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Transcriptional Control in Marine Copiotrophic and Oligotrophic Bacteria with Streamlined Genomes

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ABSTRACT

Bacteria often respond to environmental stimuli using transcriptional control, but this may not be the case for marine bacteria such as "*Candidatus* Pelagibacter ubique," a cultivated representative of the SAR11 clade, the most abundant organism in the ocean. This bacterium has a small, streamlined genome and an unusually low number of transcriptional regulators, suggesting that transcriptional control is low in *Pelagibacter* and limits its response to environmental conditions. Transcriptome sequencing during batch culture growth revealed that only 0.1% of protein-encoding genes appear to be under transcriptional control in *Pelagibacter* and in another oligotroph (SAR92) whereas >10% of genes were under transcriptional control in the copiotrophs *Polaribacter* sp. strain MED152 and *Ruegeria pomeroyi*. When growth levels changed, transcript levels remained steady in *Pelagibacter* and SAR92 but shifted in MED152 and *R. pomeroyi*. Transcript abundances per cell, determined using an internal RNA sequencing standard, were low (<1 transcript per cell) for all but a few of the most highly transcribed genes in all four taxa, and there was no correlation between transcript abundances per cell and shifts in the levels of transcription. These results suggest that low transcriptional control contributes to the success of *Pelagibacter* and possibly other oligotrophic microbes that dominate microbial communities in the oceans.

IMPORTANCE

Diverse heterotrophic bacteria drive biogeochemical cycling in the ocean. The most abundant types of marine bacteria are oligotrophs with small, streamlined genomes. The metabolic controls that regulate the response of oligotrophic bacteria to environmental conditions remain unclear. Our results reveal that transcriptional control is lower in marine oligotrophic bacteria than in marine copiotrophic bacteria. Although responses of bacteria to environmental conditions are commonly regulated at the level of transcription, metabolism in the most abundant bacteria in the ocean appears to be regulated by other mechanisms.

Investigations of metabolic regulation in bacteria have focused on isolates growing under laboratory conditions that differ substantially from those experienced by most microbes in the ocean. These laboratory studies have typically examined copiotrophic bacteria isolated and grown using high concentrations of organic substrates (1–3). Although possible during phytoplankton blooms and in particles (4, 5), such high concentrations are not common, so copiotrophic bacteria are much less abundant than oligotrophic bacteria (6). In most of the ocean, concentrations of organic compounds and inorganic nutrients are very low and impose strong selective pressure for oligotrophic bacteria (6). It remains unclear if the regulation mechanisms seen for copiotrophic bacteria are also used for regulating the metabolism of oligotrophic bacteria, the most abundant type of microbe in the ocean.

Some of the most abundant bacteria are in the SAR11 clade of *Alphaproteobacteria*, which accounts for as much as 30% of bacterial abundance in surface waters and occurs everywhere in the global ocean (7, 8). It has been proposed that the ecological success of these oligotrophs is the result of superior competitiveness for nutrient uptake (9) and highly efficient use of limiting resources (10). A cultivated representative of the SAR11 clade, "*Candidatus* Pelagibacter ubique," has one of the smallest genomes (1.3 Mbp) among free-living bacteria, only half to a quarter of the size of that in most bacteria, with a low proportion of noncoding DNA (11). These genomic features are consistent with the genome-streamlining hypothesis, which suggests that in very large microbial populations that frequently experience resource limitation, selection efficiently eliminates cellular and genomic complexity (12–14). One consequence of such reductive evolution in *Pelagibacter* appears to be the loss of most transcriptional regulatory elements (15, 16). The *Pelagibacter* genome includes only four two-component regulators involved in N and P limitation, osmotic stress, and cellular oxidation-reduction pathways and only two sigma factors, including the growth regulator *rpoD* and the heat shock factor sigma-32 (16). In contrast, bacterial genomes typically have tens to hundreds of transcriptional regulators (10). The numbers of two-component regulators differ among bacteria, but it is common for bacterial genomes to contain 10 or more such elements (17).

The paucity of transcriptional regulatory elements in the *Pelagibacter* genome and other evidence suggest that transcriptional regulation in this SAR11 representative is low. In one of the few studies directly examining transcription in *Pelagibacter*, Steindler et al. (18) demonstrated significant albeit small shifts in transcription in *Pelagibacter* exposed to light compared to darkness. The transcriptional response may have been small because the light

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and dark treatments had no impact on growth rates and the change in respiratory oxygen consumption was small (18). In a proteomic study, Sowell et al. (19) concluded that *Pelagibacter* responds adaptively to stationary phase by increasing the abundance of a few proteins rather than by remodeling the entire proteome. Unfortunately, transcriptional control was not explicitly examined in that proteomic study, and of the entire set of proteins potentially expressed by *Pelagibacter*, only 65% were detected (19). The intriguing results of that proteomic study and the work by Steindler et al. (18) point to the need for more study of transcriptional control in *Pelagibacter*.

