

Supplementary material

Biochemical Characteristics of the Novel Haloalkane Dehalogenase DatA Isolated from the Plant Pathogen *Agrobacterium tumefaciens* C58

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a b c
TTG AAG GAA CAC CGT CAC ATG ACT GAG AAA TCA CCA CAT TCC GCC TTT GGT GAC GGC GCG AAA GCG TAT GAT GTG CCG
L K E H R H M T E K S P H S A F G D G A K A Y D V P
(M) (M)

FIG. S1. Prediction of the start codon of the *datA* gene. The start codon of *datA* has been predicted differently as a (B9JPJ9 and Q8U671) or c (*atu6064* of AE009425; AE008690-65). However, we predicted it as b on the basis of comparison with other haloalkane dehalogenases and the existence of most probable SD sequence upstream of our predicted start codon (underlined). The nucleotide sequence of our newly annotated *datA* gene has been deposited to database under accession number AB478945, and was used in this study.

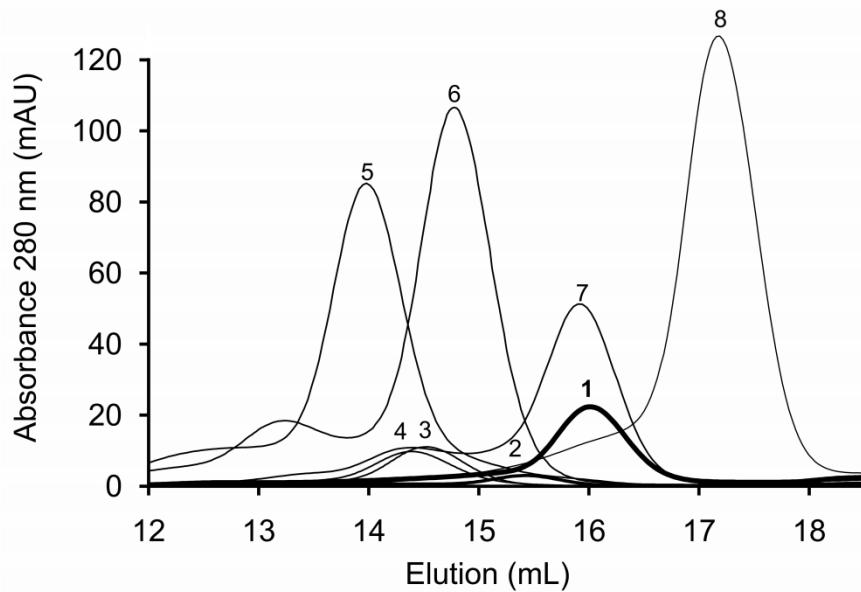


FIG. S2. Gel filtration chromatogram of DatA, DhaA, DbjA, DbeA, and four proteins from the calibration kit. (1) DatA (34 kDa), (2) DhaA (35 kDa), (3) DbjA (30 kDa), (4) DbeA (34 kDa), (5) conalbumin (75 kDa), (6) ovalbumin (67 kDa), (7) carbonic anhydrase (29 kDa), and (8) ribonuclease (14 kDa). All proteins were analyzed at 4°C, using 50 mM Tris-HCl containing 150 mM of NaCl of pH 7.5 as the elution buffer. The concentrations of samples were approximately 2 mg ml⁻¹.

