Metabolite diversity among representatives of divergent *Prochlorococcus* ecotypes

Supplemental information

Table S1.

Table of growth rates at each light intensity for each strain. Growth rates are averages of the five transfers before the onset of the experiment.

Strain	Light intensity (μmol photons m^{-2} s^{-1})	Phosphate levels	Average growth rate (day^{-1})
MIT9301	50	low phosphate	0.29
MIT9301	10	phosphate-replete	0.39
MIT9301	50	phosphate-replete	0.67
MIT0801	10	phosphate-replete	0.40
MIT9313	5	phosphate-replete	0.29
MIT9313	10	phosphate-replete	0.43

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Table S2.

Complete set of metabolites within the targeted metabolomics method used in the current project and the extraction efficiency information from Johnson et al. (1) which have been updated with unpublished data. Metabolites marked with 'yes' in intracellular and/extracellular columns were found in at least one *Prochlorococcus* strain under any light condition. We report all detected metabolites, but only concentrations for those with extraction efficiencies above 1%. Intracellular concentrations of oxidized glutathione are not available due to interference by an unknown compound. The concentration data for each metabolite is available at MetaboLights (http://www.ebi.ac.uk/metabolights/) as study accession number MTBLS567.

metabolite	extraction efficiency (%)	intracellular	extracellular
2,3-dihydroxybenzoic acid	100.9		
2,3-dihydroxypropane-1-sulfonate	0.6		yes
3-mercaptopropionic acid	88.6		
3-methyl-2-oxobutanoic acid	10.1		yes
3-methyl-2-oxopentanoic acid	50.1	yes	yes
4-aminobenzoic acid	18.5	yes	yes
4-hydroxybenzoic acid	88		yes
4-methyl-2-oxopentanoic acid	43.4	yes	yes
5'-methylthioadenosine	80.9	yes	yes
4-amino-5-aminomethyl-2-methylpyrimidine	0		
D-glucosamine 6-phosphate	0		
dimethylsulfoniopropionate	0		
γ-aminobutyric acid	0		
4-Amino-2-methyl-5-pyrimidinemethanol	0		
nicotinamide adenine dinucleotide	20.9	yes	
putrescine	0		yes
acetyltaurine	0		
adenine	0		
adenosine	6.5		
adenosine 5'-monophosphate	0.2	yes	
alpha-ketoglutaric acid	0	yes	
arginine	0	yes	

aspartic acid	0	yes	
glycine betaine	0	yes	
biotin	52.8		
caffeine	23.5		
chitobiose	0.3	yes	
chitotriose	5.6		
choline	0		
ciliatine	0		
citrate	0.9		
citrulline	0	yes	
cyanocobalamin	79.1		
cysteine	0		
cytosine	0		
desthiobiotin	6.5		
dihydroxyacetone phosphate	0	yes	
ectoine	0		
folic acid	41.2		
fosfomycin	0		
fumaric acid	0		
glucose 6-phosphate	0	yes	
glutamic acid	0	yes	
glutamine	0	yes	
glyphosate	15.1		
glutathione	1.1	yes	yes
glutathione oxidized	1.5	not available	yes
guanine	0		
guanosine	7.7	yes	yes
hemin	0	yes	
indole 3-acetic acid	16.8		
inosine	8.1		
inosine 5'-monophosphate	0	yes	
isoleucine	1.41	yes	yes
kynurenine	41.7		yes
leucine	3.3	yes	
malic acid	0.7		
methionine	0	yes	
muramic acid	0		
n-acetyl glucosamine	0		
n-acetyl glutamic acid	1.1	yes	
n-acetyl muramic acid	2.8		
ornithine	0		

orotic acid	0		
pantothenic acid	51.9	yes	yes
phenylalanine	39.7	yes	yes
phosphoenolpyruvate	0		
phycocyanobilin	0		
proline	0	yes	
pyridoxine	6.8		
riboflavin	87.6		
S-(5'-adenosyl)-L-homocysteine	43.3	yes	
S-adenosyl-L-methionine	0	yes	
alanine (isom. sarcosine)	0		
serine	0		
sn-glycerol 3-phosphate	0		
taurocholic acid	92.8		
spermidine	0	yes	yes
succinic acid	0		yes
syringic acid	32.9		
taurine	0		
thiamine	2		
thiamine monophosphate	0		
threonine / homoserine	0		
thymidine	52.6		yes
tryptamine	21.3		
tryptophan	46.7	yes	yes
tyrosine	2.1	yes	yes
uracil	0	yes	
uridine 5'-monophosphate	0	yes	
valine	0	yes	yes
xanthine	0.3		yes
xanthosine	10.4		yes

21

22

Table S3.

