LETTERS

SAR11 marine bacteria require exogenous reduced sulphur for growth

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Sulphur is a universally required cell nutrient found in two amino acids and other small organic molecules. All aerobic marine bacteria are known to use assimilatory sulphate reduction to supply sulphur for biosynthesis, although many can assimilate sulphur from organic compounds that contain reduced sulphur atoms[1–3]. An analysis of three complete '*Candidatus* Pelagibacter ubique' genomes, and public ocean metagenomic data sets, suggested that members of the ubiquitous and abundant SAR11 alphaproteobacterial clade are deficient in assimilatory sulphate reduction genes. Here we show that SAR11 requires exogenous sources of reduced sulphur, such as methionine or 3-dimethylsulphoniopropionate (DMSP) for growth. Titrations of the algal osmolyte DMSP in seawater medium containing all other macronutrients in excess showed that 1.5×10^8 SAR11 cells are produced per nanomole of DMSP. Although it has been shown that other marine alphaproteobacteria use sulphur from DMSP in preference to sulphate[1,2], our results indicate that '*Cand.* P. ubique' relies exclusively on reduced sulphur compounds that originate from other plankton.

SAR11 is the most abundant and ubiquitous clade of heterotrophic marine bacteria in the oceans, often comprising 30% of the surface bacterial plankton community[4]. '*Cand.* P. ubique' strain HTCC1062, as well as other cultivated strains of SAR11, consistently grow to about 1.12×10^6 cells ml^{-1} ($n = 41$, s.d. $\pm 4.04 \times 10^5$, range 2.5×10^5 to 3.5×10^6 cells ml^{-1}) on natural, autoclaved sea water amended with ammonium, phosphate and various organic carbon compounds, under dark or light conditions. These cell densities are similar to SAR11 population densities observed in coastal Oregon sea water, suggesting that, when growing in seawater culture, SAR11 strains are limited by compounds found naturally in the environment. Iron is also thought to sometimes limit biomass production in marine environments[5], but the addition of 53.6 μM $FeCl_3$ did not affect '*Cand.* P. ubique' yields (1.22×10^6 cells ml^{-1}, $n = 2$, s.d. $\pm 3.89 \times 10^5$), nor did additions of 18 trace elements used in culturing marine bacteria[6] (data not shown).

The reconstruction of metabolic pathways from complete genome sequences of two '*Cand.* P. ubique' strains, HTCC1002 and HTCC1062, revealed an apparently incomplete set of genes for assimilatory sulphate reduction in both genomes, suggesting that SAR11 might require reduced sulphur compounds for growth. The canonical assimilatory reduction pathway for *Escherichia coli* shown in Fig. 1a consists of *cysDNCHIJ*, which occurs in two operons (Fig. 1b). Variants of the canonical pathway exist. *Corynebacterium*

