

Supplementary Material

A novel MFS-MDR transporter, MdrP, employs D223 as a key determinant in the Na⁺ translocation coupled to norfloxacin efflux

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Juquan Jiang

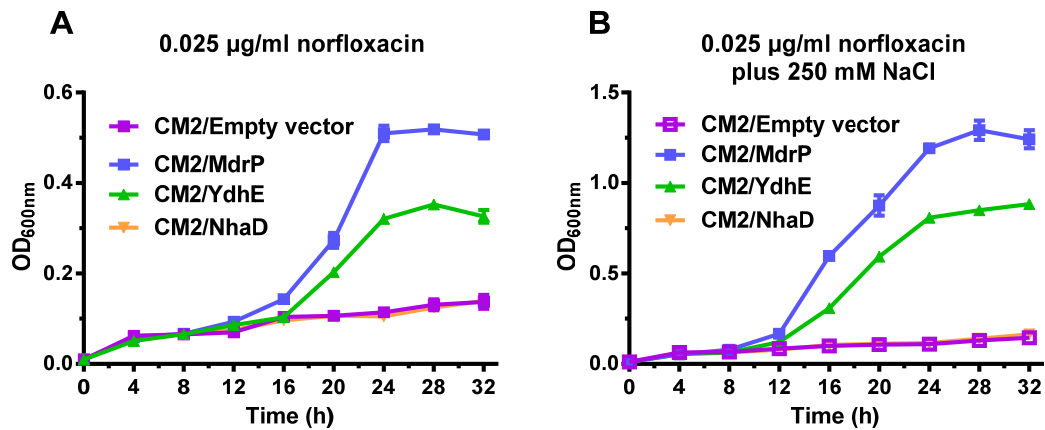
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Supplemental Table 1. Primers used in this study.

Primers	Primer sequence (5' to 3')	The source
MdrP-F	TCTAGAGTGTCTTTGGAAACGGGCAG	This study
MdrP-R	GGTACCTTAATGATGATGATGATGCTTGTCGTCGTCGTCTAT GTTTCGCAACCGTTCTTTTATC (6×His tag, underlined)	This study
YdhE-F	TTGTCACCCACCAGCGCGGA	This study
YdhE-R	ATAATATGACCTGACATTAA	This study
NhaD-F	TATACCTACAAAGCAACGGA	This study
NhaD-R	AAAGCGCTTACCGCTGGATG	This study
D67A-F	GGCTATTTTGCTG <u>CAC</u> GGTTCGGGC (Mutagenic bases, underlined)	This study
D67A-R	<u>TGCAGCAAAATAGCCGCCAAATAAA</u> (Mutagenic bases, underlined)	This study
R71A-F	GACCGGTTTCGGGG <u>CCA</u> AGCGGATGC (Mutagenic bases, underlined)	This study
R71A-R	<u>GGCCCCGAACCGGTCAGCAAAATAG</u> (Mutagenic bases, underlined)	This study
R71K-F	GACCGGTTTCGGGA <u>AAGA</u> AGCGGATGC (Mutagenic bases, underlined)	This study
R71K-R	<u>CTTCCCGAACCGGTCAGCAAAATAG</u> (Mutagenic bases, underlined)	This study
D127A-F	GCAATGATTGCC <u>GCT</u> GTTCATTCCTG (Mutagenic bases, underlined)	This study
D127A-R	<u>AGCGGCAATCATTGCTTGGCTCGCT</u> (Mutagenic bases, underlined)	This study
D127E-F	GCAATGATTGCC <u>GAGGTC</u> ATTCCTG (Mutagenic bases, underlined)	This study
D127E-R	<u>CTCGGCAATCATTGCTTGGCTCGCT</u> (Mutagenic bases, underlined)	This study
D127N-F	GCAATGATTGCC <u>AATGTC</u> ATTCCTG (Mutagenic bases, underlined)	This study
D127N-R	<u>ATTGGCAATCATTGCTTGGCTCGCT</u> (Mutagenic bases, underlined)	This study
E188A-F	TTTTATACAGAG <u>GCG</u> ACTTTATCGG (Mutagenic bases, underlined)	This study
E188A-R	<u>CGCCTCTGTATAAAAGCGTAATAAC</u> (Mutagenic bases, underlined)	This study
D223A-F	ATCATTTTAAAAG <u>CCCGTGTCTTTT</u> (Mutagenic bases, underlined)	This study
D223A-R	<u>GGCTTTTAAAATGATGCCGTAATCT</u> (Mutagenic bases, underlined)	This study
D223E-F	ATCATTTTAAAAG <u>GAGCGTGTCTTTT</u> (Mutagenic bases, underlined)	This study
D223E-R	<u>CTCTTTTAAAATGATGCCGTAATCT</u> (Mutagenic bases, underlined)	This study
D223N-F	ATCATTTTAAAAA <u>AATCGTGTCTTTT</u> (Mutagenic bases, underlined)	This study
D223N-R	<u>ATTTTTTAAAATGATGCCGTAATCT</u> (Mutagenic bases, underlined)	This study
D244A-F	TTCATGCAATTG <u>GCGCTCGTTATTC</u> (Mutagenic bases, underlined)	This study
D244A-R	<u>CGCCAATTGCATGAAAGTCTGCGCC</u> (Mutagenic bases, underlined)	This study
D244E-F	TTCATGCAATTG <u>GAACTCGTTATTC</u> (Mutagenic bases, underlined)	This study

