Supporting Information

A genetically engineered *Pseudomonas putida* for 1,2,3-trichloropropane bioremediation

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Figures

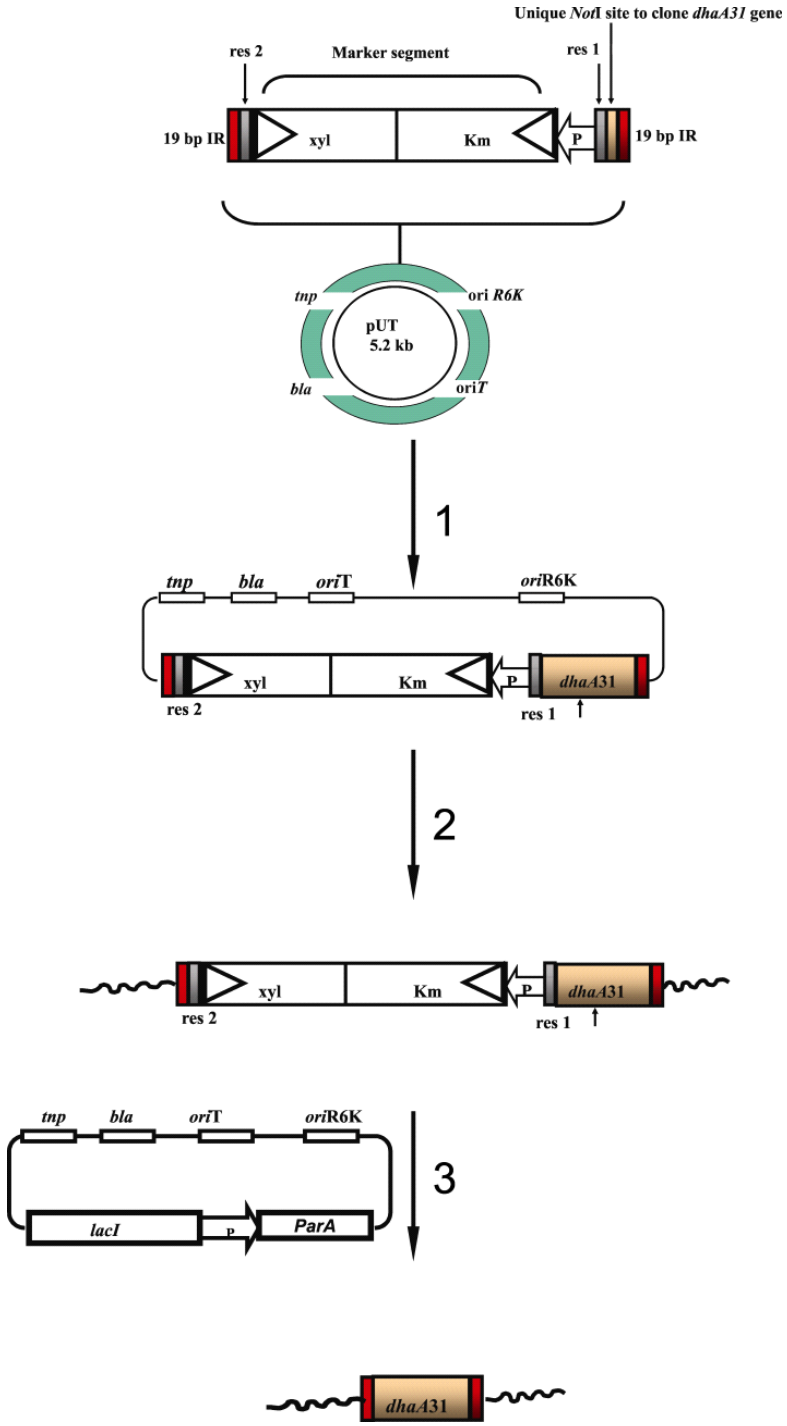

Unique NotI site to clone dhaA31 gene
res 2
Marker segment
res 1
19 bp IR
xyl
Km
P
19 bp IR
tnp
ori R6K
pUT
5.2 kb
bla
oriT
1
tnp
bla
oriT
oriR6K
xyl
Km
P
dhaA31
res 2
res 1
2
xyl
Km
P
dhaA31
res 2
res 1
tnp
bla
oriT
oriR6K
lacI
P
ParA
3
dhaA31

