

Supplementary Material

**An uncharacterized major facilitator superfamily
transporter from *Planococcus maritimus* exhibits dual
functions as a $\text{Na}^+(\text{Li}^+, \text{K}^+)/\text{H}^+$ antiporter and a multidrug
efflux pump**

Heba Abdel-motaal, Lin Meng, Zhenglai Zhang, Amro Abdelazez, Shao Li, Tong Xu, Fankui Meng, Shaima Abozaed, Rui Zhang, Juquan Jiang^{*}

^{*} Correspondence: Juquan Jiang: jjqdainty@163.com

Table S1. Strains, plasmids and primers used in the study.

Strains, plasmids or primers	Relevant phenotype, genotype or primer sequence	The source or usage
Strains		
<i>Planococcus maritimus</i> DSM17275 ^T	Type strain of <i>Planococcus maritimus</i> , a slightly halophile	Ordered from German Collection of Microorganisms and Cell Cultures (DSMZ)
<i>Escherichia coli</i> KNabc	<i>nhaA</i> ::Km ^R , <i>nhaB</i> ::Em ^R , <i>chaA</i> ::Cm ^R	Donated by Dr. Terry A. Krulwich
<i>Escherichia coli</i> DH5α	F ⁻ , φ80dlacZ ΔM15, Δ(<i>lacZYA-argF</i>)U169, <i>deoR</i> , <i>recA1</i> , <i>endA1</i> , <i>hsdR17(rK⁻, mK⁺)</i> , <i>phoA</i> , <i>supE44</i> , λ ⁻ , <i>thi</i> ⁻¹ , <i>yrA96</i> , <i>relA1</i>	Takara Biotechnology (Dalian) Co., Ltd., China
<i>Escherichia coli</i> CM2	A major multidrug efflux system-deficient <i>E. coli</i> DH5α mutant (Δ <i>acrAB</i>), which was obtained by homologous recombination	This study
Plasmids		
pUC18	Cloning vector	Takara Biotechnology (Dalian) Co., Ltd., China
pUC-PM29	pUC18 carrying a 3.2 kb DNA fragment including 5'-end truncated <i>uvrD</i> and <i>mdrP</i>	This study
pET19b	Over-expression vector	Novagen Ltd., USA
pET- <i>mdrP</i>	pET19 carrying <i>mdrP</i> gene	This study
pET-truncated <i>uvrD</i>	pET19 carrying 5'-end truncated <i>uvrD</i>	This study
pKD46	λ Red recombinase expression plasmid with ampicillin resistance	Donated by Dr. W. Todd Lowther
pKD3	Template plasmid for a chloramphenicol resistance gene	Donated by Dr. W. Todd Lowther
Primers		
<i>mdrP</i> _F	5'-CATATGAAGATCAAGGATTGGAA-3' (<i>Nde</i> I, undelined)	For the subcloning of <i>mdrP</i> gene
<i>mdrP</i> _R	5'-GGATCCTTATATGTTTCGCAACCGTT-3' (<i>Bam</i> HI, underlined)	
<i>uvrD</i> _F	5'-CATATGCTCGAGGAATTGGTCTCATT-3' (<i>Nde</i> I, undelined)	For the subcloning of 5'-end truncated <i>uvrD</i> gene
<i>uvrD</i> _R	5'-GGATCCTTATATCAGTTCCAGCAATT-3' (<i>Bam</i> HI, underlined)	
<i>acrAB</i> _KF	5'-ATGAACAAAAACAGAGGGTTTACGCCTCTGGCGGTCCATATGAATATCCTCCTTA-3' (<i>Eco</i> RI, underlined)	For the knockout of <i>acrAB</i> genes
<i>acrAB</i> _KR	5'-TCAATGATGATCGACAGTATGGCTGTGCTCGATATCTGTGTAGGCTGGAGCTGCTTC-3' (<i>Eco</i> RI, underlined)	
<i>acrAB</i> _VF	5'-TTAGGAAGGAAGCGGGACA-3'	For the verification of <i>acrAB</i> knockout
<i>acrAB</i> _VR	5'-AGTGGATCGCCAGGGAATT-3'	

