

Supplementary Information for

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

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

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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

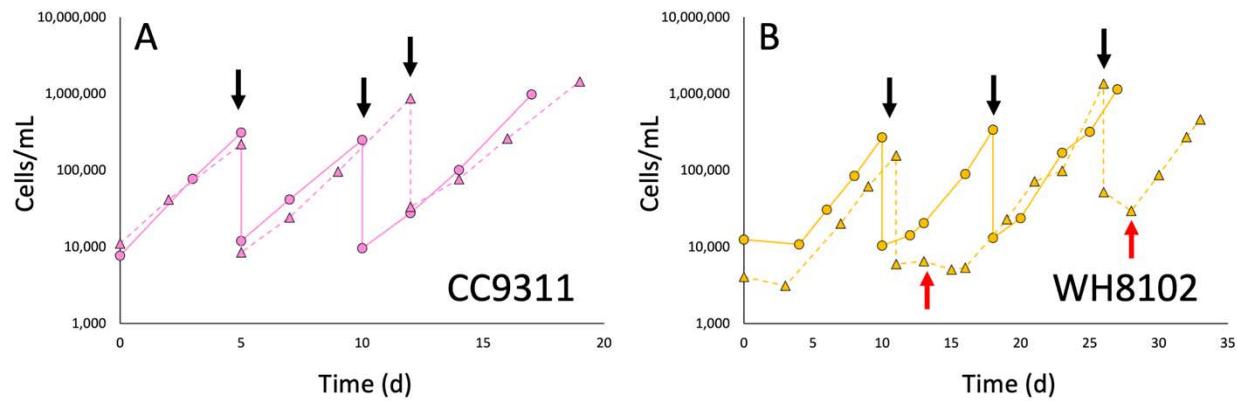


Figure S1. Growth dynamics of *Synechococcus* CC9311 (A) or *Synechococcus* WH8102 (B) under 400 (solid line) or 800 (dashed line) ppm pCO₂ conditions. Each graph shows a representative culture series across three subsequent transfer cycles; cultures were transferred into fresh media by 26-fold dilution 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.

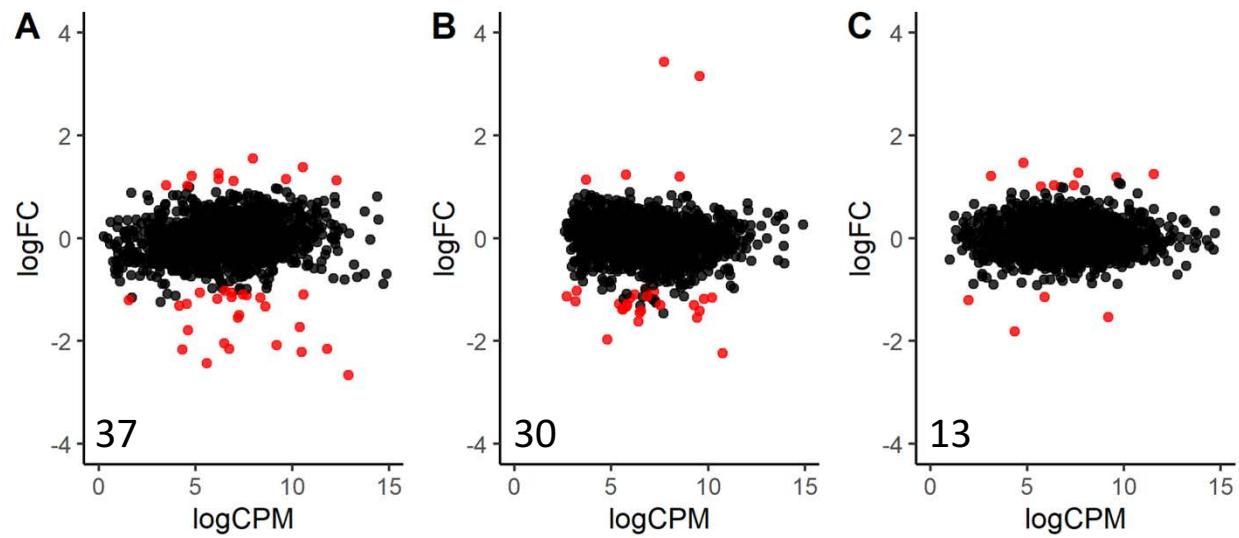


Figure S2. Differential gene transcription in *Prochlorococcus* MIT9312 (A), *Synechococcus* WH8102 (B) and *Synechococcus* CC9311 (C) between growth at 400 and 800 ppm pCO₂. Transcription for each gene is shown on the x-axis as the average abundance in log₂ counts per million (CPM), and the log₂ of 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.

