

Supplementary Information for

Community context and pCO₂ remodel the transcriptome of the “helper” bacterium

***Alteromonas* in co-culture with picocyanobacteria**

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This document includes:

Legends for Supplemental tables

Supplemental Figs. S1 to S12

Supplemental Tables (all are .csv files available with the supplemental material)

Table S1. Differentially regulated genes between pCO₂ conditions for *Prochlorococcus* MIT9312, *Synechococcus* CC9311, and *Synechococcus* WH8102

Table S2. Differentially regulated genes in *Alteromonas* EZ55 between pCO₂ conditions

Table S3. Differentially regulated genes in *Alteromonas* EZ55 between coculture conditions

Table S4. Differentially regulated genes in *Alteromonas* EZ55 between pCO₂ conditions that are affected by co-culture condition (i.e., the interaction between pCO₂ and coculture)

Table S5. Differentially regulated stress-related genes in *Alteromonas* EZ55 in response to the general response to coculture, and coculture with specific cyanobacteria at either ambient (400 ppm) or elevated (800 ppm) pCO₂.

Table S6. Differentially regulated Transporter-related genes in *Alteromonas* EZ55 in response to the general response to coculture, and coculture with specific cyanobacteria at either ambient (400 ppm) or elevated (800 ppm) pCO₂.

Table S7. Differentially regulated Chemotaxis-related genes in *Alteromonas* EZ55 in response to the general response to coculture, and coculture with specific cyanobacteria at either ambient (400 ppm) or elevated (800 ppm) pCO₂.

Table S8. Differentially regulated Metabolism-related genes in *Alteromonas* EZ55 in response to the general response to coculture, and coculture with specific cyanobacteria at either ambient (400 ppm) or elevated (800 ppm) pCO₂.

Table S9. Differentially regulated genes in *Alteromonas* EZ55 in response to coculture with *Prochlorococcus* sp. MIT9312 at 400 ppm pCO₂

Table S10. Differentially regulated genes in *Alteromonas* EZ55 in response to coculture with *Synechococcus* sp. CC9311 at 400 ppm pCO₂

Table S11. Differentially regulated genes in *Alteromonas* EZ55 in response to coculture with *Synechococcus* sp. WH8102 at 400 ppm pCO₂

Table S12. Differentially regulated genes in *Alteromonas* EZ55 in response to coculture with *Prochlorococcus* sp. MIT9312 at 800 ppm pCO₂

Table S13. Differentially regulated genes in *Alteromonas* EZ55 in response to coculture with *Synechococcus* sp. CC9311 at 800 ppm pCO₂

Table S14. Differentially regulated genes in *Alteromonas* EZ55 in response to coculture with *Synechococcus* sp. WH8102 at 800 ppm pCO₂

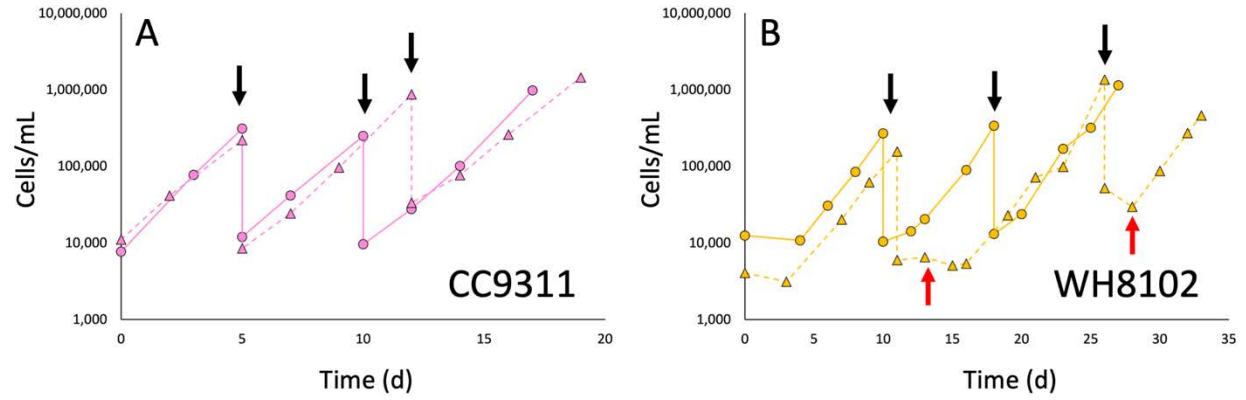


Figure S1. Growth dynamics of *Synechococcus* CC9311 (A) or *Synechococcus* WH8102 (B) under 400 (solid line) or 800 (dashed line) ppm pCO₂ conditions. Each graph shows a representative culture series across three subsequent transfer cycles; cultures were transferred into fresh media by 26-fold dilution whenever they crossed 2.6×10^5 cells mL⁻¹. Transfers were performed at the points indicated by black arrows. The red arrows in (B) shows an extended lag period (left arrow) and a moderate cell die-off (right arrow) illustrative of the growth problems experienced by WH8102 at 800 ppm pCO₂.

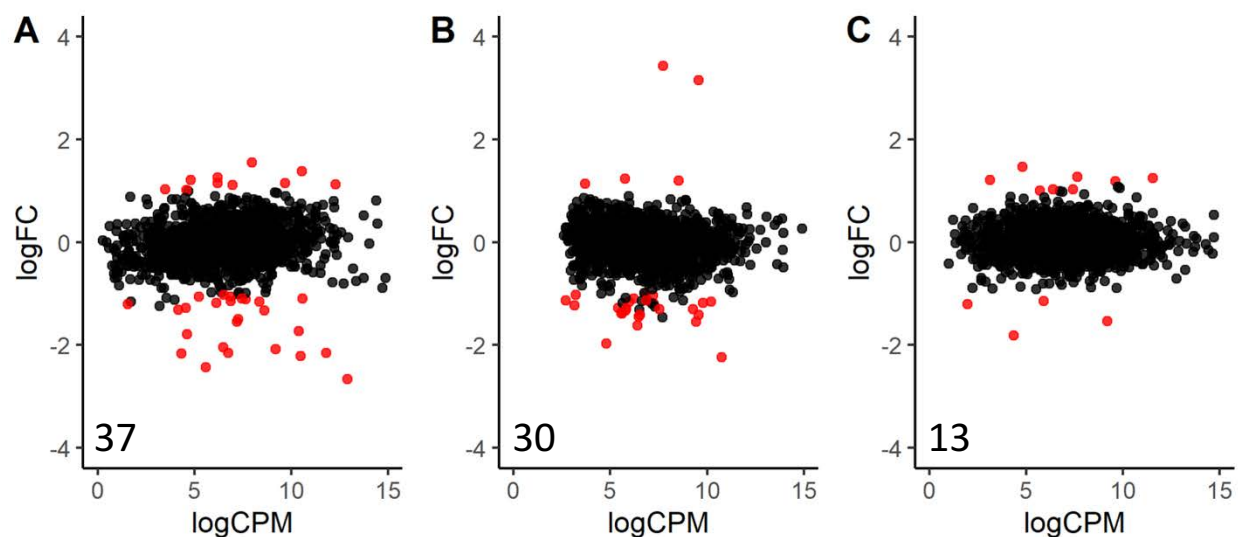


Figure S2. Differential gene transcription in *Prochlorococcus* MIT9312 (A), *Synechococcus* WH8102 (B) and *Synechococcus* CC9311 (C) between growth at 400 and 800 ppm pCO₂. Transcription for each gene is shown on the x-axis as the average abundance in log₂ counts per million (CPM), and the log₂ of the fold change (FC) on the y-axis. Genes which are significantly differentially transcribed ($p < 0.05$ and $|\log_2(\text{FC})| > 1$) are highlighted in red. Values in the bottom left corner of each plot indicate the number of genes significantly differentially regulated in each case.

