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Supplemental Information

Context-Specific Requirement of Forty-Four Two-Component Loci in *Pseudomonas aeruginosa* Swarming

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Figure S1

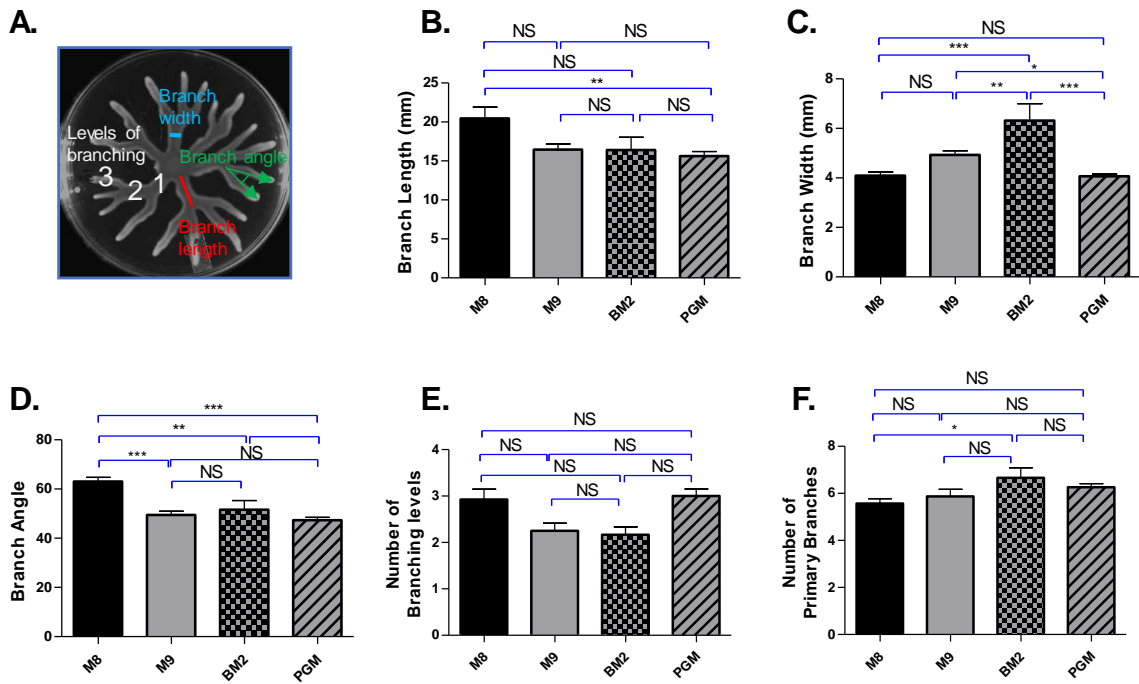


Figure S1: Macroscale features of *P. aeruginosa* swarm, Related to Figure 1 and Figure 2. (A) swarm features are indicated. Histograms for (B) branch or dendrite length, (C) branch width, (D) branch angle, (E) branching levels, and (F) number of primary branches. Pairwise comparison between every two media was performed by Tukey test. ($P > 0.05$, ns; $P < 0.05$, *; $p < 0.01$, **, $P < 0.001$ ***).