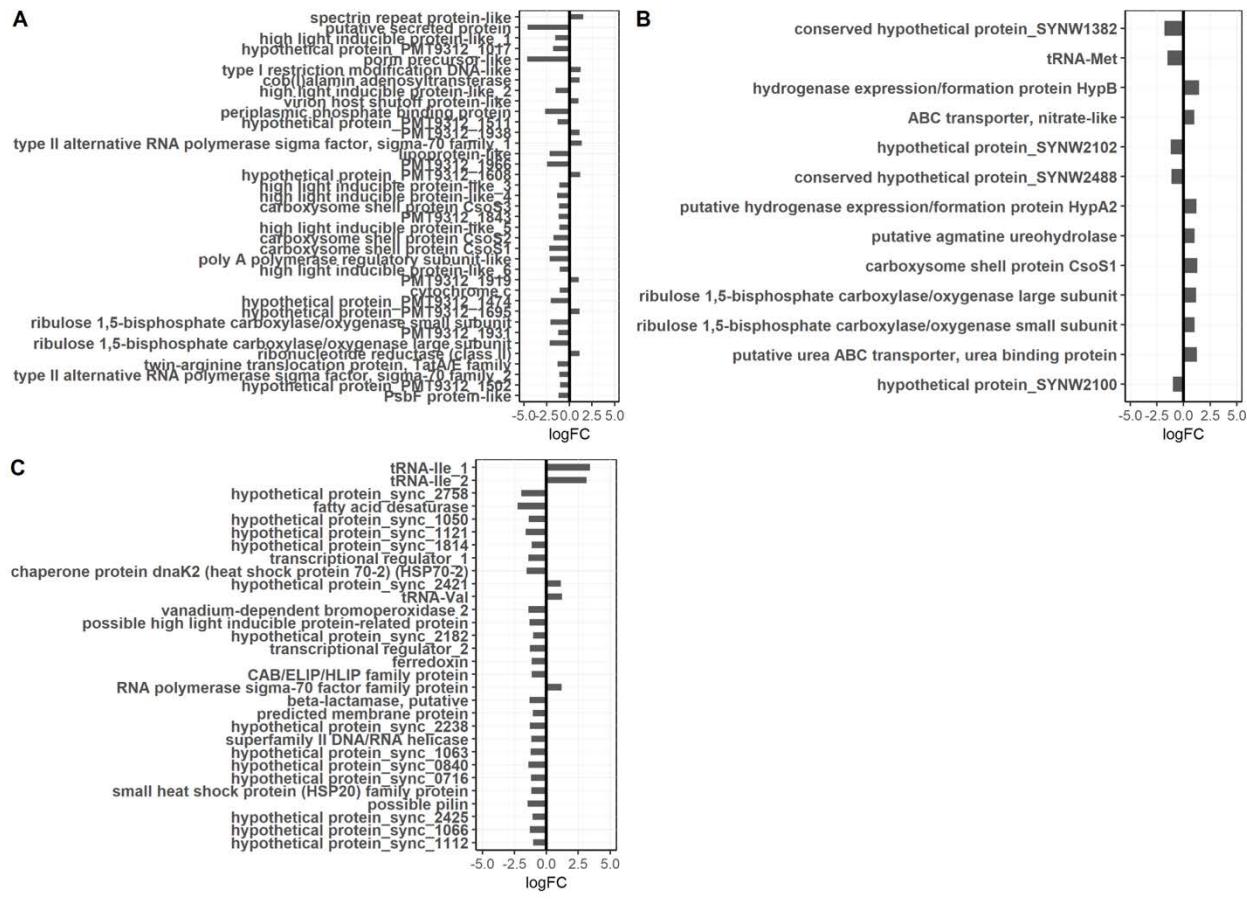


Figure S3. Differentially transcribed individual gene products in *Prochlorococcus* MIT9312 (A), *Synechococcus* WH8102 (B) and *Synechococcus* CC9311 (C) between growth at 400 and 800 ppm pCO₂. Positive or negative transcribed for each gene product is shown on the x-axis as the log₂ of the fold change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

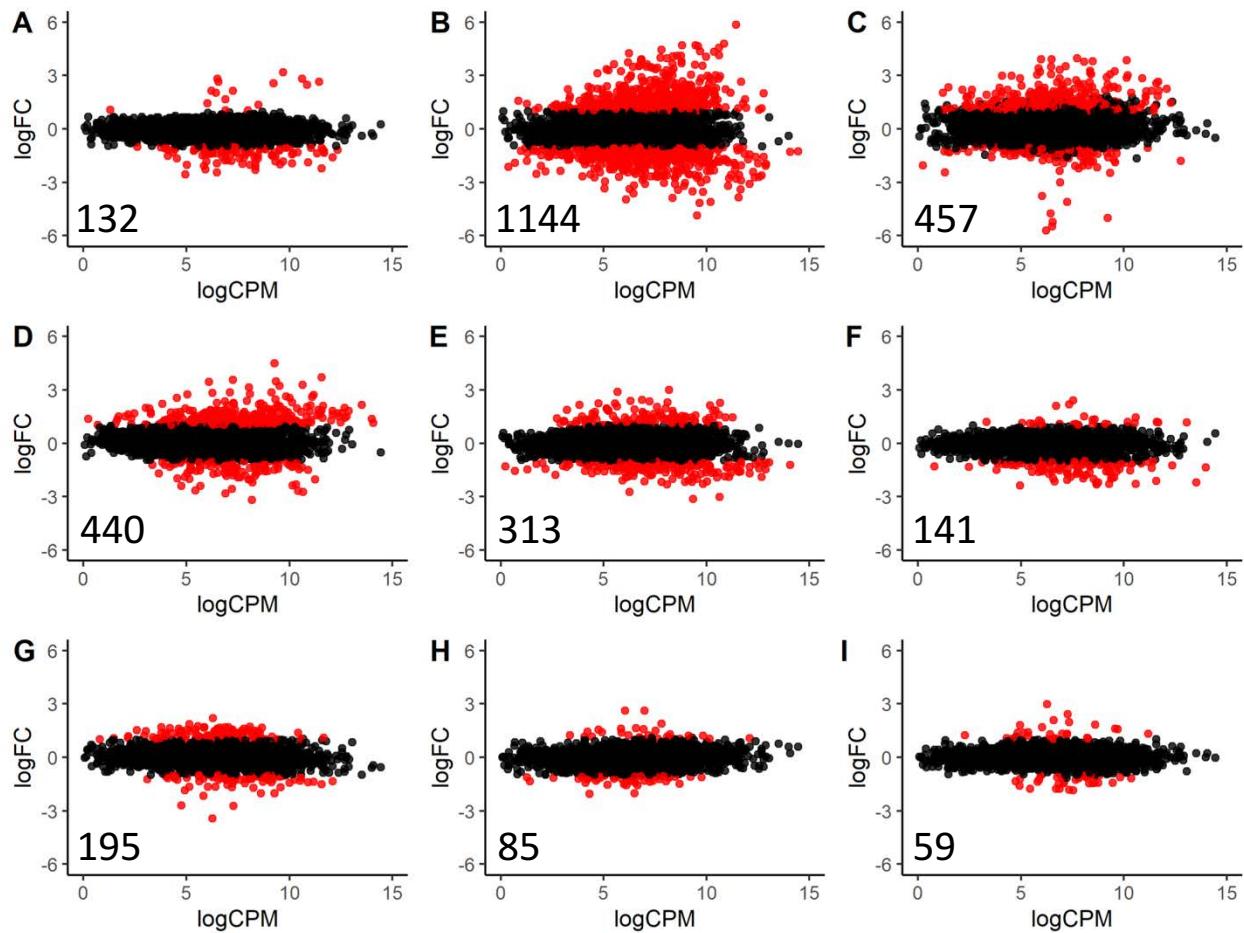


Figure S4. Differential gene transcribed in *Alteromonas* EZ55 under various experimental conditions. Transcription for each gene is shown on the x-axis as the average abundance in \log_2 counts per million (CPM) and the \log_2 of the fold change (FC) on the y-axis. Genes which are significantly differentially 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.

