

Table S7. Physiological Properties of *P. temperata* NC19 mutants and their parental wild type.

	<u>Swim Ring Formation</u> <sup>1</sup>		<u>Swarm Ring Formation</u> <sup>2</sup>	<u>Insect Mortality</u> <sup>4</sup>		<u>Symbiosis</u> <sup>5</sup>	<u>Dye Absorption</u> <sup>6</sup>			<u>Extracellular Products</u> <sup>7</sup>				<u>Pigmentation</u>
	+ NaCl	- NaCl		LT <sub>50</sub>	LT <sub>100</sub>		EB	NTBA	Mac	Protease	DNase	Hemolytic	Antibiotic <sup>e</sup>	PP3
<b><u>Wild type</u></b>														
NC 19 Primary (Parental)	36.9 ± 3.3	3.0 ± 0.0	10.7 ± 1.5	38	46	+	+	+	+	++	++	+	+	Dark
NC 19 Secondary	3.0 ± 0.0	3.0 ± 0.0	N.D. <sup>3</sup>	35	43	-	-	-	-	+	+	-	-	Light
<b><u>Non-motile</u></b>														
P3-C8	3.0 ± 0.0	3.0 ± 0.0	11.0 ± 1.1	+	+	+	+	+	+	++	++	+	+	Dark
P4-27	3.0 ± 0.0	3.0 ± 0.0	10.0 ± 1.1	delay	delay	+	+	+	+	++	+	+	+	Dark
P4 C8	3.0 ± 0.0	3.0 ± 0.0	11.0 ± 1.1	+	+	+	+	+	+	++	++	+	+	Dark
P5-G7	3.0 ± 0.0	3.0 ± 0.0	10.0 ± 1.0	+	+	+	+	+	+	++	+	+	+	Dark
P7 F6	3.0 ± 0.0	3.0 ± 0.0	10.0 ± 1.0	+	+	+	+	+	+	++	+	+	+	Dark
P12 -18	3.0 ± 0.0	3.0 ± 0.0	10.0 ± 1.1	+	+	+	+	+	+	++	+	+/-	+	Dark
P20 F12	3.0 ± 0.0	3.0 ± 0.0	9.0 ± 1.0	enhan	enhan	+	+	+	+	++	++	-	+	Dark
P26 E6	3.0 ± 0.0	3.0 ± 0.0	7.0 ± 1.1	+	+	+	+	+	+	++	++	-	+	Dark

P26-E8	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$8.0 \pm 1.1$	+	+	+	+	+	+	++	++	+	+	Dark
P30-B7	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$9.0 \pm 1.1$	+	+	+	+	+	+	++	+	+	+	Dark
P39-C1	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$10.0 \pm 1.1$	+	+	+	+	+	+	++	++	+	+	Dark
P39E6	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$11.0 \pm 1.1$	+	+	+	+	+	+	++	++	+	+	Dark
P41 G10	$4.0 \pm 0.5$	$3.0 \pm 0.0$	$8.0 \pm 1.1$	+	+	+	+	+	+	++	++	+	+	Dark
P59 D11	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$9.0 \pm 1.1$	+	+	+	+	+	+	++	++	+	++	Dark
P59-G8	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$8.0 \pm 1.1$	+	+	+	+	+	+	++	+	+	+	Dark
P72-C6	$2.0 \pm 0.0$	$2.0 \pm 0.0$	$7.8 \pm 1.1$	+	+	+	+	+	+	++	++	+	++	Dark
P76-H11	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$11.0 \pm 1.1$	+	+	+	+	+	+	+++	+	+	+	Dark
P84-B1	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$10.0 \pm 1.0$	+	+	+	+	+	+	++	++	+	+	Dark
P84-H2	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$10.0 \pm 1.1$	+	+	+	+	+	+	++	++	+	+	Dark
P86-G6	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$9.0 \pm 1.1$	delay	delay	+	+	+	+	+++	+	+	++	Dark
P87-G4	$4.0 \pm 0.0$	$3.0 \pm 0.0$	$9.0 \pm 1.1$	+	+	-	+	+	+	++	++	+	+	Dark
P88-D7	$3.0 \pm 0.0$	$3.0 \pm 0.0$	$11.0 \pm 1.0$	+	+	+	+	+	+	+++	+	+	++	Dark
P90-G1	$1.3 \pm 0.6$	$3.0 \pm 0.6$	$12.3 \pm 4.0$	+	+	+	+	+	+	++	+	+	+	Dark
<b><u>Defective Motility</u></b>														
P5-11	$8.0 \pm 1.1$	$1.0 \pm 0.0$	$10.0 \pm 1.2$	+	+	+	-/+	-/+	-	++	+	-	-	Light

P13-7	18.7 ± 1.5	1.0 ± 0.0	15.0 ± 1.7	delay	delay	+	-	-	-	-	++	-	-	Light
P28-A10	22.0 ± 1.1	3.0 ± 0.0	11.0 ± 1.1	+	+	+	+	+	+	+++	++	+	+/-	Dark
P31-B7	18.0 ± 1.3	3.0 ± 0.0	9.0 ± 1.0	+	+	+	+	+	+	++	++	+	+	Dark
P31-B12	11.0 ± 1.1	3.0 ± 0.0	12.0 ± 1.3	+	+	+	+	+	+	++	++	+	+	Dark
P43-B3	5.0 ± 2.0	3.0 ± 0.0	8.0 ± 1.1	+	+	+	+	+	+	+++	++	+	+	Dark
P47-F1	5.0 ± 1.5	3.0 ± 0.0	8.0 ± 1.1	+	+	+	+	+	+	++	++	+	+	Dark
P58-C8	5.0 ± 2.0	3.0 ± 0.0	10.0 ± 1.0	enhan	enhan	enhan	+	+	+	++	++	+	+	Dark
P64-C3	18.0 ± 1.2	3.0 ± 0.0	12.0 ± 1.1	+	+	delay	-	-	-	++	++	+	+	Dark
P64-D5	10.0 ± 1.1	3.0 ± 0.0	15.0 ± 1.1	delay	delay	+	-	-	-	++	++	+	+	Dark
P88-D8	22.0 ± 1.4	3.0 ± 0.0	15.0 ± 1.1	+	+	+	-/+	-/+	-/+	++	++	+	+/-	Dark
P84-E4	12.0 ± 1.1	3.0 ± 0.0	8.0 ± 1.1	+	+	+	+	+	+	++	+	+	+/-	Dark
<b><u>Swim –NaCl motility</u></b>														
P1-4	48.3 ± 6.1	37.7 ± 4.0	10.3 ± 4.0	+	+	+	+	+	+	++	++	+	+	Dark
P4-9	42.3 ± 4.9	20.0 ± 3.0	25.3 ± 2.5	+	+	+	+	+	+	++	++	+	+	Dark
P6-22	42.7 ± 2.6	30.0 ± 10.0	10.7 ± 2.9	+	+	+	+	+	+	++	+	+/-	+	Dark
P30-H5	27.0 ± 3.0	27.0 ± 2.5	15.6 ± 2.5	+	+	+	+	+	+	++	++	+/-	+	Dark