Figure S2

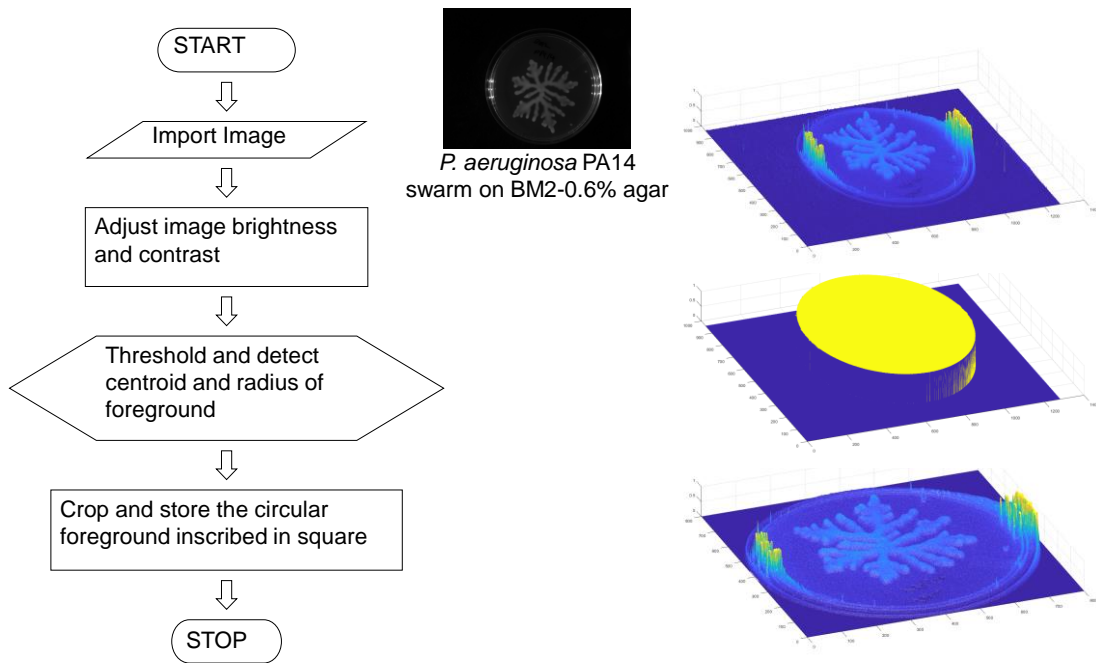


Figure S2. MATLAB workflow for swarm image processing, Related to Figure 3 and Figure 4.

Figure S3.

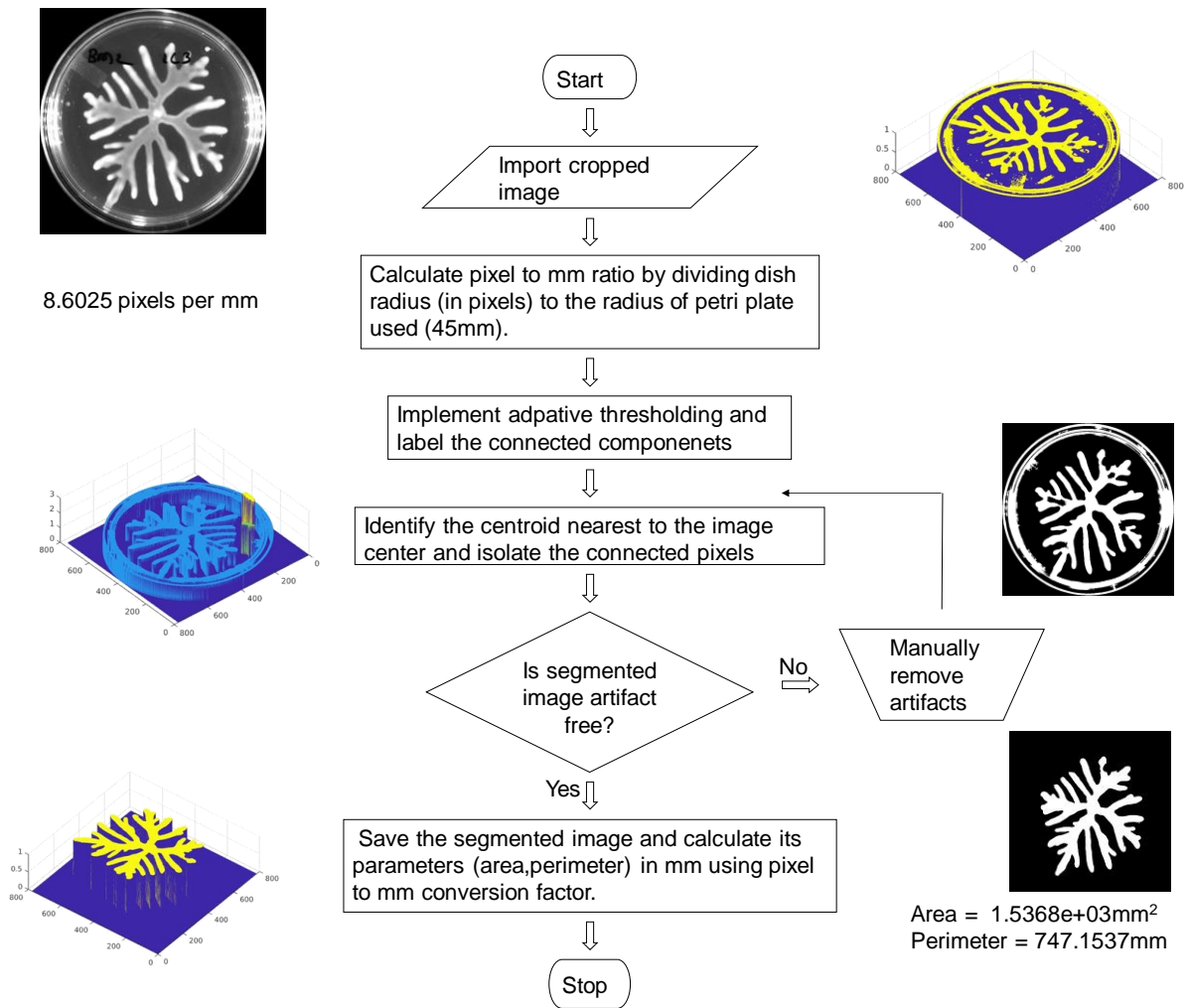


Figure S3. MATLAB workflow for feature extraction from processed swarm image, Related to Figure 3 and Figure 4.

