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SAR11 lipid renovation in response to phosphate starvation

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Phytoplankton inhabiting oligotrophic ocean gyres actively reduce their phosphorus demand by replacing polar membrane phospholipids with those lacking phosphorus. Although the synthesis of nonphosphorus lipids is well documented in some heterotrophic bacterial lineages, phosphorus-free lipid synthesis in oligotrophic marine chemoheterotrophs has not been directly demonstrated, implying they are disadvantaged in phosphate-deplete ecosystems, relative to phytoplankton. Here, we show the SAR11 clade chemoheterotroph *Pelagibacter* sp. str. HTCC7211 renovates membrane lipids when phosphate starved by replacing a portion of its phospholipids with monoglucosyl- and glucuronosyl-diacylglycerols and by synthesizing new ornithine lipids. Lipid profiles of cells grown with excess phosphate consisted entirely of phospholipids. Conversely, up to 40% of the total lipids were converted to nonphosphorus lipids when cells were starved for phosphate, or when growing on methylphosphonate. Cells sequentially limited by phosphate and methylphosphonate transformed >75% of their lipids to phosphorus-free analogs. During phosphate starvation, a four-gene cluster was significantly up-regulated that likely encodes the enzymes responsible for lipid renovation. These genes were found in *Pelagibacterales* strains isolated from a phosphate-deficient ocean gyre, but not in other strains from coastal environments, suggesting alternate lipid synthesis is a specific adaptation to phosphate scarcity. Similar gene clusters are found in the genomes of other marine α-proteobacteria, implying lipid renovation is a common strategy used by heterotrophic cells to reduce their requirement for phosphorus in oligotrophic habitats.

marine phosphorus cycle | lipids | glucuronic acid | cyanobacteria | methylphosphonate

Microbes primarily assimilate phosphorus (P) in its +5 valence state (phosphate; P_i), which comprises ~3% of total cellular mass as a structural constituent of nucleic acids and phospholipids, and is intimately involved in energy metabolism and some transport functions (via ATP hydrolysis) (1). In oligotrophic ocean gyres, P_i concentrations are extremely low (0.2–1.0 nM in the Sargasso Sea; ref. 2) and the availability of P_i can limit bacterial and primary production (2–5). Microbes inhabiting these low P_i environments have evolved numerous strategies to maintain growth and enhance their competitiveness for trace amounts of P_i. These mechanisms are commonly induced by P_i starvation and include one or more of the following: (*i*) expression of high affinity P_i transporters (6); (*ii*) reduction of cellular P_i quotas (7, 8); (*iii*) utilization of alternate phosphorus sources (9, 10); and (*iv*) polyphosphate storage and breakdown (11, 12). Such strategies facilitate survival in the face of P_i insufficiency.

Polar membrane lipids are a substantial cellular sink for phosphate in bacteria. Structural lipids consist of glycerol esterified to hydrophobic fatty acid chains (diacylglycerol) and a hydrophilic polar head group, which commonly contains P_i (phospholipids). When grown with sufficient P_i, phospholipids in Gram-negative marine bacteria frequently include phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and diphosphatidylglycerol (13). However, when P_i is low or limiting, many microbes replace phospholipids with those that lack phosphorus (14–16). Nonphosphorus polar head groups are structurally diverse and include sulfoquinovose (sulfolipids) (17), various monosaccharides and disaccharides (glycolipids; reviewed in ref. 18), ornithine, or other amino acids (reviewed in ref. 19). When P_i stressed, the marine cyanobacteria *Prochlorococcus* and *Synechococcus* reduce their P_i demand by 0.5–8.6 attomoles P per cell by substituting phosphorus-containing lipids with sulfolipids; depending on the strain, this reduction equates to 10–86% of the P bound in their nucleic acids (7). There is indirect evidence natural populations of marine chemoheterotrophic bacteria also use nonphosphorus lipids in response to P_i deprivation. Bacterioplankton collected in the Sargasso Sea had greater concentrations of nonphosphorus lipids than those from adjacent regions of the North Atlantic where P_i was relatively abundant (20).

