

Supplementary Information for:

Quorum Sensing regulates ‘swim-or-stick’ lifestyle in the phycosphere

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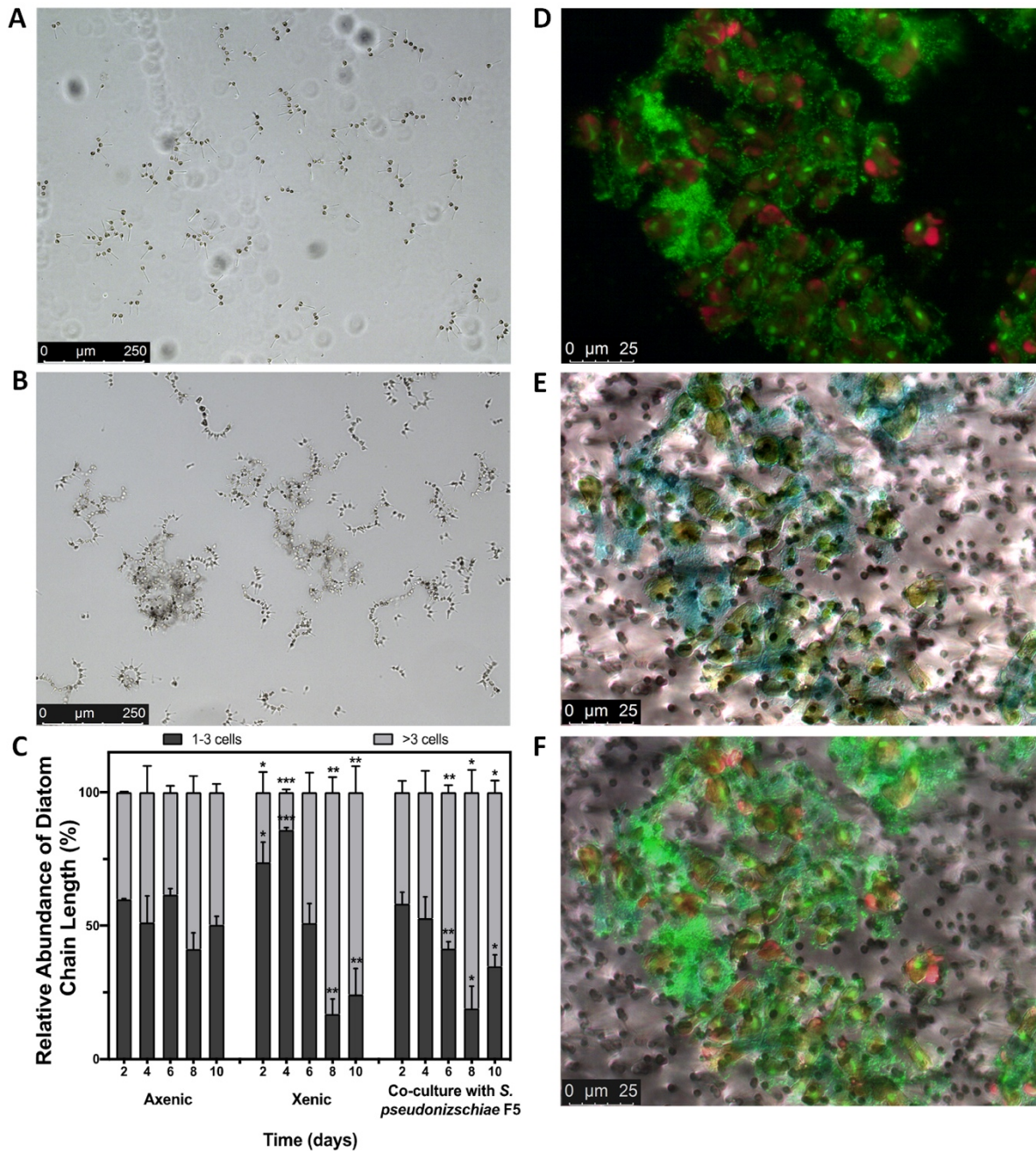
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Supplementary Figures S1-S5

