Supporting information

for

Arsenobetaine: an ecophysiological important organoarsenical confers cytoprotection against osmotic stress and growth temperature extremes

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Running title: Stress protection by and synthesis of arsenobetaine

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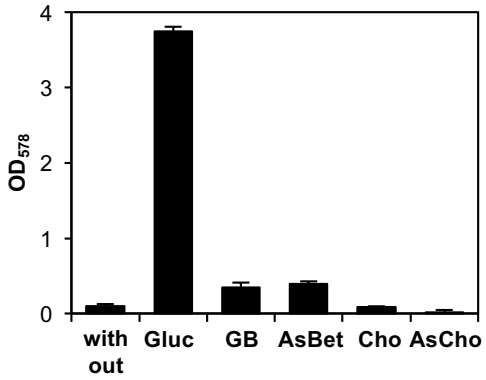

Fig. S1. Glycine betaine, choline or their arsenic derivatives, cannot be used as sole carbon source by *B. subtilis*. Cells of the wild-type strain JH642 were cultivated in shake flasks containing SMM without a carbon source, or with 28 mM (final concentration) of either glucose (Gluc), glycine betaine (GB), arsenobetaine (AsBet) choline (Cho) or arsenocholine (AsCho), respectively. Growth yields of the cultures were determined after 12 h of incubation in a shaking water bath set to 220 rpm at 37 °C. The data represent the mean and the standard deviation of three independently grown cultures.

