

Supplementary Information for

Prevalent reliance of bacterioplankton on exogenous vitamin B1 and precursor availability

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Tables S1 to S11
References for SI reference citations

Other supplementary materials for this manuscript include the following:

SI Datasets: Dataset S1 to S6

Supplementary Information.

Methods.

Correlation analyses. Spearman rank correlations between B1-related gene abundance and corresponding environmental data from times of LMO metagenome sampling (1) were calculated using the spearmanr function in the scipy python package with nan-policy set to 'omit'. p-values were corrected with the step-down method using Bonferroni adjustments (implemented as "holm" in the python package statsmodels). The data analyzed were % normalized gene abundance values and raw environmental values, except that DOC values >400 μM were set to NA (as these were identified as extreme outliers).

ThiE amino acid tree generation. Nineteen ThiE amino acid sequences were retrieved from UNIPROT and aligned using the Clustal Omega program (<http://www.uniprot.org/align/>). A Maximum Likelihood tree was generated in MEGA7 (2) from the aligned sequences using a Poisson correction model (3). The tree with the highest log likelihood is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair-wise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A total of 239 positions were used in for the tree generation process.

Calculation of B1 diffusive flux to 1 μm diameter bacterioplankton cell, after Zehr, Weitz, and Joint 2017 (4).

B1 molecules per μm^3 of water (x), assuming a concentration (p) of 1 pM B1:

$$x = 0.001 \text{ (nmol/L)} * 10^{-9} \text{ (mol/nmol)} * 10^{-3} \text{ (L/cm}^3\text{)} * 10^{-12} \text{ (cm}^3/\mu\text{m}^3\text{)} * 6.02 \times 10^{23} \text{ (molecules/mol)}$$

$$x = 6 \times 10^{-4} \text{ B1 molecules}/\mu\text{m}^3, \text{ which is } 6 \times 10^8 \text{ B1 molecules}/\text{cm}^3$$

Daily diffusive flux (J) of B1 to a (assumed spherical) bacterioplankton cell with a radius (r) of 1 μm and a diffusion coefficient (D) for citrate (in place of B1(5)):

$$J \approx 4\pi * D * r * p * 86400 \text{ s/d}$$

$$J \approx 4\pi * (3.22 \times 10^{-6} \text{ cm}^2/\text{s}) * (1 \times 10^{-3} \text{ cm}) * (6 \times 10^8 \text{ B1 molecules}/\text{cm}^3) * 86400 \text{ s/d}$$

$$J \approx 2.16 \times 10^6 \text{ B1 molecules/d}$$

Cell demand for Vibrio anguillarum PF430-3 is maximally 5.3×10^2 B1 molecules/cell (Table S7). Dividing the daily flux of B1 by cell demand (given one division per day) yields an estimate of excess B1 molecules encountered by the cell:

$$\text{Ratio} = (2.16 \times 10^6 \text{ B1 molecules/d}) / (5.3 \times 10^2 \text{ B1 molecules/d}) = \sim 4.0 \times 10^3 \text{ excess B1 molecules reaching the cell via diffusion}$$