Mean (± one standard deviation) cell-specific concentrations of glycine betaine (fg cell^{-1}) in cultures of three strains of *Prochlorococcus* grown at a range of light intensities. *Biomass of cells from each strain are from Cermak et al. (2) in which the authors used a microfluidic mass sensor. We assumed 50% of the cell was carbon (3). These values were used to calculate the percent of each strain's intracellular carbon content that can be attributed to the carbon in glycine betaine. †Measured value for NATL2A, a related LLI strain of *Prochlorococcus*.

Strain	Clade	Growth light intensity (µmol photons m^{-2}s^{-1})	Median cellular biomass (fg)*	Glycine betaine (fg cell^{-1})	% of biomass
MIT9301	HLII	10	60 ± 3	4.5 x 10^{-4} (± 8.0 x 10^{-4})	0.002%
MIT9301	HLII	50	60 ± 3	0	
MIT0801	LLI	10	91 ± 5†	0	
MIT9313	LLIV	5	158 ± 6	3.0 (± 1.3)	4%
MIT9313	LLIV	10	158 ± 6	4.7 (± 2.4)	6%

Supplemental figures:

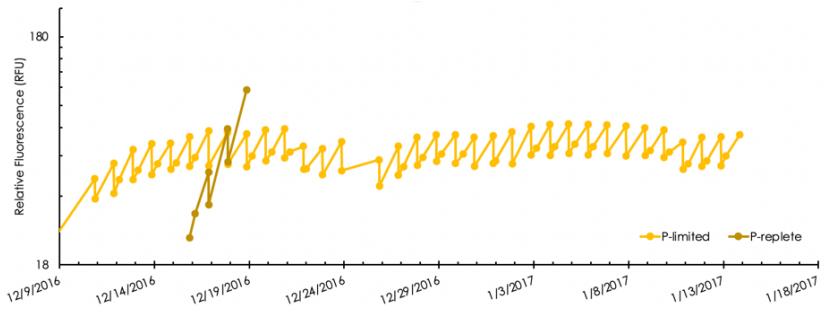


Figure S1: The yellow curve shows a culture of *Prochlorococcus* MIT9301 maintained in a semi-continuous state of P-limitation through daily dilutions with media containing 20-fold less phosphorus than the replete media. The brown curve shows a culture that from that point forward was diluted (at the same dilution rate) in media replete with phosphorus.

Figure S2. Intracellular metabolites from Figure 3, plotted on a log scale as discrete amol per cell values for each metabolite.

