

**The GbsR family of transcriptional regulators: functional characterization of the
OpuAR repressor**

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Erhard Bremer^{1,2*}**

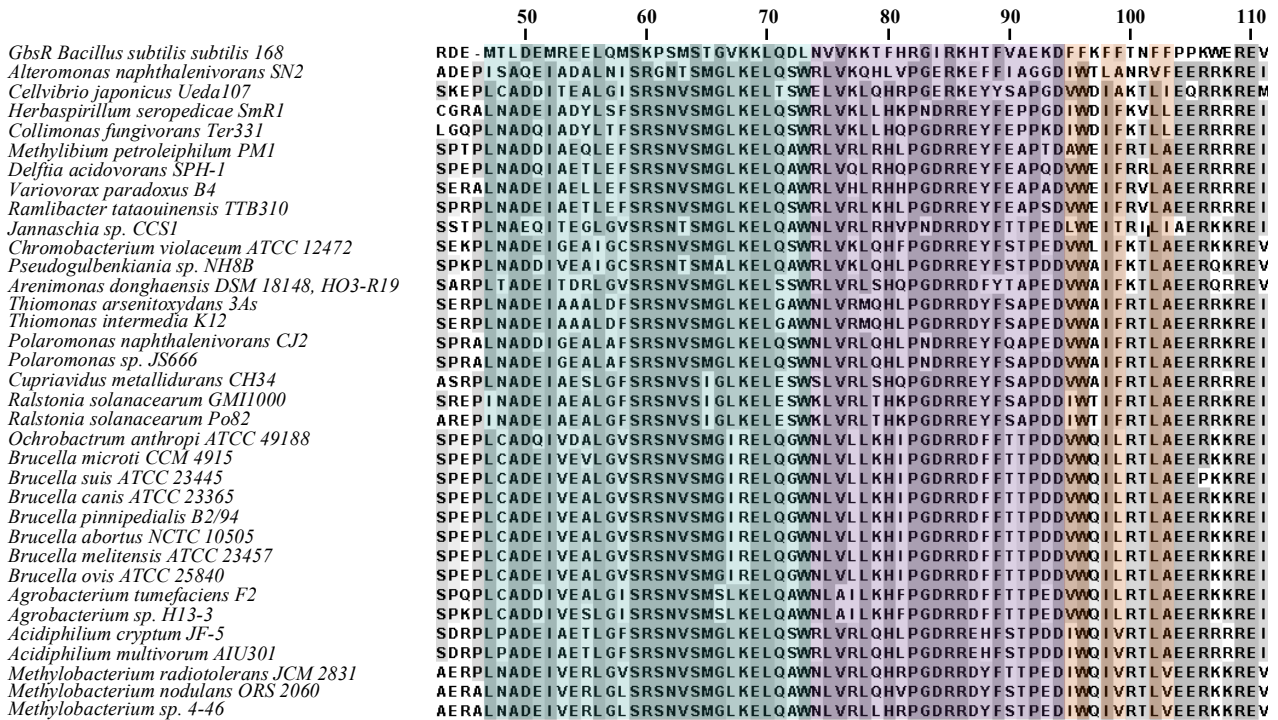