Supplementary Figures

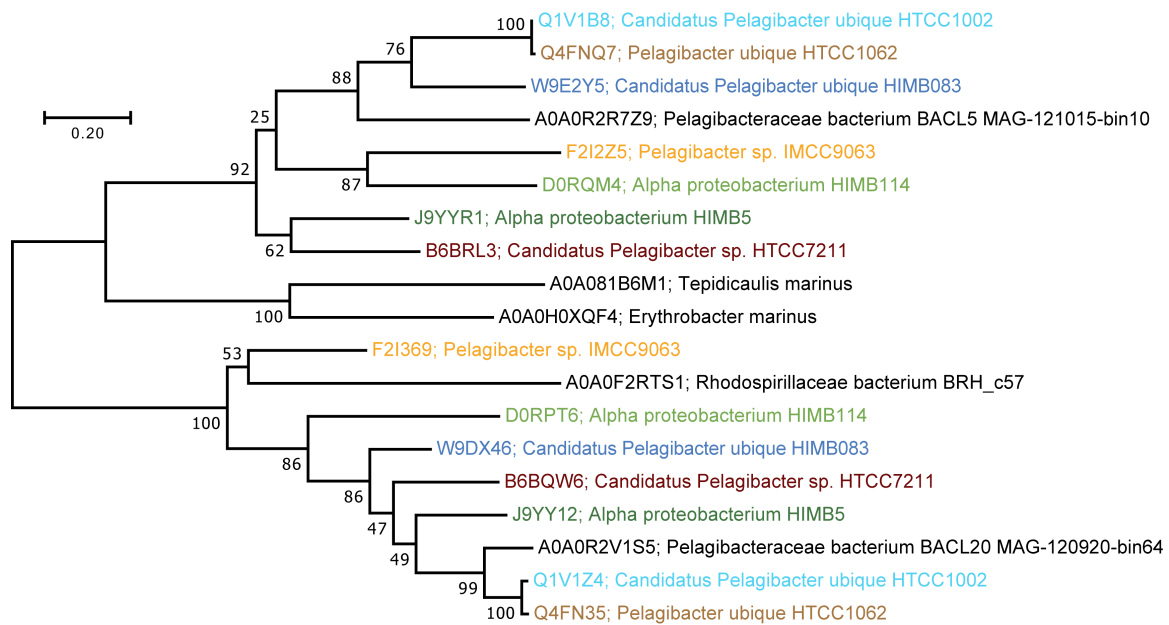


Figure S1. An unrooted maximum likelihood tree of thiamine monophosphate synthase (ThiE) amino acid sequences from *Pelagibacterales* spp. and *Alphaproteobacterial* relatives. The clustering of ThiE sequences highlights multiple isoforms of ThiE exist within (e.g. HTCC1002) and between (BACL5 MAG versus BACL20 MAG) the populations. UNIPROT ID's are listed in front of strain names. Colored isolate names are those recognized to possess multiple ThiE sequences. Bootstrap values >50 (%) noted at nodes are derived from 1000 replicate trees. The plotted scale represents the number of residue (amino acid) substitutions (0.20) per site.

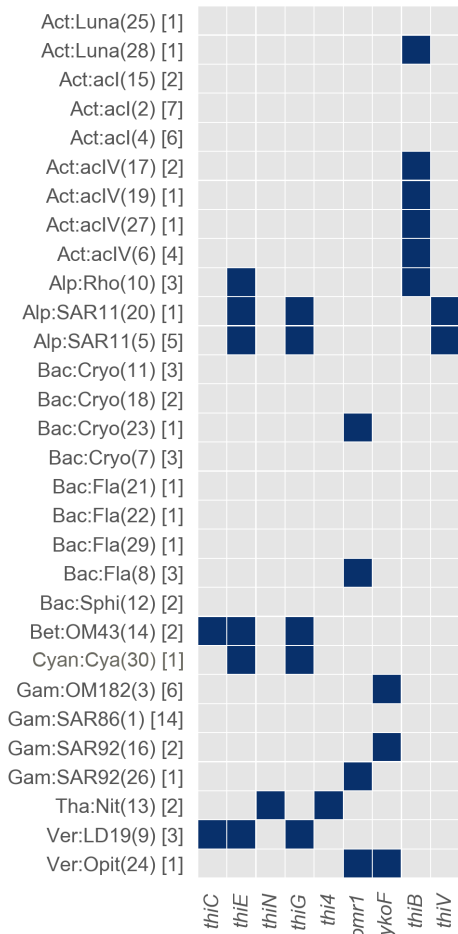


Figure S2. Different B1-related transporters were detected in LMO MAG clusters – including one cluster with multiple transporter types (Ver:Opit(24) – possessing *omr1* and *ykoF* genes). Blue squares indicate detection of the protein-encoding gene.

