```
P42615    ----MQIPRMSLRQLAW---SGAVLLLVGTLLLAWSAVRQQESTLAIRAVHQGTTMPDGF   53    YQJB_ECOLI
Q3YXD0    ----MQIPRMSLRQLAW---SGTVLLLVGTLLLAWSAVRQQESTLAIRAVHQGTTMPDGF   53    Q3YXD0_SHISS
A8APZ0    ----MVKPHITLRQLAW---TTSFLVLMSVLFLVWSTIRQQESTLAIRAIHQGVSMPDGF   53    A8APZ0_CITK8
Q8XF92    ----MLKPRITARQLIW---ISAFLLMLTILMMTWSTLRQQESTLAIRAVNQGASMPDGF   53    Q8XF92_SALTI
A6TED5    ---MMVMKRPSLRQFSW---LLGGSLLLGALFWLWLAVQQQEATLAIRPVGQGIGMPDGF   54    A6TED5_KLEP7
A4WES8    ----MVISPLALRRLSY---GLIALVLLSALILVWTALQRQESTLAIRPVSQGASVPDGF   53    A4WES8_ENT38
A7MIS1    --------------------MLSAGALLAAMLLCWAALQKHETTLEIRAVSQGATIPDGF   40    A7MIS1_ENTS8
Q7CGC4    ----MINFRGRFGRPLWHYLVLPVVLLLLAVILLTPMIVQTESTLKIRPNQQGLSLPDGF   56    Q7CGC4_YERPE
Q6D9H4    MSIRWLSPTLSPRKIGR--ILLLLALPIIALTQSQPPRHSQDDAMLHIKPYDGAALPDGF   58    Q6D9H4_ERWCT
                                     :  :          : ::      :*  :****

P42615    SIWHHLDAHGIPFKSITPKNDTLLITFDSSDQSAAAKAVLDRTLPHGYIIAQQDNNSQAM   113   YQJB_ECOLI
Q3YXD0    SIWHHLDAHGIPFKSITPKNDTLLITFDSSDQSAAAKAVLDRTLPHGYIIAQQDNNSQAM   113   Q3YXD0_SHISS
A8APZ0    SIWHHLDANGIRFKSITPQNDALLITFDSSAQSAAAKAVLDRTLPHGYIIAQQNGDSQAV   113   A8APZ0_CITK8
Q8XF92    SVLHHLDANGIHFKSITPKNDMLLITFDSPAQSAAAKTVLDQTLPHGYVVAQQDDDNETV   113   Q8XF92_SALTI
A6TED5    SVWHHLDANGIRFKSITPQKDGLLIKFDSTAQGAAAKEVLGRALPHGYIIALLEDDNSPT   114   A6TED5_KLEP7
A4WES8    YIWHHLDANGIQFKSITPQDDVLLIKFDSSAQSAAAKVVLDRSLPRGYIIALQDDQSQTA   113   A4WES8_ENT38
A7MIS1    SVWHHLDANGIRFKSITPQDDILLIKFDTSDQSEAARKVLYRSLPRGFVIAQQEEKSLTP   100   A7MIS1_ENTS8
Q7CGC4    YLYQHLNGRGIHIKSIIPENDSLVVSLEFPEQQMQAIEVLQDVLPAGYAIVASESKKR-H   115   Q7CGC4_YERPE
Q6D9H4    YVYQRLNEKGIAIKSITPEQDSLIVRLASPEQSIAARDVLRLSLPK-VTITAQQATSPTP   117   Q6D9H4_ERWCT
           : ::*: .** :*** *:.* *:: :  . *   *  **   **    :.  :  .

P42615    QWLTRLRDNSHRFG   127   YQJB_ECOLI
Q3YXD0    QWLTRLRDNSHRFG   127   Q3YXD0_SHISS
A8APZ0    QWLTRLRDNPHRFG   127   A8APZ0_CITK8
Q8XF92    QWLSRLRESSHRFG   127   Q8XF92_SALTI
A6TED5    AWLSRLRDAPHRLG   128   A6TED5_KLEP7
A4WES8    VWLTRLRDTSHRFG   127   A4WES8_ENT38
A7MIS1    AWLTRLRHDIQRLG   114   A7MIS1_ENTS8
Q7CGC4    RLLPVFRSNQQNLG   129   Q7CGC4_YERPE
Q6D9H4    FWQQKLTQKQSKLG   131   Q6D9H4_ERWCT
               :     .:*
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Figure S1. Multiple sequence alignment of MzrA (YqjB) sequences from *Enterobacteriaceae*. Amino acid sequences were aligned using the CLUSTAL W (1.83) program (http://ch.EMBnet.org/). Amino acids that are either identical (*), have similar side chains (:) or similar overall characteristics (.) are shown in the last line.

