gamma	No	10, 32, 43	0.66 h-1
MED21 AF025564435	97,7 NR_043080 <i>Tenacibaculum lutimaris</i> strain TF-26 16S ribosomal RNA gene, partial sequence	98,0 U64023; <i>Flexibacter</i> sp., Southern California Bight	Bacterioidetes	No	ND	
MED25 AF025568400	94,9 NR_114025 <i>Gaetbulibacter marinus</i> strain NBRC 102040 16S ribosomal RNA gene, partial sequence	91,0 AF141547; Uncultured Cytophagales clone CRE-PA79, columbia river, estuary, or adjacent coastal ocean	Bacterioidetes	No	5, 29, 35	0.33 h-1
MED28 AF182015425	96,6 NR_025229 <i>Tenacibaculum skagerrakense</i> strain D30 16S ribosomal RNA gene, partial sequence	95,0 AF367855 Bacterium KHN36A, Southern California Bight	Bacterioidetes	No	10, 28, 39	0.52 h-1
SCB23 U64000 487	99,6 NR_042490 <i>Salinicola salarius</i> strain M27 16S ribosomal RNA gene, partial sequence	99,0 U78786 <i>Haererehalobacter ostenderis</i>	Gamma	No	15, 25, 46	0.37 h-1
SCB26 U64003 413	99,1 NR_025817 <i>Erythrobacter seohaensis</i> strain SW-135 16S ribosomal RNA gene, partial sequence	99,0 AF26106; <i>Agrobacterium</i> sp. SKA40 Skagerrak	Gamma	No	15, 30, 42	0.35 h-1
SCB32 U64009 431	99,5 NR_043936 <i>Sulfitobacter marinus</i> strain SW-265 16S ribosomal RNA gene, partial sequence	99,0 AF007257; <i>Sulfitobacter</i> sp. GAI-21	Alpha	Yes	5, 25, 40	0.2 h-1
SCB33 U64010 442	97,5 NR_042667 <i>Alteromonas genovensis</i> strain LMG 24078 16S ribosomal RNA gene, partial sequence	99,0 Y18232; <i>Alteromonas macleodii</i> strain CH-518, Mediterranean Sea	Gamma	Yes	5, 35, 44	1.2 h-1
SCB34 U64011 439	100,0 NR_025691 <i>Sulfitobacter dubius</i> strain KMM 3554 16S ribosomal RNA gene, partial sequence	99,0 AF025549; <i>Roseobacter</i> sp. MED6, Mediterranean Sea	Alpha	Yes	5, 38, 42	0.52 h-1
SCB36 U64013 500	99,6 NR_118846 <i>Winogradskyella rapida</i> strain SCB36 16S ribosomal RNA gene, partial sequence	98,0 AF125323; <i>Flexibacter</i> sp. D8 Southern California Bight	Bacterioidetes	No	5, 30, 39	0.58 h-1

