

Supplemental Material for:
**“MzrA-EnvZ Interactions in the Periplasm Influence the EnvZ/OmpR Two-Component
 Regulon”**

Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Characteristics	Reference or Source ^a
Strains		
MC4100	F ⁻ <i>araD139</i> Δ (<i>argF-lac</i>) <i>U139</i> <i>rspL150</i> <i>relA1</i> <i>flbB5301</i> <i>ptsF25</i> <i>deoC1</i> <i>thi-1</i> <i>rbsR</i>	(7)
RAM1292	MC4100 Δ <i>ara714</i>	(37)
BTH101	F ⁻ <i>cya99</i> <i>araD139</i> <i>galE15</i> <i>galK16</i> <i>rpsL1</i> (Str ^r) <i>hsdR2</i> <i>mcrA1</i> <i>mcrB1</i>	Euromedex
RAM483	RAM474 <i>ompF315</i> ; Dex ⁺ Δ <i>lamB106</i>	(24)
RAM1503	RAM1292 / pBAD24 (Amp ^r)	(13)
RAM1505	RAM1292 / pBAD24- <i>mzrA</i> (FLAG)	(13)
RAM1508	RAM1292 Δ <i>mzrA</i> :: <i>scar</i>	(13)
RAM1543	BTH101 / pKT25 (Kan ^r) pUT18C (Amp ^r)	(13)
RAM1544	BTH101 / pKT25- <i>mzrA</i> and pUT18C	(13)
RAM1545	BTH101 / pKT25- <i>mzrA</i> and pUT18C- <i>envZ</i>	(13)
RAM1563	RAM1292 / pBAD24- <i>mzrA</i> :: <i>phoA</i>	(13)
RAM1774	RAM1292 / pBAD24- <i>mzrA</i> [Δ 105-127](FLAG)	This Study
RAM1775	RAM1292 / pBAD24- <i>mzrA</i> [Δ 73-127](FLAG)	This Study
RAM1776	RAM1292 / pBAD24- <i>mzrA</i> [Δ 2-31](FLAG)	This Study
RAM1777	RAM1292 / pBAD24- <i>mzrA</i> [Δ 13-28](FLAG)	This Study
RAM1778	RAM1292 / pBAD24- <i>mzrA</i> [Δ 1-5](FLAG)	This Study
RAM1779	RAM1292 / pBAD24- <i>mzrA</i> [TM]:: <i>mzrA</i> [Δ TM](FLAG)	This Study
RAM1780	RAM1292 / pBAD24- <i>narX</i> [TM]:: <i>mzrA</i> [Δ TM](FLAG)	This Study
RAM1781	RAM1292 / pBAD24- <i>phoA</i> [SS]:: <i>mzrA</i> [Δ TM](FLAG)	This Study
RAM1782	RAM1292 / pBAD24- <i>ompF</i> [SS]:: <i>mzrA</i> [Δ TM](FLAG)	This Study
RAM1783	RAM1292 / pBAD24- <i>ompF</i> [SS]:: <i>mzrA</i> [Δ TM], [Δ 105-127](FLAG)	This Study
RAM1784	RAM1292 / pBAD24- <i>mzrA</i> [TM]	This Study
RAM1785	RAM1292 / pBAD24- <i>narX</i> [TM]	This Study
RAM1786	RAM1292 / pBAD24- <i>phoA</i> [SS]	This Study
RAM1787	RAM1292 / pBAD24- <i>ompF</i> [SS]	This Study
RAM1788	RAM1292 / pBAD24- <i>mzrA</i> [Δ TM](FLAG)	This Study
RAM1789	RAM1292 / pBAD24- <i>mzrA</i> [D51A](FLAG)	This Study
RAM1790	RAM1292 / pBAD24- <i>mzrA</i> [K67A](FLAG)	This Study
RAM1791	RAM1292 / pBAD24- <i>mzrA</i> [D74A](FLAG)	This Study
RAM1792	RAM1292 / pBAD24- <i>mzrA</i> [Q85A](FLAG)	This Study
RAM1793	RAM1508 / pBAD24- <i>mzrA</i> :: <i>phoA</i>	This Study
RAM1794	RAM1508 / pBAD24- <i>mzrA</i> [D51A]:: <i>phoA</i>	This Study
RAM1795	RAM1508 / pBAD24- <i>mzrA</i> [K67A]:: <i>phoA</i>	This Study
RAM1796	RAM1508 / pBAD24- <i>mzrA</i> [D74A]:: <i>phoA</i>	This Study
RAM1797	RAM1508 / pBAD24- <i>mzrA</i> [Q85A]:: <i>phoA</i>	This Study
RAM1798	RAM1508 / pBAD24- <i>mzrA</i> [I78F]:: <i>phoA</i>	This Study
RAM1799	RAM1508 / pBAD24- <i>mzrA</i> [D51A,I78F]:: <i>phoA</i>	This Study
RAM1800	RAM483 <i>araCBAD</i> :: <i>kan</i> ^r	This Study
RAM1801	RAM1800 / pBAD24- <i>mzrA</i> :: <i>phoA</i>	This Study
RAM1802	RAM1800 / pBAD24- <i>mzrA</i> [D51A]:: <i>phoA</i>	This Study
RAM1803	RAM1800 / pBAD24- <i>mzrA</i> [I78F]:: <i>phoA</i>	This Study
RAM1804	RAM1800 / pBAD24- <i>mzrA</i> [D51A,I78F]:: <i>phoA</i>	This Study
RAM1805	BTH101 / pKT25 <i>mzrA</i> [D51A] and pUT18C	This Study

