SUPPLEMENTARY INFORMATION

The naked bacterium: emerging properties of a surfome-streamlined *Pseudomonas putida* strain

by

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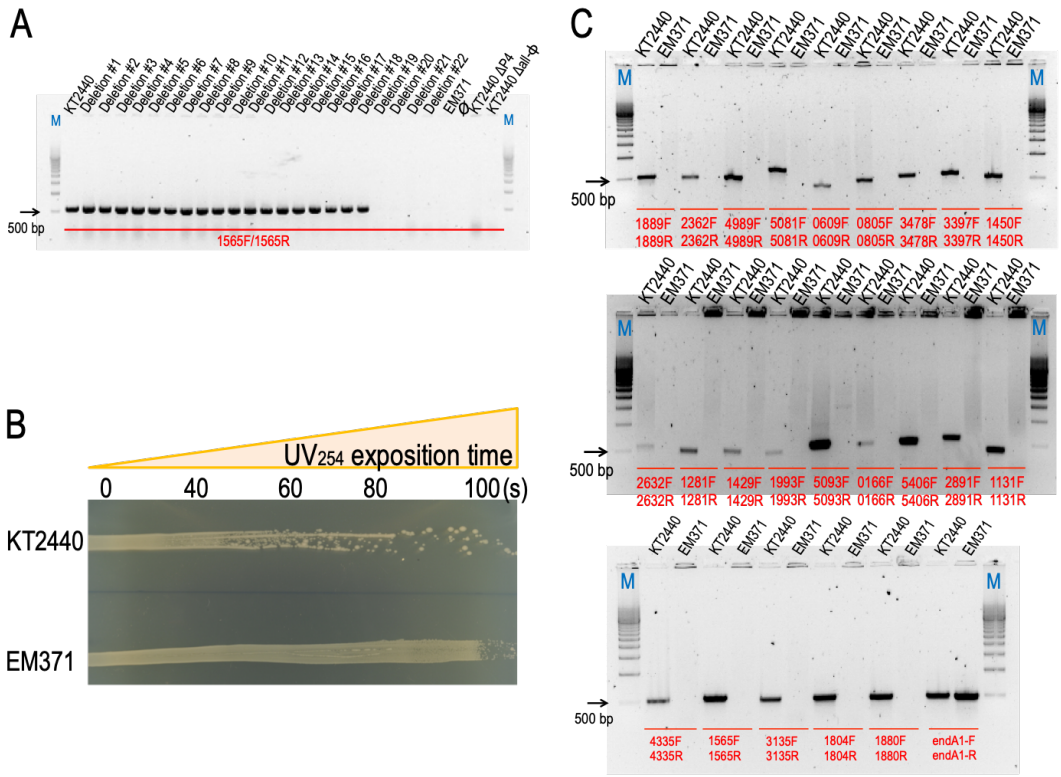
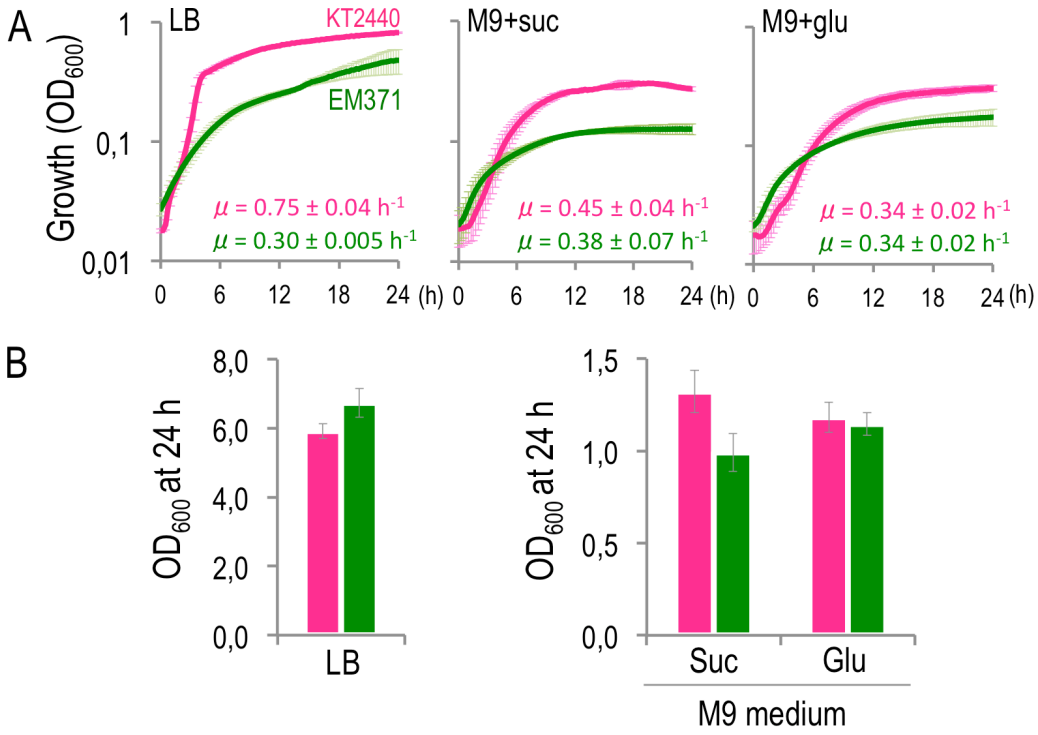
SUPPLEMENTARY FIGURES

Supplementary Figure S1. Genomic regions deleted in *P. putida* KT2440 to construct the EM371 surface-naked strain.



