Supplementary Information

From substrate specificity to promiscuity: hybrid ABC transporters for osmoprotectants

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Table S1: Bacterial strains

Strain	Relevant genotype	Reference/origin
JH642	*trpC2 pheA1*	J. Hoch
CCB2	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery)3* Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*::*opuB*	This study
CCB3	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*::*opuC*	This study
SBB2	Δ*(opuA*::*tet*)3 Δ(*opuD*::*kan*)2 Δ(*opuB*::*ery*)1	S. Broy
SBB5	Δ(*opuA*::*tet*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2	S. Broy
TMB107	Δ(*opuA*::*tet*)3	This study
TMB108	Δ*(opuC*::*spc)*3	This study
TMB112	Δ(*opuC*::*spc*)3 Δ (*opuB*::*ery*)3	This study
TMB116	Δ(*opuB*::*ery*)1	This study
TMB118	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery)3* Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2	This study
RMKB7	Δ(*opuD*::*neo*)2	(Kappes *et al.*, 1996)
GNB40	Δ(*gbsR*::*neo*)1 Δ(*treA*::*ery*)2	(Nau-Wagner *et al.*, 2012)
GNB48	Δ(*gbsR*::*neo*)1 Δ(*treA*::*ery*)2 *amyE*::Φ(*gbsA'-treA*)	G. Nau-Wagner
GNB51	Δ(*gbsR*::*neo*)1 Δ(*treA*::*ery*)2 *amyE*::Φ(*gbsA'-treA*)-*gbsR*	G. Nau-Wagner
STHB53	Δ(*gbsR*::*spc*)2 Δ(*treA*::*ery*)1 *amyE*::Φ(*opuBA'-treA*)	S. Ronzheimer
LTB1[1]	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*:: *opuB*::*opuCC** (M211/I)	This study
LTB3[1]	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*:: *opuB*::*opuCC** (M211I), g*bsR*¶ [G39/E (M1)]	This study
LTB4[1]	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*:: *opuB*::*opuCC** (M211I), *gbsR*¶ [T79/A (M2)]	This study
LTB5[1]	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*:: *opuB*::*opuCC** (M211I), *gbsR*¶ [R85/S (M3)]	This study
LTB10	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*:: *opuB*::*opuCC*	This study
LTB11[1]	Δ(*gbsR*::*neo*)1 Δ(*treA*::*ery*)2 *amyE*::Φ(*gbsA'-treA*), *gbsR*¶ [G39/E (M1)]	This study
LTB12[1]	Δ(*gbsR*::*neo*)1 Δ(*treA*::*ery*)2 *amyE*::Φ(gbsA'-*treA*), *gbsR*¶ [T79/A (M2)]	This study
LTB14	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*::*opuC*Δ*opuCC*	This study
LTB15	Δ(*opuA*::*tet*)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*::*opuB*Δ*opuBC*	This study

LTB16	Δ(*opuA*::tet)3 Δ(*opuB*::*ery*)3 Δ(*opuC*::*spc*)3 Δ(*opuD*::*kan*)2 *amyE*::*opuC*Δ*opuCC::BC*	This study
LTB17[1]	Δ(*gbsR*::*neo*)1 Δ(*treA*::*ery*)2 *amyE*::Φ(*gbsA'-treA*), *gbsR*¶ [R85/S (M3)]	This study
LTB18	Δ(*gbsR*::*spc*)2 Δ(*treA*::*ery*)1 *amyE*::Φ(*opuBA'-treA*), *ytoI*::*gbsR*	This study
LTB19[1]	Δ(*gbsR*::*spc*)2 Δ(*treA*::*ery*)1 *amyE*::Φ(*opuBA'-treA*), *ytoI*::*gbsR*¶ [G39/E (M1)]	This study
LTB20[1]	Δ(*gbsR*::*spc*)2 Δ(*treA*::*ery*)1 *amyE*::Φ(*opuBA'-treA*), *ytoI*::*gbsR*¶ [T79/A (M2)]	This study
LTB21[1]	Δ(*gbsR*::*spc*)2 Δ(*treA*::*ery*)1 *amyE*::Φ(*opuBA'-treA*), *ytoI*::*gbsR*¶ [R85/S (M3)]	This study

[1]The *opuCC* gene marked by a star (*) carries a point mutation [ATG to ATA] that leads to singe amino acid substitution [Met^{211} to Ile] in the OpuCC substrate-binding protein. The suppressor derivatives from strain LTB1 carry point mutations in the *gbsR* repressor gene (Nau-Wagner *et al.*, 2012) and these are indicated by (¶). The following mutants were isolated: M1 [GbsR-Gly^{39}/Glu, strain LTB3]; M2 [GbsR-Thr^{79}/Ala, strain LTB4]; and M3 [GbsR-Arg^{85}/Ser, strain LTB5].