Metatranscriptomic analyses of *Pelagibacter* populations in the oceans suggest that levels of transcriptional control may exceed those seen in cultivated *Pelagibacter* bacteria. For example, in coastal northern California waters, populations of archaea and bacteria, including *Pelagibacter*, displayed highly similar temporal synchronies of gene expression over a 2-day period (20). In those waters, *Pelagibacter* appeared to have tightly coordinated, genome-wide transcriptional regulation as revealed by high covariance between major metabolic pathways, including positive correlations between transcripts for ribosomal and oxidative phosphorylation proteins and negative correlations between transcripts for ribosomal and transport proteins, including members of the ATP binding cassette (ABC) transporter family (20). In another study, transcription in SAR11 populations closely related to *Pelagibacter* was not as steady as might have been expected from the pure culture work but oscillated over diel cycles in the Pacific Ocean, further suggesting the importance of transcriptional control in these bacteria (21). In short, in contrast to the laboratory experiments, field work suggests that *Pelagibacter* has the capacity for highly dynamic transcriptional responses, potentially linked to shifts in nutrient availability over small time scales (20).

In this study, we tested the hypothesis that levels of transcription vary less in *Pelagibacter* than in copiotrophic marine bacterial taxa. Levels of transcription were assessed for *Pelagibacter* (HTCC1062) and three other marine bacteria with different growth rates and potentially dissimilar levels of transcriptional control. In addition to *Pelagibacter*, we examined another oligotroph, the gammaproteobacterium SAR92 (HTCC2207), which also grows slower than copiotrophic bacteria and has a small (1.6-Mbp) genome (22, 23). It survives only at the low concentrations of organic substrates tolerated by oligotrophs (23, 24). Two copiotrophs were examined, *Ruegeria pomeroyi* (DSS-3) and *Polaribacter* (MED152), both with moderately sized genomes (4.1 Mbp and 2.9 Mbp, respectively) and capable of rapid growth on high concentrations of organic substrates (25–28). These copiotrophs appear to have complex life strategies incorporating such adaptations as particle attachment, motility, and environmental sensing, which likely require much transcriptional control. We found large differences between oligotrophs and copiotrophs in levels of transcription, highlighting the potential for contrasting control mechanisms between bacteria using different adaptive strategies in the ocean.

MATERIALS AND METHODS

Growth media and conditions. *Pelagibacter* and SAR92 were grown in a defined medium containing artificial seawater salts (AMS1; 29) with additions of pyruvate, glycine, methionine, and other organic substrates plus vitamins as described in reference 18. *R. pomeroyi* was grown using YTSS medium, which includes 0.4 g liter^{-1} yeast extract and 0.25 g liter^{-1} tryptone added to artificial seawater salts (Sigma-Aldrich). MED152 was grown in a medium with 5 g of peptone liter^{-1} and 1 g of yeast extract liter^{-1} added to the AMS1 salt solution. Cultures were maintained at 19°C in the dark and were aerated by bubbling with air filtered with a 0.2-μm-pore-size filter or by rotary shaking. Strains were grown in triplicate cultures and were sampled for transcriptomic analysis (one per culture) on the schedule indicated in Fig. 1.

Growth rates and cell C content. Bacterial abundance was monitored by flow cytometry using a BD FACSCalibur instrument, and samples were stained with SYBR green I (Invitrogen). Samples were stained at a concentration of 1:2,000 of the manufacturer-supplied solution for 30 min. Growth rates were calculated from the rate of change of bacterial abundance over time.

Particulate organic carbon levels were determined on samples filtered onto precombusted GF/F (Whatman) filters, rinsed with artificial seawater, and stored in a desiccated state at −20°C. The organic carbon was measured using an automated Perkin-Elmer 240B CHN analyzer.

Nucleic acid extraction. Bacterial biomass was collected from triplicate cultures of each strain by vacuum filtration using 0.2-μm-pore-size Durapore (Millipore) filters. The filters were stored at −80°C in RLT buffer (Qiagen) until DNA and RNA were extracted using an AllPrep DNA/RNA (Qiagen) kit following the manufacturer's instructions.

RNA sequencing abundance standard. Internal standard RNA molecules were used to obtain absolute quantification of transcripts based on the number of standard molecules added at the beginning of sample processing and those recovered in the sequence library (30, 31). RNA standards were prepared using *in vitro* transcription (RiboMax large-scale RNA production systems; Promega) from plasmid templates pTXB1 (New England BioLabs) and pFN18K (Promega), yielding single-stranded RNA transcripts of 917 nucleotides (nt) and 970 nt, respectively. The RNA standards were added immediately before nucleic acid extraction at a concentration of 0.5% (by mass) of the total RNA yield in the sample (30, 31).