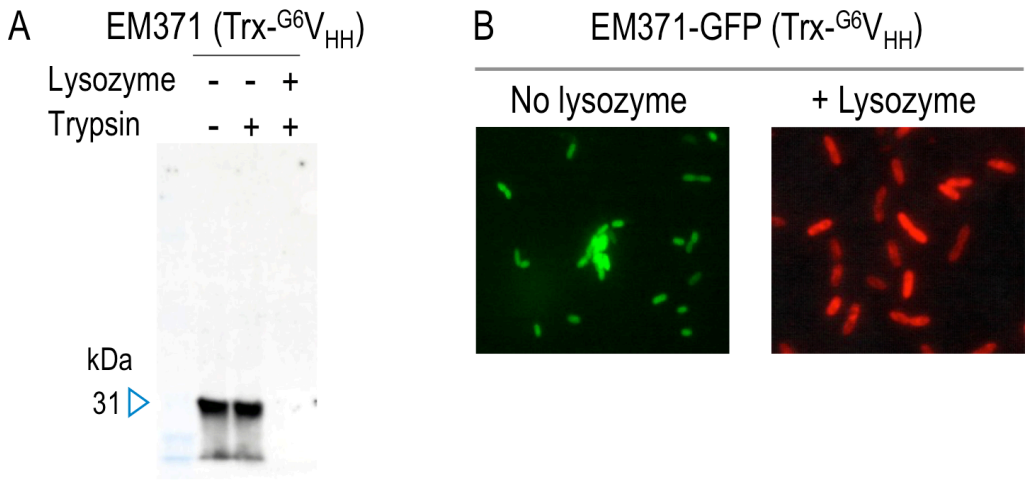
(A) Agarose gel electrophoresis of the diagnostic PCR to identify the deletion step at which the spontaneous excision of prophage 4 occurred [1]. PCR products from different intermediate deletion steps and KT2440 ΔP4 and Δall-**ϕ** strains as negative controls indicated on top. To confirm the absence of P4 we used the oligos 1565F and 1565R (Table S2) that produced a ~500 bp in strains harboring the prophage. The symbol Ø refers to a blank sample without template. (B) Qualitative UV resistance test. For this experiment, cells were spread onto an LB agar plate and irradiated with UV light for the time indicated. (C) electrophoresis of the diagnostic PCRs to verify the different genomic regions eliminated. Analytical PCR amplifications using either *P. putida* KT2440 or EM371 as DNA template. Oligos, shown in red in the gel, were designed to hybridize within an internal deleted gene (Table S2). Therefore, amplifying only in the wild type a ~500 bp DNA fragment. The oligos endA1-F/endA1-R were used as a *P. putida* control since amplify a fragment of a non-deleted gene in EM371. M corresponds to the 500-bp Molecular Ruler *EZ* load™ (Bio-Rad, Berkeley, CA, USA).

Supplementary Figure S2. Growth profiles of *P. putida* KT2440 and the EM371 naked surface-strain under different metabolic regimes.



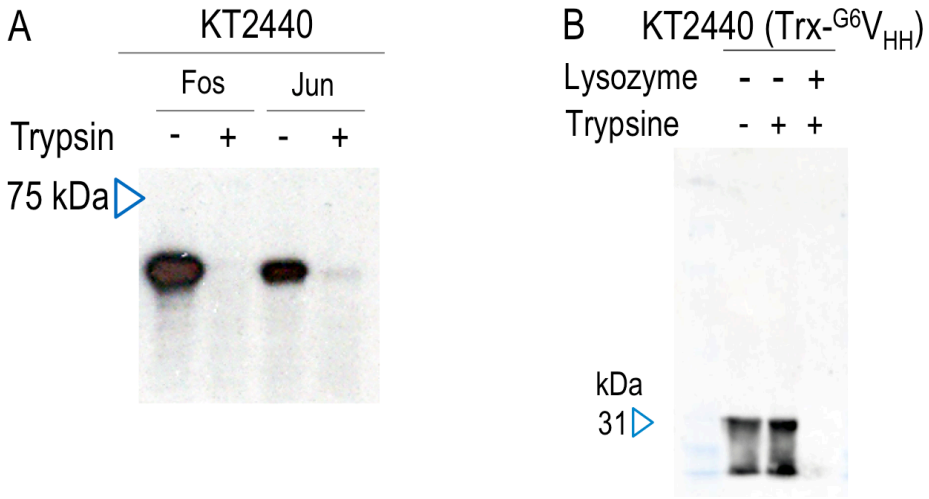
(A) growth curves on rich media (LB), M9 minimal medium with either 0.2 % (w/v) succinate (gluconeogenic condition) or glucose (glycolytic) as sole carbon source. The experiment was performed in Spectramax M2 using a 96-well plates in which the OD_{600} was monitored every 15 minutes through 24h. The parental strain is depicted in pink while the EM371 in green. The specific growth rates (μ) of both strains are located within each plot. (B) end-point cell density (OD_{600}) values of the parental (KT2440 in pink) and EM371 (green) strains taken after 24 h of growth in shaken flasks at 30 ºC in LB and M9 with either succinate of glucose as sole carbon source. The average and standard deviation of three independent experiments are shown.

Supplementary Figure S3. Permeabilization and trypsin treatment of *P. putida* EM371 with the control Trx-G^6V_{HH} plasmid.



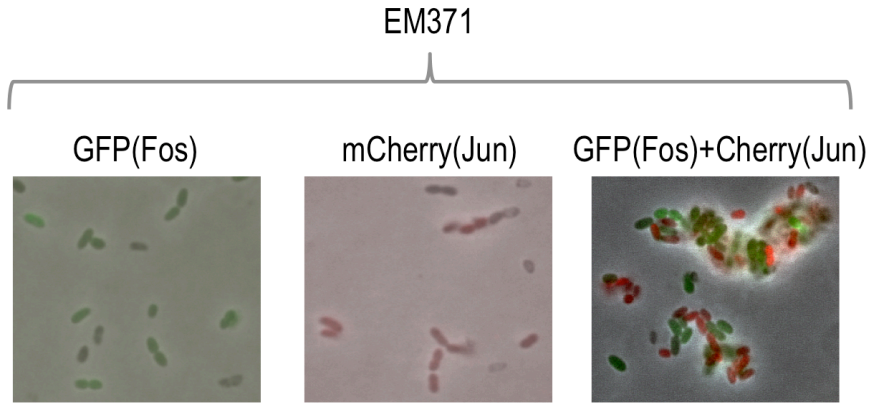
(A) induced cells were permeabilized (+) or not (-) with lysozyme. Following that, samples were incubated (+) or not (-) with trypsin. Then, digestion of the cytoplasmic protein was monitored by western blot using the anti-E-tag antibody and the anti-mouse IgG conjugated with peroxidase. This experiment allows to confirm that the recombinant control protein (Trx-G^6V_{HH}) is effectively degraded by trypsin only when cells are permeabilized with lysozyme. (B) Immunofluorescence experiment to visualize the effect of membrane permeabilization. Induced EM371-GFP tagged cells expressing the cytoplasmic control protein (Trx-G^6V_{HH}) were treated with a primary anti-E-tag antibody and then with the anti-mouse IgG Alexa fluor 594. Only in permeabilized cells (+ lysozyme) the cytoplasmic control protein is detected (red) while in non-permeabilized bacteria the antibodies do not enter and only the GFP fluorescence is observed.

Supplementary Figure S4. Expression and localization of the Fos and Jun chimeric proteins in *P. putida* KT2440.



