

1 **Positive selection inhibits gene mobilisation and transfer in soil bacterial**
2 **communities**

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4 **Supplementary Information**

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16 **Supplementary Table 1: Numbers of clones sequenced from each population**

plasmid treatment	culture	mercury treatment	species ^a	# clones sequenced from replicate					
				a	b	c	d	e	f
pQBR57	single-species	0	Pf	1	2	2	2	2	2
pQBR57	single-species	0	Pp	1	1	1	1	1	1
pQBR57	single-species	16	Pf	1	1	1	1	1	1
pQBR57	single-species	16	Pp	1	1	1	2	1	2
pQBR57	co-cultured	0	Pf	1	2	2	2	1	2
pQBR57	co-cultured	0	Pp	1	2	2	2	2	2
pQBR57	co-cultured	16	Pf	1	1	1	1	1	1
pQBR57	co-cultured	16	Pp	2	2	2	2	2	2
plasmid-free	single-species	0	Pf	1	0	1	1	0	0
plasmid-free	single-species	0	Pp	1	0	1	1	0	0
plasmid-free	single-species	16	Pf	1	0	1	1	0	0
plasmid-free	single-species	16	Pp	1	0	1	1	0	0
plasmid-free	co-cultured	0	Pf	1	0	0	1	0	0
plasmid-free	co-cultured	0	Pp	1	0	0	1	0	0
plasmid-free	co-cultured	16	Pf	1	0	0	1	0	0
plasmid-free	co-cultured	16	Pp	1	0	0	1	0	0

17 ^aPf = *Pseudomonas fluorescens* SBW25; Pp = *Pseudomonas putida* KT2440.

18 **Supplementary Table 2:** Focal Tn4652 insertions in co-cultured *P. fluorescens*.

population	transfer	total clones ^a	clones with pQBR57 ^a	pQBR57 with Tn4652 ^b	focal chromosomal Tn4652 ^b
d	1	31	5	0/5	NA
d	3	32	3	0/3	NA
d	5	32	2	0/1	NA
d	7	32	1	0/1	NA
d	9	32	0	NA	NA
d	11	32	0	NA	NA
d	13	32	0	NA	NA
d	15	32	0	NA	NA
d	17	32	0	NA	NA
d	23	32	0	NA	NA
d	29	32	0	NA	0/32
d	35	32	0	NA	0/32
d	41	32	23	23/23	0/32
d	47	32	22	6/6	0/32
d	53	32	20	6/6	0/32
d	59	32	26	7/7	1/32
d	65	32	13	4/4	7/32
e	1	31	2	0/2	NA
e	3	32	3	1/3	NA
e	5	32	1	1/1	NA
e	7	32	1	1/1	NA
e	9	32	0	NA	0/32
e	11	32	3	3/3	0/32
e	13	32	14	14/14	0/32
e	15	40	30	29/29	0/32
e	17	32	30	8/8	0/32
e	23	32	31	8/8	0/32
e	29	32	31	8/8	0/32
e	35	32	32	8/8	0/32
e	41	32	31	8/8	0/32
e	47	32	32	8/8	0/32
e	53	32	32	8/8	0/32
e	59	32	32	8/8	0/32
e	65	32	32	8/8	18/32
f	1	31	5	0/2	NA
f	3	32	2	0/2	NA
f	5	32	2	0/2	NA
f	7	32	2	0/1	NA
f	9	32	1	1/1	NA
f	11	32	0	NA	NA
f	13	32	2	0/2	NA
f	15	32	0	NA	NA
f	17	32	2	1/2	0/32
f	23	32	0	NA	0/32
f	29	32	1	1/1	0/32
f	35	32	16	5/5	0/32
f	41	32	30	8/8	1/32
f	47	32	24	6/6	0/32
f	53	32	29	7/7	1/31
f	59	32	22	7/7	0/32
f	65	32	8	4/4	1/32

^aIdentified previously ¹⁷. ^bNumber of clones positive/tested for each insertion; NA indicates no clones tested.

