

S1 Table. Mutations identified in the evolved strains compared to the parental MG1655.

Selective Agent	Population	Strain	Position	Mutation	Annotation	Gene		
WT spent media	WT2	WT2_2	611,871	G→T	intergenic (-154/-167)	<i>fepA / fes</i>		
			903,861	G→T	E16* (<u>G</u> AA→ <u>I</u> AA)	<i>ybjQ</i>		
			1,195,443	C→T	H366H (<u>C</u> AC→ <u>C</u> AT)	<i>icd</i>		
			1,195,455	C→T	T370T (<u>A</u> CC→ <u>A</u> CI)	<i>icd</i>		
			1,195,468	T→C	L375L (<u>I</u> TA→ <u>C</u> TA)	<i>icd</i>		
			1,195,470	A→G	L375L (TT <u>A</u> →TT <u>G</u>)	<i>icd</i>		
			1,982,212	C→G	G294G (GG <u>G</u> →GG <u>C</u>)	<i>araG</i>		
			2,808,978	A→C	T63P (<u>A</u> CG→ <u>C</u> CG)	<i>mprA</i>		
			4,275,901	A→G	D137G (<u>G</u> AC→ <u>G</u> GC)	<i>soxR</i>		
		WT2_3	611871	G→T	intergenic (-154/-167)	<i>fepA / fes</i>		
			2808978	A→C	T63P (ACG→CCG)	<i>mprA</i>		
			3578318	Δ3,133 bp	IS1-mediated	<i>[yhhX]-yrhA</i>		
			4294404	+GC	intergenic (+587/+55)	<i>gltP / yjcO</i>		
	WT3	WT3_2	1,208,643	C→G	pseudogene (200/501 nt)	<i>stfE</i>		
				2,150,268	C→G	E77Q (<u>G</u> AA→ <u>C</u> AA)	<i>yegK</i>	
				2,809,057	C→T	T89M (<u>A</u> CG→ <u>A</u> IG)	<i>mprA</i>	
				3,577,701	Δ3,750 bp	IS 1-mediated	<i>yhhX-yrhA</i>	
				WT3_3	2,809,057	C→T	T89M (<u>A</u> CG→ <u>A</u> IG)	<i>mprA</i>
					3,577,701	Δ3,750 bp	IS 1-mediated	<i>yhhX-yrhA</i>
ΔpvdJ spent media	pvdJ1	pvdJ1_2	2,809,237	Δ1 bp	coding (446/531 nt)	<i>mprA</i>		
			pvdJ1_3	2,809,237	Δ1 bp	coding (446/531 nt)	<i>mprA</i>	
	pvdJ2	pvdJ2_1		1,208,643	C→G	pseudogene (200/501 nt)	<i>stfE</i>	
				2,809,237	Δ1 bp	coding (446/531 nt)	<i>mprA</i>	
				4,294,404	+GC	intergenic (+587/+55)	<i>gltP / yjcO</i>	
		pvdJ2	2,809,237	Δ1 bp	coding (446/531 nt)	<i>mprA</i>		

		_2				
			3,577,292	Δ4,159 bp	IS 1-mediated	[yhhW]–yrhA
			4,294,404	+GC	intergenic (+587/+55)	gltP / yjcO
		pvdJ2_3	1,040,609	G→C	G257A (G <u>G</u> A→G <u>C</u> A)	appA
			2,809,237	Δ1 bp	coding (446/531 nt)	mprA
			3,578,830	Δ2,622 bp	IS 1-mediated	yhhY–yrhA
	pvdJ3	pvdJ3_1	485,346	IS 1 (–) +8 bp	coding (362-369/648 nt)	acrR
			2,809,117	A→C	D109A (G <u>A</u> T→G <u>C</u> T)	mprA
		pvdJ3_2	485,346	IS 1 (–) +8 bp	coding (362-369/648 nt)	acrR
			1,525,626	G→C	intergenic (+450/-300)	yncH / rhsE
			2,809,117	A→C	D109A (G <u>A</u> T→G <u>C</u> T)	mprA
			4,205,587	A→C	intergenic (-32/-583)	purH / rrsE
		pvdJ3_3	2,809,117	A→C	D109A (G <u>A</u> T→G <u>C</u> T)	mprA
			3,583,704	G→T	G381G (GG <u>C</u> →GG <u>A</u>)	ggt
			3,726,206	C→G	A397P (G <u>C</u> G→ <u>C</u> CG)	xylB
			4,176,250	T→G	V162G (G <u>I</u> T→G <u>G</u> T)	nusG
			4,205,587	A→C	intergenic (-32/-583)	purH / rrsE
			4,294,404	+GC	intergenic (+587/+55)	gltP / yjcO
pyocyanin	pyo1	pyo1_1	2,310,612	G→A	Q54* (C<u>A</u>G→<u>I</u>AG)	ompC
			4,112,442	G→A	A18A (GC<u>C</u>→GC<u>I</u>)	fpr
			4,112,479	G→A	T6I (A <u>C</u> A→A <u>I</u> A)	fpr
		pyo1_2	1,040,609	G→C	G257A (G <u>G</u> A→G <u>C</u> A)	appA
			1,754,820	G→T	E367* (G <u>A</u> A→ <u>I</u> AA)	pykF
			1,755,411	C→G	intergenic (+277/-34)	pykF / lpp
			2,310,612	G→A	Q54* (C<u>A</u>G→<u>I</u>AG)	ompC
			3,309,402	IS4 (+) +12 bp	intergenic (-212/+24)	pnp / rpsO
			4,112,442	G→A	A18A (GC<u>C</u>→GC<u>I</u>)	fpr
		pyo1_3	1,754,820	G→T	E367* (G <u>A</u> A→ <u>I</u> AA)	pykF
			1,755,411	C→G	intergenic (+277/-34)	pykF / lpp

			2,310,612	G→A	Q54* (C AG→ I AG)	<i>ompC</i>
			4,112,442	G→A	A18A (G CC→ G CI)	<i>fpr</i>
	pyo2	pyo2_ 1	729,877	T→C	C358R (I GT→ C GT)	<i>rhcC</i>
			2,310,234	+TC	coding (538/1104 nt)	<i>ompC</i>
			4,112,492	C→A	A2S (G CT→ I CT)	<i>fpr</i>
		pyo2_ 2	1,525,626	G→C	intergenic (+450/-300)	<i>yncH / rhsE</i>
			2,310,234	+TC	coding (538/1104 nt)	<i>ompC</i>
			4,112,492	C→A	A2S (G CT→ I CT)	<i>fpr</i>
		pyo2_ 3	729,877	T→C	C358R (I GT→ C GT)	<i>rhcC</i>
			2,310,234	+TC	coding (538/1104 nt)	<i>ompC</i>
			4,112,492	C→A	A2S (G CT→ I CT)	<i>fpr</i>
			4,294,403	+G	intergenic (+586/+56)	<i>gltP / yjcO</i>

The mutations in **bold** were transferred to the parental WT strain and tested further.