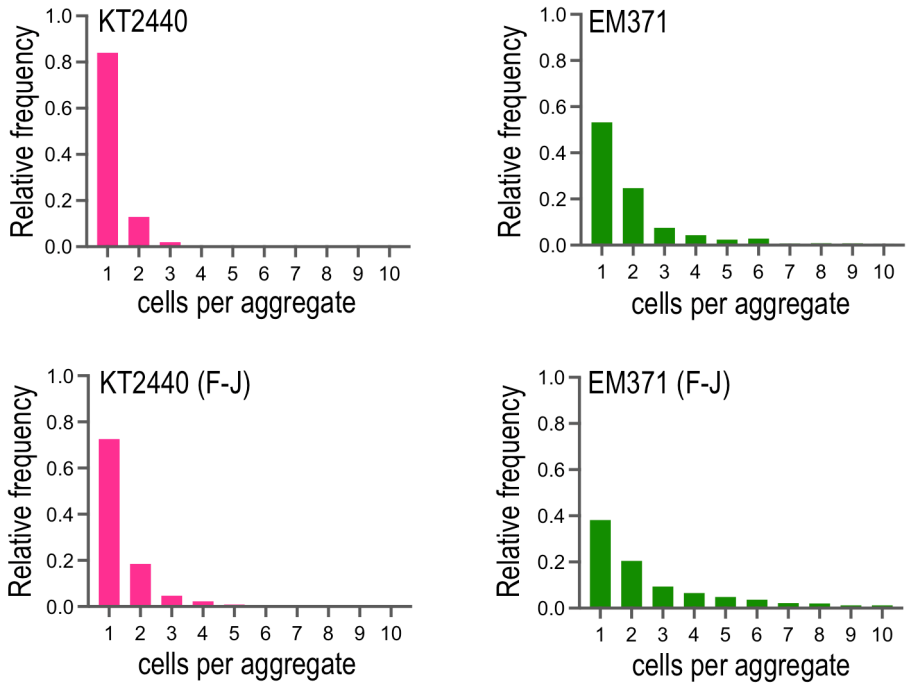
Western blot of induced whole cell extracts containing either pSEVA238-AT-Fos or the pSEVA238-AT-Jun plasmids. Also, induced cells were treated (+) or not (-) with 10 µg ml^{-1} trypsin. The western blot was revealed with anti-E-tag as the primary antibody and reveled with anti-mouse IgG conjugated with peroxidase. (B) permeabilization and trypsin treatment of KT2440 harboring the Trx-G^6V_{HH} plasmid. Western blot of induced cells permeabilized (+) or not (-) with lysozyme. Then, samples were incubated (+) or not (-) with trypsin.

Supplementary Figure S5. Fluorescent microscopy images of the induced aggregation experiments using EM371 as the bacterial chassis.



The first image corresponds to EM371-GFP induced cells harboring the hybrid autotransporter with Fos as passenger. The second is induced EM371-mCherry with Jun as passenger. The third one corresponds to a 1:1 aggregation experiment mixing induced cells with the Fos (GFP) and Jun (mCherry) domains and let them stand at room temperature for 20 minutes. Pictures are obtained after merging phase contrast with the fluorescent images of GFP and Red channels.

Supplementary Figure S6. Normalized frequency distribution of cells per aggregate.



Microscopy images analyzed as described before and the number of cells per aggregate plotted as a normalized histogram. The x-axis was truncated in 10 cells per aggregate for visualization purposes, although the distribution in the case of EM371 (F-J) extends beyond this value (up to 172) in very low frequencies, as can be observed in Fig. 9B.

REFERENCES

(1) Martinez-Garcia, E., Jatsenko, T., Kivisaar, M., de Lorenzo, V. (2015) Freeing *Pseudomonas putida* KT2440 of its proviral load strengthens endurance to environmental stresses. *Environ. Microbiol. 17*, 76-90.

SUPPLEMENTARY TABLES S1, S2, S4

Supplementary Table S1. Genomic coordinates of the 23 deletions introduced in *Pseudomonas putida* KT2440 to construct the naked strain EM371.

Deletion[a]	Gene or gene cluster	Number of genes targeted	Coordinates deleted (bp)		Extension of the deletion (bp)
			Start	End	
1	*PP_1887 - PP_1891*	5	2,124,630	2,133,604	8,975
2	*PP_2357 - PP_2363*	7	2,690,661	2,697,071	6,411
3	*PP_4986 - PP_4992*	7	5,680,665	5,690,342	9,678
4	*PP_5080 - PP_5083*	4	5,801,595	5,805,208	3,614
5	*PP_0607 - PP_0611*	5	715,011	717,278	2,268
6	*PP_0803 - PP_0806*	4	921,843	945,621	23,779
7	*PP_3472 - PP_3484*	13	3,938,978	3,948,639	9,662
8	*PP_3396 - PP_3399*	4	3,846,911	3,852,454	5,544
9	*PP_1449 - PP_1450*	2	1,652,242	1,658,493	6,252
10	*PP_2629 - PP_2638*	9	3,009,136	3,022,981	13,846
11	*PP_1277 - PP_1288*	12	1,458,140	1,474,220	16,081
12	*PP_1427 - PP_1430*	4	1,627,273	1,631,066	3,794
13	*PP_1993*	1	2,259,413	2,262,148	2,736
14	*PP_5093*	1	5,816,943	5,817,953	1,011
15	*PP_0164 - PP_0168*	7	187,240	220,543	33,304
16	*PP_5404 - PP_5407*	4	6,161,924	6,168,519	6,596
17	*PP_2891 - PP_2893*	3	3,288,248	3,294,391	6,144
18	*PP_1131*	1	1,294,520	1,294,984	465
19	*PP_4329 - PP_4397*	68	4,919,155	4,988,330	69,176
20[b]	*PP_1532 - PP_1584*	57	1,737,987	1,777,401	39,415
21	*PP_3132 - PP_3142*	12	3,547,056	3,559,123	12,068
22	*PP_1804*	1	2,027,211	2,028,215	1005
23	*PP_1879 - PP_1882*	4	2,103,369	2,114,285	10,917

[a] The deletion number identifies the order in which the specific gene or gene cluster was deleted. The information regarding genomic coordinates of each locus was derived from the reported sequence of *P. putida* KT2440 (GenBank #: AE015451[1] and its updated sequence AE015451.2[2]).

[b] Spontaneous excision of prophage 4 (*PP_1532-PP_1584*), that occurred sometime after deletion #18 [3], during the construction process.

Supplementary Table S2. Oligonucleotides used in this study.