Sequencing and analysis. RNA libraries were prepared from each replicate culture for sequencing using a Ribo-Zero rRNA removal kit (Bacteria) and a TruSeq RNA Sample Prep kit following Illumina protocols. No steps were taken to remove structural RNA other than rRNA. Sequences were obtained using an Illumina HiSeq 2500 instrument, generating paired-end reads using the 2-by-150-cycle protocol. Sequence data were analyzed by directed assembly against the corresponding genome sequences using Rockhopper software (http://cs.wellesley.edu/~btjaden/Rockhopper/) (32). Transcript abundance levels were estimated using the reads per kilobase per million (RPKM) measure, which sums the number of reads for a gene and divides by the transcript's length (33). Tests for differential gene transcription were performed using the DESeq algorithm (34). Briefly, Rockhopper estimates the transcript expression variance, obtains a smooth estimate of the variance using local regression, and then performs a statistical test of differential expression under two or more conditions. The negative binomial distribution is used as the statistical model in order to compute a *P* value indicating the probability that such a difference of observations in two samples was seen by chance alone. Finally, a false-discovery rate is calculated using the Benjamini-Hochberg procedure (35) to correct for multiple comparisons.

Accession number(s). Transcriptome sequencing data have been deposited in the Gene Expression Omnibus under accession number GSE66443.

RESULTS

To test the hypothesis that transcriptional control is low in oligotrophic bacteria, we examined the transcriptomes of *Pelagibacter*, SAR92, MED152, and *R. pomeroyi* during growth on defined media in batch cultures. As expected, the lowest maximum growth rates were seen in *Pelagibacter* and SAR92, both averaging ~0.4 day^{-1} (Fig. 1). In contrast, MED152 had a maximum growth rate of 2.6 day^{-1}, more than 6-fold higher than the growth rates of the

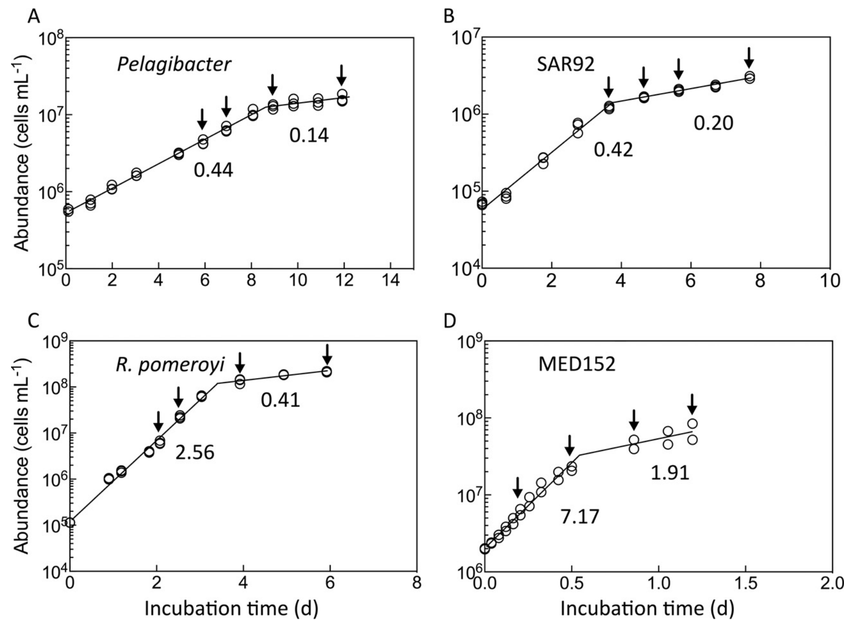

FIG 1 Abundance of four marine bacterial taxa, including *Pelagibacter* (A), gammaproteobacterium SAR92 (B), *R. pomeroyi* (C), and *Polaribacter* MED152 (D). Abundances were determined in triplicate (A, B, and C) or duplicate (D) cultures. Arrows indicate the times when transcripts were sampled. Fast-growth and slow-growth phases of the batch cultures are distinguished by the change in slope of the solid line, which was calculated by segmented regression analysis. *Pelagibacter*, *R. pomeroyi*, and MED152 were sampled twice during the fast and slow phases of growth. SAR92 was sampled once during the fast phase of growth and three times during the slow phase of growth. Values adjacent to the growth curves are growth rates (per day [d^{-1}]) calculated for the fast and slow phases of growth.

oligotrophs. The most rapidly growing strain was *R. pomeroyi*, with a maximum growth rate of 7 day^{-1}, 20-fold higher than the growth rates of the oligotrophs (Fig. 1). The growth rates of the two oligotrophs shifted 2- to 3-fold between the fast- and slow-growth phases, whereas the growth rates of MED152 and *R. pomeroyi* shifted 4-fold and 6-fold, respectively (Fig. 1).