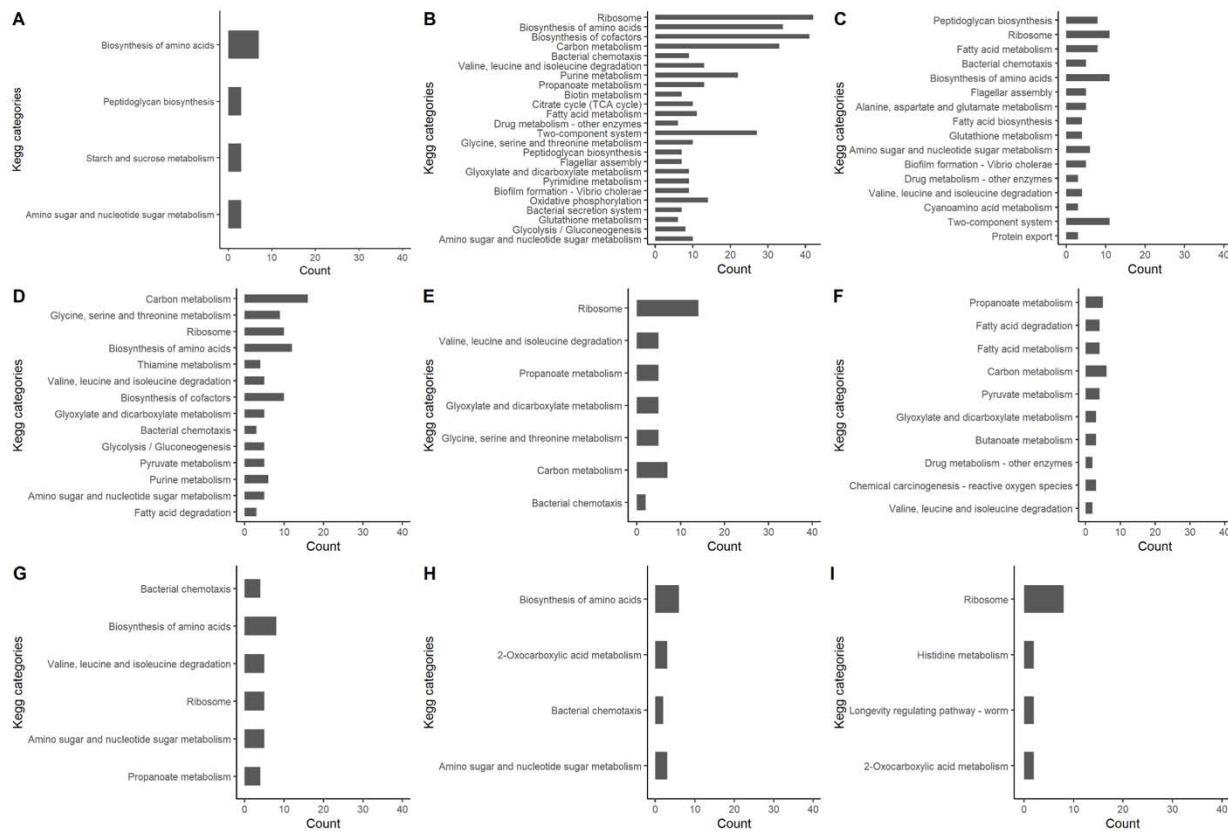


Figure S5. Plots for over-representation analysis (ORA) of significantly-changed KEGG pathways ($p < 0.01$) in *Alteromonas* EZ55 under different experimental conditions. ORA was implemented using hypergeometric tests according to Wu et al., (2021). (A) General response to 800 ppm pCO₂; (B) general response 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.