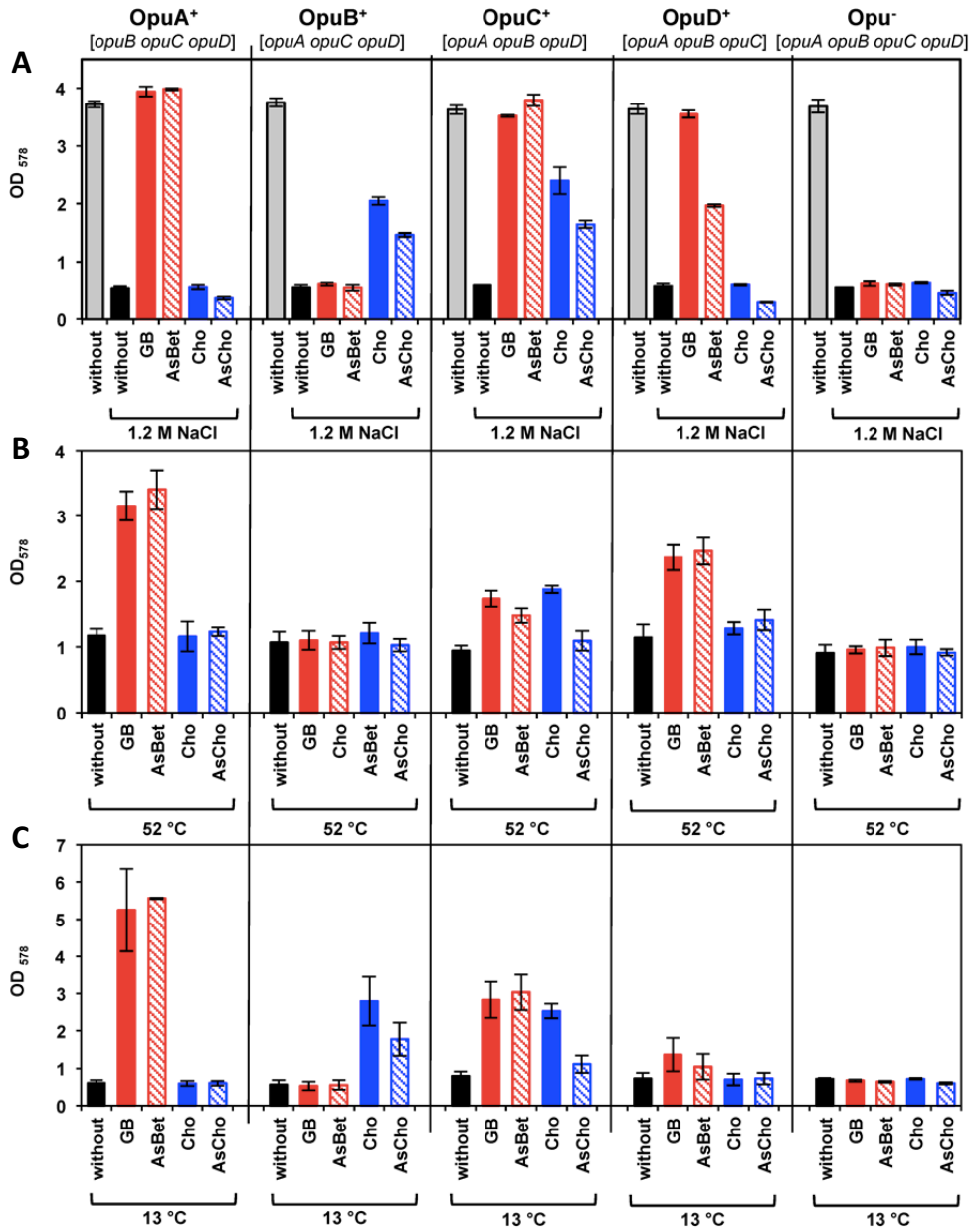

Fig. S2. Genetic identification of the Opu uptake systems responsible for the uptake of glycine betaine (GB), arsenobetaine (AsBet), choline (Cho) or arsenocholine (AsCho) under osmotic or temperature stress conditions. (A) Growth yields of cultures grown in the absence (grey bars), or presence of 1.2 M NaCl without (black bars) or with (colored bars) the addition of 1 mM of the indicated compatible solutes. The growth yields of the cultures were measured after 14 hours of incubation at 37° C. (B) Growth yields of cultures grown at 52° C without (black bars) or with (colored bars) the addition of 1 mM of the indicated compatible solutes; the growth yields were measured after 16 hours of incubation. (C) Growth yields of cultures grown at 13° C without (black bars) or with (colored bars) the addition of 1 mM of the indicated compatible solutes measured after seven days of cultivation. The growth yields were measured for a set of *opu* mutants derived either from the *B. subtilis* wild-type strain JH642 (A, B), or from the wild-type strain 168 (C). The used strains express only one of the indicated Opu transporters; all strains possess the osmotically controlled proline-specific uptake system OpuE. The values shown are the means and standard deviations of three independently grown cultures.