Oligotrophic bacteria belonging to the SAR11 clade (*Pelagibacterales*) of α-proteobacteria are numerically dominant chemoheterotrophs in marine euphotic zones worldwide (21). *Pelagibacterales* cells are small (volume of 0.01 μm^3; ref. 22) and contain streamlined genomes (23, 24). The reduced cell and genome size likely stem from natural selection to reduce the overhead cost of

Significance

Nonphosphorus lipids produced by heterotrophic bacteria have been measured in marine ecosystems without an understanding of their origins or role. This work shows SAR11 chemoheterotrophic bacteria synthesize multiple nonphosphorus lipids in response to phosphate depletion. Because this process results in a reduced cellular P:C ratio, it impacts our understanding of ocean processes related to cellular elemental stoichiometry by showing how different environmental parameters alter P:C ratios in heterotrophs. Also, SAR11 grown with excess organophosphonate synthesized phosphorus-free lipids. This finding contrasts the contemporary view of organophosphorus utilization because organophosphate-derived phosphorus did not equally substitute for inorganic phosphate in lipids. Considering lipid phosphorus content was lower in cells using organophosphonate, phosphorus-based productivity estimates may vary as a function of phosphorus source.

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replication in oligotrophic ocean gyres where P and N may periodically limit growth (25, 26). Despite their abundance in low P_i environments, it remains unknown whether *Pelagibacterales* synthesize phosphorus-free lipids to reduce their P quota. The genome of *Pelagibacter ubique* str. HTCC1062 lacks genes predicted to encode proteins used in sulfolipid, betaine, or ornithine lipid biosynthesis, suggesting this strain is unable to modulate lipid composition in response to P_i availability (7, 8). Previous laboratory experiments partially supported this prediction by showing that *P. ubique* lacks nonphosphorus lipids when grown under P_i replete conditions; however, lipids from P_i limited cells were not examined in that research (7). Relative to *P. ubique*, a Sargasso Sea isolate, *Pelagibacter sp*. str. HTCC7211 (str. HTCC7211) contains extended genetic inventory associated with P_i acquisition, storage, and metabolism (10). When P_i limited, str. HTCC7211 induces a suite of these genes, including both inorganic (*pstSCAB*) and organophosphate (*phnCDEE$_2$*) ABC transporters and the C–P lyase complex (*phnGHIJKLNM)*, required for phosphonate degradation (10). Laboratory experiments have distinctly linked the expression of these genes to the utilization of both phosphate esters and phosphonates (including methylphosphonate; MPn) (10). This finding indicates organophosphate utilization is one strategy str. HTCC7211 employs to evade P_i growth limitation.

While examining gene expression profiles of P_i starved str. HTCC7211 cultures, we observed the unexpected up-regulation of a four gene cluster proximal to the collection of P uptake genes on the str. HTCC7211 chromosome. Two of the genes were annotated as "putative hemolysins," one as a "glycosyltransferase," and a "metallophosphatase." Comparative genomic examination of these genes led us to hypothesize that the four genes might be involved in the restructuring of lipid polar head groups and the synthesis of nonphosphorus lipids. Herein, we present the results of laboratory experiments designed to test the potential for synthesis of nonphosphorus lipids in response to P_i stress by *Pelagibacter*.

Results

Previously we reported P_i starvation in str. HTCC7211 significantly induced the expression of 31 genes, including those encoding P_i and organophosphorus transport and catabolism proteins (10). Proximal to the genes encoding a high-affinity P_i transporter (*pstSCAB*), a four-gene cluster (HTCC7211_00011000–HTCC7211_00011030) was significantly up-regulated in P_i starved cultures, relative to P_i replete cultures (2.7- to 8.2-fold, reported in ref. 10). Two of these genes (HTCC7211_00011000 and HTCC7211_00011010) are oriented in a probable operon (+ strand; Fig. 1) and annotated as "putative hemolysins" belonging to COG3176. The other two genes (HTCC7211_00011020 and HTCC7211_00011030) are located downstream of HTCC7211_00011010 in a second probable operon (- strand; Fig. 1). HTCC7211_00011020 is annotated as a group 1 glycosyltransferase belonging to COG438 (RfaG). HTCC7211_00011030 is annotated as a metallophosphoesterase belonging to COG2908. Protein domain signatures in each predicted amino acid sequence were identified with InterProScan5 (27). HTCC7211_00011000 contains an acyl-CoA *N*-acyltransferase domain (IPR016181; residues 26–174). HTCC7211_00011010 contains a phospholipid/glycerol acyltransferase domain (IPR002123; residues 49–180). HTCC7211_00011020 contains a glycosyltransferase subfamily 4, N-terminal domain (IPR028098; residues 5–164), and a glycosyl transferase, family 1 domain (IPR001296; residues 177–285). HTCC7211_00011030 contains a calcineurin-like phosphoesterase domain, *lpxH* type domain (IPR024654; residues 10–249).