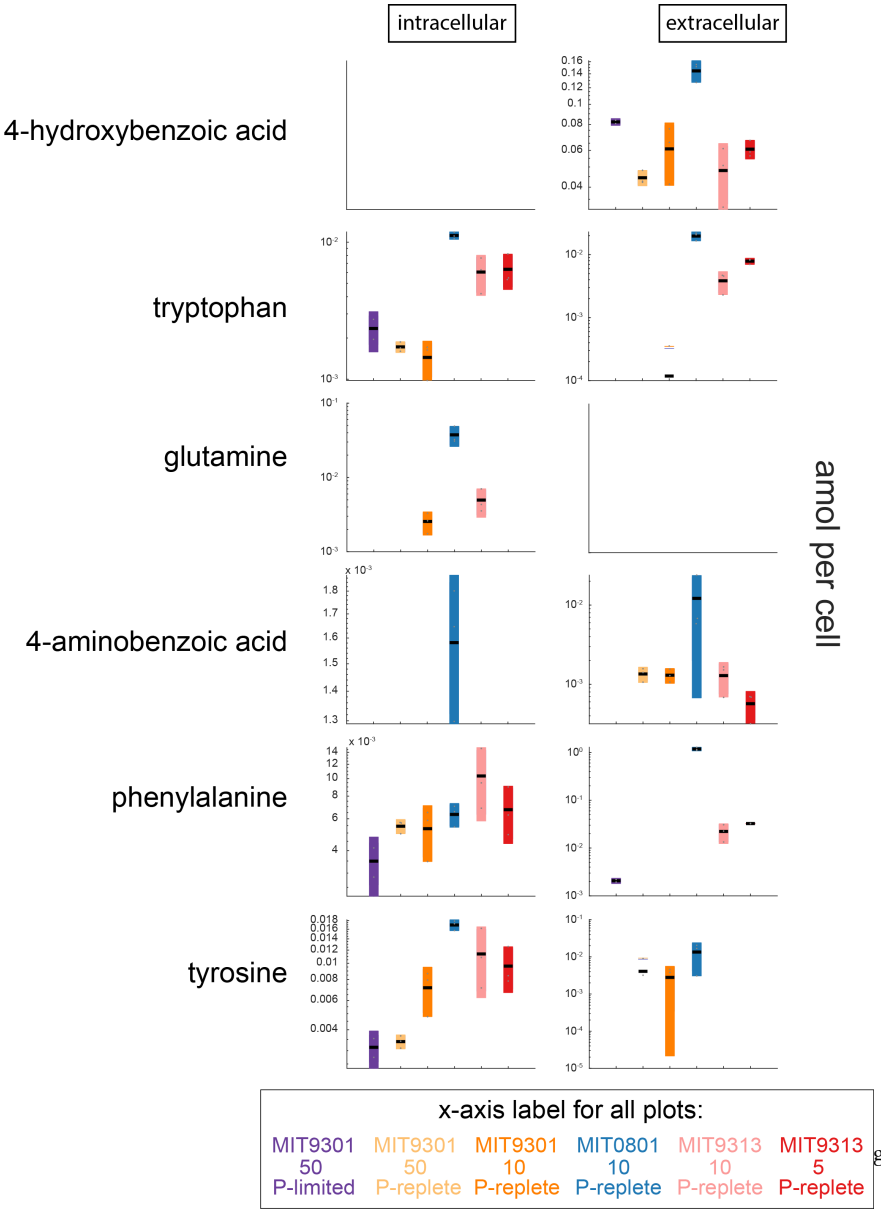


Figure S3. Intracellular metabolites from Figure 5, plotted as discrete amol per cell values for each metabolite.