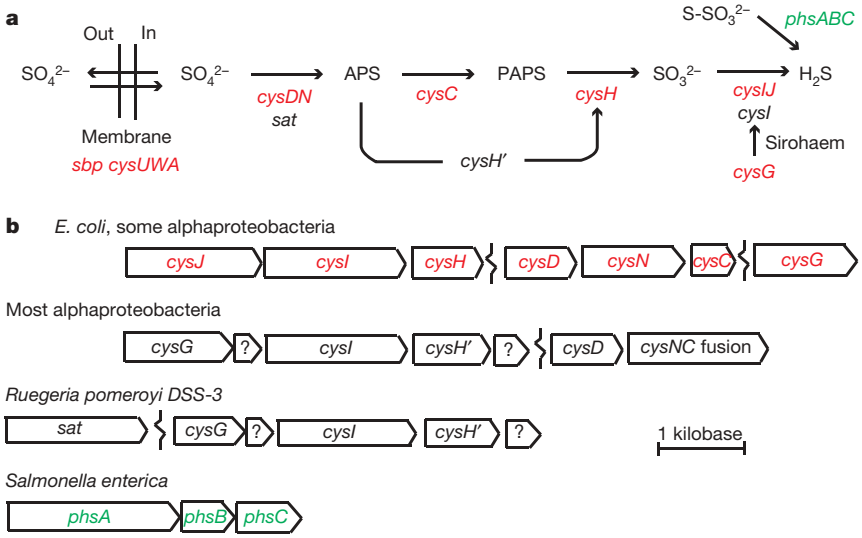

Figure 1 | Sulphur metabolism pathways and comparative genomics. **a**, Pathways of sulphate transport, assimilatory sulphate reduction, thiosulphate reduction and sulphite oxidation. *E. coli* genes for canonical assimilatory sulphate reduction and sulphate transport are shown in red, alternative assimilatory sulphate reduction genes are shown in black, *Salmonella* genes for canonical thiosulphate reduction are shown in green. APS, adenosine 5′-phosphosulphate; PAPS, 3′-phosphoadenosine 5′-phosphosulphate. **b**, Operon organization of assimilatory sulphate reduction pathway from selected organisms.

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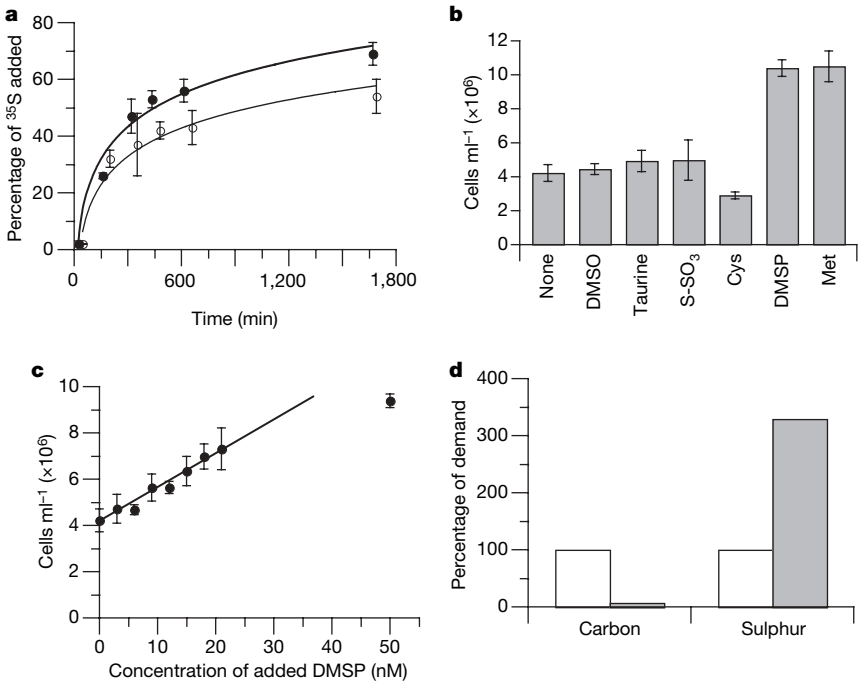

Figure 2 | ^{35}S radiotracer uptake and growth responses to additions of sulphur compounds. **a**, Incorporation of ^{35}S from ^{35}S[DMSP] into protein. Filled symbols, ^{35}S uptake ($R^2 = 0.9734$); open symbols, ^{35}S incorporation into trichloroacetic acid-insoluble fraction ($R^2 = 0.9586$). **b**, Maximum cell density for additions of 100 nM sulphur compounds. Control is sea water and excess carbon, nitrogen, phosphorus, iron and vitamins. The sulphur compounds are shown in order from most oxidized to most reduced. Cysteine and thiosulphate results varied depending on the batch of sea water, with cysteine showing a slight inhibition in this experiment. **c**, Molar growth yield for DMSP with other nutrients in excess. The regression equation is $y = 150{,}797x + (4 \times 10^6)$; $R^2 = 0.9683$. **d**, Calculated carbon and sulphur supply from 1 nM DMSP enrichment compared with the biomass demand, as described in the text. Error bars in **a**–**c** show s.d. for triplicate samples.