Table S1: Bacterial strains and plasmids

Strain or Plasmid	Description	Reference or Source
MC4100	F- *araD139* Δ(argF-lac)U139 *rspL150 relA1 fibB5301 ptsF25 deoC1 thi*-1 *rbsR*	(Casadaban, 1976)
JM109	e14$_{-}$(McrA$_{-}$) *recA1 endA1 gyrA96 thi-1 hsdR17* ($r_K^- m_K^+$) *supE44 relA1* Δ(*lac-proAB*) [F′ *traD36 proAB lacI$_q$Z*Δ*M15*].	Promega
RAM1292	MC4100 Δ*ara714*	(Werner and Misra, 2005)
BTH101	F- *cya99 araD139 galE15 galK16 rpsL1* (*Str ^{r}*) *hsdR2 mcrA1 mcrB1*	EUROMEDEX
BL21(DE3)	F–, *omp*T, *hsd*S_B(r_B-, m_B-), *dcm*, *gal*, λ(DE3)	Novagen
RAM1318	MC4100 Δ*bamB*::scar *degP*::Tn*10*	(Charlson *et al*., 2006)
RAM1503	RAM1292 / pBAD24	This Study
RAM1504	RAM1292 / pBAD24-*mzrA*	This Study
RAM1505	RAM1292 / pBAD24-*mzrA*(FLAG)	This Study
RAM1506	RAM1292 / pBAD33	This Study
RAM1507	RAM1292 / pBAD33-*mzrA*(FLAG)	This Study
RAM1508	RAM1292 Δ*mzrA:*:scar	This Study
RAM1509	RAM1508/ pBAD33	This Study
RAM1510	RAM1508/ pBAD33-*mzrA*(FLAG)	This Study
RAM1511	RAM1508 Δ*omr*::*lacZ*	This Study
RAM1512	RAM1292 Δ*yqj*::*lacZ*	This Study
RAM1513	RAM1512 *zii*::Tn*10*	This Study
RAM1514	RAM1512 *cpxA* zii*::Tn*10*	This Study
RAM1515	RAM1512 Δ*cpxR*::Cm^r *zii*::Tn*10*	This Study
RAM1516	RAM1292 *zii*::Tn*10*	This Study
RAM1517	RAM1292 *cpxA* zii*::Tn*10*	This Study
RAM1518	RAM1516 Δ*mzrA*::scar	This Study
RAM1519	RAM1517 Δ*mzrA*::scar	This Study
RAM1520	RAM1516 Δ*envZ*::Cm^r	This Study
RAM1521	RAM1517 Δ*envZ*::Cm^r	This Study
RAM1522	RAM1292 Δ*omr*::*lacZ*	This Study
RAM1523	RAM1522 / pBAD24	This Study
RAM1524	RAM1522 / pBAD24-*mzrA*	This Study
RAM1525	RAM1522 zii::Tn*10*	This Study
RAM1526	RAM1522 *cpxA* zii*::Tn*10*	This Study
RAM1527	RAM1522 Δ*cpxR*::Cm^r *zii*::Tn*10*	This Study
RAM1528	RAM1525 Δ*mzrA*::scar	This Study
RAM1529	RAM1526 Δ*mzrA*::scar	This Study
RAM1530	RAM1292 *ompR101*	This Study
RAM1531	RAM1530 / pTrc99A	This Study
RAM1532	RAM1530 / pTrc99A-*mzrA(*FLAG)	This Study
RAM1533	RAM1530 Δ*omr*::*lacZ*	This Study
RAM1534	RAM1292 *ompR472*	This Study
RAM1535	RAM1534 / pTrc99A	This Study
RAM1536	RAM1534 / pTrc99A-mzrA(FLAG)	This Study
RAM1537	RAM1292 Δ*envZ*::scar	This Study
RAM1538	RAM1537 / pTrc99A	This Study
RAM1539	RAM1537 / pTrc99A-mzrA(FLAG)	This Study
RAM1540	RAM1537 Δ*omr*::*lacZ*	This Study
RAM1541	RAM1292 *envZ*[R397L]	This Study