Figure S4. Swarming of 44 TCS mutant strains on six different growth media, Related to Figure 4 and Figure 5.

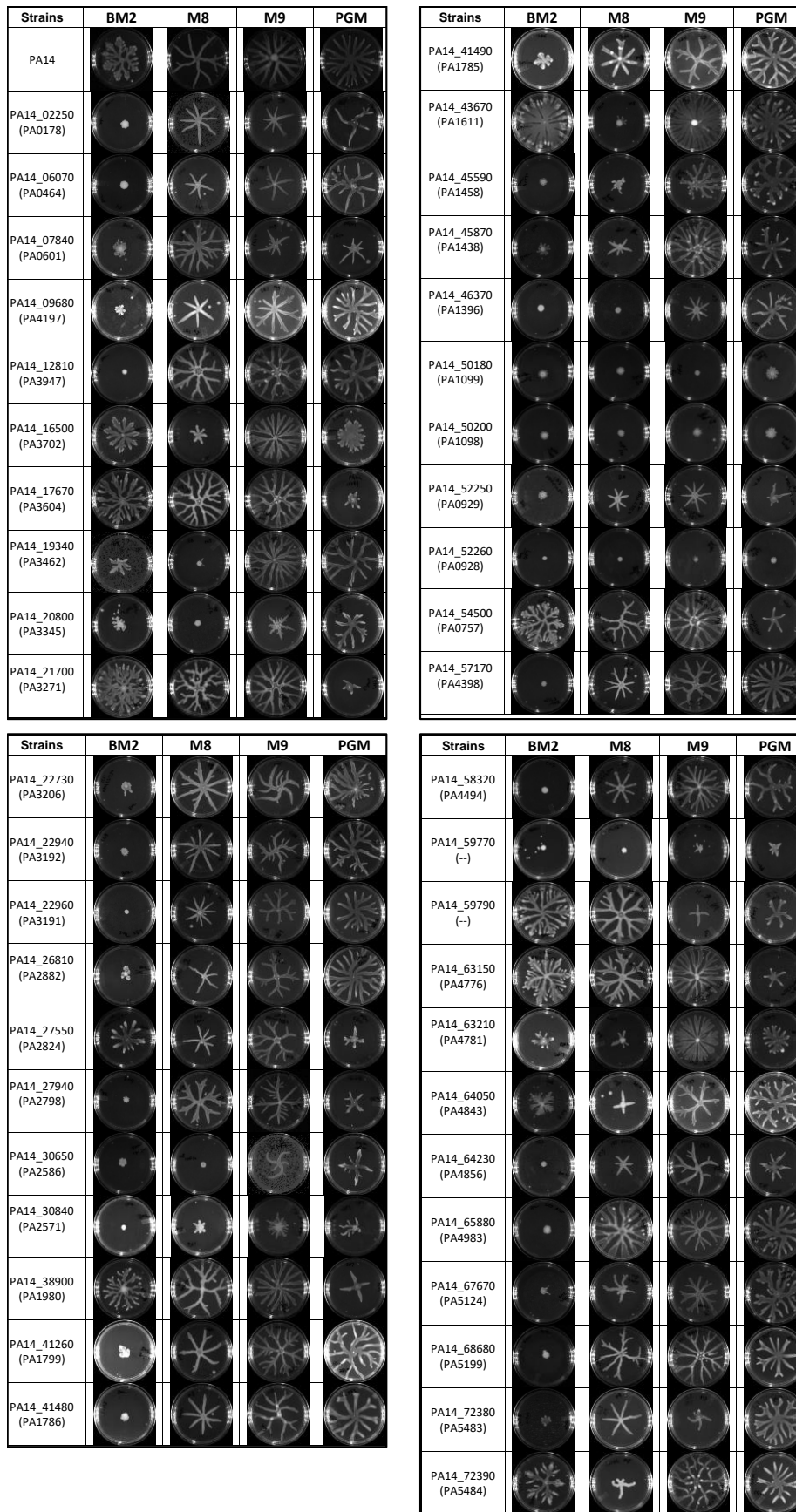


Table S1. Perimeter values# for swarms of TCS mutants.