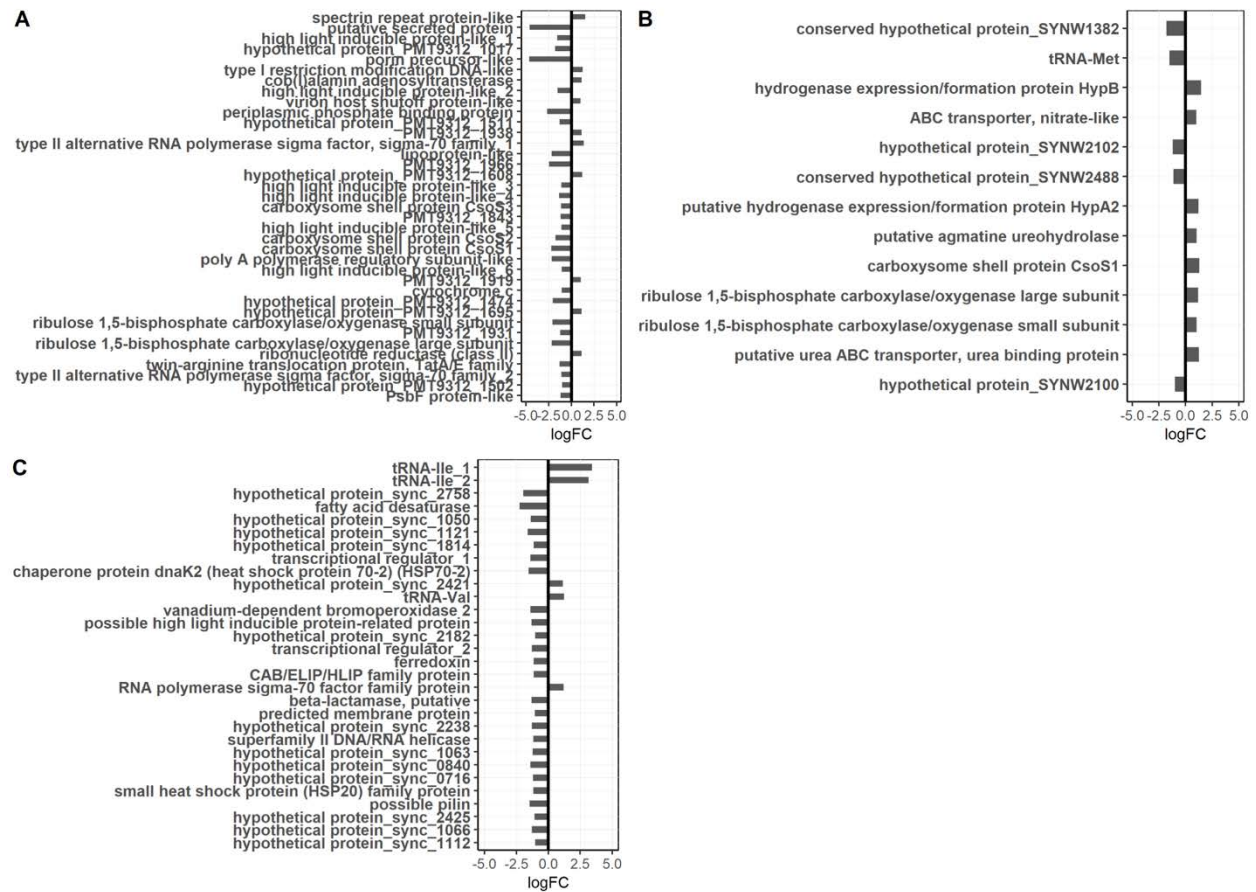


Figure S3. Differentially transcribed individual gene products in *Prochlorococcus* MIT9312 (A), *Synechococcus* WH8102 (B) and *Synechococcus* CC9311 (C) between growth at 400 and 800 ppm pCO₂. Positive or negative transcribed for each gene product is shown on the x-axis as the log₂ of the fold change (FC).