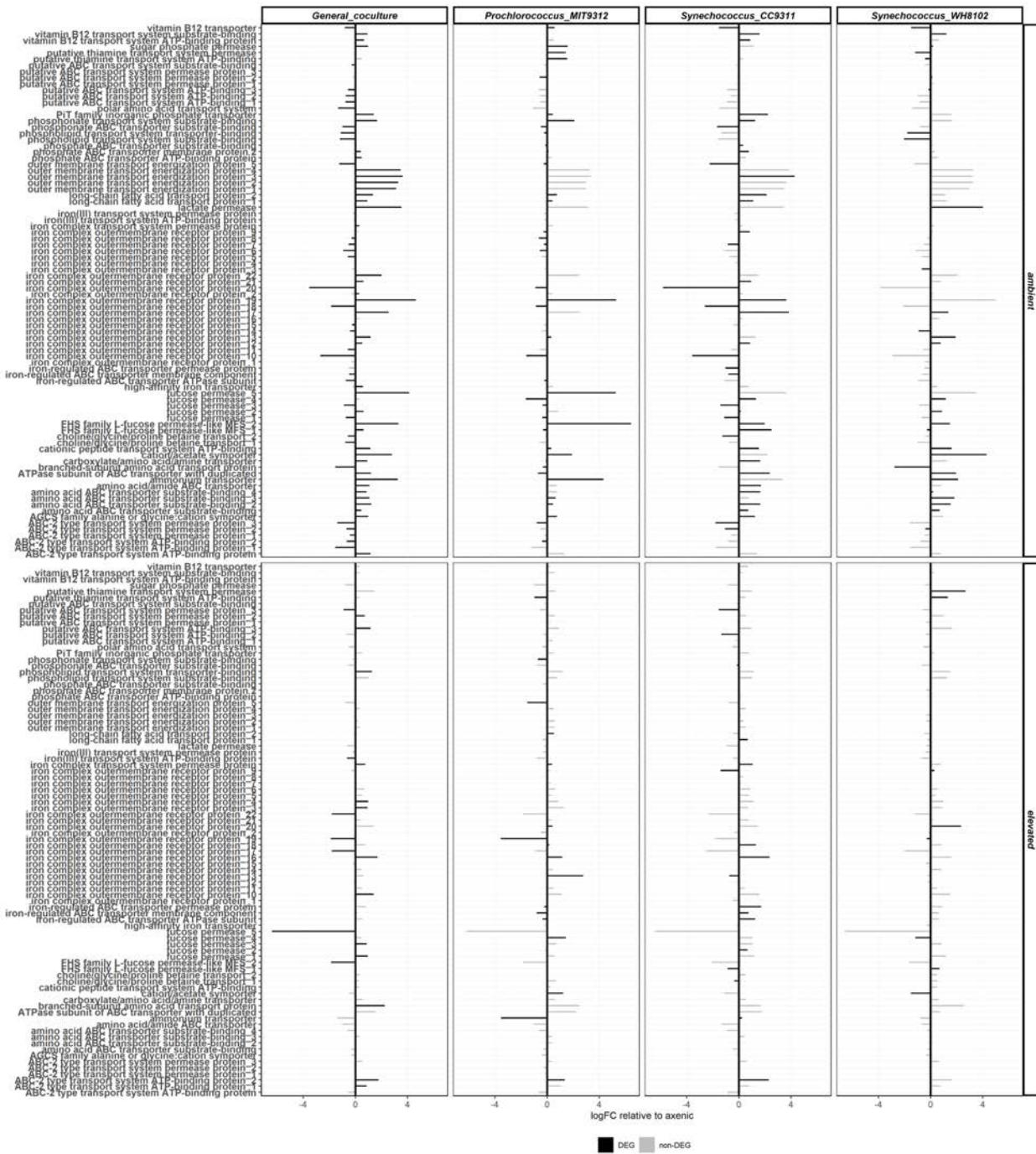


Figure S6. Transporter-related genes in EZ55 representing the general response to co-culture (column 1), and co-culture with specific cyanobacteria (columns 2-4) at 400 or 800 ppm pCO₂, relative to axenic conditions at the same pCO₂. Log₂ fold change (logFC) is plotted relative to axenic EZ55 under the 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.

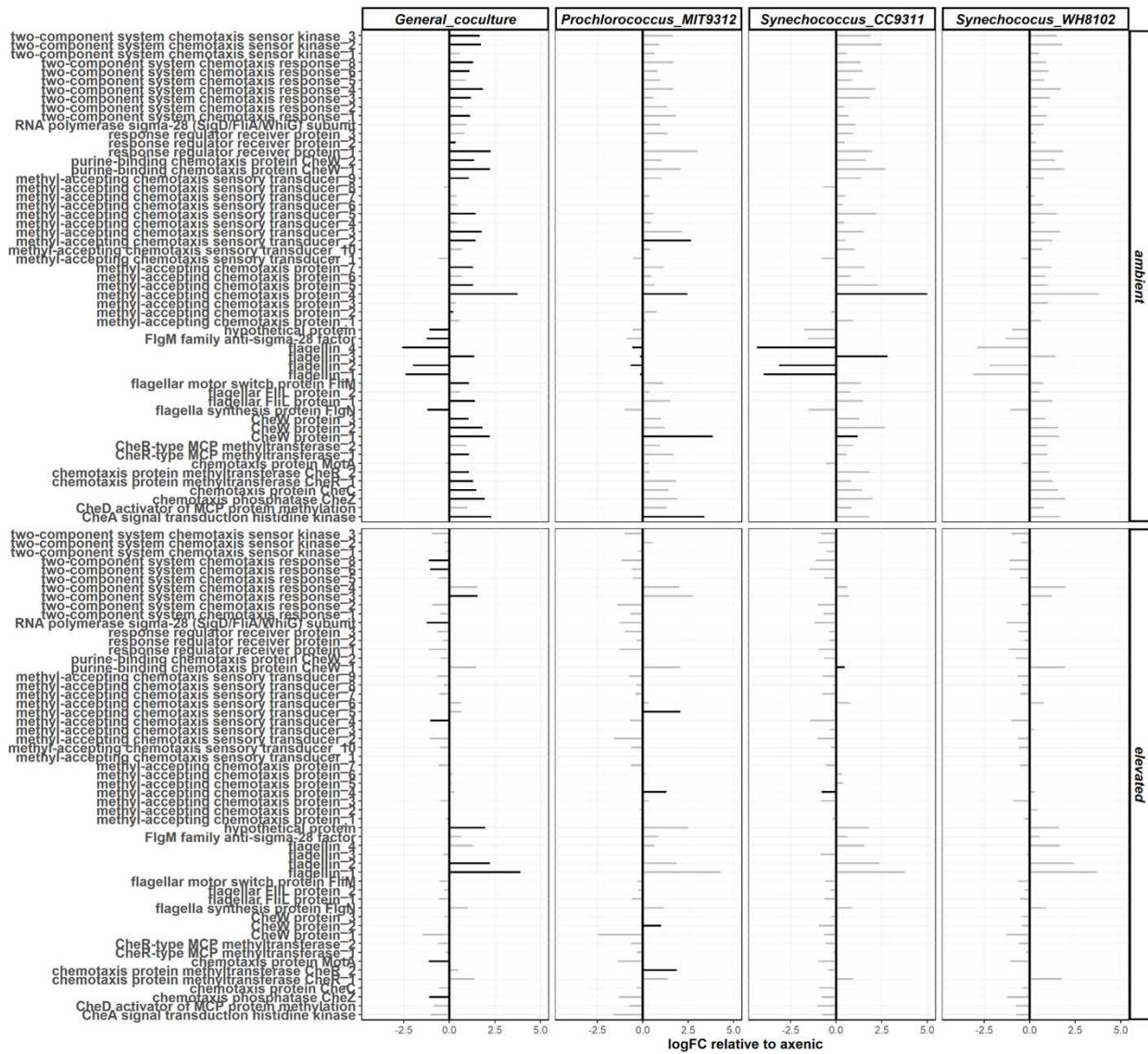


Figure S7. Summary plot of chemotaxis and two-component sensory system gene products in *Alteromonas* EZ55 representing the general response to co-culture (column 1), and co-culture with specific cyanobacteria (columns 2-4) at either 400 or 800 ppm pCO₂, relative to transcription under axenic conditions 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 $\log FC > 1$) 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.