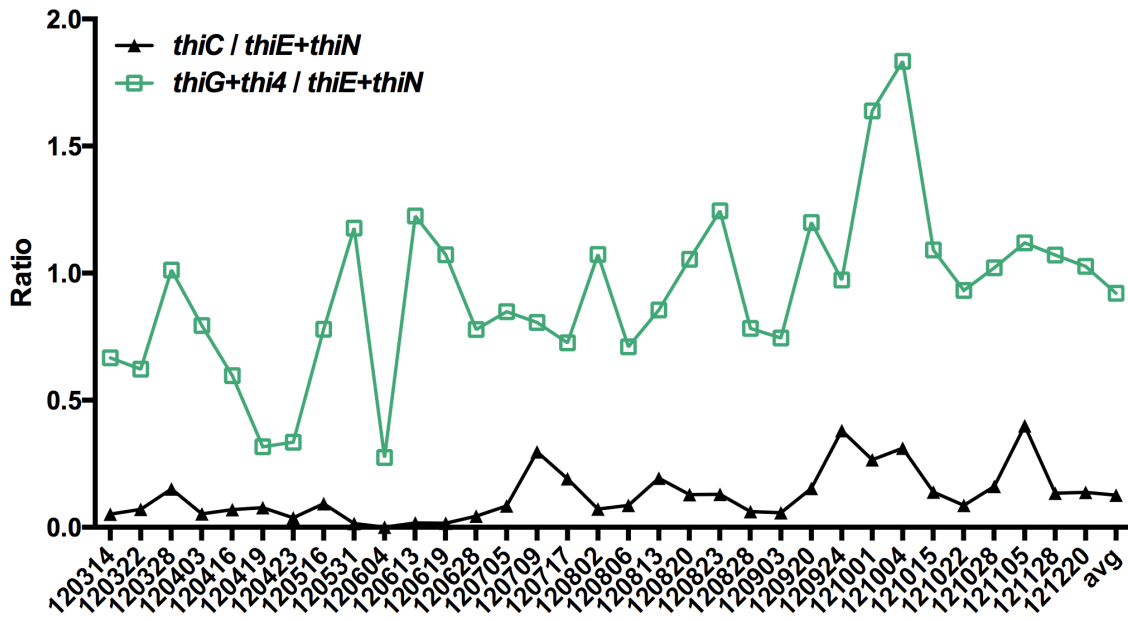


Figure S3. Ratios of pyrimidine B1 biosynthesis gene *thiC* to thiamine monophosphate genes (*thiE* + *thiN*), and thiazole B1 biosynthesis genes (*thiG* + *thi4*) to thiamine monophosphate genes (*thiE* + *thiN*) across LMO metagenomes (timestamp format is: YR:MO:DA; e.g. March 14, 2012 = 120314). The final data point on the far right represents the average (avg) ratio across all metagenomes.

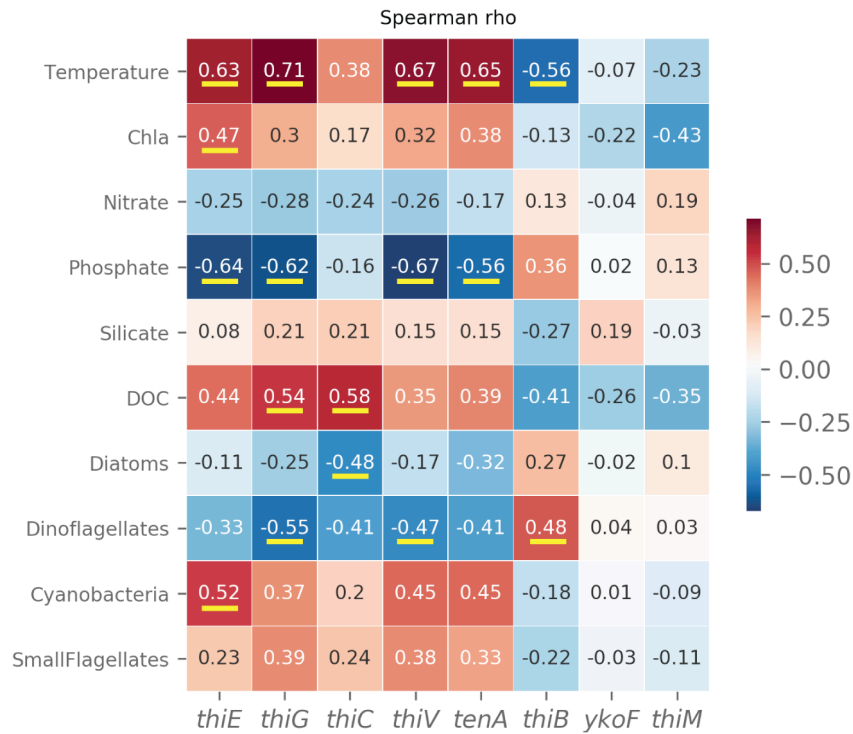


Figure S4. Spearman rank correlations between abundance (% normalized gene abundance) of B1 biosynthesis, salvage, and uptake genes identified in the LMO metagenomes, and measured environmental parameters (1). Values indicate the type of correlation (Spearman rho; red being positive and blue being negative). Values with a yellow underline are significant correlations with respective p values <0.05.