P56-C3	$42.0 \pm 4.4$	$35.3 \pm 4.1$	$10.8 \pm 3.3$	+	+	+	+	+	+	++	++	+/-	+	Dark
<b><u>Hyperswarmers</u></b>														
P2-A9	$43.3 \pm 3.5$	$9.3 \pm 0.6$	$48.3 \pm 5.8$	+	+	+	+	+	+	++	++	+	+	Dark
P5-A7	$36.0 \pm 3.6$	$6.2 \pm 0.6$	$22.5 \pm 3.5$	+	+	+	+	+	+	++	++	+	+	Light
P7-A8	$40.3 \pm 2.5$	$1.0 \pm 0.0$	$20.3 \pm 0.6$	+	+	+	+	+	+	++	++	+	+	Dark
P7-A11	$41.3 \pm 1.2$	$3.3 \pm 0.6$	$74.0 \pm 3.6$	+	+	+	+	+	+	++	++	+	+	Dark
P8-C2	$27.0 \pm 2.6$	$3.4 \pm 3.4$	$69.4 \pm$ $13.4$	+	+	+	+	+	+	+++	+	+	+	Dark
P8-H6	$35.2 \pm 4.5$	$2.0 \pm 0.6$	$18.7 \pm 4.8$	+	+	+	+	+	+	+++	++	+	+	Dark
P11-A2	$43.3 \pm 1.2$	$3.7 \pm 0.6$	$66.7 \pm 6.4$	+	+	+	+	+	+	+++	++	+	+	Dark
P11-A6	$53.0 \pm 1.5$	$3.0 \pm 1.0$	$51.0 \pm$ $55.0$	+	+	+	+	+	+	++	++	+	++	Dark
P12-A1	$37.3 \pm 0.6$	$7.0 \pm 2.6$	$79.4 \pm 3.4$	delay	delay	enhan	+	+	+	++	+	+	++	Dark
P14-G2	$24.0 \pm 3.4$	$20.0 \pm 5.2$	$23.0 \pm 1.7$	+	+	+	+	+	+	++	++	+	+	Dark
P20-D5	$45.0 \pm 3.4$	$20.0 \pm 3.4$	$27.7 \pm 2.5$	+	+	+	+	+	+	++	++	+/-	+	Dark
P38-B11	$36.3 \pm 1.5$	$2.7 \pm 0.6$	$16.5 \pm 4.9$	+	+	+	+	+	+	++	+	+	+	Dark
P38-F2	$18.0 \pm 3.4$	$6.0 \pm 1.4$	$45.0 \pm 3.2$	+	+	+	+	+	+	++	+	+	+	Dark
P46-G10	$40.9 \pm 1.7$	$2.7 \pm 0.6$	$30.0 \pm 5.6$	+	+	+	+	+	+	+++	+	+	+	Dark

P50-A6	$42.0 \pm 1.0$	$3.0 \pm 1.0$	$36.7 \pm 5.8$	delay	delay	+	+	+	+	+++	+	+	++	Dark
P57-G9	$29.0 \pm 6.5$	$20.7 \pm 0.6$	$69.0 \pm 7.9$	+	+	+	+	+	+	++	+	+	++	Dark
P61-A3	$29.0 \pm 3.4$	$7.0 \pm 3.4$	$22.6 \pm 2.6$	+	+	+	+	-	-	-	++	+/-	-	Light
P63-H9	$29.0 \pm 3.4$	$80.0 \pm 0.0$	$55.4 \pm 3.9$	+	+	+	+	+	+	++	++	+	+	Dark
P64-B10	$32.0 \pm 4.5$	$9.0 \pm 3.2$	$80.0 \pm 0.0$	+	+	+	+	+	+	+++	+	+	++	Dark
P78-C2	$49.0 \pm 0.6$	$50.0 \pm 13.4$	$24.9 \pm 11.8$	+	+	+	+	+	+	+++	++	+	+	Dark
P80-G7	$38.3 \pm 2.6$	$25.0 \pm 3.5$	$55.0 \pm 5.5$	+	+	+	+	+	+	++	+	+	+	Dark
P81-G6	$45.3 \pm 2.9$	$3.7 \pm 0.6$	$53.3 \pm 30.5$	+	+	-	+	+	+	++	+	+	+	Dark
P81-G7	$51.5 \pm 5.9$	$7.8 \pm 1.3$	$3.7 \pm 0.6$	+	+	+	+	+	+	++	+	+	-	Dark
P81-H5	$31.3 \pm 1.2$	$3.7 \pm 0.6$	$31.0 \pm 3.6$	+	+	+	+	+	+	+++	+	+	+	Dark
P81-H7	$33.0 \pm 0.6$	$8.0 \pm 2.6$	$68.0 \pm 10.4$	+	+	delay	+	+	+	++	+	+	+	Dark
P81-H8	$58.6 \pm 3.0$	$19.0 \pm 1.7$	$67.0 \pm 19.0$	+	+	+	+	+	+	++	+	+	+	Dark
P82-A5	$27.0 \pm 3.4$	$3.0 \pm 0.0$	$80.0 \pm 0.0$	+	+	+	+	+	+	+	+	+	+	Dark
P82-B9	$52.9 \pm 4.5$	$1.7 \pm 2.8$	$16.7 \pm 4.7$	+	+	+	+	+	+	++	+	+	+	Dark
P82-G9	$40.0 \pm 3.4$	$14.0 \pm 5.2$	$25.3 \pm 3.2$	+	+	+	+	+	+	++	+	+	+	Dark

P83-A9	$35.0 \pm 3.0$	$10.0 \pm 3.4$	$41.7 \pm 5.8$	delay	delay	+	+	+	+	++	+	+	+	Dark
P83-B10	$64.0 \pm 2.0$	$14.7 \pm 1.5$	$69.0 \pm 7.9$	enhan	enhan	+	+	+	+	+++	+	+	++	Dark
P83-H5	$27.0 \pm 3.4$	$10.0 \pm 4.8$	$70.0 \pm 6.4$	+	+	+	+	+	+	++	++	+	+	Dark
P90-G8	$45.1 \pm 5.2$	$6.0 \pm 0.0$	$22.0 \pm 5.3$	+	+	+	+	+	+	++	+	+	+	Dark
<b><u>Hyperswimmers</u></b>														
P2-H12	$47.0 \pm 3.4$	$3.0 \pm 0.0$	$50.0 \pm 16.4$	+	+	+	+	+	+	++	+	-	+	Dark
P5-2	$45.0 \pm 4.3$	$3.0 \pm 0.0$	$12.7 \pm 1.2$	+	+	+	+	+	+	++	++	+	+	Dark
P7-E1	$44.0 \pm 2.4$	$3.0 \pm 0.0$	$25.0 \pm 3.4$	+	+	+	+	+	+	++	++	+/-	+	Dark
P8-D3	$42.0 \pm 1.1$	$4.3 \pm 0.6$	$12.0 \pm 3.4$	+	+	+	+	+	+	+++	++	+	+	Dark
P10-D9	$57.0 \pm 1.0$	$4.3 \pm 0.6$	$53.3 \pm 8.2$	+	+	+	+	+	+	++	++	+/-	+	Dark
P32-G7	$47.0 \pm 5.4$	$3.0 \pm 0.0$	$10.0 \pm 3.4$	+	+	+	+	+	+	++	++	+	+	Dark
P35-B12	$47.0 \pm 4.4$	$3.0 \pm 0.0$	$10.0 \pm 3.4$	+	+	+	-/+	+	+	-	-	-	-	Light
P35-C12	$49.0 \pm 3.5$	$3.0 \pm 0.0$	$12.0 \pm 5.4$	+	+	+	+	+	+	+++	++	+	+	Dark
P62-H6	$44.3 \pm 3.4$	$7.3 \pm 0.6$	$30.0 \pm 12.5$	enhan	enhan	+	+	+	+	++	++	+	+	Dark
P75-A4	$53.3 \pm 2.5$	$3.5 \pm 0.7$	$33.3 \pm 14.2$	delay	delay	+	+	+	+	++	+	+	+	Dark