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D244E-R	<u>TTCCAATTGCATGAAAGTCTGCGCC</u> (Mutagenic bases, underlined)	This study
D244N-F	TTCATGCAATTGA <u>ACCT</u> CGTTATTC (Mutagenic bases, underlined)	This study
D244N-R	<u>GTT</u> CAATTGCATGAAAGTCTGCGCC (Mutagenic bases, underlined)	This study
E341A-F	TTCACATTTGCC <u>GC</u> ATTGATGGTAG (Mutagenic bases, underlined)	This study
E341A-R	<u>TGCGGCAAATGTGAAA</u> ACTGCCATC (Mutagenic bases, underlined)	This study
R361A-F	CCGGAGTCCATGG <u>CC</u> GGACAGTATT (Mutagenic bases, underlined)	This study
R361A-R	<u>GGCC</u> ATGGACTCCGGCGCCAGTTTA (Mutagenic bases, underlined)	This study
R361K-F	CCGGAGTCCATG <u>AAAG</u> GACAGTATT (Mutagenic bases, underlined)	This study
R361K-R	<u>TTT</u> CATGGACTCCGGCGCCAGTTTA (Mutagenic bases, underlined)	This study



Supplemental Figure 1. Growth curves of *E. coli* CM2 transformants in the LBO media plus 0.025 µg/ml norfloxacin without or with the addition of NaCl.

Pre-cultures of *E. coli* CM2 transformants were grown in LBO broths to OD_{600 nm} of 1.0, and then 1% of pre-cultures were inoculated in the fresh LBO broths plus 0.025 µg/ml norfloxacin without the addition of NaCl (A) or with the addition of 250 mM NaCl (B). Growth curves were plotted by evaluating OD_{600 nm} of *E. coli* CM2 transformants in triplicate within 32 h. Blue filled square stands for CM2/MdrP; green filled upward triangle stands for CM2/YdhE as the positive control of a H⁺-coupled norfloxacin efflux transporter; brown filled downward triangle stands for CM2/NhaD as the positive control of a Na⁺/H⁺ antiporter; purple open square stands for CM2/Empty vector as a negative control.

