

The MFS efflux pump EmrKY contributes to the survival of *Shigella* within macrophages

Pasqua M., Grossi M., Scinicariello S., Aussel L., Barras F., Colonna B. and Prosseda G.

Fig S1

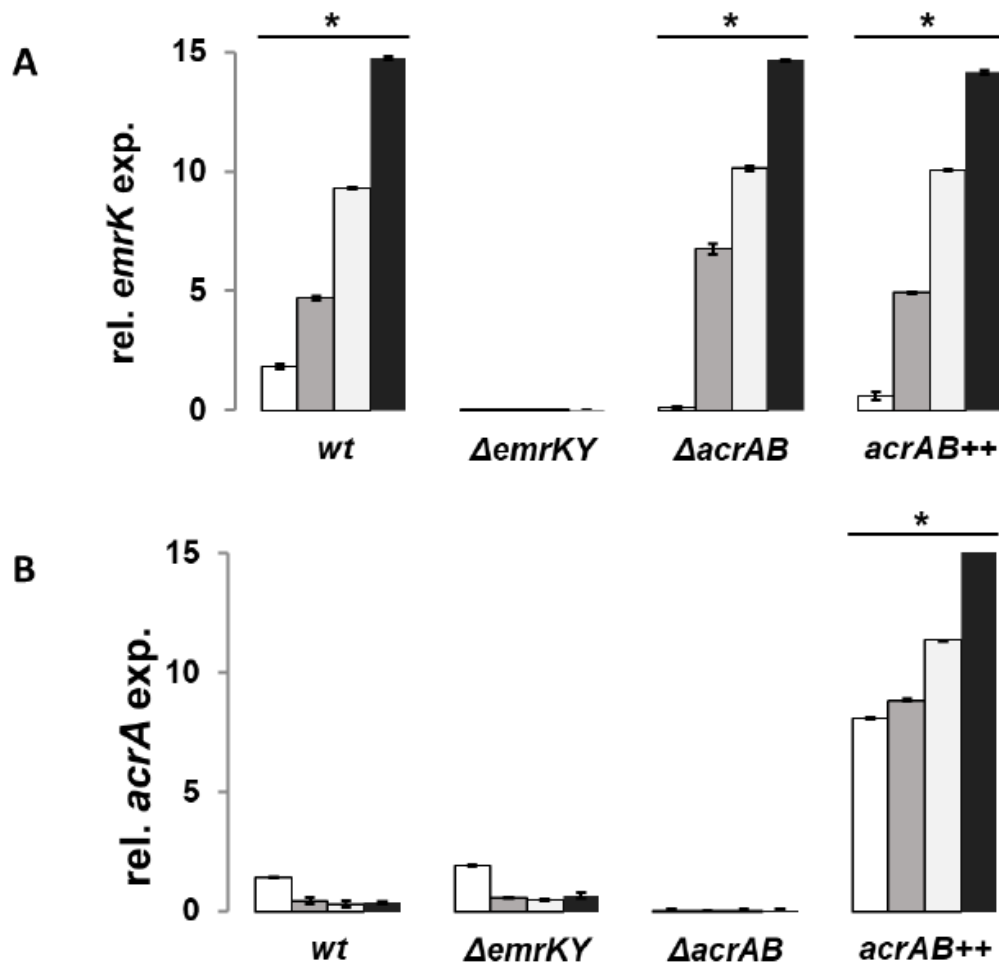


Figure S1. *emrK* expression during *S. flexneri* infection of U937 is independent from *acrA* transcription and *vice versa*. Quantitative analysis of (A) *emrK* and (B) *acrA* transcripts was performed by Real Time PCR assay using RNA extracted from intracellular *S. flexneri* M90T and its derivatives lacking EmrKY (M90T $\Delta emrKY$) or AcrAB (M90T $\Delta acrAB$) EP or carrying the pACacrAB plasmid. The *emrK* and *acrA* expression was followed in each infecting strain from 0h up to 3h post-infection. At least three experiments were carried out and the results are shown relative to the expression in bacteria grown in RPMI. Statistical significance was determined by a one-tailed ANOVA, and p values are as follows: * p<0.05, ** p<0.01. Error bars represent SD.

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Table S1. Strains and plasmids used in this study

Strain	Relevant characteristics	Source/Reference
DH10b	<i>E. coli K12</i>	[44]
M90T	<i>S. flexneri 5a</i>	[30]
M90T Δ <i>mdtJI</i>	M90T mutant defective in <i>mdtJI</i> operon	[27]
M90T Δ <i>emrKY</i>	M90T mutant defective in <i>emrKY</i> operon	This study
M90T Δ <i>acrAB</i>	M90T mutant defective in <i>acrAB</i> operon	This study
M90T Δ <i>evgA</i>	M90T mutant defective in <i>evgA</i> gene	This study
M90T Δ <i>evgS</i>	M90T mutant defective in <i>evgS</i> gene	This study
M90T Δ <i>fnr</i>	M90T mutant defective in <i>fnr</i> gene	This study
Plasmid name	Relevant characteristics	Source/Reference
pKD4	<i>kan</i> -containing plasmid, template for PCR	[45]
pKD46	Red recombinase expression plasmid	[45]
pGIP7	pACYC184 derivative carrying Ptac promoter	[47]
pZEP08	GFP reporter plasmid; Ap ^R , Cm ^R	[46]
pAC <i>acrAB</i>	pGIP7 derivative carrying <i>S. flexneri acrAB</i> operon	This study
pZ <i>emrKYgfp</i>	pZEP08 derivative carrying the <i>S. flexneri emrKY</i> promoter region	This study
pZ <i>mdtJIgfp</i>	pZEP08 derivative carrying the <i>S. flexneri mdtJI</i> promoter region	This study

