Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment

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Since the recognition of prokaryotes as essential components of the oceanic food web[1], bacterioplankton have been acknowledged as catalysts of most major biogeochemical processes in the sea. Studying heterotrophic bacterioplankton has been challenging, however, as most major clades have never been cultured[2] or have only been grown to low densities in sea water[3,4]. Here we describe the genome sequence of *Silicibacter pomeroyi*, a member of the marine Roseobacter clade (Fig. 1), the relatives of which comprise ~10–20% of coastal and oceanic mixed-layer bacterioplankton[2,5,6,7]. This first genome sequence from any major heterotrophic clade consists of a chromosome (4,109,442 base pairs) and megaplasmid (491,611 base pairs). Genome analysis indicates that this organism relies upon a lithoheterotrophic strategy that uses inorganic compounds (carbon monoxide and sulphide) to supplement heterotrophy. *Silicibacter pomeroyi* also has genes advantageous for associations with plankton and suspended particles, including genes for uptake of algal-derived compounds, use of metabolites from reducing microzones, rapid growth and cell-density-dependent regulation. This bacterium has a physiology distinct from that of marine oligotrophs, adding a new strategy to the recognized repertoire for coping with a nutrient-poor ocean.

The genome sequence of the coastal bacterioplankter *S. pomeroyi* DSS-3 was determined by the random whole-genome shotgun method and found to contain 4,283 predicted coding sequences (CDS, Table 1). The megaplasmid contains 10.4% of the CDS, with biases towards genes involved in energy metabolism, transport and regulation (see Supplementary Table S1). Compared with other α-Proteobacteria for which complete genome sequences are available, *S. pomeroyi* has the highest proportion of genes coding for signal transduction (1.6% versus 0.23 ± 0.39% (±s.d.) for 14 other α-Proteobacteria) and transport/binding proteins (12.1% versus 8.1 ± 3.0%) (see Supplementary Fig. S1), potentially reflecting an enhanced ability to sense and respond to conditions outside the cell.

Two operons encoding aerobic carbon monoxide dehydrogenases for the oxidation of CO to CO_2 are present in the *S. pomeroyi* genome (*coxSML*). The absence of ribulose bisphosphate carboxylase or other complete pathways for autotrophy suggests that the bacterium gains energy, but not carbon, through carboxidotrophy. As all previously characterized carboxidotrophs are autotrophic[8], CO oxidation was experimentally confirmed in *S. pomeroyi* and found to occur at CO concentrations typical of surface sea water (10 nM in coastal and 2 nM in oceanic regions) (see Supplementary Fig. S2). *Silicibacter*-like lithoheterotrophs might act as a microbial CO sink in the surface ocean—this poorly characterized process consumes 10–60 teragrams of C (as CO) annually and buffers the partial pressure of this greenhouse gas in the ocean[9]. A cluster encoding reduced inorganic sulphur oxidation (*soxRSVWXYZABCDF*) provides another mechanism for lithoheterotrophic growth in *Silicibacter*. The presence of a reduced inorganic sulphur compound was experimentally confirmed to enhance biomass production in acetate-grown *S. pomeroyi* cultures by 45% relative to cultures receiving no sulphur (see Supplementary Fig. S3). Analysis of codon usage in the *S. pomeroyi* genome indicates that both *coxL* and *soxB* have codon adaptive indices (CAIs) higher than >90% of the genes (0.36 and 0.35, respectively). CAIs have been found to correlate positively with levels of transcription in other genomes.

Because cultured bacteria might not be appropriate analogues of their uncultured relatives, the importance of lithoheterotrophy among marine bacterioplankton was assessed by an analysis of gene stoichiometry in the Sargasso Sea environmental shotgun library[10]. *coxL* was present in the library at an abundance equivalent to one per 14 bacterial cells and *soxB* at one per 10 cells (Table 2). As the Roseobacter clade accounted for only 3% of Sargasso 16S ribosomal RNA genes (see Supplementary Table S2), lithoheterotrophic strategies involving CO and inorganic sulphur compounds probably have distribution beyond this taxonomic group. Both *coxL* and *soxB* are also represented in a smaller coastal environmental genomics library[11]. CO is ubiquitous in marine surface waters owing to photo-oxidation of dissolved organic matter (DOM)[9], and sulphide has been measured in reducing microzones of marine snow[12] and might be generated from the degradation of organic sulphur compounds. Energy derived from oxidation of reduced inorganic compounds could allow greater heterotrophic efficiency for marine bacterioplankton by decreasing the fraction of assimilated organic carbon respired and hence increasing bacterial growth yield. Alternatively, lithotrophy could enhance anaplerotic CO_2