TCS SI No.	Locus ID	MEDIA				TCS SI No.	Locus ID	MEDIA			
		PGM	M9	M8	BM2			PGM	M9	M8	BM2
	PA14	100±11	100±11	100±9	100±24	57	PA14_43350	72	132	128	116
	<i>rhIR</i>	3	3	4	4	58	PA14_43670	104	161	11	174
1	PA14_00430	42	118	110	113	59	PA14_45590	71	67	15	8
2	PA14_02250	30	32	67	6	60	PA14_45870	52	140	24	16
3	PA14_02260	69	86	66	68	61	PA14_45880	78	195	121	159
4	PA14_05320	119	95	42	116	62	PA14_46370	51	30	4	5
5	PA14_05330	61	193	97	30	63	PA14_46980	97	113	27	36
6	PA14_06060	103	37	45	168	64	PA14_47390	108	54	29	58
7	PA14_06070	56	32	32	6	65	PA14_48160	142	111	83	167
8	PA14_07820	93	76	111	88	66	PA14_49170	53	46	50	97
9	PA14_07840	23	25	136	18	67	PA14_49180	25	185	32	37
10	PA14_09680	84	56	40	9	68	PA14_49420	45	111	110	44
11	PA14_09690	90	137	31	67	69	PA14_49440	121	155	70	157
12	PA14_10770	48	72	38	99	70	PA14_50180	9	3	8	13
13	PA14_11120	127	122	106	196	71	PA14_50200	7	7	6	10
14	PA14_11630	83	107	68	148	72	PA14_52240	88	49	99	126
15	PA14_11680	81	44	22	44	73	PA14_52250	15	33	27	7
16	PA14_12780	106	62	31	60	74	PA14_52260	4	3	3	3
17	PA14_12810	99	118	122	4	75	PA14_54500	18	120	85	177
18	PA14_12820	71	128	103	21	76	PA14_54510	108	49	61	15
19	PA14_13740	62	67	53	180	77	PA14_55780	110	110	133	39
20	PA14_16350	61	117	34	112	78	PA14_55810	88	159	159	111
21	PA14_16500	31	133	20	83	79	PA14_56950	88	90	140	40
22	PA14_17670	12	109	150	176	80	PA14_57140	94	126	157	152
23	PA14_19340	80	138	7	29	81	PA14_57170	93	77	50	5
24	PA14_20780	92	78	135	125	82	PA14_58300	106	106	61	155
25	PA14_20800	43	27	5	17	83	PA14_58320	59	97	50	5
26	PA14_20820	83	21	30	41	84	PA14_59770	10	7	4	4
27	PA14_21700	10	108	137	147	85	PA14_59780	77	71	120	72
28	PA14_22730	75	60	102	11	86	PA14_59790	25	12	140	213
29	PA14_22940	86	60	80	6	87	PA14_59800	78	71	26	36
30	PA14_22960	66	42	49	3	88	PA14_60250	50	102	101	105
31	PA14_24340	84	108	83	181	89	PA14_60260	64	131	140	184
32	PA14_24350	79	63	24	130	90	PA14_62530	128	107	89	201
33	PA14_24710	145	69	41	48	91	PA14_62540	118	59	60	142
34	PA14_24720	98	136	124	148	92	PA14_63150	17	142	142	153
35	PA14_26810	118	50	36	11	93	PA14_63160	94	37	133	34
36	PA14_27550	16	70	41	58	94	PA14_63210	33	114	18	24
37	PA14_27800	65	72	83	140	95	PA14_64050	81	50	17	51
38	PA14_27810	92	114	91	129	96	PA14_64230	28	18	74	4
39	PA14_27940	17	75	89	5	97	PA14_65860	119	108	109	178
40	PA14_29360	44	114	138	149	98	PA14_65880	82	69	183	6
41	PA14_29740	107	96	174	157	99	PA14_67670	89	52	34	9
42	PA14_30650	29	26	4	6	100	PA14_67680	113	86	172	133
43	PA14_30700	75	88	67	102	101	PA14_68230	105	79	39	119
44	PA14_30830	67	78	89	142	102	PA14_68250	83	128	99	101
45	PA14_30840	21	34	13	4	103	PA14_68680	55	114	87	6
46	PA14_31950	64	106	157	39	104	PA14_69470	77	121	110	179
47	PA14_31960	50	56	124	43	105	PA14_69480	56	28	150	81
48	PA14_32580	57	92	38	197	106	PA14_70750	58	108	136	118
49	PA14_36420	109	112	72	166	107	PA14_70760	103	119	145	205
50	PA14_37690	35	105	55	23	108	PA14_70790	21	71	71	112
51	PA14_38900	20	104	116	116	109	PA14_72380	93	16	46	12
52	PA14_40570	44	129	153	117	110	PA14_72390	56	131	21	91
53	PA14_41260	89	115	67	10	111	PA14_72720	40	131	154	46
54	PA14_41270	65	160	59	27	112	PA14_72740	112	87	38	55
55	PA14_41480	74	66	59	6	113	PA14_73020	33	94	5	65
56	PA14_41490	97	53	55	19						

Table S1. Perimeter values[#] for swarms of TCS mutants, Related to Figure 4 and Figure 5.