P83-A2	42.0 ± 1.1	3.0 ± 0.0	10.0 ± 3.4	+	+	+	+	+	+	++	++	+	+	Dark
P93-A4	47.3 ± 6.6	5.0 ± 1.3	34.0 ± 13.9	enhan	enhan	+	+	+	+	++	+	+	+	Dark

<sup>1</sup> Swim-migration assay was measured after 48 h incubation at 28 C and swim-migration medium contained 0.3% Bactoagar. Values represent the average swim ring diameter with the standard deviation indicated by ±. Results are the average of 3 measurements per independent experiment.

<sup>2</sup> Swarm-migration medium contained 1.25 % Bactoagar and swarm ring diameter was measured at 48 h incubation at 28 C. Values represent the average swim or swarm ring diameter with the standard deviation indicated by ±. Results are the average of 3 measurements per independent experiment.

<sup>3</sup> N.D., not determined

<sup>4</sup> Insect pathogenesis was determined on *G. mellonella* larvae as described in the Methods. LT<sub>50</sub> and LT<sub>100</sub> values represent the time (h) required to kill 50 and 100% of larvae to die. Numerical values are given for parental wild type and secondary-phase.

Symbols: (Enhan) enhanced LT<sub>50</sub> and LT<sub>100</sub> values that are < 5 h from the parental wild type value; (Delay) delayed LT<sub>50</sub> and LT<sub>100</sub> values that are > 5 h from the parental wild type value; and (+) LT<sub>50</sub> and LT<sub>100</sub> values that are similar to the parental wild type value.

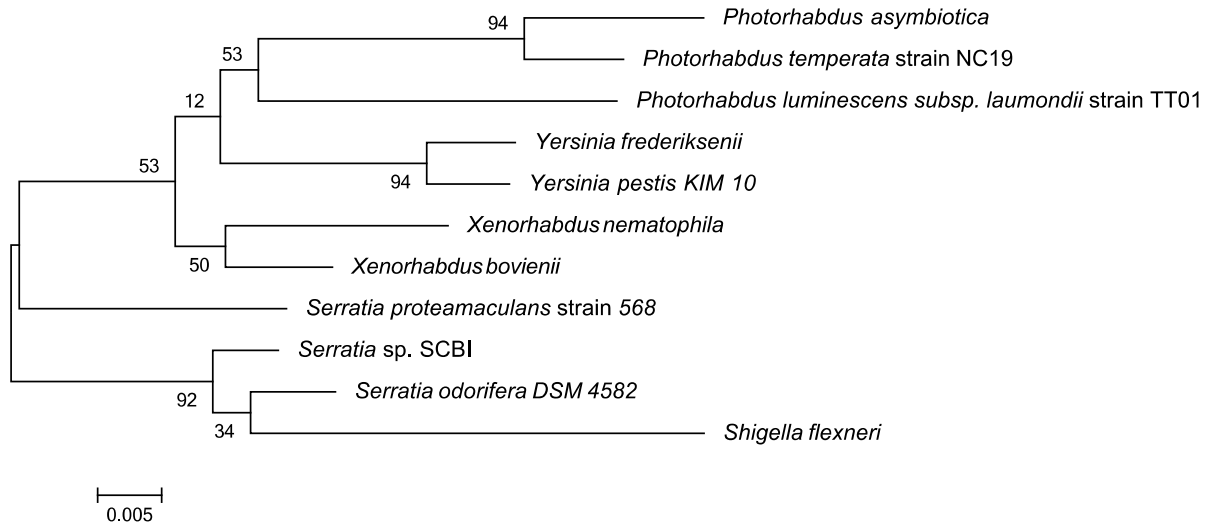
<sup>5</sup> Symbiosis was determined by the nematode assay and determined by the development of mature IJs. Symbols represent: (+) capable of normal nematode development similar to the parental wild type, (-) incapable of supporting nematode development, (enhan)

enhanced, nematode development occurred 2 days earlier than parental wild type, and (delay) delayed, nematode development occurred 2 days later than parental wild type.