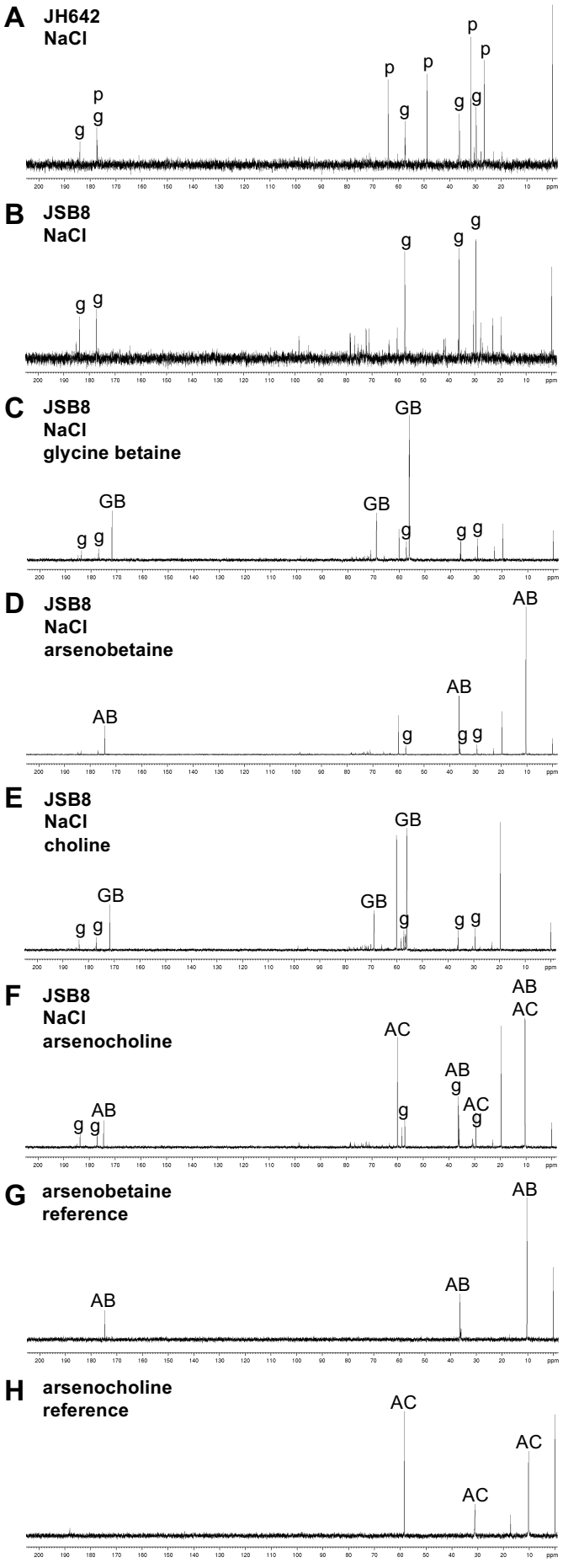

Fig. S3. Detection of accumulated arsenobetaine and arsenocholine in the cytoplasm of *B. subtilis*. ^{13}C-NMR spectra of ethanolic cell extracts of the *B. subtilis* wild-type strain JH642 and its *proHJ* mutant derivative JSB8. Cells were grown in SMM containing 1.2 M NaCl either without compatible solute (A, B, in the presence of glycine betaine (C), arsenobetaine (D), choline (E), or arsenocholine (F). These solutes were added to the cultures at a final concentration of 1 mM. ^{13}C-NMR spectra of arsenobetaine (G) and arsenocholine (H) were recorded as a reference. The resonance signals for glycine betaine (GB), arsenobetaine (AB), arsenocholine (AC) or glutamate (g) are indicated. The ^{13}C-NMR spectrum of an extract of the *B. subtilis* wild type strain JH642 grown at 1.2 M NaCl (A) serves as reference for the detection of glutamate (g) and proline (p). Strain JSB8 is a *proHJ* mutant and is therefore unable to synthesize osmostress protective proline pools.