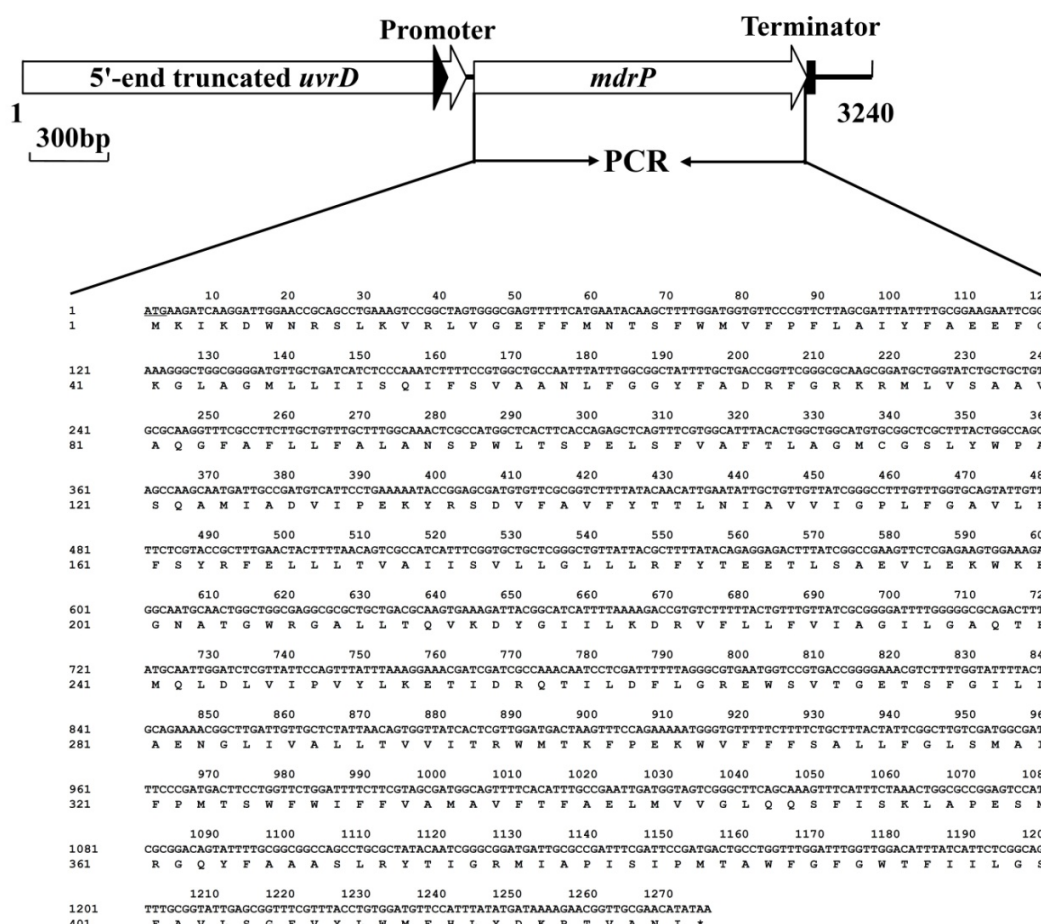


Fig. S1. Mapping of the inserted DNA fragment in the recombinant plasmid pUC-PM29 and subcloning of *mdrP* gene.

One 5'-end truncated *uvrD* and one intact *mdrP* are included in this 3.2-kb DNA fragment. The former begins from No. 1 bp of this DNA fragment whereas the latter is preceded by respective promoter-like sequence (filled arrow) and a respective Shine-Dalgarno (SD) sequence (filled rectangle). The sequence of *mdrP* gene from its initiation codon (ATG, underlined) to stop codon (TAA, marked with the star) was subcloned by PCR amplification and fused in frame with an N-terminal His₆ tag into an expression vector pET19b.

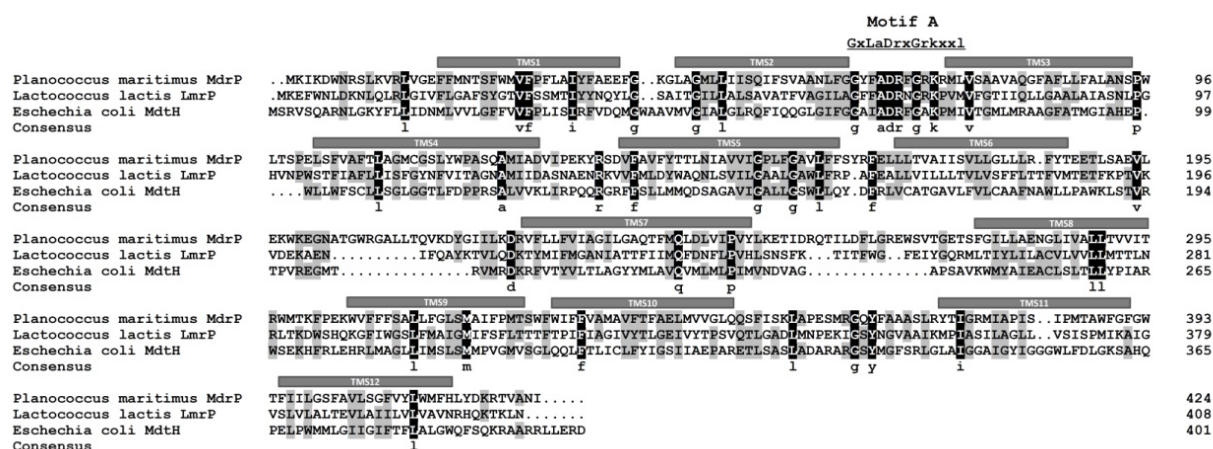


Fig. S2. Alignment of MdrP with the solely two characterized DHA1 family multidrug efflux pumps.

MdrP was aligned with the solely two characterized DHA1 family multidrug efflux pumps, *Lactococcus lactis* LmrP (28 %, accession version No. CAA61918.1) and *Escherichia coli* MdtH (22 %, accession version No. P69367.1). Shading homology corresponds to 100 % (black), ≥ 50 % (grey) and < 50 % (white) amino acid identity, respectively. The twelve putative transmembrane segments (TMSs) are marked with grey bars above the alignment. The consensus sequence of Motif A was highlighted above the alignment between TMS2 and TMS3.