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Table S2. Oligonucleotides used in this study

Name	Sequence (5'-3')	Used for
KYF	TCACTGGATAAAACTGATGCCACTATCGCACTC AATAGTGTAGGCTGGAGCTGCTTC	<i>emrKY</i> deletion
KYR	TGTCACAGAACGCCAGTAATAGCAAACCGCAT ACATCAATTCCGGGGATCCGTCGACC	<i>emrKY</i> mutation
ABF	ACTTTTGACCATTGACCAATTTGAAATCGGACA CTCGAGGTTTACATATGTGTAGGCTGGAGCTGC TTCG	<i>acrAB</i> mutation
ABR	TAGGCATGTCTTAACGGCTCCTGTTAAGTTAA GACTTGGACTGTTCAAGGATTCGGGGATCCGTC GACC	<i>acrAB</i> mutation
AF	AAAGACCACAACAGAGAAGAAAAAATAGGG TAAAAACTTCATATGAATATCCTCCTTA	<i>evgA</i> mutation
AR	CTGTATTACTACAGGGAGAAGGGAGATGCTTC ATTGCAAAGTGTAGGCTGGAGCTGCTTC	<i>evgA</i> mutation
SF	ATCTTTACACATTCGCGCAACGTAACAAAATC GGCTAATCGTGTAGGCTGGAGCTGCTTC	<i>evgS</i> mutation
SR	AAGATGTGCTGGTAAATAGCTCCCACATTAGA ACATTGTGGAATATCCTCCTTAGTTCC	<i>evgS</i> mutation
<i>pacrABF</i>	NNN GGATCCATGACCAATTTGAAATC	Cloning <i>acrAB</i> genes
<i>pacrABR</i>	NNN GGATCCATACGTGGTTAATACT	
KPF	NNNCCCGGGTTACGAATTGCTGCGATAGC	Cloning <i>emrKY</i> promoter
KPR	NNNTCTAGATGACCAATAGGCATAGGCAC	
JPF	NNNCCCGGGCTCGGCGGCAAAACGGATA	
JPR	NNNTCTAGAGACGCTCGCCCATTTTCATTG	Cloning <i>mdtII</i> promoter
<i>acrAF</i>	GTCTATCACCTACGCGCTATCTT	Real Time PCR
<i>acrAR</i>	GCGCGCACGAACATACC	Real Time PCR
<i>acrDF</i>	GTACCCTGGCGATTTTTTCATT	Real Time PCR
<i>acrDR</i>	CGGTCCTCGCACATTCA	Real Time PCR
<i>bcrF</i>	TGTTTTTCTGTTTCGTGATGAGGAT	Real Time PCR
<i>bcrR</i>	GGAACATATTTAACGCCGCAAT	Real Time PCR
<i>emrAF</i>	CGTTCCAGCCACCAATATGTG	Real Time PCR
<i>emrAR</i>	CCGTAAATATCGGTGGTGTATGG	Real Time PCR
<i>emrDF</i>	GTGGATCCCCGACTGGTTT	Real Time PCR
<i>emrDR</i>	CCCGGCACCGAAAAAGA	Real Time PCR

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emrEF	CACCAGGATGAAAACGGTGAGT	Real Time PCR
emrER	TGACCTCTGCAAGTATTGCGC	Real Time PCR
emrKF	GCGTTAAACGTACGGATATTAAGA	Real Time PCR
emrKR	ACTGTTTCGCCGACCTGAAC	Real Time PCR
fsrF	ACGCTGATTTTACCCTACGCC	Real Time PCR
fsrR	AGCGTAGACCAGAATGGCAGAG	Real Time PCR
macAF	CGACGCCGGAAAAGGTAAA	Real Time PCR
macAR	ATGCACTTGCGCGGTCATA	Real Time PCR
mdfAF	CTTGCTGTTAGCGCGTCTGA	Real Time PCR
yceLF	TTTTCACCCCTGATTTGTCTGTTTTAT	Real Time PCR
yceLR	CAGCGAAAGCACTTAAGGTTTCA	Real Time PCR
mdtJF	TGATGAAAATTGCCGGGTAA	Real Time PCR
mdtJR	CGCTTACGGGTACCTGATTTTA	Real Time PCR
ydhEF	CCGGTTATCGCGCAATTAAT	Real Time PCR
ydhER	GAAACCTTGTCGCACCTGATG	Real Time PCR
yidYF	TATCCCGCCGGGATTGATAT	Real Time PCR
yidYR	CGCTTCGCTGGCATTGA	Real Time PCR
nusAF	TGAAGCCGCACGTTATGAAG	Real Time PCR
nusAR	TCAACGTAATCGCCCAAGTT	Real Time PCR
evgAF	TCTTGCTATCGCAGCAATTCG	Real Time PCR
evgAR	AAGTGTTTCCACCCGCTGAAC	Real Time PCR