RAM1806	BTH101 / pKT25- <i>mzrA</i> [I78F] and pUT18C	This Study
RAM1807	BTH101 / pKT25- <i>mzrA</i> [D51A,I78F] and pUT18C	This Study
RAM1808	BTH101 / pKT25- <i>mzrA</i> and pUT18C <i>mzrA</i>	This Study
RAM1809	BTH101 / pKT25- <i>mzrA</i> [D51A] and pUT18C- <i>mzrA</i>	This Study
RAM1810	BTH101 / pKT25- <i>mzrA</i> [I78F] and pUT18C- <i>mzrA</i>	This Study
RAM1811	BTH101 pKT25- <i>mzrA</i> [D51A,I78F] and pUT18C- <i>mzrA</i>	This Study
RAM1812	BTH101 / pKT25- <i>mzrA</i> [D51A] and pUT18C- <i>envZ</i>	This Study
RAM1813	BTH101 / pKT25- <i>mzrA</i> [I78F] and pUT18C- <i>envZ</i>	This Study
RAM1814	BTH101 / pKT25- <i>mzrA</i> [D51A,I78F] and pUT18C- <i>envZ</i>	This Study
RAM1815	RAM1292 Δ <i>malE</i> ::Kan ^r	This Study
RAM1816	RAM1815 / pMAL-p2x	This Study
RAM1817	RAM1815 / pMAL-p2x- <i>mzrA</i> [Δ TM]-FLAG	This Study
RAM1818	RAM1815 / pMAL-p2x- <i>mzrA</i> [Δ TM], [Δ 105-127]-(FLAG)	This Study
Plasmids		
pBAD24	Amp ^r ; Expression vector; arabinose inducible	(16)
pKT25	Kan ^r ; Harbors the T25 fragment of adenylate cyclase from <i>B. pertussis</i>	Euromedex
pUT18	Amp ^r ; Harbors the T18 fragment of adenylate cyclase from <i>B. pertussis</i>	Euromedex
pUT18C	Amp ^r ; Harbors the T18 fragment of adenylate cyclase from <i>B. pertussis</i>	Euromedex
pMAL-p2x	Amp ^r ; Expression vector; IPTG inducible for periplasmic MalE fusion	New England Biolabs
pBAD24 <i>mzrA</i> - <i>phoA</i>	Amp ^r ; pBAD24 with the <i>mzrA</i> :: <i>phoA</i> gene fusion	(13)
pKT25 <i>mzrA</i>	Kan ^r ; pKT25 with the <i>mzrA</i> gene	(13)
pKT25 <i>envZ</i>	Kan ^r ; pKT25 with the <i>envZ</i> gene	(13)
pUT18C <i>mzrA</i>	Amp ^r ; pUT18C with the <i>mzrA</i> gene	(13)
pUT18C <i>envZ</i>	Amp ^r ; pUT18C with the <i>envZ</i> gene	(13)
pBAD24- <i>mzrA</i> [Δ 105-127]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [Δ 105-127](FLAG) gene	This Study
pBAD24- <i>mzrA</i> [Δ 73-127]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [Δ 73-127](FLAG) gene	This Study
pBAD24- <i>mzrA</i> [2-31]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [Δ 2-31](FLAG) gene	This Study
pBAD24- <i>mzrA</i> [13-28]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [Δ 13-28](FLAG) gene	This Study
pBAD24- <i>mzrA</i> [1-5]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [Δ 1-5](FLAG) gene	This Study
pBAD24- <i>mzrA</i> [TM]- <i>mzrA</i> [Δ TM]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [TM]:: <i>mzrA</i> [Δ TM](FLAG) gene	This Study
pBAD24- <i>narX</i> [TM]- <i>mzrA</i> [Δ TM]-FLAG	Amp ^r ; pBAD24 containing the <i>narX</i> [TM]:: <i>mzrA</i> [Δ TM](FLAG) gene	This Study
pBAD24- <i>phoA</i> [SS]- <i>mzrA</i> [Δ TM]-FLAG	Amp ^r ; pBAD24 containing the <i>phoA</i> [SS]:: <i>mzrA</i> [Δ TM](FLAG) gene	This Study
pBAD24- <i>ompF</i> [SS]- <i>mzrA</i> [Δ TM]-FLAG	Amp ^r ; pBAD24 containing the <i>ompF</i> [SS]:: <i>mzrA</i> [Δ TM](FLAG) gene	This Study
pBAD24- <i>ompF</i> [SS]- <i>mzrA</i> [Δ TM],[Δ 105-127]-FLAG	Amp ^r ; pBAD24 containing the <i>ompF</i> [SS]:: <i>mzrA</i> [Δ TM],[Δ 105-127](FLAG) gene	This Study
pBAD24- <i>mzrA</i> [TM]	Amp ^r ; pBAD24 containing the <i>mzrA</i> [TM] gene fragment	This Study
pBAD24- <i>narX</i> [TM]	Amp ^r ; pBAD24 containing the <i>narX</i> [TM] gene fragment	This Study
pBAD24- <i>phoA</i> [SS]	Amp ^r ; pBAD24 containing the <i>phoA</i> [SS] gene fragment	This Study
pBAD24- <i>ompF</i> [SS]	Amp ^r ; pBAD24 containing the <i>ompF</i> [SS] gene fragment	This Study
pBAD24- <i>mzrA</i> [Δ TM]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [Δ TM](FLAG)gene fragment	This Study
pBAD24- <i>mzrA</i> [D51A]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [D51A](FLAG) gene	This Study
pBAD24-	Amp ^r ; pBAD24 containing the <i>mzrA</i> [K67A](FLAG) gene	This Study

