

Supporting Information

Phenotypic Plasticity During Organofluorine Degradation Revealed by Adaptive Evolution

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>*Dechloromonas aromatica* RCB defluorinase (DEF2) gene sequence (codons optimized for expression in *E. coli*)

ATGTTTGACTCATCTTATGTTACTCGTGATGTTGACGTGGGCGCAACGCGTATTCACGTTCCGGGTTCGT
GAGAATGAGGGGCGCCCTCCACTCTTATTACTTTCATGGTTATCCAGAGACCCATGCAATGTGGCACAAG
GTCGCTTCATTACTGCAAGATCGCTTTTCCCTCGTGCTCCCTGACCTGCGGGGGTATGGGGACTCGGGG
ATGCCTGCGTCGCAGGCGGATGCGGGTAACCAGTCGAAGCGCGTTATGGCGCAGGATATGGCAGAGTTG
ATGACCGCACTGGGGTATCAGCGGTTTCATGTGGCAGCCACGATCGGGGCGCCCGTGTGCTGCATCGT
CTCTGTTTAGATCATCCAGGTCGTATTCAAACAGCCTGCATCATGGATATCGCGCCGACGGCTACAAC
TTCGCACTCACTAATCAGGCCTTAGCGACTTCCTATTTTCATTGGTTCTTTTAAATCCAAGCGGCACCA
TTGCCTGAGAATATGATCGCTGCTGATCCTGCCTCTTGGCTCAAGGGCTGCCTTAGCCGTTGGAGTATG
GGCAACGAAGAAGCATTTCGATCCTGCCGTTGTGAGTGAGTATGTGCGGTGTTTTTCTAATCCGGAGGCT
ATTGCTGCTCTTGCATGATTATCGGGCTGCGGCTGGTATTGACCTTCAGCATGACGGCGAAGATGCC
GAACGTCGCATTAGTTGCCCGCTGTTAGTTCTGTGGGGCAACAAAGGGTTCGTTGGCCGTAACATGAT
GTCGTTGCTTTGTGGCGCGAAAAAGCCCTTGACGTATCAGGCAAAGGTCTCCCATGTGGGCACCTTTCTC
CCTGAAGAGCTGCCGAACGATGTAGCTCGGGAACTCGTGCAGTTCATCGCTCGGCACTCTTCATAA

>*Dechloromonas aromatica* RCB defluorinase (DEF2) gene sequence (codons optimized for expression in *P. putida*)

ATGTTTCGACTCGAGCTACGTGACGCGCGACGTGGACGTCCGGTGCAACGCGCATTCACGTTCCGGGTCCGG
GAAAATGAAGGCCGTCCTCTGTTGCTGCTGCATGGTTATCCCGAAACCCATGCGATGTGGCACAAG
GTGGCCTCGCTCCTCCAGGATCGGTTTCAGCCTGGTCCCTCCCGACCTCCGTGGCTACGGGGATTCCGGG
ATGCCAGCTAGCCAAGCAGACGCAGGGAACCAGTCGAAACGGGTCATGGCTCAAGACATGGCTGAGCTC
ATGACCGCGCTGGGGTATCAACGTTTCCATGTGGCAGCTCATGACCGGGGCGCTCGTGTGTTGCACCGG
CTGTGCCTCGATCACCTGGTCCGATTCAAACGGCGTGTATTATGGATATTGCCCTACCGCAACGACC
TTTGCCTCACCAACCAAGCGCTCGCAACCTCGTACTTTTCATTGGTTTTTTCTCATCCAGGCTGCCCTC
CTGCCGAAAACATGATCGCAGCGGACCCCGCTAGCTGGTTGAAAGGCTGCTTGTCCCGCTGGTTCGATG
GGTAACGAGGAAGCGTTTCGACCCGGCGGTGGTCTCGGAGTATGTGCGGTGTTTTTCCAACCCTGAGGCG
ATCCGCTGCTCGTGCATGATTATCGCGCTGCTGCTGGCATCGACCTGCAGCATGACGGTGAAGATGCT
GAACGTCGATTAGCTGCCCCCTGTTGGTCTTGTGGGGTAACAAGGGCTTCGTGGGTGTAATTACGAT
GTGGTGGCGCTCTGGCGCGAGAAAGCATTTGGACGTGAGCGGTAAAGGGCTCCCTTGTGGCCACTTTTTG
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Figure S1