We searched for orthologs to HTCC7211_00011000–HTCC7211_00011030 in α-proteobacterial genomes by using the Hal pipeline (28) and found all four genes differentially distributed across the clade (Fig. 1 and Dataset S1). HTCC7211_00011000 clustered with amino acid sequences of characterized enzymes from *Ensifer* (*Sinorhizobium) meliloti* 1021 (OlsB) and *Rhodobacter capsulatus* SB 1003 (OlsB) (Fig. 1). OlsB catalyzes the first step in ornithine lipid formation (29, 30) (Fig. S1). The second step of ornithine lipid biosynthesis is catalyzed by the protein product of *olsA* (Fig. S1), which is frequently found downstream of *olsB* (30, 31). Unexpectedly, the amino acid sequence of HTCC7211_00011010 did not cluster with OlsA sequences from characterized enzymes, despite all three having phospholipid/glycerol acyltransferase domain (IPR002123) signatures. HTCC7211_00011020 clustered with an *Agrobacterium fabrum* C58 gene (Fig. 1) recently demonstrated to encode a bifunctional glycosyltransferase that forms both monoglucosyl- and glucuronosyl-diacylglycerols (MGDG and GADG, respectively) by the addition of glucose or glucuronic acid moieties to diacylglycerols (32) (Fig. S2). HTCC7211_00011030 clustered with an *E. meliloti* gene shown to encode a phospholipase C (PlcP) (Fig. 1) that initiates lipid renovation during P_i starvation by cleaving phosphorus-containing polar head groups from diacylglycerols (8) (Fig. S2).

We hypothesized that HTCC7211_00011000–HTCC7211_00011030 encode proteins involved in the renovation of membrane lipids during P_i stress. Renovation is defined here as the combined result of: (*i*) the synthesis of new ornithine lipids; and (*ii*) the exchange of phospholipids for MGDG and/or GADG. We based this hypothesis on the increased expression of these genes under P_i limiting conditions (10), and the characterized functions of orthologous genes in other α-proteobacteria (Fig. 1). We tested this hypothesis by analyzing the lipid polar head group composition over time in P_i replete and P_i deplete growth conditions (Fig. 2). Total lipids extracted per sample ranged from 2.8 to 9.7 attomoles·cell^{-1} and varied as a function of growth state and P source (Dataset S2 and Fig. S3).

Strain HTCC7211 cells growing exponentially with excess P_i (Fig. 2*A*) exclusively contained phospholipids (PG and PE) (Fig. 2*B*). In carbon-limited stationary phase, a low proportion of GADG lipids were detected (<1%; Fig. 2*B*). Cells incubated in growth medium without added P_i did not grow appreciably (Fig. 2*C*), but renovated 26–38% of total cellular phospholipids with multiple P-free lipids (GADG > MGDG > ornithine lipid; Fig. 2*D*). Although cells renovated membrane lipids in the absence of P_i, total cellular lipid content decreased, relative to P_i replete cells, suggesting cell size was slightly reduced (Fig. S3).

Lipid profiles of cells resuspended into growth medium with MPn as the sole P source were measured. Exponentially growing cells using MPn as the sole P source (Fig. 2*E*) replaced phospholipids with P-free lipids (Fig. 2*F*). In this growth condition, up to 40% of total cellular phospholipids were renovated to P-free analogs. Similar to P_i starved profiles, GADG was the most abundant P-free lipid (Fig. 2*F*). However, after an initial drop in abundance, the total percent of phospholipids increased to 87% as the cells entered carbon-limited stationary phase. Total cellular lipid contents in MPn-replete cells were comparable to those of P_i replete cultures, suggesting cell size was conserved during renovation (Fig. S3).

Cells harvested in midlogarithmic phase, grown with MPn as the sole P source for >20 generations, contained a standing stock of 11% P-free lipids (Fig. S4).

P_i starved cells exposed to MPn levels sufficient to sustain >1 generation, but insufficient for maximal growth (that is, cells were MPn-limited; Fig. 3*A*), contained 77% P-free lipids in MPn-limited stationary phase (Fig. 3*B*). A lesser degree of conversion was observed when P_i starved cells were exposed to P_i sufficient for >1 generation, but insufficient for maximal growth (Fig. 3 *A* and *C*).