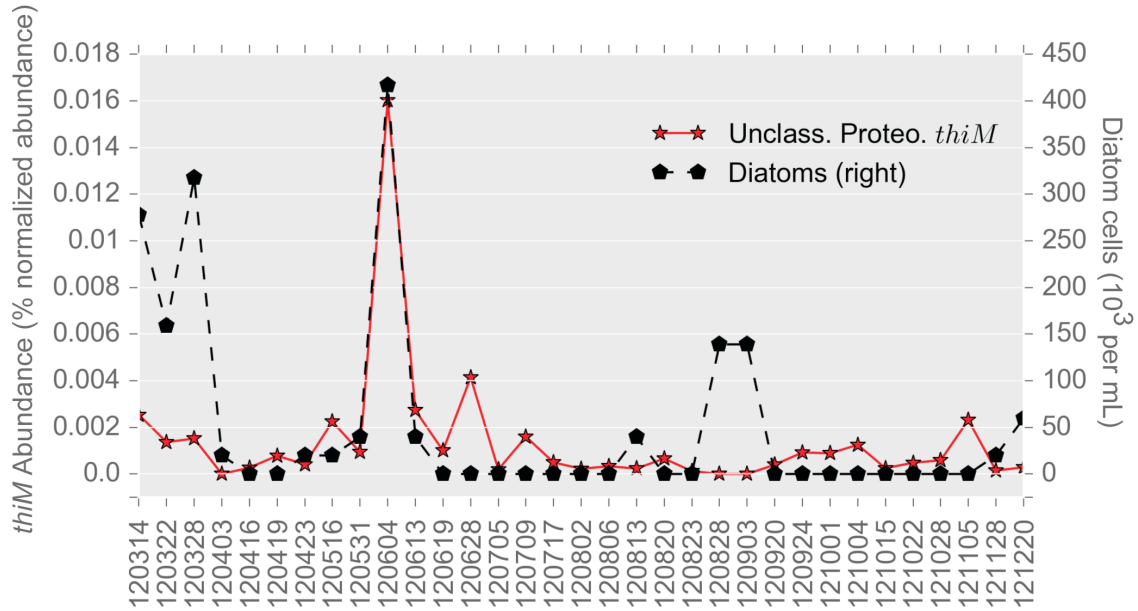


Figure S5. Abundance (% normalized abundance) of unclassified Proteobacterial *thiM* genes and total diatom cell abundances across the LMO dataset. A pronounced short-term peak in *thiM* abundance occurred in early summer, coinciding a notable peak in diatom cell abundance. Sampling dates (in two digit year, month, and day format) linked to respective metagenomes are presented along the x-axis.

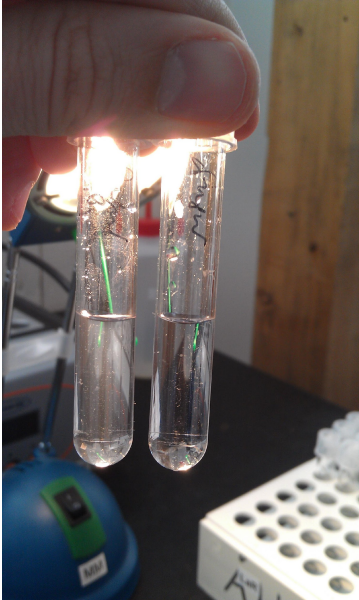


Figure S6. A photograph of the B1-auxotrophic *V. anguillarum* PF430-3 Δ ThiE mutant line growing to high concentrations when provided 1 nM B1 (left) versus a no addition negative control (right). Further experiments confirmed PF430-3 Δ ThiE grows only upon intact B1 (Table S5).

Supplementary Tables

Table S1. Protein models used in surveys for protein sequences attributed to B1 biosynthesis, uptake, or salvage.

<i>Protein Target</i>	<i>Protein Model</i>
ThiN	PF10120
Thi4	TIGR00292
ThiC	TIGR00190
ThiD	TIGR00097
ThiE	TIGR00693
TMP-TENI	PF02581
TenA	PF03070
ThiM	TIGR00694
TenA	TIGR04306
ThiaminaseI	TIGR04541
CytX	TIGR02358
ThiB 1	TIGR01254
ThiB 2	TIGR01276
ThiP	TIGR01253
ThiQ	TIGR01277
ThiT	TIGR02357
ThiW	TIGR02359
YkoF	PF07615
SSUA/Thi5/NMT1	PF09084
SSSP	PF00474
ThiPerm	PF02133
ThiG	PF05690
ThiY	thiY_custom
ThiV	thiV_custom
Omr1	omr1_custom
ThiM	PF02110
PnuC	TIGR01528

Table S2. Results from a B1-auxotrophy survey of 25 bacterial strains isolated from Baltic Sea surface waters. B1 was added in all surveys at a final concentration of 500 pM. A = Auxotrophy; P = Prototrophy.

