OpuF: a new *Bacillus* compatible solute ABC transporter with a substrate-binding protein fused to the trans-membrane domain

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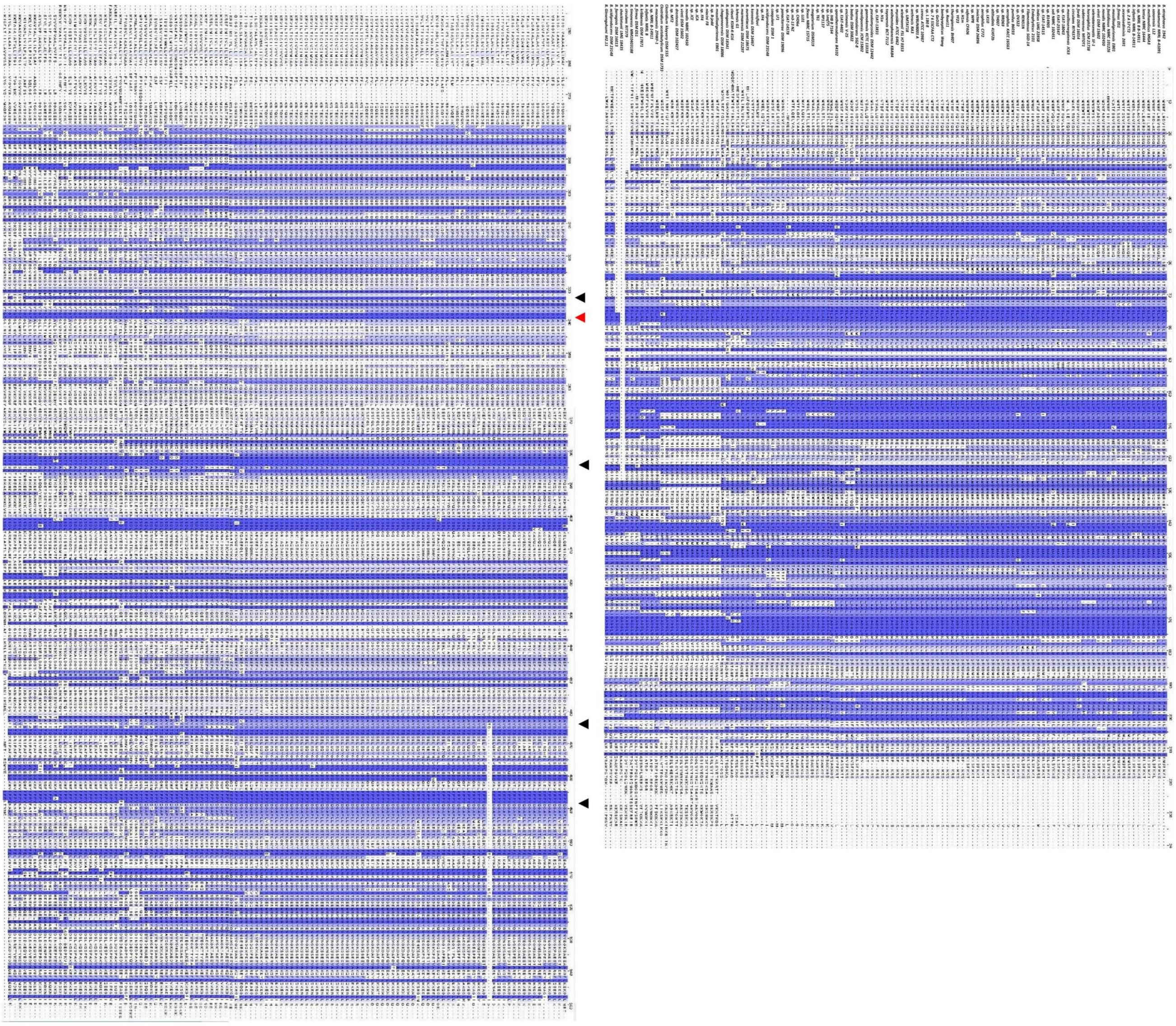

FIG S1. Alignment of the amino acid sequences of 112 OpuFB-type proteins that are present in the genus *Bacillus*. The OpuFB hybrid protein consists of a N-terminal TMD (right panel) and a C-terminal SBP (left panel) domain. Black arrows point out the four amino acid residues forming the aromatic cage that probably binds the trimethyl-ammonium (or dimethylsulfonium) head group of various ligands. The red arrow highlights the amino acid at position 74 of the mature SBP portion, that probably is involved in the closure of the two lobes of the SBP and which might contribute in setting the substrate specificity of the SBP. In all proteins a threonine (T) is present at this position.