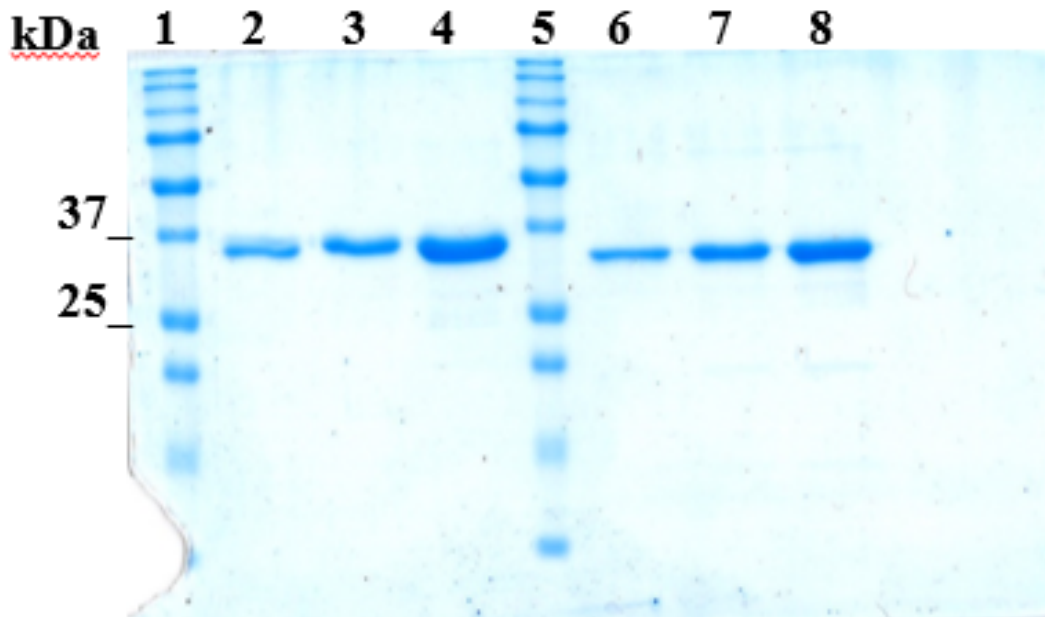


Figure S2 12.5% polyacrylamide gel stained with Coomassie blue showing the relative purities of the DEF1 and DEF2 preparations. Lanes 1 and 5 are All Blue Precision Plus Protein molecular weight standards (Bio-Rad). Total protein amounts of 1, 2, 4 μg of each enzyme were loaded into lanes 2, 3, 4 (DEF2) or 6, 7, 8 (DEF1), respectively.

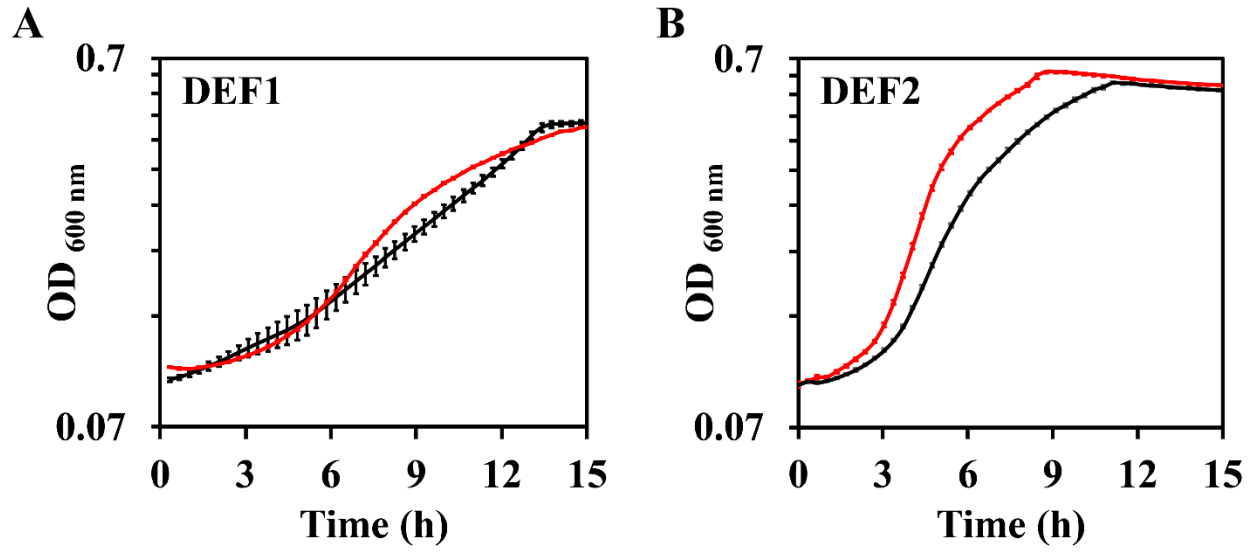


Figure S3 Growth of post-adapted (red) or pre-adapted (black) recombinant *Pp12663* strains expressing (A)DEF1 or (B)DEF2 on 10 mM mandelic acid as sole carbon and energy in a 96-well plate.