Table S2: DNA primers

Primer name	Primer sequences 5'-3'
opuCF	AGCTGATCATCCCTTCAAATGGC
opuCR	AGCGTTTTCTCCTTTACAAAAAAACATTTAG
opuBF	CGGTTTCATCCTTTCAGCTAACAATTC
opuBR	TACGATTTAAAGAGAAAAAAGAGGCTGGAC
pXF	CATGTTTGACAGCTTATCATCGGC
pXR	GGACCCAAATGCAGCTGTGGAAAT
pXRb	CCATTATGTACTATTTCGATCAGACCAGTT
opuCCF	ATGACAAAAATCAAATGGCTTGGCG
opuCCR	TTAGTCAAAATAATGATGTTTCTCTAAAAATTCCTTTGC
opuCClessF	TTTCAACAGTGCCAACTCCTTACGATAC
opuCClessR	GAAAAGAGGTGGATCATATGGAAGTACTACAGCAG
opuBCF	ATGAAAAGAAAATATCTCAAATTAATGATAGGTTTAGCAC
opuBCR	TCACGATTCGAAATAGCGATGTTTTTCTAAATATTCC
opuBClessF	GAGCCGCCTCCTTATGACAATTCCTTC
opuBClessR	AAGGGGGAAGAGGTCAATGAACGTGC
GbsR TreA Mfrag for	AAACCCGGGGGGACTTTGACAGTTTAAAAACC
MAL-C2 GbsR rev	AAAGGATCCGTTTCCCAGGCGTTTTCTGCT
Q5_OpuCCMut_TzuC_F	AAACCGCATCCATTTTCCCGTTTTTG
Q5_OpuCCMut_TzuC_R	TGGCTTATTCAACGGATG
Q5_GbsRMut1_GzuA_F	ATTTTATATGAGACGATGTATATGAGGGATGAG
Q5_GbsRMut1_GzuA_R	CCCGACACTGCGGGTAAT
Q5_GbsRMut2_AzuG_F	AGTGAAAAAAGCATTTCACCGG
Q5_GbsRMut2_AzuG_R	ACATTTAAGTCTTGAAGCTTTTTG
Q5_GbsRMut3_CzuA_F	CCGGGGCATCAGCAAGCATAC
Q5_GbsRMut3_CzuA_R	TGAAATGTTTTTTTCACTACATTTAAGTCTTG
gbsR-F	AAAGCTAGCGGTTTTTAAACTGTCAAAGTCCC
gbsR-R	AAAGCTAGCCTGCTTTACTTTGTTTCGACCG
OpuC-Seq1	CAGAAAATTTAAAGGAAACCTGCGGAGG
OpuC-Seq2	CCCACGATATGGATGAAGCGATTAAGC
OpuC-Seq3	CTGACCAGGGGAGGAGCTCTTTTAAGATG
OpuC-Seq4	GAATATATCATTGGCGGTGCCGTGCCTGTC
OpuC-Seq5	GGAGCATATTAACACCGTGTCTGACCTG
OpuC-Seq6	GCTCGGCATCCTGATAGCCAGATACAGAAG
OpuC-Seq7	CATCCCTTCAAATGGCAATTGATGGTGTC
opuAA-P1	AGTAGAGACATGAAACTGATCCTGTAAAAG