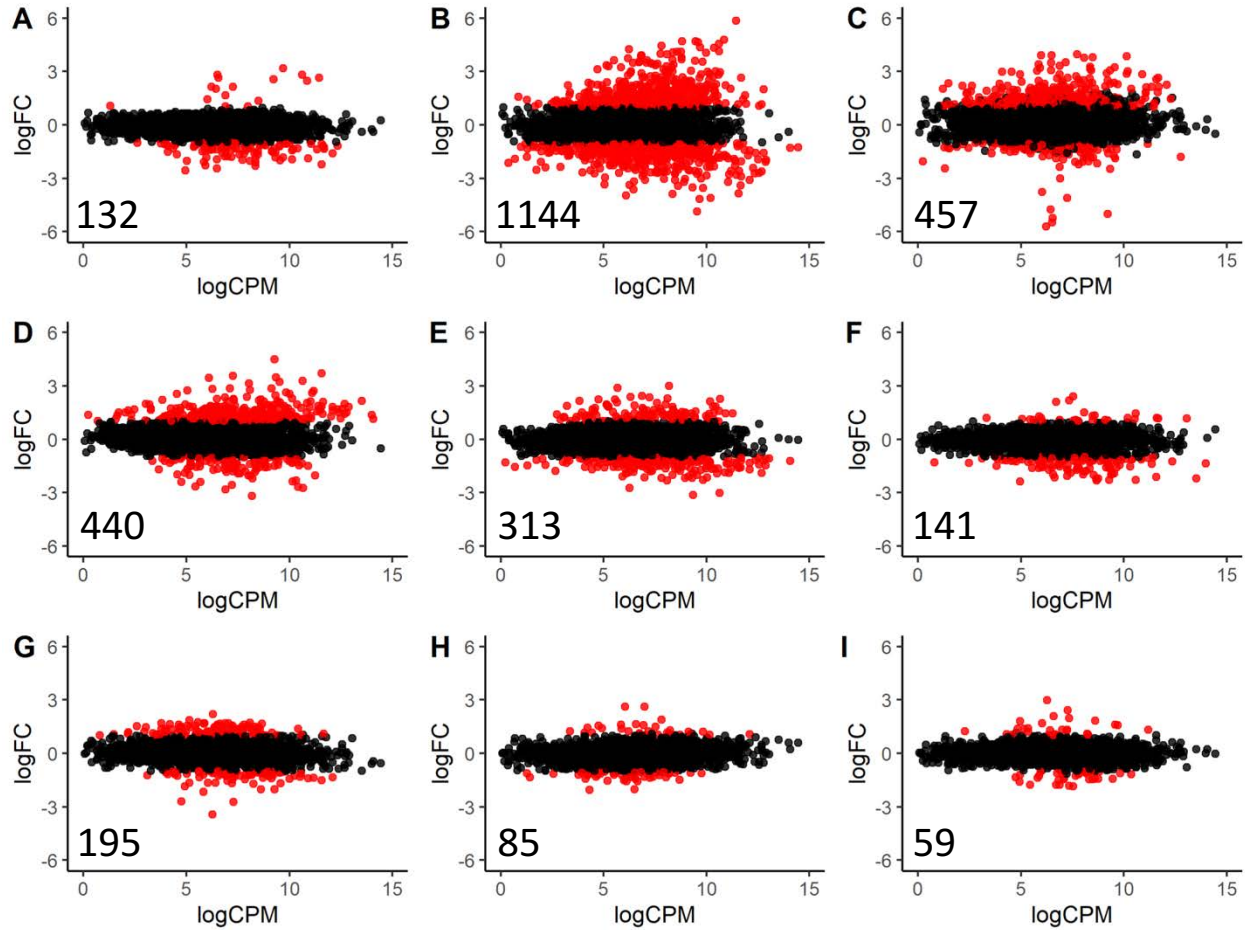


Figure S4. Differential gene transcribed in *Alteromonas* EZ55 under various experimental conditions. Transcription for each gene is shown on the x-axis as the average abundance in log₂ counts per million (CPM) and the log₂ of the fold change (FC) on the y-axis. Genes which are significantly differentially transcribed ($p < 0.05$ and $|\log_2(\text{FC})| > 1$) are highlighted in red. Values in the bottom left corner of each plot indicate the number of genes significantly differentially regulated in each case. (A) General response to 800 ppm pCO₂; (B) general response to co-culture with cyanobacteria; (C) genes that are differentially transcribed between pCO₂ conditions in one co-culture context but not the other; (D-F) genes that are differentially transcribed only in coculture with MIT9312, CC9311, or WH8102, respectively, at 400 ppm pCO₂; (G-I) genes that are differentially transcribed only in coculture with MIT9312, CC9311, or WH8102, respectively, at 800 ppm pCO₂.

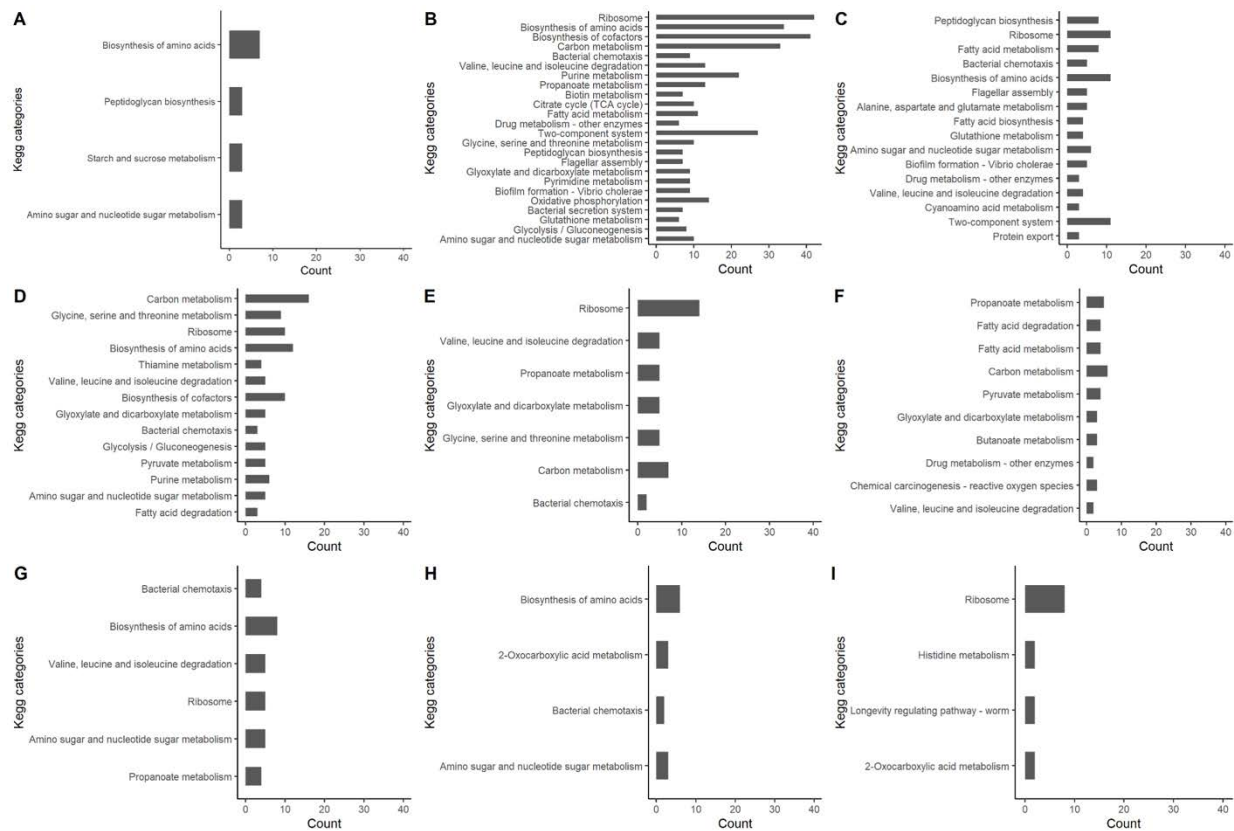


Figure S5. Plots for over-representation analysis (ORA) of significantly-changed KEGG pathways ($p < 0.01$) in *Alteromonas* EZ55 under different experimental conditions. ORA was implemented using hypergeometric tests according to Wu et al., (2021). (A) General response to 800 ppm pCO₂; (B) general response to co-culture with cyanobacteria; (C) genes that are differentially transcribed between pCO₂ conditions in one co-culture context but not the other; (D-F) genes that are differentially transcribed only in coculture with MIT9312, CC9311, or WH8102, respectively, at 400 ppm pCO₂; (G-I) genes that are differentially transcribed only in coculture with MIT9312, CC9311, or WH8102, respectively, at 800 ppm pCO₂.

