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SI Methods

Transporters. The low number of transporters was apparent in most of the main transporters families. Thus, together with *Pelagibacter ubique*, MED152 has the lowest number of ATPdependent transporters [a majority belonging to the ATPbinding Cassette (ABC) superfamily] of the marine bacteria sequenced so far (Table 1). The same holds true for the secondary transporters, which account for 50–70% of the transporters in the marine bacterial genomes sequenced until now. MED152 has only a total of 62 secondary transporters – similar to the number found in *Pelagibacter ubique*– while other bacteria may have from 100–200. In particular, the number of transporters in the drug/metabolite transporter (DMT) superfamily and in the major facilitator superfamily [MFS; used for transport of a diverse set of ''small solutes in response to chemiosmotic ion gradients'' (1)] was low, while the number of multidrug/ oligosaccharidyl-lipid/polysaccharide (MOP) flippase superfamily transporters was similar to that in other marine bacteria (despite its smaller genome).

Like *Pelagibacter ubique*, MED152 (and other marine flavobacteria) lacks phosphotransferase system (PTS) genes for active transport of sugars. PTS genes are present in all major bacterial groups except cyanobacteria and epsilonproteobacteria (2). The lack thereof is consistent with the aerobic lifestyle of MED152 (PTS are particularly important for sugar uptake under anaerobic conditions).

Comparative Analysis of Transporters. Transporter families in MED152 were compared to the following marine genomes in the TransportDB database (details on transporter families and substrate specificity of particular transporters in specific bacteria are available at www.membranetransport.org): alphaproteobacteria *Bradyrhizobium japonicum* USDA 110, ''*Candidatus* Pelagibacter ubique'' HTCC1062, *Caulobacter crescentus* CB15, *Hyphomonas neptunium* ATCC 15444, *Jannaschia* sp. CCS1, *Loktanella vestfoldensis* SKA53, *Maricaulis maris* MCS10, *Roseobacter* sp. MED193, *Roseobacter* sp. TM1040, *Roseovarius nubinhibens* ISM, and *Silicibacter pomeroyi* DSS-3; gammaproteobacteria *Aeromonas hydrophila* subsp. *hydrophila* ATCC 7966, *E. coli* K12-MG1655, *Marinomonas* sp. MED121, *Photobacterium profundum* SS9, *Pseudoalteromonas haloplanktis* TAC125, *Pseudomonas aeruginosa* PAO1, *Shewanella oneidensis* MR-1, and *Vibrio* sp. MED222; Bacteroidetes/Chlorobi phylum *Bacteroides fragilis* NCTC9343, *Bacteroides fragilis* YCH46, *Bacteroides*

- 1. Pao SS, Paulsen IT, Saier MH (1998) Major facilitator superfamily. *Microbiol Mol Biol Rev* 62:1–34.
- 2, Barabote RD, Saier MH (2005) Comparative genomic analyses of the bacterial phosphotransferase system. *Microbiol Mol Biol Rev* 69:608–634.
- 3. Parsons TR, Maita Y, Lalli CM (1984) *A Manual of Chemical and Biological Methods for Seawater Analysis* (Pergamon Press, Oxford, UK).
- 4. Latasa M, *et al.* (2001) Losses of chlorophylls and carotenoids in aqueous acetone and methanol extracts prepared for RPHPLC analysis of pigments. *Chromatographia* 53:385–391.

thetaiotaomicron VPI-5482, *Chlorobium tepidum* TLS, *Dokdonia* sp. MED134, *Leeuwenhoekiella blandensis* MED217, and *Polaribacter* sp. MED152; and Firmicutes *Bacillus subtilis* 168.

