Engineering the soil bacterium *Pseudomonas putida* for arsenic removal

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Supplemental materials:

Abbreviations: polymerase chain reaction, PCR; sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE); high pressure liquid chromatography (HPLC); inductively coupled plasma mass spectroscopy (ICP-MS).

Supplemental Figure Legends:

Supplemental Fig. 1S. **Methylation of As(III) by purified CrArsM**. **A:** Transformation of 10 μM As(III) into soluble methylated species was determined by high pressure liquid chromatography (HPLC) coupled to inductively coupled mass spectroscopy (ICP-MS) using a C18 reverse phase column after incubation for various times with 1 μM purified CrArsM. Curve 1, standards. Curve 2: 0 time; Curve 3: 7 h; Curve 4: 14 h. **B:** Production of volatile species determined by anion exchange HPLC-ICP-MS analysis. Curve 1, 0 time; Curve 2: 7 h; Curve 3: 14 h.

Supplemental Fig. 2S. **Construction of plasmid pBAM1-P_{km}-CrArsM**. The gene for CrArsM with a C-terminal six-histidine tag was cloned from a *C reinhardtii* cDNA library using forward primer *arsM*-F 5'-GGAATTCCATATGGTGGAGCCGGCTTCC ATCGCGGAGCTT-3' (*Nde*I site underlined) and reverse primer *arsM*-R 5'-CCGCTCGAGTTAATG ATGATGATGATGATGGCAGCAGGCGCCGCCGGGG-3' (*Xho*I site underlined). The polymerase chain reaction (PCR) fragment was gel purified and cloned into vector plasmid pET28a vector with the indicated restriction enzymes, generating plasmid pET28a-*CrArsM*.

The kanamycin promoter was cloned from pBAM1 vector using forward primer 5'-GAAGATCTTGTCTC TTATACACATCTGACG-3' (*Bgl*II site underlined) and reverse primer 5'-GGAATTCCATATGAA CACCCCT TGTATTAC-3' (*Nde*I site underlined). The T7 promoter in pET28a-*CrArsM* was replaced with the kanamycin promoter, generating plasmid pET28a-P_{km}-*CrArsM*. Finally, a fragment contained the kanamycin promoter and *arsM* was excised by digestion with *Bgl*II and *Xho*I and ligated into vector plasmid pBAMI vector that had been digested with *BamH*I and *Sal*I, generating plasmid pBAM1-P_{km}-*CrArsM*. Functional elements of the plasmid include relevant restriction sites, transposase (tnpA), origin of replication (R6K), the origin of transfer region (oriT), mosaic element O (ME-O), and mosaic element I (ME-I), as shown.

Supplemental Fig. 3S. **Purification of CrArsM from *E. coli*.** A culture of *E. coli* BL21(DE3) pET28a-CrArsM was induced overnight with 0.3 mM isopropyl β-D-1-thiogalactopyranoside and lysed by a single passage through a French pressure cell. Following high speed centrifugation, the cytoplasmic solution was chromatographed on a Ni-NTA column, and the fractions analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) stained with Coomassie Blue. Lane 1: Standard protein markers. Lane 2-8: Successive column fractions eluted with an imidazole gradient. Lane 9, Cells of *E. coli* BL21(DE3) pET28a-CrArsM without induction. Lane 10, Cells induced with 0.3 mM isopropyl β-D-1-thiogalactopyranoside.

Supplemental Fig. 4S. **Immunoblot detection of CrArsM**. Cultures of both *E. coli*

BL21(DE3) pET28a-CrArsM induced with 0.3 mM isopropyl β-D-1-thiogalactopyranoside and *P. putida* pBAM1-Pkm-CrArsM were grown for 6 h in LB medium, centrifuged and suspended in 1/10 the original volume of SDS sample gel buffer and boiled for 5 m. Portions (5 µl) were separated by SDS-PAGE on a 12% polyacrylamide gel, transferred to a nitrocellulose membrane and electrophoresed at 25 V overnight. After transfer, the membrane was blocked with 5% nonfat dried milk in physiological saline buffer for 4 hrs with shaking. The membrane was incubated with the primary His-tag monoclonal antibody (anti-mouse cat. No. 70796-3 diluted 1:1000 (Novagen-EMD Millipore) in 5% blocking buffer for 1 hrs at room temperature. The membrane was washed four times with the same buffer containing 0.05% Tween 20 for 25 min for each time. The membrane was then incubated with secondary antibody (peroxidase-coupled anti-mouse IgG, Sigma Chemical Co.) diluted 1:5000 for 1 hrs at room temperature and washed with the same buffer 4 times for 25 min each time. The proteins were detected using a Western Lightning™ Ultra chemiluminescent substrate kit (ECL Kit, 2 ml/membrane). In a separate tube, the solutions were mixed in a 1:1 ratio and poured onto the membrane for 1 min before exposure.