RAM1542	RAM1541 Δ*omr*::*lacZ*	This Study
RAM1543	BTH101 / pKT25 pUT18C	This Study
RAM1544	BTH101 / pKT25*mzrA* and pUT18C	This Study
RAM1545	BTH101 / pKT25*mzrA* and pUT18C*envZ*	This Study
RAM1546	BTH101 / pKT25*mzrA* and pUT18*envZ*	This Study

RAM1547	BTH101 / pKT25*mzrA* and pUT18C*ompR*	This Study
RAM1548	BTH101 / pKT25*mzrA* and pUT18*ompR*	This Study
RAM1549	BTH101 / pKT25*envZ* and pUT18*envZ*	This Study
RAM1551	RAM1292 Δ*ppiA*::*lacZ*	This Study
RAM1552	RAM1551/ pBAD24	This Study
RAM1553	RAM1551/ pBAD24-*mzrA*	This Study
RAM1554	RAM1292 Δ*ompF*::*lacZ*	This Study
RAM1555	RAM1554 / pBAD24	This Study
RAM1556	RAM1554 / pBAD24-*mzrA*	This Study
RAM1557	RAM1292 *mgrB*::λp*lac*Mu55	This Study
RAM1558	RAM1557 / pBAD24	This Study
RAM1559	RAM1557 / pBAD24-*mzrA*	This Study
RAM1560	RAM1292 *mgtA*::λp*lac*Mu55	This Study
RAM1561	RAM1560 / pBAD24	This Study
RAM1562	RAM1560 / pBAD24-*mzrA*	This Study
RAM1563	RAM1292 / pBAD24-*mzrA::phoA*	This Study
RAM1564	RAM1292 / pBAD24-*mzrA*[Δ13-28]*::phoA*	This Study
RAM1565	RAM1292 *dsbA*$^-$::Kan^r / pBAD24-*mzrA::phoA*	This Study
RAM1566	RAM1292 / pBAD24-*mzrA::lacZα*	This Study
RAM1567	RAM1292 / pBAD24-*mzrA*[Δ13-28]*::lacZα*	This Study
RAM1568	JM109 pBAD24	This Study
RAM1569	JM109 pBAD24-*mzrA*	This Study
RAM1570	JM109 pBAD24-*mzrA::lacZα*	This Study
RAM1571	JM109 pBAD24-*mzrA*[13-28]*:: lacZα*	This Study
RAM1572	RAM1540 / pBAD24	This Study
RAM1573	RAM1540 / pBAD24-*mzrA*	This Study
RAM1574	RAM1292 Δ*rseA*::scar	This Study
RAM1575	RAM1574 Δ*yqj*::*lacZ*	This Study
RAM1576	RAM1292/ pTrc99A	This Study
RAM1577	RAM1292/ pTrc99A-*mzrA*(FLAG)	This Study
RAM1578	RAM1318/ pBR322	This Study
RAM1579	RAM1318/ pBR322 *exuT'exuRyqjABCDE'*	This Study
RAM1580	RAM1292 λRS88 *cpxP*'-*lacZ* *nadA*::Tn*10*	This Study
RAM1581	RAM1580/ pBAD24	This Study
RAM1582	RAM1580/ pBAD24-*mzrA*(FLAG)	This Study
RAM1583	RAM1530 Δ*yqj*::*lacZ*	This Study
RAM1584	RAM1537 Δ*yqj*::*lacZ*	This Study
RAM1585	RAM1541 Δ*yqj*::*lacZ*	This Study
RAM1586	RAM1292 *envZ11*	This Study
RAM1587	RAM1586 Δ*yqj*::*lacZ*	This Study
Plasmids		
pBAD24	Amp^r; Expression vector; arabinose inducible	(Guzman *et al*., 1995)
pBAD33	Cm^r; Expression vector; arabinose inducible	(Guzman *et al*., 1995)
pTrc99A	Amp^r; Expression vector; IPTG inducible	GE Healthcare
