

Supplemental Materials

Adaptive laboratory evolution restores solvent tolerance in plasmid-cured *Pseudomonas putida* S12; a molecular analysis

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Table S1. Background mutations on plasmid-cured *P. putida* S12 strains (due to the Mitomycin C treatment)

Strain	Minimum	Maximum	Length	Amino Acid Change	CDS Position	Change	Codon Change	Coverage	Polymorphism Type	Protein Effect	Variant Frequency	Variant P-Value (approximate)
S12.6	1,256,943	1,256,943	1			G -> C		295	SNP (transversion)		99.7%	1.9E-1086
S12.22	2,812,212	2,812,212	1			A -> G		188	SNP (transition)		34.0%	2.1E-93
S12.6	3,316,341	3,316,341	1	T -> P	157	T -> G	ACC -> CCC	267	SNP (transversion)	Substitution	99.3%	1.0E-1007
S12.10	3,316,341	3,316,341	1	T -> P	157	T -> G	ACC -> CCC	368	SNP (transversion)	Substitution	99.7%	4.6E-1393
S12.22	3,316,341	3,316,341	1	T -> P	157	T -> G	ACC -> CCC	238	SNP (transversion)	Substitution	100.0%	4.0E-905

Strain	Parent Strain	Name	Minimum	Maximum	Length	Amino Acid Change	CDS Position	Change	Codon Change	Coverage	Polymorphism Type	Protein Effect	Variant Frequency	Variant P-Value (approximate)
S12-10e35	S12-10	A	2709165	2709165	1	A -> T	565	G -> A	GCC -> ACC	217	SNP (transition)	Substitution	93.1%	2.1E-686
S12-10e35	S12-10	C	2735364	2735363	0		233	(C)3 -> (C)4		253	Insertion (tandem repeat)	Frame Shift	99.2%	1.8E-903
S12-10e35	S12-10	G	2812212	2812212	1			A -> G		216	SNP (transition)		30.1%	4.6E-97
S12-10e35	S12-10	C	2920552	2920552	1	V -> A	986	T -> C	GTG -> GCG	269	SNP (transition)	Substitution	99.3%	2.9E-1014
S12-10e35	S12-10	G	2922216	2922216	1		27	A -> G	GCT -> CCC	268	SNP (transition)	None	91.0%	1.4E-852
S12-10e35	S12-10	T	2966285	2966285	1	A -> T	229	C -> T	GCT -> ACT	285	SNP (transition)	Substitution	92.3%	4.6E-971
S12-10e35	S12-10	T	3056701	3056701	1	G -> D	688	C -> T	GGC -> GAC	267	SNP (transition)	Substitution	97.0%	2.2E-872
S12-10e35	S12-10	G	3097502	3097501	0		984	(G)6 -> (G)7		295	Insertion (tandem repeat)	Frame Shift	100.0%	1.0E-1062
S12-10e35	S12-10	G	3101879	3101878	0		843	(G)6 -> (G)7		257	Insertion (tandem repeat)	Frame Shift	99.6%	2.5E-922
S12-10e35	S12-10	T	3109593	3109593	1			C -> T		225	SNP (transition)		100.0%	1.0E-810
S12-10e35	S12-10		3146925	3146925	1			(G)8 -> (G)7		234	Deletion (tandem repeat)		100.0%	4.0E-375
S12-10e35	S12-10	T	3249350	3249350	1	A -> V	68	C -> T	GCC -> GTC	232	SNP (transition)	Substitution	98.3%	2.4E-797
S12-10e35	S12-10	T	3297187	3297187	1	T -> I	833	C -> T	ACC -> ATC	258	SNP (transition)	Substitution	99.1%	8.2E-794
S12-10e35	S12-10	G	3303300	3303300	1	V -> A	2,699	A -> G	ATC -> GCG	266	SNP (transition)	Substitution	99.2%	5.6E-973
S12-10e35	S12-10	G	3307552	3307551	0		140	(G)6 -> (G)7		261	Insertion (tandem repeat)	Frame Shift	97.3%	1.6E-933
S12-10e35	S12-10	G	3316341	3316341	1	T -> P	157	T -> G	ACC -> CCC	253	SNP (transversion)	Substitution	100.0%	4.0E-962
S12-10e35	S12-10	G	3316345	3316344	0		154	(G)3 -> (G)4		250	Insertion (tandem repeat)	Frame Shift	99.6%	4.0E-897
S12-10e35	S12-10	G	3419216	3419216	1		798	A -> G	CAA -> CAG	263	SNP (transition)	None	99.6%	2.5E-996
S12-10e35	S12-10	G	3452892	3452892	1	Y -> H	1,156	A -> G	TAC -> CAC	253	SNP (transition)	Substitution	99.6%	2.5E-958
S12-10e35	S12-10	A	3479624	3479624	1	X -> E	788	M -> A	GMG -> GAG	222	SNP	Substitution	99.5%	5.6E-65
S12-10e35	S12-10	G	3528742	3528741	0		316	(G)6 -> (G)7		268	Insertion (tandem repeat)	Frame Shift	99.3%	2.5E-958
S12-10e35	S12-10	T	3537413	3537413	1	A -> V	329	C -> T	GCC -> GTC	262	SNP (transition)	Substitution	89.3%	4.2E-856