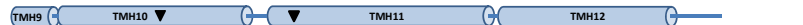
A novel MFS drug/Na⁺ antiporter



<i>Planococcus maritimus</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Planococcus plaktoidis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Planococcus rufitarsis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Planococcus donghaiensis</i> pMdrP	MNTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	68
<i>Planomicrobium okanokoites</i> pMdrP	MNTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLISVASVAGFAEL	68
<i>Planomicrobium glaciei</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Planococcus massiliensis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Psychrobacillus psychrophilus</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	88
<i>Paenisporeosarcina quiquiliarum</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	88
<i>Paenisporeosarcina indica</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	88
<i>Bacillus clausii</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Bacillus campisalis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Bacillus weihenensis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Halobacillus mangrovi</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Ficitobacillus clausii</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Anoxybacillus oxydans</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Anoxybacillus puschinoensis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Listeria welshimeri</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Brochothrix thermosphacta</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Subopercularia subopercularis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Brevibacillus brevis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Bradyrhizobium japonicum</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Paenibacillus glucanolyticus</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Ocellulibacillus massiliensis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Oceanobacillus kinsella</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Oceanobacillus massiliensis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Pontibacillus halophilus</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Terribacillus halophilus</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Bacillus coquilhanii</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Lentzea jiangshensis</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87
<i>Bacillus cereus</i> pMdrP	MKIKDNNRSILKRLVIGEFNNMTSY.....VFFPFLIATYAEK.....GKAGLLMLISQISVVAANVGGC.....QFRGRKMLVSVASVAGFAEL	87



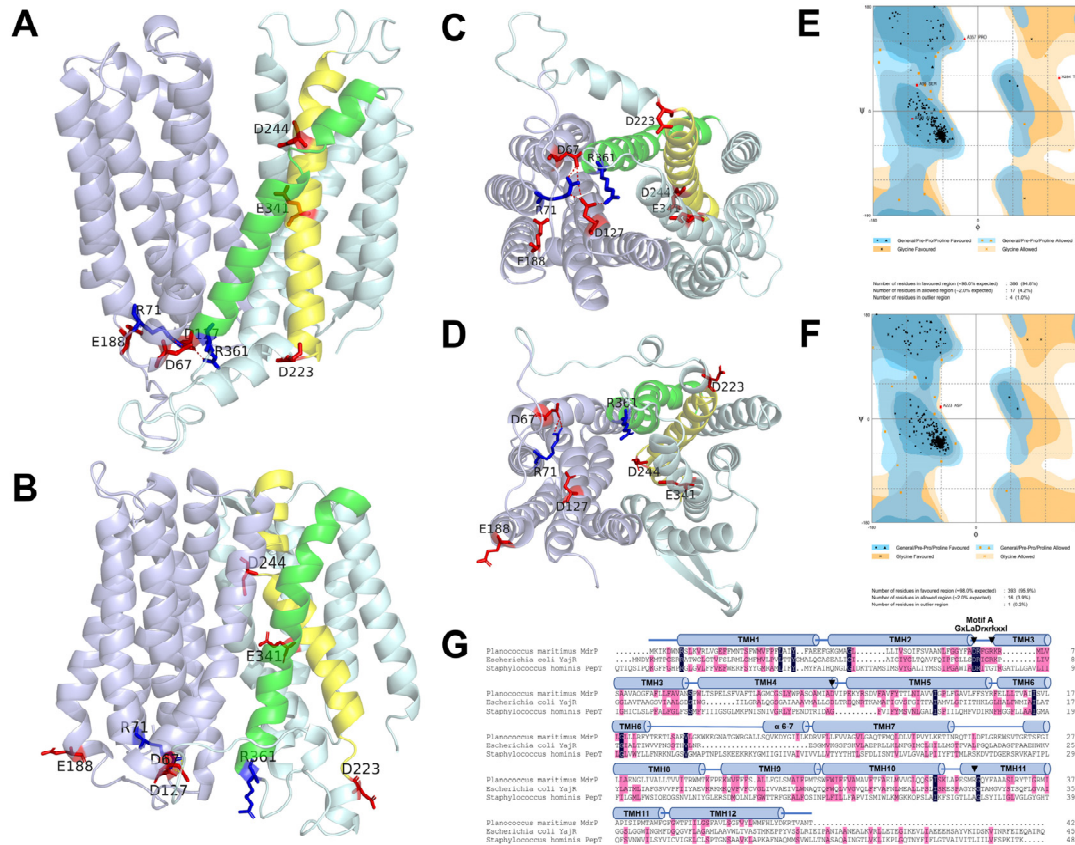
Planococcus maritimus MdirP	LFALANSPWMLSPESLVSFAITLTMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Planococcus plaktortidis pMdirP	LFANANSPFLKPTKLTIDISLTMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Planococcus rifeoekensis pMdirP	LFANANSPFLKPTKLTIDISLTMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Planococcus rifeoekensis pMdirP	LFANANSPFLKPTKLTIDISLTMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Planomicrobium okenoekites pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Planomicrobium glaciei pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Planococcus massiliensis pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Planococcus massiliensis pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Paeniporocarcina quatuorquarum pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Paeniporocarcina indica pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Bacillus encimensis pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Bacillus campalis pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Bacillus campalis pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Halobacillus mangrovei pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Fictibacillus encimensis pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Anaerobacillus mayae pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Anaerobacillus mayae pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Listeria welshimeri pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Brochothermus thermopacta pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Rubeoparvum massiliense pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Rubeoparvum massiliense pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Bradyrhizobium japonicum pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Paenibacillus gluconolyticus pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Gorillibacterium massiliense pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Gorillibacterium massiliense pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Oceanobacillus massiliensis pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Pontibacillus halophilus pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Terribacillus halophilus pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Bacillus cereus pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203
Bacillus cereus pMdirP	LFALANSPWLESELCFIATITMAGCSLSL	...LAVD	LPKYSRSEVAIVPTTL	...IAVVL	SLFGLVLFSSYRRELLIVATISVLGLLIRTYE	...LSAEVLKWEKK	A	203

