

Polar marine diatoms likely take up a small fraction of dissolved dimethylsulfoniopropionate relative to bacteria in oligotrophic environments

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1. Details on the methodology

1.1 Antibiotic treatment

The three algal species were treated with a combination of three antibiotics (kanamycin, streptomycin, penicillin) to produce monospecific axenic algae cultures (Andersen 2005). After four antibiotic treatments, axenicity of algae cultures was confirmed by DAPI staining (Porter & Feig 1980). Furthermore, inoculating 1 mL of *T. gravida* and *C. neogracilis* cultures into 100 mL sterile peptone bacterial growth media and visually checking for bacterial growth after 1 month at 4 °C (Andersen 2005) did not reveal any bacterial growth based on visual inspection of the cultures. The algae were then acclimated to the ESAW medium for more than 7 growth cycles. Axenicity was further validated for cultures at the start of the dimethylsulfoniopropionate (DMSP) uptake experiments.

1.2 Synthesis and activity of the radioactive DMSP source

A carrier-free ³⁵S-DMSP source was synthesized by Ronald P. Kiene and Jessie Motard Côté at the University of South Alabama (Alabama, USA). The ³⁵S-DMSP was synthesized from ³⁵S-methionine by enzymatic deamination and decarboxylation to 3-methiolpropionate using L-amino acid oxidase, with subsequent methylation of 3-methiolpropionate in acidic methanol. The resulting ³⁵S-DMSP was purified by ion exchange chromatography and HPLC (> 97% radiochemical purity).

2. Benefits of DMSP uptake in *Thalassiosira gravida*

DMSP synthesis is a costly process relative to the biosynthesis of other biomolecules although the energy cost of DMSP biosynthesis is generally small relative to the total energy cost for growth in phytoplankton (Lavoie et al. 2016). What are the benefits associated with DMSP uptake from the environment in *T. gravida*? Control *T. gravida* cells (solid circles, Fig. 1 of the main text body) took up dissolved DMSP (DMSP_d) at a rate of 4.8 amol DMSP cell⁻¹ h⁻¹ when exposed to 69 nM DMSP_d. Using a mean measured cell radius of 4.45 μm and assuming spherical cells of 369 μm³/cell or fL/cell, this uptake rate is equivalent to 13 μmol DMSP L_{cell}⁻¹ h⁻¹ (or 1.95 x 10⁻²⁰ mol DMSP μm⁻² h⁻¹ using a cell surface of 248.8 μm² cell⁻¹). This uptake rate represents around 2% of the net DMSP production rate of *T. gravida* (≈ 24 mmol DMSP L_{cell}⁻¹ x 0.72 d⁻¹ = 720 μmol DMSP L_{cell}⁻¹ h⁻¹) and would allow the cells to save only a small fraction of the total cellular energy required for DMSP biosynthesis. The running cost and resource costs due to DMSP membrane transport systems utilization and synthesis will further reduce the benefits of external DMSP uptake in *T. gravida*.

3. Minimum DMSP uptake rates measurable with our methodology

For *C. neogracilis* and *C. gelidus*, the ^{35}S signal did not increase significantly after 5 min to 5.5 h of exposure relative to the ^{35}S signal embedded in the filter of culture medium without algae. A fast DMSP adsorption step was not detected in the two *Chaetoceros* species in contrast with *T. gravida* perhaps because of differences between species in the presence and density of cell membrane DMSP binding sites. Based on a t-test of independent samples, we calculated the threshold of ^{35}S -DMSP signal in filters of DMSP-exposed algae that would be significantly higher (at $p=0.049$) than that embedded in the filters and computed the minimum detectable DMSP uptake rate under the conditions of our experiments. For *C. neogracilis*, we calculate that the lowest DMSP uptake rates was lower than 6.5×10^{-21} mol DMSP cell $^{-1}$ h $^{-1}$. Taking the mean measured cell radius of *C. neogracilis* (1.9 μm) and assuming spherical cells, the cell volume is 28.7 μm^3 or fL. DMSP uptake rates in the presence of 1 nM DMSP_d on a cell volume basis are lower than 2.3×10^{-7} mol DMSP L_{cell} $^{-1}$ h $^{-1}$, i.e., which is at least 57-fold lower than the DMSP uptake rates measured in *T. gravida*, which, because it produced DMSP_d, was exposed to a 69 nM DMSP_d. Based on the threshold signal for ^{35}S -DMSP uptake, we estimate that DMSP uptake by *C. gelidus* was less than 1.4×10^{-20} mol DMSP cell $^{-1}$ h $^{-1}$ (7.9×10^{-8} mol DMSP L_{cell} $^{-1}$ h $^{-1}$) if DMSP uptake occurs at all in this species.