[#]Deep blue (non-swarmer), perimeter < 20% of PA14 WT; light blue (weak swarmer), perimeter >20% to < mean - SD of PA14 WT; grey (wild type), mean \pm SD of PA14 WT; yellow (hyper-swarmer) > mean + SD of PA14 WT.

Table S2. Description of TCS class of bacterial swarm regulators of *P. aeruginosa* PA14

Sl. No	PA14 ID	PAO1 ID	Gene name ^{&}	Domain features [*]
	TCS pairs			
1	PA14_22940	PA3192	<i>gltR</i>	REC-wHTH
2	PA14_22960	PA3191	<i>gtrS</i>	HAMP-HisK
3	PA14_41480	PA1786	<i>nasS</i>	(no hit found)
4	PA14_41490	PA1785	<i>nasT</i>	REC-ANTAR
5	PA14_50180	PA1099	<i>fleR</i>	REC-AAA-Fis
6	PA14_50200	PA1098	<i>fleS</i>	PAS-HisK
7	PA14_72380	PA5483	<i>algB</i>	REC-AAA-Fis
8	PA14_72390	PA5484	<i>kinB</i>	HAMP-HisK
	TCS pairs[#]			
9	PA14_02250	PA0178	<i>cheA</i>	HPt-HisK-cheW
10	PA14_06070	PA0464	<i>creC</i>	HAMP-HisK
11	PA14_07840	PA0601	<i>agtR</i>	REC-HTH
12	PA14_09680	PA4197	<i>bfiS</i>	PAS-PAC-PAS-HisK
13	PA14_12810	PA3947	<i>rocR</i>	REC-EAL
14	PA14_16500	PA3702	<i>wspR</i>	REC-GGDEF
15	PA14_22730	PA3206	<i>cpxA</i>	HAMP-HisK
16	PA14_26810	PA2882		CBS-HisK
17	PA14_30840	PA2571		HisK
18	PA14_38900	PA1980	<i>eraR</i>	REC-HTH
19	PA14_41260	PA1799	<i>parR</i>	REC-wHTH
20	PA14_45590	PA1458		Hpt-HisK-CheW
21	PA14_45870	PA1438		HAMP-HisK
22	PA14_52250	PA0929	<i>pirR</i>	REC-wHTH
23	PA14_54500	PA0757	<i>tctE</i>	HAMP-HisK
24	PA14_57170	PA4398		HAMP-PAS-HisK
25	PA14_58320	PA4494	<i>RoxS</i>	HisK
26	PA14_63150	PA4776	<i>pmrA</i>	REC-wHTH
27	PA14_65880	PA4983	<i>aruR</i>	REC-wHTH
28	PA14_67670	PA5124	<i>ntrB</i>	HisK
29	PA14_68680	PA5199	<i>envZ/amgS</i>	HAMP-HisK
30	PA14_59770	N/A	<i>rcsB</i>	REC-HTH
31	PA14_59790	N/A	<i>pvrR</i>	REC-EAL
	Hybrids			
32	PA14_19340	PA3462		HisK-REC
33	PA14_21700	PA3271		Na:Solute_symport-HisK-REC
34	PA14_27550	PA2824	<i>sagS</i>	HisK-REC
35	PA14_43670	PA1611		HAMP-HisK-REC
36	PA14_46370	PA1396		HisK-REC
37	PA14_52260	PA0928	<i>GacS</i>	HAMP-HisK-REC-HPt
38	PA14_64230	PA4856	<i>RetS</i>	HisK-REC-REC
	Orphans			
39	PA14_17670	PA3604	<i>erdR</i>	REC-HTH
40	PA14_27940	PA2798		REC
41	PA14_63210	PA4781		REC-HD-GYP
42	PA14_64050	PA4843	<i>gcbA</i>	REC-REC-GGDEF
43	PA14_30650	PA2586	<i>gacA</i>	REC-HTH
	HPt Protein			
44	PA14_20800	PA3345	<i>hptB</i>	HPt

Table S2. Description of TCS class of bacterial swarm regulators of *P. aeruginosa* PA14, Related to Figure 5. * Domain descriptions are based on prosite (<https://prosite.expasy.org/scanprosite/>) protein domain searches. # TCS genes with known interacting partner. However, the partner did not enrich in this screen. & Those in bold letters are newly proposed *bsw* names, based on this study. Sensor kinases are shown in grey. All others are response regulators or histidine phosphor transfer protein.

Table S3. Crystal Violet based quantification[#] of biofilm formation in *P. aeruginosa* PA14.