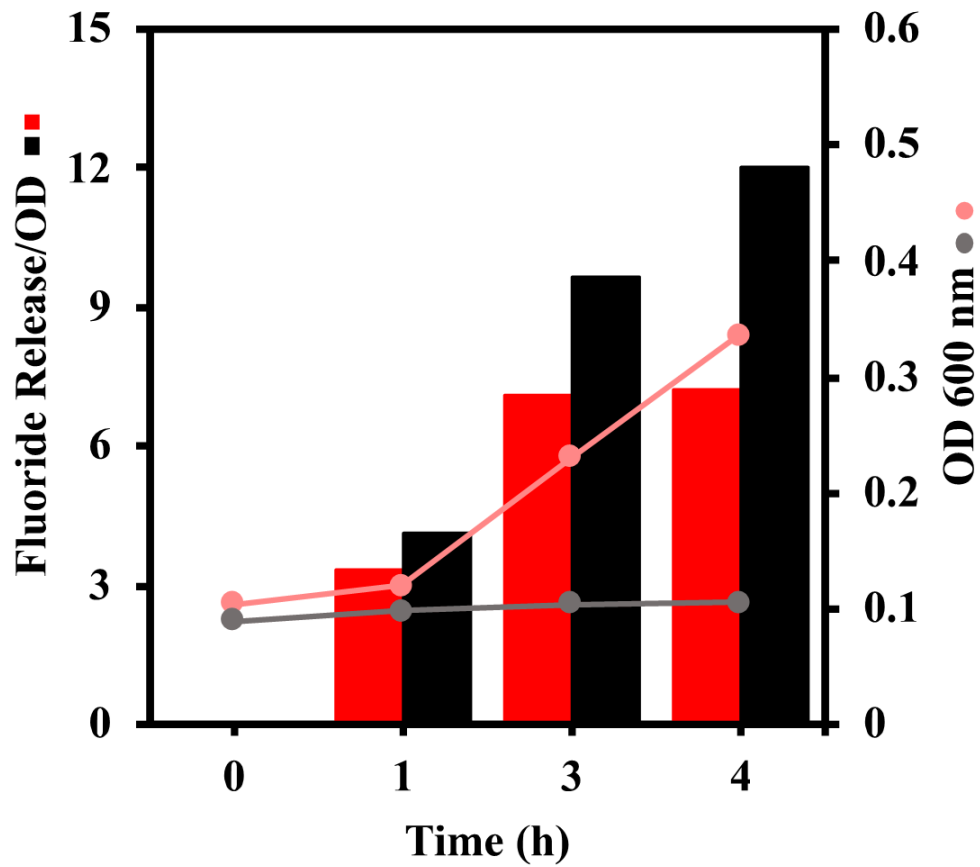


Figure S4 Initial fluoride release normalized to OD_{600} , for post-adapted (black columns) or pre-adapted (red columns) DEF1 strains growing on α -fluorophenylacetic acid. OD_{600} is also shown for the post-adapted (pink line) or pre-adapted (gray line) strains.