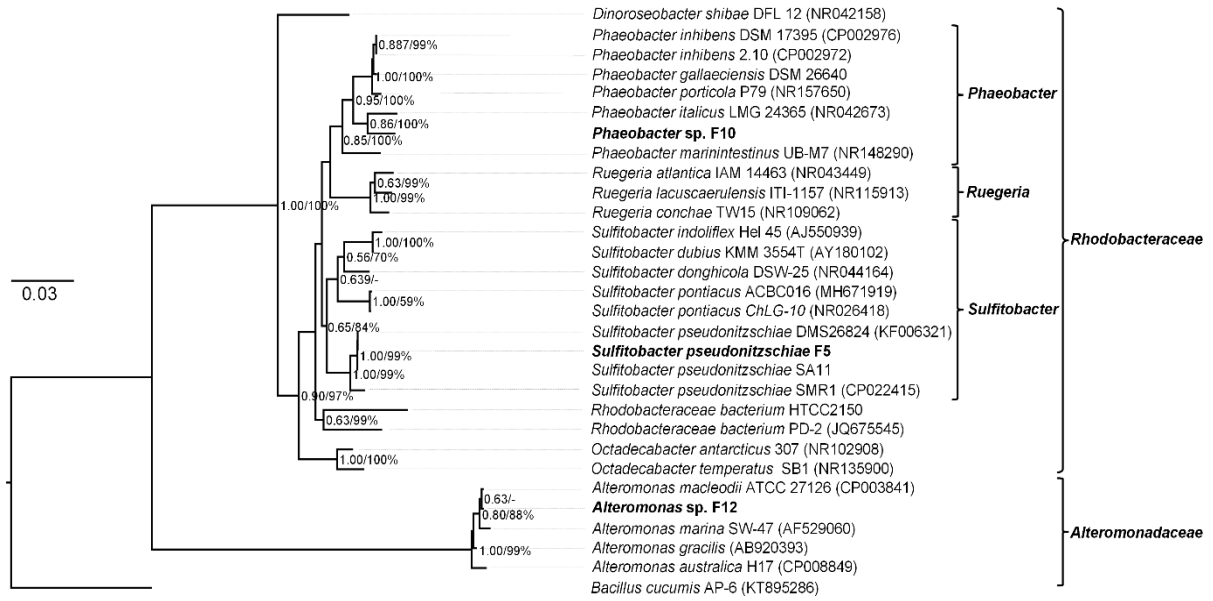
Supplementary Tables S1-S4

References

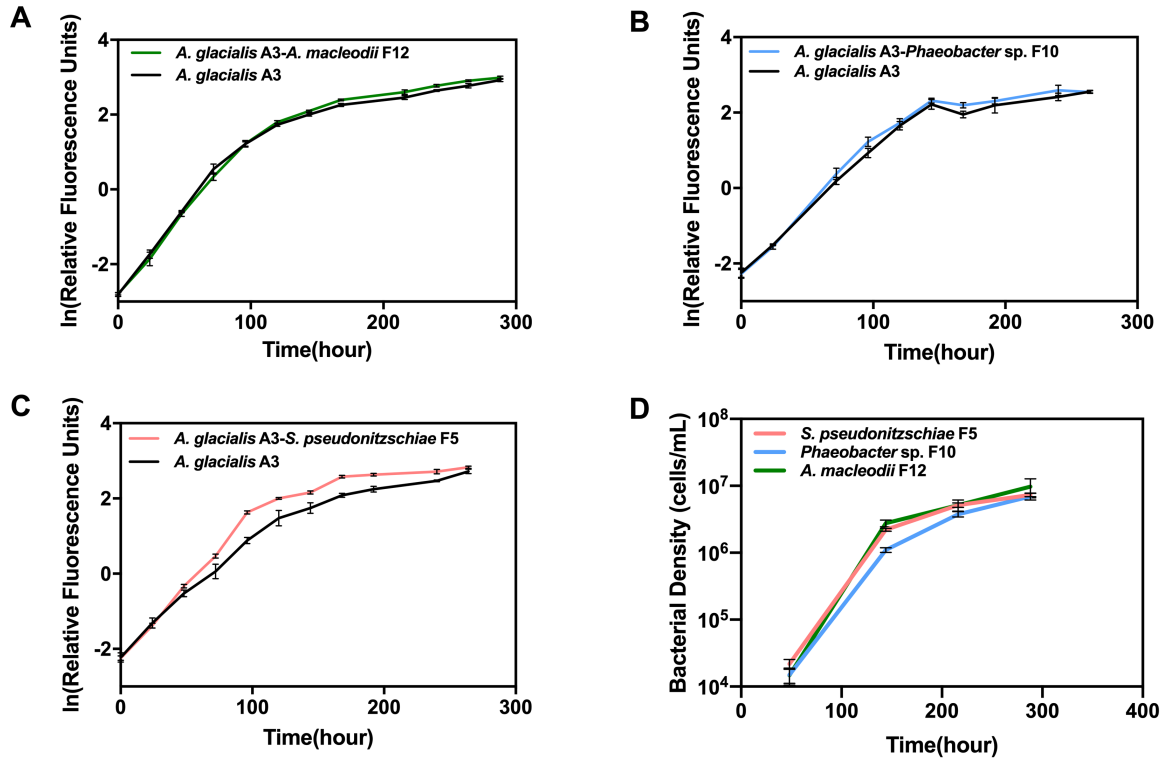


Supplementary Fig. S1. Bacteria influence chain length and display strong attachment to *Asterionellopsis glacialis* A3. Bright-field microscopic images of axenic (A) and xenic (B) *A. glacialis* A3 at equivalent cell densities ($\sim 1.5 \times 10^5$ cells/mL) displaying a general trend towards longer chains in xenic cultures. (C) The relative abundance of different diatom chain lengths in cultures of axenic, xenic *A. glacialis* A3 and co-cultures of *A. glacialis* and *S. pseudonitzschiae* F5. Error bars represent standard