Tet-opuAA-P2	CATAGCTGTTTCCTGTGTGAAATTGTTATCGGTTTAACATCCGTACTAAAGTTGATTTAC
Tet-opuAC-P3	CAACTTTTTATCTCTCTTCGTTCTTTGTTCGGCTTGTTGTTCCTCAATATATGAAAAATG
opuAC-P4	TAATGCTGCTAAAAAAACAACCTGAGCTTC
REV-opuAA-Tet-P2	GTAAATCAACTTTAGTACGGATGTTAAACCGATAACAATTTCACACAGGAAACAGCTATG
REV-opuAC-Tet-P3	CATTTTTCATATATTGAGGAACAACAAGCCGAACAAAGAACGAAGAGAGATAAAAAGTTG
opuC-P1	ATGATGCAAAAAGCAGCTCTCTTATTTTAG
Spc-opuC-P2	TCATAGCTGTTTCCTGTGTGAAATTGTTATTATACTTTTGACACTTGTTCCAATTTCAGC
Spc-opuC-P3	CTTGCCAGTCACGTTACGTTATTAGTTATAAAAACCACCTCTATTTAAATACAACAGAGG
opuC-P4	CCAATAATTAAAAAGATACCAACACCAAGC
opuC-Spc-REV-P2	GCTGAAATTGGAACAAGTGTCAAAAGTATAATAACAATTTCACACAGGAAACAGCTATGA
opuC-Spc-REV-P3	CCTCTGTTGTATTTAAATAGAGGTGGTTTTTATAACTAATAACGTAACGTGACTGGCAAG
opuB-P1	GCTAGTCAGAATAATCAACAAAAAATGGAT
Ery-opuB-P2	TCTTTAATAATTCATCAACATCTACACCGCGGTTAATCATTTTCATTGTTGTCGTTTTTC
Ery-opuB-P3	CATTCAATTTTGAGGGTTGCCAGAGTTAAACAAACGCAACAAACGGAACTGCGATTATTC
opuB-P4	TGAATGAGTTTACCGAAAGCATTGATAAAG
opuB-Ery-REV-P2	GAAAAACGACAACAATGAAAATGATTAACCGCGGTGTAGATGTTGATGAATTATTAAAGA
opuB-Ery-REV-P3	GAATAATCGCAGTTCCGTTTGTTGCGTTTGTTTAACTCTGGCAACCCTCAAAATTGAATG

Table S3: Plasmids

Plasmid	Genotype/Description	Resistance	Reference
pX	integration vector *amyE*::*cat*::*amyE*	*bla, cat*	(Kim *et al.*, 1996)
pJB007	*gbsR, gbsAB*	*cat*	(Boch *et al.*, 1996)
pJMB1	*amyE*::*treA*	*bla, cat*	M. Jebbar
pFSB1	*amyE*::*bgaB*	*bla, cat*	F. Spiegelhalter
pGNB2	*amyE*::Φ*gbsA'-bgaB*, *gbsR*	*bla*, *cat*	(Nau-Wagner *et al.*, 2012)
pGNB13	*amyE*::Φ*gbsA'-treA*, *gbsR*	*bla, cat*	G. Nau-Wagner
pBB287	integration vector *ytoI*::*tet*::*ytoI*	*tet*	D. Rudner,
pDG1515	tetracycline resistance cassette	*tet*	(Guerout-Fleury *et al.*, 1995)
pDG646	erythromycin resistance cassette	*ery*	(Guerout-Fleury *et al.*, 1995)
pDG1726	spectinomycin resistance cassette	*spc*	(Guerout-Fleury *et al.*, 1995)
pChen1	*opuB* operon with native promoter cloned into pX-*amyE*-site	*bla, cat*	This study
pChen3	*opuC* operon with native promoter cloned into pX-*amyE*-site	*bla, cat*	This study
pChen5[1]	*opuB* with native promotor (pChen1) but *opuBC* replaced by *opuCC** (M211/I) (*opuB::opuCC**)	*bla, cat*	This study
pChen6	*opuC* with native promoter (pChen3) but *opuCC* replaced by *opuBC* (*opuC::opuBC*)	*bla, cat*	This study
pChen10	*opuB* with native promoter (pChen1) but *opuBC* removed from start to stop codon (*opuB*Δ*opuBC*)	*bla, cat*	This study
pChen11	*opuC* with native promoter (pChen3) but *opuCC* removed from start to stop codon (*opuC*Δ*opuCC*)	*bla, cat*	This study
pLT1	pChen5 with correct *opuCC* (*opuB::opuCC*)	*bla, cat*	This study
pLT2[1]	site directed mutagenesis of *gbsR* in pGNB13: *gbsR*¶ [G39/E (M1)]	*bla, cat*	This study
pLT3[1]	site directed mutagenesis of *gbsR* in pGNB13: *gbsR*¶ [T79/A (M2)]	*bla, cat*	This study

pLT4[1]	site directed mutagenesis of *gbsR* in pGNB13: *gbsR*¶ [R85/S (M3)]	*bla, cat*	This study
pLT5	*gbsR* with native promoter cloned into pBB287-*ytoI*-site	*tet*	This study
pLT6[1]	site directed mutagenesis of *gbsR* in pBB287: *gbsR*¶ [G39/E (M1)]	*tet*	This study
pLT7[1]	site directed mutagenesis of *gbsR* in pBB287: *gbsR*¶ [T79/A (M2)]	*tet*	This study
pLT8[1]	site directed mutagenesis of *gbsR* in pBB287: *gbsR*¶ [R85/S (M3)]	*tet*	This study

[1]The *opuCC* gene marked by a star (*) carries a point mutation [ATG to ATA] that leads to singe amino acid substitution [Met^{211} to Ile] in the OpuCC substrate-binding protein. The suppressor derivatives from strain LTB1 carry point mutations in the *gbsR* repressor gene (Nau-Wagner *et al.*, 2012) and these are indicated by (¶). The M1 (plasmid pLT2), M2 (plasmid pLT3), and M3 (plasmid pLT4) suppressor mutants, harbor point mutations in *gbsR* that lead to single amino acid substitutions at either positions 39 [Gly/Glu] (M1), 79 [Thr/Ala] (M2), or 85 [Arg/Ser] (M3) in the 180 amino acid-comprising GbsR regulatory protein (Nau-Wagner *et al.*, 2012).