<i>mzrA</i> [K67A]-FLAG pBAD24- <i>mzrA</i> [D74A]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [D74A](FLAG) gene	This Study
pBAD24- <i>mzrA</i> [Q85A]-FLAG	Amp ^r ; pBAD24 containing the <i>mzrA</i> [Q85A](FLAG) gene	This Study
pBAD24- <i>mzrA</i> [D51A]- <i>phoA</i>	Amp ^r ; pBAD24 with <i>mzrA</i> [D51A]:: <i>phoA</i> gene fusion	This Study
pBAD24- <i>mzrA</i> [K67A]- <i>phoA</i>	Amp ^r ; pBAD24 with <i>mzrA</i> [K67A]:: <i>phoA</i> gene fusion	This Study
pBAD24- <i>mzrA</i> [D74A]- <i>phoA</i>	Amp ^r ; pBAD24 with <i>mzrA</i> [D74A]:: <i>phoA</i> gene fusion	This Study
pBAD24- <i>mzrA</i> [Q85A]- <i>phoA</i>	Amp ^r ; pBAD24 with <i>mzrA</i> [Q85A]:: <i>phoA</i> gene fusion	This Study
pBAD24- <i>mzrA</i> [I78F]- <i>phoA</i>	Amp ^r ; pBAD24 with <i>mzrA</i> [I78F]:: <i>phoA</i> gene fusion	This Study
pBAD24- <i>mzrA</i> [D51A,I78F]- <i>phoA</i>	Amp ^r ; pBAD24 with <i>mzrA</i> [D51A, I78F]:: <i>phoA</i> gene fusion	This Study
pKT25 <i>mzrA</i> [D51A]	Kan ^r ; pKT25 with the <i>mzrA</i> [D51A] gene	This Study
pKT25 <i>mzrA</i> [I78F]	Kan ^r ; pKT25 with the <i>mzrA</i> [I78F] gene	This Study
pKT25 <i>mzrA</i> [D51A,I78F]	Kan ^r ; pKT25 with the <i>mzrA</i> [D51A, I78F] gene	This Study
pMAL-p2x- <i>mzrA</i> [ΔTM]-FLAG	Amp ^r ; pMAL-p2x containing the <i>mzrA</i> [ΔTM](FLAG) gene fragment	This Study
pMAL-p2x- <i>mzrA</i> [ΔTM],[Δ105-127]-FLAG	Amp ^r ; pMAL-p2x containing the <i>mzrA</i> [ΔTM], [Δ105-127](FLAG) gene	This Study

^a Numbered references are shown in the main text.

Table S2. Primers used for cloning and mutagenesis.