4. Summary of bioinformatics analysis on the presence of DMSP catabolism enzymes, DMSP catabolite assimilation enzymes, and transporters capable of taking up DMSP

Protein sequences for 52 DMSP degradation enzymes, DMSP degradation product assimilation enzymes, and transporter systems capable of DMSP transport were collected from NCBI databases (NCBI 2017), which are based on the following studies (Haardt et al. 1995, Kappes et al. 1999, Nau-Wagner et al. 1999, Todd et al. 2010, Frossard et al. 2012, Alcolombri et al. 2015, Dickschat et al. 2015, Lidbury et al. 2016, Sun et al. 2016, Wicht 2016, Eyice et al. 2017). Transcriptomes for *T. gravida* St. GMp14c1, *T. rotula* GSO102, *T. rotula* CCMP3096, *C. curvisetus*, and *C. debilis* MM31A-1 from the Marine Microbial Eukaryotic Transcriptome (MMET) database (Keeling et al. 2014) were interrogated using the Phylo-MetaRep database and its tools (Goll et al. 2010) with an expect E-value cutoff of e^{-5} to allow detection of partial translated transcript records that would otherwise evade notice. These species were selected since they are the most closely related species to those used in our uptake experiments for which transcriptomic/genomic resources were available (Kooistra et al. 2010, Whittaker et al. 2012). Putative homologues were further analyzed by reciprocal BLASTP (Altschul et al. 1990) performed against NCBI databases to eliminate paralogous enzymes or obvious bacterial sequences such as those from α -proteobacteria present in the *C. curvisetus* databases. Other *Thalassiosira* MMET databases were interrogated as above. Enzymes with non-DMSP metabolism roles were examined with the aid of the KEGG pathway databases (Kanehisa et al. 2017).

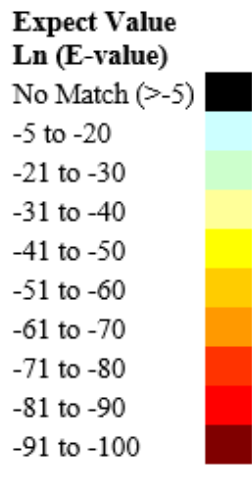
Experimental growth conditions and additional details for each of the Marine Microbial Eukaryotic Transcriptome Species from Keeling et al. (2014) may be found in the Excel spreadsheet “Callum_Final_biosample_ids.xls”, downloadable from <ftp://ftp.imicrobe.us/projects/104/>. Further specifics on the transcriptomes may be found at <https://www.imicrobe.us/#/projects/104>.

Fig. S1. Summary of bioinformatics investigation of DMSP catabolism enzymes, DMSP catabolite assimilation enzymes, and transporters capable of taking up DMSP in four selected diatom species of the *Thalassiosira* and *Chaetoceros* genus. Protein sequences of transporters and enzymes capable of acting on DMSP or its catabolites were collected from NCBI. These sequences were then used to BLASTP query the PhyloMetaRep database containing the Marine Microbial Eukaryotic Transcriptomes for *Thalassiosira gravida*, St. GMp14c1, *Thalassiosira rotula* GSO102, *Thalassiosira rotula* CCMP3096, *Chaetoceros curvisetus*, *Chaetoceros debilis* MM31A-1 using a cutoff E-value of e^{-5} . Retrieved sequences were then subjected to best reciprocal BLASTP tests to eliminate paralogues and obvious contaminating bacterial sequences. The colour key shows the level of *Thalassiosira/Chaetoceros* protein similarity to the query sequence, where the lowest similarity is black and the highest similarity is red. Black areas indicate an absence of *Thalassiosira/Chaetoceros* sequences below the E-value cutoff level. **A)** DMSP catabolic enzymes and DMSP catabolite assimilation enzymes. MO: monooxygenase. **B)** Transporters capable of DMSP transport.

§ For OpuAA, OpuCA, and OsmV, a protein containing an ATPase domain was detected but the other components of the OpuA, OpuC, and OsmV transporter systems were not detected. Therefore, the detected ATPase domain is likely a component of a protein complex unrelated to osmolyte uptake.

1: The *T. gravida* Strain GMp14c1 BCCT-like transporter was identified by using the *T. rotula* CCMP3096 BCCT-like protein sequence in a second round of BLASTP searches of the PhyloMetaRep database since the *T. rotula* CCMP3096 BCCT-like protein fell below the E-value cutoff level in the first round due to it being an incomplete sequence.

2 : Similarly, the *T. rotula* GSO102 BCCT-like protein sequence was not identified using the *Escherichia coli* CaiT sequence as a query probably because that BCCT-like protein is incomplete and furthest related to CaiT, since the BCCT-like protein sequence was found (although with small similarity) using the BetP, OpuD, and DddT sequence.



