

Supplemental Material

Table S1. Comparison and quality data of structures for MrpA domain models.

Domain	Ramachandran plot quality (%)			
	Core	Allowed	General	Disallowed
MrpA_N	91.6	6.9	1.5	0.0
MrpA_C	93.0	5.7	1.7	0.0

Ramachandran plot qualities shows percentage (%) of residues belonging to the core, allowed, generally allowed and disallowed region of the plot.

Figure S1. Agarose gel electrophoresis of restriction-digested plasmids isolated from Na⁺/H⁺ antiporter-deficient *E. coli* strains transformed with pET21a containing the *mrpA* insert. Colony 9 (EP432) and colony 13 (KNabc) were used for further experiments.

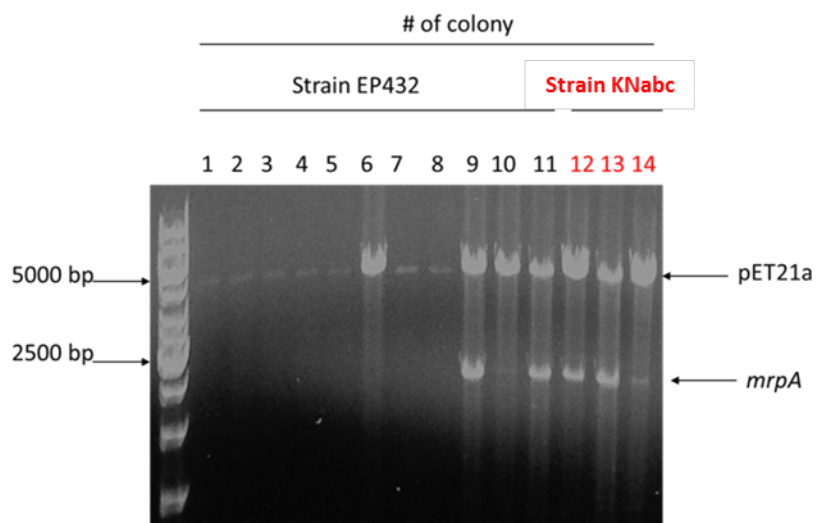


Figure S2. Ramachandran plot for the MrpA_N domain.

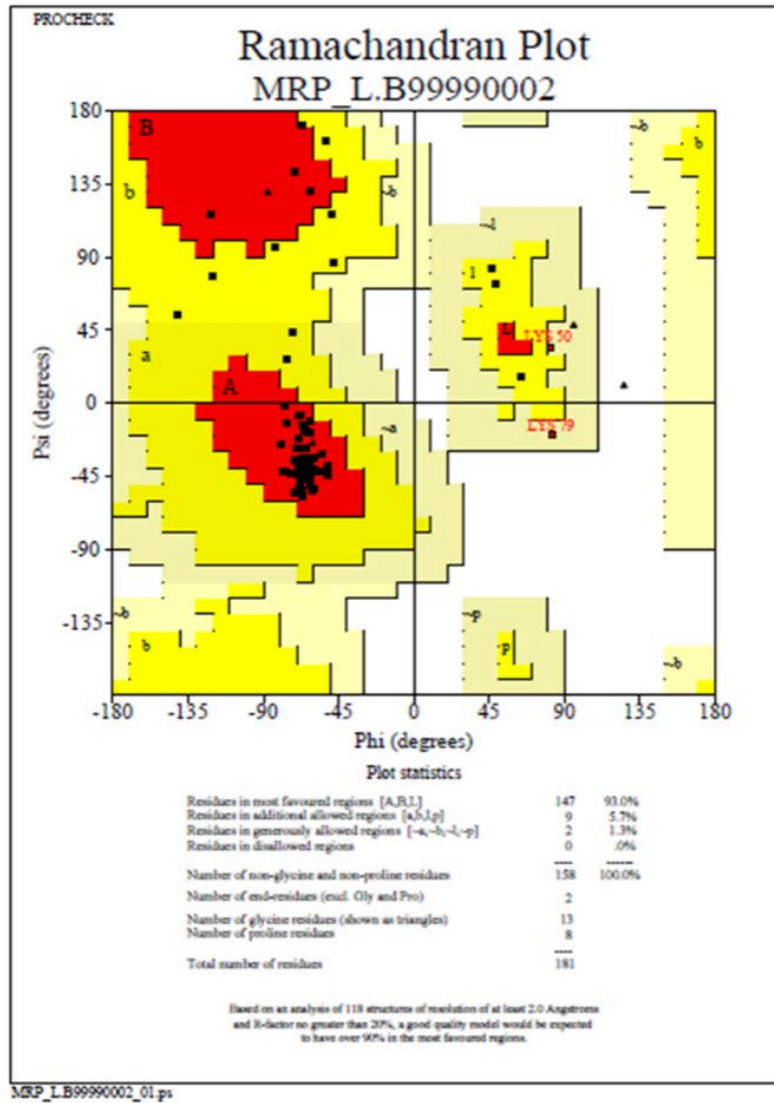


Figure S3. Ramachandran plot for the MrpA_C domain.