Name	Sequence (5' → 3')[a]	Usage / Ref
TS1(1887)EcoRI-F	CG**GAATTC**CGGTCTTTTGGCAATTGTCAAT	Deletion of *PP_1887* - *PP_1891*
TS1(1887)-R	TTGGCTCCATCAAAGAATCGAG	Deletion of *PP_1887* - *PP_1891*
TS2(1891)-F	*CTCGATTCTTTGATGGAGCCAA*GACATAACCTG ATTGGGTAAATGGG	Deletion of *PP_1887* - *PP_1891*
TS2(1891)XmaI-R	TCCC**CCCGGG**GGCCAGCGGCTGCACGAGGCC GAAC	Deletion of *PP_1887* - *PP_1891*
TS1(2357)-SacI	ATCC**GAGCTC**CTGCGTAGCTGGGGTAGCCGG	Deletion of *PP_2357* - *PP_2363*
TS1(RR1)-R	*TTCGGGGGTATTGACCGCCGT*AAAGCCATTTTT TTTGTATTAGCG	Deletion of *PP_2357* - *PP_2363* [9]
TS2(RR1)-F	ACGGCGGTCAATACCCCCGAA	Deletion of *PP_2357* - *PP_2363* [9]
TS2(RR1)BamHI-R	CG**GGATCC**CAGCGGCCGCAGTGTCCAG	Deletion of *PP_2357* - *PP_2363* [9]
TS1(4985)-EcoRI-F	CG**GAATTC**ATTCACCGCTGCATGTGCA	Deletion of *PP_4986* - *PP_4992*
TS1(4985)-R	*CAATCCTACTGGTAGGCGTCT*TTAAAAGTCGCC CCACAACTG	Deletion of *PP_4986* - *PP_4992*
TS2(4993)-F	AGACGCCTACCAGTAGGATTG	Deletion of *PP_4986* - *PP_4992*
TS2(4993)BamHI-R	CG**GGATCC**TGCGCATCAGGATCACGTCCAG	Deletion of *PP_4986* - *PP_4992*
TS1(5080)XmaI-F	TCCC**CCCGGG**AGCGCTCGAGAATATCGATCAC C	Deletion of *PP_5080* - *PP_5083*
TS1(5080)-R	*GTAACAGACAGCAAAGGAGTCGCG*TTCTGTGC GAAATTTGATACTTG	Deletion of *PP_5080* - *PP_5083*
TS2(5083)-F	CGCGACTCCTTTGCTGTCTGTTAC	Deletion of *PP_5080* -

		PP_5083
TS2(5083)BamHI-R	CG**GGATCC**GGGGTCGACGCCGTAGTGGTTGAG	Deletion of *PP_5080* - *PP_5083*
TS1(0607)EcoRI-F	CG**GAATTC**CAGCCGCGTCATCGATGCGCTG	Deletion of *PP_0607* - *PP_0611*
TS1(0607)-R	*TGCACCGGGCATCATTGAACCT*CGCGGTCATTG CAGGAGCGGTC	Deletion of *PP_0607* - *PP_0611*
TS2(0611)-F	AGGTTCAATGATGCCCGGTGCA	Deletion of *PP_0607* - *PP_0611*
TS2(0611)BamHI-R	CG**GGATCC**GGGTTGCGCACATTGGCCACACC	Deletion of *PP_0607* - *PP_0611*
TS1(0803)EcoRI-F	CG**GAATTC**CCAGCACCTGCACCAGGGTGTTG	Deletion of *PP_0803* - *PP_0806*
TS1(0803)-R	*CGGATATCAGCAGGGAGCATCC*TCCAGGCATT CCTGGGTTCTGTG	Deletion of *PP_0803* - *PP_0806*
TS2(0806)-F	GGATGCTCCCTGCTGATATCCG	Deletion of *PP_0803* - *PP_0806*
TS2(0806)XbaI-R	GC**TCTAGA**GATCGGCCGCTCGGCACTCAAGGC	Deletion of *PP_0803* - *PP_0806*
TS1(3472)EcoRI-F	CG**GAATTC**TTCATTTCGGCTAGAAGCAAAGATT	Deletion of *PP_3472* - *PP_3484*
TS1(3472)-R	*GGCAGTATTTGGGAGTCGACCTTG*TTGACTGTT AGGGAAGCTCTGATC	Deletion of *PP_3472* - *PP_3484*
TS2(3484)-F	CAAGGTCGACTCCCAAATACTGCC	Deletion of *PP_3472* - *PP_3484*
TS2(3484)-BamHI-R	CG**GGATCC**CTTCGACAGGGCGCAGTACACTGA A	Deletion of *PP_3472* - *PP_3484*
TS1(3396)EcoRI-F	CG**GAATTC**GTATTCGCTCAGCTCGCTGTCCAG	Deletion of *PP_3396* - *PP_3399*
TS1(3396)-R	*CACCGCGGTTCAAGGGGGAGCGGT*GAGTCAAT	Deletion of *PP_3396* -

	TCCTCCTGCAAACGGGC	*PP_3399*
TS2(3399)-F	ACCGCTCCCCCTTGAACCGCGGTG	Deletion of *PP_3396* - *PP_3399*
TS2(3399)-BamHI-R	CG**GGATCC**GTTGACCAGGCAGGCATCCGGCACG	Deletion of *PP_3396* - *PP_3399*
TS1(1449)EcoRI-F	CG**GAATTC**GGCTACTTCACCGTGCTGACCAATAC	Deletion of *PP_1449* - *PP_1450*
TS1(1449)-R	*CGAGTACGAAAACCGTACATCAT*GACGCCATCGCGGCCCTGGCCATTTG	Deletion of *PP_1449* - *PP_1450*
TS2(1450)-F	ATGATGTACGGTTTTCGTACTCG	Deletion of *PP_1449* - *PP_1450*
TS2(1450)BamHI-R	CG**GGATCC**GCTGAAAACCTACCGCATCTCGCT	Deletion of *PP_1449* - *PP_1450*
TS1(2629)EcoRI-F	CG**GAATTC**TCACCTTGGTAGTGACCCGGCCAGA	Deletion of *PP_2629* - *PP_2638*
TS1(2629)-R	*CCCTTGCAACGCAGCGATTGCCG*TTATACAGCATCAAGGCAGAATGAAATC	Deletion of *PP_2629* - *PP_2638*
TS2(2638)-F	CGGCAATCGCTGCGTTGCAAGGG	Deletion of *PP_2629* - *PP_2638*
TS2(2638)BamHI-R	CG**GGATCC**AAGGCCAGCAACTCACGCAGGTTG	Deletion of *PP_2629* - *PP_2638*
TS1(1277)EcoRI-F	CG**GAATTC**GTCACCGGTGAGTCACTGTGCCAGA	Deletion of *PP_1277* - *PP_1288*
TS1(1277)-R	*CGTGATAAACACATGAGGTGATAGCG*ATGCTGACTCGCCCCTGGGCTGAC	Deletion of *PP_1277* - *PP_1288*
TS2(1288)-F	CGCTATCACCTCATGTGTTTATCACG	Deletion of *PP_1277* - *PP_1288*
TS2(1288)XmaI-R	TCCC**CCCGGG**ATATCTCGCAACAGTTACGTCCTTTA	Deletion of *PP_1277* - *PP_1288*
TS1(1427)EcoRI-F	CG**GAATTC**TGTTGTCCAGCACTGCAGCGACGC	Deletion of *PP_1427* -