pBAD33*mzrA*-FLAG	Cm^r; pBAD33 containing the *mzrA*(FLAG) gene	This Study
pBAD24*mzrA*	Amp^r; pBAD24 containing the *mzrA* gene	This Study
pBAD24*mzrA*-FLAG	Amp^r; pBAD24 containing the *mzrA*(FLAG) gene	This Study
pBAD24*mzrA*-*phoA*	Amp^r; pBAD24-*mzrA*::*phoA*	This Study
pBAD24*mzrA*[Δ 13-28]-*phoA*	Amp^r; pBAD24-*mzrA*[Δ13-28]::*phoA*	This Study
pBAD24*mzrA*-*lacZα*	Amp^r; pBAD24-*mzrA*:: *lacZα*	This Study

pBAD24*mzrA*[Δ 13-28]- *lacZα*	Amp^r; pBAD24-*mzrA*[Δ13-28]::*lacZα*	This Study
pTrc99A *mzrA*-FLAG	Amp^r; pTrc99A containing the *mzrA*(FLAG) gene	This Study
pKD3	Cm^r; Plasmid carrying Cm^r gene cassette	(Datsenko and Wanner, 2000)
pKD4	Kan^r; Plasmid carrying Kan^r gene cassette	(Datsenko and Wanner, 2000)
pKD46	Amp^r; Red recombinase expression plasmid	(Datsenko and Wanner, 2000)
pCP20	Cm^r and Amp^r; FLP recombinase expression plasmid, temperature sensitive	(Datsenko and Wanner, 2000)
pKG136	*ahp* FRT *lacZY*$^+$ t_{his} oriR6K	(Ellermeier *et al*., 2002)
pKT25	Kan^r; Harbors the T25 fragment of adenylate cyclase from *B. pertussis*	EUROMEDEX
pUT18	Amp^r; Harbors the T18 fragment of adenylate cyclase from *B. pertussis*	EUROMEDEX
pUT18C	Amp^r; Harbors the T18 fragment of adenylate cyclase from *B. pertussis*	EUROMEDEX
pKT25*mzrA*	Kan^r; pKT25 with the *mzrA* gene	This Study
pKT25*envZ*	Kan^r; pKT25 with the *envZ* gene	This Study
pKT25*ompR*	Kan^r; pKT25 with the *ompR* gene	This Study
pUT18C*mzrA*	Amp^r; pUT18C with the *mzrA* gene	This Study
pUT18C*envZ*	Amp^r; pUT18C with the *envZ* gene	This Study
pUT18C*ompR*	Amp^r; pUT18C with the *ompR* gene	This Study
pUT18C*cpxA*	Amp^r; pUT18C with the *cpxA* gene	This Study
PUT18*envZ*	Amp^r; pUT18 with the *envZ* gene	This Study
pUT18*ompR*	Amp^r; pUT18 with the *ompR* gene	This Study
pUT18*cpxA*	Amp^r; pUT18 with the *cpxA* gene	This Study
pBR322	Amp^r;Tet^r cloning vector	(Bolivar, *et al.* 1977)
pBR322-*exuT' exuRyqjABCDE'*	Amp^r;pBR322 with *exuT'exuRyqjABCDE'* genes	This Study

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Table S2: Primers used for cloning and deletion

Deletion Primers

Primer Name	Sequence (5'-3')
yqjA Fwd	GTTAACCGATTCACCAGGAATAATGAATGGAACTTTTGTGTAGGCTGGAGCTGCTTCG
mzrA Fwd	GAAATATGGAAATCGGGGGTAAGGGATGCAAATATGTAGGCTGGAGCTGCTTCG