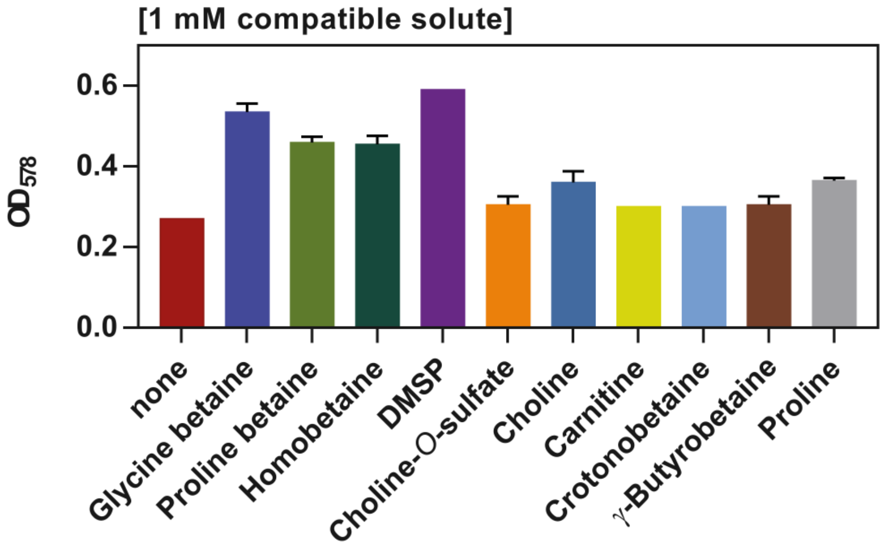

FIG S2. Protection of *B. infantis* against high osmolarity challenges. Cultures of *B. infantis* were grown in MOPS-buffered basal medium containing 0.9 M NaCl in the absence or presence of the indicated compatible solutes (1 mM final concentration). Growth yields were determined after 29 hours of incubation at 37 °C by measuring of the OD_{578}. The shown values are representative data derived from one biological replicate, with each of the conditions measured twice.