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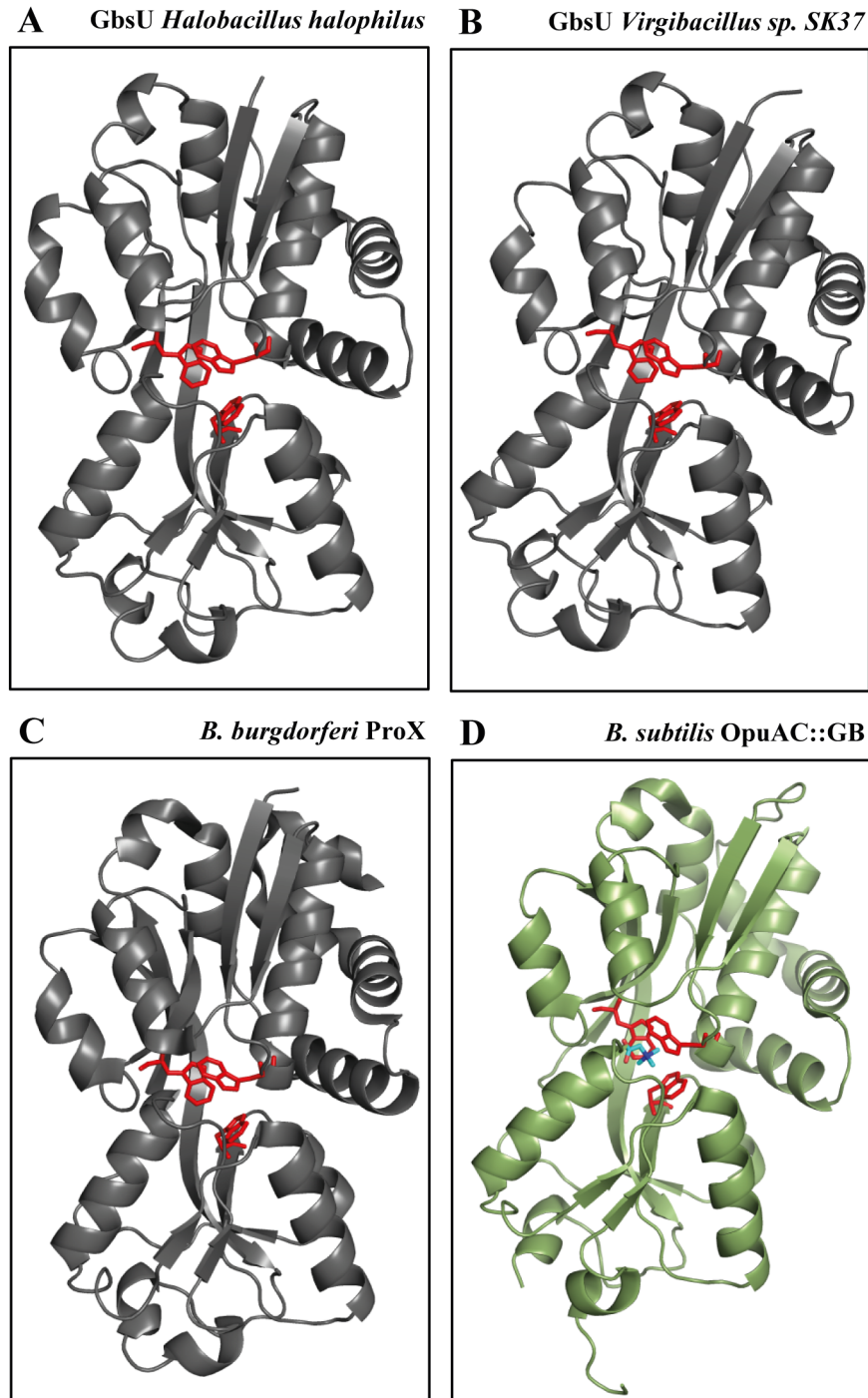
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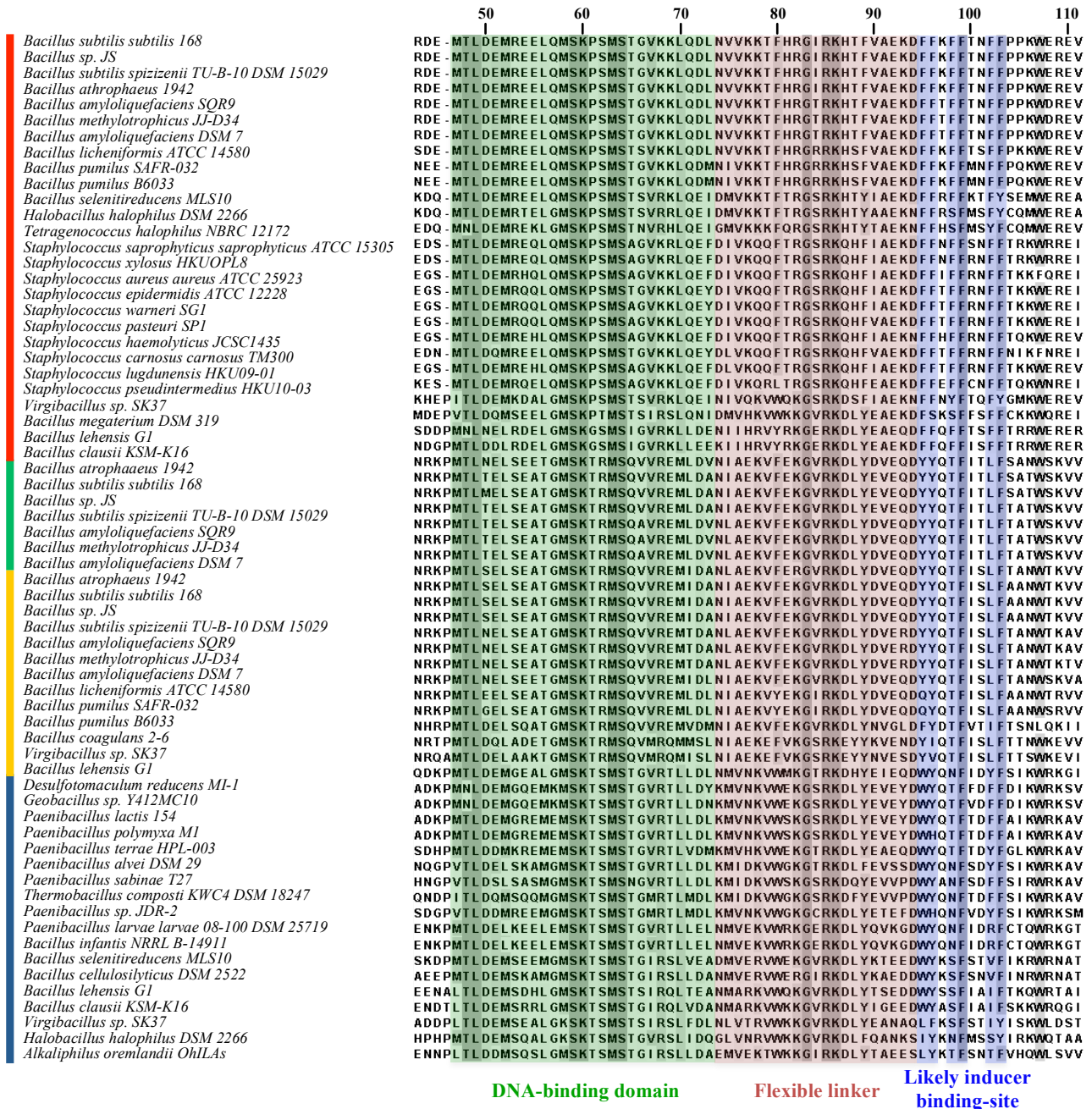