Isolate name Accession # seq length	2016 similarity % Identity	2003 similarity % Identity	2016 /2003 Phylum	Motility	Temp. °C Min*, Opt, Max	Growth rate μ at Opt
SCB37 U64014 478	99,4 NR_113943 Dokdonia genika strain NBRC 100811 16S ribosomal RNA gene, partial sequence	92,0 AF141518; Uncultured Cytophagales clone CRE-PA37 , columbia river, estuary, or adjacent coastal ocean	Bacteriodes	No	5, 25, 35	0.54 h-1
SCB38 U64015 478	100,0 NR_044346 Joostella marina strain En5 16S ribosomal RNA gene, partial sequence	89,0 AB016515 Flavobacterium columnare	Bacteriodes	No	5, 31, 38	0.66 h-1
SCB40 U64017 490	99,4 NR_074656 Zunongwangia profunda strain SMA-87 16S ribosomal RNA gene, complete sequence	98,0 AJ002569; Marine bacterium (isolate DPT1.3), Ligurian Sea	Bacteriodes	No	5, 32, 43	0.51 h-1
SCB41 U64018 444	96,9 NR_042909 Muricauda aquimarina strain SW-63 16S ribosomal RNA gene, partial sequence	97,0 AF386740; Flavobacterium sp. 7BT	Bacteriodes	Yes	10, 30, 48	0.56 h-1
SCB46 U64023 436	96,5 NR_043080 Tenacibaculum lutimaris strain TF-26 16S ribosomal RNA gene, partial sequence	98,0 AF025564 Cytophagales str. MED21), Mediterrean Sea	Bacteriodes	No	10, 25, 29	0.41 h-1
SCB48 U64025 384	95,6 NR_104546 Ruegeria faecimaris strain HD-28 16S ribosomal RNA gene, partial sequence	95,0 AF359535; Marine bacterium ATAM407_56	Alpha	Yes	5, 29, 35	0.43 h-1
SCB49 U64026 388	93,6 NR_133883 Ulvibacter marinus strain IMCC12008 16S ribosomal RNA, partial sequence	87,0 AF125323 Flexibacter sp. D8), Southern California Bight	Bacteriodes	No	5, 28, 35	0.33 h-1
SCB51 AF02639 321	98,5 NR_114268 Vibrio azureus strain NBRC 104587 16S ribosomal RNA gene, partial sequence	99,0 AF025960 Vibrio campbellii (99%), Andaman Sea	Gamma	Yes	10, 25, 32	0.9 h-1
BAL3 U63935 418	99,5 NR_037108 Brevundimonas mediterranea strain V4.BO.10 16S ribosomal RNA gene, partial sequence	99,0 AJ227801 Brevundimonas sp., strain V4.BO.10	Alpha	Yes	5, 35, 46	0.48 h-1
BAL4 U63936 451	100,0 NR_025538 Flavobacterium gelidilacus strain R-8899 16S ribosomal RNA gene, partial sequence	99,0 AF321015 CFB group bacterium GOBB3-210	Bacteriodes	Yes	5, 18, 29	0.19 h-1
BAL5 U63937 409	97,9 NR_118315 Sphingobium baderi strain LL03 16S ribosomal RNA gene, partial sequence	98,0 AF270948; Uncultured eubacterium clone IAFR208, Soil	Alpha	Yes	10, 25, 36	0.27 h-1
BAL11 U63939 407	98,2 NR_109486 Sphingobium boeckii strain 469 16S ribosomal RNA gene, partial sequence	98,0 AF182026 Sphingomonas sp. BAL53, Baltic Sea	Alpha	Yes	5, 28, 38	0.24 h-1
BAL17 U63943 425	96,4 NR_113710 Flavobacterium hydatis strain NBRC 14958 16S ribosomal RNA gene, partial sequence	96,0 AJ252635; Agricultural soil bacterium clone SC-I-41,	Bacteriodes	No	5, 25, 35	0.36 h-1
BAL18 U63944 491	99,8 NR_114911 Pseudomonas extremaustralis strain 14-3 16S ribosomal RNA, complete sequence	99,0 AF313466 Pseudomonas veronii	Gamma	Yes	5, 25, 37	0.52 h-1
BAL22 U63946 419	96,0 NR_114254 Flavobacterium cheniae strain NBRC 103934 16S ribosomal RNA gene, partial sequence	99,0 U63954 Flavobacterium sp., Baltic Sea	Bacteriodes	No	5, 36, 46	1.75 h-1
BAL29 U63950 308	95,5 NR_025602 Algoriphagus aquimarinus strain LMG 21971 16S ribosomal RNA gene, partial sequence	95,0 AF182020;Flectobacillus sp. BAL49 , Baltic Sea	Bacteriodes	Yes	5, 25, 35	0.19 h-1
BAL44 U63958 339	98,0 NR_043014 Sphingopyxis baekryungensis strain SW-150 16S ribosomal RNA gene, partial sequence	96,0 AF173973; Sphingomonas subarctica, North Sea	Alpha	Yes	5, 15, 29	0.05 h-1
BAL46 U63960 396	97,0 NR_118315 Sphingobium baderi strain LL03 16S ribosomal RNA gene, partial sequence	97,0 U63937; Sphingomonas sp., Baltic	Alpha	Yes	15, 25, 37	0.2 h-1
BAL49 AF182020452	98,7 NR_025602 Algoriphagus aquimarinus strain LMG 21971 16S ribosomal RNA gene, partial sequence	95,0 U85891; Marine psychrophile IC025 , Antarctic Sea ice	Bacteriodes	Yes	5, 25, 35	0.22 h-1
BAL50 AF182021402	95,5 NR_043481 Flavobacterium saliperosum strain AS 1.3801 16S ribosomal RNA gene, partial sequence	94,0 AF361197 Uncultured Cytophagales bacterium clone 15 Potential	Bacteriodes	Yes	5, 25, 35	0.36 h-1

FIG. S1. Phylogenetic tree of 16S rRNA gene sequences showing the placement of the isolates among all genome-sequenced Gammaproteobacteria in RefSeq. 16S rRNA sequences were retrieved from RefSeq using the biomart package (Drost & Paszkowski, 2017) for R (R Core Team, <https://www.R-project.org>). Alignments were made in muscle v3.8.31 (Edgar, 2004), the tree was constructed using FastTree v2.1.8 (Price et al. 2010) and visualized in iTOL (Letunic & Bork, 2016). Taxonomy was assigned in iTOL using the NCBI taxids. Branches are collapsed to broader groups to facilitate better overview.



References

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