Strain ID	Class Affiliation	Accession #	Proto./Auxo.?
BAL116	<i>Actinobacteria</i>	DQ063056	A
BAL246	<i>Actinobacteria</i>	DQ063182	A
BAL115	<i>Actinobacteria</i>	DQ063055	A
BAL248	<i>Actinobacteria</i>	DQ063184	A
BAL206	<i>Actinobacteria</i>	DQ063144	P
BAL209	<i>Actinobacteria</i>	AY962021	A
BAL96	<i>Alphaproteobacteria</i>	DQ063036	A
BAL108	<i>Alphaproteobacteria</i>	DQ063048	P
BAL100	<i>Alphaproteobacteria</i>	DQ063040	P
BAL163	<i>Alphaproteobacteria</i>	DQ063103	P
BAL176	<i>Bacteroidetes</i>	DQ063116	P
BAL241	<i>Bacteroidetes</i>	DQ063177	P
BAL180	<i>Bacteroidetes</i>	DQ063120	P
BAL64	<i>Bacteroidetes</i>	DQ063004	P
BAL268	<i>Bacteroidetes</i>	DQ063204	A
BAL242	<i>Bacteroidetes</i>	DQ063178	P
BAL162	<i>Betaproteobacteria</i>	DQ063102	P
BAL253	<i>Betaproteobacteria</i>	DQ063189	P
BAL110	<i>Betaproteobacteria</i>	DQ063050	P
BAL109	<i>Betaproteobacteria</i>	DQ063049	P
BAL361	<i>Gammaproteobacteria</i>	KC140295	P
BAL286	<i>Gammaproteobacteria</i>	AY972873	P
BAL66	<i>Gammaproteobacteria</i>	DQ063006	A
BAL144	<i>Gammaproteobacteria</i>	DQ063084	P
BAL67	<i>Gammaproteobacteria</i>	DQ063007	P

Table S3. Of 330 total surveyed complete bacterioplankton genomes, only 14 lack *thiE* (Bacteria) or *thiN* (Archaea) – encoding for thiamine monophosphate synthase.

<i>IMG_taxid</i>	<i>Habitat</i>	<i>Kingdom</i>	<i>Species</i>	<i>thiE</i>	<i>thiN</i>
2504643007	Fresh	Bacteria	<i>Candidatus Rhodoluna laticola</i>	0	0
644736368	Fresh	Bacteria	<i>Flavobacteriaceae bacterium 3519-10</i>	0	0
650377973	Fresh	Bacteria	<i>Sphaerochaeta globosa</i>	0	0
2508501070	Fresh	Bacteria	<i>Sphaerochaeta pleomorpha</i>	0	0
2503982041	Marine	Bacteria	<i>Alpha Proteobacterium HIMB59</i>	0	0
2516143106	Marine	Bacteria	<i>Francisella sp. FSC1006</i>	0	0
2511231080	Marine	Bacteria	<i>Glaciecola nitratreducens</i>	0	0
639633025	Marine	Bacteria	<i>Gramella forsetii</i>	0	0
648028027	Marine	Bacteria	<i>Maribacter sp. HTCC2170</i>	0	0
649633077	Marine	Bacteria	<i>Oceanithermus profundus</i>	0	0
641228500	Marine	Bacteria	<i>Petrotoga mobilis</i>	0	0
638341218	Marine	Bacteria	<i>Polaribacter sp. MED152</i>	0	0
646311950	Marine	Bacteria	<i>Robiginitalea biformata</i>	0	0
2556921662	Marine	Bacteria	<i>Spirochaeta sp. L21-RPul-D2</i>	0	0

Table S4. A table of bacterioplankton isolates with publically available complete genome sequences that possess >1 copy of thiazole synthase *thiG* or *thi4*. The detection of genes is highlighted in blue. Respective symbols indicate: * = *thiG* is not common in Archaea, and manual inspection revealed that positive hits were to pyrroloquinoline-quinone synthase; this false positive occurred only in this Archaea, as well as *Thermoplasma volcanium* (one sequence; not shown here); ^ = *Sinorhizobium meliloti* possesses a second truncated *thiG* sequence located on a plasmid - we presume the short *thiG* sequence does not lead to a fully functional ThiG protein.

<i>IMG_taxid</i>	<i>Habitat</i>	<i>Kingdom</i>	<i>Species</i>	<i>thiG</i>	<i>thi4</i>
650716056	Marine	Archaea	<i>Methanotorris igneus</i>	0	2
650716079	Marine	Archaea	<i>Pyrococcus sp. NA2</i>	0	2
650716080	Marine	Archaea	<i>Pyrococcus yayanosii</i>	0	2
2511231148	Marine	Archaea	<i>uncultured marine group II euryarchaeote</i>	2*	0
2512564006	Marine	Bacteria	<i>Octadecabacter arcticus</i>	2	0
650716086	Marine	Bacteria	<i>Sinorhizobium meliloti</i>	2^	0

Table S5. Multiple B1-related transporters were detected in LMO metagenomes; their confirmed or predicted functions are included. Highlighted transporters were also detected in the LMO metagenomes, however, their respective protein models pickup proteins other than B1-related transporters and hence were not used in our analyses. * = see PFAM protein family PF09084 (<http://pfam.xfam.org/family/PF09084>).