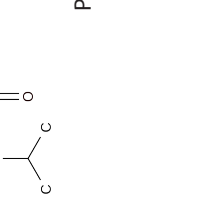
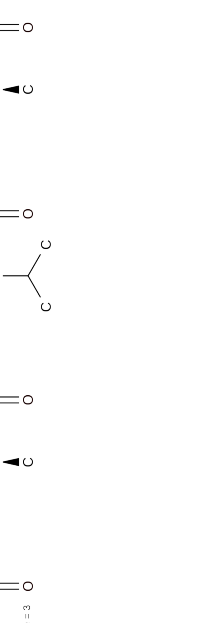
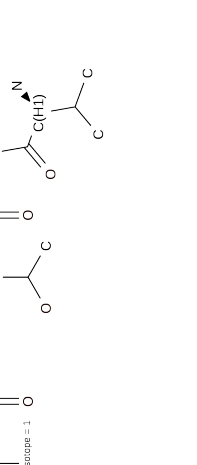
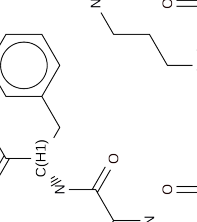
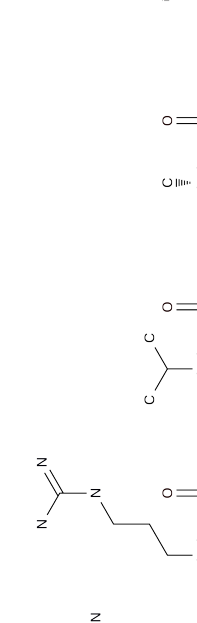
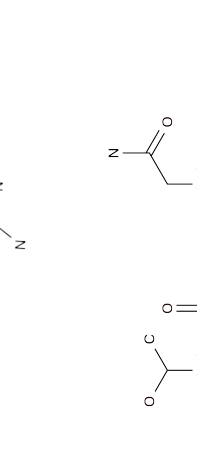
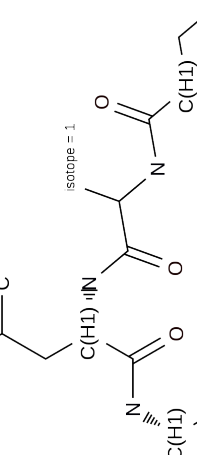
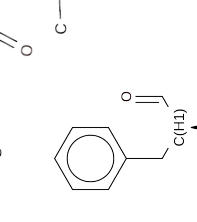
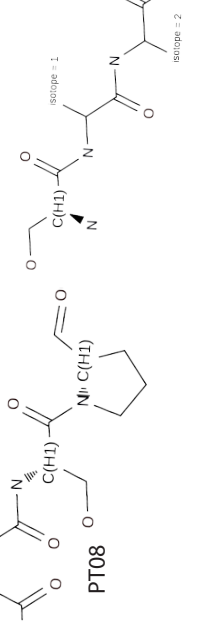
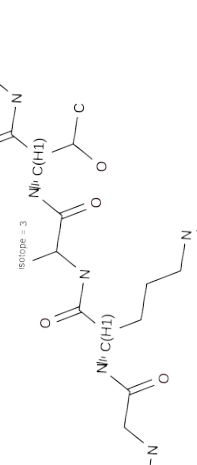
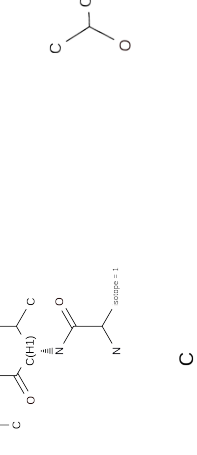
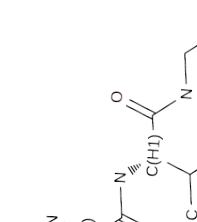
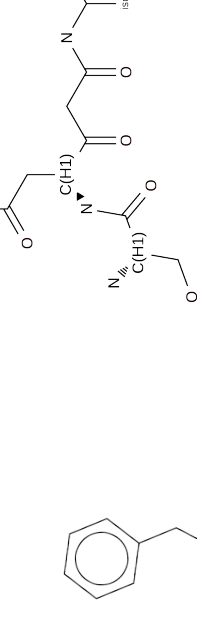
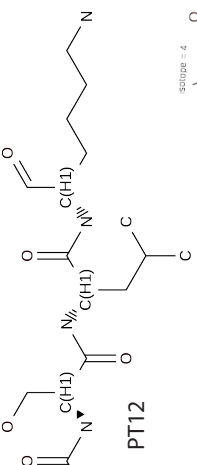
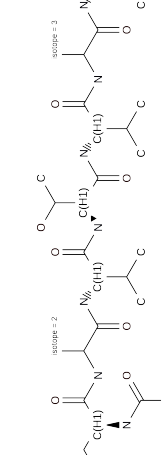
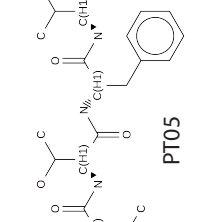
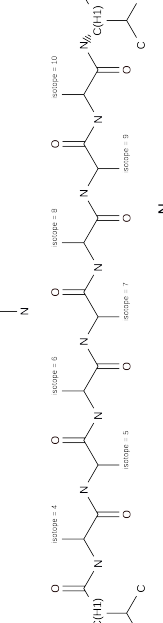
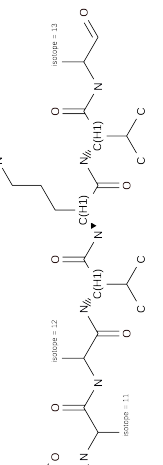
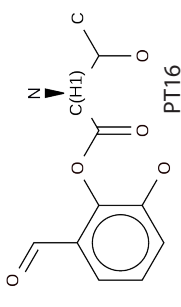
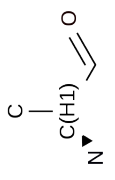
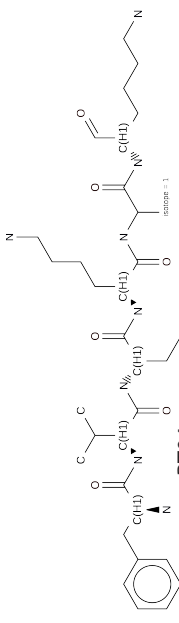
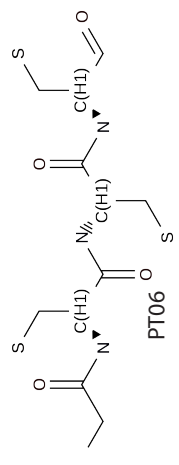
<sup>6</sup>Dye absorption on EMB and MacConkey plates were noted at 48 h. A positive result on EMB plates was indicated by metallic green colonies, and a negative result by dull purple colonies. A positive result on MacConkey plates was indicated by bright red colonies and a negative result by colorless or pink colonies.

<sup>7</sup>DNase and Protease activities were determined by measuring the size of the halo (mm) surrounding the bacterial colony, 24 h after inoculation. Hyper positive (++++) > 4 mm halo, strongly positive (++) 2-4 mm halo, positive (+) 1-2 mm halo, weakly positive (-/+) < 1 mm halo, and negative (-) no halo. Hemolytic activity was determined by clearing zones around the bacterial colony. Strong annular hemolysis (+), weakly positive (-/+) and negative (-) no hemolysis. Antibiotic production was determined by measuring the size of the halo (mm) surrounding the bacterial colony, 1 day after inoculation of the tester bacterium (*Micrococcus luteus*). Strongly positive (++) > 5 mm halo, positive (+) 2-5 mm halo, weakly positive (-/+) < 2 mm halo, and negative (-) no halo.

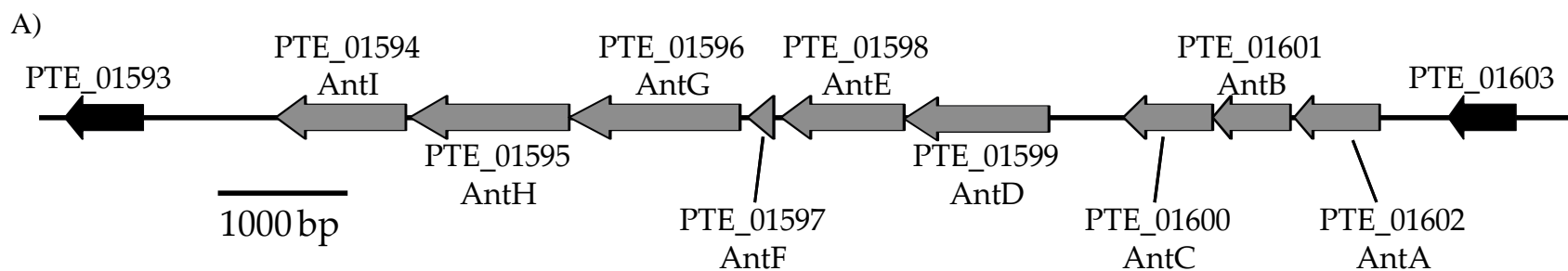




**Figure S1.** Maximum-likelihood phylogenetic tree of the 16S rDNA for *P. temperata* and closely related species. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (1). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.