		PP_1430
TS1(1427)-R	*CCCTTTCGCCTTGCGAAACTGC*GAACACTCCTC AGTGAACTCGAAGG	Deletion of *PP_1427 - PP_1430*
TS2(1430)-F	GCAGTTTCGCAAGGCGAAAGGG	Deletion of *PP_1427 - PP_1430*
TS2(1430)BamHI-R	CG**GGATCC**TGGGGCAGGTCCATCTTGTTCAGG	Deletion of *PP_1427 - PP_1430*
TS1(1993)EcoRI-F	CG**GAATTC**GCGCAAGGCCGTGAAGCGGTCAGC	Deletion of *PP_1993*
TS1(1993)-R	*CCAAGAGGCCTGACCTGCTTGCA*GACCTCTTCC CTTGTATGAATCGTC	Deletion of *PP_1993*
TS2(1993)-F	TGCAAGCAGGTCAGGCCTCTTGG	Deletion of *PP_1993*
TS2(1993)BamHI-R	CG**GGATCC**CGAATCGGGTCGTTGTAGATGAC	Deletion of *PP_1993*
TS1(5093)EcoRI-F	CG**GAATTC**AGGCAATGCCGCCAGCAGCCGCGG T	Deletion of *PP_5093*
TS1(5093)-R	*GGCATTCTACCTGCTTGAAGGCCT*TACCTGCAA AGGCCCTTTCGCG	Deletion of *PP_5093*
TS2(5093)-F	AGGCCTTCAAGCAGGTAGAATGCC	Deletion of *PP_5093*
TS2(5093)BamHI-R	CG**GGATCC**CAACTTGCGCAGGCTGGCGAAGGC	Deletion of *PP_5093*
TS1(0164)EcoRI-F	CG**GAATTC**GGATATACCCGCGGGCGCTGGCGA T	Deletion of *PP_0164 - PP_0168*
TS1(0164)R	*CGCTGTGCGGCCCCGGTGGTCTG*GGCAAAGCC GACATAGCGCACTACGG	Deletion of *PP_0164 - PP_0168*
TS2(0168)-F	CAGACCACCGGGGCCGCACAGCG	Deletion of *PP_0164 - PP_0168*
TS2(0168)XmaI-R	TCCC**CCCGGG**CAAGGCGGCTGACATTTTTCACT C	Deletion of *PP_0164 - PP_0168*
TS1(2891)EcoRI-F	CG**GAATTC**ACTGGCTTTCCAGCAGTGCCTG	Deletion of *PP_2891 - PP_2893*

TS1(2891)R	*TTGATGGCCGCCACGCTGCGGGC*TCAGTGCCT TGGAGGTGCCCCAG	Deletion of *PP_2891 - PP_2893*
TS2(2893)F	GCCCGCAGCGTGGCGGCCATCAA	Deletion of *PP_2891 - PP_2893*
TS2(2893)BamHI-R	CG**GGATCC**ATTGACCTGCGTGAAATTACC	Deletion of *PP_2891 - PP_2893*
TS1(1131)EcoRI-F	CG**GAATTC**ACTGCCCTGAGCATTGGGG	Deletion of *PP_1131*
TS1(1131)R	*GGGTTTTTTATTACTGCACTACGG*GGGTAAAGT CTCCATAAGTCAGG	Deletion of *PP_1131*
TS2(1131)F	CCGTAGTGCAGTAATAAAAAACCC	Deletion of *PP_1131*
TS2(1131)BamHI-R	CG**GGATCC**TGCTGGTGCTGGTGGCGATGAA	Deletion of *PP_1131*
TS1(3132)EcoRI-F	CG**GAATTC**GGTACAGCGCCTGGCGCAGCGT	Deletion of *PP_3132 - PP_3142*
TS1(3132)R	*GCTTGAAGCAGGAGCGCTTCGC*GACTTCAATCT CTGACTGATTGG	Deletion of *PP_3132 - PP_3142*
TS2(3142)F	GCGAAGCGCTCCTGCTTCAAGC	Deletion of *PP_3132 - PP_3142*
TS2(3142)BamHI-R	CG**GGATCC**CGAGTTGCGCCTGGTGGCTGAG	Deletion of *PP_3132 - PP_3142*
TS1(1804)EcoRI-F	CG**GAATTC**CAAGCACGAAGCGGAGCAGGGG	Deletion of *PP_1804*
TS1(1804)R	*GATGCCTTGCGTGCTTGGCCATC*GGGCGGCGT GCACCCAGAAAAG	Deletion of *PP_1804*
TS2(1804)F	GATGGCCAAGCACGCAAGGCATC	Deletion of *PP_1804*
TS2(1804)BamHI-R	CG**GGATCC**GAAATACTGGCGCATGATCAGGCG	Deletion of *PP_1804*
TS1(1879)EcoRI-F	CG**GAATTC**AACCCAGGCTGAATCGTGAGGTT	Deletion of *PP_1879 - PP_1882*
TS1(1879)R	*ACAAGCAGAGTGAGAACGTTCATT*CCCGATGCA	Deletion of *PP_1879 -*