Table 1 **General features of the *S. pomeroyi* genome**

Feature		
Total number of coding sequences		4,283
Number of rRNA operons (16S, 23S, 5S)		3
Number of tRNA genes		53
Number of structural RNA genes		2
Proteins similar to proteins of known function and role (% of total proteins)		59.7
Proteins of unknown function (% of total proteins)		16.8
Conserved hypothetical proteins (% of total proteins)		15.3
Hypothetical proteins (% of total proteins)		8.2
	Main chromosome	Megaplasmid
Molecule length (bp)	4,109,442	491,611
G+C content (%)	64.2	62.8
Number of coding sequences	3,838	445
Average size of coding sequences (bp)	966	997
Percentage of coding sequence	90.2	90.3

fixation via pyruvate carboxylase, as has been found for *Sulfitobacter pontiacus*—a member of the Roseobacter clade—in the presence of thiosulphate[13]. Either alternative would influence the pathways and efficiencies of carbon flow through the marine microbial loop. Along with recent discoveries of bacterial photoheterotrophy in marine surface waters[14,15], they imply that mixed metabolic schemes might be particularly successful in the ocean.

High numbers of peptide transporters (16 ATP-binding cassette (ABC)-type systems), branched chain amino acid transporters (ten ABC-type systems) and amino acid efflux proteins (seven RhtB family systems) suggest that proteins are an important carbon source for *Silicibacter.* Six ABC-type transporter systems for putrescine and spermidine are present (no other sequenced genome has more than three); these compounds are produced in marine phytoplankton and zooplankton to regulate cell proliferation and bloom formation[16]. Transport systems for algal osmolytes feature significantly in the *S. pomeroyi* genome, and include five systems for transporting glycine betaine and/or dimethylsulphoniopropionate (DMSP) (OpuA and OpuD). Only the marine bacterium *Oceanobacillus iheyensis* has more such systems (with 16, see Supplementary Table S3). There is also a transport system and degradation pathway for taurine (*tauABCR*, *xsc*, *pta*, *tpa*). Organic matter from living plankton and detrital particles in the surface mixed layer is composed of ≥50% protein[17], and osmolytes such as DMSP can comprise 20% of the cytoplasm of some marine phytoplankton[18]. Several genome features suggest that organic matter derived from plankton and marine snow are important substrates for *Silicibacter*-like organisms. Members of the Roseobacter clade are commonly found in association with algal cells[19] and have a depth distribution that matches that of phytoplankton[5]. *S. pomeroyi* is motile and is potentially able to position itself in favourable microniches in association with plankton and particles (31 genes encode elements for motility via a polar complex flagellum[20]). However, there are no open reading frames (ORFs) with strong homology to known chemotaxis proteins or methyl-accepting chemotaxis transducers, suggesting either that the organism is motile but not chemotactic or that chemotaxis occurs by an unknown mechanism, either of which is unusual.

Analysis of flanking genes of an uncommonly high number of TRAP transporter systems (26 systems; no other sequenced genome has more) indicates an ability of *S. pomeroyi* to transport carboxylic acids of the type produced in surface waters during photo-oxidation of DOM[21], including glyoxylate and acetate. Other secondary transporters target malonate and formate. Carboxylic acids together with CO form a pool of biologically labile DOM photoproducts that can support 20% of bacterioplankton biomass production in near-surface coastal waters at temperate latitudes[21]. While it is anticipated that many marine bacteria assimilate selected photoproducts, *Silicibacter*-like organisms are genetically positioned to make thorough use of the mixture of compounds formed by DOM photo-oxidation.

Two putative quorum-sensing systems (*luxI*/*luxR* homologue pairs) are present on the *S. pomeroyi* chromosome. The presence of these *luxI* homologues is consistent with detection of at least three acylhomoserine lactone (AHL)-type signal molecules in culture extracts and distinct AHL-type activities for each homologue when expressed in *Escherichia coli* (see Supplementary Fig. S4). The presence of two quorum-sensing systems is in contrast to what is expected for an oligotrophic marine bacterium with conservative metabolic strategies. Quorum sensing would provide