Bicarbonate Uptake. For the bicarbonate uptake experiment, MED152 was grown at 16°C in Marine Broth (Difco) diluted 1:8 with artificial seawater (35 practical salinity units, prepared from Sea Salts; Sigma) in light (180 μ mol photons m⁻² s⁻¹) or dark conditions (duplicate 200 ml cultures in each condition). Cultures were maintained without shaking to reduce aggregation and flock formation. After 50 h incubation (during exponential growth), four subsamples from one culture of each incubation condition (light and dark) were transferred to 25 ml glass bottles. From each original incubation condition, two subsamples (treated as duplicates) were placed in transparent bottles and two subsamples were placed in dark bottles (bottles covered with aluminum foil and black plastic). All bottles were then incubated under white light (180 μ mol photons m⁻² s⁻¹; no light entered the dark bottles) for 2 h with 20 μ l of H¹⁴CO₃⁻ (3 μ Ci; DHI). Controls from each treatment were treated with 10% trichloroacetic acid (final concentration). After incubation, 3 ml aliquots from each replicate bottle were filtered through $0.2 \mu m$ pore size filters (25 mm diameter, Supor-200, Pall), and the filters were exposed to HCl 0.7 M fumes for 2 h. Finally, the filters were placed in vials with 10 ml scintillation mixture (Perkin–Elmer) and kept at least 24 h in the dark before counting. Bicarbonate uptake rates were calculated according to the standard radioactive carbon assimilation technique procedure (3). At the time of the experiment, bacterial abundance was $\approx 5.1 \times 10^8$ cells ml^{-1} , as determined by epifluorescence microscopy of SYBR Gold stained cells.

Pigment Analysis. MED152 was grown in Marine Broth 2216 (Difco) and filtered onto Whatman GF/F filters. Pigments were extracted by placing the filters in 3 ml of 90% acetone (with 0.01% of butylated hydroxytoluene to prevent chlorophyll allomerization) and vortexing them vigorously for 45 s. After 24 h at -20°C, samples were sonicated for 1 min and vortexed again for 45 s. The extracts were cleared by filtration through Poretics 0.8 μ m polycarbonate filters. For pigment chromatography, 150 μ l of a mixture of 0.5 ml extract plus 0.1 ml H₂O was injected into a Thermo HPLC system and run under the conditions described in ref. 4. Standards of myxoxanthophyll, zeaxanthin and β -carotene (DHI) were used for pigment identification and quantification.

- 5. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positionspecific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.
- 6. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540–552.
- 7. Felsenstein J (1989) PHYLIP—Phylogeny Inference Package (Version 3.2). *Cladistics* 5:164–166.

Fig. S1. Glycolysis/gluconeogenesis.

AS

IAS

Fig. S3. TCA cycle and glyoxylate shunt.

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Fig. S4. Proposed carbon flow in MED152 in the presence and absence of light. When the cell is exposed to light (*A*), the prevailing role of the main metabolic pathways is biosynthetic. In the dark (*B*), the cell must respire more organic carbon than in the light to meet its energy requirements.

SVNAC

 \boldsymbol{S}

Fig. S6. Evolutionary relationships of cryptochrome/photolyase protein family from the marine Bacteroidetes and representatives from other organisms. Subfamilies are indicated on the right. A multiple alignment was constructed with the software package CLUSTAL W 1.74 (5). The alignment was edited with Gblocks (Version 0.91b) to identify conserved regions (6) with a minimum block of ten and without gaps. The tree was constructed based on a Kimura's distance matrix and the Neighbor-Joining method using the PHYLIP package (Version 3.2) (7). The statistical significance of the tree topology was evaluated by bootstrap analysis with 1000 iterative constructions of the neighbor-joining tree. The numbers at the nodes are bootstrap values higher than 50%. The scale barrepresents the Kimura distance.

SVNG

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A

0.05

Fig. S8. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of MED152 and closely related type strains for each species. A multiple alignment was constructed with the software package CLUSTAL W (Version 1.83; ref. 5). The alignment was edited with Gblocks (Version 0.91b) to identify conserved regions (6) with a minimum block of 10 and without gaps. The tree was constructed based on a Jukes-Cantor distance matrix and the Neighbor-Joining method using the PHYLIP package (Version 3.2) (7). The sequence of *Cytophaga hutchinsonii* (M58768) served as the outgroup. Bootstrap values greater than 70% confidence are shown at branching points (percentage of 1,000 resamplings). GenBank accession numbers are given in parentheses. The scale bar represents Jukes-Cantor distance.