Transcriptome sequence analysis revealed substantial differences among the four bacteria in how transcript levels changed over time. Transcriptomes from *Pelagibacter* in the fast- and slow-growth phases were indistinguishable (Fig. 2A). On average, transcript levels in *Pelagibacter* changed only 1.15-fold between the two growth phases (Fig. 2A). Similarly, the transcriptome of SAR92 changed very little between growth phases—only 1.21-fold between the phases of fast and slow growth (Fig. 2B). In contrast, the transcriptomes of MED152 and *R. pomeroyi* changed substantially between the fast- and the slow-growth phases in these batch cultures (Fig. 2C and D). On average, transcript levels in MED152 were up- or downregulated 1.66-fold between the two growth phases (Fig. 2C). Transcript levels in *R. pomeroyi* shifted even more—6.25-fold (Fig. 2D). In each taxon, a transcript for nearly every gene in the genome was seen in every transcriptome sample. The maximum number of genes not observed in a transcriptome sample ranged from 4 for *Pelagibacter* to 36 for *R. pomeroyi*. Therefore, shifts in transcript levels were not impacted by changes in the number of transcribed genes. These results suggest that transcriptional control is lower in the two oligotrophs than in the two copiotrophic bacteria examined here.

The fraction of genes for which levels of transcription differed between phases of growth was smaller for the two oligotrophs than for the two copiotrophic bacteria. The percentages of genes with different transcription levels differed significantly among the four marine bacterial taxa examined here (analysis of variance [ANOVA]; $P < 0.05$). In *Pelagibacter* and SAR92, the transcription level shifted >2-fold between the fast- and slow-growth phases for only 0.3% and 0.7% of genes, respectively (Fig. 3). In fact, the expression levels of only two *Pelagibacter* genes and one SAR92 gene changed more than 2-fold (Fig. 2A). In contrast, transcriptional control appeared to influence 10-fold to 100-fold more genes in MED152 and *R. pomeroyi* than in *Pelagibacter* and SAR92, respectively (ANOVA; $P < 0.05$). Transcription levels were shifted >2-fold for 3.5% of genes in MED152 and for 47% of genes in *R. pomeroyi* (Fig. 3).

Shifts in levels of transcription appeared higher for copiotrophs than for oligotrophs even after accounting for differences in growth rates among bacterial taxa. Overall, shifts in transcription were more prevalent in copiotrophs than oligotrophs, impacting 6-fold to 35-fold more protein-encoding genes in copiotrophs than oligotrophs (Table 1). Upregulated protein-encoding genes were 15-fold to 20-fold more prevalent in copiotrophs than oligotrophs when shifts in expression levels were normalized to changes in growth rate (Table 1). The extent of differential gene expression was smaller for downregulated genes than for upregulated genes. Among the four taxa, 2-fold to 20-fold more protein-encoding genes were downregulated in copiotrophs than in oligotrophs.

In all four taxa that we examined, a few genes were always highly transcribed while most genes were transcribed at very low levels. When cells shifted from the slow-growth phase to the fast-growth phase, significantly more genes were highly expressed in copiotrophs than in oligotrophs (Fisher's exact test; $P = 0.0006$; $n = 113$) (Fig. 4). In *Pelagibacter*, during fast and slow growth, only six and eight genes, respectively, were expressed at levels >10% of

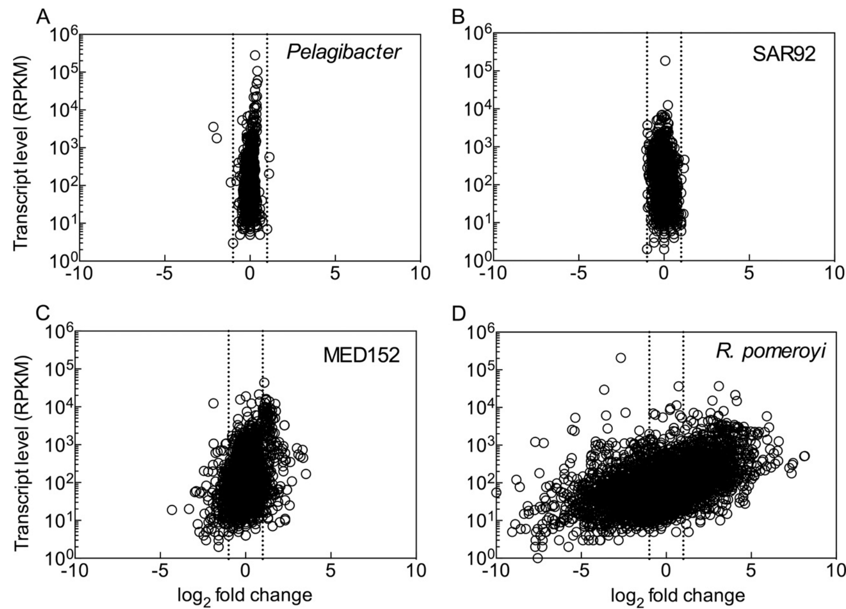

FIG 2 Transcript levels in the fast-growth and slow-growth phases of batch culture in four marine bacterial taxa, including *Pelagibacter* (A), gammaproteobacterium SAR92 (B), *Polaribacter* MED152 (C), and *R. pomeroyi* (D). Transcript levels during the fast-growth phase are plotted versus the $\log_2$ fold change in transcript level between the fast-growth phase and slow-growth phase of batch culture growth. Transcript level data represent the numbers of reads mapping to a gene normalized by the gene length and the total numbers of mapped reads (RPKM). The dotted lines indicate a 2-fold change in the level of transcription between the fast- and slow-growth phases.