Previous measurements of the lipid composition of *P. ubique* str. HTCC1062 growing in P_i replete natural seawater medium showed that only PE and PG were present in lipid extractions (7). We conducted similar experiments with P_i replete and P_i starved str. HTCC1062 cells grown in synthetic medium. The lipids of str. HTCC1062 cells growing in P_i replete conditions were

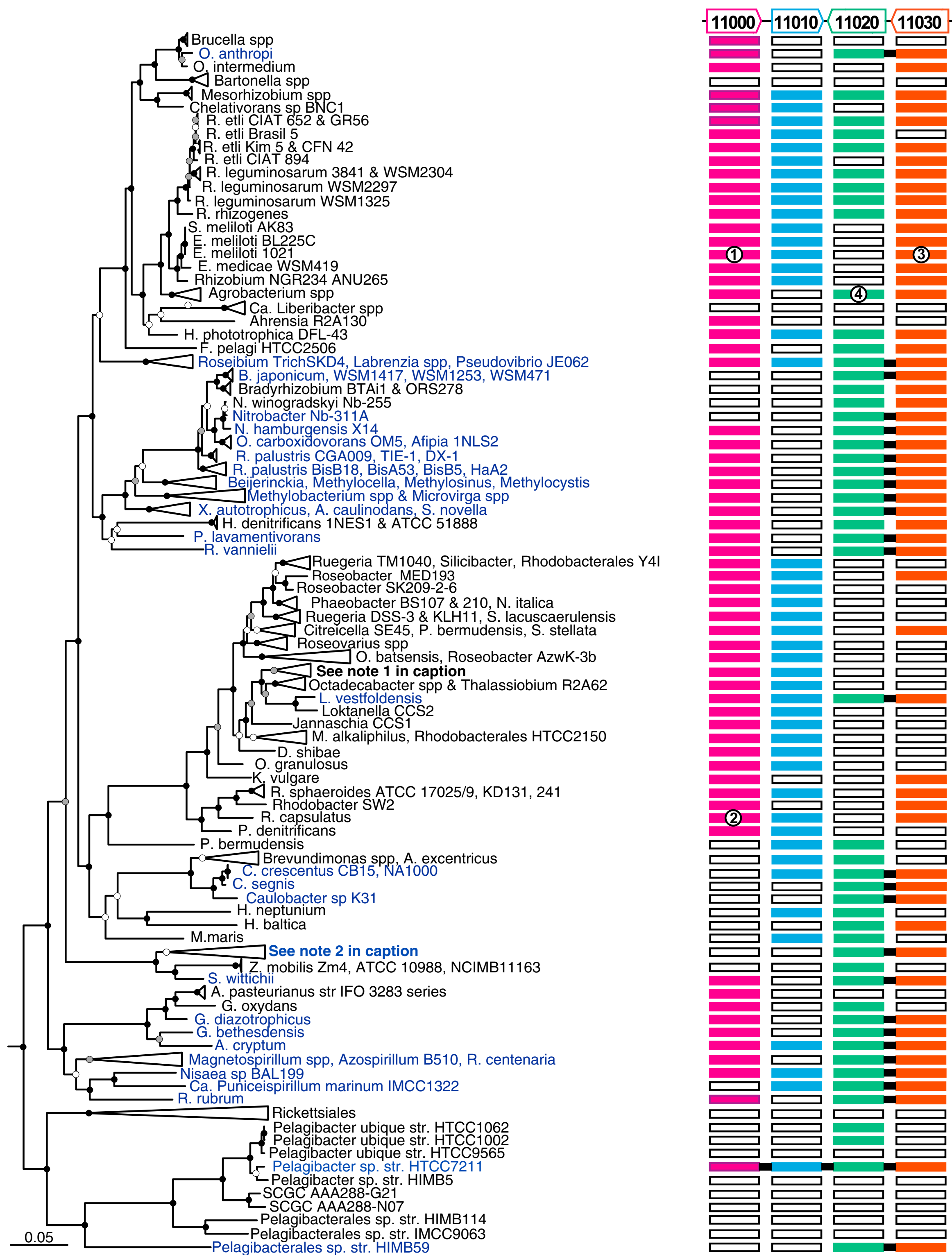

Fig. 1. Distribution of nonphosphorus lipid synthesis genes in α-proteobacteria. Tree inferred from concatenated 16S/23S rRNA gene sequences. Most monophyletic groups where all members had the same patterns of gene distribution were collapsed. The outgroup taxa can be found in Dataset S1. Node labels represent Shimodaira–Hasegawa confidence test values: black filled, ≥0.9; gray filled, 0.7–0.9; white filled, ≤0.7. *Pelagibacter* sp. str. HTCC7211 gene orientation is depicted above colored bars; "HTCC7211_000" for each gene identifier has been omitted. The colored bars are a visual representation of Hal clustering results. Bars indicate ortholog presence (filled), absence (open), and chromosomal synteny (black line between bars), or lack of synteny (no black line between bars), for the four genes. Those taxa with adjacent glycosyltransferases and metallophosphoesterases are colored blue in the tree for ease of identification. Numbers inside of colored boxes indicate genes for which functions have been characterized, as follows: 1, SMc01116 (OlsB, *E. meliloti*); 2, RCAP_rcc02997 (OlsB, *R. capsulatus*); 3, SMc00171 (phospholipase C, *E. meliloti*); 4, atu2297 (GADG/MGDG glycosyltransferase, *A. fabrum,* in collapsed node). Note 1: *Sulfitobacter* spp., *O. indolifex*, *R. denitrificans* and *litoralis*, *Rhodobacterales* HTCC2083. Note 2: *Erythrobacter* HTCC2594/NAP1, and *C. bathyomarinum* JL354, *N. aromaticivorans* DSM 12444, *Sphingobium* spp, *Sphingopyxis alaskensis* RB2256, *Sphingomonas* SKA58.