deviation (SD) of triplicate cultures. Statistical significance is denoted by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ for xenic and coculture samples compared to axenic cultures at each time point. (D-F) Micrographs of xenic *A. glacialis* A3 cells and attached bacteria: (D) epifluorescence field, (E) bright field, and (F) merged image of (D) and (E). Red color corresponds to diatom chlorophyll *a*

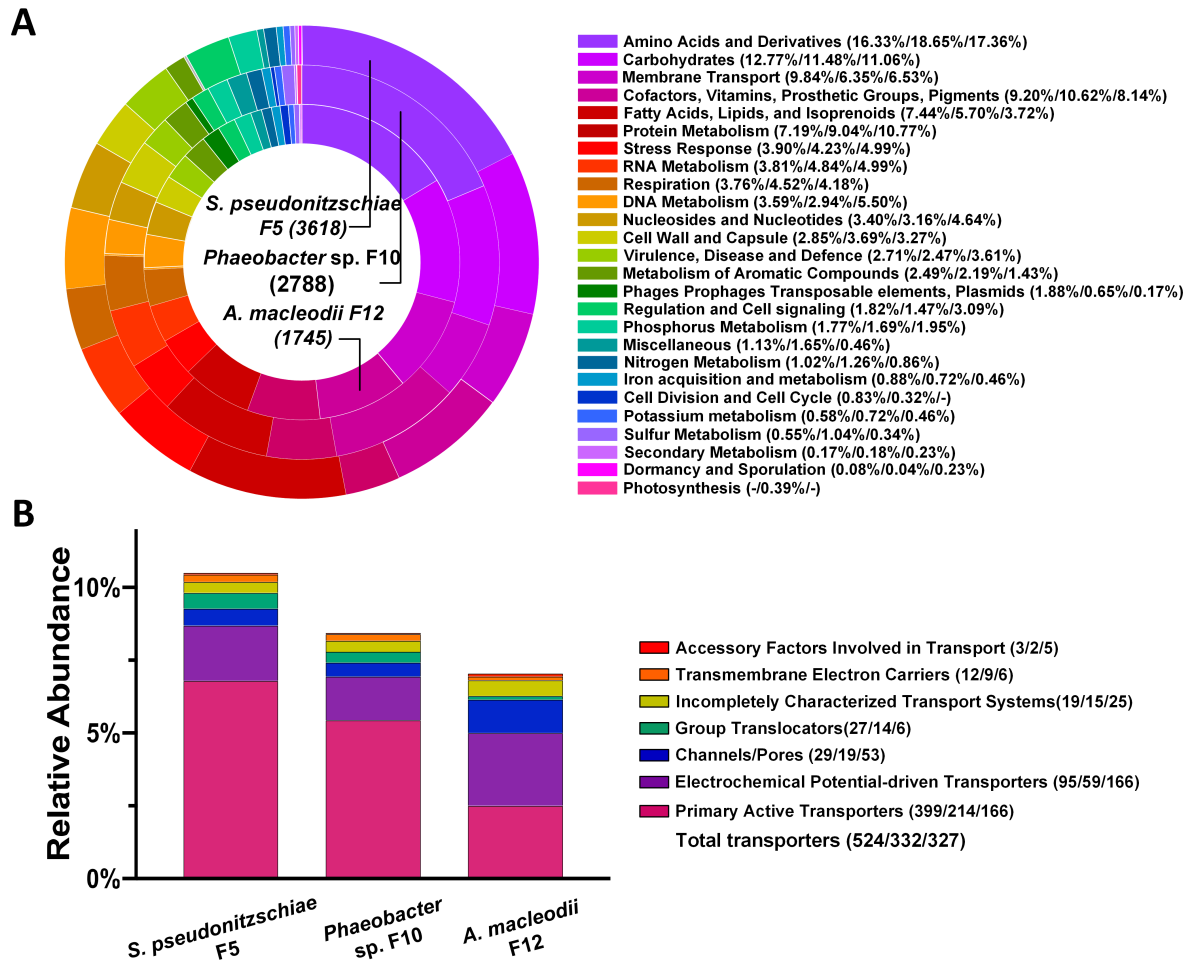
autofluorescence, while green color indicates nucleic acids of diatoms and bacteria. Blue color in (E) and (F) corresponds to diatom transparent exopolymeric particles (TEP) stained with alcian blue.