Supplementary Figure S1 | Amino acid sequence alignment of CydE-type proteins. The amino acid sequences of 34 CydE-type proteins (Xia et al., 2018) were compared to the amino acid sequence of the *B. subtilis* GbsR protein (Nau-Wagner et al., 2012) by an alignment using the MAFFT server (Kato et al., 2017). Depicted are only the N-terminal domains of these proteins, containing the amino acids corresponding to the winged helix-turn-helix DNA-binding motif (light blue), the inter-domain linker (purple) and of the likely inducer-binding site (reddish) of GbsR. Highly conserved amino acids are shaded in grey

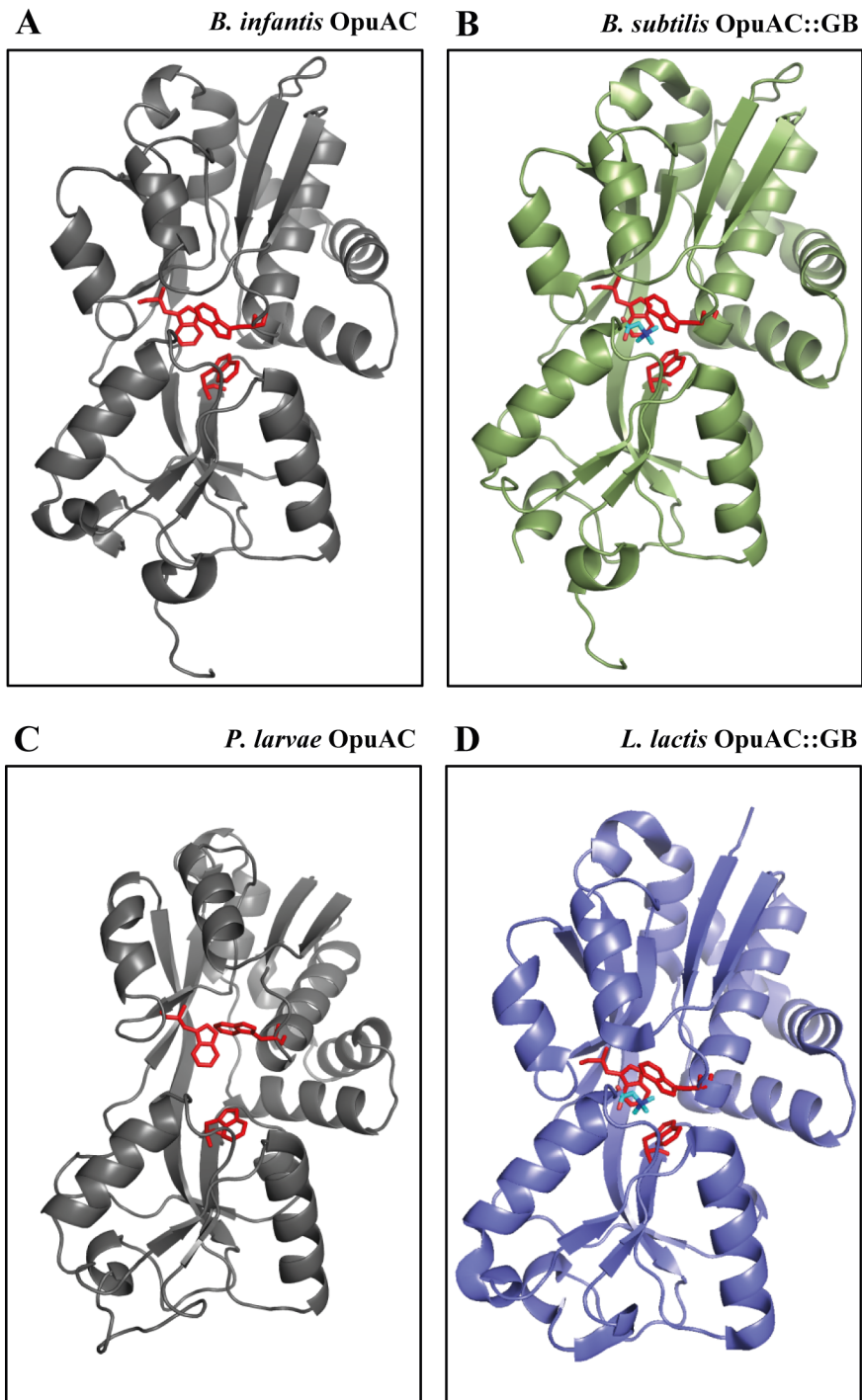


Supplementary Figure S2 | In silico models of the GbsU substrate-binding proteins.

Depicted are the substrate-binding proteins encoded within the putative glycine betaine synthesis gene clusters of (A) *Halobacillus halophilus* DSM 2266 and (B) *Virgibacillus sp. SK37* based on the crystal structure of (C) ProX of *Borrelia burgdorferi* [PDB entry 3TMG; (SSGCID, Gardberg, A., Fox, D., Staker, B., Stewart, L.; *to be published.*)]. All of these substrate-binding proteins show a ligand-binding site similar to the OpuAC protein of *B. subtilis*. Its crystal structure in complex with the ligand glycine betaine [PDB entry 2B4L (Horn et al., 2006)] is depicted in panel D. Aromatic amino acids likely to be involved in ligand-binding of the *H. halophilus* DSM 2266 and *Virgibacillus sp. SK37* GbsU proteins are highlighted in red. Glycine betaine, the substrate of the OpuAC protein of *B. subtilis* (Horn et al., 2006) is shown in blue.



Supplementary Figure S3 | Amino acid sequence alignment of GbsR-type proteins associated with osmotic stress. The amino acid sequences of 27 GbsR-type proteins associated with glycine betaine synthesis (red), 18 OpuAR-type proteins (blue), 7 GbsR-type proteins associated with OpuB-type transporters (green) and 13 GbsR-type proteins associated with OpuC-type ABC transporters (yellow) were aligned using the MAFFT server (Kato et al., 2017). Depicted are the N-terminal domains of these proteins. The amino acids corresponding to the winged helix-turn-helix DNA-binding motif (green), the inter-domain linker (reddish), and of the likely inducer-binding site (blue) are highlighted. Highly conserved amino acids are shaded in grey.