A

	<i>Thalassiosira gravida</i> . GMp14c1	<i>Thalassiosira rotula</i> GSO102	<i>Thalassiosira rotula</i> CCMP3096	<i>Chaetoceros curvisetus</i>	<i>Chaetoceros debilis</i> MM31A-1
DddL					
DddP					
DddQ					
DddW					
DddY					
DddA					
DddC					
DddD					
DddK					
Alma1					
Alma2					
Alma3					
Alma4					
Alma5					
Alma6					
Alma7					
DmdA					
DmdB					
DmdC					
DmdD					
AcuI/yhdH					
AcuN					
AcuK					
PrpE					
Cystathionine γ -synthase					
Tmm DMS MO					
SfnG DMSO, MO					
DMS MO					
DMSdehydA					
DMSdehydB					
DMSdehydD					
DMSdehydG					
MTOX					

B

	<i>Thalassiosira gravida</i> , St. GMP14c1	<i>Thalassiosira rotula</i> GSO102	<i>Thalassiosira rotula</i> CCMP3096	<i>Chaetoceros</i> <i>curvisetus</i>	<i>Chaetoceros</i> <i>debilis</i> MM31A-1
OpuAA	§	§	§	§	§
OpuAB					
OpuAC					
OpuCA	§	§	§	§	§
OpuCB					
OpuCC					
OpuCD					
ProX					
ProW					
OsmV	§		§		
OsmX					
OsmY					
OsmW					
ProP					
BetP	1				
CaiT	1	2			
OpuD	1				
DddT	1				

Apart of the OpuD/DddT-like putative DMSP transporter discussed in the main text body, the results of Fig. S1 show other interesting features. Indeed, a putative ProP-like DMSP transporter was found in all transcriptomes of *Thalassiosira* spp. and *Chaetoceros* sp. (Gouesbet et al. 1994) (Fig. S1 A). The presence of a ProP homologue in both *Chaetoceros* and *Thalassiosira* species taken together with their differing ability to take up DMSP suggests that these ProP homologues are incapable of DMSP transport at the outer cell membrane *in vivo*. Moreover, the apparent absence of any enzyme capable of degrading DMSP (by demethylation or cleavage of DMSP) (Fig. S1 B), suggest that there are no DMSP catabolites for any further enzymes to act on. Therefore, the putative DMSP catabolite assimilation enzymes downstream of DMSP demethylation/cleavage are likely involved in biochemical pathways that are unrelated to DMSP metabolism. Finally, we found evidence for the presence in *Thalassiosira* spp. and *Chaetoceros* spp. of a putative monooxygenase (MO) (tmm) as in the bacterium *Ruegeria pomeroyi* (Lidbury et al. 2016) (Fig. S1 B). This MO could catalyze the oxidation of DMS, which is mainly produced via DMSO reduction in diatoms (Spiese et al. 2009). Interestingly, DMSO production from added DMS has been experimentally measured in *Emiliana huxleyi* strain 373 (unpublished results of Ronald P. Kiene), and one can hypothesize that such an MO could catalyze DMS conversion to DMSO in this species. Note that we found no DMSP lyase in the diatom transcriptomes investigated (Fig. S1 B) and hence DMS production cannot occur from the DMSP lyase pathway. This result suggests that the several diatoms investigated do not express a lyase, which is in line with the reported low potential of diatoms to produce DMS (Baumann et al. 1994, Matrai et al. 1995, Stefels et al. 2007). Indeed, DMSO reduction to DMS has been suggested as a major source of DMS in several diatoms (Spiese et al. 2009). Alternatively, other substrates than DMS such as trimethylamine (TMA) could be metabolized by the putative MO.

5. Alignment of amino acid sequences of Betaine/Carnitine/Choline transporters

Fig. S2. Multiple alignment of amino acid sequences of Betaine/Carnitine/Choline (BCCT) Transporters from *Thalassiosira* species. Amino acid sequences were collected from the Phylo MetaRep and NCBI nonredundant protein databases and protein sequence alignment was performed using T-Coffee and BoxShade. Amino acids that are identical or similar between species are shaded black or gray, respectively. The fraction of the protein sequence that must agree for shading was set to 0.5 due to incomplete protein sequences for several transporter proteins. Gaps (depicted by dashes) were introduced in the sequences to maximize alignment. The abbreviated species name and PhyloMetaRep or NCBI accession numbers are as follows: T. gravida GMp14c1: *Thalassiosira gravida* GMp14c1 (MMETSP0492|Thalassiosira-gravida-GMp14c1-20140214|1870_1); T. rotula GSO102: *Thalassiosira rotula* GSO102 (MMETSP0910|Thalassiosira-rotula-GSO102-20130909|11489_1); T. rotula CCMP3096: *Thalassiosira rotula* CCMP3096 (MMETSP0403|Thalassiosira-rotula-CCMP3096-20130909|86170_1); T. punctigerae 2005C2: *Thalassiosira punctigerae* Tpunct2005C2 (MMETSP1067|MMETSP1067-20121228|36635_1); Thalassiosira NH16: *Thalassiosira* Strain NH16 (MMETSP1071|MMETSP1071-20121207|6016_1); T. antarctica CCMP982: *Thalassiosira antarctica* CCMP982 (MMETSP0903|Thalassiosira-antarctica-CCMP982-20140214|4194_1); T. oceanica CCMP1005: *Thalassiosira oceanica* CCMP1005 (MMETSP0970|Thalassiosira-oceanica-CCMP1005-20130912|13493_1); T. miniscula CCMP1093: *Thalassiosira miniscula* CCMP1093 (MMETSP0737|Thalassiosira-miniscula-CCMP1093-20140214|20636_1); T. pseudonana 1335: *Thalassiosira pseudonana* CCMP1335 (THAPSDRAFT_262307); C. glutamicum BetP: *Corynebacterium glutamicum* ATCC 13032 glycine betaine transporter BetP (P54582.1); E. coli BL21 CaiT: *Escherichia coli* BL21(DE3) CaiT carnitine BCCT transporter (CAQ30560.1); B. subtilis 168 OpuD: *Bacillus subtilis* subsp. *subtilis* str. 168 glycine betaine transporter OpuD (P54417.1); Halomonas HTNK1 DddT: *Halomonas* sp. HTNK1 BCCT family DMSP transporter (ACV84066.1).