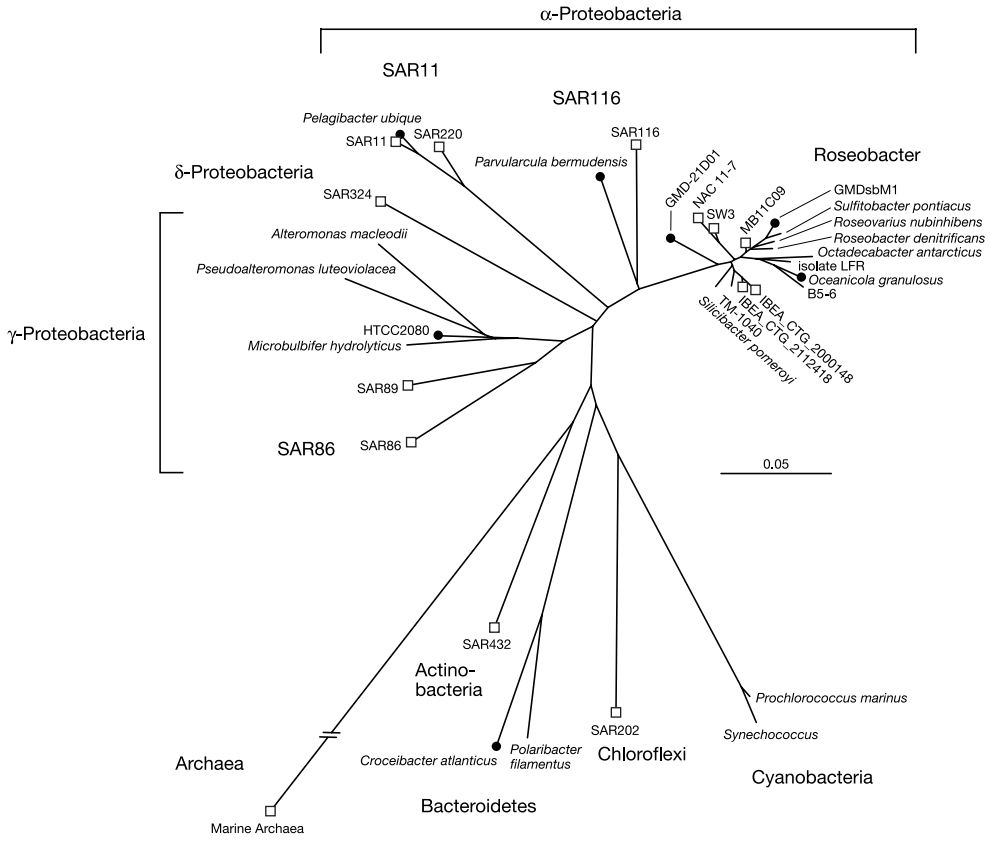

Figure 1 Phylogenetic tree of 16S rRNA gene sequences from the Roseobacter clade and other major marine taxa. Sequences include those from uncultured bacterioplankton (open square) and from cultured bacterioplankton isolated at very low nutrient concentrations (filled circle). Scale bar shows Jukes–Cantor evolutionary distance.

Table 2 **Stoichiometry of selected genes in the Sargasso Sea shotgun library***

Query gene	Frequency in cells†						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
16S rRNA	1 in 0.31	1 in 0.48	1 in 0.46	1 in 0.55	1 in 0.65	1 in 0.33	1 in 0.54
Roseo 16S rRNA	1 in 68	1 in 114	1 in 80	1 in 69	0	1 in 5	1 in 44
coxL	1 in 11	1 in 22	1 in 10	1 in 15	0	1 in 3	1 in 41
soxB	1 in 12	1 in 9	1 in 9	1 in 8	1 in 14	1 in 4	1 in 16
nirS	1 in 225	0	0	0	0	0	0

*Samples 1–7 from the unassembled Sargasso Sea shotgun library[10].
†Calculated as: number of target genes divided by number of *recA* homologues for the target genes 16S rRNA, *coxL*, *soxB* and *nirS* (rows 1, 3, 4 and 5). For Roseobacter 16S rRNA genes (row 2), calculated as: (number of 16S rRNA genes × 0.47) divided by the number of *recA* homologues. This assumes that Roseobacter clade cells contain an average of 2.1 rRNA operons based on the 16S rRNA:*recA* gene ratio in the complete Sargasso data set (this table). *recA* is typically a single-copy gene. BLAST searches against trace files identified 225, 217, 228, 231, 28, 16 and 84 *recA* homologues in Sargasso samples 1–7, respectively.

regulatory flexibility to manage a complex suite of metabolic pathways, possibly associated with switching between particle-associated (high population density, high substrate availability) and free-living (low population density, low substrate availability) stages[22]. The presence of three rRNA operons in the *S. pomeroyi* genome is consistent with the potential to respond rapidly to changes in resource availability[23], and a pathway for polyhydroxyalkanoic acid synthesis (*phaZCP*, *phbAB*) provides a mechanism for carbon and energy storage when substrates are abundant.