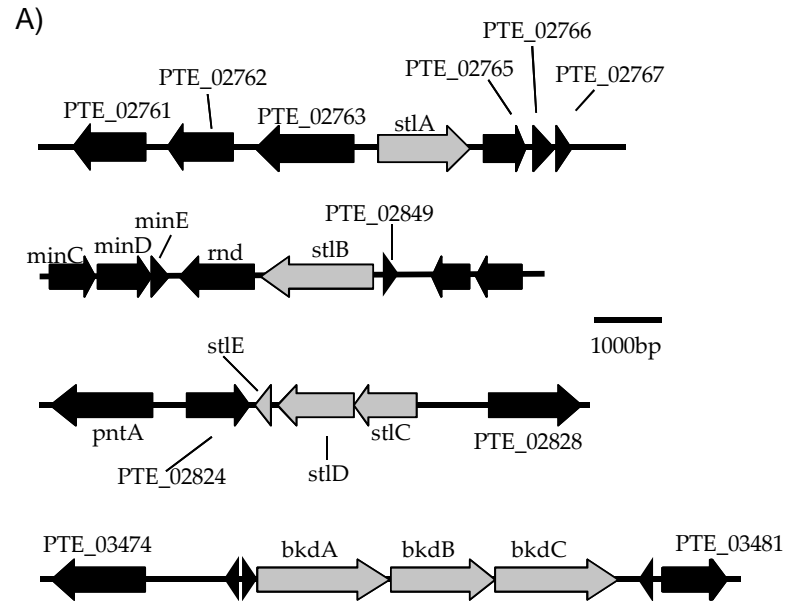
**Figure S2.** Predicted *Photorhabdus* chemical structures based on bioinformatic analysis of *Photorhabdus* biosynthetic gene clusters. Compound numbers correlate with the “Predicted structure” data in Table 2.



B)

AA% identity	AntI (plu4186)	AntH (plu4187)	AntG (plu4188)	AntF (plu4189)	AntE (plu4190)	AntD (plu4191)	AntC (plu4192)	AntB (plu4193)	AntA (plu4194)
<i>P. asymbiotica</i>				PAU_01705 (24%; 47% +ive)	PAU_01706 (30%; 50% +ive)	PAU_01706 (37%; 56% +ive)			PAU_01704 (30%; 51% +ive)
<i>P. temperata</i>	PTE_01594 (87%; 94% +ive)	PTE_01595 (77%; 89% +ive)	PTE_01596 (87%; 95% +ive)	PTE_01597 (83%; 93% +ive)	PTE_01598 (83%; 93% +ive)	PTE_01599 (88%; 94% +ive)	PTE_01600 (91%; 94% +ive)	PTE_01601 (84%; 92% +ive)	PTE_01602 (91%; 97% +ive)

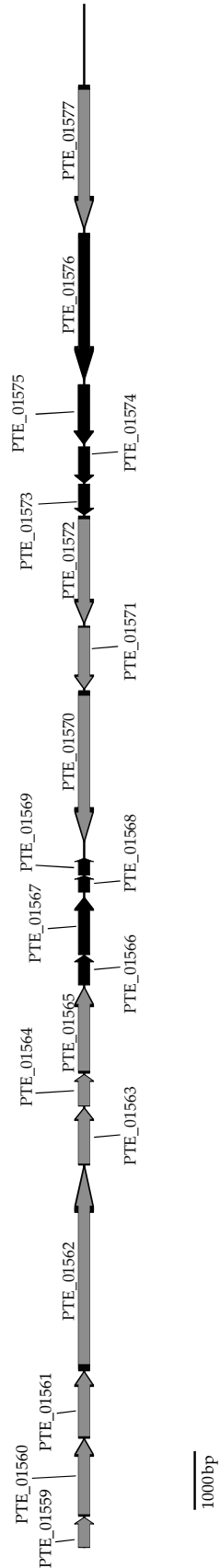
**Figure S3.** The *antABCDEFGHI* locus in *P. temperata* NC19. (A) Map of the genes involved in anthraquinone biosynthesis. (B) Amino acid sequence identity between anthraquinone biosynthetic proteins found in *P. luminescens* TTO1.



B)

AA % identity	BkdA (plu1883)	BkdB (plu1884)	BkdC (plu1885)	stlA (plu2234)	stlB (plu2134)	stlC (plu2163)	stlD (plu2164)	stlE (plu2165)
<i>P. temperata</i>	PTE_03477 (94%; 97% +ive)	PTE_03478 (83%; 89% +ive)	PTE_03479 (91%; 96% +ive)	PTE_02764 (93%; 97% +ive)	PTE_02848 (94%; 97% +ive)	PTE_02827 (85%; 91% +ive)	PTE_02826 (96%; 97% +ive)	PTE_02825 (85%; 91% +ive)
<i>P. asymbiotica</i>	PAU_02673 (95%; 98% +ive)	PAU_02672 (87%; 90% +ive)	PAU_02671 (89%; 94% +ive)	PAU_02187 (89%; 95% +ive)	PAU_02433 (93%; 97% +ive)	PAU_02402 (80%; 90% +ive)	PAU_02401 (85%; 92% +ive)	PAU_02400 (88%; 91% +ive)

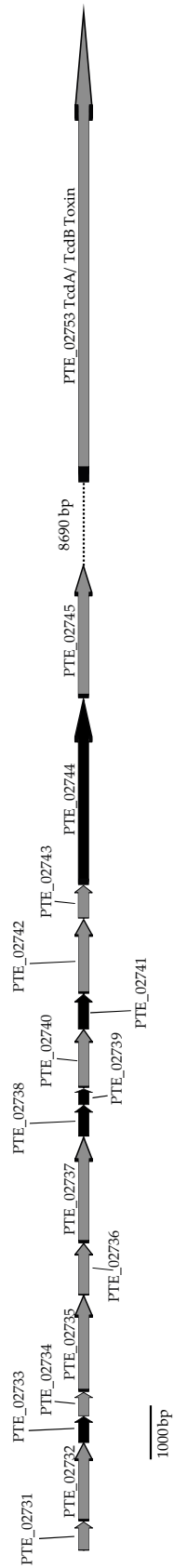
**Figure S4.** The genetic loci involved in stilbene production in *P. temperata* NC19. (A) Map of the genes involved in stilbene biosynthesis. (B) Amino acid sequence identity between stilbene biosynthetic proteins found in *P. luminescens* TTO1. Eight genes presented are not expected to be sufficient for stilbene production. Other genetic loci are also involved, but these genes have not been fully described (see ref. 2). These loci are not grouped together on the chromosome suggesting independent regulation.



<i>P. temperata</i> Locus Tag	Gene Name <sup>a</sup>	<i>P. luminescens</i> Locus Tag	AA% identity	<i>P. asymbiotica</i> Locus Tag	AA% identity
PTE_01577	vgrG	plu0355	34	PAU_03805	89.3
PTE_01576	-	-	-	PAU_03804	88.8
PTE_01575	-	-	-	PAU_03803	81.8
PTE_01574	-	-	-	PAU_03802	90.5
PTE_01573	impF	plu3222	29	PAU_03801	80.2
PTE_01572	COG3519	plu0369	27	PAU_03800	91.6
PTE_01571	COG3520	plu2296	26	PAU_03799	87.3
PTE_01570	clpV1	plu2287	46	PAU_03798	89.4
PTE_01569	-	plu0444	40	PAU_02212	71.4
PTE_01568	-	plu0441	59	-	-
PTE_01567	-	plu0367	27	PAU_03793	77.8
PTE_01566	-	plu2299	23	PAU_03792	90.6
PTE_01565	iglB	plu2300	47	PAU_03791	96.4
PTE_01564	iglA	plu2301	40	PAU_03790	95.1
PTE_01563	ImpA	plu2292	31	PAU_03789	78.9
PTE_01562	icmf	plu0359	24	PAU_03788	87.9
PTE_01561	dotU	plu0364	35	PAU_03787	87.1
PTE_01560	COG3522	plu0365	30	PAU_03786	88
PTE_01559	COG3521			PAU_03785	76.4

**Figure S5.** Organization of T6SS operon 1. The genes involved in T6SS are shown in gray and flanking genes in black. (Table) Amino acid sequence identity between T6SS proteins found in *P. temperata* with *P. luminescens* and *P. asymbiotica*, respectively.