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T.rotula GSO102 -----
T.gravida GMp14c1 -----
T.rotula CCMP3096 |MTNPD SLK--IEEIHQPDP E V P V-A-----QEKEEE P Q P D P E V P F F A Q E K T K E G H
T.oceanica CCMP1005 |MASTEAPSEHLQETA-----L-----R---KES-----EGNEL
T.punctigerae 2005c2 |MIREKEEVGLV E E P L A E Q P S E P E A I M E A P E P T Y Y Q Q S E A--E-----I M E E S P D K H G D N A
Thalassiosira NH16 |MGGENPEVE-EHT P X E D L P K A E-----E V-----H--SEDELADIX
T.antarctica CCMP982 |MTGEYPEVA-VEEP H D K E P E A E-----D A--E-----I A E E S L D D D A D D D
T.miniscula CCMP1093 |MTGEDPEVL-A-EPT E E P P E V-----V--K-----I V E E A H D D Y A D E D
T.pseudonana 1335 -----
C.glutamicum BetP |MTTSDPNPK-P-IV E D A Q P E Q I-----T-----A T E E L A G--L L E N
E.coli BL21 CaiT |MKNEKRKT-----
B.subtilis 168 OpuD |MLKHIS-----
Halomonas HTNK1 DddT |MNTPNKPG-----

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T.rotula GSO102 -----
T.gravida GMp14c1 -----
T.rotula CCMP3096 |EIE--STKV-DGESESPSSYPMRSTTVKLP L F K D P I S F N A-AV S F I A L V V W G I A I Y C M V
T.oceanica CCMP1005 |-----GKT--NSQS Q L E K Y P V R E T V C-----G Q L R F N W-L V T F S S L A L L W G I S I Y C M T
T.punctigerae 2005c2 |A-M--PANVAHLEPKANKTYPMRETA F S L P F F G G D I R F N W-L V S I L G L G V I W G I A I F C M T
Thalassiosira NH16 |P-KDSSEEVDSVEKPEKKSFP M R E M T L S L P F I K G E L R F N W-L V S I I G L G V I W G V S I F C M T
T.antarctica CCMP982 |P-K--SQKVDPVEQTARKTYPMREVSLSL P F L K E D L R F N W-L V S I L G L A F I W G L S I F C M T
T.miniscula CCMP1093 |P-K--SLKENDGEETATKVFPMREVCV-----G P L R F N W-L V S I L G L G V I W G V S I Y C M T
T.pseudonana 1335 -----
C.glutamicum BetP |P-T--NLEGKLADA---EE-----E I I L E G E D T Q A S L N W S-V I V P A L V I V L T V V W G I G
E.coli BL21 CaiT |-----G I E P K-V F F P P L I I V G I L C W L T V R
B.subtilis 168 OpuD |-----S V F W I V I A I T A A A-----V L W G V I
Halomonas HTNK1 DddT |-----D S M D L R-V F I P S V L V I L G I I V P L I M

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T.rotula GS0102
T.gravida Gmp14c1
T.rotula CCMP3096
T.oceanica CCMP1005
T.punctigeræ 2005c2
Thalassiosira NH16
T.antarctica CCMP982
T.miniscula CCMP1093
T.pseudonana 1335
C.glutamicum BetP
E.coli BL21 CaiT
B.subtilis 168 OpuD
Halomonas HTNK1 DddT