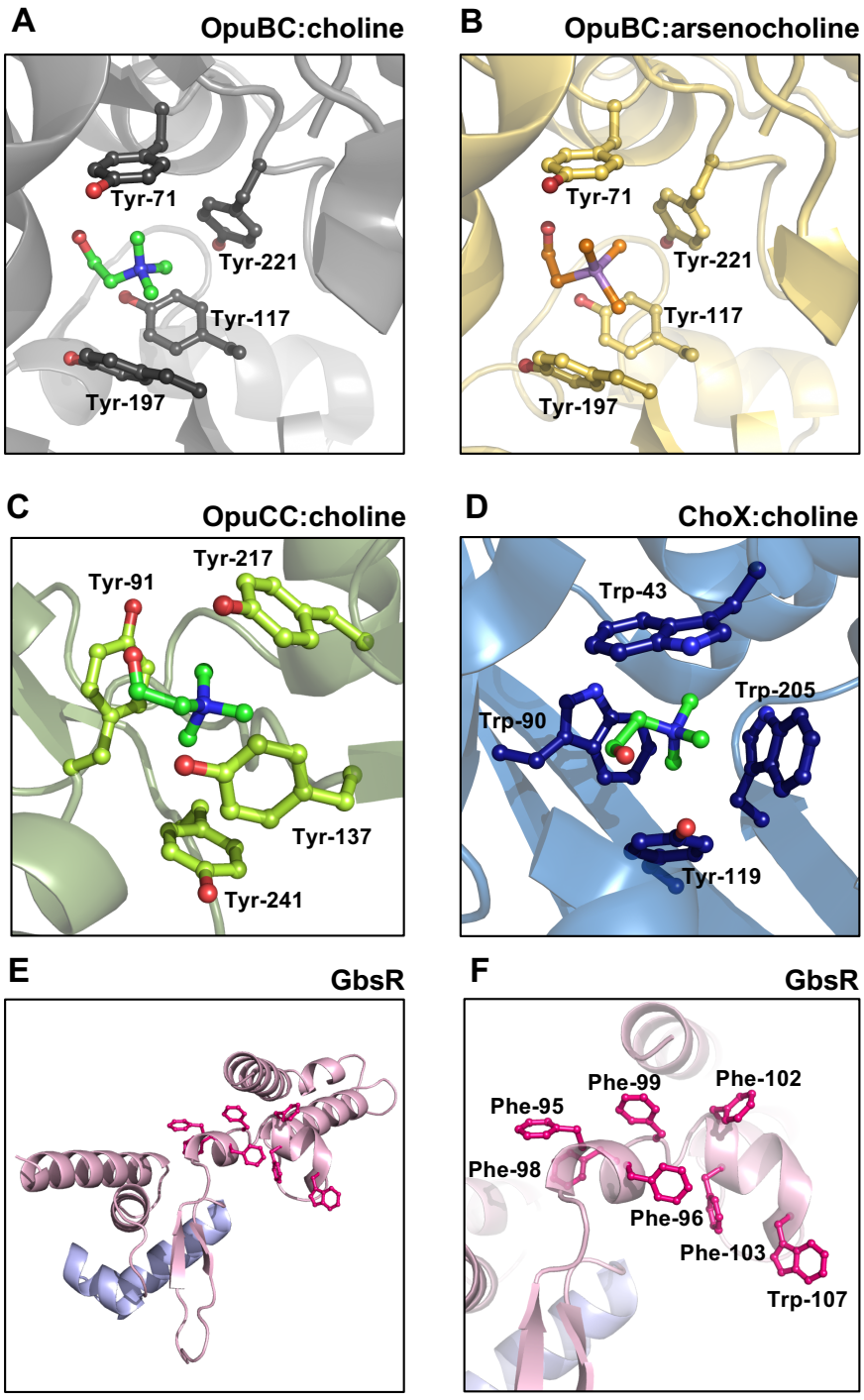

FIG. S4. Architecture of aromatic ligand binding boxes for choline and arsenocholine. Shown are the crystal structures of the ligand binding protein OpuBC of *B. subtilis* in complex with either (A) choline (PDB code: 3R6U), or (B) arsenocholine (PDB code: 5NXY), and of the ligand binding protein OpuCC of *B. subtilis* (C) (PDB code: 3PPQ), and of ChoX from *Sinorhizobium meliloti* (D) (PDB code: 2REG) with bound choline, respectively. The aromatic residues involved in cation-Π interactions with the various ligands are presented as sticks. In (E) and (F), an *in silico* derived model of a monomer of the dimeric GbsR repressor protein from *B. subtilis* is shown. This *in silico* model was obtained through a comparison with the crystal structure of the *Methanococcus jannaschii* Mj223 protein (pdb code: 1KU9). An overview (E) and the central region (F) of the model is shown. Those aromatic amino acid residues that might interact via cation-Π interactions with the fully methylated headgroups of either choline or arsenocholine are represented as sticks. The DNA-binding region present in the N-terminal domain of the GbsR repressor protein is shown in light blue.