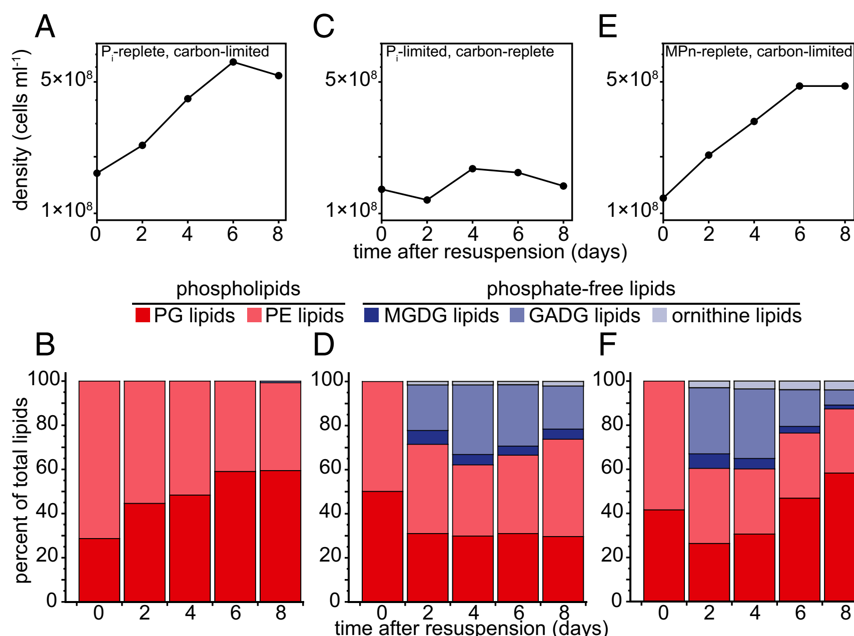

Fig. 2. *Pelagibacter* sp. str. HTCC7211 renovates lipid composition in response to P_i limitation. Growth curves show cell densities in suspensions that were subsampled for lipid analyses during time courses: excess P_i (*A*); no P_i (*C*); or excess MPn (*E*). Time course of lipid composition of str. HTCC7211 cells in AMS1 containing: excess P_i (*B*); no P_i (*D*); or excess MPn (*F*).

comprised exclusively of phospholipids (PG and PE). Non-phosphorus lipids were not detected under conditions of P_i starvation (Fig. S5).

Discussion

Similar to phytoplankton (7) and terrestrial α-proteobacteria (8), we show *Pelagibacter sp.* str. HTCC7211, but not *P. ubique* str. HTCC1062, modulates its phospholipid composition in response to P availability (Fig. 2). We identify the genes likely to confer the ability to renovate polar lipid head groups (Fig. 1) and link to studies of their regulation (10). Published metagenomic analysis show that three of these genes are overrepresented in the Sargasso Sea (HTCC7211_00011010–HTCC7211_00011030) relative to *Pelagibacterales* populations inhabiting the comparatively P-replete North Pacific subtropical gyre (11). In these studies, a metric called "multiplicity per cell" was used to infer that ∼28% of the *Pelagibacterales* population contained HTCC7211_00011010; ∼95% contained HTCC7211_00011020 and all cells contained HTCC7211_00011030 (∼117%, indicating one or more copies in some genomes). These data suggest that the capacity to convert phospholipids to glycolipids, as conferred by orthologs to HTCC7211_00011020 (MGDG/GADG glycosyltransferase) and HTCC7211_00011030 (phospholipase C), is widespread in *Pelagibacterales* lineages inhabiting the Sargasso Sea and is likely a specific adaptation to P scarcity. These findings likely explain reports of MGDG production by chemoheterotrophic bacteria from the Sargasso Sea (20).