SPDEA-NTTISQWFDKVDVETWFYIVANPVLTFVFWVA-ERYGDIKLGAKDAEPEFSN
SPE-AAMVTLGEWYSDCLLYETWFYILGNPMTFFFYIA-WRYGHIKLGPKNAEPEFSN
DPN-A-KAVLDTWYNTTLYETWFYILGNPVMFFFIFWVA-YRYGHIKLGPKNAEPEFSN
NPADA-KTELKSWYDNTLYETWFYILGNPVMFFFIFWVA-YRYGHIKLGPKNAEPEFSN
HPD-A-KALISGWYDNTLYETWFYILGNPVMFFFIFWVA-YRYGHIKLGPKNAEPEFSN
NPE-A-SAELGKWDYDNTLYETWFYILGNPVMFFFIFWVA-YRYGHIKLGPKNAEPEFSN
-----MFFFVWVA-YRFGSIKLGKDAEPEFSN
FKDSF-TNFASSALSAVDNLGWAFILFGTVFVFFIVVAASKFGTIRLGRIDEAPEFRT
DLDA-NVVTNAVFSYVTNVWGAPEWYVVMVLFGWVWVFGPYAKKRLG--NEPPEFST
SPDSL-QNVSQSAQAFITDSFGWYLLVVSIFVGFCLFLIFSPIGKIKLGPDEKPEFGL
FPDSC-TLLVNAAFATATGNEGWLMLAGLSIVLFLIGLALSREFGNVRGEPDDKPEFSY

T.rotula GS0102
T.gravida Gmp14c1
T.rotula CCMP3096
T.oceanica CCMP1005
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T.pseudonana 1335
C.glutamicum BetP
E.coli BL21 CaiT
B.subtilis 168 OpuD
Halomonas HTNK1 DddT

ASYFAMLFSAGVGVGMFFYGVSEPLWHTRPDNYYSNAGYSQNEIAQWLSLVTMYHWGFA
MTYFAMLFSAGVGVGLFFGVSEPLFHLTGNYR-DNPGYRSEDEMAAWSLTIISLYHWGFA
VSYFAMLFSAGVGVGLFFYGVSEPLFHQGNYY-TEAGYRQNDIDQWALNTMYHWGFA
FSYFAMLFSAGVGVGLFFYGVSEPLFHQGNYY-TEAGYRQNDIDQWALNTMYHWGFA
VSYFAMLFSAGVGVGLFFYGVSEPLTHAASNYITAPGYRQNDIDQWALNTMYHWGFA
VSYFAMLFSAGVGVGLFFGVSEPLTHAASNYITAPGYRQNDIDQWALNTMYHWGFA
ASYFAMLFSAGVGVGLFFYGVSEPLWHTGNSRY-AMSGYRQNEIDQWALNTMYHWGFA
VSWISMNEAAGMGIQLMFYGTTEPLTFYRNG-V---PGHDEHN--VGVAMSTMFHWHTLH
ASWIFMNFASCTSAAVLFGSIEIYYYISTPPE---GLEPNSTGAKELCLAYSIFHWGFL
LSWFAMLFSAGMGIQLVFYGAAPISHYAISSP---SETETPQAFRDALRYTFEHWGLH
FSWVAMTEAGGIGIATVNAWVEPIYYFTSPPPE---GVEPSSNAAEWALSYGQEHWGFT

T.rotula GS0102
T.gravida Gmp14c1
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E.coli BL21 CaiT
B.subtilis 168 OpuD
Halomonas HTNK1 DddT

AWAPYLVAIASGLASYRFGFLPL-TIRSFYPIFGDY-CWGWIGDLIDSIVMTIAGVC
AWSPYLVAIASGLASYRFGFLPL-TIRSFYPIFGDY-CWGWIGDLIDSIVMTIAGVC
GWSPYLVAIASGLASYRFGFLPL-TIRSFYPIFGDY-CWGWIGDFIDANIVMTIAGVC
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GWSPYLVAIASGLASYRFGFLPL-TIRSFYPIFGDY-CWGWIGDFIDANIVMTIAGVC
AWSPYLVAIASGLASYRFGFLPL-TIRSFYPIFGDY-CWGWIGDFIDANIVMTIAGVC
PWAIYAVGLAIAYSTRVGRKQ-LSSAFVPLIGEKGAEGWIKLIDILAIATVFGTA
PWATYSELSVAFAYFFVRKMEVIRPSSLVPLVGEKHKVGLGFTIVDNYIVVALIFAMG
AWAIYAVGLAIAYSTRVGRKQ-LSSAFVPLIGEKGAEGWIKLIDILAIATVFGTA
PWAIYAVGLAIAYSTRVGRKQ-LSSAFVPLIGEKGAEGWIKLIDILAIATVFGTA

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T.miniscula CCMP1093
T.pseudonana 1335
C.glutamicum BetP
E.coli BL21 CaiT
B.subtilis 168 OpuD
Halomonas HTNK1 DddT