Transporter	Detected at LMO?	Function
<i>thiPerm</i>	Yes	Transporter of nucleobase or vitamin; Performed by cation symport - facilitated diffusion (6)
<i>thiV</i>	Yes	HMP transport; Possibly B1+HMP in select organisms; driven by electrochemical sodium gradient (6, 7)
<i>thiB</i>	Yes	B1 or phosphorylated B1 transporter; ATP-dependent transport (8)
<i>omr1</i>	Yes	TonB dependent transporter; H ⁺ gradient driven transport (9)
<i>thiY</i>	Yes	Pyrimidine precursor binding; Binds the formylaminopyrimidine N-formyl-4-amino-5-aminomethyl-2-methylpyrimidine (FAMP); ATP-dependent (10)
<i>ykoF</i>	Yes	B1 and/or HMP transport; ATP-dependent (6, 11)
<i>cytX</i>	No	Putative Hydroxymethylpyrimidine transporter (6)
<i>pnuC</i>	No	Nicotinamide mononucleotide transport, some may function in B1 transport (12)
<i>thiT</i>	No	B1 transporter; lower affinity for phosphorylated B1; energy coupling factor transport, ATP-dependent transport (13)
<i>thiW</i>	No	Putative thiazole precursor transporter (6)
SSSP	Yes	Broad family of sodium solute transporters; driven by electrochemical sodium gradient (14)
SSUA/Thi5/NMT1	Yes	A broad family of pyrimidine precursor binding proteins-including biosynthesis proteins in prokaryotes and eukaryotes*

Table S6. A summary of B1-related lifestyles and respective genotypes present amongst endemic LMO bacterioplankton as well as bacterioplankton isolates. *= Transporter component *thiY* is implicated in B1 transport (15), but also pyrimidine precursor transport (6, 16). # = evidence presented in this study; ## = evidence provided in Table S11.

<i>Auxotroph possessing a partial pathway, has thiamine monophosphate synthetase (thiE or thiN)</i>		
<u>Lifestyle</u>	<u>Genotype</u>	<u>Examples & References</u>
Pyrimidine Auxotroph	Missing <i>thiC</i>	Most bacterioplankton [#] ; <i>Pelagibacter ubique</i> HTCC1062 (7)
Thiazole Auxotroph	Missing <i>thiG</i> (Bacteria) or <i>thi4</i> (Archaea)	<i>Methylococcus capsulatus</i> ^{##} , others [#] ; White or pigmented isolates with unknown identity (17)
Dual Auxotroph	Missing <i>thiC</i> and <i>thiG</i> or <i>thi4</i>	<i>Rhodobacterales</i> [#] ; <i>Dokdonia</i> MED134 (9)
<i>Auxotroph lacking thiamine monophosphate synthetase</i>		
<u>Lifestyle</u>	<u>Genotype</u>	<u>Examples</u>
Thiamine Obligate Requirer	Missing <i>thiE</i> (Bacteria) or <i>thiN</i> (Archaea)	<i>Cellvibrionales</i> , <i>Sphingobacterales</i> , and others [#] (17); <i>Pelagibacter sp.</i> , HIMB59 (7)
<i>Exogenous compound use</i>		
<u>Lifestyle</u>	<u>Genotype</u>	<u>Examples</u>
Pyrimidine AmMP Scavenger	Possesses <i>tenA</i>	<i>Pelagibacterales</i> [#] , <i>Actinobacteria</i> [#] , and Unclassified taxa [#] ; <i>Pelagibacterales sp.</i> IMCC9063 (7); White/pigmented isolates with unknown identity (17)
Pyrimidine Scavenger	Possesses <i>thiV</i> or <i>thiY</i>	<i>Pelagibacter ubique</i> HTCC1062 (7); <i>Alphaproteobacteria</i> [#]
Thiazole Precursor Scavenger	Possesses <i>thiM</i>	Unclassified Proteobacteria [#] ; <i>Roseobacter sp.</i> GIA101 and other diverse strains (18); White or pigmented isolates with unknown identity (17)
Intact B1 Scavenger (including B1 prototrophs)	Possesses <i>thiB</i> ; <i>ThiPerm</i> ; <i>thiY</i> *; <i>omr1</i>	<i>Vibrio anguillarum</i> PF430-3 [#] ; <i>Pelagibacter sp.</i> HIMB59 (7); <i>Dokdonia</i> MED134 (9); Diverse lineages [#]

Table S7. Growth of *Vibrio anguillarum* PF430-3 $\Delta thiE$ upon addition of B1 and related compounds in B1-deplete medium. Positives indicate turbidity in triplicate cultures containing washed/resuspended cells (diluted 1:1000 at start of the survey). Negatives indicate no turbidity. AmMP = 4-amino-5-aminomethyl-2-methylpyrimidine; NEG CON = negative control, no compound addition.