The complete four-gene cluster conferring glycolipid and ornithine lipid biosynthesis in str. HTCC7211 is not found in the same syntenic arrangement in other α-proteobacteria (Fig. 1). However, tandem genes encoding phospholipase C and the MGDG/GADG glycosyltransferase were identified in a number of α-proteobacteria, including the marine strains *Pelagibacterales* sp. str. HIMB59, "*Candidatus* Puniceispirillum marinum" str. IMCC1322 (SAR116), *Erythrobacter litoralis* HTCC2594, and *Loktanella vestfoldensis* SKA53 (Fig. 1). This finding expands our knowledge of the distribution of glycolipid synthesis genes to cosmopolitan marine bacteria and implies lipid renovation in response to P_i scarcity may be a relatively common feature of marine chemoheterotrophic bacteria.

HTCC7211_00011010 likely catalyzes the final step of ornithine lipid biosynthesis in str. HTCC7211. Ornithine lipids are synthesized by the *N*-acylation of ornithine with a hydroxy-fatty acyl group (by OlsB) (29), followed by an *O*-acylation of lyso-ornithine (by OlsA) to form ornithine lipid (Fig. S1). In most ornithine lipid synthesizing bacteria, *olsA* and *olsB* genes form an operon. We identified a probable *olsB* gene in the str. HTCC7211 genome (Fig. 1; HTCC7211_00011000) but were unable to identify an ortholog to known *olsA* genes with all vs. all BLASTP and Markov clustering (MCL). To further examine this question, we searched the str. HTCC7211 genome with a hidden Markov model (HMM) trained on amino acid sequences of characterized *olsA*s from *E. melilotii* and *R. capsulatus* plus the best BLAST hits to each (*SI Methods*). The analysis returned low E-value hits to HTCC7211_0001770 and did not provide additional evidence that HTCC7211_00011010 is an *olsA* ortholog. Thus, the primary amino acid sequence of HTCC7211_00011010 does not likely encode an ortholog to OlsA. However, OlsA has homology to glycerol acyltransferases, the protein domain we identified in HTCC7211_00011010. These similarities, plus the syntenic arrangement akin to other *olsBA* pairs, suggest HTCC7211_00011010 has an activity similar to that of characterized OlsAs. Regarding the str. HTCC7211 ornithine lipid synthesis gene cluster, we did not find other α-proteobacteria with the same chromosomal arrangement, implying this arrangement may be unique to a subset of organisms in the *Pelagibacterales.*

The use of lipids that lack P, together with the utilization of assorted organophosphorus sources (10), may enable certain *Pelagibacterales* members to cope with patchiness in the dissolved organic phosphorus pool and maintain growth in low P_i waters. In P_i replete conditions, the lipid polar head groups of str. HTCC7211 and str. HTCC1062 had a molar P:C ratio of ∼0.4 (Fig. 4). Under conditions of P_i deprivation or growth on MPn, the average molar P:C ratio decreased to ∼0.2 in str. HTCC7211 (Fig. 4). From the change in composition of polar head groups during P_i starvation, we calculated that lipid renovation reduces

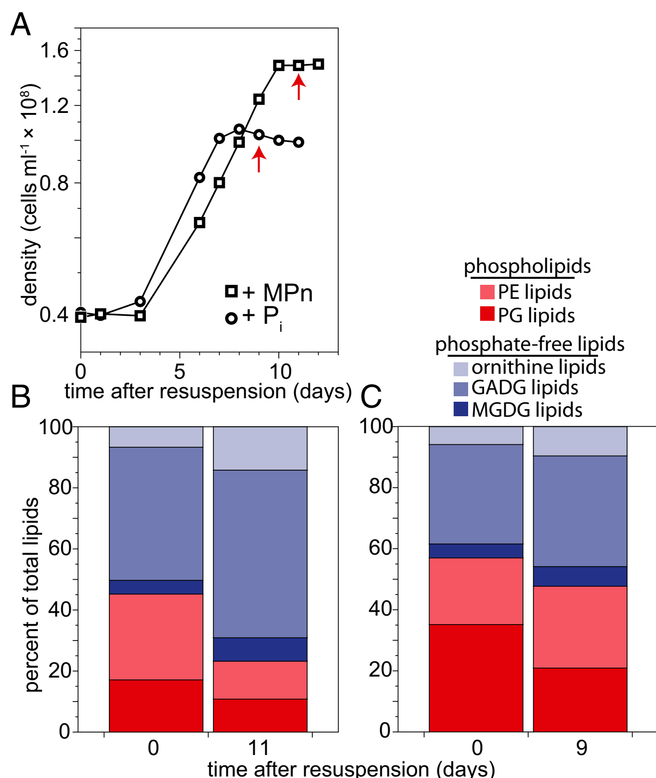

Fig. 3. Majority of lipids are phosphate-free analogs under conditions of methylphosphonate starvation. (*A*) Bacterial growth curves of previously P_i starved str. HTCC7211 cells in growth medium with growth-limiting amounts of P_i or MPn. Lipid profiles before and after reaching MPn (*B*) or P_i (*C*) limitation. Red arrows in *A* point to P limited sample points for *B* and *C*.