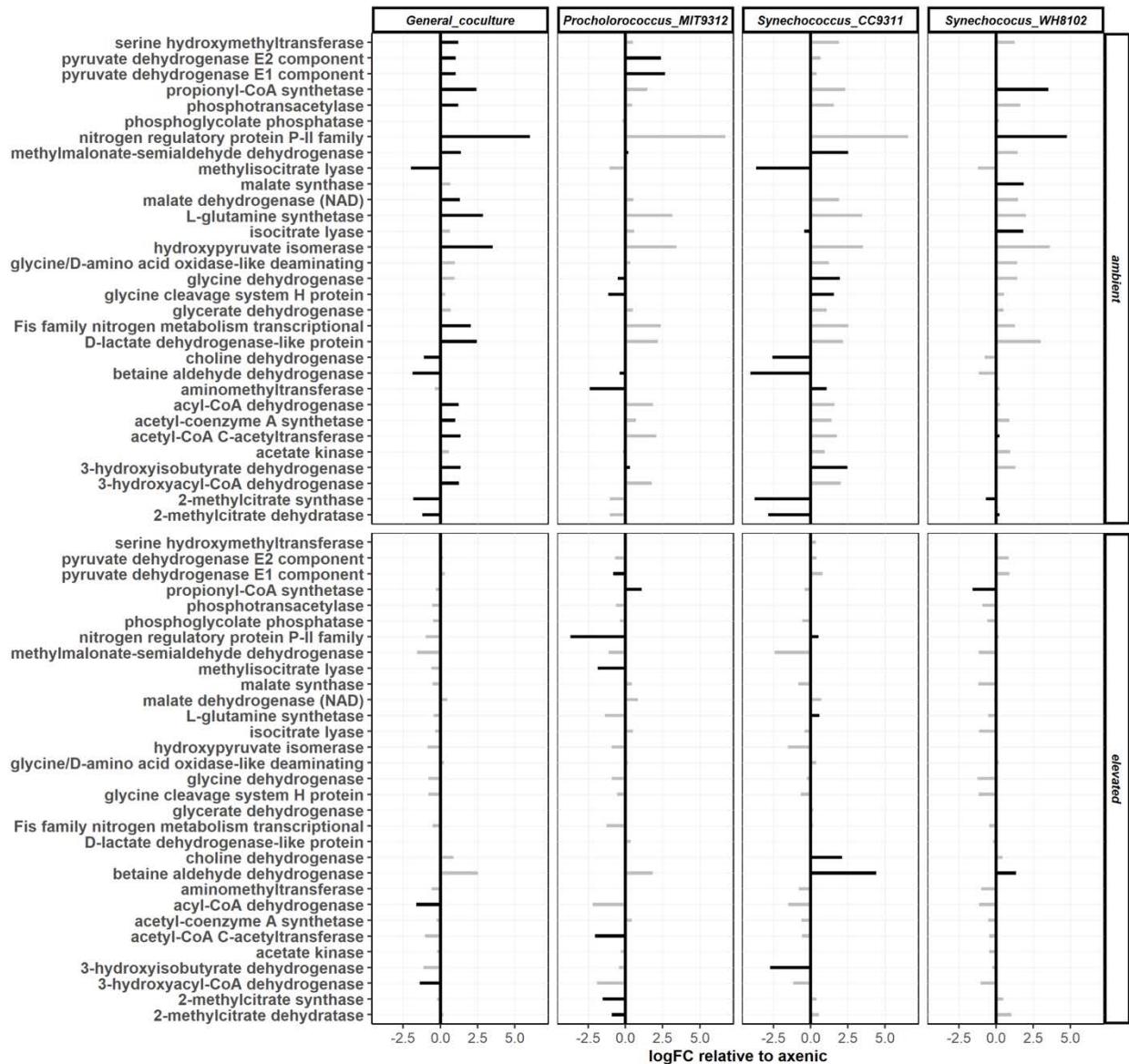


Figure S8. Summary plot of metabolism-related gene products in *Alteromonas* EZ55 representing the general response to co-culture (column 1), and co-culture with specific cyanobacteria (columns 2-4) at either 400 or 800 ppm pCO₂, relative to transcription under axenic conditions at the same pCO₂. Log₂ 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 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.