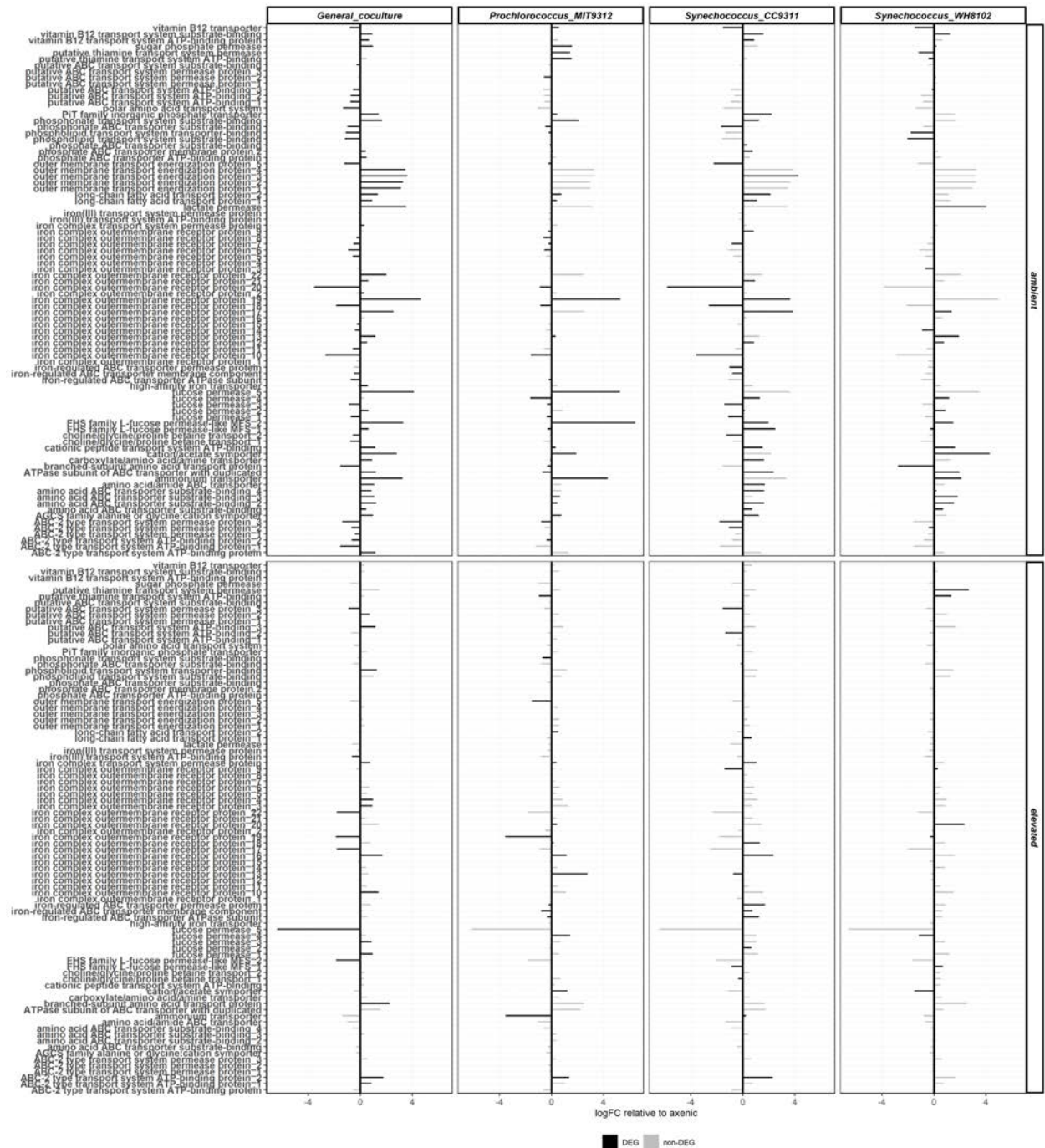


Figure S6. Transporter-related genes in EZ55 representing the general response to co-culture (column 1), and co-culture with specific cyanobacteria (columns 2-4) at 400 or 800 ppm pCO₂, relative to axenic conditions at the same pCO₂. Log₂ fold change (logFC) is plotted relative to axenic EZ55 under the same pCO₂ condition. DEG products are highlighted in black. Black bars in column 1 indicate the average co-culture response is significantly different from the axenic response at the same pCO₂; black bars in columns 2-4 indicate significant difference between the specific cyanobacterial response and the general coculture response shown in column 1.

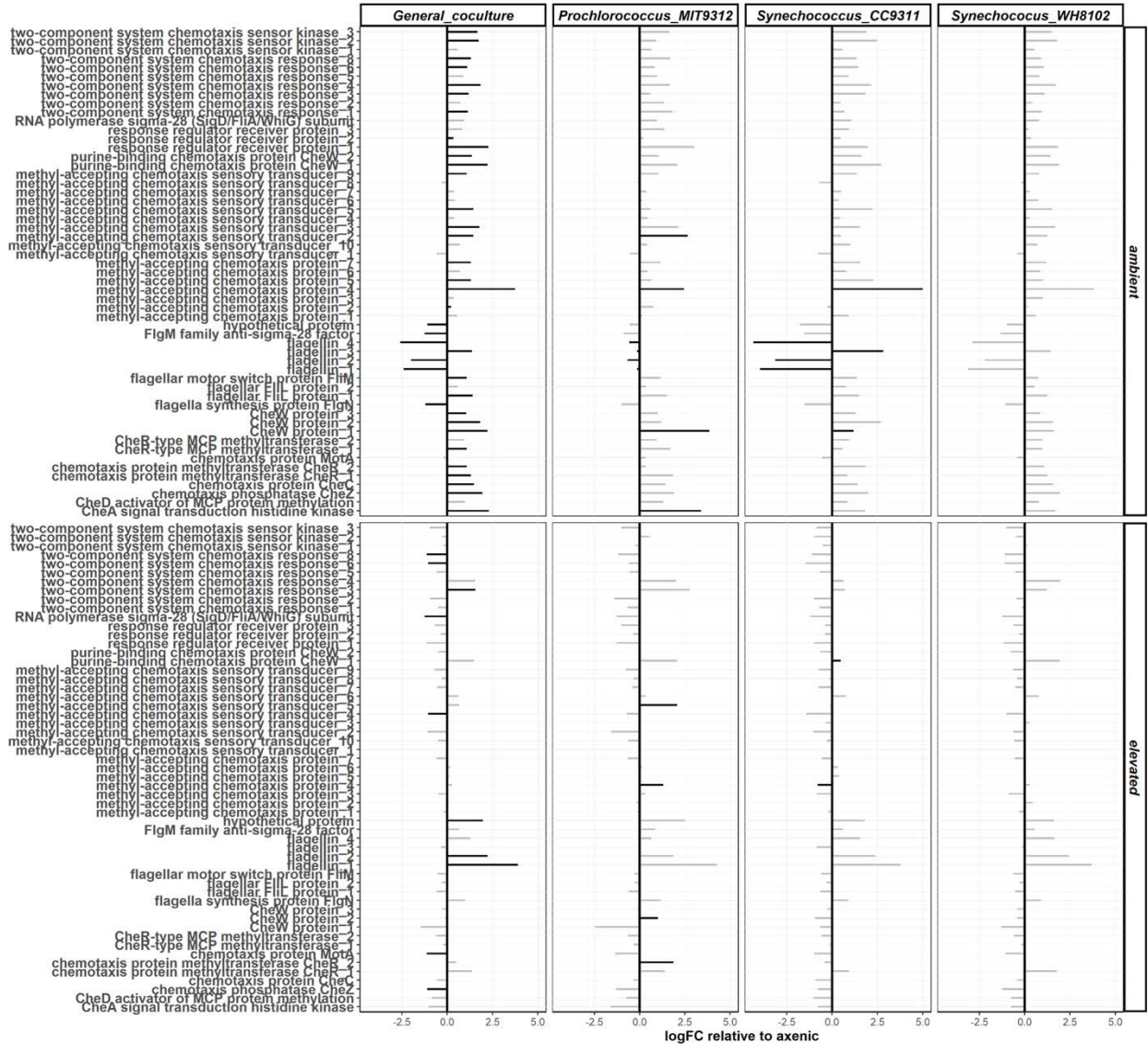


Figure S7. Summary plot of chemotaxis and two-component sensory system gene products in *Alteromonas* EZ55 representing the general response to co-culture (column 1), and co-culture with specific cyanobacteria (columns 2-4) at either 400 or 800 ppm pCO₂, relative to transcription under axenic conditions at the same pCO₂. Log₂ fold change (logFC) is plotted on the x-axis relative to axenic *Alteromonas* under the same pCO₂ condition. Differentially transcribed gene products (p < 0.05 and logFC > 1) are highlighted in black. Black bars in column 1 indicate the average co-culture response is significantly different from the axenic response at the same pCO₂; black bars in columns 2-4 indicate significant difference between the specific cyanobacterial response and the general coculture response shown in column 1.

