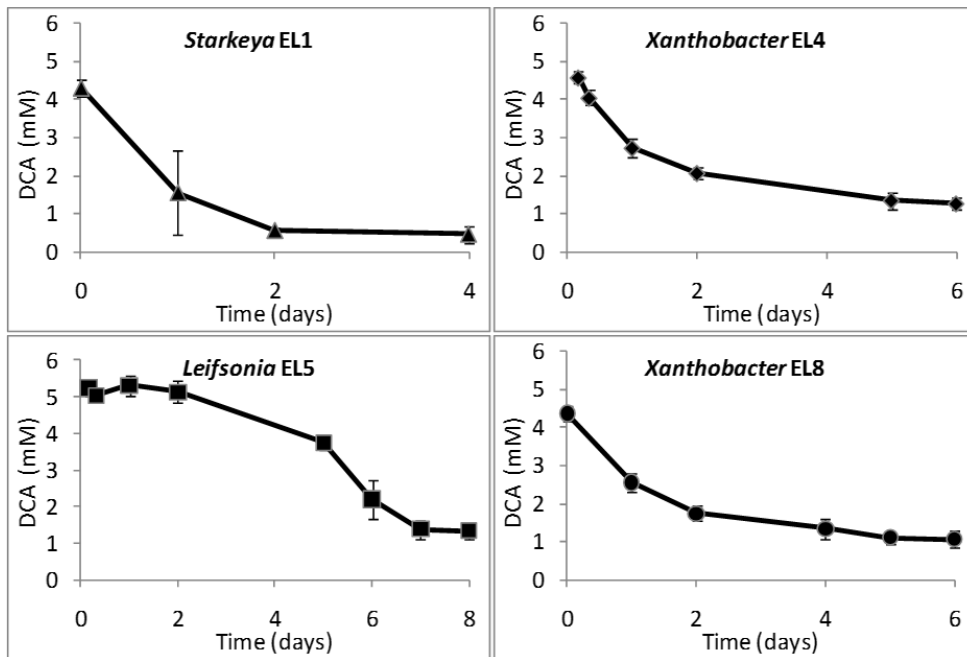


1 **A new catabolic plasmid in *Xanthobacter* and *Starkeya* spp.**
2 **from a 1,2-dichloroethane-contaminated site**

3

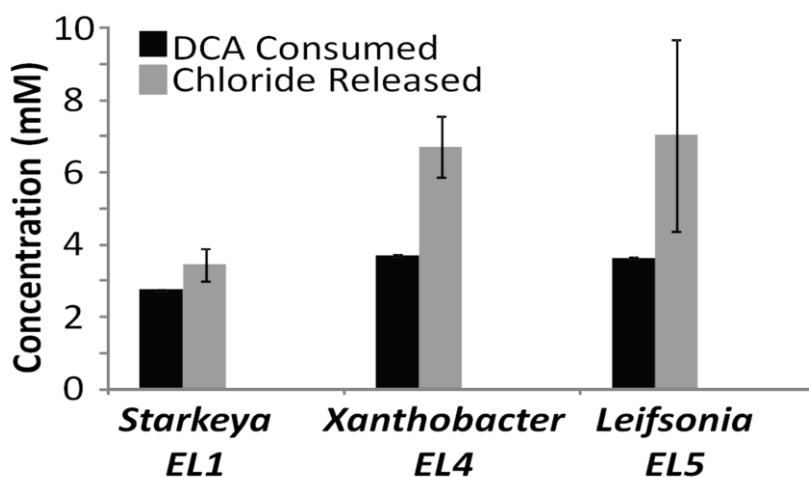
4 **Supplemental Material**



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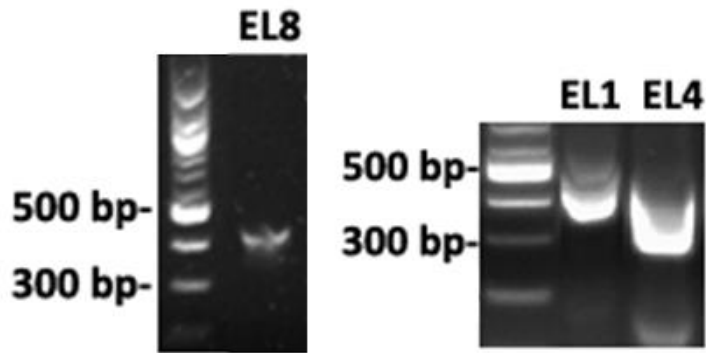
6 **Supplementary Figure 1:** Biodegradation of DCA by isolates EL1, EL4, EL5, and EL8. Values
7 shown are the average of three replicates, and the error bars indicate one standard deviation.
8 No significant DCA losses occurred from abiotic controls (data not shown).