mzrA Rev	GTGAGTGATATGTTTCGGATAACAGGAAGTTACATATGAATATCCTCCTTAG
envZ Fwd	CTTTGTACCGGACGGCTCTAAAGCATGAGGCGATTGCGCTTCTGTAGGCTGGAGCTGCTTCG
envZ Rev	ACCTTCGCCTCCCGTTTATTTACCCTTCTTTTGTCGTGCCCATATGAATATCCTCCTTAG
omrB Fwd	CCGCTGGTGGCGTTTGGCTTCAGGTTGCTAAAGTGGTGATTGTAGGCTGGAGCTGCTTCG
omrA Rev	ACACGAGATAAAGAACGCGAGCGACAGTAAATTAGGTGCGCATATGAATATCCTCCTTAG
cpxR Fwd	TTTCTGCCTCGGAGGTATTTAAACAATGAATAAAATCCTGTGTAGGCTGGAGCTGCTTCG
cpxR Rev	AGATGGCGAAGATGCGCGCGGTTAAGCTGCCTATCATGAAGCCATCATATGAATATCCTCCTTAG
rseA Fwd	GGATACTGGATAAGGGTATTAGGCATGCAGAAATGTGTAGGCTGGAGCTGCTTCG
rseA Rev	CCAAAGTTGCTTCATTACTGCGATTGCGTTCCTAACATATGAATATCCTCCTTAG

Expression Vector Primers (restriction sites shown in lower case)

Primer Name	Sequence(5'-3')	Cloning Vector
*mzrA Eco*RI Fwd	AGGAGgaattcACCATGCAAATACCTCGC	pBAD24
*mzrA Xba*I Fwd	TGCAtctagaAATATGGAAATCGGGGGTAAGGGATGCAAATA	pBAD24 and pBAD33
*mzrA HinD*III Rev	CGATCaagcttGAGTGATATGTTTCGGATAAC	pBAD24
mzrA-FLAG *HinD*III Rev	CGATCaagcttACTTATCGTCGTCGTCCTTGTAGTCACCGAAGCGATGAGAGTTATC	pBAD24 and pBAD33
*mzrA Xba*I Rev	CAGTtctagaTCCGAAGCGATGAGAGTTATC	pBAD24
*phoA Xba*I Fwd	ATAtctagaATGCCTGTTCTGGAAAACCGG	pBAD24*mzrA*
*phoA Sal*I Rev	ATATgtcgacTTATTTCAGCCCCAGAGCG	pBAD24*mzrA*

Two Hybrid System Primers (restriction sites shown in lower case)

Primer Name	Sequence (5'-3')	Cloning Vector
mzrA E2HS *Xba*I Fwd	AGCTGtctagaGCAAATACCTCGCATGTCGC	pUT18C and pKT25
mzrA E2HS *Kpn*I Rev	GCAGTggtaccCCGAAGCGATGAGAGTTATCCCG	pUT18C and pKT25
envZ E2HS *Xba*I Fwd	AGCTGtctagacAGGAGGTTGCGCTTCTCGCCACG	pUT18, pUT18C and pKT25
envZ E2HS *Kpn*I Rev	GCAGTggtaccCCTTCTTTTGTCGTGCCCTGC	pUT18, pUT18C and pKT25
ompR E2HS *Xba*I Fwd	AGCTGtctagaGCAAGAGAACTACAAGATTCTGG	pUT18, pUT18C
cpxA E2HS *Xba*I Fwd	ACGTGtctagaGATAGGCAGCTTAACCGCGCG	pUT18, pUT18C, and pKNT25
cpxA E2HS *Kpn*I Fwd	GCAGTggtaccCGACTCCtCTTATACAGCGGC	pUT18, pUT18C, and pKNT25

Site-Directed Mutagenesis Primers (alterations shown in lower case)

Primer Name	Sequence (5'-3')	Cloning Vector
mzrA Δ13-28 SDM Fwd	CGCATGTCGCTTCGCCAGCTAGCCTCCGCGGTTCGCCAGCAAGAGTC	pBAD24*mzrA::phoA,* pBAD24*mzrA::lacZα*
mzrA Δ13-28 SDM Rev	GACTCTTGCTGGCGAACCGCGGAGGCTAGCTGGCGAAGCGACATGCG	pBAD24*mzrA::phoA,* pBAD24*mzrA::lacZα*
EnvZ R397L Fwd	GCGGCGACAGcGCaCtCACCATTAGCGGCACGGGAT	pETEnvZc

EnvZ R397L Rev	TAGGG CCCTAATCCCGTGCCGCTAATGGTGaGtGCgCTGTC GCCGC	pETEnvZc