[illegible]

Planococcus maritimus MdrP	IPFMSFMS	IFVFMAVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	424
Planococcus plakortidis pmdrP	IPFMSFMS	IFVFMAVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	424
Planococcus rifeotoensis pmdrP	IPFMSFMS	IFVFMAVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	424
Planococcus rufus pmdrP	IPFMSFMS	IFVFMAVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	424
Planomicrobium okanoikeis pmdrP	LPFTSFTS	IFVVIAMGITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	423
Planomicrobium glaciei pmdrP	LPFTSFTS	IFVVIAMGITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	423
Planococcus massiliensis pmdrP	LPFTSFTS	IFVVIAMGITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	423
Psychrobacter psychrophilus pmdrP	IPFMSFMS	IFVFMAVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	423
Pseudoporaosarcina aquasquillorum pmdrP	IPFMAVNI	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	425
Pseudoporaosarcina indica pmdrP	IFSFIDFL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	425
Pseudoporaosarcina indica pmdrP	IFSFIDFL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	425
Bacillus enciensis pmdrP	MFALDITD	GFVVGMAVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	422
Bacillus campalis pmdrP	MFSGQVNI	GFILLAMVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	422
Bacillus thuringiensis pmdrP	MFSGQVNI	GFILLAMVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	422
Halobacillus mangrovei pmdrP	LYGNTGTL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	418
Fictibacillus enciensis pmdrP	LYGNTGTL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	418
Anaerobacillus mayae pmdrP	MGGHTITD	GFILLAMVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	428
Bacillus thuringiensis pmdrP	LYGNTGTL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	418
Listeria welshimeri pmdrP	IFGISTGP	VAIFALITP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	428
Brochothermus thermopacta pmdrP	IFGSSISV	GFILLAMVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	417
Rubeoparvum massiliense pmdrP	LMASFNPL	GFILLAMVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	417
Brachyobacterium massiliense pmdrP	LMASFNPL	GFILLAMVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	417
Brachyobacterium japonicum pmdrP	LMASFNPL	GFILLAMVITPL	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	417
Pseudobacillus gluconolyticus pmdrP	ALGHNTNI	LFVFFVTITP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	392
Gorillibacterium massiliense pmdrP	ALGHNTNI	LFVFFVTITP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	392
Planococcus maritimus MdrP	ALGHNTNI	LFVFFVTITP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	423
Oceanobacillus massiliensis pmdrP	TVSYNNIL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	440
Pontibacillus halophilus pmdrP	ALAYSNNIL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	440
Terribacillus halophilus pmdrP	ALAYSNNIL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	440
Bacillus cereus pmdrP	ALAYSNNIL	VFLIAMIATP	LMVVLGQSFISKL	DMRMGRQ	PAASALRY	TIGRMATIS	PIPTAAWFG	GWTFIL	LGSAVLS	GFVVLAMH	IFKTRAVNI	429