9



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11 **Supplementary Figure 2:** Chloride production from DCA by isolates EL1, EL4 and EL5. Values
12 shown are the average of three replicates, error bars indicate one standard deviation. Due to
13 the high phylogenetic similarity of EL4 and EL8, EL4 was chosen as a representative
14 *Xanthobacter* sp. for this experiment.

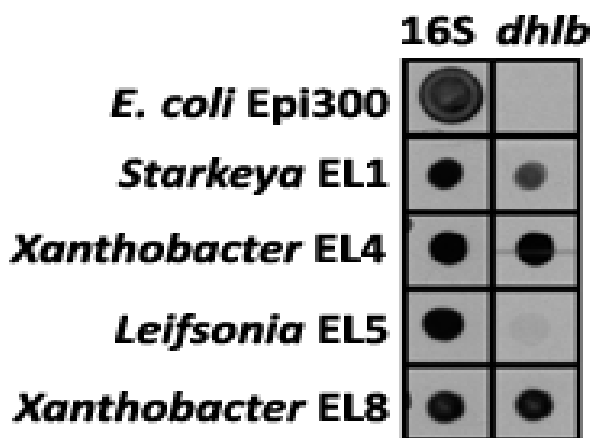


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17 **Supplementary Figure 3.** Detection of *dhIA* genes in isolates EL8, EL1, and EL4 by PCR. The
 18 corresponding PCRs from strain EL5 (not shown) yielded many bands, but none were the
 19 correct size. Sequencing of cloned PCR products arising from *dhIA* PCRs using strain EL5 DNA
 20 indicated that none of these were *dhIA* or any other recognisable dehalogenase.

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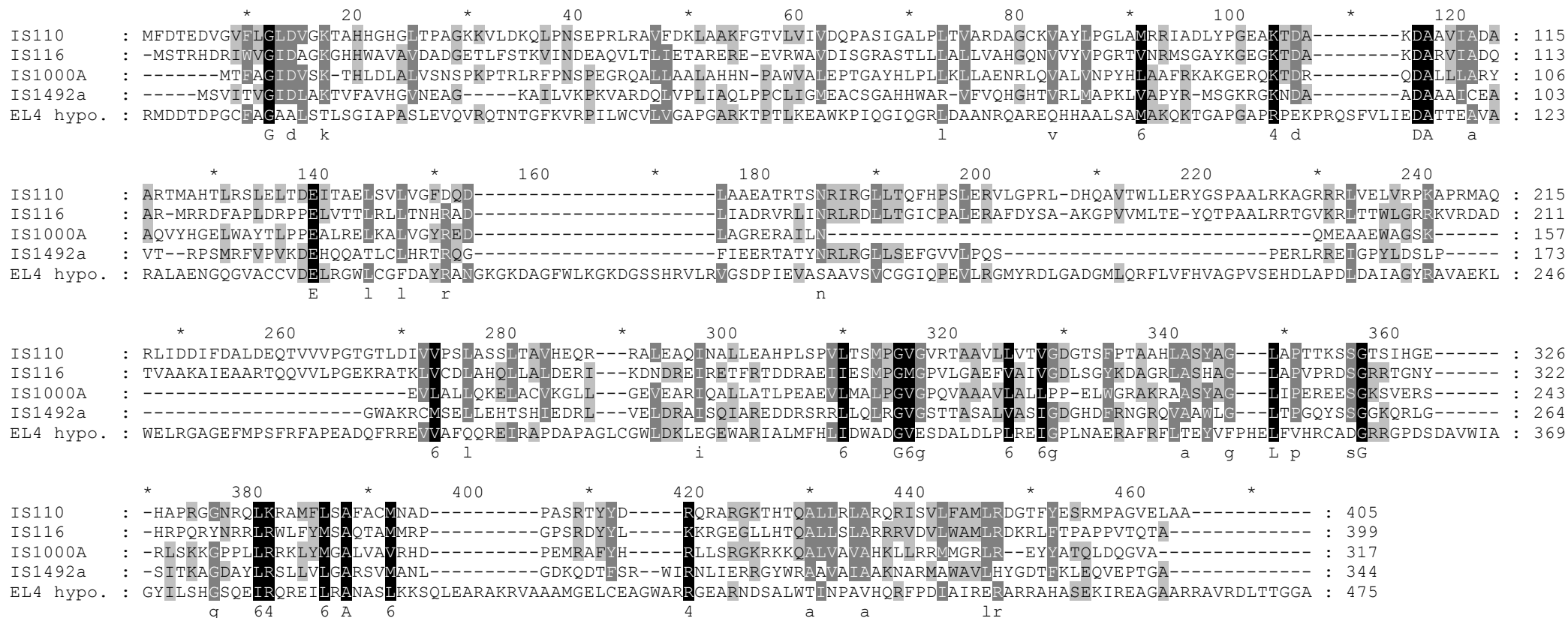