Lane 1, Standard protein markers. Lane 2, vector plasmid pET28a in BL21(DE3), Lane 3, vector plasmid pBAM1 in *P. putida*. Lane 4, *arsM* in plasmid pET28a expressed in BL21(DE3) with IPTG. Lane 5, *arsM* chromosomally expressed in *P. putida*.

Fig. 1S_A

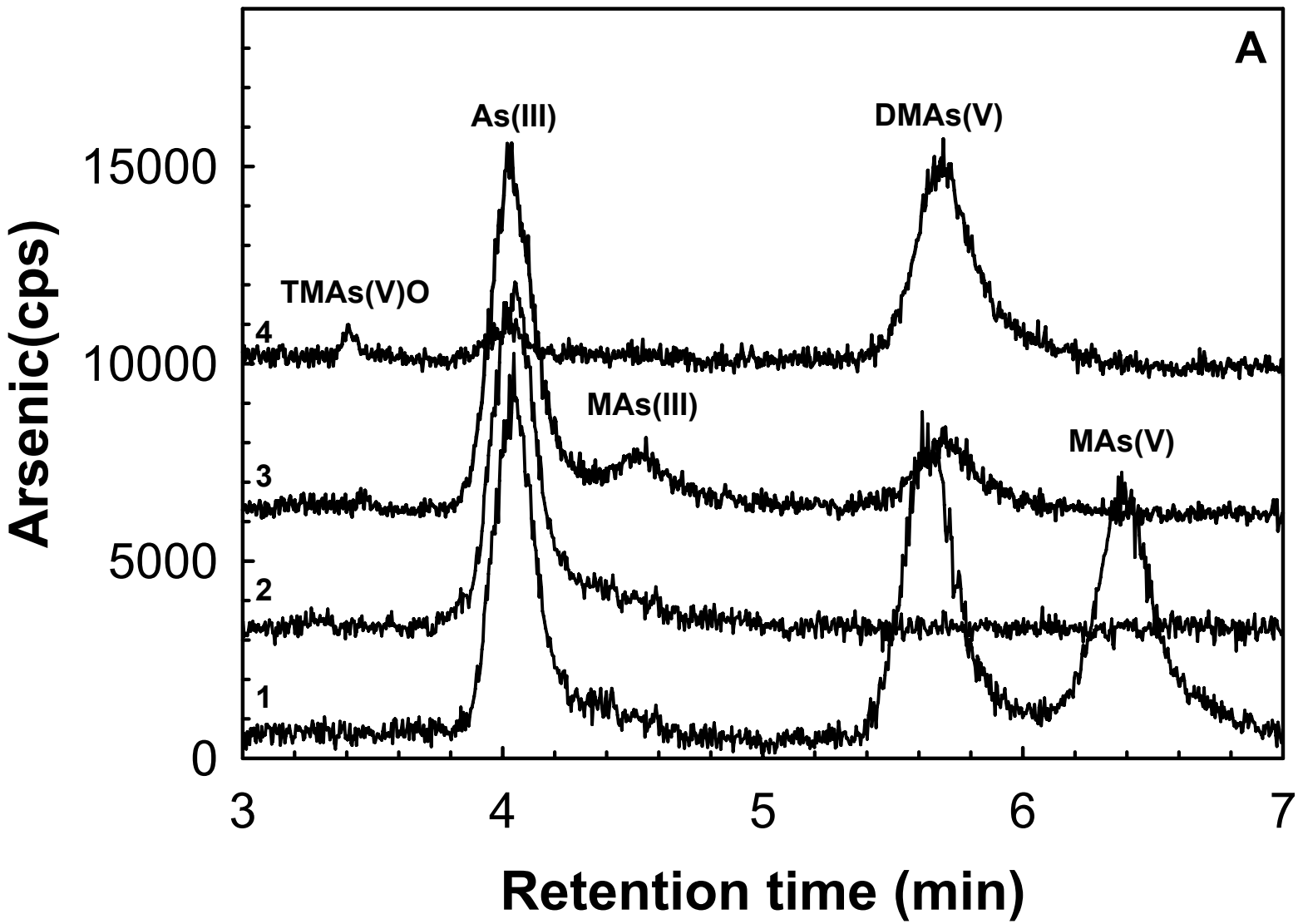
A
As(III)
DMAs(V)
TMAs(V)O
MAs(III)
MAs(V)
4
3
2
1
15000
10000
5000
0
3
4
5
6
7
Arsenic(cps)
Retention time (min)