that seen with the most highly transcribed gene (Fig. 4A); in SAR92, during the two growth phases, only one gene was transcribed at levels >10% of that seen with the most highly transcribed gene (Fig. 4B). During both growth phases, for *R. pomeroyi*, eight and five genes, respectively, were transcribed at levels >10% of that seen with the most highly transcribed gene (Fig. 4C). In contrast, in MED152, the highly transcribed genes appeared to shift substantially between the fast and slow phases of growth; 75 and 9 genes, respectively, were transcribed at levels >10% of those seen with the most highly transcribed genes (Fig. 4D).

We expected that genes encoding ribosomal proteins would be among the most highly transcribed because rRNA accounts for >95% of total RNA in bacteria (36). This was true for the two copiotrophs but not for the two oligotrophs examined here (Table 2). Genes for 30S and 50S ribosomal proteins were among the most highly transcribed in MED152 during the fast and slow phases of growth. In *R. pomeroyi*, ribosomal protein genes were also among the most highly transcribed during fast growth but not during the slow-growth phase. In contrast, no transcripts for ribosomal proteins were seen among the top 10 most highly transcribed genes in *Pelagibacter* and SAR92 (Table 2). Instead, transcripts for a porin and transporters dominated the most highly transcribed genes in *Pelagibacter*. In SAR92, the most highly expressed genes encoded a dioxygenase, a TonB receptor, and a flagellar protein (Table 2).

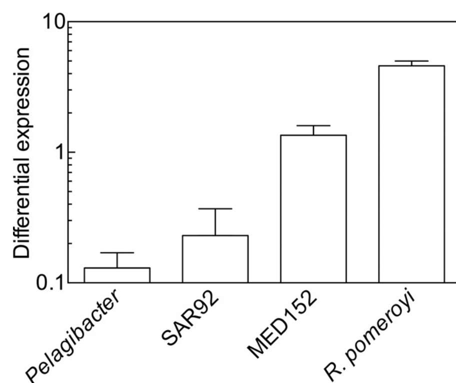

FIG 3 Percentage of protein-encoding genes normalized to shifts in growth rate in four marine bacterial taxa transcribed at significantly (false-discovery rate [FDR] < 0.05) higher (2-fold) levels during the fast-growth phase compared to the slow-growth phase of batch culture. Error bars are standard deviations (SD); $n = 3$ cultures.

Transcript abundances per cell, determined using an internal RNA sequencing standard (30), were low for even the most highly transcribed genes, and there was no correlation between transcript abundances per cell and levels of transcriptional control. Transcript abundances were low in the two oligotrophic taxa with little transcriptional control but also in the copiotrophic taxa with more transcriptional control. However, the transcriptomes of *Pelagibacter*, SAR92, and *R. pomeroyi* were very different from that of MED152. In *Pelagibacter*, SAR92, and *R. pomeroyi*, the abundance of most transcripts was <0.01 transcripts per cell, and there were two or more transcripts per cell for four genes at most in these three bacteria (Fig. 5). In contrast, levels of transcripts per cell were much higher in MED152; approximately 40% of genes in this bacterium were represented by two or more transcripts per cell (Fig. 5).

Total transcript abundances per cell were similar in the oligotroph strains. *Pelagibacter* and SAR92 contained 22.6 ± 12.3 and 57.1 ± 26.6 transcripts/cell, respectively. In comparison, total numbers of transcripts per cell differed 100-fold between the copiotrophs MED152 and *R. pomeroyi*, which contained 2,969 ±

TABLE 1 Percentages of protein-encoding genes whose transcription rates increased significantly by more than 2-fold as normalized to the growth rate during the fast versus slow growth phases[a]

	% of genes upregulated		% of genes downregulated		% of genes differentially expressed (total)	
Bacterial taxa	Mean	SD	Mean	SD	Mean	SD
Pelagibacter	0.01	0.03	0.12	0.04	0.13	0.04
SAR92	0.05	0.02	0.18	0.14	0.23	0.14
MED152	0.91	0.30	0.44	0.25	1.35	0.25
R. pomeroyi	2.24	0.13	2.36	0.41	4.60	0.41

[a] The growth rates of oligotrophs *Pelagibacter* and SAR92 and copiotrophs MED152 and *R. pomeroyi* shifted 2- to 3-fold and 4- to 6-fold, respectively, between the fast- and slow-growth phases. False-discovery rate (FDR), <0.05; $n = 3$.