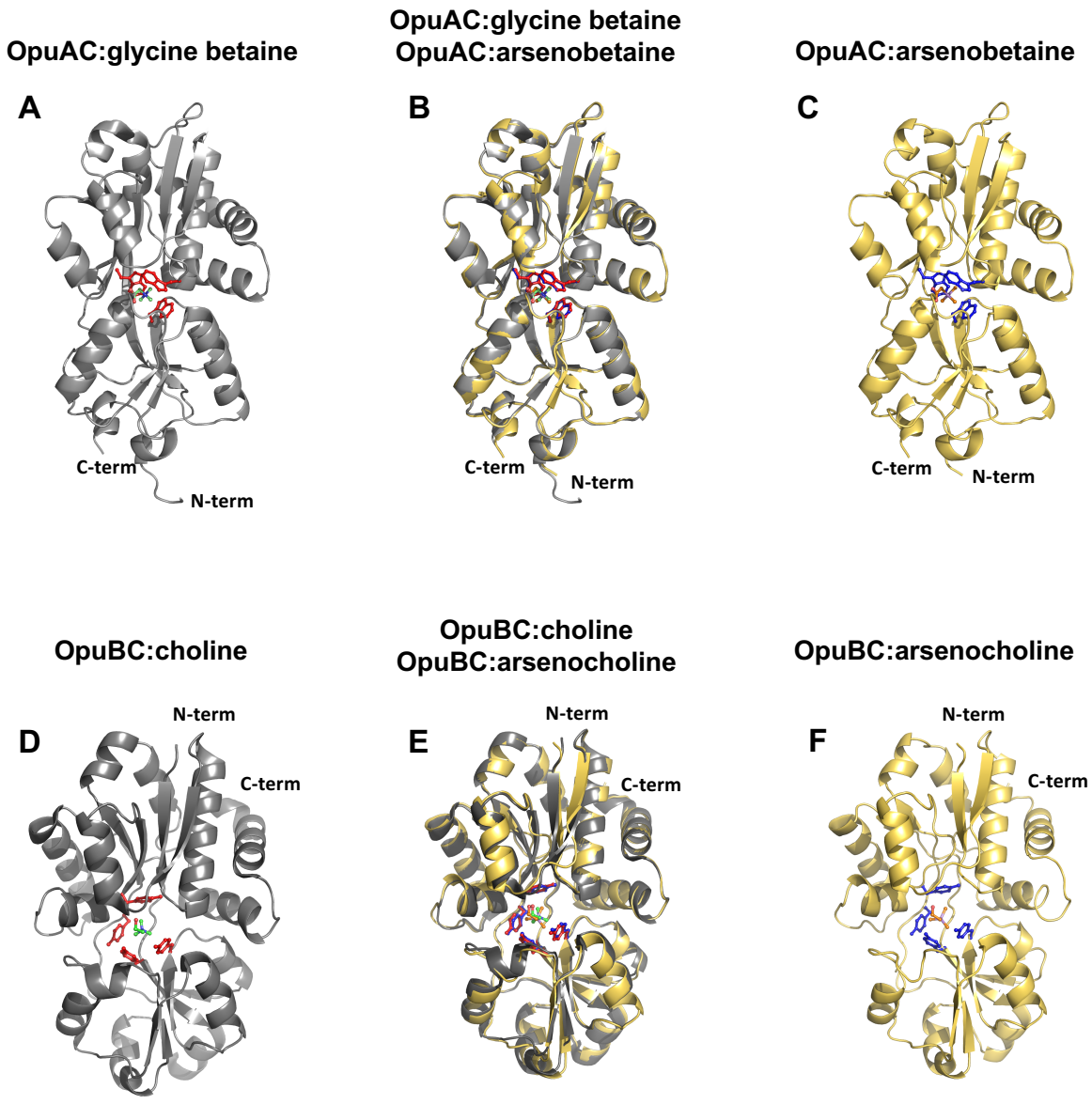

FIG. S5. Crystal structures of the solute binding proteins OpuAC and OpuBC. The OpuAC protein structures in complex with either (A) bound glycine betaine (green) or (C) arsenobetaine (orange); an overlay of both structures is shown in (B). The three tryptophan residues forming the aromatic ligand-binding boxes for either glycine betaine or arsenobetaine are presented as sticks. The OpuBC protein structures in complex with either (D) choline (green) or (F) arsenocholine (orange); an overlay of both structures is shown in (E). The four tyrosine residues forming the aromatic ligand-binding boxes for either choline or arsenocholine are presented as sticks. Structural data for these crystal structures are deposited at the RCSB protein data base with the following PDB codes: 2B4L (OpuAC::glycine betaine), 5NXX (OpuAC::arsenobetaine), 3R6U (OpuBC:choline) and 5NXY (OpuBC:arsenocholine).