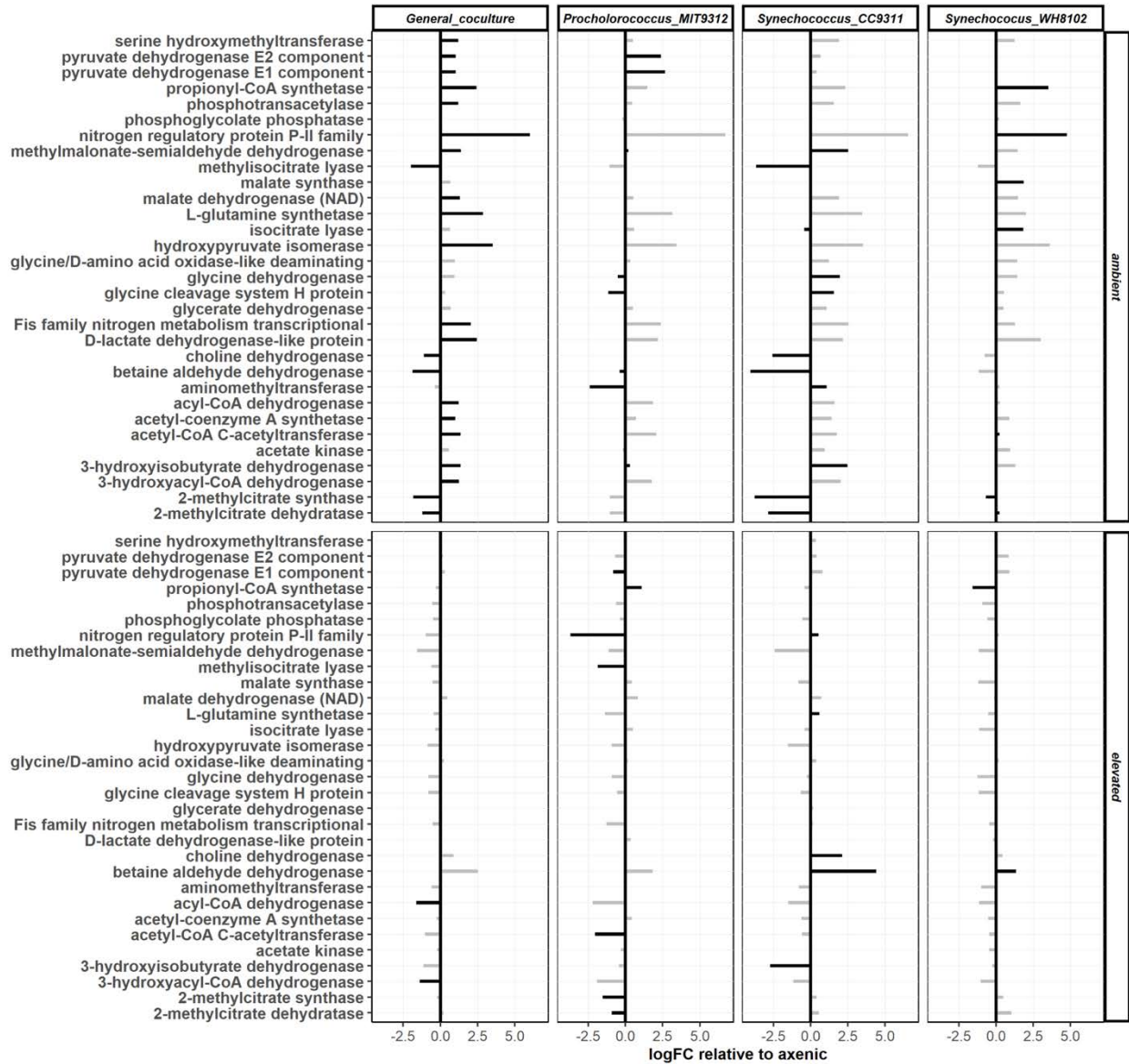


Figure S8. Summary plot of metabolism-related gene products in *Alteromonas* EZ55 representing the general response to co-culture (column 1), and co-culture with specific cyanobacteria (columns 2-4) at either 400 or 800 ppm pCO₂, relative to transcription under axenic conditions at the same pCO₂. Log₂ fold change (logFC) is plotted on the x-axis relative to axenic *Alteromonas* under the same pCO₂ condition. Differentially transcribed gene products ($p < 0.05$ and $\log_{2}FC > 1$) are highlighted in black. Black bars in column 1 indicate the average co-culture response is significantly different from the axenic response at the same pCO₂; black bars in columns 2-4 indicate significant difference between the specific cyanobacterial response and the general coculture response shown in column 1.

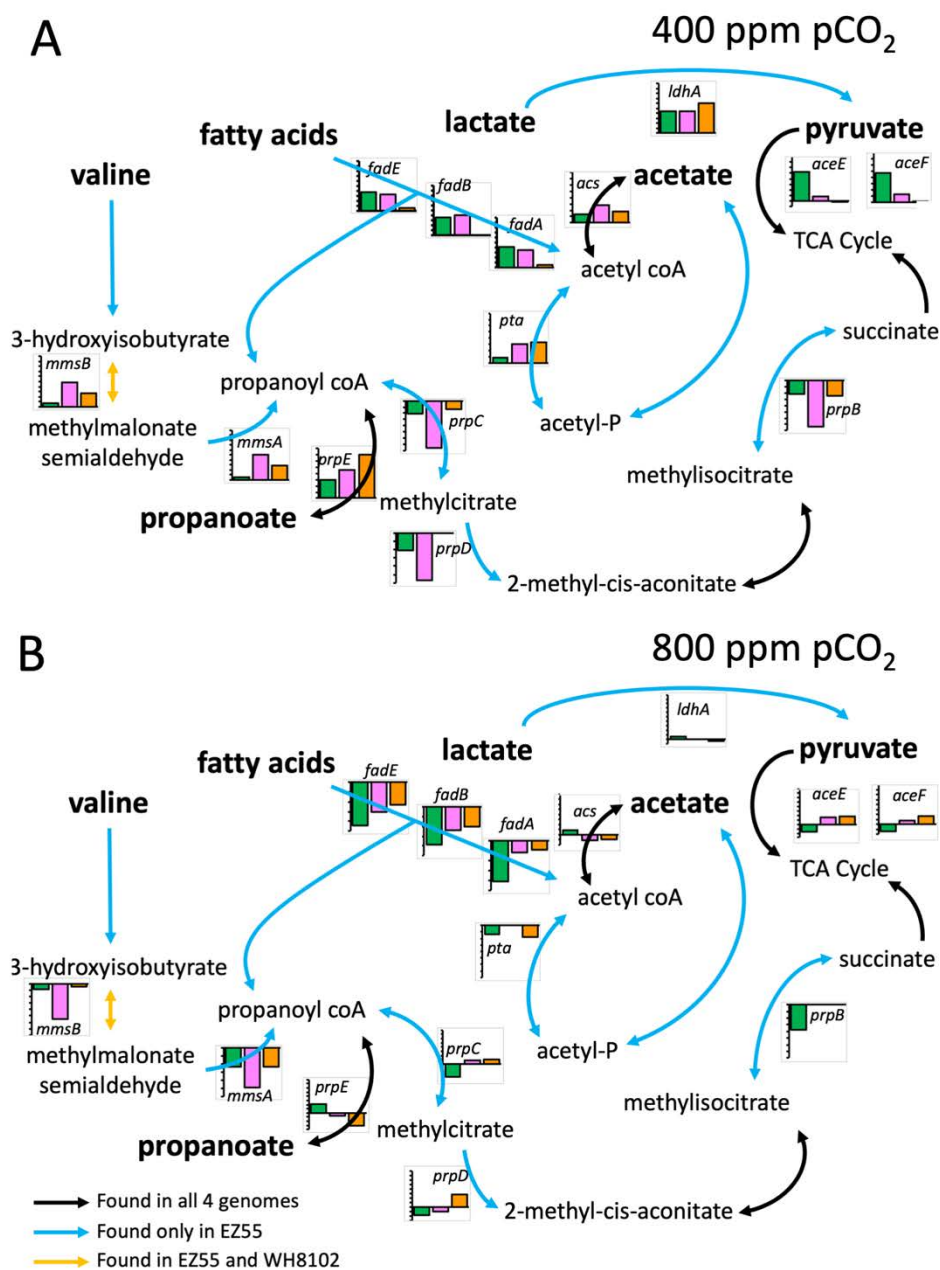


Figure S9. Reconstruction of metabolic pathways involving genes not directly involved in the recovery of photorespiratory byproducts that were significantly differentially regulated in *Alteromonas* EZ55 between axenic and co-culture conditions at 400 (A) or 800 (B) ppm pCO₂. Small inset graphs indicate the log fold change of transcription, relative to axenic culture, in co-culture with MIT9312 (green bars), CC9311 (pink bars), or WH8102 (orange bars). Gene names correspond to the related genes in *E. coli*. Only genes that are differentially transcribed between axenic and co-culture in at least one condition are shown. Metabolites in bold print are hypothesized to be phytoplankton exudates used by EZ55 under certain conditions. Arrow colors indicate which of the four genomes are capable of performing the indicated reaction based on annotated gene functions; see inset legend in panel (B) for explanation.