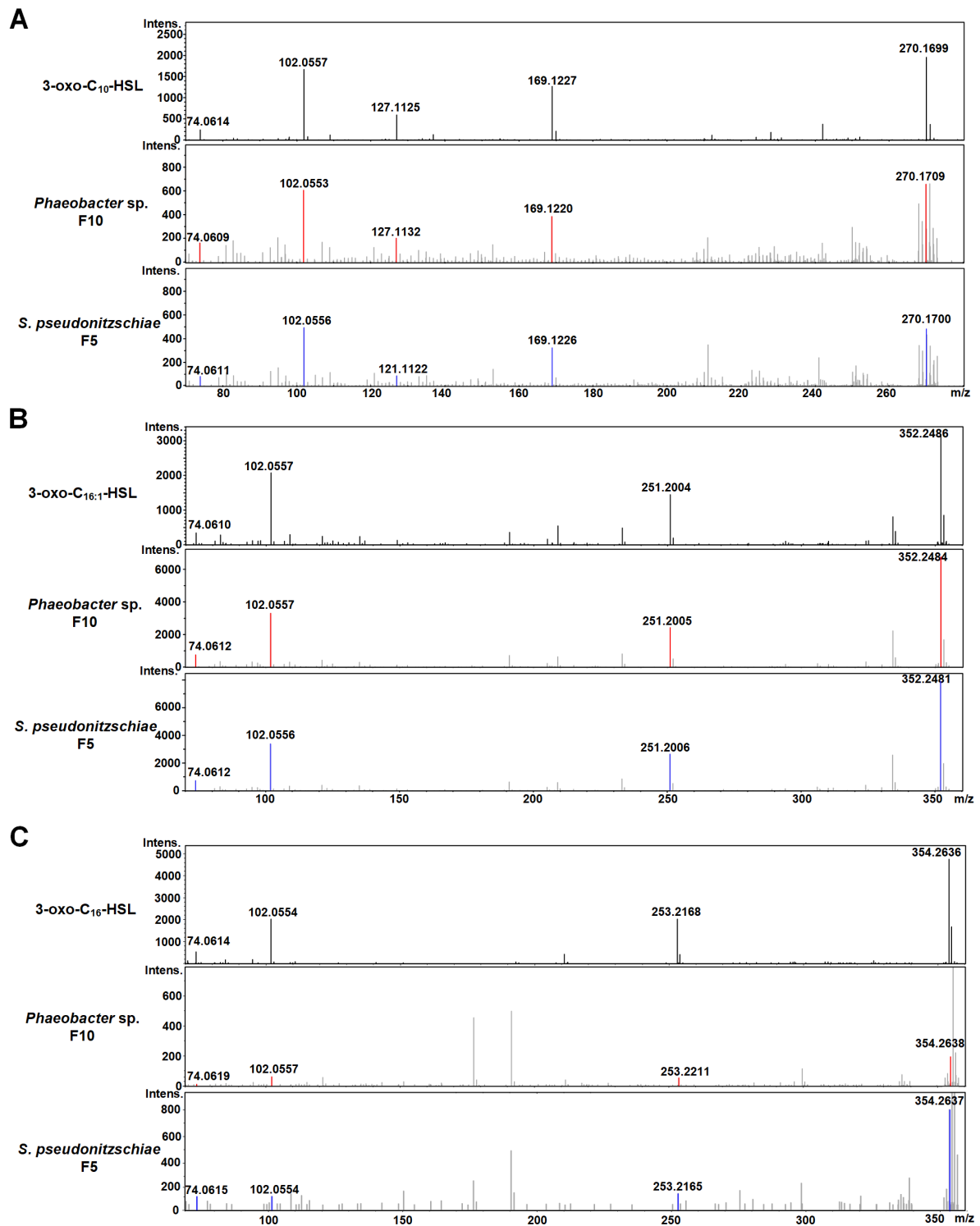
Supplementary Fig. S2. Bayesian phylogenetic tree of the 16S rRNA gene of cultivable bacteria from the *A. glacialis* A3 microbial consortium. Three bacteria isolated from *A. glacialis* A3 are highlighted in bold. Genera and families of the different bacteria are indicated to the right. Posterior probabilities and ML bootstrap values are listed adjacent to branches, respectively. GenBank accession numbers are denoted in parentheses.