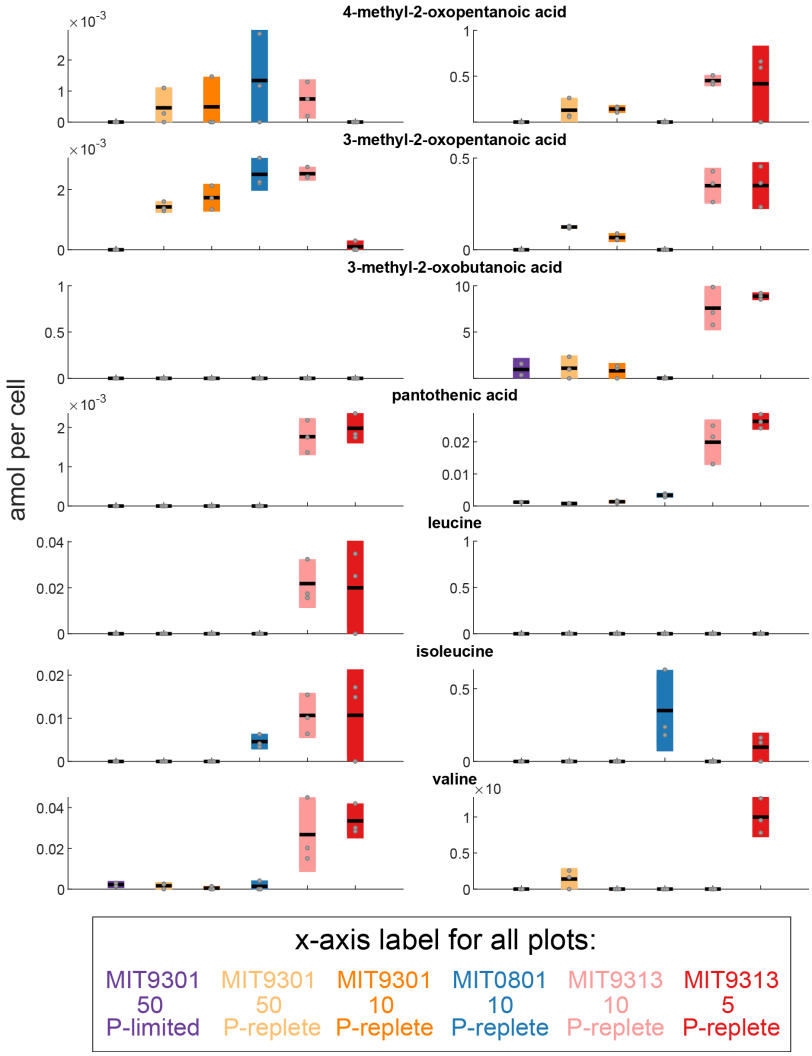
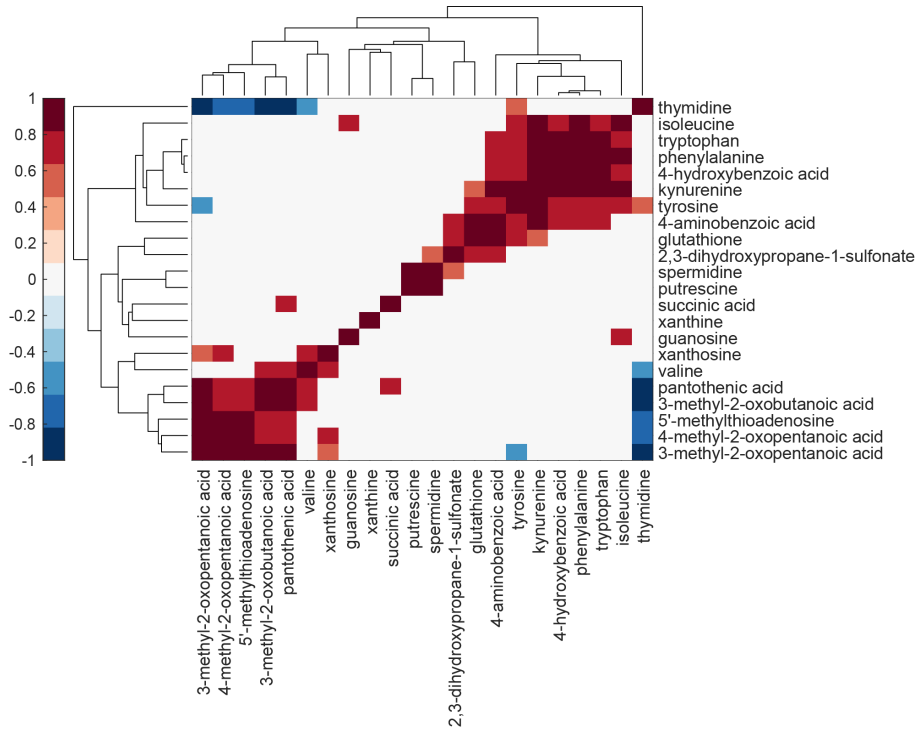


Figure S4. Clustergram of correlations for all extracellular metabolites collected from cells grown in replete conditions. Statistically significant positive (red) and negative (blue) correlations are Pearson correlations with p-values adjusted using a False Discovery Rate of 5%.



References cited

1. Johnson WM, Kido Soule MC, Kujawinski EB. 2017. Interpreting the impact of matrix on extraction efficiency and instrument response in a targeted metabolomics method. Limnology and Oceanography Methods 15:417-428.
2. Cermak N, Becker JW, Knudsen SM, Chisholm SW, Manalis SR, Polz MF. 2017. Direct single-cell biomass estimates for marine bacteria via Archimedes' principle. ISME J 11:825-828.
3. Atlas RM. 1988. Microbiology. Macmillan Publishing Company, New York, NY.