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Table S1. Summary of MED152 genome

Table S2. Key enzymes and metabolic pathways identified in MED152

Pathway/enzyme (gene copy when greater than one) ORFs

Main pathways Pyruvate carboxylase. Anaplerotic pathway MED15204060 PEP carboxylase. Anaplerotic pathway and the state of the MED152_09950 MED152_09950

Glyoxylate bypass. Anaplerotic pathway and the state of the MED152_00580 Glyoxylate bypass. Anaplerotic pathway MED15200580 (*aceA*), MED15200575 (*aceB*) Malic enzyme. Anaplerotic pathway MED15206685 Phosphate acetyltransferase. Acetate metabolism MED152005555 Acetate kinase. Acetate metabolism

Acetate CoA ligase. Acetate metabolism

MED152_13179 Acetate CoA ligase. Acetate metabolism Acyl-CoA dehydrogenase. β -Oxidation (8) MED152_01705, MED152_04870, MED152_05285, MED152_05295, MED152_05785, MED152_07525, MED152_07535, MED152_10560 Enoyl-CoA hydratase. β -Oxidation (4) MED152_05285, MED152_06790, MED152_09440, MED152_11649 Electron transfer protein. β -Oxidation MED152_11949 (*etfA*), MED152_11944 (*etfB*) Glycerol kinase. Lipid degradation
Glycerol-3-phosphate dehydrogenase (NAD(P)⁺). Lipid degradation (2) MED152_05430, MED152_08715 Glycerol-3-phosphate dehydrogenase (NAD(P)⁺). Lipid degradation (2) MED152.05430, MED15205430, MED15205430, MED15208690 FAD-linked glycerol-3-phosphate dehydrogenase. Lipid degradation Glycogen biosynthesis Glycogen biosynthesis MED15205855 (*glgA*), MED15205860 (*glgC*), MED15205865, MED152_05870, MED152_05875 (glgB), MED152_05880, MFD15205885 **Electron transport chain** Na-translocating NADH:quinone oxidoreductase MED15211704 (*nqrA*), MED15211709 (*nqrB*), MED15211714 (*nqrC*), MED15211719 (*nqrD*), MED15211724 (*nqrE*), MED15211729 (*nqrF*) Type II NADH dehydrogenase (FAD-dependent)
Succinate dehydrogenase Succinate dehydrogenase MED15208110 (*sdhA*), MED15208115 (*sdhB*), MED15208105 (*sdhC*) Cytochrome c oxidase MED15210275 (*coxM*), MED15210280 (*coxN*), MED15206775 (*coxO*), MED15206770 (*coxP*) Cytochrome oxidase, cbb3 type MED15203190 (*ccoNO*), MED15203195 (*ccoS*) Cytochrome c class I MED15210265 Cytochrome c
Cytochrome c assembly protein and the MED15210245 cytochrome c assembly protein and the MED152 of SAS20 Cytochrome c assembly protein Cytochrome oxidase assembly protein and the material method of the MED152.06660 MED152.06660 Cytochrome oxidase assembly factor Cytochrome oxidase assembly factor 60 KD inner membrane protein OxaA homolog
SenC (3) MED152_03785 MED152_03785, MED152_06550, MED152_06755 **Nitrogen assimilation** Glutamate synthase **MED152_05815** (*gltB*), MED152_05820 (*gltD*) Ammonium channel (*amtB*) (2)