Supplementary Fig. S3. Effects of bacterial isolates on the specific growth of *A. glacialis* A3. Co-culture of *A. glacialis* A3 with **(A)** *Alteromonas macleodii* F12, **(B)** *Phaeobacter* sp. F10, and **(C)** *S. pseudonitzschiae* F5. **(D)** Cell density of each bacterium co-cultured with A3. Error bars represent S.D. of six replicates.



Supplementary Fig. S4. Genome-wide analysis of annotated genes for *S. pseudonitzschiae* F5, *Phaeobacter* sp. F10, and *A. macleodii* F12. **(A)** Number of total annotated genes for each bacterium is listed in parentheses on the pie chart. Proportion of genes in each category belonging to *S. pseudonitzschiae* F5 (left), *Phaeobacter* sp. F10 (middle) and *A. macleodii* F12 (right) are listed next to each category. **(B)** Normalized number of total putative transporters to the genome size of each bacterium. Number of genes in each category belonging to *S. pseudonitzschiae* F5 (left), *Phaeobacter* sp. F10 (middle) and *A. macleodii* F12 (right) are listed next to each category.



Supplementary Fig. S5. UHPLC-MS/MS spectra of three AHLs from purchased standards, supernatants of *S. pseudonitzschiae* F5 or *Phaeobacter* sp. F10. MS/MS spectra of **(A)** 3-oxo-C₁₀-HSL, **(B)** 3-oxo-C_{16:1}-HSL and **(C)** 3-oxo-C₁₆-HSL are shown and specific fragment ion peaks are labeled with their m/z values in standards (black), *S. pseudonitzschiae* F5 (red), and *Phaeobacter* sp. F10 (blue).

Supplementary Table S1. Putative IAA biosynthesis pathway in *S. pseudonitzschiae* F5

Gene annotation	Feature ID	% ID*	Bit score*
IAM hydrolase [EC:3.5.1.4]	fig 1402135.21.peg.35 60	97.4	523
Tryptophan 2-monooxygenase	fig 1402135.21.peg.35 59	100	1148
Putative IpyA decarboxylase	fig 1402135.21.peg.23 26	98.9	946
Aldehyde dehydrogenase (NAD ⁺)	fig 1402135.21.peg.26 28	99.8	981
Nitrile hydratase subunit alpha	fig 1402135.21.peg.36 3	99.5	375
Nitrile hydratase subunit beta	fig 1402135.21.peg.36 2	100	452

*Identity (% ID) and bit score refer to identity of homologs in *S. pseudonitzschia* F5 compared to *S. pseudonitzschia* SA11.

Supplementary Table S2. Homolog genes of *S. pseudonitzschiae* F5, *Phaeobacter* sp. F10, and *A. macleodii* F12 depicted in Figure 2 and their similarity to homologs in the genome of the model bacterium *Ruegeria pomeroyi* DSS-3. Complete annotation of each genome is provided as a separate supplementary file.