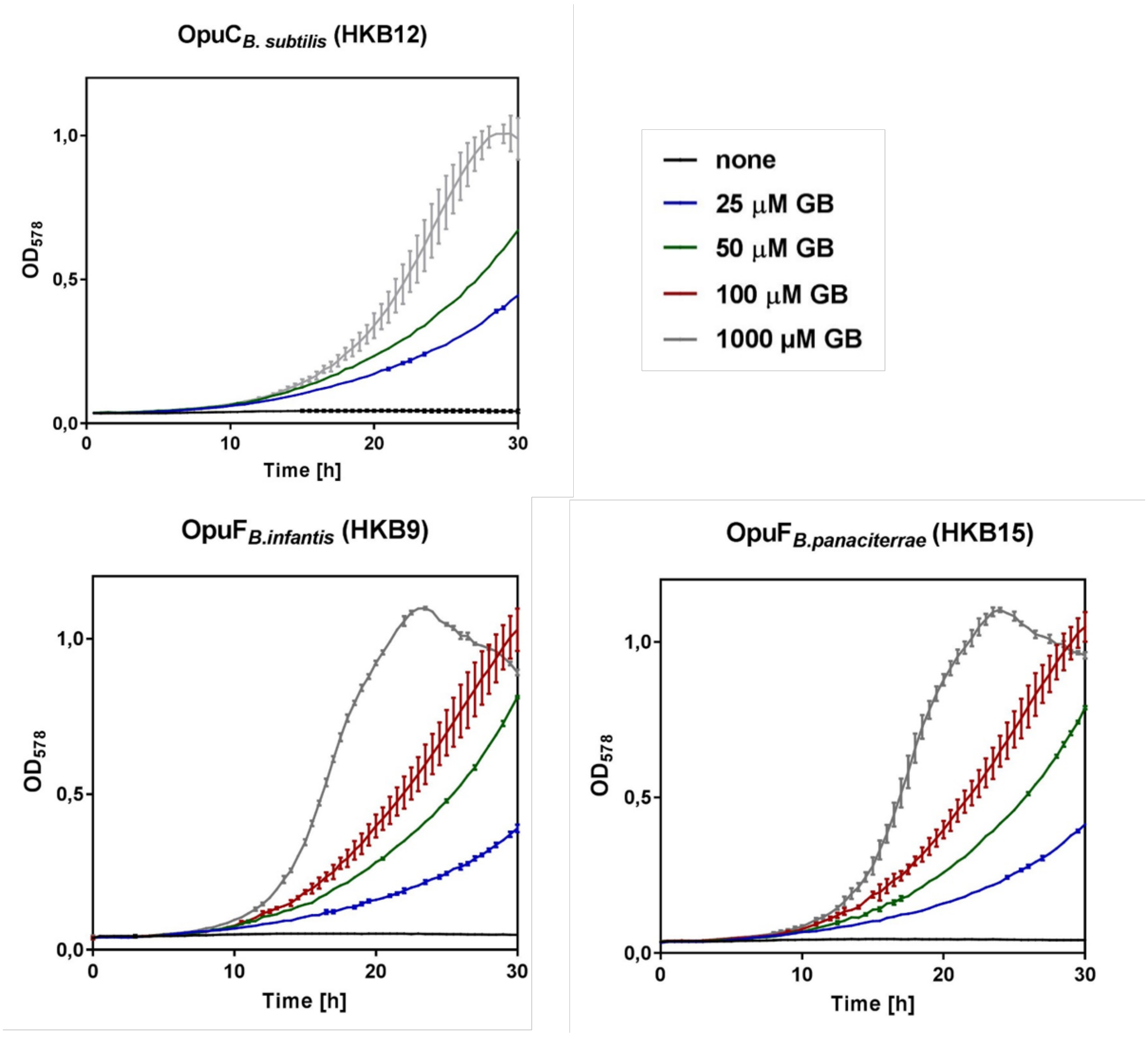

FIG S3. Growth curves of the *B. subtilis* strains possessing either the wild-type OpuC system or one of the studied OpuF transport systems. Cells were cultured in SMM containing 1.2 M NaCl in the absence or presence of either 25 µM, 50 µM, 100 µM, or 1 mM glycine betaine and their growth was monitored in a microtiter plate reader (EPOCH 2, BioTek) at 37° C. The shown values represent data derived from two independent biological replicates.

