

SUPPLEMENTAL FIGURES AND TABLES

A *Rhizobiales*-specific unipolar polysaccharide adhesin contributes to *Rhodopseudomonas palustris* biofilm formation across diverse photoheterotrophic conditions

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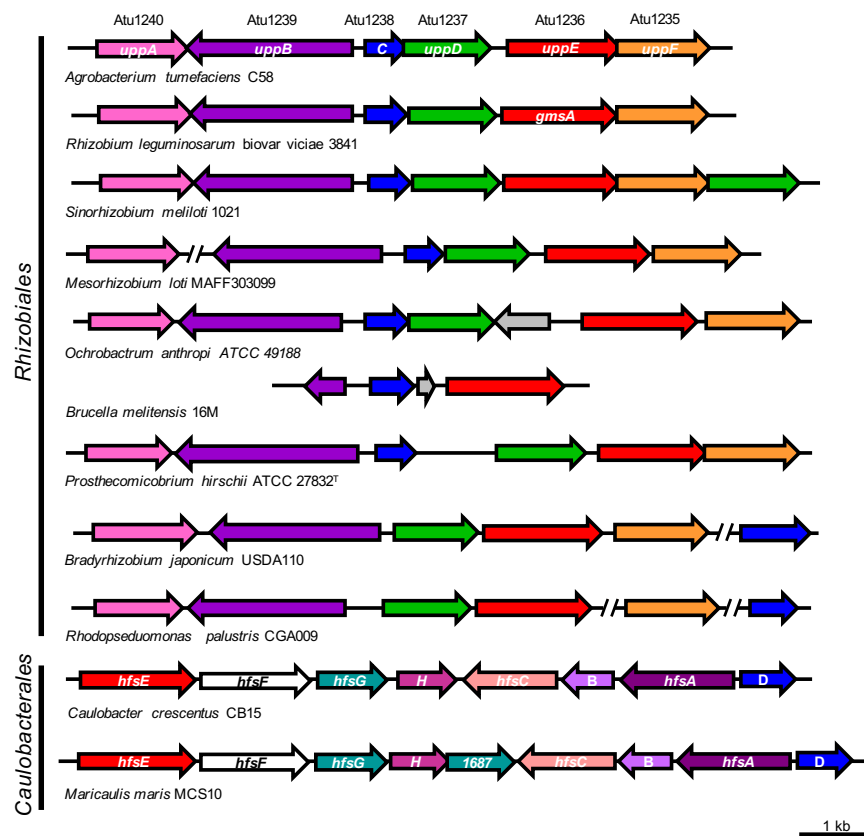


FIG S1. Conservation and synteny of core *upp* and *hfs* gene clusters. Genes (arrows) are colored based on predicted homology and function such that genes of the same color are putative homologs. Genes without a predicted role in polysaccharide biosynthesis and export are shown in grey. Query cover, % Identities/Positives, and E values for *hfs* and *upp* homologs can be found in Dataset S1 and S2, respectively.