Four transporters for ammonium (*amt*) and one for urea (*urtABC*) are present in the *S. pomeroyi* genome, as are genes for ammonium (*glnA*, *gdhA*) and urea (*ureABCDEFG*) assimilation. Genes for assimilating nitrate and nitrite were not identified. Experiments confirmed that ammonium and urea are used as nitrogen sources, whereas nitrate and nitrite are not (see Supplementary Fig. S5). Nitrite is toxic at high concentrations (>500 μM), potentially because NO accumulates through the partial denitrification pathway (NO_2 to N_2) encoded on the *S. pomeroyi* megaplasmid (*norFEDQBC*, *nnrS*, *nirSECF,D-L* fusion,*GHJN*, *nosRZDFYL*). Like CO and other DOM photodegradation products, ammonium and urea are regenerated in marine surface waters. Thus, nutrient, energy and carbon acquisition strategies in *S. pomeroyi* coincide with Roseobacter depth distributions (highest abundance in surface waters)[2,5]. Although a single cultured organism cannot represent the breadth of features embodied in a diverse taxon that is broadly distributed over distinct hydrographical regions of the ocean[24], the emerging picture of *S. pomeroyi* physiology fits distribution patterns previously observed for Roseobacter clade members.

This genome sequence from a major bacterioplankton clade reveals an organism equipped to take advantage of transient occurrences of high-nutrient niches within a bulk low-nutrient environment. Living and dead plankton and microscale 'hot spots' of the surface ocean[25] might provide such niches. Lithoheterotrophic growth could allow *Silicibacter*-like bacterioplankton to use a greater proportion of organic carbon for biomass production as it becomes available. Although most ecologically relevant marine heterotrophs were previously assumed to be oligotrophs that subsist on dilute organic substrates dissolved in sea water[26], an 'opportunitroph' strategy might be a successful alternative. The available metagenomic data from coastal[11] and oceanic[10] sites (Table 2) indicate that such a strategy is not atypical among marine bacterioplankton. The genome sequence of this cultured marine microbe provides a window into the ecological strategies that maintain a significant fraction of the prokaryotes in the ocean. □

Methods

Isolation of *S. pomeroyi* (ATCC700808)

Sea water from southeastern US coastal waters (salinity = 31) was diluted with filter-sterilized sea water and enriched with 10 μM DMSP for two weeks. A small white colony was selected from a low-nutrient seawater plate spread with 0.1 ml of the enriched sea water[20].

Sequencing and annotation

The complete genome sequence of *S. pomeroyi* was determined using the whole-genome shotgun method as described[27], with libraries of 1–2 kb and 12–15 kb. Closure of physical and sequencing gaps was performed by primer walking, sequencing of transposon-tagged libraries of large-insert clones and multiplex polymerase chain reaction (PCR). Assembly was performed with the TIGR Assembler[27], and repeats were identified using RepeatFinder. G+C skew and oligoskew analyses[27] identified a putative origin of replication, and base pair 1 was assigned adjacent to the glucose-inhibited division protein A gene (*gidA*).

An initial set of ORFs predicted to encode proteins was identified using GLIMMER. ORFs consisting of <30 codons or containing overlaps were eliminated, and frameshifts and point mutations were corrected or designated authentic. Functional assignment of genes, identification of membrane-spanning domains, determination of paralogous gene families and identification of regions of unusual nucleotide composition were performed[27]. Phylogenomic analysis was used to assist with functional predictions[27], and comparative genome analyses were performed using the Comprehensive Microbial Resource (http://www.tigr.org/tigr-scripts/CMR2/CMRHomePage.spl). CAI index was calculated using the CAIC package[28].

Carbon monoxide oxidation

Three millilitres of culture (absorbance at 600 nm (A_{600}) of 1) were incubated in triplicate 160-ml serum bottles at 30° C, shaking at 250 r.p.m. CO was added at 10 p.p.m. and uptake was monitored to lower limits of 0.13 ± 0.05 p.p.m. using headspace gas chromatographic (GC) analysis (see Supplementary Fig. S2).

Thiosulphate assay

One millilitre of overnight culture was added to 100 ml of basal medium plus acetate (2, 5 or 10 mM) with or without 10 mM thiosulphate. Cultures were incubated at 30 °C and shaken at 250 r.p.m. in the dark and A_{600} was measured (see Supplementary Fig. S3).

Nitrogen assays

Cultures were grown as described for thiosulphate assays but with 20 mM acetate and 10 mM nitrogen supplied as urea, nitrate, nitrite or ammonium (see Supplementary Fig. S5).