Fig S1. Integration of the *dhaA31* gene into the genome of strain MC4. Steps: 1) Construction of pUT31B by cloning the *dhaA31* gene with the *dhlA* promoter into the unique NotI site, which is present between the insertion sequences of the pUT delivery vector (1); 2) Introduction of pUT31B into strain MC4 by triparental mating (1-3). The exconjugants obtained were tested for kanamycin resistance, yellow coloration with catechol, haloalkane dehalogenase activity and growth on DCP as sole carbon source; 3) The resolvase gene *parA* on vector pJMSB8 was introduced into MC4 derivatives by triparental mating to remove kanamycin resistance gene and the *xylE* marker (4). The resulting colonies were confirmed to have lost the marker segment.

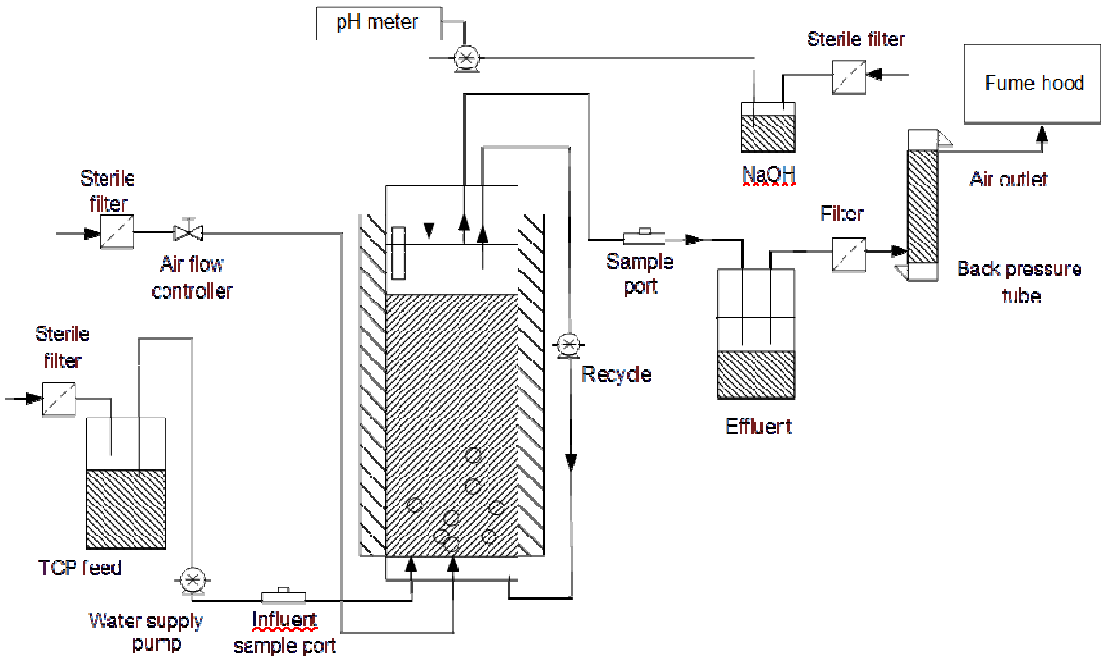


Fig S2. Process flow diagram of fixed-bed bioreactor.