S12-10e35	S12-10	G	3690651	3690651	1	Y -> C	2,177	A -> G	TAC -> TGC	237	SNP (transition)	Substitution	91.1%	3.0E-794
S12-10e35	S12-10	T	3748119	3748119	1			C -> T		246	SNP (transition)		99.2%	2.5E-830
S12-10e35	S12-10	C	3858978	3858978	1		639	T -> C	CGT -> CGC	242	SNP (transition)	None	100.0%	2.5E-920
S12-10e35	S12-10	G	3865096	3865096	1	D -> A	3,089	T -> G	GAC -> GCC	221	SNP (transversion)	Substitution	99.5%	1.4E-768
S12-10e35	S12-10	G	3946605	3946604	0			(G)7 -> (G)8		214	Insertion (tandem repeat)		95.3%	8.8E-624
S12-10e35	S12-10	G	4176584	4176583	0		474	(G)5 -> (G)6		230	Insertion (tandem repeat)	Frame Shift	95.7%	1.3E-784
S12-10e35	S12-10	T	4221641	4221641	1	R -> C	1,192	C -> T	CGT -> TGT	253	SNP (transition)	Substitution	99.6%	4.0E-933
S12-10e35	S12-10	G	4463943	4463942	0		275	(G)7 -> (G)8		243	Insertion (tandem repeat)	Frame Shift	99.6%	4.0E-896
S12-10e35	S12-10	G	4666198	4666198	1		1,179	A -> G	GGT -> GGC	232	SNP (transition)	None	95.7%	2.5E-809
S12-10e35	S12-10	G	4711920	4711919	0		284	(G)5 -> (G)6		262	Insertion (tandem repeat)	Frame Shift	99.6%	2.5E-940
S12-10e35	S12-10	A	4742974	4742974	1	G -> D	476	G -> A	GGC -> GAC	234	SNP (transition)	Substitution	95.3%	2.3E-810
S12-10e35	S12-10	T	4743032	4743032	1		534	C -> T	CGC -> CGT	220	SNP (transition)	None	33.2%	1.2E-212
S12-10e35	S12-10	G	4836001	4836000	0		1,033	(G)6 -> (G)7		247	Insertion (tandem repeat)	Frame Shift	91.9%	4.2E-824
S12-10e35	S12-10	G	4928278	4928278	1	F -> L	943	A -> G	TTC -> CTC	256	SNP (transition)	Substitution	99.6%	1.0E-969
S12-10e35	S12-10	C	5043280	5043279	0		721	(C)8 -> (C)9		208	Insertion (tandem repeat)	Frame Shift	99.0%	1.6E-783
S12-10e35	S12-10	C	5146750	5146750	1	T -> A	508	T -> C	ACG -> GCG	227	SNP (transition)	Substitution	88.5%	2.2E-734
S12-10e35	S12-10	C	5167012	5167012	1	V -> A	233	T -> C	GTC -> GCC	233	SNP (transition)	Substitution	99.1%	3.4E-832
S12-10e35	S12-10	A	5424887	5424887	1		2,610	G -> A	AGC -> AGT	269	SNP (transition)	None	97.8%	3.3E-918
S12-10e35	S12-10	G	5530225	5530225	1	S -> P	220	A -> G	TCG -> CCG	245	SNP (transition)	Substitution	98.8%	6.4E-891
S12-10e35	S12-10	G	5594662	5594662	1		882	A -> G	GAA -> GAG	219	SNP (transition)	None	99.1%	6.7E-803
S12-10e35	S12-10	C	5602034	5602034	1	L -> P	728	T -> C	CTC -> CCC	225	SNP (transition)	Substitution	99.1%	4.1E-800
S12-10e35	S12-10	A	5610231	5610231	1	E -> K	1,015	G -> A	GAA -> AAA	249	SNP (transition)	Substitution	98.0%	1.0E-851
S12-10e35	S12-10		5711294	5711294	1			(G)12 -> (G)11		136	Deletion (tandem repeat)		96.3%	3.1E-144
S12-22e30	S12-22	A	142041	142041	1	P -> L	269	G -> A	CCC -> CTC	249	SNP (transition)	Substitution	98.8%	6.3E-911
S12-22e30	S12-22	C	1595974	1595974	1	D -> E	1,230	G -> C	GAC -> GAG	310	SNP (transversion)	Substitution	99.7%	6.2E-1142
S12-22e30	S12-22	C	2150602	2150602	1			G -> C		259	SNP (transversion)		100.0%	6.3E-985
S12-22e30	S12-22		3316341	3316341	1		157	-T		253	Deletion	Frame Shift	100.0%	4.0E-456
S12-22e30	S12-22		RPPX_RS14645 - RPPX_RS14650									insertion of mobile element ISS12		

Figure S1. Volcano plot of differential gene expression in wild-type, plasmid-cured, and ALE-derived *P. putida* S12 strains in the presence of 0.1% (vol/vol) toluene growing on LB.

Blue area indicates the significantly upregulated genes and beige area indicates the significantly downregulated genes (cut-off: Log_2 Fold Change ≥ 1 for up-regulated genes or ≤ -1 for down-regulated genes and FDR-adjusted p-value ≤ 0.05). The black dots represent the significantly up-/down-regulated genes.