2,805 and 41.8 ± 49.4 transcripts/cell, respectively. In comparison, model bacteria such as *Escherichia coli* have about 1,500 mRNA molecules per cell (37, 38). The differences among the four bacteria were also large (about 100-fold) when transcript abundances were normalized to cell C content. Oligotrophs *Pelagibacter* and SAR92 contained 4 ± 5 and 2 ± 9 transcripts/fg C, respectively, and copiotrophs MED152 and *R. pomeroyi* contained 16 ± 59 and 0.2 ± 12 transcripts/fg C, respectively. The transcript abundances normalized to cell C content in these marine bacteria were similar to the 3.9 transcripts/fg C calculated for *E. coli* with a C content of 350 fg C/cell (39). Overall, our results revealed similarities among transcript abundances in marine bacteria and *E. coli* as well as differences among marine bacteria that may have implications for the impact of transcriptional control on these marine microbes.

DISCUSSION

Regulation of metabolism and growth in bacteria may occur at the level of transcription or through posttranscriptional or other regulatory mechanisms. Changes in transcription promptly result in shifts in transcript levels because mRNA is degraded so rapidly; the half-life of mRNA in laboratory-grown *E. coli* is only about 5 min (38, 40–42) whereas it is 2.4 min in cultures of the marine cyanobacterium *Prochlorococcus* MED4 (43). Therefore, analysis of transcript levels under changing environmental conditions reveals the extent of transcriptional control in bacteria. In this study, we found differences among the four bacterial taxa in how transcript levels changed as growth rate changed, suggesting that transcriptional control is lower in the two oligotrophic bacteria than in the two copiotrophic bacteria examined here.

The low level of transcriptional control seen in *Pelagibacter* and SAR92 supports the hypothesis that bacteria with streamlined genomes have alternative ways of responding to the environment compared to other bacteria with larger genomes such as MED152 and *R. pomeroyi*. When transcriptional control is not used, the amount of DNA dedicated to transcriptional regulators can be minimized (10). The paucity of these regulators is consistent with our results showing minimal transcriptional control in *Pelagibacter* and in the other oligotroph, SAR92. Similarly, Lankiewicz et al. (44) demonstrated using quantitative PCR that ribosome abun-

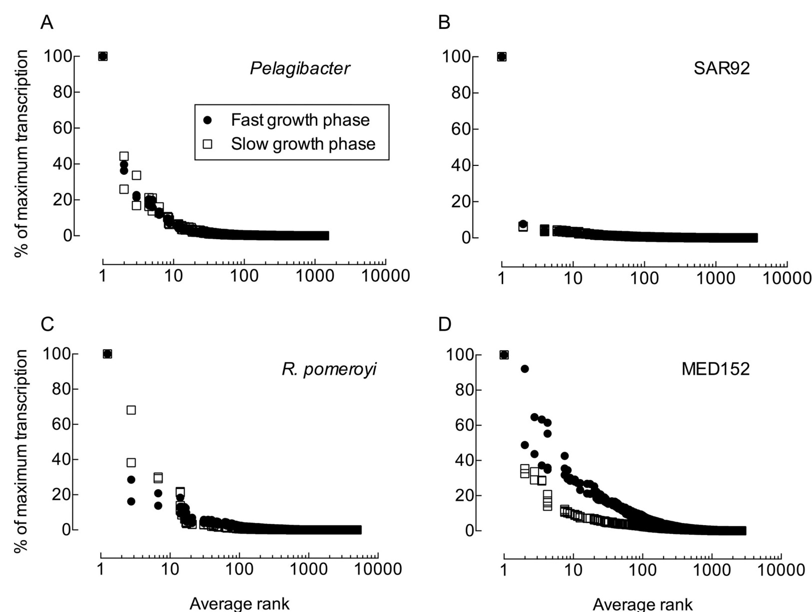

FIG 4 Rank abundance of (A) *Pelagibacter*, (B) gammaproteobacterium SAR92, (C) *R. pomeroyi*, and (D) *Polaribacter* MED152 transcripts during the fast-growth and slow-growth phases of batch culture. The rank for each transcript was determined from the average abundance determined for four samples collected during the fast-growth and slow-growth phases of batch culture for *Pelagibacter*, *R. pomeroyi*, and MED152. The SAR92 culture was sampled once during the fast-growth phase and three times during the slow-growth phase. Maximum transcription was set to 100% for the transcript with a rank value of 1.