TSLGLGATQMVAGLQRLGWVDSQKEDL-TSEYNIIVWLMTAAATISVILGLSFIGIKTLAN
TSLGLGATQMSVGLQMLGWVEEVG-DSPEVLYVTIIVWITAFATLSVLSGLGVGKILYLSQ
TSLGLGTMQATGMMRLGWIDPNA-NI-TSVYVSTIWIITAFATLSVLSGLGVGKILYLSQ
TSLGLGTMQATGMMRLGWIDPTS-NP-TTVYIATIIWITAFATLSVLSGLGVGKILYLSV
TSLGLGTMQATGMMRLGWIDPNS-NS-TTIYVIIIWITAFATLSVLSGLGVGKILYLSL
TSLGLGTMQAAAGIIRLGWVDPPT-NM-TNIYVAIIWITAFATLSVLSGLGVGKILYLSV
TSLGLGTMQATGLQRLGWVDPKEDL-MNVYIITIIWITAFATLSVLSGLGVGKILYLSL
CSLGLGATQMGAGLSAANIIDPS-DW-TI--VGIIVSVITLAFIFSAISGKGIQYLSN
TSLGLGATQINGGLNYLFGIP-HT-LQ-LD--AIIITCWIILNAICVACGLQKGVRIASD
TSLGLGATQINGGLNYLFGIP-NA-FI-VQ--LVLIITVTVLFLLSAWSGLGKGIKILYLSN
TSLGLAVPLVSQLVSGLFGIA-PS-FM-LD--VGIILLWTAIFASVWFLGAKGIRILSD

T.rotula GS0102
T.gravida Gmp14c1
T.rotula CCMP3096
T.oceanica CCMP1005
T.punctigeræ 2005c2
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T.antarctica CCMP982
T.miniscula CCMP1093
T.pseudonana 1335
C.glutamicum BetP
E.coli BL21 CaiT
B.subtilis 168 OpuD
Halomonas HTNK1 DddT

IAFSLGTLIFLSFVMEKSYLLNLLIVQTAGIYLYQYNIHQPFWTFDAFGALKENEGRAVD
AGFLFCCLIFLFCFTMEKSYLLNLLIVQSTGDYLVQWCIHQPFWTFDAFAGLKEGEGRASD
IGFGLGCLIFLSFVMEKSYLLNLLIVQTGTYLQWAIHQPFWTFDAFGGLREGEGRAID
LGFGLGCLIFLSFVMEKSYLLNLLIVQTGVYLVQWNIHQPFWTFDAFGGLEEGEGRAID
VGFGLGCLIFLSFVMEKSYLLNLLIVQTGVYLVQWNIHQPFWTFDAFAGLKEGEGRASD
AGFCLGCLIFLFCFVMEKSYLLNLLIVQSTGVYLVQWNIHQPFWTFDAFASLTPGEGRAVD
ANMVLALLAIFVFGVPLVSLNLLIPGSIQNYLS-NFQWAGRTAMS-----
VRSYLSFLMLGWVFIWVSGASIFMNYFTDSVGMMLM-YPRMFLFYTDPI-----
TNMVLALLAIFVFGVPLVSLNLLIPGSIQNYLS-NFQWAGRTAMS-----
INVWLAIFLFAFTFLVGDSELMIEGWNSLGNMMS-HFVEMSLWTDPIV-----

T.rotula GS0102 -----VFYMAWVVAWACFVGFARISKNRTRRTVIVSVFICPTLYCLIWFS
T.gravida Gmp14c1 -----
T.rotula CCMP3096 EHSSAQWFTIGAWTVFYMAWVVAWACFVGFARISKNRTRRTVIVSVFICPTLYCLIWFS
T.oceanica CCMP1005 GKSAPAAWVGWWTVFYMAWVVSWSACFVGFARISKNRKRSVIVGCVICPTLYAILWFG
T.punctigerae 2005c2 -----
Thalassiosira NH16 GNTASPKWVGWWTVFYMAWVVAWACFVGFARISKNRTRRSVITIGVFMAPTIVYGLLWFS
T.antarctica CCMP982 EKSAPSEWVGWWTVFYMAWVVSWSACFVGFARISKNRTRRSVIVGCVFMAPTAYALIWFS
T.miniscula CCMP1093 EKSAPSEWVGWWTVFYMAWVVAWACFVGFARISKNRTRRSVIVGCVFMAPTAYALVWMS
T.pseudonana 1335 GKSAPSEWVGAWTVFYMAWVVSWSACFVGFARISKNRTRRSVIVGCVFMAPTLYALLWFP
C.glutamicum BetP ADGTAGEWVGSWTFYMAWVVSWSACFVGFARISRGRSIREFILGVLLVPAGVSTVWFS
E.coli BL21 CaiT ---AKGGFPQGWTVFYMAWVVIYAIQMSIFLARISRGRTRREFICFGVVGITASTWILWT
B.subtilis 168 OpuD NDPEKREWVNSWTFYMAWVVSWSACFVGFARISRGRTRREFILGVLLVTEPCILTFWFS
Halomonas HTNK1 DddT ---CGSTWQDWTVFYMAWVVIYAPMGLFVARISRGRTRREFILIAEMVFGALGCWVIFA