	AAAAAAAAACGCCACC	*PP_1882*
TS2(1882)F	AATGAACGTTCTCACTCTGCTTGT	Deletion of *PP_1879 - PP_1882*
TS2(1882)XmaI-R	TCCC**CCCGGG**AACGAAGAAGAAACGACGCTTG GAC	Deletion of *PP_1879 - PP_1882*
1889F	GGTAGTTGTCGGCTTCGGTA	Diagnose deletion of *PP_1887 – PP_1891*
1889R	TATCGCTATTCGACCCAAGG	Diagnose deletion of *PP_1887 – PP_1891*
PP2362F	GGACATGCAACTGAGCAAAA	Diagnose deletion of *PP_2357 – PP_2363*
PP2362R	TCCACACCAGAGAACCACTG	Diagnose deletion of *PP_2357 – PP_2363*
4989F	GGCAACATCTTCAGCCTTTC	Diagnose deletion of *PP_4986 – PP_4992*
4989R	GATCGACTCGACCATGTCAC	Diagnose deletion of *PP_4986 – PP_4992*
5081F	TTAGTGGTGATTGCCGAACA	Diagnose deletion of *PP_5080 – PP_5083*
5081R	GCTTGGTGAGGGTGATGAAC	Diagnose deletion of *PP_5080 – PP_5083*
0609F	CCAACTTGGTTTTGGTTTGG	Diagnose deletion of *PP_0607 – PP_ 0611*
0609R	ATCGTCCTGGAGCTGGTAGA	Diagnose deletion of *PP_0607 – PP_ 0611*
0805F	GACACAGCCTATGCCTGGAT	Diagnose deletion of *PP_0803 - PP_0806*
0805R	CCCCTTGGTAGATGGGAACT	Diagnose deletion of *PP_0803 - PP_0806*
3478F	ATGAAAGAGGGCCAGTACGA	Diagnose deletion of

		PP_3472 – PP_3484
3478R	AGATGGTGGCTTTCATGTCC	Diagnose deletion of *PP_3472 – PP_3484*
3397F	TCCGATGTGGTACAAGGACA	Diagnose deletion of *PP_3396 - PP_3399*
3397R	GTGACCGCTTCGGTGATATT	Diagnose deletion of *PP_3396 - PP_3399*
1450F	GTCGAAGGCTTTGTCGAAAC	Diagnose deletion of *PP_1449 – PP_1450*
1450R	ACAATACCCGCTCGACACTC	Diagnose deletion of *PP_1449 – PP_1450*
2632F	GTTGCTGGTGGGTTACCTGT	Diagnose deletion of *PP_2629 – PP_2638*
2632R	GGTAATGTCGCCGATGAAGT	Diagnose deletion of *PP_2629 – PP_2638*
1281F	AAGTACGAAGGCTCGGACAA	Diagnose deletion of *PP_1277 – PP_1288*
1281R	GCCGACCTTGTATTCCTTCA	Diagnose deletion of *PP_1277 – PP_1288*
1429F	GCTGAAACTGATGGGTTGGT	Diagnose deletion of *PP_1427 – PP_1430*
1429R	CACTTTGCCCTTGGGTGTAT	Diagnose deletion of *PP_1427 – PP_1430*
1993F	TGAAGTCGGCACAGAATCAG	Diagnose deletion of *PP_1993*
1993R	CCAACCTTCAGCTGGTTGAT	Diagnose deletion of *PP_1993*
0166F	GATATGGGGCAGTTCAAGGA	Diagnose deletion of *PP_0164 – PP_0168*
0166R	AATTTCGTCCTGCAGTTGCT	Diagnose deletion of

		PP_0164 – PP_0168
5406F	CATCTCCTTTCCAACCCAGA	Diagnose deletion of Tn*7* [12]
5406R	CGTGCATACCAAACAACAGG	Diagnose deletion of Tn*7* [12]
2891F	GCAGGCACTCGGCTACTATC	Diagnose deletion of *PP_2891 – PP_2893*
2891R	GTGGTTTACGGGTTTCCAGA	Diagnose deletion of *PP_2891 – PP_2893*
1131F	GTAAATCCGCTTTGCTGGTG	Diagnose deletion of *PP_1131*
1131R	CGGAAGATTTCGTTCTCCTG	Diagnose deletion of *PP_1131*
4335F	TACCGAGGAACACGAAAACC	Diagnose deletion of the flagellum [13]
4335R	TTGGCAGGTTGTCAGTGAAG	Diagnose deletion of the flagellum [13]
1565F	CTGACCGAGGATCAGATGGT	Diagnose deletion of the prophage 4 [3]
1565R	CCGGGTTGAACTTCACGTAG	Diagnose deletion of the prophage 4 [3]
3135F	CTCAATACCGATGCCTTCGT	Diagnose deletion of *PP_3132 – PP_3142*
3135R	TGATGCTTGCGGAAGTACAG	Diagnose deletion of *PP_3132 – PP_3142*
1880F	GAGCCCACAATCACCAGTTT	Diagnose deletion of *PP_1879 – PP_1882*
1880R	CACCCAGTTCAGTGTCATGG	Diagnose deletion of *PP_1879 – PP_1882*
1804F	CGGCAGTGCTGACCAGTGTGTTG	Diagnose deletion of *PP_1804*

1804R	CACCCGAAAGTATTAACCACC	Diagnose deletion of *PP_1804*
5093F	GATCAACCCTCGCTCCCTCAGC	Diagnose deletion of *PP_5093*
5093R	CCTGGCAACGCTGCTCGACCAG	Diagnose deletion of *PP_5093*
endA1-F	CGCTTTTCGCAGCAGCCTGCCTG	Diagnose presence of *endA-1* [12]
endA1-R	GAAGTAGGTGCGGGCGATCATGCC	Diagnose presence of *endA-1* [12]
2357-2363-junction-F	GATACGCTACGCAGCGCAGCAA	Sequence boundaries of *PP_2353 - PP_2363* deletion
2357-2363-junction-R	CAGCAGCGCTGGTTCCGTGTG	Sequence boundaries of *PP_2353 - PP_2363* deletion
Tn7-junction-F	CCGACCTGGGAAGGTCGACTTT	Sequence boundaries of Tn*7* deletion [12]
Tn7-junction-R	GATGACTTCCTAGGCCATTACTTA	Sequence boundaries of Tn*7* deletion [12]
Junction-F	CGCCAAGCCTCGCTACCCGGCCTGCT	Sequence boundaries of flagella deletion [13]
Junction-R	CAGTTGATTCTGGTGGTGCACCCG	Sequence boundaries of flagella deletion [13]
curli1-junction-F	CTGCGGTCATCCCAATTAATG	Sequence boundaries of curli operon 1 deletion
curli1-junction-R	GGCAGGAAGCGCAACGCCAAG	Sequence boundaries of curli operon 1 deletion
curli2-junction-F	GCCACACCACGAACGACATCGG	Sequence boundaries of curli operon 2 deletion
curli2-junction-R	CAGACCAGCACATCGCCGTGGC	Sequence boundaries of