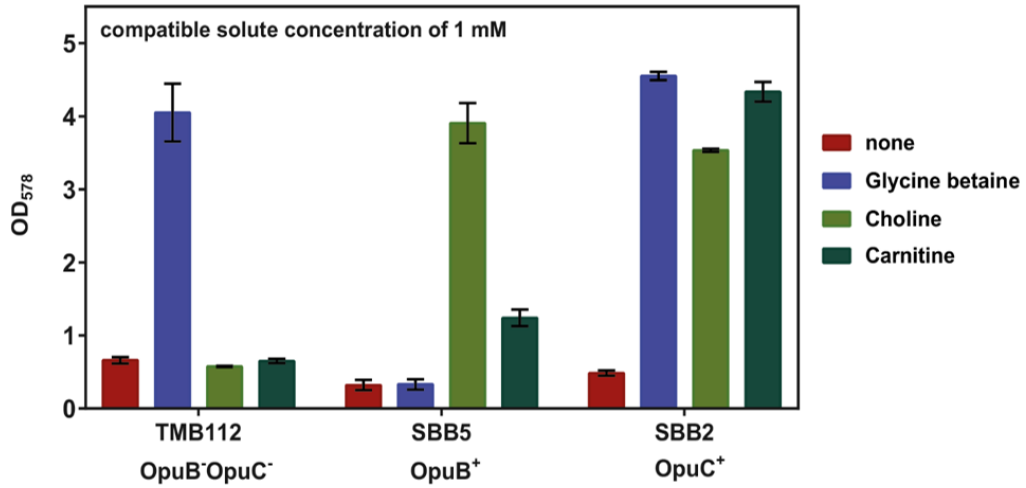

Fig. S1 Osmostress protection of *B. subtilis* strains. Strains SSB5 and SSB2 possess the gene for the OpuB or OpuC ABC transporter at their authentic position in the genome but lack otherwise the OpuA and OpuD compatible solute transporters; these are however present in strain TMB112. Strains were grown in SMM containing 1.2 M NaCl either in the absence or the presence of the indicated osmostress protectants. The growth yield of the cultures was determined by measuring their OD_{578} after 17 hours of incubation at 37°C. The shown values represent data from two independent biological experiments and the reported errors represent the corresponding standard deviation.

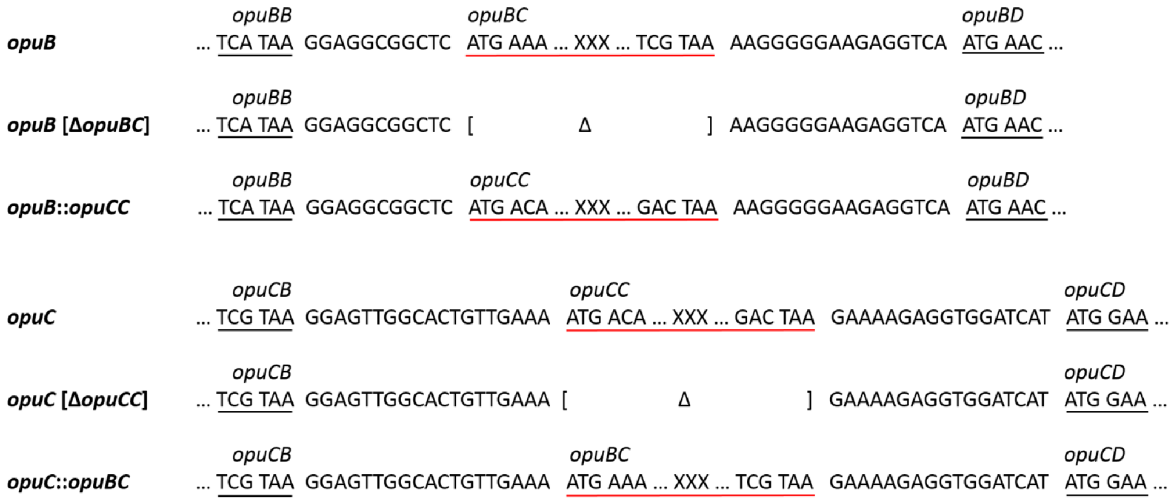

Fig. S2 Deletion and insertion junctions of the *opuB* and *opuC* mutant operons. The DNA sequences of the junctions for the constructed *opuBC* and *opuCC* deletions and those of the insertions of the foreign *opuBC* and *opuCC* genes into the *opuC* and *opuB* operons are shown. Coding regions are underlined.