Table S1. *B. subtilis* strains used in this study

Strain	relevant genotype	source or reference
JH642	*trpC2 pheA1*	J. Hoch; BGSC* 1A96
168	*trpC2*	BGSC* 1A1
JBB5	JH642 ∆(*gbsAB*::*neo*)2	(Boch et al., 1996)
GNB45	JH642 Δ(*treA*::*erm*)*2* [*amyE*::(Δ*gbsR*)2-Φ(*gbsA´-treA*)1]	Nau-Wagner
GNB46	JH642 Δ(*treA*::*erm*)2 Δ(*gbsAB*::*neo*)2 [*amyE*::(Δ*gbsR*)2- Φ(*gbsA´-treA*)1]	Nau-Wagner
GNB48	JH642 Δ(*treA*::*erm*)2 (*gbsR*::*neo*)1 [*amyE*::(Δ*gbsR*)2-Φ(*gbsA´-treA*)1]	Nau-Wagner
RMKB20	JH642 ∆(*opuA::erm*)4 *opuC-20*::Tn*10*(*spc*) ∆(*opuD::neo*)2	(Kappes et al., 1996)
RMKB22	JH642 ∆(*opuA::erm*)4 *opuB-20*::Tn*10*(*spc*) ∆(*opuD::neo*)2	(Kappes et al., 1996)
RMKB24	JH642 ∆(*opuA::erm*)4 ∆(*opuBD::tet*)23 *opuC-20*::Tn10(*spc*) ∆(*opuD::neo*)*2*	(Kappes et al., 1996)
RMKB33	JH642 ∆(*opuA::erm*)4 ∆(*opuBD::tet*)23 *opuC-20*::Tn10(*spc*)	(Kappes et al., 1996)
RMKB34	JH642 ∆(*opuB::tet*)23 *opuC-20*::Tn*10*(*spc*) ∆(*opuD::neo*)2	(Kappes et al., 1996)
JGB23	168 ∆(*opuA::erm*)4 ∆(*opuBD::tet*)23 *opuC20*::Tn*10* (*spc*)	(Hoffmann et al., 2011)
JGB24	168 ∆(*opuA::erm*)4 ∆(*opuBD::tet*)23 ∆(*opuD::neo*)2	(Hoffmann et al., 2011)
JGB25	168 ∆(*opuBD::tet*)23 *opuC20::Tn10* (*spc*) ∆(*opuD::neo*)2	(Hoffmann et al., 2011)
JGB26	168 ∆(*opuA::erm*)4 *opuC20::Tn10* (*spc*) ∆(*opuD*::*neo*)2	(Hoffmann et al., 2011)
JGB27	168 ∆(*opuA::erm*)4 ∆(*opuBD::tet*)23 *opuC20*::Tn*10* (*spc*) ∆(*opuD*::*neo*)2	(Hoffmann et al., 2011)
SOB9	168 ∆(*gbsAB*::*neo*)2	this work
JSB8	JH642 ∆(*proHJ::tet*)1	(Brill et al., 2011)
TMB118	JH642 Δ(*opuA::tet*)3 Δ(*opuC::spc*)3 Δ(*opuD::neo*)2 Δ(*opuB::erm*)3	(Teichmann et al., 2017)
MBB9	JH642 (*treA::neo*)1 *amyE*::[Φ(*opuAA-treA*)1 *cat*]	(Hoffmann et al., 2013)

*BGSC: Bacillus Genetic Stock Center (Columbus, OH, USA).

Table S2. Data collection, phasing and refinement statistics for OpuAC::arsenobetaine and OpuBC:arsenocholine complexes

	OpuAC::arsenobetaine	**OpuBC::arsenocholine**
Space group	P2(1)	P2(1)
Unit cell parameters		
a, *b*, *c* (Å)	88.10 30.0 106.1	38.2, 117.4, 68.7
α, β, γ (deg)	90.0 95.6 90.0	90.0,104.0, 90.0
Data collection and processing		
Wavelength (Å)	0.8726	0.8726
Resolution (Å)	19.94 - 2.2 (2.3 - 2.2)	20 – 1.9 (2.0-1.9)
Mean redundancy	2.1 (2.0)	1.9 (1.9)
Unique reflections	27772	31660
Completeness (%)	96.1 (93.0)	95.1 (93.7)
I/δ	8.2 (1.2)	17.2 (9.7)
R_{merge} [b]	4.2 (17.8)	3.1 (6.9)
Refinement		
*R*F*c* (%)	18.3	16.6
*R*free *d* (%)	27.2	20.6
Overall B factor from Wilson	20.69	18.3
RMSD from ideal		
Bond lengths (Å)	0.008	0.007
Bond angles (deg)	1.045	1.055
Ramachandran plot		
Most favored (%)	96.8	98.0
Allowed (%)	2.8	2.0
Disallowed (%)	0.4	0.0
Model content		
Monomers/ASU	2	1
Protein residues	528	271
Ligand	2	1

Supporting references

Boch J., Kempf B., Schmid R., and Bremer E. (1996) Synthesis of the osmoprotectant glycine betaine in *Bacillus subtilis*: characterization of the *gbsAB* genes. *J Bacteriol* **178**: 5121-5129.

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