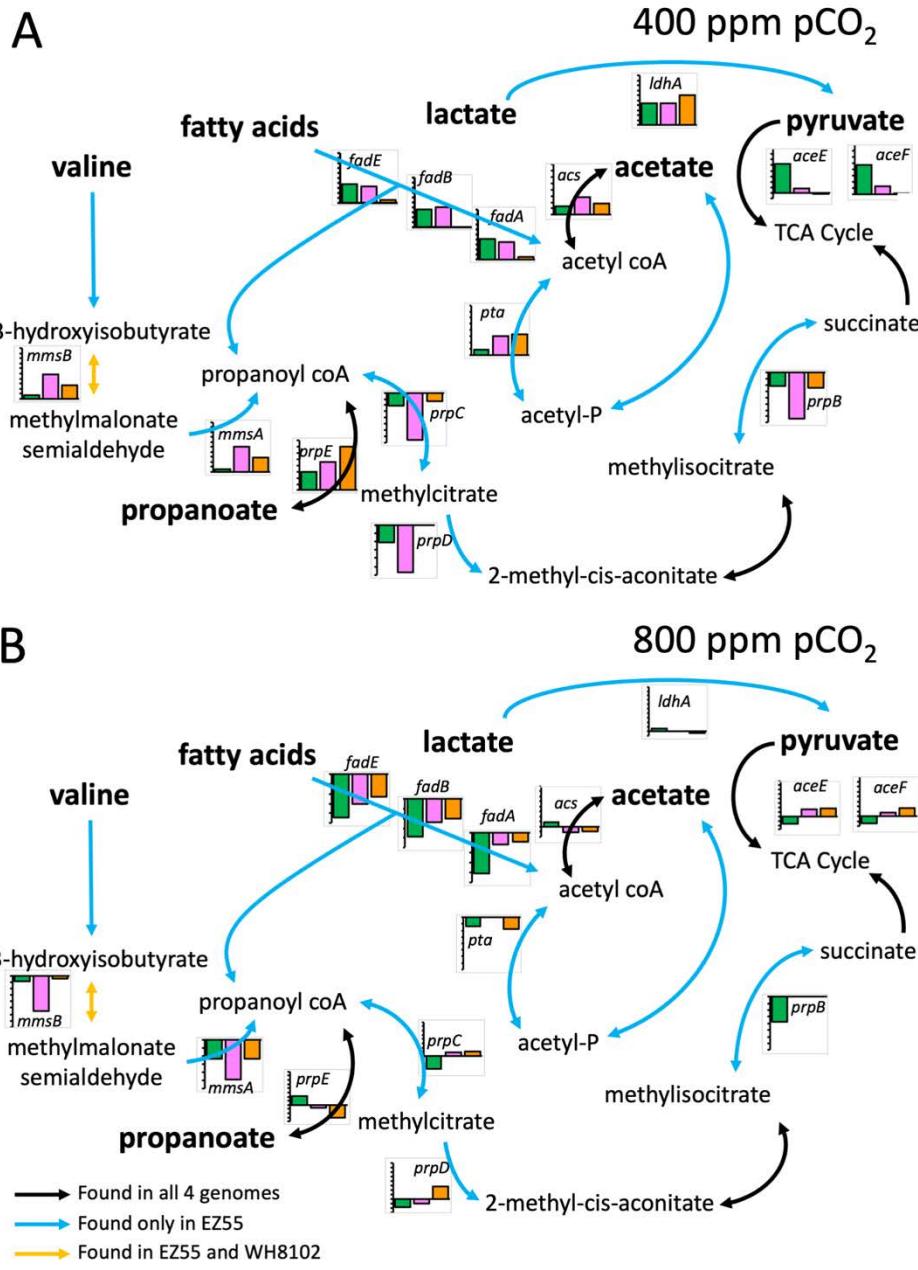
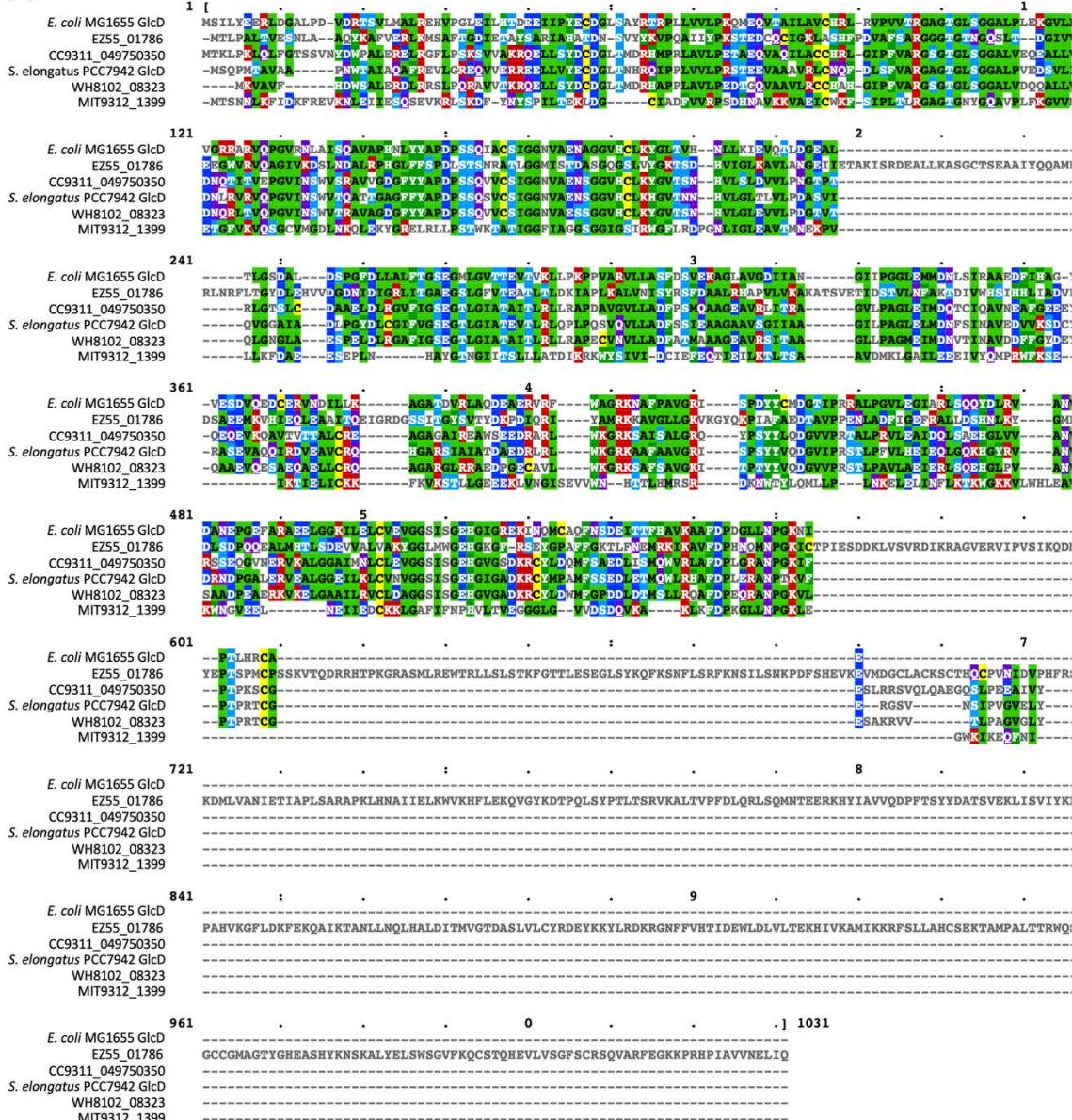
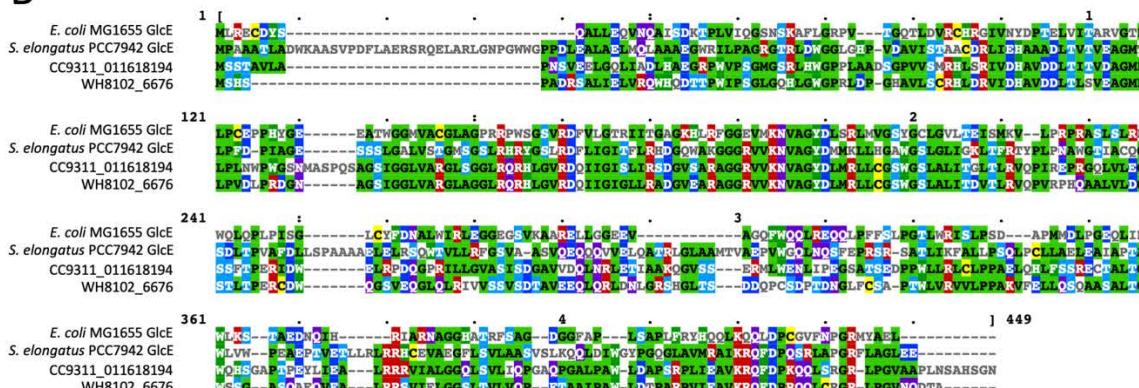