Fig. 1S_B

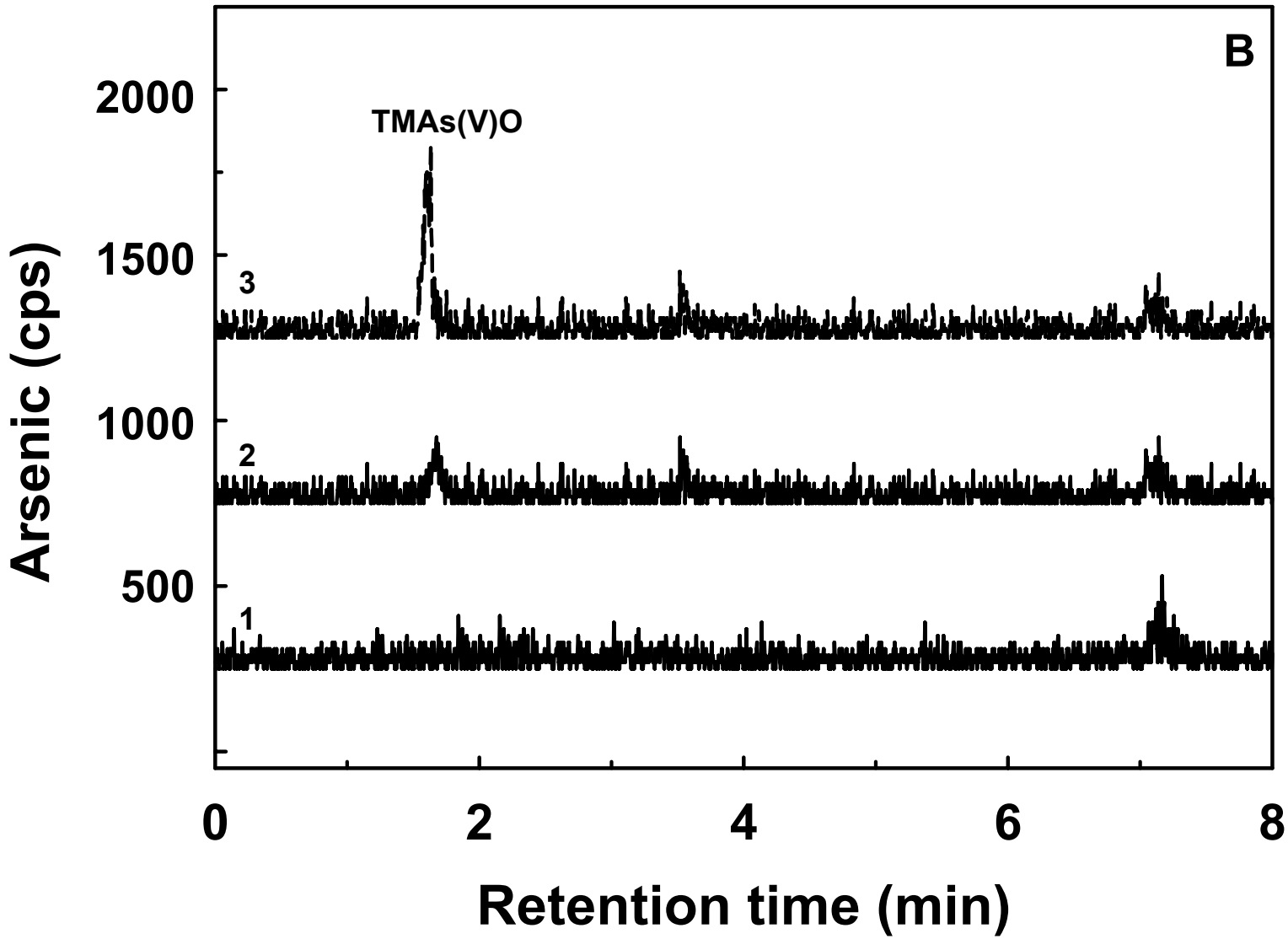
B
TMAs(V)O
3
2
1
2000
1500
1000
500
Arsenic (cps)
0
2
4
6
8
Retention time (min)