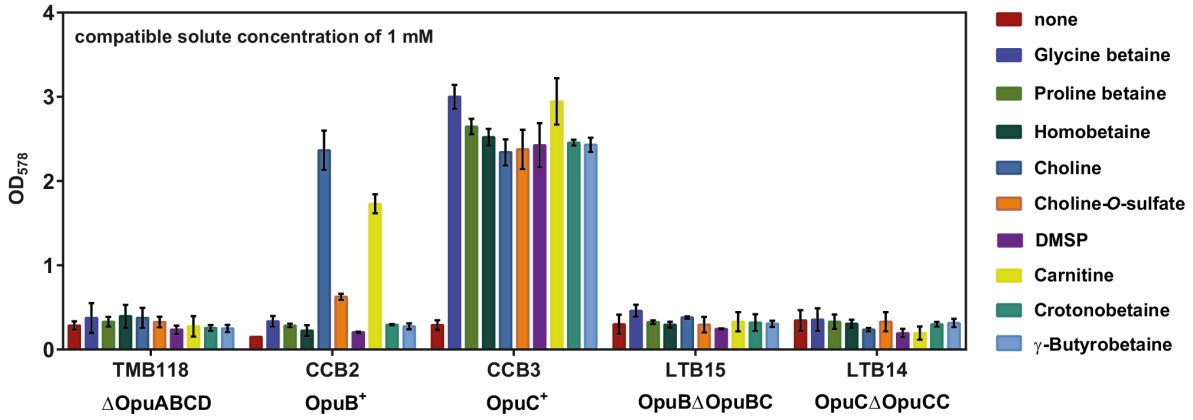

Fig. S3 Import of compatible solutes under osmotic stress conditions via the OpuB and OpuC wild-type ABC transporters, and the corresponding mutant systems lacking their substrate-binding protein. Cells of *B. subtilis* strains were grown in SMM containing 1.2 M NaCl in the absence or presence of 1 mM of various osmostress protectants. The growth yield of the cultures was determined by measuring their OD_{578} after 17 hours of incubation at 37°C. The shown values represent data from two independent biological experiments and the reported errors represent the corresponding standard deviation.

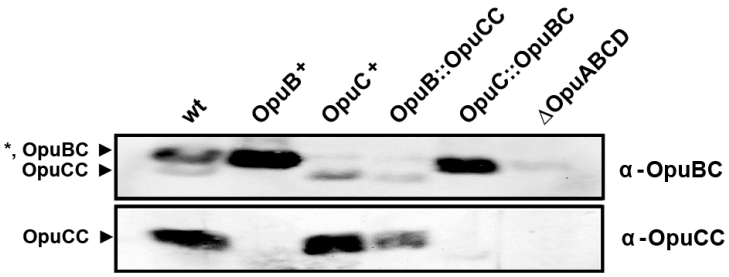

Fig. S4 Detection of the OpuBC and OpuCC ligand-binding proteins by immuno-blot analysis. Proteins of total cell lysates of *B. subtilis* strains grown in SMM with 1.2 M NaCl were separated by SDS-PAGE followed by Western Blot analysis. Sample normalization was achieved by adjusting the cell suspensions to the same OD_{578}. Proteins transferred to the blotting membrane were probed either with a polyclonal antiserum raised against OpuBC or a serum raised against OpuCC.

It should be noted that the used polyclonal anti-sera recognize their substrates with different specificities. The OpuBC anti-serum recognizes effectively OpuBC, and weakly the amino acid sequence-related OpuCC protein. It also cross-reacts with an unknown *B. subtilis* protein (marked by a star∗), a contaminating activity that was already present in the pre-serum. The OpuCC anti-serum does not recognize the OpuBC protein (Kappes *et al.*, 1999).