		curli operon 2 deletion
4986-4992 junction-F	GCACGACCTGCCCGAGGCCCAG	Sequence boundaries of *PP_4986 – PP_4992* deletion
4986-4992 junction-R	GGGTCGGGGTGGTGCATTGCG	Sequence boundaries of *PP_4986 – PP_4992* deletion
1887-1891 junction-F	ATACCTCGATGGTGCGCTGGGA	Sequence boundaries of *PP_1887 – PP_1891* deletion
1887-1891 junction-R	GCAACGGGCCAGTGACCTGCTC	Sequence boundaries of *PP_1887 – PP_1891* deletion
2629-2638 junction-F	CAGCACGACCCAGGCAATGAAG	Sequence boundaries of *PP_2629 – PP_2638* deletion
2629-2638 junction-R	CGTTCGATGCGTACGCCTGTGC	Sequence boundaries of *PP_2629 – PP_2638* deletion
5093-junction-F	CTATTCATAAGAGCTTCATCTATG	Sequence boundaries of *PP_5093* deletion
5093-junction-R	GCTCATGTCAGGTCCTTGTGGAAA	Sequence boundaries of *PP_5093* deletion
1993-junction-F	TGCCGGAAGCCAACGCCGAACG	Sequence boundaries of *PP_1993* deletion
1993-junction-R	TGGCCATGGCGGGTAACACGCAGG	Sequence boundaries of *PP_1993* deletion
1427-1430 junction-F	GTGAGGTGGAACTCGAAGCCGC	Sequence boundaries of *PP_1427 – PP_1430* deletion
1427-1430 junction-R	CAGGTAATTGTCGAACCAAGAGT	Sequence boundaries of *PP_1427 – PP_1430* deletion

1277-1288 junction-F	GCCCAGGCCACACACAGCCGCCAG	Sequence boundaries of *PP_1277 – PP_1288* deletion
1277-1288 junction-R	CGCGAAGAGGTCAGCCGCCGTGAT	Sequence boundaries of *PP_1277 – PP_1288* deletion
5080-5083 junction-F	GATCGGGTAGCTACGCTCGCCCA	Sequence boundaries of *PP_5080 – PP_5083* deletion
5080-5083 junction-R	CAGCTCACGCTCGATTTGCAGGGC	Sequence boundaries of *PP_5080 – PP_5083* deletion
1804-junction-F	TGGCCGAGTTCCGTCGGGTGAAT	Sequence boundaries of *PP_1804* deletion
1804-junction-R	GTCATCATCAATGAACGCCACCG	Sequence boundaries of *PP_1804* deletion
0607-0611 junction-F	GAAGTCGAAGGCACCATGGGCCA	Sequence boundaries of *PP_0607 – PP_0611* deletion
0607-0611 junction-R	TCCAGGCGCCTGCGCTGAGCACA	Sequence boundaries of *PP_0607 – PP_0611* deletion
0803-0806 junction-F	GTGTGCACCGACTGCTGCAAGGCC	Sequence boundaries of *PP_0803 – PP_0806* deletion
0803-0806 junction-R	TGCACGTGCCACCTTTGCGCGA	Sequence boundaries of *PP_0803 – PP_0806* deletion
1449-1450 junction-F	AGCAGCTGTACGTGAAGCTGCAG	Sequence boundaries of *PP_1449 - PP_1450* deletion
1449-1450 junction-R	GGCGGCCTTCACCGAAACCATC	Sequence boundaries of *PP_1449 - PP_1450* deletion

0164-0168 junction-F	CAGGCCCATGGACGAAAGATGG	Sequence boundaries of *PP_0164 – PP_0168* deletion
0164-0168 junction-R	CACATGGTGGTCGAGGTGGGCGC	Sequence boundaries of *PP_0164 – PP_0168* deletion
2891-2893 junction-F	GAAGCTGTCTACCAACCCCCAGC	Sequence boundaries of *PP_2891 – PP_2893* deletion
2891-2893 junction-R	CGAAATATCCCAGGCGGACAC	Sequence boundaries of *PP_2891 – PP_2893* deletion
1131-junction-F	GCCGGGTGCAGAAGGTCACGG	Sequence boundaries of *PP_1131* deletion
1131-junction-R	CGGCGTCCTGGCCCTGCTCGGC	Sequence boundaries of *PP_1131* deletion
3132-3142 junction-F	CGTCGACCAGGGCGCTGTACAA	Sequence boundaries of *PP_3132 – PP_3142* deletion
3132-3142 junction-R	CGCCGAGGGGCAACGCCTGGC	Sequence boundaries of *PP_3132 – PP_3142* deletion
1879-1882 junction-F	TCACGGCGGTAACAGGGGTTCG	Sequence boundaries of *PP_1879 – PP_1882* deletion
1879-1882 junction-R	CCAACTCAGGAAAACCTTGTCA	Sequence boundaries of *PP_1879 – PP_1882* deletion
3104-3110 Junction-F	GATGGAAGAGCTGACTTACG	Sequence boundaries of *PP_3104 – PP_3110* deletion
3104-3110 Junction-R	CGAGCTCCAGAAAGAAATC	Sequence boundaries of *PP_3104 – PP_3110* deletion

PP1532-XmaF	TCCC**CCCGGG**GACCAGGCGGTGCGACAGCA	Deletion of prophage 4 [3]
PP1586-BamR	CG**GGATCC**CCAACACGAAGCTGAAGCTGGC	Deletion of prophage 4 [3]
pEMG-F1	CCATTCAGGCTGCGCAACTGTTG	To sequence TS1-TS2 in pEMG [9]
pEMG-R1	CTTTACACTTTATGCTTCCGGC	To sequence TS1-TS2 in pEMG [9]
pSW-F	GGACGCTTCGCTGAAAACTA	Diagnose curation of the plasmid pSW-I [9]
pSW-R	AACGTCGTGACTGGGAAAAC	Diagnose curation of the plasmid pSW-I [9]

[a] Recognition site for the restriction enzymes specified are indicated in boldface in the DNA sequence, and complementary sequences used in splicing by overlap extension (SOEing) PCR amplifications are shown in italics.

Supplementary Table S3. List of surface-associated proteins identified by activated magnetic nanoparticles.

Please, see additional Excel Table S3.

Supplementary Table S4. Bacterial strains and plasmids used in this work.