the P content of str. HTCC7211 by 2.7–3.9 amoles $P \cdot cell^{-1}$ (Dataset S2). The genome of str. HTCC7211 is 1,456,888 bp long and contains 4.8 attomoles P. Thus, the savings in lipid P is ∼56–81% of the P contained in the genome. A similar savings was also observed when cells were grown with MPn (Fig. 4), indicating that growth with organophosphorus sources may have unexpected effects on cellular stoichiometry. Although HTCC7211 appeared to use MPn-derived P for phospholipid synthesis in pure culture studies (Fig. 2*F*), we measured a standing stock of ∼11% nonphosphorus lipids in cultures grown exclusively on MPn (Fig. S4). Moreover, when MPn-adapted cells were starved for MPn (Fig. 3*A*), the majority of lipids were converted to non-P lipids (Fig. 3*B*). Previous studies showed that str. HTCC7211 used a broad spectrum of organophosphorus compounds. Although we only show the lipid composition with MPn, our data suggests deposition of P into, and withdrawal from, structural lipids may act as a P reserve for cells, effectively enabling survival in low P_i ecosystems where the bioavailability of P_i or organophosphorus is variable or patchy.

We have demonstrated that some *Pelagibacterales* strains can modify their lipids in response to P stress, replacing phospholipids with GADG, MGDG, and ornithine lipids, thereby reducing cellular demands for P. The genes associated with this adaptive response are found in hypervariable genomic regions and appear to be most abundant in *Pelagibacterales* strains that inhabit P_i limited ocean regions, like the Sargasso Sea. This discovery is significant because it shows that, like phytoplankton, chemoheterotrophic bacterioplankton have mechanisms that cause them to deviate from expected elemental ratios, thereby changing estimates of the impacts of P limitation on productivity and biomass accumulation. In this case, the mechanism identified is associated with specific genes that previously had poorly assigned functions. Because many genes in microbial genomes have incompletely assigned functions, studies like this improve the accuracy of annotations and are important to improving the long-term impact of genome data on the prediction geochemical processes. In future work, it will be important to understand why cells prefer phospholipids and to identify changes in fitness associated with the substitution of glycolipids.

Methods

Organism Source and Growth Conditions. *P. ubique* str. HTCC1062 and *Pelagibacter sp.* str. HTCC7211 were revived from frozen stocks and cultivated on AMS1 synthetic growth medium without added phosphate as described (10, 33, 34). Cell growth was monitored by flow cytometry as described (35). Phosphorus was added as NaH_2PO_4 or methylphosphonate (MPn), as indicated in the text.

Cell Harvesting for Lipid Profiles. Strain HTCC7211 cells, grown in AMS1 with excess P_i (100 μM), were harvested in late-logarithmic growth-phase (approximately 1.0×10^8 $cells \cdot ml^{-1}$) by centrifugation (17,664 × *g* for 1.0 h at 20 °C). Pellets were washed twice with growth medium and resuspended in one of the following conditions: (*i*) P_i replete (100 μM); (*ii*) MPn-replete medium (100 μM); or (*iii*) P_i deplete growth medium (no P_i added). Suspensions were monitored for growth (reported in Fig. 2 *A*, *C*, and *E*) and subsampled (40 mL) by centrifugation (48,298 × for 1.0 h at 4 °C) at $t = 0$, 2, 4, 6, and 8 d. The supernatant was removed and cell pellets were frozen at −80 °C until lipid extraction.

To study the effect of sequential P_i and MPn limitation on lipid composition, str. HTCC7211 cells were grown with excess P_i to late-logarithmic growth phase and harvested by centrifugation. Pellets were washed with growth medium, resuspended in growth medium without added P (to approximately 4×10^7 $cells \cdot mL^{-1}$), and incubated for 4 d to induce the P_i starvation response. After starvation, P-starved cells were used to inoculate growth media containing a growth-limiting amount of P_i (1.2 μM) or MPn (1.5 μM). The carbon and nitrogen constituents of this medium support cell yields in excess of 5.0×10^8 $cells \cdot mL^{-1}$. Therefore, P_i or MPn were growth-limiting, as calculated from the cellular P quotas of 11 amol $P \cdot cell^{-1}$ (P_i-grown) or 10 amol $P \cdot cell^{-1}$ (MPn-grown) (10). Cell suspensions were subsampled by centrifugation at $t = 0$ d and after P-limited stationary phase had been reached, as determined from direct cell counts: 9 d for P_i-grown cells; 12 d for MPn-grown cells.