Nitrogen regulatory protein P-II MED15205805

MED15205805 Nitrogen regulatory protein P-II and the state of the MED152.05805

Slutamine synthetase type II and the state of the MED152.05925 Glutamine synthetase, type II and the synthetise of the MED152.05925
Glutamine synthetase, type III and the synthetise of the MED152.05920 Glutamine synthetase, type III and the synthetic syn Glutamate dehydrogenase **Phosphate metabolism** Phosphate permease and the set of the matrix of the MED152.09510
Polyphosphate kinase (3) The matrix of the matrix of the MED152.00300, MED152_00300, MED152_00305, MED152_05065 Exopolyphosphatase MED152_04590 H⁺-translocating pyrophosphatase MED152_11924 Soluble pyrophosphatase MED152_11929 **Sulfur assimilation** APS/PAPS pathway MED15206170 (*cysD*), MED15206175 (*cysH*), MED15206165 (*cysN*), MED15206160 (*cysI*), MED15209765 (*cysJ*), MED15200895 (*cysK*), MED15206130 (*cysM*), MED15206135 (*cysE*) Sulphate permease (3) **MED152_09030, MED152_10290, MED152_12914 Iron assimilation** Fe³⁺-binding periplasmic protein (2) and the state of the MED15201595, MED15211574 ABC-type Fe³⁺-siderophore transporter (2) MED15200690, MED15200690, MED15200695
MED15203840, MED15211859 Mn^{2+}/Fe^{2+} transporter, NRAMP family (2) Ferritin-like protein (2) The state of the state of the MED15201695, MED15203075 ATP-dependent Fe²⁺ transport system FeoAB MED15206540, MED15206540, MED15206545
Fur (2) MED15201940 MED152_00170, MED152_01940 Repressor MED152_10760 **Bicarbonate uptake** SulP type transporter (BicA) and the state of the state of the MED152_09030 MED152_09030 SbtA MED15203855

The following additional pathways were complete and are not shown on the table: glycolysis, gluconeogenesis, TCA cycle, pentose phosphate pathway, purine biosynthesis and salvage, pyrimidine biosynthesis and salvage, thimidylate biosynthesis, amino acid metabolism, fatty acid biosynthesis, NAD biosynthesis, riboflavin and FAD biosynthesis, siroheme biosynthesis, quinone biosynthesis, H⁺-ATPase, pyridoxal phosphate biosynthesis, pantothenate and CoA biosynthesis. Complete cobalamine, biotin and thiamine biosynthetic pathways were not found. Entner–Doudoroff pathway is incomplete.

Carbonic anhydrase

Table S3. Key stress response-related genes in MED152

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ORFs with an asterisk also appear in Table S8 showing transporters.

Table S4. Genes coding for replication, repair, and recombination mechanisms in MED152

Table S5. Distribution of the anaplerotic enzymes pyruvate and PEP carboxylases, as well as carbonate anhydrase in cultured marine heterotrophic bacteria that contain the proteorhodopsin gene

The presence of the proteorhodopsin gene is based on hits against PF01036. Further, only those peptides that contained an Asp85 and Asp96, or a different carboxylate residue, were considered (Asp85 and Asp96 functions as the H⁺ acceptor and donor, respectively, during the rhodopsin photocycle in the H⁺ pump type of rhodopsins). Number of pluses indicates the number of genes with the same putative function. Pyruvate carboxylase (EC 6.4.1.1) catalyzes the following reaction: pyruvate + ATP + HCO₃ <=> ADP + phosphate + oxaloacetate. PEP carboxylase (EC 4.1.1.31) catalyzes the reaction: PEP + HCO₃ + H₂O <=> phosphate + oxaloacetate. Carbonate anhydrase (EC 4.2.1.1) catalyzes the reaction: $HCO_3^- \leq >> CO_2 + H_2O$.

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Table S6. Domains and peptides with a putative role in light absorption and response

Table S7. Distribution of putative light sensors in proteorhodopsin-containing marine bacteria (as defined in Table S5)

Presence of light sensors are predicted by hits to specific PFAMs or TIGRFAMs. DNA photolyase/cryptochrome subfamilies are based on phylogenetic analysis as in Fig. S6, as well as on custom-built PFAMs. Of 80 Moore genomes, 30 contain at least one BLUF domain, 18 contain at least one phytochrome domain, and 12 contain three cryptochrome/photolyase peptides (most contain one or two and no other contain four cryptochrome/photolyase peptides).

Table S8. Transporters identified in the genome of MED152

Table S9. Genes and domains with a potential role in adhesion