Strain or plasmid	Relevant characteristics [a]	Reference or source
Escherichia coli		
DH5α λ*pir*	Cloning host; F⁻ λ⁻ *endA1 glnX44*(AS) *thiE1 recA1 relA1 spoT1 gyrA96*(Nal^R) *rfbC1 deoR nupG* Φ80(*lacZ*Δ*M15*) Δ(*argF-lac*)*U169 hsdR17*(r_K^- m_K^+) λ*pir* lysogen	4
HB101	Helper strain; F⁻ λ⁻ *hsdS20*(r_B^- m_B^-) *recA13 leuB6*(Am) *araC14* Δ(*gpt-proA*)*62 lacY1 galK2*(Oc) *xyl-5 mtl-1 thiE1 rpsL20*(Sm^R) *glnX44*(AS)	5
Pseudomonas putida		
KT2440	Wild-type strain; mt-2 derivative cured of the TOL plasmid pWW0	6
EM371	KT2440 derivative; Δ*PP_1887-PP_1891* Δ*PP_2357-PP_2363* Δ*PP_4986-PP_4992* Δ*PP_5080-PP_5083* Δ*PP_0607-PP_0611* Δ*PP_0803-PP_0806* Δ*PP_3472-PP_3484* Δ*PP_3396-PP_3399* Δ*PP_1449-PP_1450* Δ*PP_2629-PP_2638* ΔPP_1277-PP_1288 Δ*PP_1427-PP_1430* Δ*PP_1993* Δ*PP_5093* Δ*PP_0164-PP_0168* Δ*PP_5404-PP_5407* Δ*PP_2891-PP_2893* Δ*PP_1131* Δ*PP_4329-PP_4397* Δprophage 4 Δ*PP_3132-PP_3142* Δ*PP_1804* Δ*PP_1879-PP_1882*	This work
KT2440-GFP	KT2440 GFP derivative	7
KT2440-mCherry	KT2440 mCherry derivative	3
EM371-GFP	EM371 GFP derivative	This work
EM371-mCherry	EM371 mCherry derivative	This work

Plasmid	Relevant characteristics	Reference or Source
pRK600	Helper plasmid used for conjugation; *oriV*(ColE1), RK2(*mob*$^+$ *tra*$^+$); Cm^R	[8]
pEMG	Plasmid used for deletions; *oriV*(R6K), *lacZ*α fragment with two flanking I-*Sce*I recognition sites; Km^R	[9]
pSW-I	Helper plasmid used for deletions; *oriV*(RK2), *xylS*, P*m*$\rightarrow$*I-Sce*I; Ap^R	[10]
pEMG-1887	pEMG bearing a 1.6-kb TS1-TS2 EcoRI-XmaI insert for deleting the *PP_1887-PP_1891* operon	[3]
pEMG-R1	pEMG bearing a 1.6-kb TS1-TS2 SacI-BamHI insert for deleting the *PP_2357-PP_2363* operon	[9]
pEMG-4986	pEMG bearing a 1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_4986-PP_4992* operon	[11]
pEMG-5080	pEMG bearing a 1-kb TS1-TS2 XmaI-BamHI insert for deleting the *PP_5080-PP_5083* operon	This work
pEMG-0607	pEMG bearing a 1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_0607-PP_0611* operon	This work
pEMG-0803	pEMG bearing a 1-kb TS1-TS2 EcoR*I*-XbaI insert for deleting the *PP_0803-PP_0806* operon	This work
pEMG-3472	pEMG bearing a 1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_3472-PP_3484* operon	This work
pEMG-298	pEMG bearing a 1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_3396-PP_3399* operon	[3]
pEMG-1449	pEMG bearing a 1.1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_1449-PP_1450* operon	This work
pEMG-286	pEMG bearing a 1.2-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_2629-PP_2638* operon	[3]
pEMG-1277	pEMG bearing a 1.1-kb TS1-TS2 EcoRI-XmaI insert for deleting the *PP_1277-PP_1288* operon	This work
pEMG-1427	pEMG bearing a 1.2-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_1427-PP_1430* operon	This work

Plasmid	Relevant characteristics	Reference or source
pEMG-1993	pEMG bearing a 1.1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_1993* gene	This work
pEMG-5093	pEMG bearing a 1.1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_5093* gene	This work
pEMG-0164	pEMG bearing a 1.4-kb TS1-TS2 EcoRI-XmaI insert for deleting the *PP_0164-PP_0168* operon	This work
pEMG-Tn*7*	pEMG bearing a 1.6-kb TS1-TS2 EcoRI-XmaI insert for deleting the *PP_5404-PP_5407* operon	[12]
pEMG-2891	pEMG bearing a 1.1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_2891-PP_2893* operon	This work
pEMG-1131	pEMG bearing a 1.2-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_1131* gene	This work
pEMG-flagella	pEMG bearing a 1.5-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_4329-PP_4397* flagellar operon	[13]
pEMG-3132	pEMG bearing a 1.2-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_3132-PP_3142* operon	This work
pEMG-1804	pEMG bearing a 1-kb TS1-TS2 EcoRI-BamHI insert for deleting the *PP_1804* gene	This work
pEMG-1879	pEMG bearing a 1-kb TS1-TS2 EcoRI-XmaI insert for deleting the *PP_1879-PP_1882* operon	This work
pSEVA238	Expression vector; *oriV*(pBBR1); *xylS*-P*m*	[14]
pSEVA238-AT-Jun	Expression vector; *oriV*(pBBR1); *xylS*-P*m*→Jun-*igA*β*;* Km^R	This work
pSEVA238-AT-Fos	Expression vector; *oriV*(pBBR1); *xylS*-P*m*→Fos-*igA*β*;* Km^R	This work

Plasmid	Relevant characteristics	Reference or source
pSEVA238-trx-$^{G6}V_{HH}$	Expression vector; *oriV*(pBBR1); *xylS*-P*m*→ trx-$^{G6}V_{HH}$; Km^R	This work
pME9407	Ap^R, Gm^R, *oriV*(pUC19), mini-Tn*7*, P_{tac}→*mCherry*	[15]
pBK-miniTn*7*-*gfp2*	Ap^R, Cm^R, Gm^R, *oriV*(pUC19), *mob*$^+$, mini-Tn*7*, $P_{A1/04/03}$→*gfp2*	[16]
pUX-BF13	Ap^R; *ori*R6K, *mob*$^+$, provides the Tn*7* transposition function *in trans*	[17]
pSEVA2513	Km^R, *oriV*(RSF1010), P_{EM7}	[18]
pSEVA2513-lacZ	Km^R, *oriV*(RSF1010), P_{EM7}→*lacZ*	This work

[a] Antibiotic markers: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Nal, nalidixic acid; Sm, streptomycin.

[b] Plasmid pEMG-0164 contain a deletion of a thymidine within the TS1-TS2. Since this T is located within an intergenic region between *PP_0163* and *PP_0164* (genome coordinate: 187,126) we decided to maintain it. Therefore, the naked strain contains that deletion as well.

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