A

<i>E. coli</i> MG1655 GlcD	MSILPERRDGLPDVDRTSVLMALREHVPGIEIDHDEEIPPEYEDLSAVETRLVVLVLPKQEQTAILAVCHRLRVPPVVTGAGICLGGALPLEKCVL	1
EZ55_01786	-MILPALAVESNLA--ACYK FVER KMSAPD DICTAVS RIRAHAND--SVYKVPQNLIPKSTEDCOCIKLASHPDVAFAFGGGKCTNGOSIT--DQIV	
CC9311_049750350	MTKLPAQLPGTSSVNYDMPALEREKGFIPSKSVVKROBELLSYDQDLMDHRIPLAVLPETEQVAQLACCHRLGIPFVARGSCICLGGALVQDALL	
<i>S. elongatus</i> PCC7942 GlcD	-MSQPMALAA--PNWILAO FREVLCSEVVERRELLSYDQDLMDHRIPLAVLPETEQVAQLACCHRLGIPFVARGSCICLGGALVQDALL	
WH8102_08323	-----KLVANF-----HDWSLEERDRRSIPQRAVVKRELLSYDQDLMDHRIPLAVLPETEQVAQLACCHRLGIPFVARGSCICLGGALVQDALL	
MIT9312_1399	-MTSNMRFIDKFREVKNLIESQSEVRKSKDF--YIYSPILSKDQ-----CHADPVRSDHNAKKVETICMT--SIPILKAGICNGYQAVELFKGVV	
<i>E. coli</i> MG1655 GlcD	VRRRARQPGVRLALSOVAPENITATDSSQICACGGNVANAGGVICNYGLAVH--NLIKIEVQDLGEL	121
EZ55_01786	EEEMRQAGIVKDSNDVRRHGLFFS DLSISNRSLGGMISNDAGGQSVYCRISD--HVLGKAVLANGEITETAKISRDEALLKASGCTSEAAIYQQAM	
CC9311_049750350	DNQRTPEPGVINSWYRAVVDQTYIADSSQVVCISGGNVANESGGVICNYGVTSN--HVLSDVVLNPGT	
<i>S. elongatus</i> PCC7942 GlcD	DNQRTPEPGVINSWYRAVVDQTYIADSSQVVCISGGNVANESGGVICNYGVTSN--HVLGLTLVLPDSVI	
WH8102_08323	DNQRTPEPGVINSWYRAVVDQTYIADSSQVVCISGGNVANESGGVICNYGVTSN--HVLGLTLVLPDSVI	
MIT9312_1399	ETGFKQSGCVMEDNKOELRYELRLLESWKQKIGGFVAGSGGICSRKGLRDPGNLIGLFAVANNKTV	
<i>E. coli</i> MG1655 GlcD	-----TLGSDAL--DSPGDLALFFGSEGLGVTEVAVKLRKPPVAVLLSDFSERAGLAVGDIAN--GLIPGGIEMDMLSTRADFETAG--	241
EZ55_01786	RLNRLFLTGDDHVVVDNDIDGRLLIGESLGFYTEALNDRKTIAPKALVNSYRSFDAIRHAPVLYKAKATSVETISVNFKADIVMHSHEITDVI	
CC9311_049750350	RLGSLIC--DAEDRGVFIGSECLGITAIALRLRARDVAVGLLDFFSQAACFAVRLITRA--GVLPAGIETDQCQAVNEFEEET	
<i>S. elongatus</i> PCC7942 GlcD	QVGCATA--DEPCYDLCGIFVSECLGITAIALRLRARDVAVGLLDFFSQAACFAVRLITRA--GVLPAGIETDQCQAVNEFEEET	
WH8102_08323	QLGNGLA--SSERDQGFVFIGSECLGITAIALRLRARECVNLLADFFMAAAGFAVRSIA--GLLPAGIETDQVNTINAVDPPFYD	
MIT9312_1399	-----LKKDDE--SSEPLN--HAYCNGITSLATIDIRKKYISVI--DIEFQVAILKQTS--AVDMKLGAILEETIVYQMPHFHKS	
<i>E. coli</i> MG1655 GlcD	-VESDQEDCERNIDILK--AGARDLRLADEERUV--TAGRKNFPAVGR--SDIYCDQIFPRALPGVLEGIARQQDYD--N	361
EZ55_01786	DSAEKVEEQEAAEQEIGRDCSSIGISVITDNDIQR--YAMRKKAVGLLGVKGYQKIAFAEDIAVPPEDLDFHSEFRLDSEHNY--GM	
CC9311_049750350	-QEQEKQAVIVITLALCRE--AGAGATREANSEEDRAR--MKRKSALALGRO--YETIYODGVVPRCALRVLEADQSAEHELGV--N	
<i>S. elongatus</i> PCC7942 GlcD	-RSEVADQRDVAVCRQ--EGARSTAIADIEDRR--MKRKAFAAVGR--SYIYODGVVPRSTAFVLHEQGGQKGRY--N	
WH8102_08323	-QAEQESSEQLLRCQ--AGARGRRSDPCECAV--MKRKSFAVGR--ETIYODGVVPRSTPAVLHEERSOEHLGV--N	
MIT9312_1399	-----KKTEETCKK--FKVSTALGSEKLVNGISEVVM--HATHEHRS--DNWATLQMLP--NKSEELDNFKTKKGGKLVHLE	
<i>E. coli</i> MG1655 GlcD	DNEPGFRARELGCKILECUEYGGSSCEHGICREKNOMCAQNSDEYTFHAKAATDPDGLLPCNKE	481
EZ55_01786	DSDQOQALMTSDREUVALVAKYGLMNCHEKGFRESEYGFAGFKTLFNRKKAVIDPHNOMPKCTPIESDDKLVSVRDKRAGVERVIPVSIKQDI	
CC9311_049750350	RSSEGVNERKALGGALMNCUEYGGSSCEHGVGSKKQVLDQNSDEYTFHAKAATDPDGLLPCNKE	
<i>S. elongatus</i> PCC7942 GlcD	DRNDGALERKALGCEILKCNVYGGSSCEHGIGADKKQVLPANFSSDEYTFHAKAATDPDGLLPCNKE	
WH8102_08323	SNADFERKKEGAAILRCIDAGSSCEHGVGADKKQVLDQNSDEYTFHAKAATDPDGLLPCNKE	
MIT9312_1399	KWNGSEI--NELIEDCKKLGAFIFNPVILVEGGGL--VDSQDK--KKFDRKGLNPKKE	
<i>E. coli</i> MG1655 GlcD	-----TLHRC--	601
EZ55_01786	YEETSPMCPSSKVTQDRRHTPKGRASHLEWTRLLSLSTKFGTTLESEGLSYKQKSHFLSRFKNSILSNKPDPSHEVKKVMDGCLACKSCTHCCPVNTPHPR	
CC9311_049750350	-----PFPKCC--	
<i>S. elongatus</i> PCC7942 GlcD	-----PFPKCC--	
WH8102_08323	-----PFPKCC--	
MIT9312_1399	-----PFPKCC--	
<i>E. coli</i> MG1655 GlcD	-----	721
EZ55_01786	KDMLVANIETIAPLSARAPKLHNAILKLVKHFLEKQVGYKDTPLSLSYPTLTSRVKALTVPFDLQRLSQMNTTEERKHIVAVVDQPTTSYYDATSVKELISVIYK	
CC9311_049750350	-----	
<i>S. elongatus</i> PCC7942 GlcD	-----	
WH8102_08323	-----	
MIT9312_1399	-----	
<i>E. coli</i> MG1655 GlcD	-----	841
EZ55_01786	PAHVKGFLDKFEKQAKTANLLNQLHALDITMVGTDASLVLCYRDEYKKYLRDKRGNFVHTIDEWLDVLTEKHIVKAMIKKRFSLLAHCKSEKAMPALTRWQ	
CC9311_049750350	-----	
<i>S. elongatus</i> PCC7942 GlcD	-----	
WH8102_08323	-----	
MIT9312_1399	-----	
<i>E. coli</i> MG1655 GlcD	-----	961
EZ55_01786	GCCGMAGTYGHEASHYKNSKALYELSWSGVFKQCSQDEVLVSGFSCRSQVARFEGKKPRHPPIAVVNELIQ	1031
CC9311_049750350	-----	
<i>S. elongatus</i> PCC7942 GlcD	-----	
WH8102_08323	-----	
MIT9312_1399	-----	