Lipid Analysis. Polar lipids were extracted from the cell pellets as described (36). Published methods were also used as a basis for polar lipid separation by normal-phase high performance liquid chromatography (20) and concomitant analysis by positive electrospray ionization ion-trap mass spectrometry (37). Additional structural elucidation of GADG and ornithine lipids was conducted by using the same HPLC method in conjunction with positive

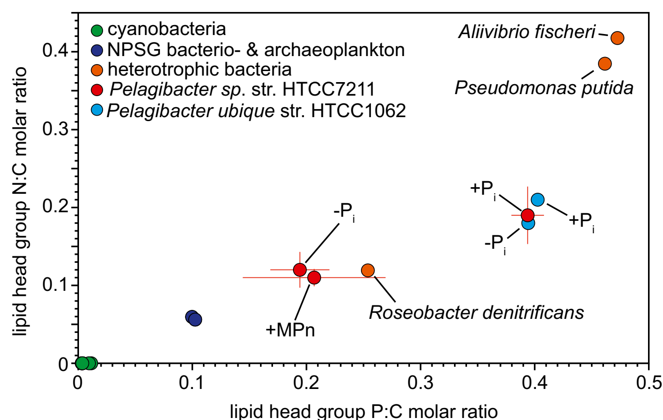

Fig. 4. Phosphorus, nitrogen, and carbon molar ratios in lipid polar head groups from str. HTCC7211 and str. HTCC1062 under different phosphorus regimes. Strain HTCC7211 data points are the mean elemental ratios ± SD from days 2–8 in Fig. 2 ($n = 4$) (Dataset S2). Cyanobacteria, non-*Pelagibacterales* heterotrophs and North Pacific Subtropical Gyre (NPSG) values were calculated from published data (44).

electrospray ionization high mass-resolution mass spectrometry (Figs. S6 and S7 and *SI Methods*).

Lipid Polar Head Group Elemental Stoichiometry. The relative elemental ratios of polar head groups under different P regimes were calculated from the molar proportion of each membrane lipid type in each treatment and the molecular formulas of each polar head group: $C_2H_8NO_4P$ for phosphoethanolamine; $C_3H_7O_7P$ for phosphoglycerol; $C_6H_9O_7$ for glucuronic acid; $C_6H_{11}O_6$ for glucose; and $C_5H_{11}N_2O_2$ for ornithine (Dataset S2).

Gene Identifiers. All gene identifiers in this study are presented as Integrated Microbial Genomes (IMG) Gene ID numbers. In previous works (10, 11, 38), the genes HTCC7211_00011000–HTCC7211_00011030 were listed with different identifiers [listed as: previous identifier (IMG Gene ID)]: PB7211_1302 (HTCC7211_00011000); PB7211_635 (HTCC7211_00011010); PB7211_960 (HTCC7211_00011020); PB7211_983 (HTCC7211_00011030).

Identification of Putative Lipid Renovation Gene Orthologs. The Hal software package (28) was used to generate orthologous protein clusters from 272 α-proteobacteria from the IMG database (39). All vs. all BLASTP was followed by MCL at 13 inflation parameters. Clusters generated with the inflation parameter of 1.5 were used to identify orthologs of the genes predicted to be involved in lipid remodeling in str. HTCC7211 (HTCC7211_00011000–HTCC7211_00011030). The complete distribution for all four genes in all α-proteobacteria can be found in Dataset S1.

α-Proteobacterial Phylogeny. Concatenated 16S-23S rRNA genes from almost all of the above α-proteobacteria and six outgroups (including members of the β-, γ-, and δ-proteobacteria) were used to manually construct a maximum likelihood tree similar to that in ref. 40. Some rRNA gene sequences from organisms included in the Hal analysis were excluded from the 16–23S tree because of poor quality or truncated rRNA genes (Dataset S1). All identifiers for taxa represented in the tree are provided in Dataset S1. The 16S and 23S rRNA genes were aligned separately with MUSCLE (41) by using default settings, and curated with Gblocks (42) using the following settings: -b1 = (n/2)+1; -b2 = (n/2) + 1; -b3 = (n/2); -b4 = 2; -b5 = h, where n = number of taxa. Alignments were normalized and concatenated with normalize_alignments.py and catPhylip.pl, respectively, included in the Hal package. The final alignment contained 261 taxa and 4,096 characters. The tree was inferred by using FastTree2 (43) with default settings.

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