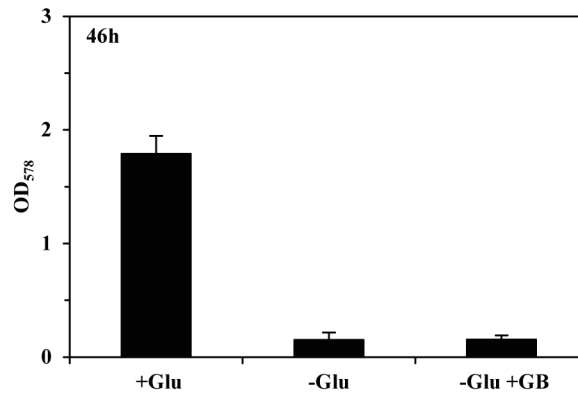


Supplementary Figure S4 | Structural arrangements of the OpuAC substrate-binding proteins. Shown are the *in silico* derived models of the OpuAC proteins of **(A)** *B. infantis* based on the crystal structure of **(B)** the *B. subtilis* OpuAC protein in complex with its ligand glycine betaine [PDB entry 2B4L (Horn et al., 2006)] and, **(C)** the modeled substrate-binding domain of the fused OpuABC protein of *P. larvae larvae* 08-100 DSM 25719 based on the crystallographic data on the **(D)** *L. lactis* OpuAC complexed with glycine betaine [PDB entry 3L6H (Wolters et al., 2010)]. Aromatic amino acids involved in the binding of glycine betaine are highlighted in red, whereas the ligand glycine betaine is depicted as blue sticks.



Supplementary Figure S5 | Clade analysis of GbsR-type proteins among *Bacteria* and *Archaea*. 179 GbsR-type proteins were identified by bioinformatics. Information on fully sequenced microbial genomes were obtained from the IMG/M Web-server and homologs of the GbsR protein of *B. subtilis* JH642 (Nau-Wagner et al., 2012) were searched for via the JGI Web-server (Chen et al., 2017) using the BLAST-P algorithm (Altschul et al., 1990). The retrieved amino acid sequences were aligned using the MAFFT Web-server (Kato et al., 2017) with bootstrapping setting (100 bootstraps) provided by the Web-server to analyze their phylogenetic relationship. The protein-homology tree was visualized using the iTOL web-tool (Letunic and Bork, 2016). GbsR homologs were grouped according to their gene neighborhood as depicted by colored boxes. If the *gbsR*-type gene was encoded in proximity of genes associated with glycine betaine synthesis or uptake systems for osmotic stress protectants it was classified as a member of the sub-group of GbsR-type proteins associated with osmotic stress (highlighted in red). Otherwise the GbsR homologs were assigned as non-associated with osmotic stress (highlighted in blue).