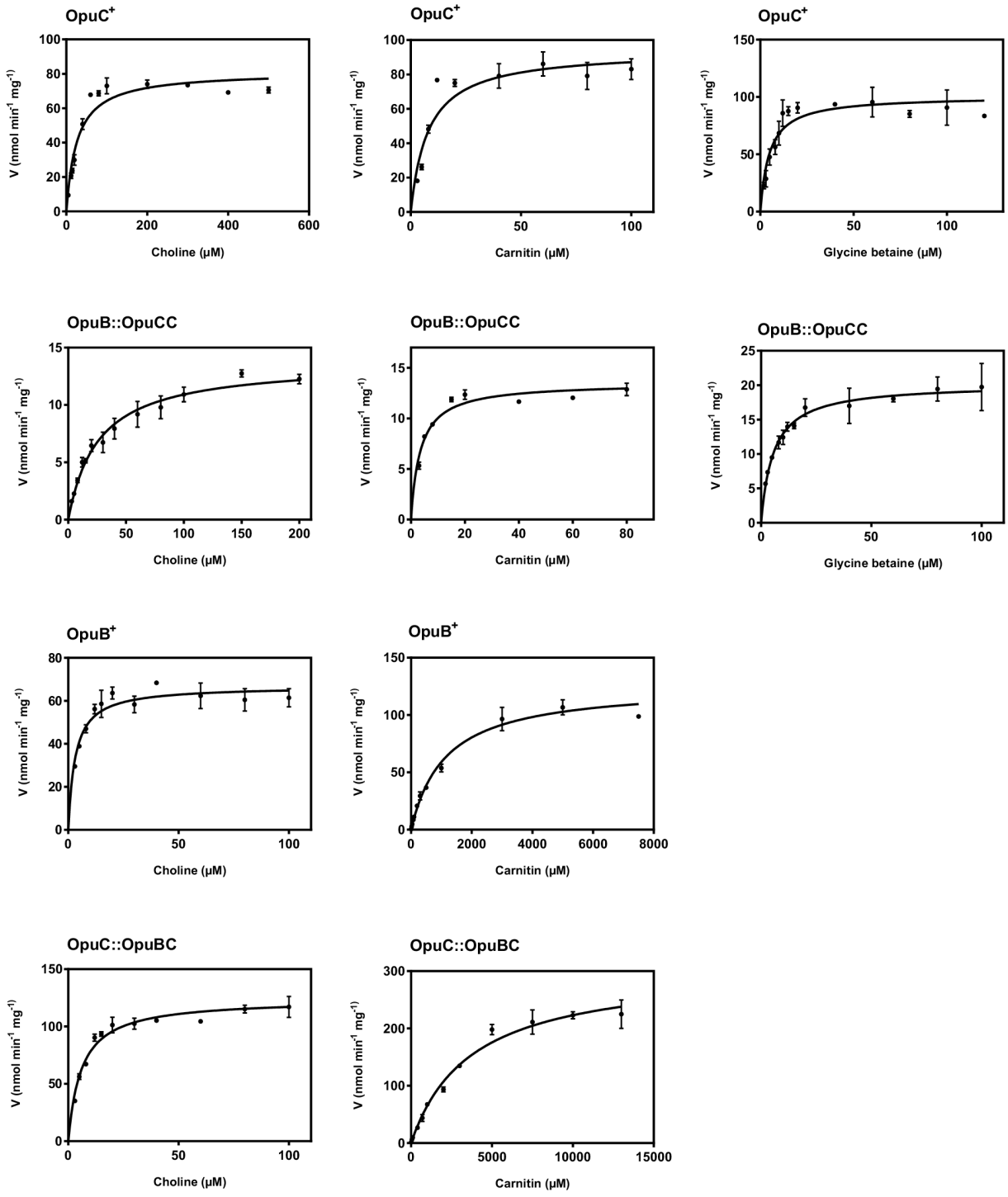

Fig. S5 Michaelis-Menten kinetics of the wild-type OpuB and OpuC ABC transporters and of the synthetically constructed hybrid transport systems OpuB::OpuCC and OpuC::OpuBC with radiolabeled glycine betaine, choline and carnitine as substrates. The substrate concentration was varied between 3 µM and 120 µM for glycine betaine, between 3 µM and 500 µM for choline and between 3 µM and 13 mM for carnitine. Each of the transport assays was conducted with two independently grown cultures, and the reported error bars represent the corresponding standard deviations.