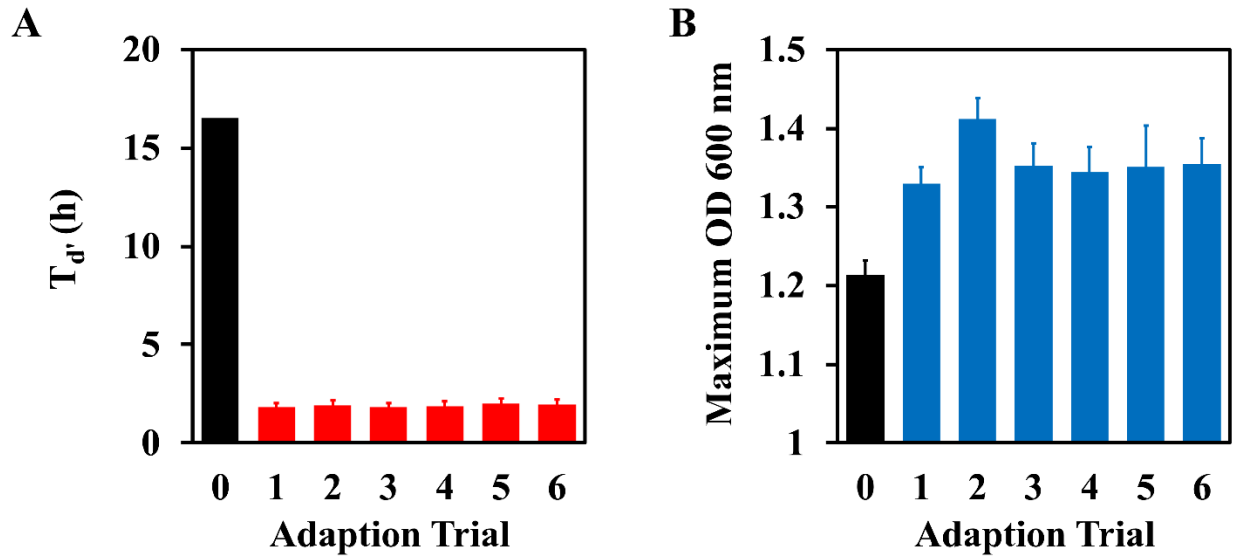


Figure S5. (A) First doubling times (T_d') and (B) maximum OD₆₀₀ from six independent experiments to adapt *Pp12633* expressing DEF1 to enhanced growth on a-FPhAA. Adaption trial zero refers to the pre-adapted strain in (A) and (B). Error bars represent the average adapted T_d' and maximum OD₆₀₀ for all transfers after maximum adaption was reached (5 transfers to maximum adaption and 6 additional transfers afterwards).

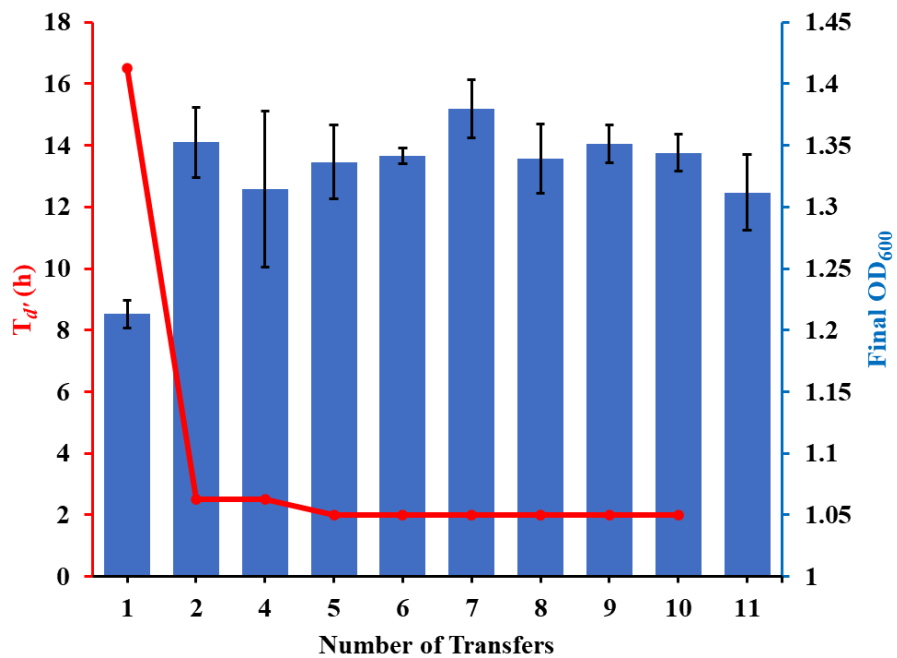


Figure S6. First doubling times (T_d') (shown in red) and final OD₆₀₀ (shown in blue) across the entire course of evolution from three independent experiments to adapt *Pp12633* expressing DEF1 to enhanced growth on α -FPhAA. Each generation represents 3-4 generations. Error bars represent the average adapted T_d' and maximum OD₆₀₀ for all transfers.

Table S1. Illumina sequencing coverage data for pre- and post-adapted DEF1 and DEF2 strains. Large standard deviations can be partially ascribed to ranges in plasmid copy number, but shouldn't be directly attributed to them. Data was generated from sequence alignment maps using bamtools as described in the methods.