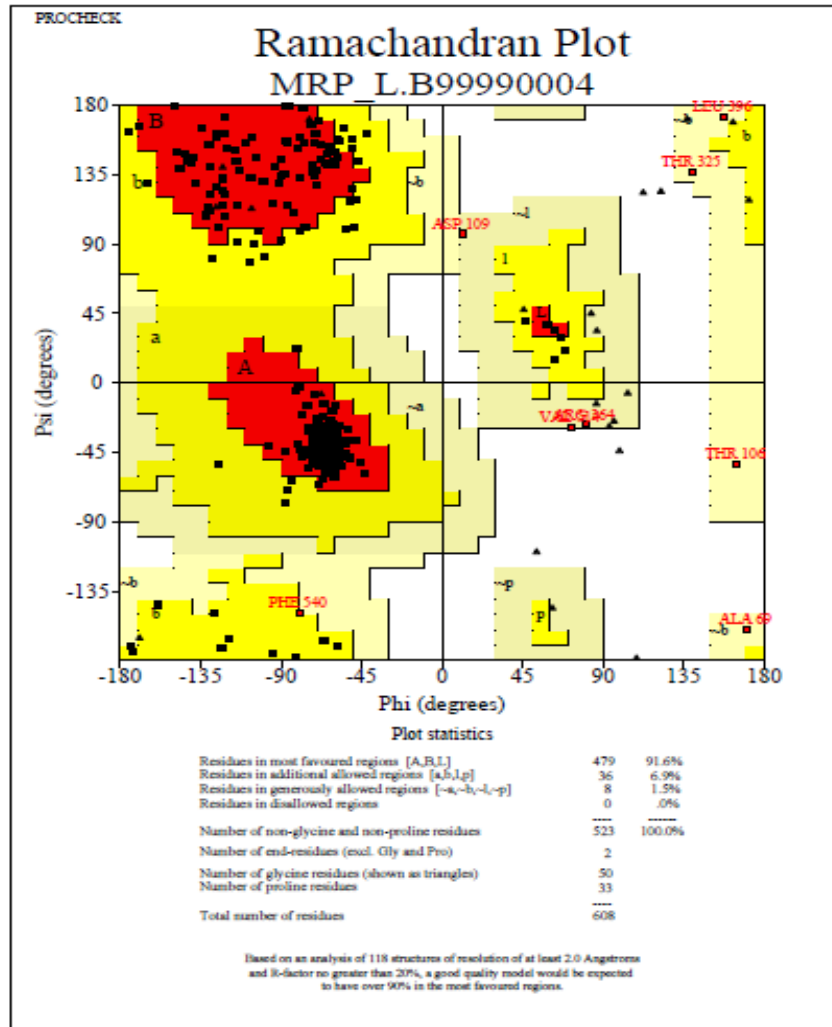


Figure S4. Multiple sequence alignment of *M. acetivorans* MrpA with MrpA-related proteins. (A) *M. acetivorans* MrpA N-terminal domain alignment. The domain is composed by 16 transmembrane (TM) helices. The C-terminus of the N-terminal domain contains the piston-like structure, which is shorter in MrpA than in NuoL of *E. coli*. (B) *M. acetivorans* MrpA C-terminal domain alignment. The domain is formed by five transmembrane (TM) segments. The predicted secondary structure of *M. acetivorans* MrpA is shown on the sequence alignment. Positions with overall identity greater than 80% are shaded in black, while sites with overall similarity greater than 80% are shaded in grey. Highly conserved charged residues proposed to be involved in MrpA function are marked with a red asterisk. The β H motifs are shown in yellow. The alignment was performed with T-Coffee (1). The sequence access numbers are: *Methanosarcina acetivorans* (WP_048174247.1), *Methanococcoides burtonii* (WP_011498313), *Bacillus subtilis* BSn5 (ADV93965), *Escherichia coli* K12 NuoL (P33607) and *Natranaerobius thermophilus* (WP_012446597).