B

<i>E. coli</i> MG1655 GlcE	MLRECDYS-----QALLEQVNOAISDRFLVIOGSNSKAFGRPV--TQOTDVRCHRCVINYDFATVIGARVGT	1
<i>S. elongatus</i> PCC7942 GlcE	MPAATLADWKAASVPDFLAERSRQELARLGNPGWNGPPDEALAEQOLAAEGWRILPACRGCRDWGGLGEP--QAVISTANCDRLTEHAADTAVVAGMT	
CC9311_011618194	MSSTAVLA-----NSVEELCOLIADHEGEFVSGMGRHNGPLAADSFPVVSRRSRIVDHAADDITAVDGMF	
WH8102_6676	MSSES-----PADRSALIEVROMHODTTWIESGLGHLGNGERI--DEAVLSCRH--DRVIDHAADDITAVVAGMT	
<i>E. coli</i> MG1655 GlcE	LPCEPPHYCE--ETWGGVACGLAGRPWPSSGSRDFVLETRITAGAEHRRFGGVKKNVAGYDLSRMVGGVYCLGLVLTENKV--LPRRSSTSR	121
<i>S. elongatus</i> PCC7942 GlcE	LPDPLFIACE--SSSLGALVSTGMSRRRRQGLRDFTIGITFTRDQGMHGGGRVKKVAGYDLMRLGLGAGSGGLGICTFRRNYPLNMCITNCO	
CC9311_011618194	LPDPLFIACE--SSSLGALVSTGMSRRRRQGLRDFTIGITFTRDQGMHGGGRVKKVAGYDLMRLGLGAGSGGLGICTFRRNYPLNMCITNCO	
WH8102_6676	LPDPLFIACE--SSSLGALVSTGMSRRRRQGLRDFTIGITFTRDQGMHGGGRVKKVAGYDLMRLGLGAGSGGLGICTFRRNYPLNMCITNCO	
<i>E. coli</i> MG1655 GlcE	WOLQPLPISG--LCMPDNALNR--EGGSGVKAARELLGEEI-----AGQFWOO--REQQLFFSPGCEMR--S--SD--APNDIPCEQID	241
<i>S. elongatus</i> PCC7942 GlcE	SDTTFVFDLSPAAAAAE--ERSQATVILRFESVA--SVQEQQVVELOETRLGLAATVVEPVWQ--NOEFERRR--S--ATLTKPALL--SO--PCLLAEE--IAPPA	
CC9311_011618194	SSDTERRDN--LRDQGERILLGVA--SDGAVDDQNR--ETATL--KQGVSS--ERMWEN--IPEGSA--TSDEPPNLLR--CLPPAP--IQRITSSRECA--ATG	
WH8102_6676	SSDTERRDN--QGSVEQGLQ--RIVISSDTAVSEQ--QR--DNRGRSHGTS--DDQPCSDP--DNGELFCSA--FVWLV--VILPPAK--VEML--QOAS--A--ATG	
<i>E. coli</i> MG1655 GlcE	WLS--T--EDNQH--RIARRAGCAARFSAQ--DGGFAP--TSARLRY--QOL--KQOLD--CGGVNPGMY--E--	361
<i>S. elongatus</i> PCC7942 GlcE	WLWN--E--EPD--TLLRLRRHCEVAGTFLVLAQVSLKQ--D--LWGYPGGLGAVIRAKKROFD--QBR--LAPGR--LAGIE--	
CC9311_011618194	WLSGAT--T--EYL--E--LRR--YIALGQ--SVL--Q--GAOPGALPAW--L--SR--LIE--AVKROFD--KQ--SRGR--LPGVA--PLNSAHSN	
WH8102_6676	WSSG--S--QAF--E--E--LRR--YIELGGS--V--LQ--E--TAIAPW--L--DAP--VIE--AVKROFD--KQ--CRGR--LPGV--NDTA--	449

[illegible]

1 [1

E. coli MGI655 LctD
E255_03347

E. coli O18:K1:H7 Lox
E255_03715

121 2

E. coli MGI655 LctD
E255_03347

E. coli O18:K1:H7 Lox
E255_03715

241 3

E. coli MGI655 LctD
E255_03347

E. coli O18:K1:H7 Lox
E255_03715

361 4] 451

E. coli MGI655 LctD
E255_03347

E. coli O18:K1:H7 Lox
E255_03715

E

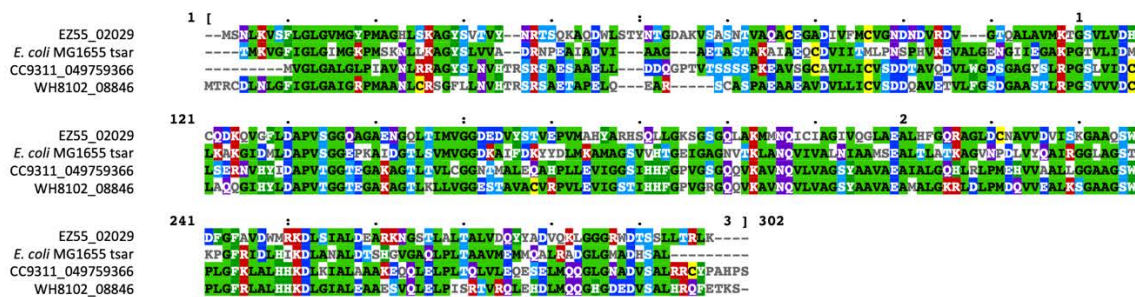


Figure S10. Alignment of hypothesized missing genes in the glycolate utilization pathways of the four strains in this study. Colored residues represent 70% consensus at the level of amino acid equivalence class. A-C) Candidates for the three subunits of bacterial glycolate dehydrogenase, GlcD, GlcE, and GlcF, respectively. For GlcD and GlcF, the putative *Alteromonas macleodii* EZ55 GlcDF fusion protein is included to show its homology with both enzymes. D) Putative lactate/glycolate oxidases. E) Putative tartronate semialdehyde reductases.