Figure S9. Reconstruction of metabolic pathways involving genes not directly involved in the recovery of photorespiratory byproducts that were significantly differentially regulated in *Alteromonas* EZ55 between axenic and co-culture conditions at 400 (A) or 800 (B) ppm pCO_2 . Small inset graphs indicate 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 differently 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**B**

C

The figure displays a series of sequence alignments for different bacterial strains, grouped by species and strain ID. Each group shows a representative sequence from *E. coli* (top) and *S. elongatus* (bottom). The alignments are color-coded to highlight conservation across the strains. The genes shown are:

- Group 1:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 2:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 3:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 4:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 5:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 6:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 7:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 8:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 9:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.
- Group 0:** E. coli MG1655 GlcF (EZZ5_01786), S. elongatus PCC7942 GlcF (CC9311_049750308), WH8102_06678.

D

Sequence alignment of LctD and Lox proteins from various *Escherichia coli* strains. The alignment highlights conserved regions (blue, red, green, purple) and secondary structure elements (alpha-helices 1-4 and beta-sheets S1-S10).

Conservation:

- Blue:** Hydrophobic residues (I, V, L, M, F, W, Y, H, C, G)
- Red:** Polar residues (R, D, E, S, T, N, Q, K, P, A)
- Green:** Acidic residues (D, E, S, T)
- Purple:** Basic residues (R, K, H, C, G)

Secondary Structure:

- Alpha-helices:** 1, 2, 3, 4
- Beta-sheets:** S1, S2, S3, S4, S5, S6, S7, S8, S9, S10

E

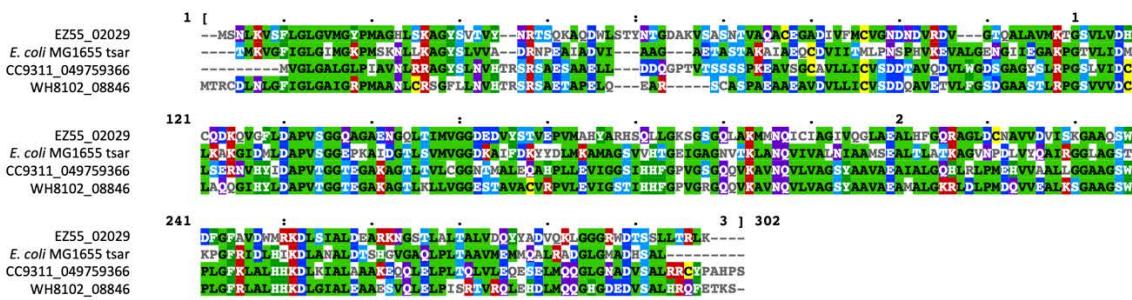


Figure S10. Alignment of hypothesized missing genes in the glycolate utilization pathways of the four strains in this study. Colored residues represent 70% consensus at the level of amino acid equivalence class. A-C) Candidates for the three subunits of bacterial glycolate dehydrogenase, GlcD, GlcE, 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.