<sup>a</sup>Nomenclature based on (3).

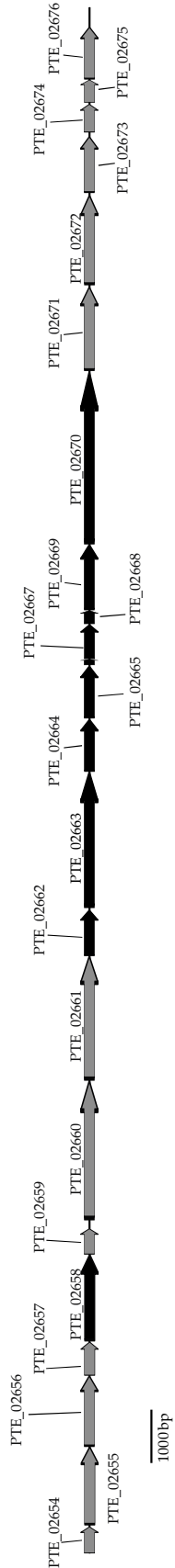


<i>P. temperata</i> Locus Tag	Gene Name	<i>P. luminescens</i> Locus Tag	AA% identity	<i>P. asymbiotica</i> Locus Tag	AA% identity
PTE_02176	icmf	plu0359	86	PAU_00275	90.2
PTE_02175	COG3515	plu0360	83.9	PAU_00276	87.7
PTE_02174	VasI	plu0361	87.5	PAU_00277	86.1
PTE_02173	-	plu0362	92.5	PAU_00278	92.1
PTE_02172	clpV1	plu0363	93.8	PAU_00279	94.9
PTE_02171	dotU	plu0364	87.1	PAU_00280	89.1
PTE_02170	COG3522	plu0365	83.3	PAU_00281	85.3
PTE_02169	COG3521	plu0366	94.1	PAU_00282	92
PTE_02168	fhaI	plu0367	92.3	PAU_00283	91.4
PTE_02167	COG3520	plu0368	89	PAU_00284	89.8
PTE_02166	COG3519	plu0369	85.7	PAU_00285	89.4
PTE_02165	COG3518	plu0370	91.1	PAU_00286	95.2
PTE_02164	iglB	plu0371	98.4	PAU_00287	98.8
PTE_02163	iglA	plu0372	97	PAU_00288	96.4
PTE_02162	-				
PTE_02161	Hcp1	plu0373	97.1	PAU_00289	97.1



**Figure S6.** Organization of T6SS operon 2. The genes involved in T6SS are shown in gray and flanking genes in black. (Table) Amino acid sequence identity between T6SS proteins found in *P. temperata* with *P. luminescens* and *P. asymbiotica*, respectively.

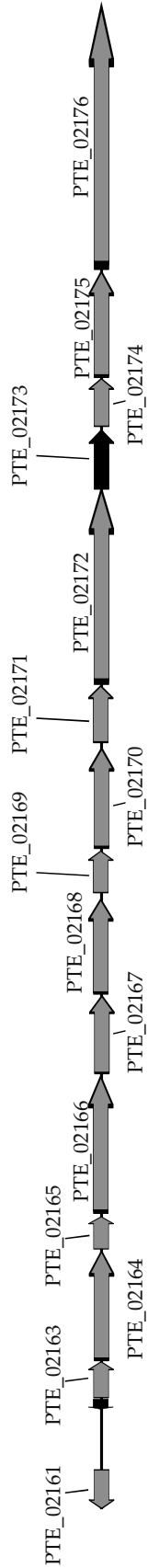
<sup>a</sup>Nomenclature based on (3)



<i>P. temperata</i> Locus Tag	Gene Name	<i>P. luminescens</i> Locus Tag	AA% identity	<i>P. asymbiotica</i> Locus Tag	AA% identity
PTE_02676	COG3515	plu4198	75.4	PAU_03814	76.6
PTE_02675	COG3518	plu3222	77.1	PAU_03815	87.5
PTE_02674	COG3521	plu4200	82.6	PAU_03816	88.5
PTE_02673	COG3520	plu4201	81.4	PAU_03817	83.5
PTE_02672	COG3519	plu4202	86.6	PAU_03818	90.8
PTE_02671	COG3515	plu4203	78.1	PAU_03819	79.6
PTE_02670	-	plu4214	77.8	PAU_03820	76.2
PTE_02669	-	-	-	-	-
PTE_02668	-	-	-	-	-
PTE_02667	-	-	-	-	-
PTE_02666	-	-	-	-	-
PTE_02665	-	-	-	-	-
PTE_02664	-	-	-	-	-
PTE_02663	-	-	-	-	-
PTE_02662	-	-	-	-	-
PTE_02661	vgrG	plu4222	74.5	PAU_03831	70.2
PTE_02660	ClpV1	plu4223	83.6	PAU_03832	82
PTE_02659	Hcp1	plu4211	92.6	PAU_03056	96.9
PTE_02658	-	plu4224	69.8	PAU_03834	76.4
PTE_02657	dotU	plu4225	68.1	PAU_03835	78.5
PTE_02656	COG3522	plu4226	86.7	PAU_03836	90.5
PTE_02655	iglB	plu3261	89.3	PAU_03837	91.7
PTE_02654	iglA	plu3262	84	PAU_03061	93.9

**Figure S7.** Organization of T6SS operon 3. The genes involved in T6SS are shown in gray and flanking genes in black. (Table) Amino acid sequence identity between T6SS proteins found in *P. temperata* with *P. luminescens* and *P. asymbiotica*, respectively.

<sup>a</sup>Nomenclature based on (3)

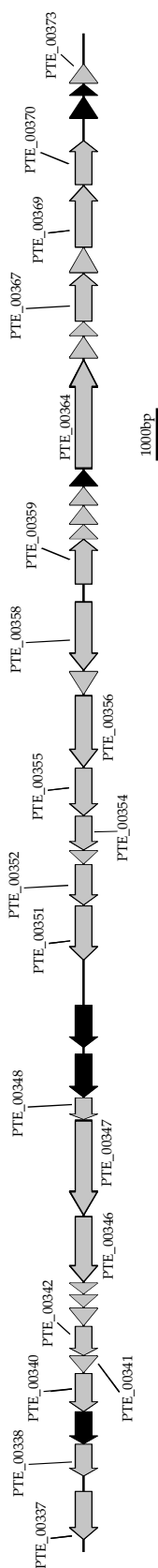


1000 bp

<i>P. temperata</i> Locus Tag	Gene Name	<i>P. luminescens</i> Locus Tag	AA% identity	<i>P. asymbiotica</i> Locus Tag	AA% identity
PTE_02745	ClpV1	plu2287	92.1	PAU_02268	90.4
PTE_02744	-	plu2288	83.4	PAU_02269	80.2
PTE_02743	dotU	plu2289	84.5	PAU_02270	86
PTE_02742	COG3522	plu2290	91.8	PAU_02271	89.9
PTE_02741	-	plu2291	82.2	PAU_02272	86.7
PTE_02740	COG3515	plu2292	81.7	PAU_02273	80.4
PTE_02739	-	plu2293	97	PAU_02274	98
PTE_02738	-	plu2294	85.1	PAU_02275	86.1
PTE_02737	vgrG	plu2295	90.9	PAU_02276	91.5
PTE_02736	COG3520	plu2296	80	PAU_02277	83
PTE_02735	COG3519	plu2297	91.7	PAU_02278	89.9
PTE_02734	COG3518	plu2298	91.8	PAU_02279	89.7
PTE_02733	-	plu2299	93.3	PAU_02280	98.8
PTE_02732	iglB	plu2300	96.8	PAU_02281	95.9
PTE_02731	iglA	plu2301	93.1	PAU_02282	93.7