Strain	PA01 ID		PGM	M9	M8	BM2	M63
PA14			1.0 ± 0.0, n=45	1.0 ± 0.0, n=45	1.0 ± 0.0, n=45	1.0 ± 0.0, n=45	1.0 ± 0.1, n=41
pelA	PA3064		0.3 ± 0.0, n=40	0.4 ± 0.0, n=41	0.2 ± 0.0, n=42	0.4 ± 0.0, n=39	0.4 ± 0.0, n=36
PA14_02250	PA0178	cheA	3.0 ± 0.4, n=9	1.7 ± 0.1, n=9	2.2 ± 0.2, n=9	2.2 ± 0.2, n=9	2.1 ± 0.2, n=9
PA14_06070	PA0464	creC	4.6 ± 0.8, n=9	2.5 ± 0.3, n=9	3.0 ± 0.6, n=9	4.6 ± 0.6, n=9	3.0 ± 0.3, n=9
PA14_07840	PA0601		1.0 ± 0.2, n=9	2.7 ± 0.3, n=12	2.9 ± 0.4, n=9	3.9 ± 0.5, n=6	2.2 ± 0.6, n=13
PA14_12810	PA3947		0.7 ± 0.1, n=6	1.2 ± 0.2, n=6	1.2 ± 0.2, n=3	2.8 ± 0.9, n=3	0.9 ± 0.1, n=6
PA14_17670	PA3604		0.6 ± 0.1, n=10	0.7 ± 0.1, n=8	1.8 ± 0.3, n=12	2.9 ± 0.4, n=15	2.2 ± 0.3, n=11
PA14_19340	PA3462		1.8 ± 0.2, n=15	2.0 ± 0.2, n=15	1.9 ± 0.2, n=15	2.4 ± 0.3, n=15	2.0 ± 0.2, n=15
PA14_21700	PA3271		2.0 ± 0.4, n=9	2.1 ± 0.3, n=9	1.5 ± 0.3, n=9	5.5 ± 0.8, n=9	2.4 ± 0.4, n=9
PA14_22730	PA3206		2.9 ± 0.2, n=15	2.3 ± 0.2, n=15	2.3 ± 0.2, n=15	3.2 ± 0.2, n=15	3.4 ± 0.5, n=14
PA14_22940	PA3192	gtlR	1.4 ± 0.1, n=9	2.0 ± 0.2, n=9	1.2 ± 0.2, n=9	3.5 ± 0.8, n=9	2.2 ± 0.2, n=9
PA14_22960	PA3191		2.5 ± 0.2, n=18	2.3 ± 0.2, n=18	2.0 ± 0.2, n=18	4.2 ± 0.8, n=12	2.1 ± 0.2, n=16
PA14_26810	PA2882		1.7 ± 0.3, n=9	1.3 ± 0.1, n=9	1.7 ± 0.2, n=9	1.0 ± 0.1, n=9	1.8 ± 0.1, n=9
PA14_27550	PA2824		1.1 ± 0.1, n=15	1.1 ± 0.2, n=15	1.1 ± 0.1, n=15	1.4 ± 0.1, n=14	1.8 ± 0.4, n=15
PA14_27940	PA2798		2.8 ± 0.5, n=12	1.9 ± 0.1, n=12	1.1 ± 0.1, n=12	1.8 ± 0.2, n=12	2.9 ± 0.4, n=12
PA14_30650	PA2586	gacA	2.1 ± 0.3, n=9	1.9 ± 0.1, n=9	2.3 ± 0.2, n=12	1.4 ± 0.2, n=9	2.5 ± 0.2, n=9
PA14_30840	PA2571		1.4 ± 0.2, n=17	1.2 ± 0.2, n=18	1.8 ± 0.4, n=12	3.4 ± 0.3, n=18	1.6 ± 0.1, n=12
PA14_41260	PA1799		0.9 ± 0.1, n=9	0.9 ± 0.1, n=9	1.1 ± 0.2, n=9	0.2 ± 0.0, n=6	0.7 ± 0.2, n=9
PA14_41480	PA1786	nasS	0.8 ± 0.1, n=9	1.0 ± 0.2, n=9	0.8 ± 0.1, n=9	0.8 ± 0.1, n=7	0.8 ± 0.2, n=8
PA14_45590	PA1458		2.7 ± 0.4, n=9	1.8 ± 0.2, n=9	1.4 ± 0.4, n=9	1.0 ± 0.2, n=8	3.7 ± 0.7, n=8
PA14_46370	PA1396		1.9 ± 0.2, n=12	2.2 ± 0.2, n=12	1.7 ± 0.3, n=11	2.5 ± 0.5, n=12	2.1 ± 0.2, n=12
PA14_50180	PA1099	fleR	0.3 ± 0.0, n=9	0.2 ± 0.0, n=9	0.3 ± 0.1, n=9	0.5 ± 0.1, n=9	0.2 ± 0.0, n=3
PA14_50200	PA1098	fleS	0.2 ± 0.0, n=9	0.2 ± 0.0, n=9	0.4 ± 0.1, n=8	0.3 ± 0.0, n=9	0.2 ± 0.0, n=3
PA14_52250	PA0929		1.4 ± 0.2, n=9	1.6 ± 0.2, n=9	1.3 ± 0.2, n=9	2.5 ± 0.3, n=6	3.1 ± 0.6, n=9
PA14_52260	PA0928	GacS	1.6 ± 0.3, n=9	3.4 ± 0.2, n=9	1.9 ± 0.3, n=9	2.8 ± 0.6, n=5	1.0 ± 0.2, n=9
PA14_54500	PA0757		0.8 ± 0.1, n=9	1.3 ± 0.1, n=9	1.4 ± 0.0, n=6	2.8 ± 0.6, n=5	2.3 ± 0.3, n=9
PA14_57170	PA4398		1.6 ± 0.3, n=12	1.2 ± 0.3, n=12	2.0 ± 0.3, n=6	2.9 ± 0.3, n=6	1.6 ± 0.4, n=12
PA14_58320	PA4494		1.5 ± 0.2, n=15	1.7 ± 0.2, n=15	1.3 ± 0.1, n=15	1.8 ± 0.2, n=12	1.7 ± 0.3, n=15
PA14_59770	NOT PRESENT	rcsB	1.9 ± 0.3, n=18	2.4 ± 0.4, n=18	2.5 ± 0.5, n=15	3.6 ± 0.5, n=12	2.5 ± 0.3, n=12
PA14_63210	PA4781		0.6 ± 0.1, n=9	0.8 ± 0.1, n=9	0.6 ± 0.1, n=9	1.0 ± 0.0, n=9	1.7 ± 0.2, n=9
PA14_64230	PA4856		3.4 ± 0.4, n=9	1.7 ± 0.1, n=9	2.5 ± 0.4, n=9	2.4 ± 0.1, n=9	3.3 ± 0.0, n=3
PA14_65880	PA4983		1.6 ± 0.3, n=12	1.8 ± 0.1, n=12	1.2 ± 0.2, n=12	1.4 ± 0.1, n=12	1.9 ± 0.2, n=12
PA14_67670	PA5124	ntrB	0.5 ± 0.0, n=15	1.5 ± 0.3, n=18	0.9 ± 0.2, n=18	1.4 ± 0.1, n=12	0.5 ± 0.1, n=15
PA14_72380	PA5483	algB	2.7 ± 1.2, n=9	1.3 ± 0.3, n=9	1.5 ± 0.3, n=8	1.3 ± 0.2, n=6	3.4 ± 1.1, n=9
PA14_72390	PA5484		1.0 ± 0.2, n=9	1.1 ± 0.1, n=9	1.1 ± 0.2, n=6	1.4 ± 0.3, n=6	0.5 ± 0.1, n=9