Gene	Annotation	<i>S. pseudonitzschiae</i> F5	% ID	Bit score	<i>Phaeobacter</i> sp. F10	% ID	Bit score	<i>A. macleodii</i> F12	% ID	Bit score
Chemotaxis										
<i>cheR</i>	Chemotaxis protein methyltransferase	fig 1402135.21.peg.3438	-\$	-	fig 6666666.382773.peg.987 fig 6666666.382773.peg.2269	-	-	fig 28108.372.peg.1943	-	-
	Chemotaxis protein histidine kinase and related kinases	fig 1402135.21.peg.1719	53.4	111	fig 6666666.382773.peg.1026	65.7	134	-	-	-
<i>cheB</i>	Chemotaxis response regulator protein-glutamate methylesterase CheB	-	-	-	fig 6666666.382773.peg.981 fig 6666666.382773.peg.2270	33.3	55.8	fig 28108.372.peg.361 fig 28108.372.peg.1933	- 34.9	- 63.2
<i>cheY</i>	Chemotaxis regulator - transmits chemoreceptor signals to flagellar motor components CheY	fig 1402135.21.peg.3437	-	-	fig 6666666.382773.peg.988	-	-	fig 28108.372.peg.1936 fig 28108.372.peg.1704	-	-
<i>cheW</i>	Positive regulator of CheA protein activity	fig 1402135.21.peg.3439	-	-	fig 6666666.382773.peg.986 fig 6666666.382773.peg.2266 fig 6666666.382773.peg.2268	-	-	fig 28108.372.peg.1930	-	-
<i>cheA</i>	Signal transduction histidine kinase CheA	fig 1402135.21.peg.3440	-	-	fig 6666666.382773.peg.985 fig 6666666.382773.peg.2265	-	-	fig 28108.372.peg.1934	-	-
<i>cheD</i>	Chemotaxis protein CheD	fig 1402135.21.peg.3435	-	-	fig 6666666.382773.peg.980	-	-	-	-	-
Flagellar Proteins										
<i>motB</i>	Flagellar motor rotation protein MotB	fig 1402135.21.peg.769 fig 1402135.21.peg.1597	55.8 53.8	626 223	fig 6666666.382773.peg.243 fig 6666666.382773.peg.909	66.5 60.7	750 232	fig 28108.372.peg.1443	-	-

	MotA/TolQ/ExbB proton channel family protein, probably associated with flagella	fig 1402135.21.peg.770	69.5 (hp)	515	fig 6666666.382773.peg.244	75.7 (hp)	569	-	-
<i>flgN</i>	Flagellar biosynthesis protein FlgN	fig 1402135.21.peg.1599	46.2 (hp)	108	fig 6666666.382773.peg.902	46.2 (hp)	105	-	-
<i>fliG</i>	Flagellar motor switch protein FliG	fig 1402135.21.peg.134	56.8	328	fig 6666666.382773.peg.2790	60.7	384	-	-
<i>flgE</i>	Flagellar hook protein FlgE	fig 1402135.21.peg.1598	65.4	566	-	-	-	fig 28108.372.peg.1939	-
<i>flgJ</i>	Flagellar protein FlgJ, putative	fig 1402135.21.peg.1600	65.7	95.5	fig 6666666.382773.peg.901	73.5	103	-	-
	Flagellar motor switch protein	fig 1402135.21.peg.210	30.8 (hp)	117	fig 6666666.382773.peg.2711	36.5 (hp)	159	-	-
<i>flgD</i>	Flagellar basal-body rod modification protein FlgD	fig 1402135.21.peg.1602	56.9	229	fig 6666666.382773.peg.899	51.5	213	-	-
Pili									
	bacterial type II/III secretion system protein	fig 1402135.21.peg.2422	33.1	185	fig 6666666.382773.peg.2057	75.4	444	-	-
	Predicted ATPase with chaperone activity, associated with Flp pilus assembly	fig 1402135.21.peg.2680	82.3 (hp)	758	fig 6666666.382773.peg.2048	84.8 (hp)	786	-	-
<i>tadC</i>	type II/IV secretion system protein, TadC subfamily	fig 1402135.21.peg.2426	-	-	fig 6666666.382773.peg.2053	75.2	504	-	-
		fig 1402135.21.peg.2685	78.1	514	fig 6666666.382773.peg.2052	72.4	484	-	-
		fig 1402135.21.peg.2684	68.9	436	-	-	-	-	-
<i>tadA</i>	type II/IV secretion system protein, TadA subfamily	fig 1402135.21.peg.2425	50.5	391	fig 6666666.382773.peg.2054	86.0	827	-	-
		fig 1402135.21.peg.2686	82.5	784	-	-	-	-	-
	Type II/IV secretion system ATPase	fig 1402135.21.peg.2687	72.5	632	fig 6666666.382773.peg.2055	74.0	649	-	-
		fig 1402135.21.peg.2424	-	-	-	-	-	-	-