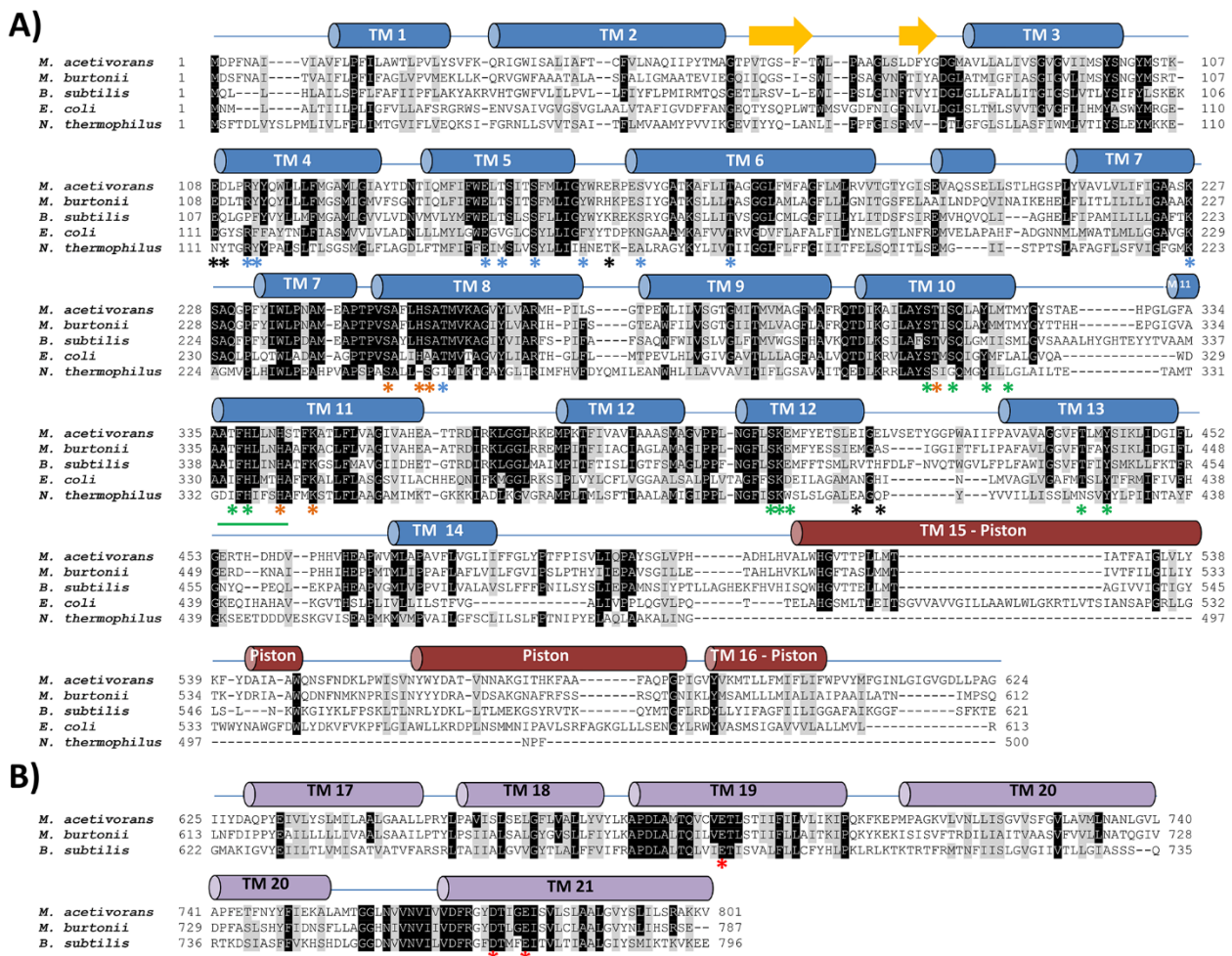


Figure S5. Alignment of amino acid sequences in clades I, II listed in Figure 4. The organism names and associated IDs are listed in the caption to Figure 4. The MrpA sequence from *M. acetivorans* (WP011024445) is bolded and underlined. Sequences were aligned with COBALT.