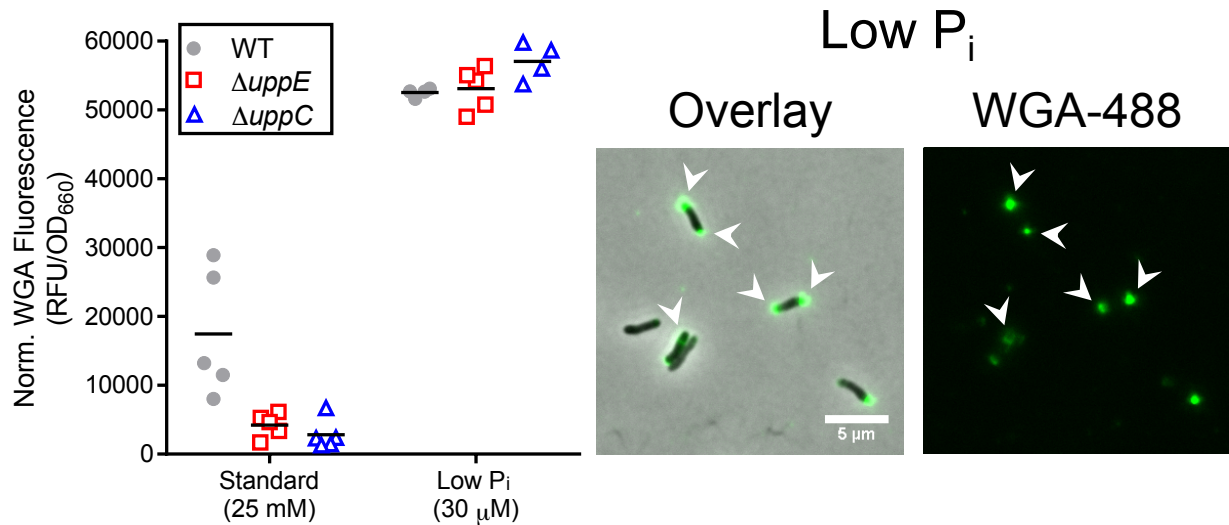


FIG S2. Increased total WGA-488 fluorescence and bipolar and cell body staining of *R. palustris* grown in low P_i photoheterotrophic conditions. (A) Normalized total WGA-488 fluorescence from batch UPP quantification via total WGA-fluorescence assay following 3 d of growth under indicated photoheterotrophic conditions. Individual values for biological replicates (n=4-5) are shown with lines indicating the means. (B) Epifluorescence microscopy of WT *R. palustris* cells stained with WGA-488 after 2 days of growth in low P_i photoheterotrophic conditions. Arrowheads indicate staining of bipolar or cell body regions. Scale bar, 5 μm.

TABLE S1. Putative orthologs of UppABCDEF in *C. crescentus* CB15 identified by reciprocal best hits approach

<i>Agrobacterium tumefaciens</i> C58 (AE007869.2) query sequence	TBLASTN best hit in <i>Caulobacter crescentus</i> CB15 (AE005673.1)				BLASTX reciprocal best hit in <i>Agrobacterium</i> <i>tumefaciens</i> C58 ((AE007869.2)			
	locus tag	Query cover	% Ident/ Positives	E value	locus tag	Query cover	% Ident/ Positives	E value
Atu1240/UppA (NP_354252.1, 409 aa)	No matches				N/A			
Atu1239/UppB (NP_354251.1, 753 aa)	CC_0164	51%	30%/51%	3.00E-37	Atu3556	99%	29%/52%	1.00E-41
Atu1238/UppC (NP_354250.1, 190 aa)	CC_0169	86%	39%/61%	5.00E-34	Atu1238/ UppC	100%	39%/61%	8.00E-38
Atu1237/UppD (NP_354249.1, 393 aa)	CC_3345	46%	24%/42%	4.00E-03	Atu2297	92%	53%/65%	2.00E-49
Atu1236/UppE (NP_354248.2, 517 aa)	CC_2425/ <i>hfsE</i>	70%	38%/57%	1.00E-71	Atu1236/ UppE	89%	41%/60%	6.00E-68
Atu1235/UppF (NP_354247.1, 413 aa)	CC_1446	11%	31%/48%	2.10	Atu3137	57%;	39%/67%	8.00E-04
BLASTP Alignment of putative homologs UppC & HfsD (AAK24403.1)		63%	33%/46%	4.00E-13				

TABLE S2. Putative orthologs of UppABCDEF in *R. palustris* CGA009 identified by reciprocal best hits approach

<i>Agrobacterium tumefaciens</i> C58 (AE007869.2) query sequence	TBLASTN best hit in <i>Rhodospseudomonas</i> <i>palustris</i> CGA009 (BX571963.1)				BLASTX reciprocal best hit in <i>Agrobacterium</i> <i>tumefaciens</i> C58 (NC_003062.2)			
	locus tag	Query cover	% Ident/ Positives	E value	locus tag	Query cover	% Ident/ Positives	E value
Atu1240/UppA (NP_354252.1, 409 aa)	RPA2753	97%	28%/44%	4.00E-24	Atu1240/ UppA	100%	28%/44%	3.00E-32
Atu1239/UppB (NP_354251.1, 753 aa)	RPA2752	53%	39%/60%	5.00E-74	Atu1239/ UppB	100%	39%/60%	3.00E-85
Atu1238/UppC (NP_354250.1, 190 aa)	RPA4833	78%	52%/72%	6.00E-48	Atu1238/ UppC	100%	52%/72%	1.00E-53
Atu1237/UppD (NP_354249.1, 393 aa)	RPA2751	79%	40%/56%	5.00E-69	Atu1237/ UppD	100%	40%/56%	7.00E-68
Atu1236/UppE (NP_354248.2, 517 aa)	RPA2750	94%	49%/62%	5.00E-143	Atu1236/ UppE	100%	49%/62%	6.00E-148
Atu1235/UppF (NP_354247.1, 413 aa)	RPA4581	76%	38%/57%	2.00E-47	Atu1235/ UppF	100%	38%/57%	2.00E-42