Table S3. Crystal Violet based quantification[#] of biofilm formation in *P. aeruginosa* PA14, Related to Figure 5.

[#] Grey cells represent values not significantly different from PA14 control ($p > 0.05$); deep blue (defective), CV stain < 50% of PA14 control; light blue (weak CV stain), 50% to mean - SD of PA14 control; light yellow, CV stain up to 2-fold of PA14 control; deep yellow, CV stain 2 to 6-fold of PA14 control. Mean values of CV stain in each strain was compared with mean value of CV stain in PA14 control by unpaired *t* test. N represents number of replicates.

TRANSPARENT METHODS

Bacterial strains and growth conditions

Pseudomonas aeruginosa PA14 was used as the wild-type strain. All the mutant strains used in this work are from the transposon insertion mutant library of PA14 (Liberati et al., 2006). Unless otherwise mentioned, all strains were grown in Luria Bertani (LB) broth under standard laboratory conditions. For selection of transposon mutants, gentamycin ($50 \mu\text{g mL}^{-1}$) antibiotic was used. No antibiotics were used in the swarm dishes. Additional growth media used in this study are BHI, BM2, M9, M8 (a modified M9 media without NH_4Cl and CaCl_2), PGM and M63 medium ($100 \mu\text{M KH}_2\text{PO}_4$, $15.14 \text{ mM } (\text{NH}_4)_2\text{SO}_4$, $0.36 \mu\text{M FeSO}_4\cdot\text{H}_2\text{O}$, 1 mM MgSO_4 and 4% arginine). Media composition for the rest is provided in Table 1.