	50	60	70	80	90	100	110																																		
<i>GbsR Bacillus subtilis subtilis</i> 168	RDE	MTLDEMREELQMSKPSMS	TGVKLLQDLNVVKKTFHRGI	RRKHTFVAEKDFKFFTFN	FFPPKWEREV																																				
<i>Staphylococcus saprophyticus saprophyticus</i> ATCC 15305	EQKSM	LDEMRYELQMSKPSMS	AGVKKLQEFDRVKQFFIRGS	RRKHFTAEKDFTFN	FFGNFFSQRNREI																																				
<i>Staphylococcus xylosum</i> HKUOPL8	EQKSM	LDEMRYELQMSKPSMS	AGVKKLQEFDRVKQFFIRGS	RRKHFTAEKDFTFN	FFGNFFSQRNREI																																				
<i>Paenibacillus mucilaginosus</i> 3016	QNKPM	LDEMRGAMESKT	SMSTGVRTLVDLKMVHKVWEKGS	RKDLHYEVMDDYQTF	FSDFSLKWRKAL																																				
<i>Terribacillus aidingensis</i> MP602	ADQPM	LDDMRDAL	EMSKTSMSTGVRAL	AEMKMMVETAYKKGM	RKDLKSEEDWYKSF	TSIFGNKWEKST																																			
<i>Halobacillus halophilus</i> DSM 2266	AEEPMT	LDDMRAL	AMSKTSMSTGVRAL	SDMKMVEPSFKKGV	RKDLKSEEDWYKSF	TSIFSSRWWKHT																																			
<i>Virgibacillus</i> sp. SK37	SDNPMT	LDDMRDAL	EMSKTSMSTGVRT	SDMKMVESTFKKGI	RKDLYRSEEDWYKSF	TSIFGNRWKQYT																																			
<i>Bacillus cellulosilyticus</i> N-4 DSM 2522	SSDPMT	LDDMRSL	AMSKTSMSTGVRT	LDVMNMVEPVFRRGV	RKDLYKAEEDWYKSF	TALFSWRWRKAS																																			
<i>Natranaerobius thermophilus</i> JW/NM-WN-LF	SDEPK	LDEL	RDMMGMSKTSMST	GVRRLEKKNMVKVWKVKG	RKHLYEGETDFNT	FLNFFIPMWRKREI																																			
<i>Natranaerobius thermophilus</i> JW/NM-WN-LF	AKKPM	SLNEIK	DAVAMSKGSVSNGL	RDLLESEMIK	VWQKGD	RKDYIYAERDFAKN	NFLIKNMRLE																																		
<i>Paenibacillus terrae</i> HPL-003	EDRPM	TL	EEMKTSNMNSKSNMS	YAVRSLTESQMI	YKLEEKQE	RKDLYWAETDF	FRTQNFAGAKLQREI																																		
<i>Bacillus clausii</i> KSM-K16	NRKPM	T	LEAL	SDET	GMSKTRMSQVVR	REMLSLNVAHKVFEKGV	RKDLVDYVEKDYQTF	SIFVANWQAVI																																	
<i>Syntrophomonas wolfei</i> Goettingen DSM 2245B	SEQPL	G	LEDE	ASELE	VSATVSIH	IRFLEGMKNVRKVVWVKG	RRDYVEAERTG	KIMTEHLQSSFINER																																	
<i>Fimbriimonas gindensis</i> Gsoil 348	TGVP	LEVNE	IMDR	LQIS	SRGNAS	MNLR	ELMDWGVVRRFR	OPGD	RKDTYVSETDP	FLLTVKIVKERKREI																															
<i>Fimbriimonas gindensis</i> Gsoil 348	TGVP	LEVNE	IMDR	LQIS	SRGNAS	MNLR	ELMDWGVVRRFR	OPGD	RKDTYVSETDP	FLLTVKIVKERKREI																															
<i>Bradyrhizobium</i> sp. BTAl	AEKPM	T	A	E	D	L	G	M	A	R	S	N	V	S	N	S	L	K	E	L	L	A	W	D	L	I	R	R	V	P	I	L	G	D							
<i>Bradyrhizobium</i> sp. ORS278	AEKPM	T	A	E	D	L	G	M	A	R	S	N	V	S	N	S	L	K	E	L	L	A	W	D	L	I	R	R	V	P	I	L	G	D							
<i>Bradyrhizobium oligotrophicum</i> S58	AEKPM	T	A	E	D	L	G	M	A	R	S	N	V	S	N	S	L	K	E	L	L	A	W	D	L	I	R	R	V	P	I	L	G	D							
<i>Bradyrhizobium japonicum</i> SEMIA 5079	AEAPM	T	A	E	D	L	G	M	A	R	S	N	V	S	N	S	L	K	E	L	L	A	W	N	L	I	R	R	V	P	I	L	G	D							
<i>Methylobacterium radiotolerans</i> JCM 2832	SERPL	T	A	E	I	A	G	L	G	I	A	R	S	N	V	S	N	S	L	K	E	L	L	A	W	N	L	I	R	R	V	P	M	G	E						
<i>Methylobacterium chloromethanicum</i> CM4	SDKPL	A	A	E	D	I	A	G	L	G	I	A	R	S	N	V	S	N	S	L	K	E	L	A	W	N	L	I	R	R	V	P	V	L	G	E					
<i>Methylobacterium extorquens</i> AM1	SDKPL	A	A	E	D	I	A	G	L	G	I	A	R	S	N	V	S	N	S	L	K	E	L	A	W	N	L	I	R	R	V	P	V	L	G	E					
<i>Methylobacterium populi</i> BJ001	SDKPL	A	A	E	D	I	A	G	L	G	I	A	R	S	N	V	S	N	S	L	K	E	L	A	W	N	L	I	R	R	V	P	V	L	G	E					
<i>Sphingomonas sanxanigenens</i> DSM 19645	SERPL	N	A	E	I	G	E	L	I	G	A	R	S	N	V	S	N	S	L	K	E	L	L	A	W	N	L	I	R	R	V	P	V	M	G						
<i>Octadecabacter temperatus</i> SB1	SI	K	P	V	N	A	E	I	S	D	Q	L	G	I	A	R	S	N	V	S	N	S	L	K	E	L	V	G	W	K	L	I	R	R	V	P	I	Q	D		
<i>Sideroxydans lithotrophicus</i> ES-1	SHVPL	N	A	D	I	T	Y	L	S	F	S	R	S	N	V	S	M	G	L	K	E	L	Q	S	W	R	L	R	S	E	Y	R	A	G	D						
<i>Comamonas testosteroni</i> TK102	SPEPL	N	A	D	I	A	E	T	L	G	I	A	R	S	N	V	S	N	S	L	K	E	L	A	W	R	L	V	Q	L	R	H	O	P	G						
<i>Enhydrobacter aerococcus</i> SK60	AGKPM	N	A	E	I	Q	E	T	L	G	V	A	R	S	N	V	S	N	S	L	K	E	L	Q	S	L	N	L	V	T	V	H	L	G	D						
<i>Niastella koreensis</i> GR20-10 DSM 17620	SPDPL	S	D	E	I	M	E	L	I	S	R	G	N	V	N	M	N	I	R	L	I	D	W	G	L	V	S	R	V	L	I	Q	G	E							