21 **Supplementary Table 3: Repetitive regions prone to spurious SNV/indel calling**

Chromosome ^a	Start	End	Notes
AE015451	194394	220642	Huge, highly repetitive gene with ~29 direct repeats
AE015451	532498	532500	Intergenic SNV present in ancestral Gm-labelled clone
AE015451	699715	699725	Intergenic region between 16S and 23S genes
AE015451	1026450	1028210	Deletion in all clones (inc. ancestor) from 1026468 to 1028201
AE015451	6170425	6170440	Region of Tn7 insertion for labelling
AM181176	895200	895500	Repeat region between PFLU_0795 and PFLU_0796
AM181176	3694000	3694600	Repeat region between PFLU_3336 and PFLU_3338
AM181176	4696690	4696700	Intergenic region between 16S and 23S genes

22 ^aChromosome AE015451 is the *P. putida* KT2440 chromosome, AM181176 is the *P. fluorescens* SBW25
 23 chromosome.

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Supplementary Table 4: Oligonucleotide primers used in this study

Primer name	Primer sequence	Target and purpose
PPUT_25728F	TAGTTGTTTCTAGGGCCCGC	With Tn5042_104312R, detection of focal Tn5042 insertion in <i>P. putida</i> from mercury-treated single-species populations a, b and c; and co-culture populations c, d and e.
PPUT_26783	CAGATCGCCAGTTCCTCC	
PPUT_18790F	TTCACGACCGCTTCAGAAAC	With Tn5042_104312R, detection of focal Tn5042 insertion in <i>P. putida</i> from mercury-treated single-species population e.
PPUT_19899R	TAGGAAGCCAGGTAGAAGCG	
PPUT_17886F	GAAGGTACATCGCCAAGCTG	With Tn5042_104312R, detection of focal Tn5042 insertion in <i>P. putida</i> from mercury-treated co-culture population f.
PPUT_18868R	CACACCTCCCTGCTCTTACC	
Tn5042_104312R	TGAGATGCAAGCGGTGTAG	Detection of focal Tn5042 insertions.
PFLU_5829752F	CTGAACAAACCGCCGTCAC	With Tn5042_110902F, detection of focal Tn5042 insertion in <i>P. fluorescens</i> from mercury-free co-culture population e.
PFLU_5830219R	CCCCTGACTATTCCCAAAGG	
Tn5042_110902F	TCTCGGAATGACACTCTGCC	Detection of focal Tn5042 insertion.
PFLU_3328819F	GTCAGACGCAAAGCCTCTTC	With Tn4652_3383009, detection of focal Tn4652 insertion in <i>P. fluorescens</i> chromosome from mercury-free co-culture population d.
PFLU_3329140R	TCAAATAAATTCCGAAACTCCCG	
PFLU_3328819F	CACTGCGCGGTAATCATC	With Tn4652_3383009, detection of focal Tn4652 insertion in <i>P. fluorescens</i> chromosome from mercury-free co-culture population e.
PFLU_3323136R	CAACTCACTGCACGCCTC	
PFLU_3327408F	TCCCACCTACCAATTCCTGAG	With Tn4652_3383009, detection of focal Tn4652 insertion in <i>P. fluorescens</i> chromosome from mercury-free co-culture population f.
PFLU_3327712R	GATGGCCGAGAGTTTCAG	
pQBR57_162616F	AAGTTGCTTGATCGCCTTCG	With Tn4652_3383009, detection of focal Tn4652 insertion in pQBR57.
pQBR57_162875R	TGTGCCGTGGAATGAATCAG	
Tn4652_3383009	TAGCCAAAGGAGGGGAGTTG	Detection of focal Tn4652 insertions.
PFLU_1891F	CGTTGCCTACTTACAGACGC	Detection of Tn6291 in <i>P. putida</i> .
PFLU_1891R	CATTGCCGTACCTGGTATGC	
PFLU_1908F	GTAGCTCTTTTGGCAGGCTC	
PFLU_1908R	CAAAGCCAGCACTGATACCG	