Swarming assay and screening

Swarming motility assays were performed as previously described (Overhage et al., 2008; Yeung et al., 2009), with additional modifications. Appropriate medium (LB, BHI, M8, M9, BM2 or PGM) was solidified with 0.6% Bacto™ agar (BD) and inoculated after 16 hours. All plates were inoculated at the centre with $2 \mu\text{L}$ of overnight bacterial culture in LB broth ($\text{OD}_{600} = 2.8 - 3.0$) and incubated at 37°C for 24 hours. All the no-swearer phenotypes were confirmed at least in three independent experiments.

Swimming Assay

For swimming tests, PGM containing 0.3% Bacto™ agar (BD) was used. A $5 \mu\text{L}$ of 2 ml overnight culture was inoculated into 5 ml LB broth (secondary culture) and incubated at 37°C for 5 hours (or $\text{OD}_{600} = 1$). Using sterile toothpicks, the secondary culture was introduced into the centre of swim agar plate by puncturing into the agar but without touching the base of the plates. Plates were incubated at 37°C for 24 hours right side up. Swimming proficiency were analysed by measuring the swim area covered.

Biofilm quantification

Biofilm formations were assayed as previously described (O'Toole, 2011). Briefly, *P. aeruginosa* strains were grown overnight in 2 mL of LB broth at 37°C . Overnight culture was then diluted with biofilm media (M63) or swarming media (M9, M8, BM2 or PGM) at a ratio of 1:100. $100 \mu\text{L}$ of the dilution were added to each well of a 96-well microtiter plate, in triplicate. Microtiter plate was then incubated at 37°C for 24 hours. After incubation, the plates were rinsed with tap water to remove the planktonic cells and air dried. 0.1% solution of crystal violet in water was added to each well of microtiter dish for staining, followed by 15 minutes incubation at room temperature. After rinsing the excess stain by vigorous shaking and tapping of the plate on paper towels, plates were dried for a few hours. Crystal violet stain in each well was solubilized in $125 \mu\text{L}$ 30% acetic acid followed by 15 minutes incubation at room temperature. Biofilm formation was quantified by measuring absorbance at 550 nm. PA14 WT was taken as reference; a mutant strain for EPS, *peIA*, was taken as control. The results are represented in table S3 as mean value (standard error of mean) and sample size (n). All strains were stained 3 - 4 independent times, with three replicates on every occasion.

Principal component analysis for swarm features

Principal component analysis (PCA) was performed on different parameters of the colony pattern of wild type PA14 in different nutrient media. Based on the gross appearance of the patterns, we divided these patterns into two classes - branching and non-branching patterns. The wild type PA14 produces non-branching patterns on LB and BHI swarm agar but produces branching patterns on BM2, M8, M9 and PGM swarm agar. We defined the ten following parameters to describe a branching pattern of PA14 swarm:

Branch length (BL): Length of all dendrites in the pattern

Branch Angle (BA): Angle between two dendrites in the pattern

Branch width (BW): Dendrite width of all branches in the pattern

Area (A): Area covered by the swarm

Perimeter (P): Perimeter of the pattern formed by the swarm

Radius of Minimum bounding circle radius (RMBC): Radius of the circle that can encircle the pattern

Number of levels (NL): Number of levels of branching a dendrite undergoes

Normalised Area (NA): Ratio of Area of the pattern and Area of the minimum bounding circle

Number of primary branches (NPB): Number of branches originating from the point of inoculation

Growing tips (GT): Total number of dendrite tips present in the swarm at 24 hours.

The non-dendritic patterns (on LB and BHI agar) are characterized by Area (A), Perimeter (P), Radius of minimum bounding circle (RMBC), and the Normalized Area (NA). We chose two independent parameters to differentiate patterns of dendritic swarm. The parameters chosen i.e. Perimeter and Normalised area are also defined for patterns on LB and BHI.

Statistical analysis: One-way ANOVA was used to analyse variance of swarming traits across swarms on different media. For comparison of means of traits, post hoc Tukey test was performed in Figure 2. In all other cases, unpaired student's t test was performed to compare mean \pm SEM as indicated.

Video S1. Video of *P. aeruginosa* PA14 swarming along with trace for area, perimeter, and circularity on BHI-0.6% agar, Related to figure 2.

Video S2. Video of *P. aeruginosa* PA14 swarming along with trace for area, perimeter, and circularity on LB-0.6% agar, Related to figure 2.

Video S3. Video of *P. aeruginosa* PA14 swarming along with trace for area, perimeter, and circularity on M8-0.6% agar, Related to figure 2.

Video S4. Video of *P. aeruginosa* PA14 swarming along with trace for area, perimeter, and circularity on M9-0.6% agar, Related to figure 2.

Video S5. Video of *P. aeruginosa* PA14 swarming along with trace for area, perimeter, and circularity on BM2-0.6% agar, Related to figure 2.

Video S6. Video of *P. aeruginosa* PA14 swarming along with trace for area, perimeter, and circularity on PGM-0.6% agar, Related to figure 2.