**Figure S8.** Organization of T6SS operon 4. The genes involved in T6SS are shown in gray and flanking genes in black. (Table) Amino acid sequence identity between T6SS proteins found in *P. temperata* with *P. luminescens* and *P. asymbiotica*, respectively.

<sup>a</sup>Nomenclature based on (3)



<i>P. temperata</i>	Annotated Product	<i>P. luminescens</i> (% identity)	<i>P. asymbiotica</i> (% identity)
PTE_00337	<b>YopT-like peptidase</b>	plu3788 (83%)	PAU_02096 (76%)
PTE_00338	apparatus protein, HrpE/YscL family	plu3787 (93.2%)	PAU_01029 (94.1%)
PTE_00340	apparatus lipoprotein, YscJ/HrcJ family	plu3785 (95.9%)	PAU_01031 (94.3%)
PTE_00341	major needle protein, YscF/MxiH/PrgI family	plu3784 (87.5%)	PAU_01032 (88.4%)
PTE_00342	<b>effector, YopR family</b>	plu3783 (73.9%)	PAU_01033 (73.4%)
PTE_00343	protein, YscG family	plu3782 (94%)	PAU_01034 (94.8%)
PTE_00344	major needle protein, YscF/MxiH/PrgI family	plu3781 (82.1%)	PAU_01035 (79.3%)
PTE_00345	protein, YseE family	plu3780 (90.3%)	PAU_01035
PTE_00346	apparatus protein, YscD/HrpQ family	plu3779 (87.7%)	PAU_01036 (87.5%)
PTE_00347	outer membrane pore, YscC/HrcC family	plu3778 (89.6%)	PAU_01037 (87.9%)
PTE_00348	chaperone, YscB family	plu3777 (90.7%)	PAU_01038 (81.4%)
PTE_00351	protein, YscU/HrpY family	plu3774 (94.6%)	PAU_01045 (94%)
PTE_00352	protein SpaR/YscT/HrcT	plu3773 (93.4%)	PAU_01046 (91.9%)
PTE_00353	protein, HrpO family	plu3772 (97.7%)	PAU_01047 (96.6%)
PTE_00354	apparatus protein, YscR/HrcR family	plu3771 (96.8%)	PAU_01048 (96.3%)
PTE_00355	apparatus protein YscQ/HrcQ	plu3770 (83.9%)	PAU_01049 (84.1%)
PTE_00356	needle length determinant	plu3769 (%)	PAU_01050
PTE_00357	protein YscO.	plu3768 (89.5%)	PAU_01051 (85%)
PTE_00358	ATPase, FliI/YscN (EC 3.6.3.15)	plu3767 (98.2%)	PAU_01052 (97.5%)
PTE_00359	outer membrane protein PopN	plu3766 (94.8%)	PAU_01053 (84.9%)
PTE_00360	<b>effector delivery regulator, TyeA family</b>	plu3765 (93.7%)	PAU_01053 (85.1%)
PTE_00361	chaperone SycN	plu3764 (95.9%)	PAU_01054 (86.2%)
PTE_00362	protein, YscX family	plu3763 (90.1%)	PAU_01055 (88.4%)
PTE_00364	protein, HrcV family	plu3761 (95.9%)	PAU_01057 (95%)
PTE_00365	regulator LcrR	plu3760 (92.3%)	PAU_01058 (90.1%)
PTE_00366	protein LcrG	plu3759 (93.5%)	PAU_01059 (83.7%)
PTE_00367	translocon protein, LcrV/PcrV family	plu3758 (80.2%)	PAU_01060 (70.1%)
PTE_00368	low calcium response chaperone LcrH/SycD	plu3757 (95.8%)	PAU_01061 (90.5%)
PTE_00369	translocon protein, YopB/IpaB/SipB family	plu3756 (75.3%)	PAU_01062 (75.2%)
PTE_00370	translocon protein, YopD/IpaC/EspB family	plu3755 (74.5%)	PAU_01063 (67.2%)
PTE_00373	chaperone YscW	plu3751 (82%)	PAU_01067 (71.5%)

**Figure S9.** Conserved T3SS homologs with gene names and % amino acid identity. The genes involved in T3SS are shown in gray and flanking genes in black. Bold text represents T3SS effectors.

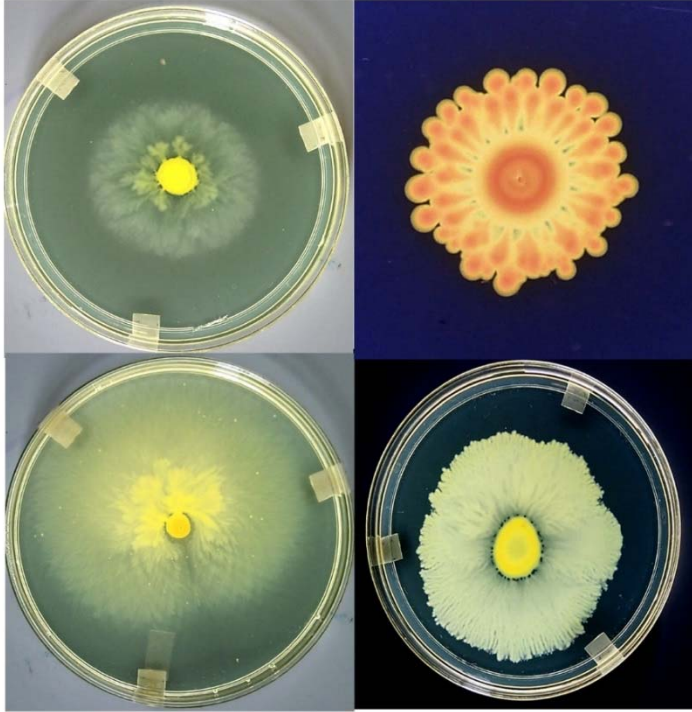


Figure S10. Hyperswarming mutants formed unique swarming patterns. Cells from an overnight broth culture (2 ul) were inoculated onto the surface of PP3 swarm plates (1.25% w/v agar) and incubated at 28°C for 24 h.