Purpose	Sequence(5'-3') ^a
For cloning	
<i>mzrA</i> EcoRI Fwd	AGGAGgaattcACCATGCAAATACCTCGC
<i>mzrA</i> -FLAG <i>Hind</i> III Rev	CGATCaagcttACTTATCGTCGTCGTCCTTGTAGTCACCGAAGCGATGAG AGTTATC
<i>mzrA</i> <i>Bsp</i> HI Fwd 2 nd met(Δ 1-5)	AGGAGtcatgaCGCTTCGCCAGCTAGCC
<i>mzrA</i> Δ 31(Δ 2-31) Fwd <i>Eco</i> RI	AGGAGgaattcACCATGCGCCAGCAAGAGTCTACGC
<i>mzrA</i> <i>Xba</i> I Fwd- Δ TM	TGCAtctagaCAaCAAGAGTCgACGCTGGCGATTCTGTCGGC
<i>mzrA</i> TM <i>Xba</i> I Rev for fusion	TGCAtctagaAACCGCGGACCAGGCCAGCAAC
<i>mzrA</i> Δ C23-Flag <i>Hind</i> III	CGATCaagcttACTTATCGTCGTCGTCCTTGTAGTCCGCAATGATGTAGCC ATGGGG
<i>mzrA</i> Δ C55-Flag <i>Hind</i> III	CGATCaagcttACTTATCGTCGTCGTCCTTGTAGTCTTTGGGGGTGATACT TTTGAAAGG
<i>narX</i> TM1 <i>Xba</i> I Rev-for Fusion	TGCAtctagaCACCAGCCAGCCAGAAACCGCC
<i>narX</i> Fwd <i>Eco</i> RI	AGGAGgaattcAccATGCTTAAACGTTGTCTCTCTCCGC
<i>phoA</i> <i>Eco</i> RI Fwd	AGGAGgaattcAccATGAAACAAAGCACTATTGCAC
<i>phoA</i> -SS <i>Xba</i> I Rev-for Fusion	TGCAtctagaGGCTTTTGTTCACAGGGGTAAAC
<i>ompF</i> -SS <i>Xba</i> I Rev-for fusion	TGCAtctagaAGCGTTTGCAGTACCTGCTACTAACAG
<i>ompF</i> Fwd <i>Bsp</i> HI	GGCATtcatgaTGAAGCGCAATATTCTGGC
For mutagenesis	
<i>mzrA</i> Δ 13-28 SDM Fwd	CGCATGTCGCTTCGCCAGCTAGCCTCCGCGGTTTCGCCAGCAAGAGTC
<i>mzrA</i> Δ 13-28 SDM Rev	GACTCTTGCTGGCGAACCAGCGGAGGCTAGCTGGCGAAGCGACATGCG
<i>mzrA</i> D51A SDM Fwd	CGTGCCGTTTCATCAAGGAcACAACGATGCCgGcCGGTTTTCAATCTGGC AcCACCTTGACGCTCATGGC
<i>mzrA</i> D51A SDM Rev	GCCATGAGCGTCAAGGTGgTGCCAGATTGAAAAACcGgCCGGCATCGT TGTtCCTTGATGAACGGCACG
<i>mzrA</i> K67A SDM Fwd	CTTGACGCTCATGGCATTTCCTTTCgcAAGTATCACCCCCAAAAAC
<i>mzrA</i> K67A SDM Rev	GTTTTTGGGGGTGATACTTgcGAAAGGAATGCCATGAGCGTCAAG
<i>mzrA</i> D74A SDM Fwd	GTATCACCCCCAAAAACGcCACTCTGTTAATTACATTTGACTCCAGC
<i>mzrA</i> D74A SDM Rev	GCTGGAGTCAAATGTAATTAACAGAGTGgCGTTTTTGGGGGTGATAC
<i>mzrA</i> Q85A SDM Fwd	CTGTTAATTACATTTGACTCCAGCGACgcGAGCGCaGCaGCAAAAGCGG TCCTCGACAG
<i>mzrA</i> Q85A SDM Rev	CTGTCGAGGACCGCTTTTGCtGcTGCgCTCgcGTCGCTGGAGTCAAATGT AATTAACAG
<i>mzrA</i> mutagenesis Fwd	CGCCAGCAAGAGTCTACGC
<i>mzrA</i> mutagenesis Rev	CGCAATGATGTAGCCATGGG
<i>mzrA</i> I78F SDM Fwd	GTATCACCCCCAAAAACGACACTCTGTTAfTTACATTTGACTCCAGCGA CCAGAGCGCCG
<i>mzrA</i> I78F SDM Rev	CGGCGCTCTGGTTCGCTGGAGTCAAATGTAAATAACAGAGTGTCGTTTT TGGGGGTGATAC

^a Restriction and mutagenesis sites are shown by lower case. Rev and Fwd mean reverse and forward primers, respectively.