and *Allochromatium* do not seem to need *cysJ*[7,8], nor apparently do most alphaproteobacteria, on the basis of alignments viewed in MicrobesOnline[9]. *Bacillus subtilis* lacks the adenylylsulphate kinase gene *cysC*[10], and uses '*sat*' genes instead of *cysDN*, an arrangement also observed in the marine alphaproteobacterium *Ruegeria* (formerly *Silicibacter*) *pomeroyi* DSS-3. *Salmonella enterica* can reduce thiosulphate to sulphide under anaerobic conditions, using *phsABC*[11]. A tBLASTx search of relevant nucleotide sequences from *Salmonella*, *Ruegeria*, *Caulobacter crescentus* and *E. coli* against HTCC1062 and HTCC1002 revealed no putative genes for assimilatory sulphate reduction (see Supplementary Information). On the basis of this evidence, we predicted that SAR11 was deficient in assimilatory sulphate reduction.

Because multiple transporters and putative degradation pathways were found for DMSP and methionine in the SAR11 genome (see Supplementary Information), we proposed that DMSP or methionine might serve as reduced sulphur sources, and designed experiments to test these hypotheses. DMSP uptake in natural SAR11 populations has been established previously by microradiography[12], and DMSP demethylation activity has been demonstrated in cloned SAR11 genes[13]. Bacteria possessing the gene for DMSP demethylation (*dmdA*) demethylate DMSP and use the remaining methanethiol group for methionine biosynthesis[1]. Bacteria possessing the marker gene for the DMSP lyase pathway (*dddD*) cleave DMSP to form 3-hydroxypropionate and dimethylsulphide (DMS), a volatile gas that oxidizes to products that cause clouds to form[14]. It is estimated that 50–90% of the DMSP metabolized by marine microorganisms is channelled into the demethylation pathway, thereby mitigating the impact of DMSP on climate[13]. On a global basis, the SAR11 clade is likely to be responsible for a significant fraction of the uptake and demethylation of DMSP because of its abundance and ubiquity in the oceans.

'*Cand.* P. ubique' cells readily incorporated ^{35}S from ^{35}S[DMSP] into cellular protein (Fig. 2a). The percentages of the added ^{35}S that were taken up (70%) and incorporated (50%) are similar to the highest values reported previously[15] (63% and 55%) for subnanomolar additions of DMSP to natural plankton communities.

Additions of 100 nM DMSP or methionine, with all other nutrients in excess, resulted in cell densities of just over 10^7 cells ml^{-1} ($n = 3$, shown in Fig. 2b), indicating that these compounds could relieve nutrient limitation. The response to additions of different concentrations of cysteine and thiosulphate to media collected on different dates was variable, as a result of factors that are not understood. The addition of cysteine sometimes increased yield (Fig. 3), but in other experiments with different batches of sea water it did not (Fig. 2b). A pathway for the conversion of cysteine to methionine

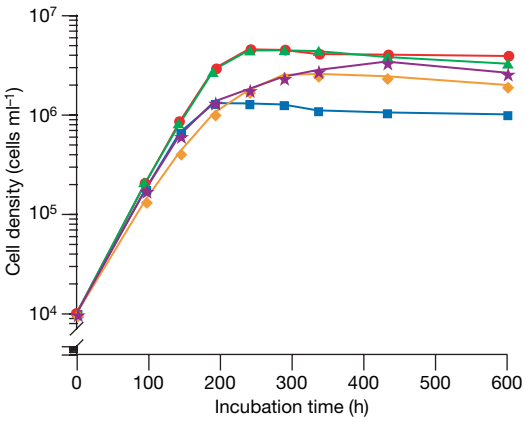

Figure 3 | Growth curves for 21-nM additions of various reduced sulphur sources. Data points show averages of duplicate measurements from two treatments. The control was sea water to which an excess of defined organic carbon compounds, nitrogen, phosphorus, iron and vitamins had been added. Blue, control; orange, cysteine; purple, thiosulphate; green, methionine; red, DMSP.