<i>1 nM additions</i>	$\Delta thiE$
HMP	-
B1	+
HET	-
NEG CON	-

<i>100 pM or 5 pM additions</i>	$\Delta thiE$
AmMP	-
HMP	-
B1	+
HET	-
NEG CON	-

Table S8. B1 and thiamine diphosphate (TDP) growth kinetics parameters for *Vibrio anguillarum* PF430-3 $\Delta thiE$. Means and standard deviations are presented for half-saturation growth constants (Ks) and pmol cell⁻¹ values. CI = 95% confidence interval and Kmax = maximum saturation growth constant. Values for Ks, Ks CI, and r² are from a hyperbolic Michaelis-Menten model generated in Prism (GraphPad; La Jolla, CA); Kmax is estimated based on manual inspection of the plotted hyperbolic model.

Provided Vitamin	Ks (pM)	Ks (CI) (pM)	r²	pmol cell⁻¹	Kmax (pM)	n
B1	0.51 ± 0.13	0.25 - 0.77	0.83	2.88E-09 ± 1.05E-09	~14	20
TDP	1.38 ± 0.29	0.78 - 1.98	0.89	4.66E-09 ± 1.05E-09	~11	16

Table S9. Nutrient components added to cFMSW for B1-auxotrophy surveys of Baltic Sea bacterioplankton isolates.

<i>Nutrient</i>	<i>Final Concentration (mM)</i>
Glucose	5.53E-01
KH ₂ PO ₄	1.48E-01
(NH ₄) ₂ SO ₄	2.50E-01
NaNO ₃	2.50E-01
Trace Metals	Farnelid et al. 2013 (19)
Cobalamin	Farnelid et al. 2013 (19)
7 Vitamin Mix	Farnelid et al. 2013 (19)
Biotin	6.67E-04
Myo-inositol	6.00E-03
Niacin	1.90E-02
L-Methionine	3.70E-02
Glycine	7.40E-02
Pyruvate	6.20E-02
Riboflavin	2.60E-03

Table S10. Maximum cell yields of PF430-3 $\Delta thiE$ in bioassays of LMO water. Yields from no B1 addition (0 pM) and added B1 (1, 2, 3, and 5 pM) were used to generate an internal standard curve (Equation) and ultimately calculate the starting B1 concentration in the initially collected LMO water (Table 1), based on PF430-3 $\Delta thiE$ yields per supplemented B1 (see Methods in the main text). Mean maximum yields in negative controls (growth on B1-deplete medium) (Neg. control) were subtracted from all other yields before calculating the internal standard curve.

	5/12/15			5/26/15			6/24/15		
<i>pM added</i>	<i>Rep1</i>	<i>Rep2</i>	<i>Rep3</i>	<i>Rep1</i>	<i>Rep2</i>	<i>Rep3</i>	<i>Rep1</i>	<i>Rep2</i>	<i>Rep3</i>
0	1.24E+05	1.07E+05	8.13E+04	3.29E+05	5.04E+05	6.92E+05	1.36E+05	2.10E+05	1.52E+05
1	3.31E+05	2.76E+05	3.13E+05	6.97E+05	6.19E+05	6.85E+05	3.00E+05	4.01E+05	2.36E+05
2	4.75E+05	4.00E+05	5.79E+05	1.12E+06	6.82E+05	6.79E+05	2.48E+05	2.67E+05	4.52E+05
3	5.03E+05	4.69E+05	4.21E+05	1.11E+06	9.90E+05	9.07E+05	5.52E+05	5.53E+05	5.04E+05
5	7.22E+05	8.39E+05	7.96E+05	1.45E+06	1.24E+06	1.63E+06	-	1.36E+06	1.51E+06
Neg. Control	4.32E+04	4.84E+04	4.01E+04	4.32E+04	4.84E+04	4.01E+04	2.49E+04	3.92E+04	2.82E+04
Equation	$y = 7.171E-06x - 0.8777; r^2 = 0.90965$			$y = 4.486E-06x - 1.7911; r^2 = 0.83354$			$y = 3.548E-06x + 0.2579; r^2 = 0.8151$		
	8/4/15			6/1/16					
<i>pM added</i>	<i>Rep1</i>	<i>Rep2</i>	<i>Rep3</i>	<i>Rep1</i>	<i>Rep2</i>	<i>Rep3</i>			
0	2.82E+05	2.67E+05	2.76E+05	5.42E+05	5.12E+05	5.82E+05			
1	3.96E+05	4.06E+05	3.74E+05	7.15E+05	8.04E+05	8.55E+05			
2	4.40E+05	6.02E+05	6.68E+05	1.01E+06	1.18E+06	8.61E+05			
3	9.51E+05	8.02E+05	7.84E+05	1.14E+06	1.31E+06	1.32E+06			
5	1.12E+06	1.59E+06	1.55E+06	1.27E+06	1.48E+06	1.44E+06			
Neg. Control	8.26E+04	9.98E+04	1.22E+05	1.53E+05	6.41E+04	7.47E+04			
Equation	$y = 3.881E-06x - 0.5151; r^2 = 0.907$			$y = 5E-06x - 2.8371; r^2 = 0.870$					