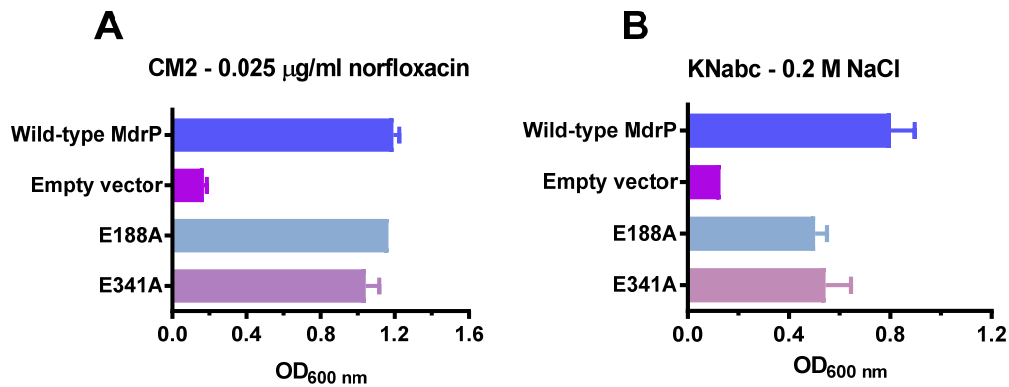
Supplemental Figure 2. Alignment of MdrP with 30 representatives of its homologs.

For the analysis of residue conservation, MdrP was aligned with 30 representatives of its homologs clustered within our recently reported phylogenetic tree (Abdel-Motaal et al., 2018). Shading homology corresponds to 100 % (black), ≥ 75 % (pink), ≥ 50 % (cyan) and ≥ 33 % (yellow) amino acid identity, respectively. The twelve putative transmembrane helices (TMHs) are marked with light blue cylinders above the alignment. The consensus sequence of the motif A was highlighted above the alignment between TMH2 and TMH3. Conserved amino acid residues (downward triangle) were selected for functional analysis via site-directed mutagenesis.



Supplemental Figure 3. Modeled structures of MdrP based on the templates of 3D structures of *E. coli* YajR and *Staphylococcus hominis* PepT.

The modeled structures of MdrP were predicted by using *E. coli* YajR 3D structure and *S. hominis* PepT 3D structures as the templates of outward-facing conformation and inward-facing one through the submission of its deduced amino acid sequence to the Phyre2 website <http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index>. TMH7-8 of PepT was considered to be a long loop and α helix 6-7 between TMH6 and TMH7 of MdrP when a modeled structure was constructed using PepT as a template. Predicted outward-facing and inward-facing conformation stereo views oriented parallel to the membrane (A&B) and from the periplasm (C&D) oriented vertical to the membrane were shown, respectively. Conserved residues between MdrP and 30 representatives of its homologs (Abdel-Motal et al., 2018) were highlighted in color and labelled with their residue names, TMH7 and TMH11 were specially colored in yellow and green, respectively. The combinations of the ϕ and ψ angles of residues in favored, allowed and outlier regions of Ramachandran plot of outward-facing and inward-facing conformation (E&F) were qualified to test the reliability of structural analysis. Also, alignment of MdrP with *E. coli* YajR and *S. hominis* PepT (G) was carried out to show their identity and coverage, and four potential conformation-related residues were highlighted with downward triangles above the alignment.



Supplemental Figure 4. Growth tests for norfloxacin or NaCl resistance of *E. coli* CM2 or KNabc expressing variants E188A and E341A.

The effect of each variant on norfloxacin resistance was tested by its complementation with *E. coli* CM2 in LB broths containing 0.025 µg/ml norfloxacin (A) or with *E. coli* KNabc in LBK broths containing 0.2 M NaCl (B). Cell growth was ended within 24 h for CM2 or 48 h for KNabc, followed by the evaluation of OD_{600 nm}. Each data point stands for the mean ± SD of three independent cultures.