Table 1 | Other aerobes that may be deficient in assimilatory sulphate reduction

Organism	Phylum	Class	Genome size* (kbp)	Habitat	Phenotype
Halobacterium NRC-1	Euryarchaeota	Halobacteria	2,571	Ponds, aquatic	Aerobe, chemo-organotroph, motile, halophile, rod-shaped
Picrophilus torridus	Euryarchaeota	Thermoplasma	1,545	Soil	Acidophile, thermophile, aerobe, heterotroph
Idiomarina iloihiensis	Proteobacteria	Gamma	2,839	Aquatic, hydrothermal vent	Halophile, rod-shaped, aerobe

* From Genomes OnLine Database (GOLD)[30]. kbp, kilobase pairs.

could not be confirmed from the genome annotation of '*Cand.* P. ubique' strains HTCC1062 and HTCC1002, suggesting that cysteine might not be able to supply the cell's full requirement for reduced sulphur (see Supplementary Information). Regardless of sulphur source (DMSP, methionine, cysteine or thiosulphate), the growth rate during the exponential phase ranged from 0.62 to 0.74 d^{-1}, in comparison with 0.70 d^{-1} for the control (Fig. 3). Extension of the exponential phase resulted in increased yields ranging from 2.56×10^6 cells ml^{-1} to 4.52×10^6 cells ml^{-1}, in contrast with 1.3×10^6 cells ml^{-1} for the control.

The yield of SAR11 cells as a function of DMSP concentration in the presence of vitamins, excess organic carbon and excess inorganic nitrogen, phosphorus and iron is reported in Fig. 2c. As shown by the slope of the linear regression, the maximum cell density increased by 1.5×10^5 cells ml^{-1} per 1 nM increase in DMSP concentration. Measurements of DMSP in the autoclaved seawater medium ranged from 0.3 to 0.7 nM, indicating that DMSP can support population sizes of 4.5×10^4 to 1.05×10^5 cells ml^{-1}. We therefore infer that SAR11 cells cultured in autoclaved sea water are likely to be using sulphur compounds in addition to DMSP to supply their sulphur requirement.

To ensure that sulphur, not carbon, from DMSP was responsible for the increase in biomass, we estimated biomass demands from a recent measurement of the '*Cand.* P. ubique' cell volume obtained by cryoelectron tomography[16]. Assuming a density of 1 g cm^{-3}, carbon as half the dry weight and a sulphur/carbon ratio of 1:90 (ref. 2), the carbon mass for a cell of 0.035 μm^3 is 5.8 fg and the sulphur mass per cell is 65 ag (65×10^{-18} g). Each 1 nM increase in DMSP concentration yielded an additional 1.5×10^5 cells ml^{-1}, or an additional 1.5×10^8 cells l^{-1}. Although the medium contained an excess of added organic carbon, we estimated carbon and sulphur demand per unit of cell biomass compared with the supply of these elements provided by the added DMSP. DMSP sulphur was more than sufficient (329%) to meet the estimated sulphur demand, but the carbon supplied by the added DMSP fell well short of the estimated carbon demand for biomass (7%), as shown in Fig. 2d. Therefore the carbon in DMSP could not have been solely responsible for the observed increase in cell yield. Excess consumption of sulphur relative to the calculated demand has been reported from studies of natural populations of marine bacteria[15,17,18] and may be due to variances in carbon-to-sulphur ratios or methods of measurement. Cell morphology and size were consistent in all media and stages of growth, up to the maximum cell density (see Supplementary Information).

The SAR11 clade is a diverse group, raising the question of whether a nutritional requirement for organic compounds containing reduced sulphur is a common feature of this clade. To address this, we searched metagenomic DNA from the Global Ocean Survey for the *cysDNCHIJ*, *sat* and *phsABC* nucleotide sequences mentioned above by using tBLASTx and a bit score cutoff of 200. This comprehensive list of all the fragments in the metagenome containing assimilatory sulphate reduction genes was then searched for any gene with a best hit to an HTCC1062 gene. Because many of the random DNA fragments in metagenomic data encode regions of two or more genes, in principle this procedure should detect instances of the insertion of *cysDNCHIJ*, *sat* and *phsABC* into the context of SAR11 genes known from the available complete genome sequences. Only one fragment, which contained a *Caulobacter cysD* gene, was found adjacent to a gene having a best hit to an HTCC1062 gene. Additional support for the conclusion that assimilatory sulphate reduction is deficient in many SAR11 populations comes from the genome sequence of a newly isolated Sargasso Sea strain of SAR11 that also has no conserved domain sequences with significant BLAST hits to canonical assimilatory sulphate reduction genes (U. Stingl, personal communication). These data strongly suggest a requirement for exogenous reduced organic sulphur that is widespread, if not universal, within the SAR11 clade.