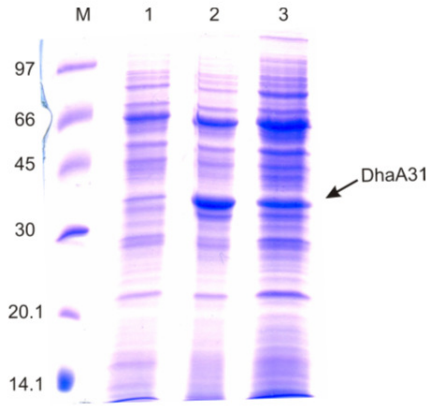


Fig. S3. Haloalkane dehalogenase expression in derivatives of *P. putida* MC4. Slots: M, marker proteins; 1, cell-free extract of strain MC4; 2, strain MC4(pIS31B); and 3, strain MC4-5222 carrying the chromosomally integrated *dhaA31* gene. All cultures were grown on DCP as carbon source.

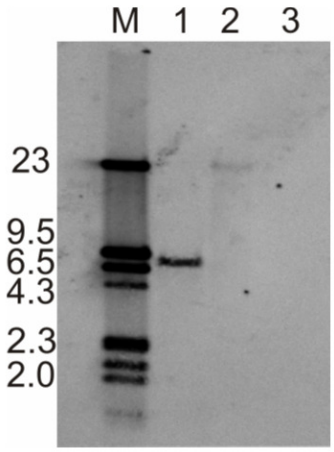
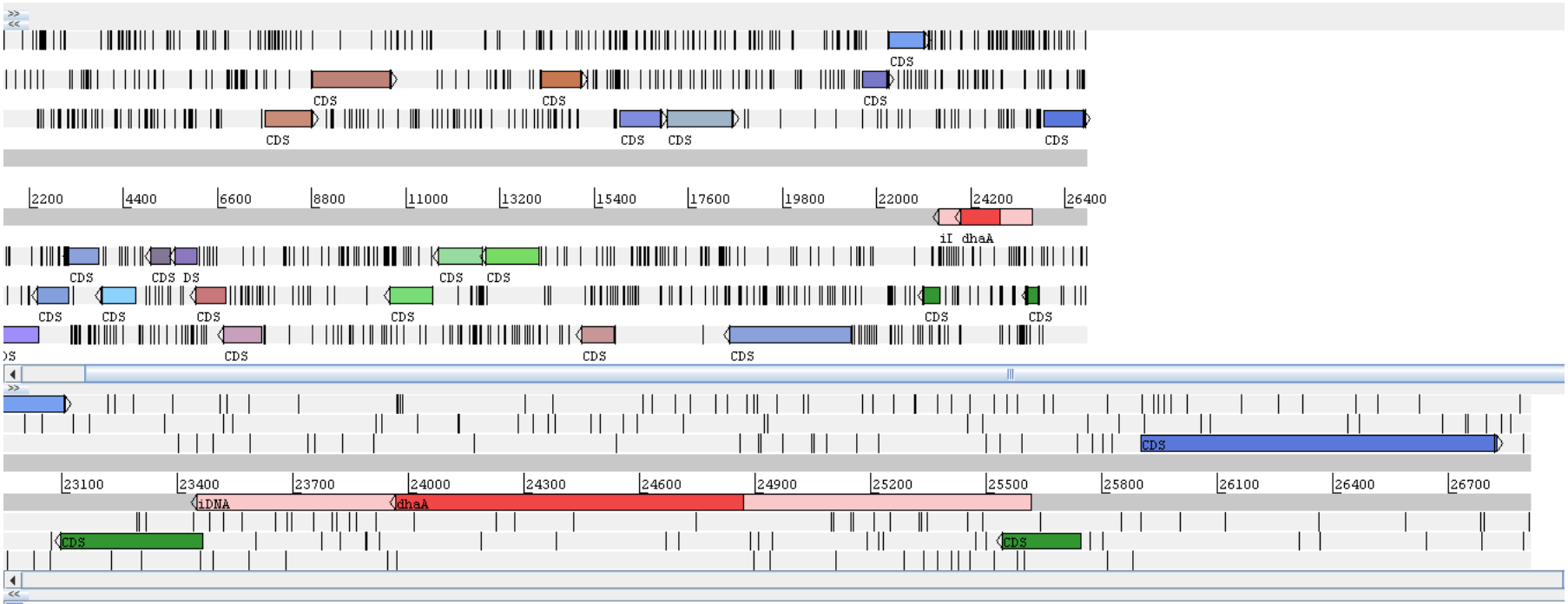


Fig. S4. Detection of chromosomally integrated *dhaA* DNA by Southern hybridization. Hybridization was performed with a 2-kb fragment (*dhlA* promoter, *dhaA31* gene and terminator) as a probe with non-radioactive DIG-labeling and SalI digested chromosomal total DNA of strain MC4-5222 (lane 1); MC4-52 (lane 2); and MC4 (lane 3). M, fragment size markers.