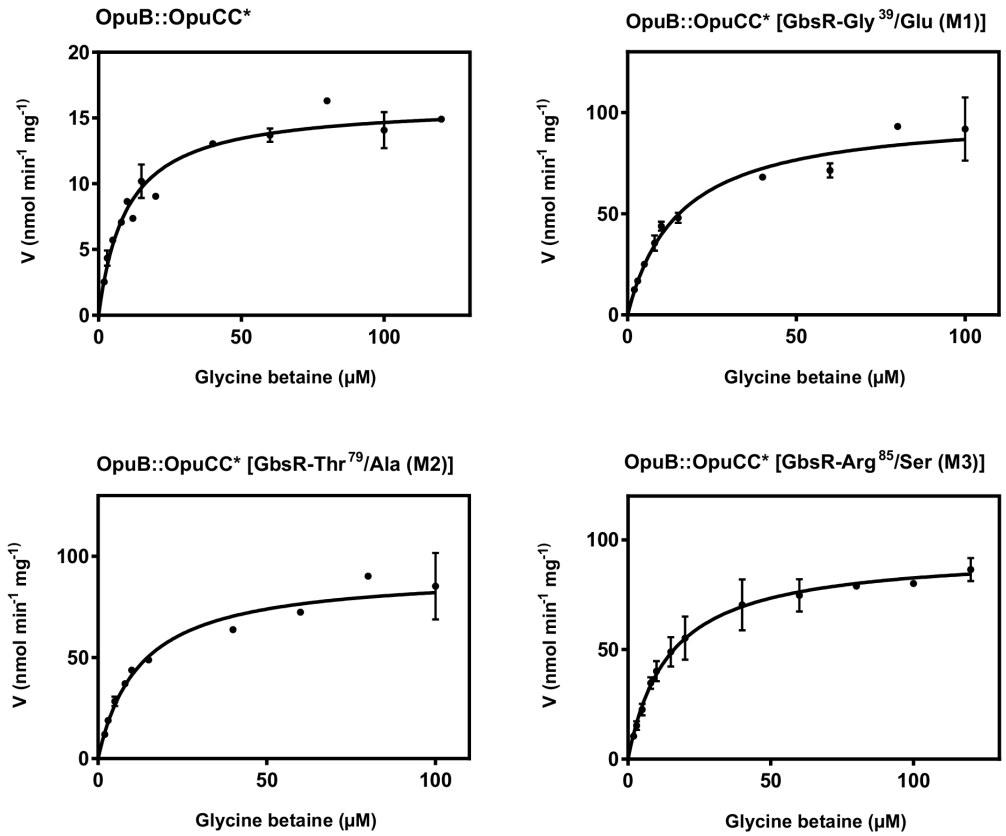

Fig. S6 Michaelis-Menten kinetics of the hybrid ABC transport system OpuB::OpuCC* and of the suppressor strains M1, M2 and M3, harboring the same OpuB::OpuCC* hybrid transporter and additional *gbsR*-mutant alleles. Measurements were conducted with radiolabeled glycine betaine as substrate. The star indicates an undesired point mutation ($Met^{211}Ile$) at a considerable distance from the OpuCC ligand-binding site. The concentration of glycine betaine varied between 3 µM and 120 µM. The OpuCC protein marked by a star (*) carries a point mutations [ATG to ATA] that leads to single amino acid substitution [Met^{211} to Ile] in the OpuCC substrate-binding protein. Each of the transport assays was conducted with two independently grown cultures, and the reported error bars represent the corresponding standard deviations.

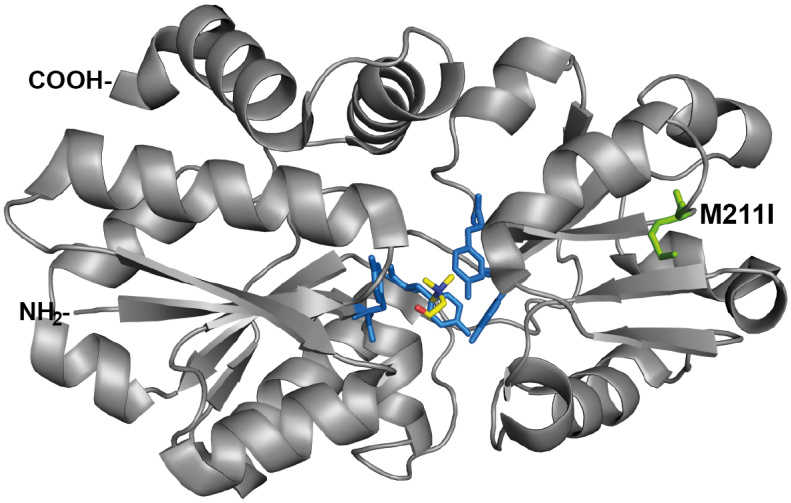

Fig. S7 Crystal structure of the substrate-binding protein OpuCC in complex with its ligand choline. The crystal structure of the OpuCC::choline complex (PDB accession code: 3PPQ) (Du *et al.*, 2011) is shown. The aromatic cage accommodating the positively charged head-group of choline is represented in blue and the choline molecule is shown in yellow. The OpuCC* amino acid substitution mutation (Met-211 to Ile) (shown in green) present in our starting plasmid pChen5 (*opuB::opuCC**) was projected onto this structure.