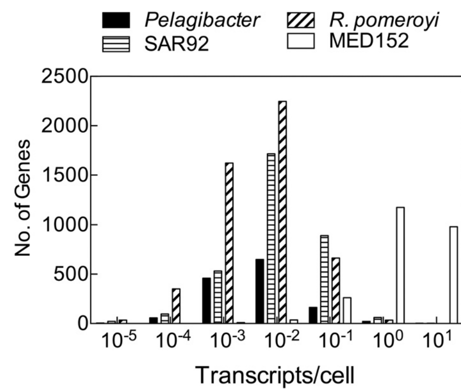

FIG 5 Frequency distribution of transcript abundance per cell for protein-encoding genes in *Pelagibacter*, gammaproteobacterium SAR92, *R. pomeroyi*, and *Polaribacter* MED152. Values are averages ($n = 4$) for the entire growth curve. The numbers on the *x* axis indicate the upper limit for each bin.

TABLE 2 The 10 most highly transcribed genes during the fast- and slow-growth phases of batch cultures of oligotrophs *Pelagibacter* and SAR92 and copiotrophs MED152 and *R. pomeroyi*[a]

	Bacterial gene product or product function and growth phase							
	Pelagibacter		SAR92		MED152		*R. pomeroyi*	
Rank	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
1	Porin	Putative porin	Biphenyl 2,3-dioxygenase	Biphenyl 2,3-dioxygenase	Elongation factor Tu	Elongation factor Tu	Porin	Hypothetical protein
2	Bacterio-rhodopsin	Bacterio-rhodopsin	Hypothetical protein	TonB receptor	OmpA protein	Hypothetical protein	Lipoprotein	Hypothetical protein
3	Spermidine/putrescine	Spermidine/putrescine	Hypothetical protein	Hypothetical protein	50S ribosomal	50S ribosomal	Hypothetical protein	Transcriptional regulator
4	ABC transporter	ABC transporter	TonB receptor	Hypothetical protein	30S ribosomal	OmpA family	Ribosomal protein	Lipoprotein, putative
5	TRAP transporter	TRAP transporter	Hypothetical protein	Cold shock protein	Hypothetical protein	TonB channel	Hypothetical protein	Cold shock protein
6	Na^+/solute symporter	Cold shock DNA binding	Flagellar protein	Elongation factor Tu	30S ribosomal	ATP synthase	Lipoprotein, putative	Membrane protein
7	Hypothetical protein	Hypothetical protein	Hypothetical protein	Homocysteine methyltransferase	30S ribosomal	30S ribosomal	Ribosomal protein	Hypothetical protein
8	Cold shock DNA-binding	Na^+/solute symporter	Homocysteine methyltransferase	Hypothetical protein	ATP synthase	30S ribosomal	50S ribosomal	Membrane porin
9	Unknown function	Iron uptake transport	TonB receptor	Flagellar protein	50S ribosomal	50S ribosomal	Cold shock CspA	Hypothetical protein
10	Aminomethyl-transferase	Unknown function	Cold shock protein	TonB receptor	TonB channel	50S ribosomal	Ribosomal protein	Lipoprotein

[a] "Fast" and "Slow" refer to the initial rapid phase of exponential growth and the slower phase of growth that followed. The growth rates of oligotrophs and copiotrophs shifted 2- to 3-fold and 4- to 6-fold, respectively, between the fast- and slow-growth phases.

dance did not change greatly in *Pelagibacter* and SAR92 when growth rates changed in the batch cultures examined here, consistent with our hypothesis that transcriptional regulation is not an important regulatory mechanism in these oligotrophs. This minimal control is probably possible only for a simple lifestyle that lacks cellular activities typically under transcriptional control, such as motility, chemotaxis, and production of hydrolytic enzymes for degrading polymeric organic materials. Minimizing transcriptional control is a step in genome streamlining and in reducing genome size which should be advantageous for oligotrophic bacteria in nutrient-limited environments, most notably, the oceans (10).

Another consequence of minimizing transcriptional control may be low transcript abundance per cell and less C, N, and P tied up in mRNA, augmenting the benefits of a small genome in oligotrophic environments. The total transcript abundance per cell was much lower for three of the four taxa examined here than for other bacteria in pure culture, which contain thousands of transcripts per cell (38, 45, 46). The two oligotrophs and *R. pomeroyi* had less than 100 transcripts per cell whereas the copiotroph MED152 had nearly 3,000 transcripts per cell. In contrast, transcript abundances are lower in natural communities of bacteria, which contain tens to hundreds of transcripts per cell (31, 47), similar to the values for *Pelagibacter*, SAR92, and *R. pomeroyi*. The low transcript abundance seen in the copiotroph *R. pomeroyi* suggests that this taxon is representative of the abundant copiotrophic bacteria in seawater. It remains unclear if the high transcript abundance of MED152 is common among marine copiotrophs.