T.rotula GS0102 FMGGIGLR-QERQAMELEKLGSD--VFQDASYVSEESE-FCYDVPOGEVS-VNRTVVFT
T.gravida Gmp14c1 -----
T.rotula CCMP3096 FMGGIGLR-QERQAMELEKLGSD--VFQDASYVSEESE-FCYDVPOGEVS-VNRTVVFT
T.oceanica CCMP1005 TFGGIGLR-QARQAAELQALGET--HMGSSDYEQSSSEKTCYDVPOSDVV-VNGTTVFT
T.punctigerae 2005c2 -----
Thalassiosira NH16 VMGGIGLR-QQRQAAELEKLGTD--FFNGQHFFQSAYSDFCFDVPQDNVYGADGALVFT
T.antarctica CCMP982 FMGGIGLR-QQRQAAELEKLGTD--FFADPNYFESTYSE-YCYDVPODDVI-VNGETVFT
T.miniscula CCMP1093 FMGGMGLR-QQRQAAELQELGRS--VNDPEARFETSYSSE-FCYDVPOEDVE-FNGATVVF
T.pseudonana 1335 IMGGIGLR-QQRQAAELKQLGAD--YFQDEAHYMASGSS-FCYDVPO-----
C.glutamicum BetP IFGGTAV-----FEQNGES--IVGDGAA-----
E.coli BL21 CaiT VLGSNTLLMDKNIINPNLIEQ--Y-GVA-----
B.subtilis 168 OpuD IFGVSAMDLOKQKGFNVAKLSTETMLHGTLDH-----
Halomonas HTNK1 DddT IWGGYATDLQISGALDVAHLDE--G-GIP-----

T.rotula GS0102 NRLLGVTPVCKFDSSNSEQAWFENVMYSFSYFDA---ENFGGFGPFLSG-LSITLAIYF
T.gravida Gmp14c1 -----
T.rotula CCMP3096 NRLLGVTPVCKFDSSNSEQAWFENVMYSFSYFDA---ENFGGFGPFLSG-LSITLAIYF
T.oceanica CCMP1005 NTLPGVTPVCLFDSNNSSESAWFENVMNSFSFPNG--DSSFAGFGPFLSG-LSITLAIYF
T.punctigerae 2005c2 -----
Thalassiosira NH16 NTLPGITPVCKLDTANSNAWFENVMFSFTYBGTGPDGNFGGFGQF-MSG-LSITLAIYF
T.antarctica CCMP982 NTLPGITPVCMQDANDSNNSWFENVMYSFSYBGTGPGDGFGGFGQF-MSG-LSITLAIYF
T.miniscula CCMP1093 NTLPGITPVCRQDPDDKTNDWFENVIMYSFSYBGTGANGDFGGFGQF-MSG-LSITLAIYF
T.pseudonana 1335 -----
C.glutamicum BetP -----EEQLFGLLHAL--P-----GGQI-MGI-LAMILLGTFE
E.coli BL21 CaiT -----RAIETWAAL--P-----LSTATWGFILCFIATVT
B.subtilis 168 OpuD -----Y--P-----LTMV-TSI-LALILIAVFF
Halomonas HTNK1 DddT -----AAVEAILNLT--P-----MSEV-VTG-VFILLCFIFL

T.rotula GS0102 VTSSDSLSLVDTILA-S---NGATEHWIQRVFWAVTEGAVACALIIAGGSKALKALQAA
T.gravida Gmp14c1 -----WAFTEGAVATGLLVAGGNGALRALQTA
T.rotula CCMP3096 VTSSDSLSLVDTILA-S---NGATEHWIQRVFWAVTEGAVACALIIAGGSKALKALQAA
T.oceanica CCMP1005 VTSSDSLSLVDTILA-S---NGSLKHHSQRVFWAFTEGAVATGLLMAGGNDALTSLOAA
T.punctigerae 2005c2 -----
Thalassiosira NH16 ITSSDSLSLVDTILA-S---NGAEKH-WLQRAFWAFTEGAVATGLLVXGGNDALGALQTA
T.antarctica CCMP982 ITSSDSLSLVDTILA-C---NGAEKH-WLQRFWAFTEGAVATGLLVAGGNDALGALQTA
T.miniscula CCMP1093 ITSSDSLSLVDTILA-S---NGAEKH-WIQRVFWALTEGAVATGLLVAGGNDALGALQTA
T.pseudonana 1335 -----
C.glutamicum BetP ITSADSASTVMGTMS-Q---HGQLEANKWVTAAGVATVAIGLTLTLLSGGDNALSNLQNV
E.coli BL21 CaiT LVNACSYTLAMSTCREV---RDGEEPLLVRIGWSILVGIICIVLLALGG---LKPIQTA
B.subtilis 168 OpuD ITSADSATFVLGMQT-S---YGSLNPAHSVKSISWGI IQSAMA AVLLYSGG---LAALQNT
Halomonas HTNK1 DddT ATTLDSAYVLA SVT-SRKLSGYQEPKRSIRLTWAFLIAGVVALIQLGG---LKAVQTS