The absence of assimilatory sulphate reduction has been reported in anaerobic photoautotrophs from the green sulphur[19], purple nonsulphur[20] and purple sulphur[21] bacteria, and in methanogenic archaea[22], all of which live in sulphide-rich environments. The loss of assimilatory sulphate reduction in pathogens was shown convincingly in the endosymbiont *Buchnera aphidicola*[23]. We searched complete genome sequences for other free-living aerobes that might also be deficient for assimilatory sulphate reduction and found two archaea and one bacterium that were missing essential genes (Table 1). Hou *et al.*[24] could not find canonical assimilatory sulphate reduction genes in the free-living aerobic marine gammaproteobacterium *Idiomarina iloihiensis*, and listed four small subunits of dissimilatory sulphate reduction genes as candidates for performing this function.

We previously reported that SAR11 has the smallest genome known for a free-living heterotrophic cell and has a low proportion of non-coding DNA[25]. These observations were attributed to the genome-streamlining hypothesis, which postulates that in very large microbial populations in which nitrogen and phosphorus are often limiting resources, selection efficiently eliminates DNA that has no function. Here we show that the absence of a conserved metabolic pathway that is not essential for autonomous replication in sea water is also a factor that contributes to the small genome size of '*Cand.* P. ubique'. Assimilatory sulphate reduction is nearly universal in aerobic marine bacteria, probably because sulphate is found at saturating concentrations in sea water, and the assimilatory sulphate reduction pathway ensures that cells will have a supply of sulphur for biosynthesis. However, the double negative charge on sulphate makes it more expensive to import, and it requires eight electrons for reduction to sulphide. Presumably, during the evolution of the SAR11 clade the loss of nutritional autonomy associated with the elimination of assimilatory sulphate reduction was offset by the advantages of a smaller genome and a metabolic strategy that acquires sulphur at a lower bioenergetic cost.

The benefits of genome reduction are probably not without a cost. The elimination of assimilatory sulphate reduction genes from SAR11 makes them dependent on reduced sulphur compounds found in the environment. One implication of our findings is that natural SAR11 populations may undergo transient periods where their growth is limited by the availability of sources of reduced sulphur, placing them at a competitive disadvantage alongside organisms that have the assimilatory sulphate reduction pathway. Understanding the factors that control plankton populations is a grail for oceanographers. It seems likely that the findings we report will focus attention on reduced sulphur compounds as potentially significant factors influencing microbial population dynamics in the oceans.

METHODS SUMMARY

Culturing and counting. Culturing techniques and seawater collection are described elsewhere[26,27]. The mixture of defined organic carbon compounds added to the medium to ensure an excess of carbon was the following: 100 nM

glycine betaine; 56 μM glucose, 44 μM ribose, 85 μM succinate, 114 μM pyruvate, 109 μM glycerol and 45 μM *N*-acetyl glucosamine; and 434 μM ethanol. Excess nitrogen and phosphorus were given as 10 μM NH_4Cl and 1 μM KH_2PO_4. Excess iron was given as 53.6 μM $FeCl_3$. Cell counts were acquired on an EasyCyte flow cytometer (Guava Technologies) after 1 h of staining in 1:2,000 diluted SYBR-Green I (Invitrogen)[28]. Sulphur compound comparisons were made at 100 nM in the same water, collected from the Oregon coast on 15 March 2007, as that used for the molar growth yield curve, which supported unusually high SAR11 cell densities in cultures. Experiments with other water samples gave similar results. Cells were incubated for three weeks to obtain the molar growth yield curve and maximum cell densities for various sulphur sources.