The abundance of transcripts has implications for understanding growth rates and transcriptional control in oligotrophic and copiotrophic bacteria. Since growth rates affect the ecological success of microbes and their contribution to food web dynamics and roles in biogeochemical processes (50), transcription is likely linked to the ecology and impact of bacteria on marine ecosystems. We examined the relationship between growth rate and transcript abundances separately for the oligotrophs and copiotrophs because, similarly to most cultivated copiotrophs, the growth rates of *R. pomeroyi* and MED152 were much higher than those of most bacteria in the ocean (50). In contrast, the growth rates of *Pelagibacter* and SAR92 are representative of the growth

rates in natural communities. There was no significant correlation between growth rate and transcript abundances for the oligotrophs ($r = 0.26$; $P > 0.05$; $n = 8$), which is consistent with low transcriptional control in *Pelagibacter* and SAR92. In contrast, growth rate and transcript abundances were highly correlated in the copiotrophs ($r = 0.92$; $P < 0.001$; $n = 8$), suggesting substantial transcriptional control of growth in *R. pomeroyi* and MED152. The ecological success of copiotrophic bacteria may depend on their capacity for rapid growth when high substrate concentrations are encountered (50), whereas oligotrophs appear to lack such transcriptional responses under conditions of changing growth rates. The transcript abundance data are another indication that transcriptional control of growth is a factor in the adaptive strategy of copiotrophic but not oligotrophic bacteria in the ocean.

The low total number of transcripts per cell for the two oligotrophs and *R. pomeroyi* is consistent with our data indicating that, for most genes, a cell of these three bacteria contains less than a single copy of the transcript. It seems likely that transcript abundance per gene for bacteria in natural communities is also as low because the total number of transcripts is low (31, 47), similar to the levels in the two oligotrophs and *R. pomeroyi*. The observation that the transcript abundance was <1 per cell for most genes seems counter to the notion that at least one transcript must be present in order to synthesize a protein. The distribution of transcript abundances among cells and RNA turnover data help to reconcile this issue. The estimates of the numbers of transcripts per cell are averages for the entire culture at one time point. It is possible that some cells do have at least one copy per cell while enough other cells have none because of RNA turnover, resulting in the observed estimate.

The results from this laboratory study have implications for examining metatranscriptomes and adaptive strategies of bacteria in the oceans, although of course there are differences between our batch cultures and natural communities. Under the batch culture conditions examined in this study, the physiological limitations causing the growth rate to slow differ from those in exponentially growing cells. Nutrient concentrations decline in batch culture, causing growth rates to slow. Under natural conditions, growth may be controlled by the nutrient supply rate under conditions of constant nutrient concentrations. These conditions may be best mimicked with continuous cultures, although oligotrophic bacteria like *Pelagibacter* are difficult to grow even in batch cultures.

In spite of the differences between batch cultures and natural communities, our results are consistent with those of a metatranscriptomic study previously conducted in the coastal Atlantic Ocean, which found that the transcriptomes of oligotrophic taxa, such as SAR11, typically are not diverse and have few transcripts of genes involved in sensing the environment and responding to stimuli, which typically requires transcriptional control (51). However, another metatranscriptomic study of waters in the Pacific Ocean suggested that the SAR11 transcriptome is diverse (20), more so than in the Atlantic (51), and is more variable than was detected in our study of *Pelagibacter*, suggesting more transcriptional control for SAR11 in the Atlantic than in the Pacific and in *Pelagibacter* pure cultures. Still another metatranscriptomic study in the Pacific Ocean demonstrated offsets in the timing of transcription maxima among SAR11 and other oligotrophs over three diel cycles (21). Oscillations in levels of transcription differed among heterotrophic bacterial groups and gene suites, suggesting population and metabolic pathway-specific patterns of transcriptional control. It is unclear why transcriptional control in SAR11 bacteria in the Atlantic Ocean and in *Pelagibacter* in culture differs from that seen in the Pacific Ocean, but the complete explanation probably involves diversity within the SAR11 clade (52).

Examination of growth and transcription in cultivated representatives of the most abundant bacteria in the ocean yielded valuable insights into differences in how metabolism is controlled in oligotrophic versus copiotrophic bacteria. Our findings suggest that the growth strategy of marine oligotrophs does not rely on transcriptional control, which is the most common type of control seen in bacteria (53) and the type seen in the two copiotrophs examined here. Understanding the linkages among environmental factors, bacterial metabolism, and ecosystem processes will require better knowledge of the control mechanisms used by oligotrophs, which dominate bacterial communities in the ocean. Although some oligotrophs and copiotrophs may deviate from the overall pattern seen here, our conclusions were drawn from data on model taxa, most importantly, a SAR11 isolate, representing some of the most abundant bacteria in the ocean (7, 8, 22, 23). The kinds of genetic controls used by oligotrophs have implications for understanding the networks of interactions among bacteria that appear to organize microbial communities (54). That the most abundant bacteria use alternatives to transcriptional control complicates the use of metatranscriptomic analyses to assess microbial activities in the ocean. New analytical methods and conceptual models are needed to better describe how the factors controlling bacterial activities link networks of bacterial communities, their metabolism, and biogeochemical processes in the ocean.

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