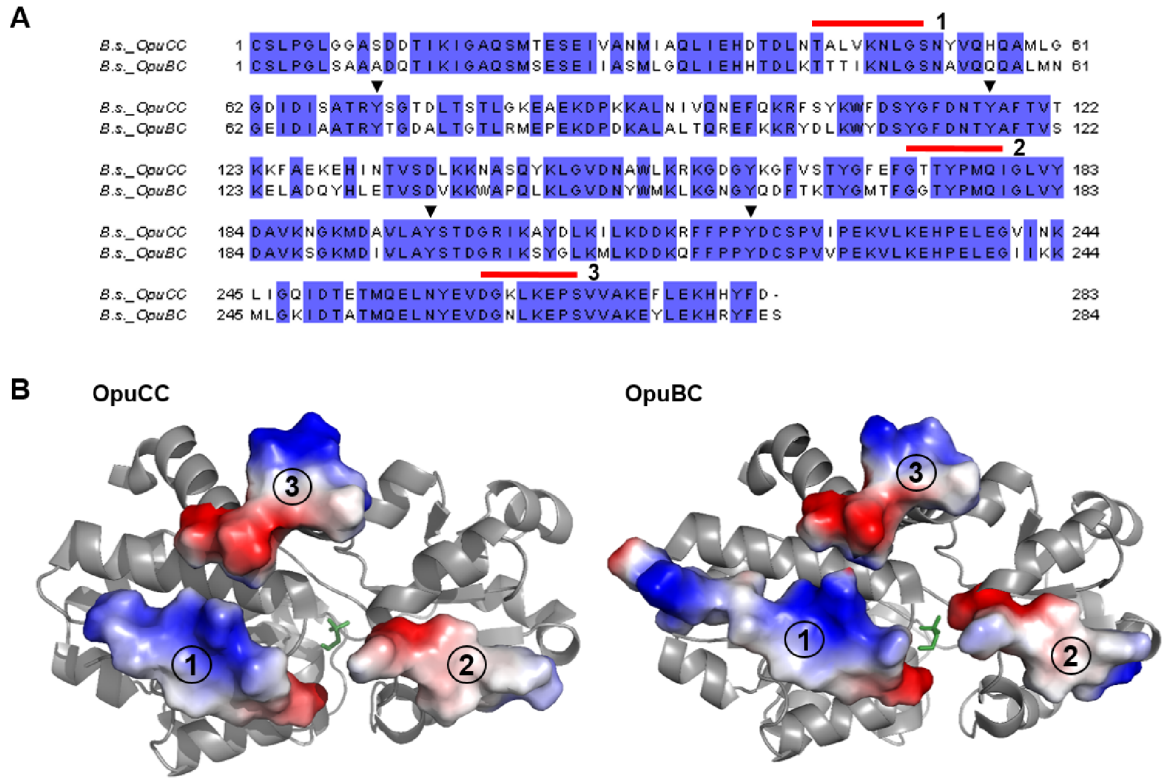

Fig. S8 Possible docking interfaces of the substrate-binding proteins OpuBC and OpuCC with their corresponding TMDs. (A) Alignment of the amino acid sequences of the biosynthetic precursors of the OpuBC and OpuCC proteins from *B. subtilis* (Kappes *et al.*, 1999). Identical amino acids are colored in blue. Highlighted in red are those regions that potentially will form the docking-interface with the integral membrane components (OpuBB/OpuBD and OpuCB/OpuCD) of the OpuB and OpuC ABC transport systems. Black arrowheads point out the four amino acid residues forming the aromatic cage that bind the trimethly-ammonium head group of various ligands of the OpuBC and OpuCC substrate-binding proteins. (B) A view onto those areas of the OpuBC and OpuCC proteins that will face the TMDs in the fully assembled ABC transport system. Crystal-structures of the OpuBC (PDB accession code: 3R6U) and OpuCC (PDB accession number: 3PPQ) protein in complex with their common ligand choline (shown as green sticks (Pittelkow *et al.*, 2011; Du *et al.*, 2011). The surfaces of possible interaction regions of the binding proteins with their cognate TMDs are highlighted; negatively charged amino acids are indicated in red and positively charged amino acids in represented in blue. Modeling of the regions that potentially interact with the OpuBB/OpuBD and OpuCB/OpuCD TMDs of the OpuB and OpuC transporters (Kappes *et al.*, 1999) was carried out with the molybdate importer (ModABC) from the archaeon *Archaeoglobus fulgidus* (PDB accession number 2ONK) (Hollenstein *et al.*, 2007) as the template.

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