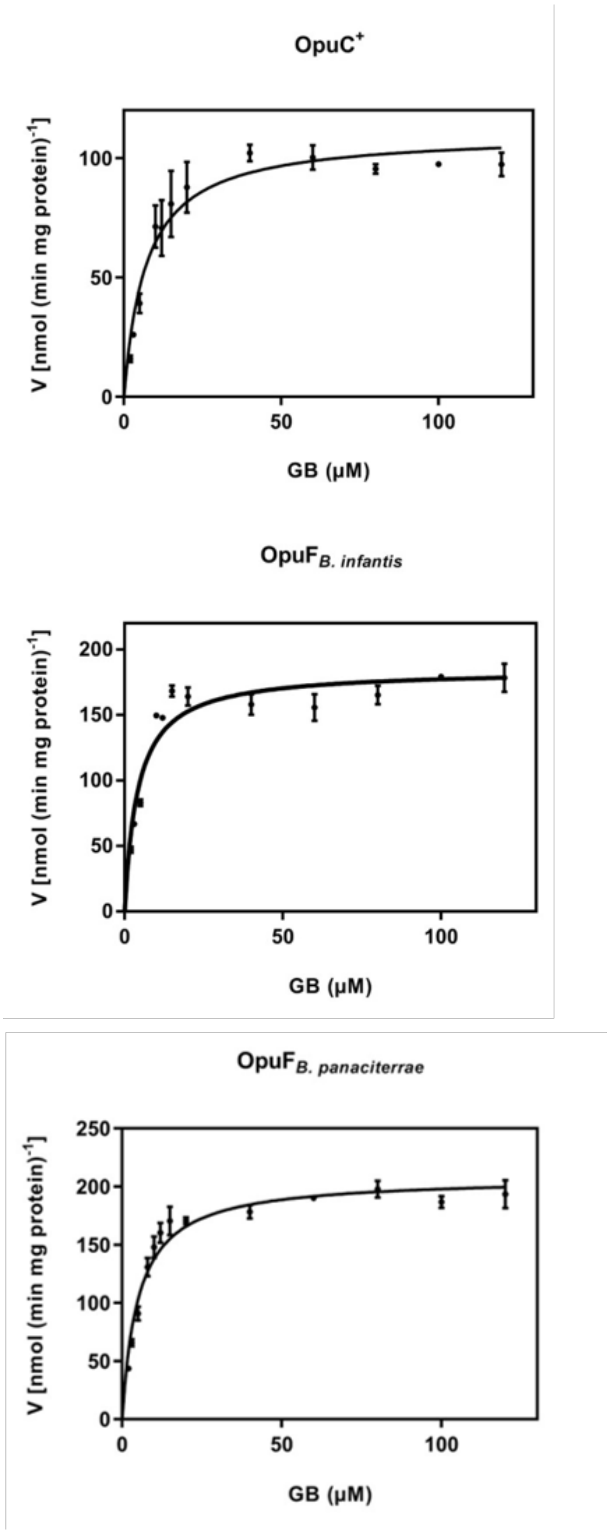

FIG S4. Michaelis-Menten kinetics of the wild-type OpuC ABC transporter from *B. subtilis* (strain HKB12) and of the OpuF systems from *B. infantis* and *B. panaciterrae* expressed in a *B. subtilis* host strain (LTB52) with radiolabeled glycine betaine as a substrate. The substrate concentration was varied between 3 µM and 120 µM. The *B. subtilis* chassis strain LTB52 is defective in the OpuA, OpuB, OpuC and OpuD compatible solute import systems, but still possesses the proline-specific OpuE importer.

TABLE S1. Identified structural analogs in PDB (OpuFB-SBP from *B. infantis*)

Rank	**Protein**	**Organism**	**PDB**	**TM-score**
1	BilE	*Listeria monocytogenes*	4z7eA	**0.973**
2	ProX	*Archaeoglobus fulgidus*	1sw5D	**0.93**
3	YehZ	*Escherichia coli*	4wepA	**0.909**
4	ProX	*Agrobacterium tumefaciens*	4ne4A	**0.906**
5	OpuCC	*Staphylococcus aureus*	3o66A	**0.885**
6	OpuCC	*Bacillus subtilis*	3ppnA	**0.823**
7	OpuBC	*Bacillus subtilis*	3r6uA	**0.756**
8	OpuAC	*Lactococcus lactis*	3l6gA	**0.719**
9	ProX	*Escherichia coli*	1r9lA	**0.663**
10	RafE	*Streptococcus peumoniae*	2heuC	**0.647**

The SWISS-model server (https://swissmodel.expasy.org/) was used to assess the structurally closest homologs of the OpuFB SBP domain of *B. infantis*. The Protein Data Base (https://www.rcsb.org/) accession codes for the retrieved proteins are given. The TM score describes the overall quality of a structural alignment between a crystalized protein and the *in silico* predicted structure of the query amino acid sequence.

TABLE S2. Distribution of OpuF-type transporter in the order Bacillales

Bacillales	**fully sequenced genomes**	**OpuFB-type Hits**
· **Allcyclobacillaceae**		
– *Alicyclibacillus*	(2)	0
– *Krypidia*	(1)	0
· **Bacillaceae**		
– *Aeribacillus*	(1)	0
– *Amphibacillus*	(1)	0
– *Anoxybacillus*	(4)	0
– *Bacillus*	(286)	**178**
– *Fictibacillus*	(2)	**3**
– *Geobacillus*	(18)	0
– *Halobacillus*	(2)	**2**
– *Lentibacillus*	(1)	**1**
– *Lysinibacillus*	(9)	**9**
– *Oceanobacillus*	(1)	**1**
– *Parageobacillus*	(5)	0
– *Terribacillus*	(1)	**1**
– *Virgibacillus*	(2)	**2**
· **Listeriaceae**		
– *Listeria*	(84)	**84**
· **Paenibacillaceae**		
– *Aneurinibacillus*	(1)	0
– *Brevibacillus*	(3)	0
– *Paenibacillus*	(50)	**2**
– *Thermobacillus*	(1)	0
· **Planococcaceae**		
– *Jeotgalibacillus*	(1)	0
– *Kurthia*	(1)	0
– *Planococcus*	(8)	0
– *Rummeliibacillus*	(1)	**1**
– *Solibacillus*	(3)	0
– *Sporosarcina*	(4)	**3**
· **Staphylococcaceae**		
– *Jeotgalicoccus*	(1)	**1**
– *Macrococcus*	(4)	**4**
– *Salinicoccus*	(2)	**1**
– *Staphylococcus*	(180)	**180**
· **Thermoactinomycetaceae**		
– *Nocibacillus*	(1)	**1**
· **unclassified**		
– *Exguobacterium*	(6)	**11**
– *Gamella*	(1)	0
	Σ 689	**Σ 485**