Acylhomoserine lactone analysis

Dichloromethane extracts of *S. pomeroyi* cultures fractionated by reverse phase thin-layer chromatography were analysed using an *Agrobacterium tumefaciens* bioassay[29]. Expression of the LuxI homologues designated SilI1 and SilI2 was engineered by fusing a PCR-amplified complete coding sequence in line with a P_{Lac} promoter in pCR2.1-TOPO (Invitrogen). The plasmids were introduced into *E. coli* XL1-Blue cells expressing the Lac repressor $lacI^Q$. Dichloromethane extracts from an *E. coli* culture expressing SilI1 contained three AHL activities with the same migration as those identified from *S. pomeroyi* cultures, and synthesis was elevated several fold by IPTG. *E. coli* expressing SilI2 had multiple IPTG-inducible AHL activities that migrated to positions different from those activities observed in extracts of *S. pomeroyi* and from *E. coli* expressing SilI1, but still in the same general area of the TLC plate (see Supplementary Fig. S4).

Environmental library BLAST analysis

Homologues of *S. pomeroyi* genes were identified in the Sargasso Sea shotgun library[10] by BLAST analysis of trace files downloaded from NCBI using a cutoff for *E* of 10^{-95} (*coxL*) and 10^{-30} (*soxB*, *nirS*, *recA*). *coxL* candidates were further screened to identify genes clustering with known CO dehydrogenases[8] (see Supplementary Fig. S6). The number of *recA* homologues was divided by the number of lithoheterotrophy homologues to estimate frequency in cells, assuming one gene copy per cell. 16S rRNA genes from the Sargasso Sea library were assigned to taxonomic groups based on similarity in Smith–Waterman alignments with representative sequences. In the Monterey Bay BAC-end sequence library[11], BLAST searches yielded one *coxL*, one *soxB*, eight *recA* and no *nirS* homologues.

Phylogenetic tree construction

A phylogenetic tree of rRNA gene sequences from cultured and uncultured members of

major marine taxa was generated using the neighbour-joining method (positions 226–878, *E. coli* numbering) excluding positions with <50% conservation. Uncultured archaeon 'KTK 31A' (GenBank accession number AJ133625) served as the outgroup.

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In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes

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Two centuries after the duck-billed platypus was discovered, monotreme chromosome systems remain deeply puzzling. Karyotypes of males[1], or of both sexes[2–4], were claimed to contain several unpaired chromosomes (including the X chromosome) that form a multi-chromosomal chain at meiosis. Such meiotic chains exist in plants[5] and insects[6] but are rare in vertebrates[7]. How the platypus chromosome system works to determine sex and produce balanced gametes has been controversial for decades[1–4]. Here we demonstrate that platypus have five male-specific chromosomes (Y chromosomes) and five chromosomes present in one copy in males and two copies in females (X chromosomes). These ten chromosomes form a multivalent chain at male meiosis, adopting an alternating pattern to segregate into XXXXX-bearing and YYYYY-bearing sperm. Which, if any, of these sex chromosomes bears one or more sex-determining genes remains unknown. The largest X chromosome, with homology to the human X chromosome, lies at one end of the chain, and a chromosome with homology to the bird Z chromosome lies near the other end. This suggests an evolutionary link between mammal and bird sex chromosome systems, which were previously thought to have evolved independently.

Monotremes (mammalian subclass Prototheria) were the earliest offshoot of the mammalian lineage, diverging 210 million years ago from therian mammals (eutherians and marsupials)[8]. Only three monotremes are extant: the platypus (*Ornithorhynchus anatinus*) and two echidna species. Their phylogenetic position, and their mix of mammalian, reptilian and specialized morphological and physiological features, makes monotremes uniquely valuable for comparative genomics and for understanding sex chromosome evolution[9,10].

The correct chromosome number ($2n = 52$) in male and female platypus was established in 1975 (ref. 3). Measurements and banding of mitotic chromosomes revealed several that lacked obvious homologues in males. The largest, defined as the X chromosome because it was present in one copy in males and two in females, shares many genes with the eutherian and marsupial X chromosome[10]. However, the presence of one or more male-specific chromosomes, and of unpaired mitotic chromosomes in females, has long been controversial[1,2,4]. No genes have been mapped to any unpaired chromosomes except for this X chromosome[10], and no male-specific sequences have ever been identified.

At male meiosis in monotremes, several chromosomes assemble in a multivalent chain, analogous to meiotic chains in invertebrates that are the result of translocation heterozygosity. The X chromosome lies at one end, but the other elements are unknown and their numbers in platypus are variously reported as eight[1] or ten[2]. How