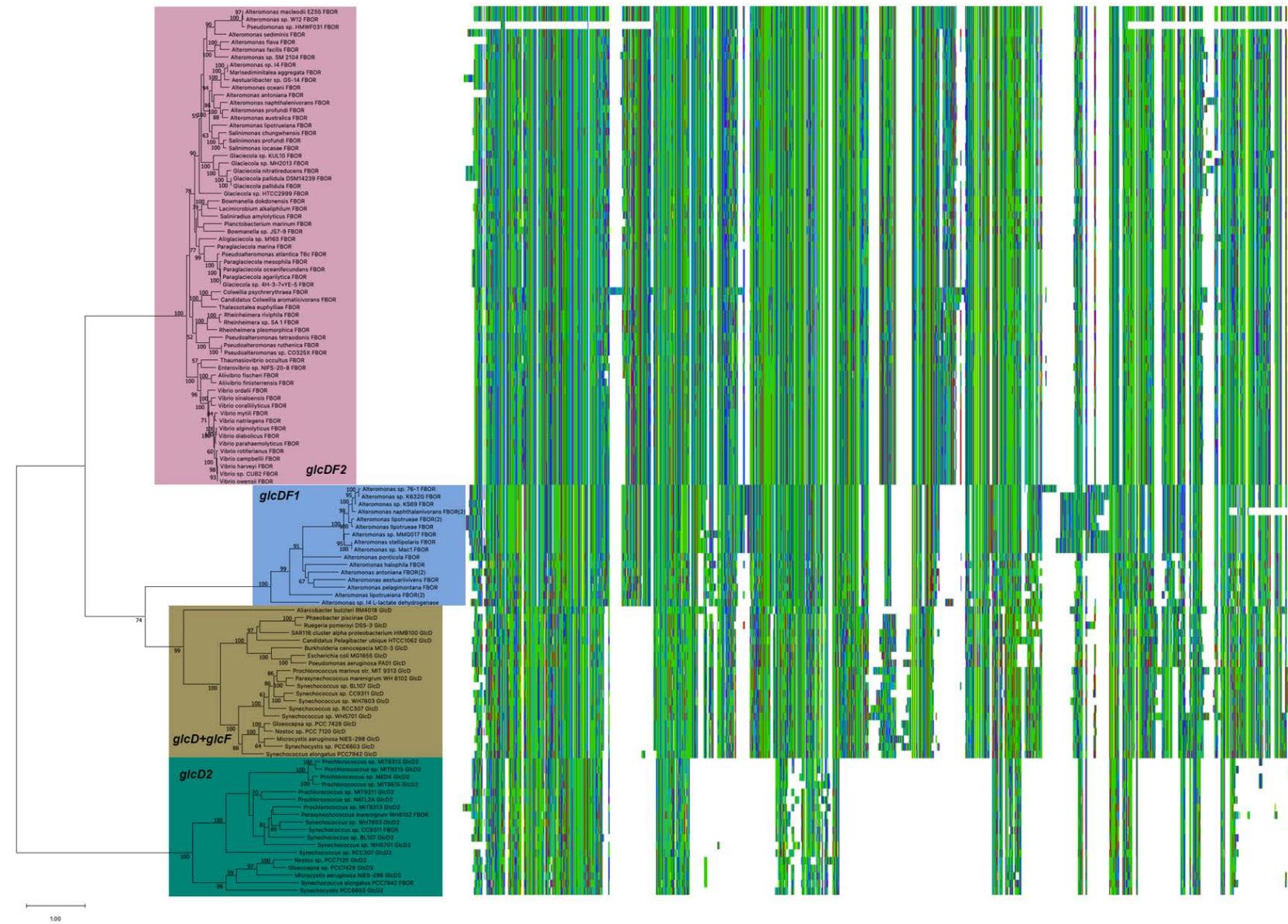


Figure S11. Maximum likelihood tree of GlcDF-like proteins. Tree was constructed in MEGA 11 using WAG+G+I+F substitution model. Node values are the percentage support from 100 bootstrap replicates. *Micromonas pusilla* peroxisomal GOX was used as an outgroup to root the tree and was subsequently pruned 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.

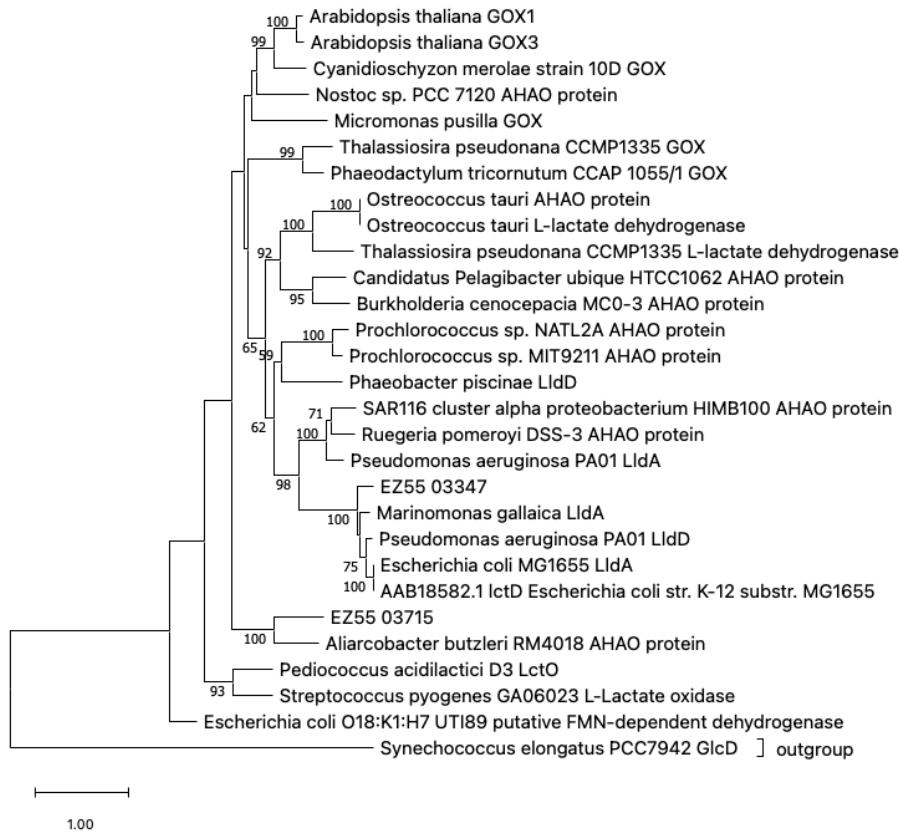


Figure S12. Maximum likelihood tree of LOX/GOX-like proteins. Tree was constructed in MEGA 11 using LG+G substitution model. Node values are the percentage support from 100 bootstrap replicates. *Synechococcus elongatus* glycolate dehydrogenase subunit GlcD was used as an outgroup to root the tree. AHAO,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