Table S11. A list of thiazole B1-auxotrophic bacterioplankton identified in our survey of 330 complete bacterioplankton genomes, based on the absence of *thiG* or *thi4* genes.

IMG_taxid	Habitat	Kingdom	Species
2506520034	Fresh	Archaea	<i>Halopiger xanaduensis</i>
2510065051	Fresh	Archaea	<i>Halostagnicola larsenii</i>
2510461022	Fresh	Archaea	<i>Natronobacterium gregoryi</i>
637000166	Fresh	Bacteria	<i>Methylococcus capsulatus</i>
640753014	Fresh	Archaea	<i>Methanoregula boonei</i>
641522637	Fresh	Bacteria	<i>Lysinibacillus sphaericus</i>
642555136	Fresh	Bacteria	<i>Leptospira biflexa</i>
642555137	Fresh	Bacteria	<i>Leptospira biflexa</i>
644736372	Marine	Archaea	<i>Halomicrobium mukohataei</i>
644736380	Marine	Bacteria	<i>Kytococcus sedentarius</i>

SI Datasets (separate files).

Dataset S1. Bacterioplankton strains that are putative dual B1 auxotrophs based on the absence of *thiC* and *thiG/thi4* from their complete genome reference sequence. *= one hit to *thiDN* fusion ~400-450 amino acids long; all others are annotated in UNIPROT as: transcriptional regulator, uncharacterized protein, or DNA binding protein; ** = all are annotated in UNIPROT as: transcriptional regulator, uncharacterized protein, or DNA binding protein; ***= hits annotated as AIR synthase, not thiamine monophosphate; # = a truncated *thiDN* protein, unclear if it is comparably functional. Detected genes are highlighted in blue.

Dataset S2. Of 330 complete isolate genomes surveyed, 109 possessed >1 copy of *thiE* or *thiN* (encoding for thiamine monophosphate synthase) and are highlighted in blue. Non-highlighted strains were conservatively not counted as having >1 thiamine synthase gene due to cryptic positive hits. *= one hit to *ThiDN* fusion ~400-450 AA long; when >1 others are annotated in UNIPROT as transcriptional regulator, uncharacterized protein, or DNA binding protein; ** = all annotated in UNIPROT as: transcriptional regulator, uncharacterized protein, or DNA binding protein; *** = annotated in UNIPROT as AIR synthase, not thiamine monophosphate; # = a truncated *ThiDN* protein was detected, unclear if it is comparably functional; ^ = one hit is annotated as Nudix hydrolase – its function as a thiamine monophosphate synthase is unclear.

Dataset S3. Isolate bacterioplankton genomes possessing >1 known B1 or precursor transporter (Table S3). The number of each transporter detected is highlighted in yellow.

Dataset S4. B1-prototrophic bacterioplankton isolates possessing at least one known B1-related transporter. Detected core B1-biosynthesis genes are highlighted in blue, while detected transporters are highlighted in yellow.

Dataset S5. A list of complete bacterioplankton genomes retrieved from the Joint Genome Institute (JGI) Genome Portal (<https://genome.jgi.doe.gov/>) used in surveys for B1-related proteins.

Dataset S6. Bacterial production data from nutrient-amendment experiments performed using near-surface LMO water. Means and standard deviations are in units of $\mu\text{gC L}^{-1} \text{d}^{-1}$. Conversion factors used to calculate final bacterial production values are provided in the methods section of the main text. P-values are those from two-tailed Welch-Corrected t-tests between control and individual treatment data.

References

1. Hugerth LW, et al. (2015) Metagenome-assembled genomes uncover a global brackish microbiome. *Genome Biol* 16:279.
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