<i>Chitinophaga pinensis</i> UQM 2034 DSM 2588	MPDPL	S	A	D	E	I	M	A	E	L	N	I	S	R	G	N	T	N	M	N	V	R	E	L	N	W	G	L	V	D	R	V	L	I	P	G	E				
<i>Halicomonabacter hydrossis</i> O DSM 1100	APQAL	S	A	D	I	M	E	L	I	S	R	G	N	N	M	N	L	R	A	L	I	D	W	G	L	V	S	K	E	L	K	A	G	E							
<i>Saprosira grandis</i> Lewin	SEAPL	S	A	D	V	M	E	L	I	S	R	S	N	S	N	S	L	K	E	L	A	W	N	L	I	D	W	G	L	V	Y	K	R	L	K	A	G	E			
<i>Spirosoma linguale</i> DSM 74	SPEAL	S	T	E	D	V	M	E	L	I	S	R	G	N	S	M	N	L	R	D	L	M	D	W	G	L	Y	K	Q	L	K	P	G	E							
<i>Spirosoma radiotolerans</i> DG5A	SPDAL	A	E	I	M	E	L	I	S	R	G	N	S	M	N	L	R	D	L	M	D	W	G	L	I	Y	K	Q	L	K	P	G	E								
<i>Emiticicia oligotrophica</i> GPTS100-15 DSM 17448	SPKSM	N	A	E	I	M	A	E	L	I	S	R	G	N	V	N	M	N	L	R	D	L	M	D	W	G	L	I	Y	K	Q	L	P	G	E						
<i>Leadbetterella byssophila</i> 4M15 DSM 17132	SEKAL	N	A	E	I	M	E	L	I	S	R	G	N	V	N	M	N	L	R	I	S	W	G	L	I	F	K	Q	L	I	P	G	E								
<i>Owenweeksia hongkongensis</i> DSM 17368	SKDAM	T	E	I	M	D	L	K	I	S	R	G	N	A	M	N	I	R	A	L	I	E	W	G	L	V	K	R	E	M	V	A	G	E							
<i>Hymenobacter</i> sp. APR13	SPGAL	S	T	E	D	I	M	E	L	I	S	R	G	N	V	N	L	N	R	A	L	M	D	W	G	L	V	R	K	E	L	R	P	G	E						
<i>Hymenobacter swuensis</i> DY53	SPGAL	S	T	E	D	I	M	E	L	I	S	R	G	N	V	N	L	N	R	A	L	M	D	W	G	L	V	R	K	E	L	R	P	G	E						
<i>Flavobacterium johnsoniae</i> UW101 ATCC 17061	SNEP	V	S	M	E	I	M	E	L	I	S	R	G	N	A	S	M	N	L	R	G	L	M	D	W	G	L	V	Y	K	E	F	K	A	G	E					
<i>Flavobacterium columnare</i> ATCC 49512	SNEA	V	S	M	E	I	M	E	L	I	S	R	G	N	A	S	M	N	L	R	A	L	M	D	W	G	L	V	Y	K	E	F	K	A	G	E					
<i>Flavobacterium indicum</i> GPTS100-9	SHEP	V	S	M	E	I	M	E	L	I	S	R	G	N	A	S	M	N	L	R	A	L	M	D	W	G	L	V	Y	K	E	F	K	A	G	E					
<i>Dokdonia</i> sp. 4H-3-7-5	SPEAL	S	M	E	D	I	M	S	E	L	I	S	R	G	N	S	M	N	L	R	S	L	I	D	W	G	L	I	F	K	E	Y	K	A	G	E					
<i>Dokdonia</i> sp. PRO95	SPEAL	S	M	E	D	I	M	S	E	L	I	S	R	G	N	S	M	N	L	R	S	L	I	D	W	G	L	I	F	K	E	Y	K	A	G	E					
<i>Cellulophaga algicola</i> IC166 DSM 14237	STKPL	S	T	E	E	I	M	E	L	I	S	R	G	N	T	S	M	N	V	R	L	I	D	W	G	L	V	T	K	E	L	V	P	G	E						
<i>Lacinutrix</i> sp. 5H-3-7-4	STKPL	S	M	E	I	M	E	L	I	S	R	G	N	T	S	M	N	L	R	L	I	D	W	G	L	V	S	K	T	I	A	G	E								
<i>Cellulophaga lytica</i> LIM-21 DSM 7489	SSEPL	S	M	E	I	M	E	L	I	S	R	G	N	T	S	M	N	L	R	Q	L	I	D	W	G	L	V	S	K	E	F	K	A	G	E						
<i>Nonlabens dokdonensis</i> DSW-6	HPDGL	S	T	E	D	I	M	E	V	Q	L	S	R	G	N	V	N	T	N	R	E	L	I	N	W	R	L	V	R	K	E	T	V	L	G	E					
<i>Opitutaceae</i> sp. TAV5	SDRPL	H	T	D	E	I	M	E	L	I	S	R	G	N	V	N	L	R	E	L	V	N	M	G	V	A	R	S	V	I	M	K	G	E							
<i>Methanothermococcus okinawensis</i> IH1	ADKPL	C	I	D	I	M	N	E	L	G	I	S	K	G	N	V	S	M	N	L	N	K	E	L	G	F	I	K	K	W	I	K	G	E							
<i>Methanocaldococcus</i> sp. FS406-22	SDEPL	T	S	D	I	M	E	L	K	I	S	K	G	N	V	S	M	S	L	K	K	E	L	G	F	V	K	K	W	I	K	G	E								
<i>Methanocaldococcus vulcanius</i> M7 DSM 12094	SDKPL	T	S	D	I	M	E	L	K	I	S	K	G	N	V	S	M	S	L	K	K	E	L	G	F	V	K	K	W	I	K	G	E								
<i>Methanocaldococcus jannaschii</i> DSM 2661	SDKPL	T	S	D	I	M	E	L	K	I	S	K	G	N	V	S	M	S	L	K	K	E	L	G	F	V	K	K	W	I	K	G	E								
<i>Methanocaldococcus fervens</i> AG86	SNKPL	T	S	D	I	M	E	L	K	I	S	K	G	N	V	S	M	S	L	K	K	E	L	G	F	V	K	K	W	I	K	G	E								
<i>Methanoterris igneus</i> K05 DSM 5666	SDKPL	C	I	A	D	I	M	E	L	G	I	S	K	G	N	V	S	M	A	L	N	K	E	L	G	F	V	R	K	W	I	K	G	E							
<i>Methanococcus aeolicus</i> Nankai-3	SHNPL	C	I	D	I	M	E	L	G	I	S	R	G	N	V	S	M	N	L	N	K	E	L	G	F	I	K	K	W	W	K	G	E								
<i>Methanocaldococcus infernus</i> ME	YERPM	S	D	E	I	V	E	L	K	I	S	K	G	N	A	S	M	N	L	K	K	E	L	G	F	V	K	R	T	W	I	E	G	E							
<i>Methanococcus voltae</i> A3	SEKPL	C	M	D	E	I	D	F	L	K	I	S	G	H	T	A	S	T	I	R	K	L	E	L	K	A	I	K	K	W	W	E	G	D							
<i>Pyrococcus abyssii</i> GE5	ANDPL	S	L	S	D	I	A	E	I	T	G	Y	S	V	S	H	V	S	A	M	K	V	L	E	G	V	L	V	O	R	I	K	K	P	D						
<i>Pyrococcus</i> sp. NA2	SNEP	S	L	S	E	I	A	E	I	T	G	Y	S	V	S	H	V	S	A	M	R	V	L	E	G	V	L	V	O	R	I	K	K	P	D						
<i>Pyrococcus</i> sp. ST04	AKEP	S	L	S	E	I	S	S	I	T	G	Y	S	L	S	H	V	S	A	M	K	V	L	E	G	V	L	V	O	R	I	K	K	P	D						
<i>Pyrococcus furiosus</i> DSM 3638	SEDP	L	S	L	G	D	I	S	E	L	T	G	Y	S	L	S	H	V	S	A	M	K	V	L	E	S	V	L	V	R	I	K	K	P	D						
<i>Pyrococcus yayanosii</i> CH1	SDEP	L	S	L	A	E	I	A	E	I	T	G	Y	S	L	S	H	V	S	A	M	K	V	L	E	G	V	L	V	O	R	I	K	K	P	D					
<i>Thermococcus onnurineus</i> NA1	AKEP	M	S	L	E	I	A	E	R	T	G	Y	S	L	S	H	V	S	T	A	L	K	S	M	E	L	G	L	V	V	R	I	K	K							