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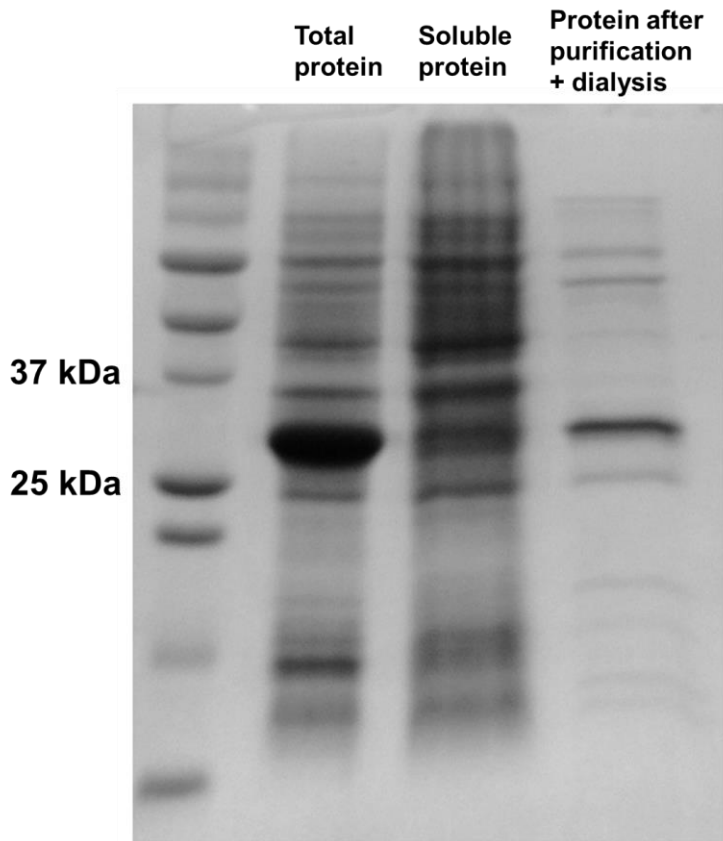
24 **Supplementary Figure 4.** Detection of *dhIB* genes in strains EL1, EL4, EL5, and EL8 by
 25 hybridization. The left column is a positive control hybridization with a 16S rDNA probe, to
 26 ensure the membrane was loaded with sufficient DNA. The *E.coli* DNA was included as a
 27 negative control, known to lack *dhIB*.

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Supplementary Figure 5. Alignment of isolate EL4 hypothetical protein upstream of *dhIB1* with transposases from the IS110 family. Note that for space considerations, 327 amino acids which did not align to the other sequences have been trimmed from the N-terminus of the EL4 protein. Residues shaded black indicate a similar amino acid is present at that position in 100% of all sequences, while dark grey shading indicates a similar amino acid in 80% of sequences, and light grey indicates amino acid similarity in 60% of sequences.



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38 **Supplementary Figure 6.** Expression and purification of His-tagged DhIB2 in *E.coli* BL21(DE3).