Defluorinase Gene	Adaption State	Coverage Area	Replicon Length	Bases Sequenced	Average Coverage	Standard Deviation
DEF1	Pre	Genome	6179370	882082477	143	40
DEF1	Pre	plasmid	5448	46269405	8493	1455
DEF1	Pre	FAcD	936	7510104	8024	576
DEF1	Pre	plasmid backbone	4512	38759301	8590	1559
DEF1	Post	Genome	6179370	916298775	148	38
DEF1	Post	plasmid	5448	21221569	3895	678
DEF1	Post	FAcD	936	3541186	3783	331
DEF1	Post	plasmid backbone	4512	17680383	3919	727
DEF2	Pre	Genome	6179370	1122908214	182	53
DEF2	Pre	plasmid	5635	29612000	5255	909
DEF2	Pre	FAcD	891	4818974	5409	407
DEF2	Pre	plasmid backbone	4744	24793026	5226	956
DEF2	Post	Genome	6179370	1089595932	176	55
DEF2	Post	plasmid	5635	12242352	2173	1025
DEF2	Post	FAcD	891	478790	537	30
DEF2	Post	plasmid backbone	4744	11763562	2480	805

Table S2 Mutations identified in the chromosomes of post-adapted strains as compared to pre-adapted strains expressing(A) DEF1 or (B) DEF 2. Mutations were identified using *breseq*.

A

Gene	Protein	Position	Nucleotide change	Mutation	Mutation Frequency (%)
<i>ispE</i>	4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase	918,632	G→T	S273I	100
<i>ftsK</i>	Located proximally to fluoride response genes <i>crcB</i> , <i>rarA</i> , <i>lolA</i>	4,047,930	12 bp deletion	E761-A764	100
<i>nhaR/sstT</i>	Transcriptional activator NhaR	3947289	C→G	Intergenic	34.6
Hypothetical	Hypothetical protein/porin	2470517	G→A	Intergenic	30.8
Hypothetical	Putative MFS transporter	3866452	C→A	Intergenic	29.3
Hypothetical	Hypothetical protein/porin	380837	C→G	A63P	29
<i>mfa/clpA</i>	Translation initiation factor/Clp protease	4052520	C→G	Intergenic	27

B

Gene	Protein	Position	Nucleotide change	Mutation	Mutation Frequency (%)
<i>mdcA</i>	Malonate decarboxylase subunit alpha	3395334	C→G	R14G	33.7
Hypothetical	fad-dependent oxidoreductase	3173615	G→C	V442L	32.1
Hypothetical	bifunctional diguanylate cyclase/phosphodiesterase	6050171	C→A	I266I	32
Hypothetical/Hypothetical	hypo/ArsR family transcriptional regulator	797758	C→G	intergenic	30.4
Hypothetical/ <i>mgo</i>	PA4642 family protein/malate dehydrogenase	929575	T→G	intergenic	27.4
Hypothetical	Lys family transcriptional regulator	3398146	C→G	P240A	26.5
<i>fliF</i>	flagellar M-ring protein FliF	4444187	G→A	K19K	25
Hypothetical	3-keto-5-aminohexanoate cleavage prot	404462	G→C	G183R	24.8
<i>benR</i>	benABC operon transcriptional activator BenR	3189798	C→G	G291G	22.9
Hypothetical	serine O-acetyltransferase	4867087	C→G	G110G	22.6

Table S3. Steady-state kinetic data for enzymes in the pathway leading from α -fluorophenylacetic acid to TCA cycle intermediates. Data and references were derived from the BRENDA Enzyme Database (brenda-enzymes.org). Data were taken from enzymes from *Pseudomonas putida* ATCC12633 where available, or from the next closest bacterium, typically another *Pseudomonas putida*. Values of k_{cat} were captured or derived. Values of K_M were captured when available.

Enzyme	Sp act ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	k_{cat} (s^{-1})	K_M (mM)	Reference
DEF1	14.5	8	N/A*	This study
DEF2	32	17	N/A	This study
Mandelate racemase	N/A	500 with (<i>R</i>)-mandelate	0.4	Mitra, et al, 1995.
(<i>S</i>)-Mandelate dehydrogenase	N/A	360	0.12	Dewant and Mitra, 2023.
Benzoylformate decarboxylase	N/A	241	0.37	Polovnikova, et al, 2003.
Benzaldehyde dehydrogenase	N/A	154	0.004	Zahniser, et al, 2017.
Benzoate dioxygenase	N/A	4.4	N/A	Rivard, et al 2015.
Benzoate 1,2-dihydrodiol dehydrogenase	0.067	0.1	N/A	Whited, et al, 1986.
Catechol 1,2 dioxygenase	N/A	50	0.002	Nakai, et al, 1980.
Muconate cycloisomerase	N/A	13.9	0.42	Ngai, et al, 1983.
Muconolactone <i>delta</i> -isomerase	860	143	0.64	Matsumura, et al, 2006.
3-Oxoadipate enol-lactonase	717	394	0.012	Ornston, 1970.
3-Oxoadipate-CoA transferase	21.3	8.6	N/A	Yeh, et al. 1981.
3-Oxoadipyl-CoA thiolase	N/A	7.8	0.15 for 3-oxoadipyl CoA	Kaschabek, et al, 2002

*N/A means not determined