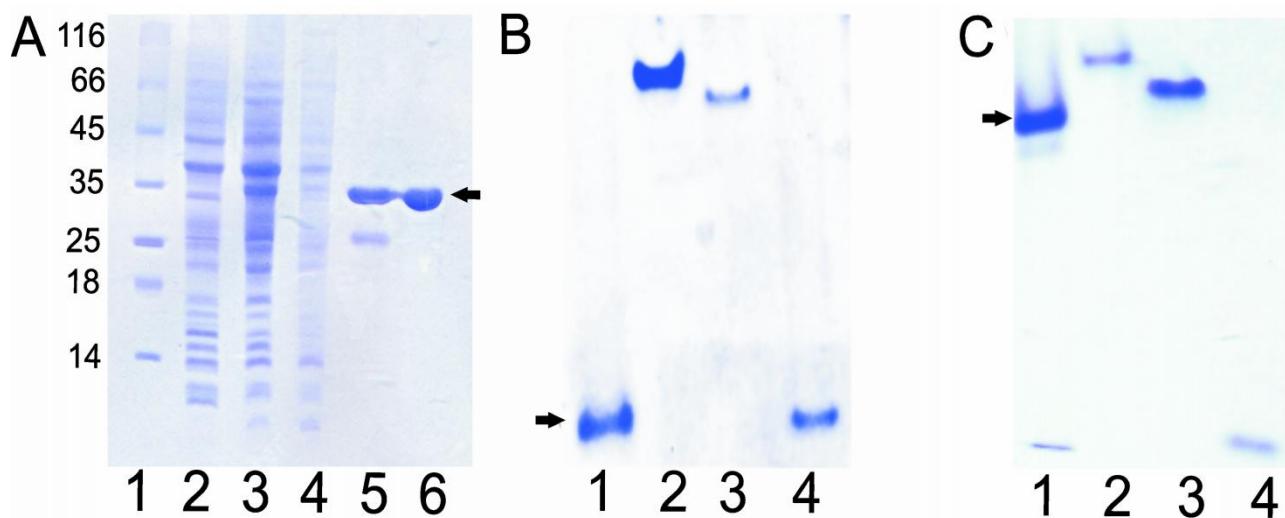


FIG. S3. (A) SDS gel electrophoresis of DataA. Lane 1, molecular weight marker; lane 2, before IPTG induction; lane 3, after IPTG induction; lane 4, crude extract; lane 5, fractions pooled from anion exchange chromatography; and lane 6, fractions pooled from affinity chromatography with Ni-nitrilotriacetic acid. Native gel electrophoresis of DataA (B) with NaCl and (C) without NaCl. Lane 1, DataA; lane 2, DbeA (dimer); lane 3, DbjA (dimer); lane 4, DhaA (monomer). DataA bands are indicated by the arrows.

TABLE S1. Protein sequence identities of biochemically characterized haloalkane dehalogenases.^a

Protein	DatA	DhaA	LinB	DmbA	DmlA	DbjA	DhmA	DmbB	DhlA	DrbA	DmbC
DatA	-	35	34	34	34	31	23	25	21	22	21
DhaA	35	-	48	43	49	47	27	27	24	22	25
LinB	34	48	-	68	42	37	25	24	21	24	25
DmbA	34	43	68	-	37	36	24	24	19	25	24
DmlA	34	49	42	37	-	55	22	21	20	23	24
DbjA	31	47	37	36	55	-	21	21	19	19	22
DhmA	23	27	25	24	22	21	-	82	35	21	28
DmbB	25	27	24	24	21	21	82	-	35	21	27
DhlA	21	24	21	19	20	19	35	35	-	20	20
DrbA	22	22	24	25	23	19	21	21	20	-	25
DmbC	21	25	25	24	24	22	28	27	20	25	-

^a Sequence identities are given in %. DatA (this study; *Agrobacterium tumefaciens* C58; accession number AB478945), DhaA (Kulakova *et al.* 1997; *Rhodococcus rhodochrous* NCIMB 13064; accession number AAC15838), LinB (Nagata *et al.* 1993; *Spingobium japonicum* UT26; accession number P51698), DmbA (Jesenska *et al.* 2005; *Mycobacterium bovis* 5033/66; accession number AJ784273), DmlA (Sato *et al.* 2005; *Mesorhizobium loti* MAFF303099; accession number NP_106032), DbjA (Sato *et al.* 2005; *Bradyrhizobium japonicum* USDA110; accession number NP_767727), DhmA (Jesenska *et al.* 2002; *Mycobacterium avium* N85; accession number AJ314789), DmbB (Jesenska *et al.* 2005; *Mycobacterium bovis* 5033/66; accession number AJ784273), DhlA (Keuning *et al.* 1985; *Xanthobacter autotrophicus* GJ10; accession number AAA88691), DrbA (Jesenska *et al.* 2009; *Rhodopirellula baltica* SH1; accession number AM696289), DmbC (Jesenska *et al.* 2009; *Mycobacterium bovis* 5033/66; accession number AM696288).

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