Radiotracer uptake. ^{35}S[DMSP] (10 μl; specific radioactivity 380,684 d.p.m. pmol^{-1}, final concentration 1.8 pM) was added to triplicate 600-ml cultures containing mid-exponential cells growing at 3.3×10^5 cells ml^{-1}. The cultures were incubated at 20 ± 0.5 °C for the duration of the experiment. For the isotope uptake measurements, duplicate 10-ml samples were filtered on 0.2-μm polycarbonate filters, rinsed twice with 10 ml of sea water, and measured by liquid-scintillation counting. To measure the incorporation of the isotope into protein, filters were also washed twice with 5 ml of ice-cold trichloroacetic acid before being counted. For each time point, cells were stained with 4,6-diamidino-2-phenylindole and counted by epifluorescence microscopy. The final cell density after 28 h was 7.6×10^5 cells ml^{-1}.

Search for candidate genomes. We began with a Genome Properties query for GenProp0149 'sulphate reduction to sulphide, assimilatory' in the Comprehensive Microbial Resource (CMR)[29]. Of the 381 genomes listed, 147 showed 'none found' for the genes present. We eliminated genomes with 'pathogen' or 'anaerobic' in the 'phenotype' field of the Genomes OnLine Database (GOLD)[30] and used the MicrobesOnline[9] homologue finder and genome browser to eliminate genomes that had assimilatory sulphate reduction operons apparently missed by CMR.