Supplementary Figure S7 | *B. infantis* cannot use glycine betaine as carbon source. Cultures of *B. infantis* NRRL B-14911 were grown in basal medium supplemented with either glucose (27.7 mM final concentration) or glycine betaine (33.24 mM final concentration) as the sole carbon source. Growth yields were measured after 46 hours of incubation at 37 °C. Cultures without the addition of a carbon source served as control.

Supplementary Table S1 | *Bacillus subtilis* strains used in this study

Strain	Relevant genotype	Source or reference
JH642	<i>trpC2 pheA1</i>	J. Hoch; BGSC* 1A96
TMB118	$\Delta(\text{opuA}::\text{tet})3 \Delta(\text{opuC}::\text{spc})3 \Delta(\text{opuD}::\text{neo})2$ $\Delta(\text{opuB}::\text{erm})3$	(Teichmann et al., 2017)
GNB37	$\Delta(\text{treA}::\text{erm})2$	(Nau-Wagner et al., 2012)
GNB40	$\Delta(\text{gbsR}::\text{neo})1 \Delta(\text{treA}::\text{erm})2$	(Nau-Wagner et al., 2012)
CAB1	$\Delta(\text{opuA}::\text{tet})3 \Delta(\text{opuC}::\text{spc})3 \Delta(\text{opuD}::\text{neo})2$ $\Delta(\text{opuB}::\text{erm})3 \text{amyE}::\text{pX}$	This study
CAB2	$\Delta(\text{opuA}::\text{tet})3 \Delta(\text{opuC}::\text{spc})3 \Delta(\text{opuD}::\text{neo})2$ $\Delta(\text{opuB}::\text{erm})3 \text{amyE}::\text{opuAR-opuA}_{B,i}$	This study
STHB04	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{gbsR}::\text{neo})1 \Delta(\text{yvaV}::\text{tet})2$	This study
STHB05	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{gbsR}::\text{neo})1 \Delta(\text{yvaV}::\text{tet})2$ $\Delta(\text{opcR}::\text{zeo})2$	This study
STHB06	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{gbsR}::\text{neo})1 \Delta(\text{opcR}::\text{zeo})2$	This study
STHB07	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{yvaV}::\text{tet})2 \Delta(\text{opcR}::\text{zeo})2$	This study
STHB08	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{opcR}::\text{zeo})2$	This study
STHB10	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{gbsR}::\text{neo})1 \Delta(\text{yvaV}::\text{tet})2$ $\Delta(\text{opcR}::\text{zeo})2 [\text{amyE}::\Phi(\text{opuAA}_{B,i}'\text{-treA});$ $\text{opuAR}^+]1$	This study
STHB11	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{gbsR}::\text{neo})1 \Delta(\text{yvaV}::\text{tet})2$ $\Delta(\text{opcR}::\text{zeo})2 [\text{amyE}::\Phi(\text{opuAA}'\text{-treA})]1$	This study
STHB65	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{gbsR}::\text{neo})1 \Delta(\text{yvaV}::\text{tet})2$ $[\text{amyE}::\Phi(\text{opuAA}'_{B,i}\text{-treA})]1$	This study
STHB66	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{gbsR}::\text{neo})1 \Delta(\text{opcR}::\text{zeo})2$ $[\text{amyE}::\Phi(\text{opuAA}'_{B,i}\text{-treA})]1$	This study
STHB67	$\Delta(\text{treA}::\text{erm})2 \Delta(\text{yvaV}::\text{tet})2 \Delta(\text{opcR}::\text{zeo})2$ $[\text{amyE}::\Phi(\text{opuAA}'_{B,i}\text{-treA})]1$	This study