	TadZ/CpaE, associated with Flp pilus assembly									
<i>tadG</i>	Flp pilus assembly protein TadG	fig 1402135.21.peg.893	39.1	132 (hp)	fig 6666666.382773.peg.2304	44.6	171 (hp)	-	-	
	ATP-dependent helicase, DEAD/DEAH box family, associated with Flp pilus assembly	fig 1402135.21.peg.2679	81.8	1344	fig 6666666.382773.peg.2047	77.4	1279	-	-	
<i>tadD</i>	Flp pilus assembly protein TadD, contains TPR repeat	fig 1402135.21.peg.2682 fig 1402135.21.peg.2683	75.8 59.1	449 218	fig 6666666.382773.peg.2050; fig 6666666.382773.peg.2051	71.7 58.2	418 210	-	-	
	Flp pilus assembly protein RcpC/CpaB	fig 1402135.21.peg.2690	68.4	395	fig 6666666.382773.peg.2058	70.9	416	-	-	
	Flp pilus assembly protein, pilin Flp	fig 1402135.21.peg.2420 fig 1402135.21.peg.2692	- -	- -	fig 6666666.382773.peg.2060	-	-	fig 28108.372.peg.1117	-	
	Type IV prepilin peptidase TadV/CpaA	fig 1402135.21.peg.2681	51.9	154	fig 6666666.382773.peg.2049	48.6	123	-	-	
Exopolysaccharides										
	bacterial sugar transferase	fig 1402135.21.peg.2078 fig 1402135.21.peg.1856	48.1 35.8	253 224	fig 6666666.382773.peg.3316	65.6	253	fig 28108.372.peg.1531	34.9	244
	polysaccharide biosynthesis/export protein	fig 1402135.21.peg.873	71.9	377	fig 6666666.382773.peg.2324	82.4	375	-	-	
<i>kpsC</i>	capsular polysaccharide export protein KpsC	fig 1402135.21.peg.874	67.0	667	fig 6666666.382773.peg.2323	70.4	667	-	-	
	Glycosyl transferase, group 2 family protein	fig 1402135.21.peg.303	54.6	628	fig 6666666.382773.peg.420	50.1	637	-	-	
<i>kpsS</i>	Capsular polysaccharide export system protein KpsS	fig 1402135.21.peg.872	75.1	430	fig 6666666.382773.peg.2325	77.7	430	-	-	

	Glycosyl transferase, group 1 family protein	fig 1402135.21.peg.2297	72.6	413	fig 66666666.382773.peg.732	74.3	413	-	-
Quorum sensing									
<i>luxRI</i> cassette (B group)	Autoinducer-binding transcriptional regulator and adjacent autoinducer synthesis	fig 1402135.21.peg.2496	67.9	304	fig 66666666.382773.peg.1880	73.6	318	-	-
		fig 1402135.21.peg.2495	68.1	344	fig 66666666.382773.peg.1879	73.2	381		
<i>luxRI</i> cassette	Autoinducer-binding transcriptional regulator and adjacent autoinducer synthesis	fig 1402135.21.peg.2198	71.2	313	-	-	-		
		fig 1402135.21.peg.2199	60.8	290					
Orphan <i>luxR</i>	Autoinducer-binding transcriptional regulator, LuxR family	fig 1402135.21.peg.3467	71.5	382;	fig 66666666.382773.peg.2975	68.3	363	-	-
	DNA-binding response regulator, LuxR family	fig 1402135.21.peg.2410	52.2	204	fig 66666666.382773.peg.3362	62.6	249	-	-
		fig 1402135.21.peg.3268	76.6	325	fig 66666666.382773.peg.2638	83.2	353		
	Transcriptional regulator, LuxR family	-	-	-	fig 66666666.382773.peg.668	44.3	108	fig 28108.372.peg.736	31.7 69.3
					fig 66666666.382773.peg.2699	38.0	108		

Further confirmation for gene annotations were acquired by blasting genes against the model *Roseobacter* group bacterium *Ruegeria pomeroyi* DSS-3 (Hit score > 50)

§ Indicates no hits were found in the *R. pomeroyi* DSS3 genome.