T.rotula GS0102 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
T.gravida Gmp14c1 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
T.rotula CCMP3096 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
T.oceanica CCMP1005 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
T.punctigerae 2005c2 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
Thalassiosira NH16 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
T.antarctica CCMP982 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
T.miniscula CCMP1093 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
T.pseudonana 1335 SIVFGLPFNLELFFMCSIVGMCNA-LEA-EQ--N-SDNPHDMLVPKESQSWDTPIFGG
C.glutamicum BetP TIVAATPELFFVIGMVAIVKDLSDVYILEY--REQQRFN-----
E.coli BL21 CaiT IIAGGCPLEFFVNIMVTSIFIKDAKQ-----NW--KD-----
B.subtilis 168 OpuD AIIAALPFSIVILLMIASVYQSLSK-----ER--REI-KKA-----
Halomonas HTNK1 DddT TIVVALPMPVILGILTSILRWRLRQ-----DF--A-----


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T.rotula GSO102      IFNIFEFIFSLGVVHKERREKGMHLPTKEQAFEFIKALELPFLTLNKVSTSAVIDPKLKD
T.gravida Gmp14c1   IFNIVEFIVSFGFRVNEKL---GIEFPPTSKQVVGFFKNLLLPFLSLRSTIYS--SIDMKGNQ
T.rotula CCMP3096   IFNIFEFIFSLGVVHKERREKGMHLPTKEQAFEFIKALELPFLTLNKVSTSAVIDPKLKD
T.oceanica CCMP1005 VFNVIENVLSFGHISKELQGFV-VDPDRREIAGFFFNLELPFVSLHATYA--ALDPGNRR
T.punctigerae 2005c2 -----
Thalassiosira NH16 IFNLIIEFIVSFGFR-----AIELPSSRHILGFLKNLVLPPFTALYSTIYS--TVDLKCKH
T.antarctica CCMP982 IFNIVEFIVSFGFRVNEEL---GIEFPPTSKHILGFFKNLLLPFLPLYSICS--SIDMKGKN
T.miniscula CCMP1093 IFNIVEFIVSFGMVNKQL---GIEFPPTSKQVVGFFKNLLLPFLPLYSICS--SIDMKGEN
T.pseudonana 1335 -----
C.glutamicum BetP -----ARLARERRVHNE-----
E.coli BL21 CaiT -----
B.subtilis 168 OpuD -----EKLDKERSPR-----
Halomonas HTNK1 DddT -----KKVLEPHIVIED-----

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T.rotula GSO102      TTNHVLTAVYTTTCFIWIVLFCFGVNYGFIALAWSLFFTNACILTNLRMTFRERLGR
T.gravida Gmp14c1   TVVNLITGAYAICHFGWIALFACGTINYGFIAFGWSAFFLNAFILASLRMTIRGKLGIS
T.rotula CCMP3096   TTNHVLTAVYTTTCFIWIVLFCFGVNYGFIALAWSLFFTNACILTNLRMTFRERLGR
T.oceanica CCMP1005 HIYRVLTTSVYTLFLFLAWIALFICGTINRGFTAFAWTAEFFVNSCILTSLRMDVRSKFGLE
T.punctigerae 2005c2 -----
Thalassiosira NH16 TKVNALITGAYALCHFQWIALFICGTINHGCVAFGWSAFFLNACILTSLRTDVVRGKLGIG
T.antarctica CCMP982 TAGNVLTGAYAICHIGWIALFVCGTINYGCVAFGWSAFFLNACILTSLRMHVVRGKLGIS
T.miniscula CCMP1093 ARANMLITVAYALCHVQWIALFICGTINYGCVAFGWSAFFLNACILTSLRMHVVRGKLGIV
T.pseudonana 1335 -----
C.glutamicum BetP -----
E.coli BL21 CaiT -----
B.subtilis 168 OpuD -----
Halomonas HTNK1 DddT -----

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T.rotula GSO102      GNVIGDFVASSFLYPQALALQAMELNSSEKTASFINGK-----HDK
T.gravida Gmp14c1   GNVIGDFVAGSFLYPQALLQMELELDYEDYDLGGDEP---HQKS-DASVP-PNHAHKA
T.rotula CCMP3096   GNVIGDFVASSFLYPQALALQAMELNSSEKTASFINGK-----HDK
T.oceanica CCMP1005 GTIIVGDFVASSFLYPQALLQMQIQILKEDDEAGEEGEDKPLAQA-EASAC-EKTEHMA
T.punctigerae 2005c2 -----X
Thalassiosira NH16 GNFVIGDFVAGSFLYPQALLQMELELQAEENNHEVEYVEE--KDEI-ELADS-LLNEHQ
T.antarctica CCMP982 GNFVIGDFVAGSFLYPQALLQMELELQAYEDYDIHEGIA--LEEK-DVTEPLHKNDHVA
T.miniscula CCMP1093 GNFVIGDFVAGSFLYPQALLQLELELQLEHEDYSFGDGNP---SEENEDVTVPLHKHGHAE
T.pseudonana 1335 -----S
C.glutamicum BetP -----HRKREAAKRRREKASGAGKR-----R
E.coli BL21 CaiT -----
B.subtilis 168 OpuD -----VKKA-----Y
Halomonas HTNK1 DddT -----H-----R-----K

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