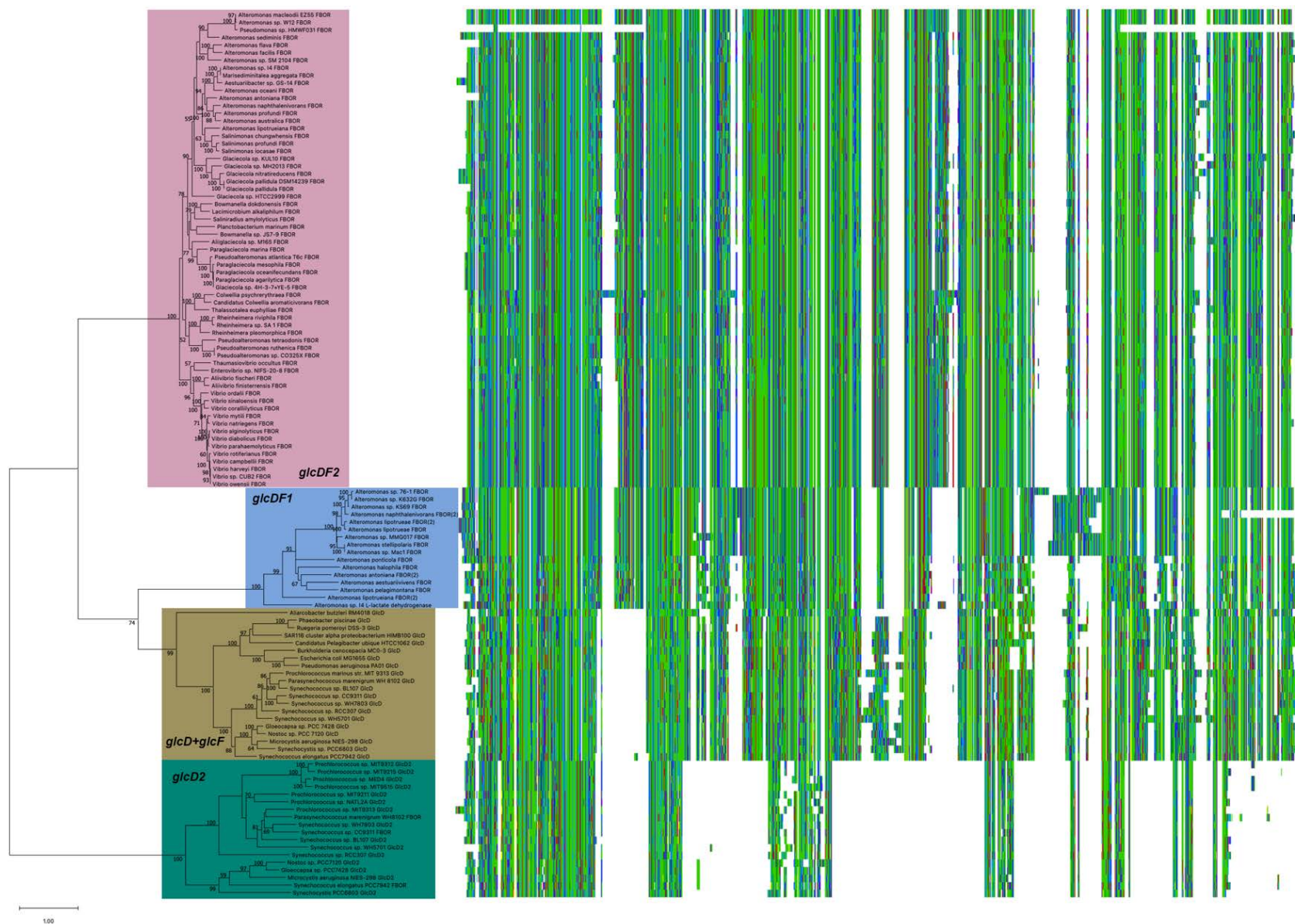


Figure S11. Maximum likelihood tree of GlcDF-like proteins. Tree was constructed in MEGA 11 using WAG+G+I+F substitution model. Node values are the percentage support from 100 bootstrap replicates. *Micromonas pusilla* peroxisomal GOX was used as an outgroup to root the tree and was subsequently pruned for easier visualization. Colored patterns to the right of the tree show the protein alignments with mview color coding, indicating the strong conservation between the separate *glcD* and *glcF* genes previously studied and the *glcDF* fusion protein described here. FBOR, FAD-binding oxidoreductase.

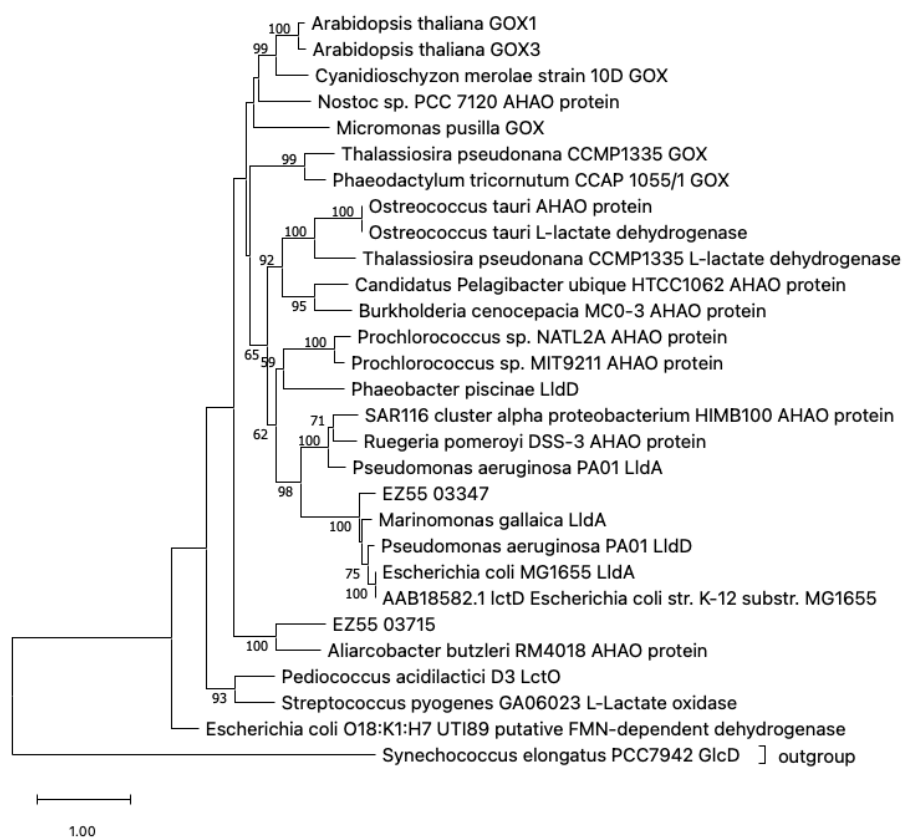


Figure S12. Maximum likelihood tree of LOX/GOX-like proteins. Tree was constructed in MEGA 11 using LG+G substitution model. Node values are the percentage support from 100 bootstrap replicates. *Synechococcus elongatus* glycolate dehydrogenase subunit GlcD was used as an outgroup to root the tree. AHAO, alpha-hydroxy-acid oxidizing