TABLE S3. Statistical analyses for comparing relative biofilm formation by WT across growth conditions in Fig. 3A

WT only	Post-hoc test						unpaired, two-tailed t test (Welch's correction)	Consensus
	Dunnett's	Holm-Sidak's	Uncorrected Fisher's LSD	Dunnett's w/o sea salts	Holm-Sidak's w/o sea salts	Dunnett's on log transformed data		
Standard vs low Pi	0.0686	0.0414	0.0209	< 0.0001	< 0.0001	< 0.0001	< 0.0001	significant
Standard vs N ₂ -fixation	0.9399	0.5633	0.5633	0.1685	0.0699	< 0.0001	< 0.0001	ns
Standard vs 1.5% sea salts	< 0.0001	< 0.0001	< 0.0001	N/A	N/A	< 0.0001	< 0.0001	significant
Standard vs chemoheterotrophy	0.0002	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	significant

Due to violation of the assumption of homogeneity of variances when performing a Two-way ANOVA for the data plotted in Fig. 3A, multiple statistical analyses were performed and compared to reach a consensus for interpreting this data set.

TABLE S4. α -proteobacterial species used for phylogenetic analysis

Alphaproteobacterial species in phylogenetic analysis	
Species	GenBank accession number/taxid
<i>Agrobacterium tumefaciens</i> C58	AE007869.2/176299
<i>Rhodopseudomonas palustris</i> CGA009	BX571963.1/258594
<i>Rhizobium leguminosarum</i> bv. <i>viciae</i> 3841	CP007045.1/216596
<i>Sinorhizobium meliloti</i> 1021	AL591688.1/266834
<i>Mesorhizobium loti</i> MAFF303099	BA000012.4/266835
<i>Bradyrhizobium japonicum</i> USDA 110	BA000040.2/224911
<i>Bradyrhizobium</i> sp. BTAi1	LN901633.1/288000
<i>Rhodopseudomonas palustris</i> BisA53	CP000463.1/316055
<i>Nitrobacter winogradskyi</i> Nb-255	CP000115.1/323098
<i>Nitrobacter hamburgensis</i> X14	CP000319.1/323097
<i>Ochrobactrum anthropi</i> ATCC 49188	CP000758.1/439375
<i>Brucella melitensis</i> 16M	AE008917.1/224914
<i>Brucella suis</i> 1330	AE014291.4/204722
<i>Bartonella henselae</i> Houston-1	BX897699.1/283166
<i>Bartonella bacilliformis</i> KC583	CP000524.1/360095
<i>Xanthobacter autotrophicus</i> Py2	CP000781.1/78245
<i>Azorhizobium caulinodans</i> ORS 571	AP009384.1/438753
<i>Methylobacterium extorquens</i> AM1	CP001510.1/272630
<i>Prosthecomicrobium hirschi</i> ATCC 27832T	LJYW00000000.1/665126
<i>Rhodomicrobium vannielii</i> ATCC 17100	CP002292.1/648757
<i>Hyphomicrobium denitrificans</i> 1NES1	CP002083.1/670307
<i>Parvibaculum lavamentivorans</i> DS-1	CP000774.1/402881
<i>Caulobacter crescentus</i> CB15	AE005673.1/190650
<i>Maricaulis maris</i> MCS10	CP000449.1/394221
<i>Phaeobacter inhibens</i> DSM 17395	CP002976.1/391619
<i>Ruegeria pomeroyi</i> DSS-3	NC_003911.12/246200

The 26 α -proteobacterial species in the phylogeny in Fig. 4 and their corresponding GenBank accession number and taxid for analysis of the conservation and distribution of core *upp* gene clusters across the order *Rhizobiales*.