Fig. 2S

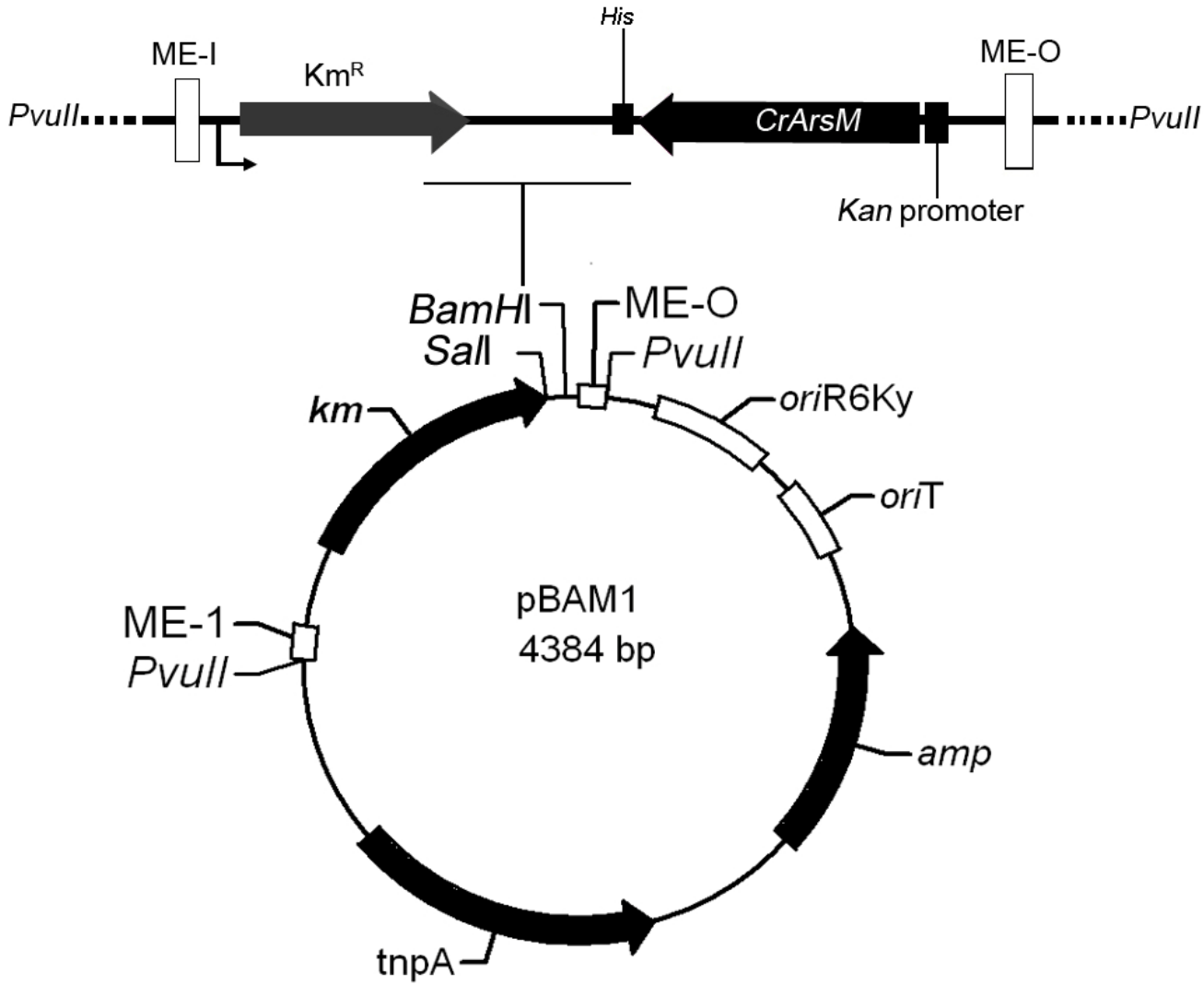
His
ME-I
Km^R
ME-O
PvuII
CrArsM
PvuII
Kan promoter
BamHI
SalI
ME-O
PvuII
km
oriR6Ky
oriT
pBAM1
4384 bp
ME-1
PvuII
amp
tnpA