<u>WP_011024445</u>	<u>1</u>	MDPFNAIIVIAVFLPFI LA WTLPLVLYSVFKQRI GWISALIA FTCFVLNAQII IPY TMA-GTPVTVGSFTWLPAAGLSLDFYGD	79
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WP_048136646	1	MDPFNAIIVIAVFLPFI LA WTLPLVLYSVFKQRI GWISALIA FTCFVLNAQVI IPY TMA-GTPVTVGSFTWLPAAGLSLDFYGD	79
WP_048125080	1	MDPFNAIIVIAVFLPFI LA WTLPLVLYSVFKQRI GWISALIA FTCFALNTQVI IPY AMA-GTPVTVGSFTWLPAAGLSLDFYGD	79
WP_048124368	1	MDPFNAILIAVFLPFI LA WTLPLVLYKVKQRI GWISALIA FAFVFNQVI IPY AMA-GTPVTVGSFTWLPAAGLSLDFYGD	79
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WP_013194388	1	MNSFTVIALAIFL PFL SAATVPLAQKIL RHRVGFASAMAFVS FLLIAYVAP VVIH -GDTFTGAVS WLP SVGV EFSV YID	79
WP_048204897	1	MDSFNAITVAIFL PFI FAGLVPVMEKLL QRVGFYAAATALT SFILIGMAAPEVLEEGHIV QGTISWIP SAGVNF SIY AD	80
WP_013037209	1	MDSFTAILAIFL PFAA FIPLLEKFL KHRIGWFAAGIA FLS FALIGIV AP EIIH -GHIIQHSIEW MP SIGAE FSIY AD	79
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WP_013898326	1	MDQSVAILAIFL PFI FALLV PVEK IK HRIGWYAS F TAL TL FLI IATAAP TI IA-GKTVQYSV KWLP SVGV DFGIY VD	79
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WP_048125080	80	GMAVLLALIVSGVGIIMS YSNGYM STKEDL PRYYQWLL LFMGAM LGI AYTDNTIQ MFIFWEL TSITS FMLIGY WRERPE	159
WP_048124368	80	GMAVLLALIVSGVGIIMS AYSNGYM STKEDL PRYYQWLL LFMGAM LGI AYTDNTIQ MFIFWEL TSITS FMLIGY WRERPE	159
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WP_013898326	555	SINYYYDKIVGNATYNAGLFSSKFIQPGPKIKLYMIPILLLAILSILIPITLLSL-----EMLPVTLNFEIPPEYELIVFL	627
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WP_023846288	627	FMIVAAALGAALLPRYIPAIISLSGLGYLVALLFIYQLAPDLALQVLVETLSTIIFLLAIVKIPQKFKEHIPSSTTLARDL	706
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WP_013037209	627	LMVVAIAAATLHRYLSAVIAISAVGYLVSLMFIYIYLKAPDLALTQFLVETLATIIFLLVIARVQTFKEKIPKNILLRDI	706
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WP_048136646	719	LISGVVSFGVLAVMLNANMGLVAPFETFNYYFMEKALAMTGGNLVNVNIVVDFRQYDTIGEISVLSLAAALGVYSLILSRA	798
WP_048125080	719	LLSGVVSFGVLAVMLNANIGVLAPFETFNYYFIEKALEMTGGNLVNVNIVVDFRQYDTIGEISVLSLAAALGVYSLILSRA	798
WP_048124368	719	LISGVVSFGLLAVMLNANIGVLAPFQFQTCYFFIQKSEMTGGNLVNVNIVVDFRQYDTLGEISVLSLAAALGVYSLIMSRG	798
WP_023846288	707	VISVAVSAMIFIMLINATQGIIVPPFESLSHYFIEKSLTLAGGHNIVNVIIVDFRQYDTLGEISVLCALAGVYNIHISRG	786
WP_013194388	707	FIISGLVSVGVFIVLINATQGIIVPPFESLSHYFIEKSVSLTGGHNIVNVIIVDFRQYDTLGEISVLCALAGVYNIHISRG	786
WP_048204897	708	TISLAVAASVAVVLLNATQGIIVPPFESLSHYFIENSLPLAGGHNIVNVIIVDFRQYDTLGEISVLCALAGVYNIHISRG	787
WP_013037209	707	MISLAVASTVLIILLNATQGIIVPPFESLSHYFLENSVPLAGGHNIVNVIIVDFRQYDTLGEISVLCALAGVYNIHISRG	786
WP_015325011	706	LIAVSVASVVFILLSATQGIIVPPFESLSHYFIEKALPLAGGHNIVNVIIVDFRQYDTLGEIVMCLAAALGVYNIHISRG	785
WP_048147233	707	LISAVGIVLVLLNATQGIIVPPFESLSHYFIEKALPLAGGHNIVNVIIVDFRQYDTLGEIVMCLAGVYNIHISRS	786
WP_011498313	707	LIAITVAASVAVVLLNATQGIIVDPFASLSHYFIDNSFLLAGGHNIVNVIIVDFRQYDTLGEISVLCALAGVYNIHISRS	786
WP_013898326	708	LISVTVASTVFIIMLYAAQGIIVPPFESVSHYFLEKSVLLTGGHNIVNVIIVDFRQYDTLGEIVMCLAAALGVYNIHISRG	787
WP_011024445	799	KKVKGKKE 806	
WP_048174247	799	KKVKGKKE 806	
WP_048136646	799	KKVKGKKE 806	
WP_048125080	799	KKVKGKKE 806	
WP_048124368	799	KKAKGKKE 806	
WP_023846288	787	EDE----- 789	
WP_013194388	787	ER----- 788	
WP_048204897	788	EEQ----- 790	
WP_013037209	787	EGE----- 789	
WP_015325011	786	ESQ----- 788	
WP_048147233	787	DSK----- 789	

WP_011498313 787 EE----- 788
WP_013898326 788 ERK----- 790

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