CDS	495	1259	c	ABC-type histidine transport system, ATPase component
CDS	1278	2390	c	Putative deacylase, succinylglutamate desuccinylase, final arginine catabolism enzyme
CDS	2399	3097	c	Arginine/ornithine ABC transporter permease
CDS	3112	3801	c	Polar amino acid ABC transporter inner membrane subunit
CDS	3893	4669	c	Arginine/ornithine ABC transporter, periplasmic arginine/ornithine binding protein
CDS	5032	5460	c	Hypothetical protein, unknown function
CDS	5599	6084	c	Putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein
CDS	6104	6757	c	Transcriptional regulator TetR family
CDS	6750	7601	c	Similar to putative 3-hydroxyacyl-CoA dehydrogenase [Pseudomonas sp. GM78]
CDS	7725	8807		Transcriptional regulator, AraC family
CDS	8813	10618		ABC-type multidrug transport system, ATPase and permease component
CDS	10622	11590	c	alpha/beta superfamily hydrolase, esterase, lipase
CDS	11764	12765	c	Putative Zn-containing NAD(P)H quinone oxidoreductase
CDS	12874	14088	c	Major facilitator superfamily, similar to putative arabinose efflux permease family protein [Pseudomonas sp. GM78]
CDS	14180	15073		LysR family transcriptional regulator
CDS	15111	15833	c	Similar to pirin-related protein [Pseudomonas sp. GM78], likely regulatory protein
CDS	16014	16949		Transcriptional regulator containing an amidase domain and an AraC-type DNA-binding HTH domain
CDS	17112	18614		Putative ATPas acting as regulator, winged helix family, DNA binding [Pseudomonas sp. GM78]
CDS	18579	21383	c	Putative ATPase involved in signal transduction
CDS	21674	22249		Similar to multimeric flavodoxin WrbA, PFAM: NADPH-dependent FMN reductase
CDS	22282	23106		Putative hydrolase or acyltransferase of alpha/beta superfamily
CDS	23096	23464	c	Gene fragment for transcriptional regulator, AraC family, 5' part missing due to insertion of dhaA31 region
iDNA	23450	25615	c	Insertion with dhaA31 gene. Inserted with a transposon.
gene	23967	24866	c	dhaA31 gene located on inserted DNA segment
CDS	25541	25744	c	Gene fragment for transcriptional regulator, AraC family, 3' part missing due to insertion of dhaA31 region
CDS	25902	26822		Highly similar to putative LysR-type transcriptional regulator [Pseudomonas sp. GM78]

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Fig S5. Artemis display of the 26,912 bp contig (contig 1007) harboring the inserted DNA fragment with the DhaA31-encoding gene on the reverse complement. The upper part shows the overall organisation of the region. The middle part is a close-up of the region where the insert (dsiplayed in pink) with the *dhaA31* gene (in red) is located. The insert is positioned in an open reading frame encoding a putative protein similar the transcriptional regulators of the AraC family. The interrupted open reading frame is shown in green. The lower part shows the annotation of the genes in the 26,912 bp contig, as obtained with the RAST server and by BLAST searches. The Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under accession number JOJW00000000. The version described in this paper is version JOJW01000000.

References

1. **de Lorenzo V, Timmis KN**. 1994. Analysis and construction of stable phenotypes in Gram-negative bacteria with Tn5-derived and Tn10-derived minitransposons. Methods Enzymol**. 235:** 386-405.
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