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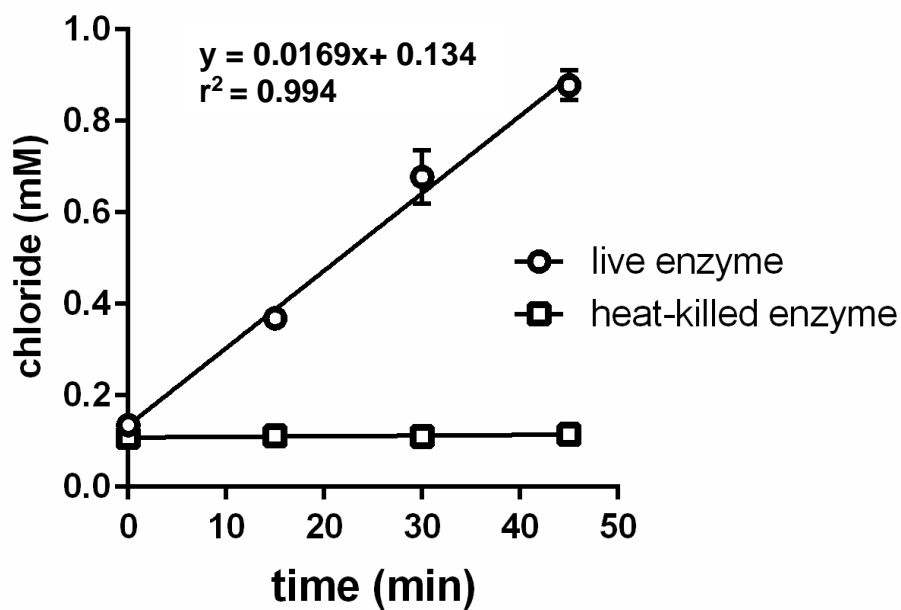
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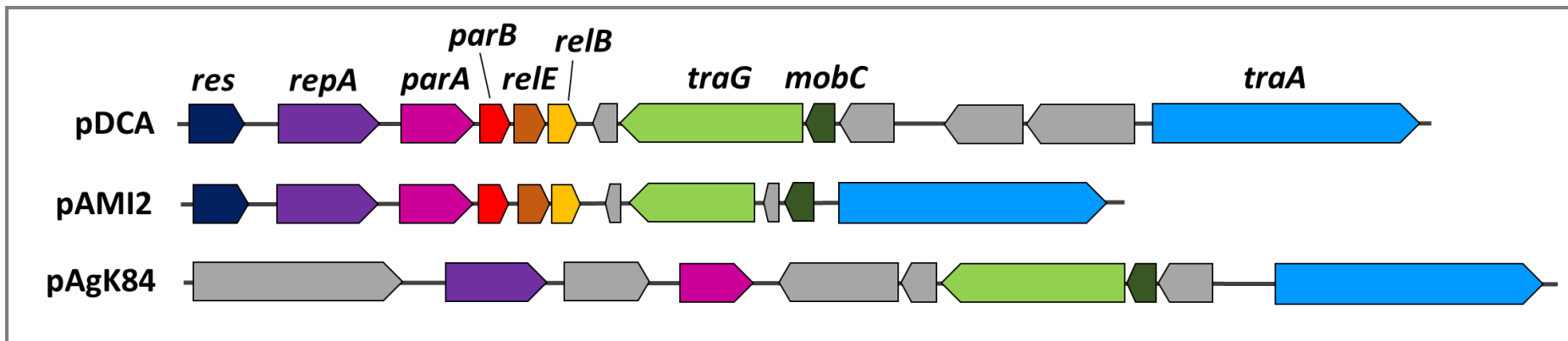
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51 **Supplementary Figure 7.** Testing the activity of purified 6xHis-DhIB2 protein via release of

52 inorganic chloride from chloroacetate. Each reaction contained 2 μ g protein.