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1. Kiene, R. P., Linn, L. J., Gonzalez, J., Moran, M. A. & Bruton, J. A. Dimethylsulfoniopropionate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. *Appl. Environ. Microbiol.* **65,** 4549–4558 (1999).
2. Cuhel, R. L., Taylor, C. D. & Jannasch, H. W. Assimilatory sulfur metabolism in marine microorganisms: sulfur metabolism, protein synthesis, and growth of *Alteromonas luteo-violaceus* and *Pseudomonas halodurans* during perturbed batch growth. *Appl. Environ. Microbiol.* **43,** 151–159 (1982).
3. Cottrell, M. T. & Kirchman, D. L. Natural assemblages of marine proteobacteria and members of the Cytophaga–Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl. Environ. Microbiol.* **66,** 1692–1697 (2000).
4. Morris, R. M. *et al.* SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420,** 806–810 (2002).
5. Street, J. H. & Paytan, A. Iron, phytoplankton growth, and the carbon cycle. *Met. Ions Biol. Syst.* **43,** 153–193 (2005).
6. Schut, F. *et al.* Isolation of typical marine bacteria by dilution culture: growth, maintenance, and characteristics of isolates under laboratory conditions. *Appl. Environ. Microbiol.* **59,** 2150–2160 (1993).
7. Neumann, S., Wynen, A., Truper, H. G. & Dahl, C. Characterization of the *cys* gene locus from *Allochromatium vinosum* indicates an unusual sulfate assimilation pathway. *Mol. Biol. Rep.* **27,** 27–33 (2000).
8. Ruckert, C. *et al.* Functional genomics and expression analysis of the *Corynebacterium glutamicum* fpr2-cysIXHDNYZ gene cluster involved in assimilatory sulphate reduction. *BMC Genomics* **6,** 121 (2005).
9. Alm, E. J. *et al.* The MicrobesOnline Web site for comparative genomics. *Genome Res.* **15,** 1015–1022 (2005).
10. Berndt, C. *et al.* Characterization and reconstitution of a 4Fe-4S adenylyl sulfate/phosphoadenylyl sulfate reductase from *Bacillus subtilis*. *J. Biol. Chem.* **279,** 7850–7855 (2004).
11. Heinzinger, N. K., Fujimoto, S. Y., Clark, M. A., Moreno, M. S. & Barrett, E. L. Sequence analysis of the *phs* operon in *Salmonella typhimurium* and the contribution of thiosulfate reduction to anaerobic energy metabolism. *J. Bacteriol.* **177,** 2813–2820 (1995).
12. Malmstrom, R. R., Kiene, R. P., Cottrell, M. T. & Kirchman, D. L. Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the North Atlantic ocean. *Appl. Environ. Microbiol.* **70,** 4129–4135 (2004).
13. Howard, E. C. *et al.* Bacterial taxa that limit sulfur flux from the ocean. *Science* **314,** 649–652 (2006).
14. Todd, J. D. *et al.* Structural and regulatory genes required to make the gas dimethyl sulfide in bacteria. *Science* **315,** 666–669 (2007).
15. Kiene, R. P. & Linn, L. J. The fate of dissolved dimethylsulfoniopropionate (DMSP) in seawater: Tracer studies using S-35-DMSP. *Geochim. Cosmochim. Acta* **64,** 2797–2810 (2000).
16. Nicastro, D. *et al.* Three-dimensional structure of the tiny bacterium *Pelagibacter ubique* studied by cryo-electron tomography. *Microsc. Microanal.* **12,** 180–181 (2006).
17. Simo, R., Archer, S. D., Pedros-Alio, C., Gilpin, L. & Stelfox-Widdicombe, C. E. Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic. *Limnol. Oceanogr.* **47,** 53–61 (2002).
18. Zubkov, M. V. *et al.* Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. *Deep-Sea Res. II* **49,** 3017–3038 (2002).
19. Lippert, K. D. & Pfennig, N. Utilisation of molecular hydrogen by *Chlorobium thiosulfatophilum*. Growth and CO_2-fixation. *Arch. Mikrobiol.* **65,** 29–47 (1969).
20. Glaeser, J. & Overmann, J. Selective enrichment and characterization of *Roseospirillum parvum*, gen. nov. and sp. nov., a new purple nonsulfur bacterium with unusual light absorption properties. *Arch. Microbiol.* **171,** 405–416 (1999).
21. Ventura, S., De Philippis, R., Materassi, R. & Balloni, W. Two halophilic *Ectothiorhodospira* strains with unusual morphological, physiological and biochemical characters. *Arch. Microbiol.* **149,** 273–279 (1988).
22. Daniels, L., Belay, N. & Rajagopal, B. S. Assimilatory reduction of sulfate and sulfite by methanogenic bacteria. *Appl. Environ. Microbiol.* **51,** 703–709 (1986).
23. van Ham, R. C. *et al.* Reductive genome evolution in *Buchnera aphidicola*. *Proc. Natl Acad. Sci. USA* **100,** 581–586 (2003).
24. Hou, S. *et al.* Genome sequence of the deep-sea gamma-proteobacterium *Idiomarina loihiensis* reveals amino acid fermentation as a source of carbon and energy. *Proc. Natl Acad. Sci. USA* **101,** 18036–18041 (2004).
25. Giovannoni, S. J. *et al.* Genome streamlining in a cosmopolitan oceanic bacterium. *Science* **309,** 1242–1245 (2005).
26. Rappe, M. S., Connon, S. A., Vergin, K. L. & Giovannoni, S. J. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* **418,** 630–633 (2002).
27. Connon, S. A. & Giovannoni, S. J. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Appl. Environ. Microbiol.* **68,** 3878–3885 (2002).
28. Stingl, U., Tripp, H. J. & Giovannoni, S. J. Improvements of high-throughput culturing yielded novel SAR11 strains and other abundant marine bacteria from the Oregon coast and the Bermuda Atlantic Time Series study site. *ISME J* **1,** 361–371 (2007).
29. Peterson, J. D., Umayam, L. A., Dickinson, T., Hickey, E. K. & White, O. The comprehensive microbial resource. *Nucleic Acids Res.* **29,** 123–125 (2001).
30. Bernal, A., Ear, U. & Kyrpides, N. Genomes OnLine Database (GOLD): a monitor of genome projects world-wide. *Nucleic Acids Res.* **29,** 126–127 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions H.J.T. conducted genome analysis, adaptation of Guava EasyCyte to marine picoplankton counting, initial experimental design, preliminary data collection and analysis, and wrote the manuscript. J.B.K. set up, performed, and collected data for many culture experiments, and assisted in EasyCyte adaptations. J.W.H.D. provided DMSP and DMSP measurements. L.J.W. performed metagenomics. S.J.G. initiated the study, suggested candidate nutrients for testing, and with M.S.S. suggested final experiments and data presentation. All authors reviewed the manuscript before submission.

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