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

Supplementary Table S2 | Primers used in this study

Primer name	Primer sequence (5'-3')
OpcR Knout P1	ATAAATTCTTCAACAACTCATTGCGG
OpcR Knout Zeo P2	CCATATCAAGATAACTTCGTATAATGTATGTTGAAGGCATTCCAAA CGTATGCATATTTT
OpcR Knout Zeo P3	CCATATCAAGATAACTTCGTATAATGTATGTTGAAGGCATTCCAAA CGTATGCATATTTT
OpcR Knout P4	GTAAAGCAATACTCGTCTGCTTTTGTTTTA
OpcR Knout Zeo P5	AAAATATGCATACGTTTGGAAATGCCTTCAACATACATTATACGAAG TTATCTTGATATGG
OpcR Knout Zeo P6	TATTTTAGAGAGCTGCATTCTTTTGTTTTCTAATGTATGCTATACGA AGTTATTCAGTCC
YvaV Knout P1	GATAAATTCCTCAACAAATTCGTCTGCC
Yvav Knout Tet P2	CCGTAATGCTATGTTAGCATTACTCTTTTCCATGTTTTCCGCGATTC TTTCTATAAAATG
YvaV Kneu2 Tet P3	AAATTGTTATCCGCTCACAATTCACACAACATATTTGAGAGCGA AGACATTTTTAAATATGTG
YvaV Knout P4	CAGTGAATAAAACCGGTAAATCTAGGTCTC
YvaV Knout Tet P5	CATTTTATAGAAAGAATCGCGGAAAACATGGAAAAGAGTAATGC TAACATAGCATTACGG
YvaV Kneu2 Tet P6	CACATATTTAAAAATGTCTTCGCTCTCAAATATGTTGTGTGGAATT GTGAGCGGATAACAATTT
OpuAR fwd	ATGGTAGGTCTCAAATGCATAACGAAGAATTGGTCGCACAG
OpuAR rev	ATGGTAGGTCTCAGCGCTCTTTCCTTTTTTTCATCCTCAGAC
OpuAR_Box1_for	CCAGGTCAAAGGGGATTGGTTCCAGAATTTTCATCGATCGCTTTTG TACG
OpuAR_Box1_rev	CGTACAAAAGCGATCGATGAAATTCTGGAACCAATCCCCTTTGAC CTGG
OpuAR_Box3_for	GGTACCAGAATTTTCATCGATTTCTTTTGTACGCAATGGAGAAAAG G
OpuAR_Box3_rev	CCTTTTCTCCATTGCGTACAAAAGAAATCGATGAAATTCTGGTAC C
OpuAR_Box1+3_for	CCAGGTCAAAGGGGATTGGTTCCAGAATTTTCATCGATTTCTTTTG TACG
OpuAR_Box1+3_rev	CGTACAAAAGAAATCGATGAAATTCTGGAACCAATCCCCTTTGA CCTGG
OpuAR_N98Fn_for	GTACAAAAGCGATCGATGAAAAACTGGTACCAATCCCCTTTGAC
OpuAR_N98Fn_rev	TCAAAGGGGATTGGTACCAGTTTTTTCATCGATCGCTTTTGTAC
OpuAR_W95F_for	GATTTATACCAGGTCAAAGGGGATTTCTACCAGAATTTTCATCGAT CG
OpuAR_W95F_rev	CGATCGATGAAATTCTGGTAGAAATCCCCTTTGACCTGGTATAAA TC
CA3-opuARA fwd	AAAGGATCCGCATCTACTCTTCCTTTTTTTC
CA3-opuARA rev	AAAGGATCCCATTAATCTAAGTCTTCGATTG
OpuAR treA Frag1/4 for	AAACCCGGGGCACCTTTTTGGAGAGCATCTAC
OpuAR treA Frag2 for	AAACCCGGGGAACCTCCGTATAAATGCATGTTTC
OpuAR treA Frag1/2 rev	AAAGGATCCCTGAAGCAGCTTCGATTGTGAC

Supplementary Table S3 | Plasmids used in this study

Plasmid	Description	Resistance	Reference
pASK-IBA3plus	Expression plasmid for <i>E. coli</i> with a AHT-inducible <i>tet</i> -promoter and a C-terminal <i>Strep</i> -tag II	<i>bla</i>	IBA (Göttingen, Germany)
pX	Integration vector for <i>B. subtilis</i> <i>amyE::cat::amyE</i>	<i>bla, cat</i>	(Kim et al., 1996)
pJMB1	<i>amyE::treA</i>	<i>bla, cat</i>	(Hoffmann et al., 2013)
pDG1515	Tetracycline resistance cassette	<i>tet</i>	(Guerout-Fleury et al., 1995)
p7Z6	Zeocin resistance cassette	<i>zeo</i>	(Yan et al., 2008)
pCA- <i>opuARA</i>	<i>opuA</i> operon and <i>opuAR</i> gene of <i>B. infantis</i> with native promoters cloned into pX- <i>amyE</i> -site	<i>bla, cat</i>	This study
pSTH33	<i>amyE::Φ(opuAA_{B,i}'-treA); opuAR⁺</i>	<i>bla, cat</i>	This study
pSTH34	<i>amyE::Φ(opuAA_{B,i}'-treA)</i>	<i>bla, cat</i>	This study
pMP_AR1	<i>B. infantis opuAR</i> gene cloned into pASK-IBA3plus	<i>bla</i>	This study
pSTH49	Site directed mutagenesis of <i>B. infantis opuAR</i> in pMP_AR1: <i>opuAR</i> [W ⁹³ F]	<i>bla</i>	This study
pSTH50	Site directed mutagenesis of <i>B. infantis opuAR</i> in pMP_AR1: <i>opuAR</i> [N ⁹⁶ F]	<i>bla</i>	This study
pOpuAR_Y94F	Site directed mutagenesis of <i>B. infantis opuAR</i> in pMP_AR1: <i>opuAR</i> [Y ⁹⁴ F]	<i>bla</i>	This study
pOpuAR_R100F	Site directed mutagenesis of <i>opuAR</i> in pMP_AR1: <i>B. infantis opuAR</i> [R ¹⁰⁰ F]	<i>bla</i>	This study
pOpuAR_Y94F_R100F	Site directed mutagenesis of <i>B. infantis opuAR</i> in pMP_AR1: <i>opuAR</i> [Y ⁹⁴ F/R ¹⁰⁰ F]	<i>bla</i>	This study

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