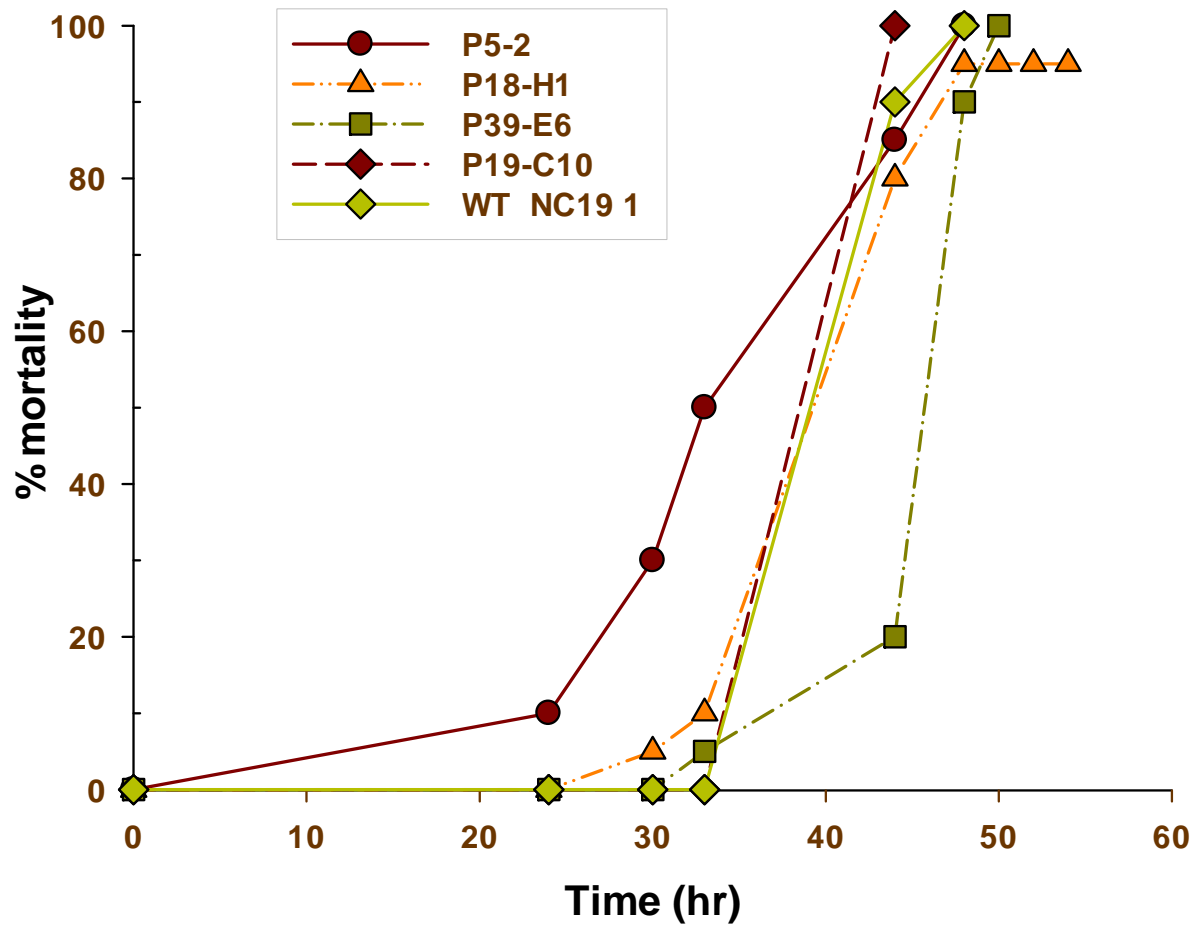


Figure S11. Insect pathogenesis assays revealed mutants with reduced and enhanced insect virulence. Lethal time was determined for each mutant by injecting 20 *G. mellonella* larvae. Insect health was monitored over time. The LT 50 value was defined as the time required for 50% of the larvae to die, while the LT100 value was defined as the time required for 100% mortality in this screen.



Figure S12. End-point RT-PCR confirmed the expression of *pte\_02637* gene in the genetically complemented P13-7 mutant. Panels: (A) 1 Kb marker ladder, (B) Control, wild type NC19 gDNA, (C) wild type NC19, (D) P13-7, (E) HNR1207 (P13-7 + pHR1)

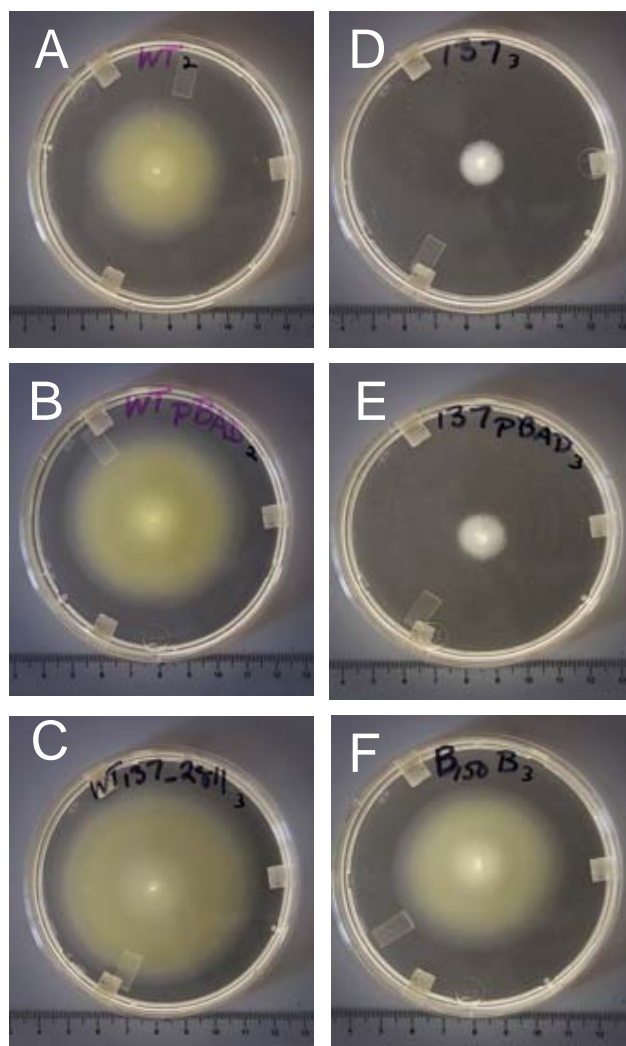


Figure S13. Genetic complementation restores swimming behavior in mutant P13-7. Swim migration plate assays were completed at 28°C for 48 h. Panels: (A) parental wild type NC 19 (B) HNR0018 (wild type NC19 + pBAD32), (C) HNR0606 (wild type NC19 + pHR1), (D) mutant P13-7, (E) HNR1318 (P13-7 + pBAD32), (F) HRN1307 (P13-7 + pHR1).

#### References

1. **Felsenstein J.** 1985. Confidence-Limits on Phylogenies - an Approach Using the Bootstrap. *Evolution* 39(4):783-791 doi:Doi 10.2307/2408678
2. **Joyce SA, Brachmann AO, Glazer I, Lango L, Schwar G, Clarke DJ, Bode HB.** 2008. Bacterial biosynthesis of a multipotent stilbene. *Angew Chem Int Edit* 47:1942-1945.
3. **Boyer F, Fichant G, Berthod J, Vandenbrouck Y, Attree I.** 2009. Dissecting the bacterial type VI secretion system by a genome wide in silico analysis: what can be learned from available microbial genomic resources? *BMC Genomics* 10:104.