Fig. 3S

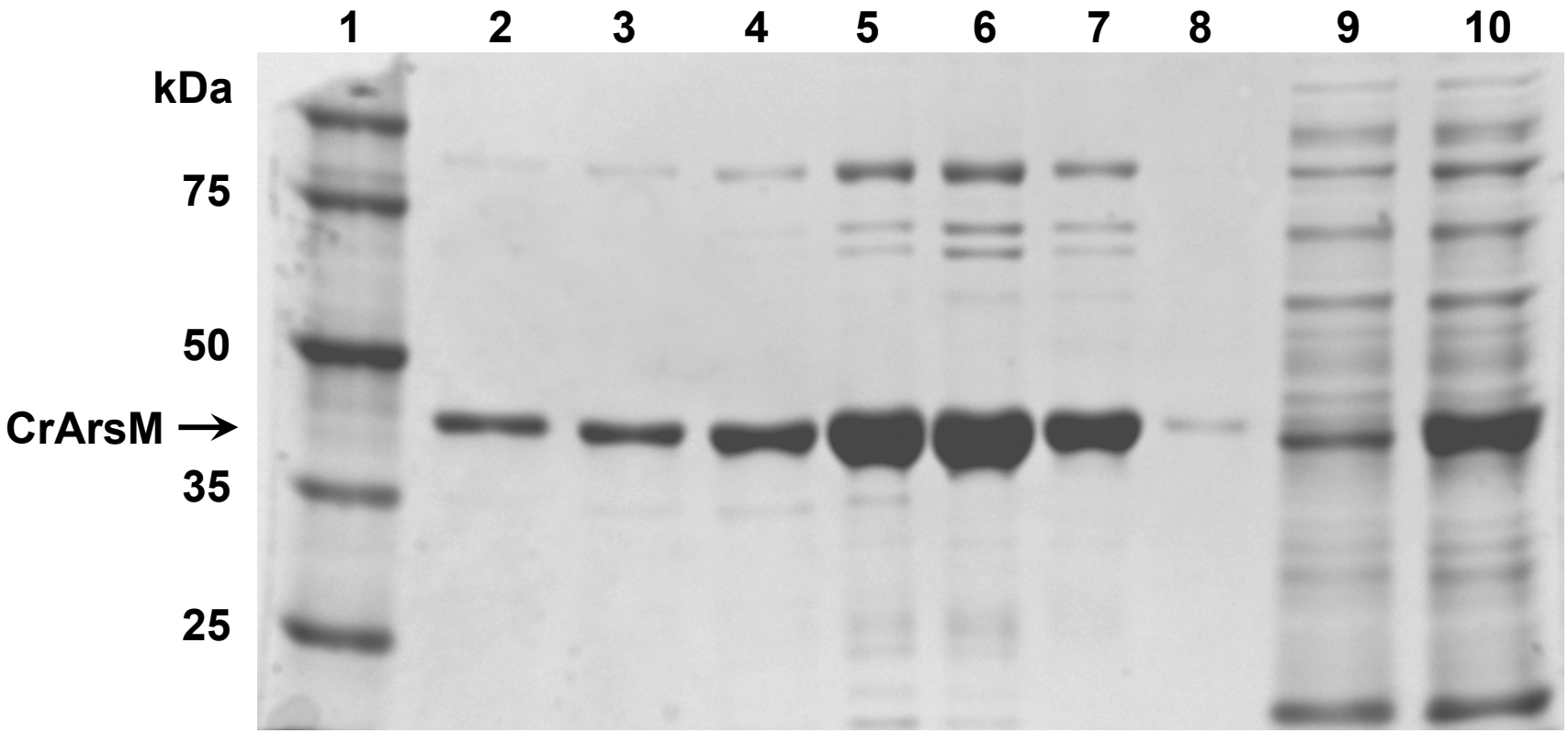

Fig. 4S