¶ Indicates that the gene annotation in *R. pomeroyi* DSS3 is 'hypothetical protein'.

Supplementary Table S3. AHL molecules listed in Figure 5.

Strains	No. of <i>luxIs</i>	AHLs	References
<i>Phaeobacter</i> sp. F10	1	3-oxo-C ₁₀ -HSL, 3-oxo-C ₁₆ -HSL, 3-oxo-C _{16:1} -HSL	This study
<i>Rhodobacterales bacterium</i> Y4I	2	C ₈ -HSL, 3-OH-C _{12:1} -HSL	[1]
<i>Phaeobacter gallaeciensis</i> DSM 26640	3	3-OH-C ₁₀ -HSL, C _{12:1} -HSL, C _{14:2} -HSL, C _{16:1} -HSL, C _{16:2} -HSL, 3-oxo-C ₁₆ -HSL, C _{18:1} -HSL, C _{18:2} -HSL,	[4]
<i>Phaeobacter gallaeciensis</i> DSM 17395	2	3-OH-C ₁₀ -HSL, C ₁₆ -HSL, C _{16:1} -HSL, C _{18:1} -HSL	[5]
<i>Phaeobacter inhibens</i> 2.10	3	3-OH-C ₁₀ -HSL, 3-oxo-C ₁₀ -HSL, C _{12:2} -HSL, C ₁₆ -HSL, C _{16:1} -HSL, C _{18:1} -HSL	[5]
<i>Ruegeria</i> sp. KLH11	3	3-OH-C ₁₄ -HSL, 3-OH-C _{14:1} -HSL, 3-OH-C ₁₂ -HSL	[9]
<i>Dinoroseobacter shibae</i> DFL 12	3	C _{14:1} -HSL, 3-oxo-C ₁₄ -HSL, C _{18:1} -HSL, C _{18:2} -HSL	[5]
<i>Rhodobacteraceae bacterium</i> PD-2	2	3-oxo-C ₈ -HSL, 3-oxo-C ₁₀ -HSL	[14]
<i>Sulfitobacter pseudonitzschiae</i> F5	2	3-oxo-C ₁₀ -HSL, 3-oxo-C ₁₆ -HSL, 3-oxo-C _{16:1} -HSL	This study
<i>Pseudomonas aeruginosa</i> PAO1	2	C ₄ -HSL, 3-oxo-C ₁₂ -HSL	[13]

Supplementary Table S4. Purchased AHLs standards used to identify AHLs produced by the roseobacters strains using HPLC-MS/MS.

AHL standards	Future ID
Acetyl-L-Homoserine lactone	C ₂ -HSL
N-butyryl-L-Homoserine lactone	C ₄ -HSL
N-hexanoyl-L-Homoserine lactone	C ₆ -HSL
N-(β-Ketocaproyl)-DL-homoserine lactone	3-oxo-C ₆ -HSL
N-phenylacetyl-L-Homoserine lactone	C ₈ -HSL
N-(3-Oxo-octanoyl)-L-homoserine lactone	3-oxo-C ₈ -HSL
N-(3-Oxodecanoyl)-L-homoserine lactone	3-oxo-C ₁₀ -HSL
N-dodecanoyl-L-Homoserine lactone	C ₁₂ -HSL
N-(3-Oxododecanoyl)-L-homoserine lactone	3-oxo-C ₁₂ -HSL
N-tetradecanoyl-L-Homoserine lactone	C ₁₄ -HSL
N-3-oxo-tetradecanoyl-L-Homoserine lactone	3-oxo-C ₁₄ -HSL
N-hexadecanoyl-L-Homoserine lactone	C ₁₆ -HSL
N-3-oxo-hexadec-11(Z)-enoyl-L-Homoserine lactone	3-oxo-C _{16:1} -HSL
N-3-oxo-hexadecanoyl-L-Homoserine lactone	3-oxo-C ₁₆ -HSL
N-octadecanoyl-L-Homoserine lactone	C ₁₈ -HSL

Reference (including full references in Figure 5 and Supplementary Table S3)

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