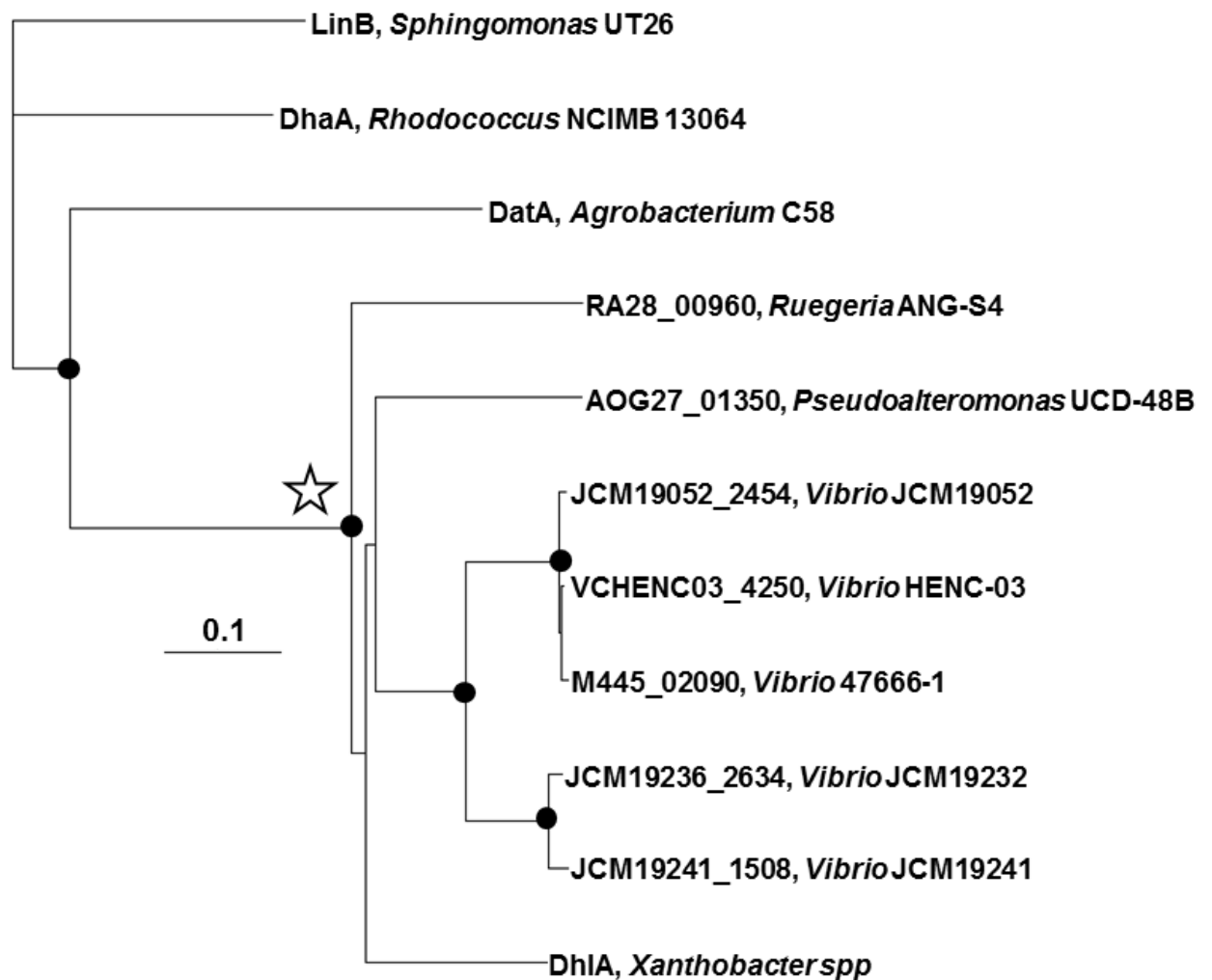


Supplementary Figure 8. Comparison of arrangement of backbone genes (replication, stability, mobilization) in pDCA, pAMI2, and pAgK84.

Note that the colour coding used in this Figure is different to that used in the plasmid Figures in the main part of the article.

Supplementary Table 1. Details of representative pDCA RepA homologs in GenBank

Plasmid name and size	Bacterium name	Accession number	% amino acid identity to RepA of pDCA
unknown	<i>Ochrobactrum rhizosphaerae</i> strain SJY1	WP_024899702	100%
unknown	<i>Paracoccus</i> sp., strain TRP	WP_010399272	92%
unknown	<i>Agrobacterium radiobacter</i> strain S2	CDN96155	90%
unknown	<i>Nitratireductor aquibiodomus</i>	WP_025031071	90%
unknown	<i>Enterobacter cloacae</i>	KVJ95762	89%
unknown	<i>Sphingobium ummariense</i> strain RL-3	EQB33664	88%
pSKYE1, 37 kb	Unknown (uncultured)	BAH89412	88%
unknown	<i>Mesorhizobium</i> sp., strain Root552	KQZ25757	83%
pAMI2, 19 kb	<i>Paracoccus aminophilus</i> strain JCM 7686	ABI48961	79%
unknown	<i>Rhizobium</i> sp., strain NT-26	CCF22396	66%
pAgK84, 44 kb	<i>Agrobacterium radiobacter</i> strain K84	ACM31469	53%
unnamed, 1300 kb	<i>Methylobacterium extorquens</i> strain AM1	ACS43739	78%
unknown	<i>Sphingomonas</i> sp., strain LH128	EJU14995	42%
pTAR, 44 kb	<i>Agrobacterium tumefaciens</i> strain 1D1422	AAD15307	34%
unnamed, 33 kb	<i>Zymomonas mobilis</i> strain CP4	ACE07206	32%



1

2 **Supplementary Figure 9.** Phylogeny of DhIA from *Xanthobacter* spp, compared to uncharacterised
3 homologs in marine bacteria (*Vibrio*, *Ruegeria*, *Pseudoalteromonas*), and representative biochemically-
4 characterised enzymes (LinB, DhaA, and DatA). The tree was prepared from a 249 aa alignment
5 (including gaps), generated in ClustalX, which gave a consensus tree via the neighbour-joining
6 method. The tree was visualised with TreeView, and manually annotated using GIMP. Filled
7 circles at nodes indicate bootstrap values >95%, and nodes with no symbol have bootstrap
8 values <75%. The star indicates a node which separates a clade including DhIA and the
9 'marine' dehalogenases in the tree from all other haloalkane dehalogenase enzymes in
10 GenBank (represented here by LinB, DhaA, and DatA).