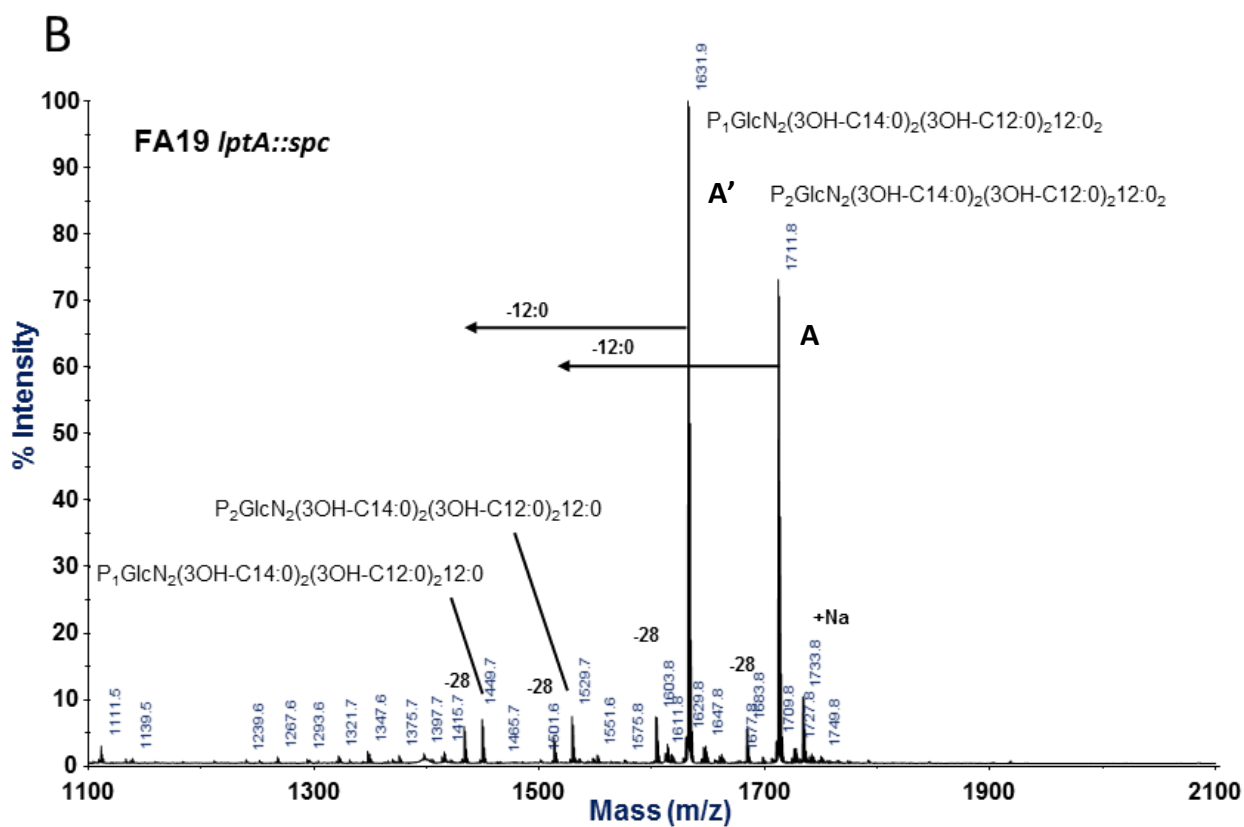
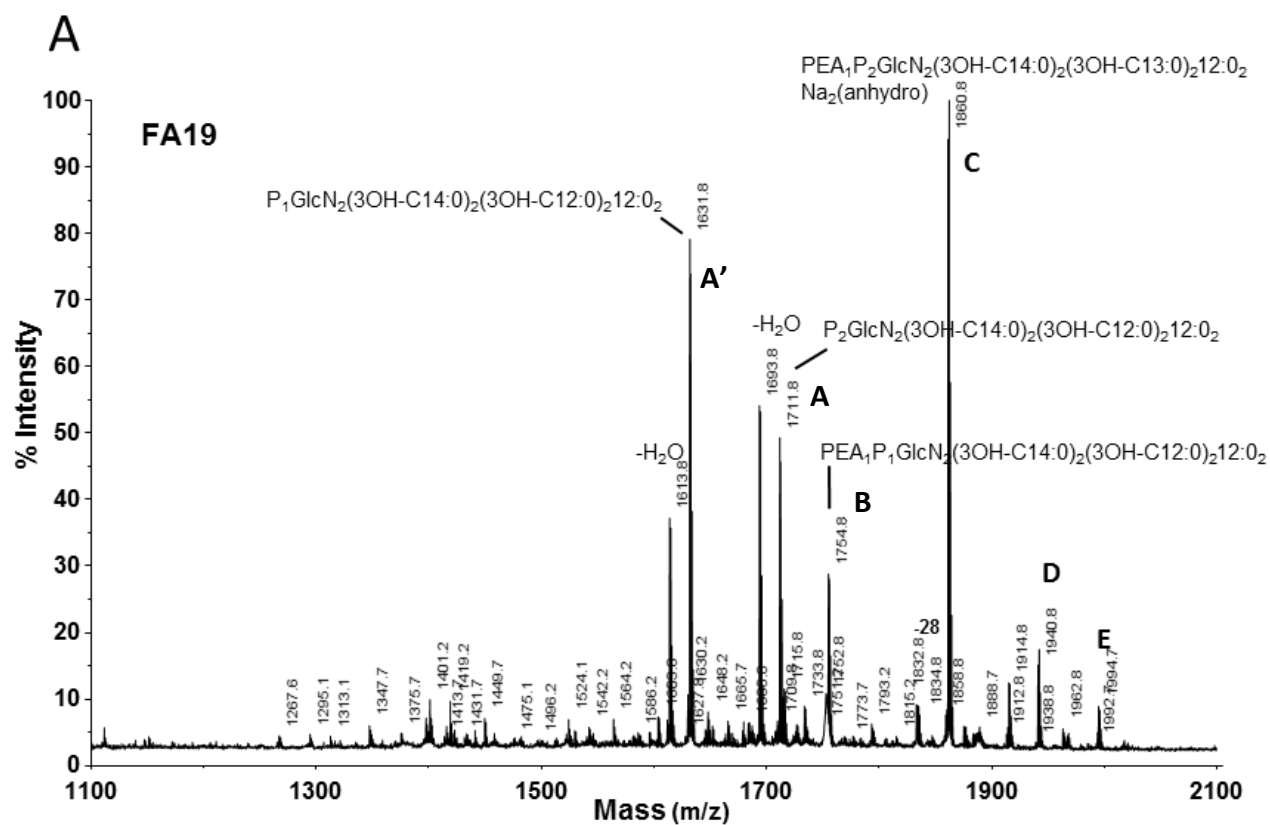
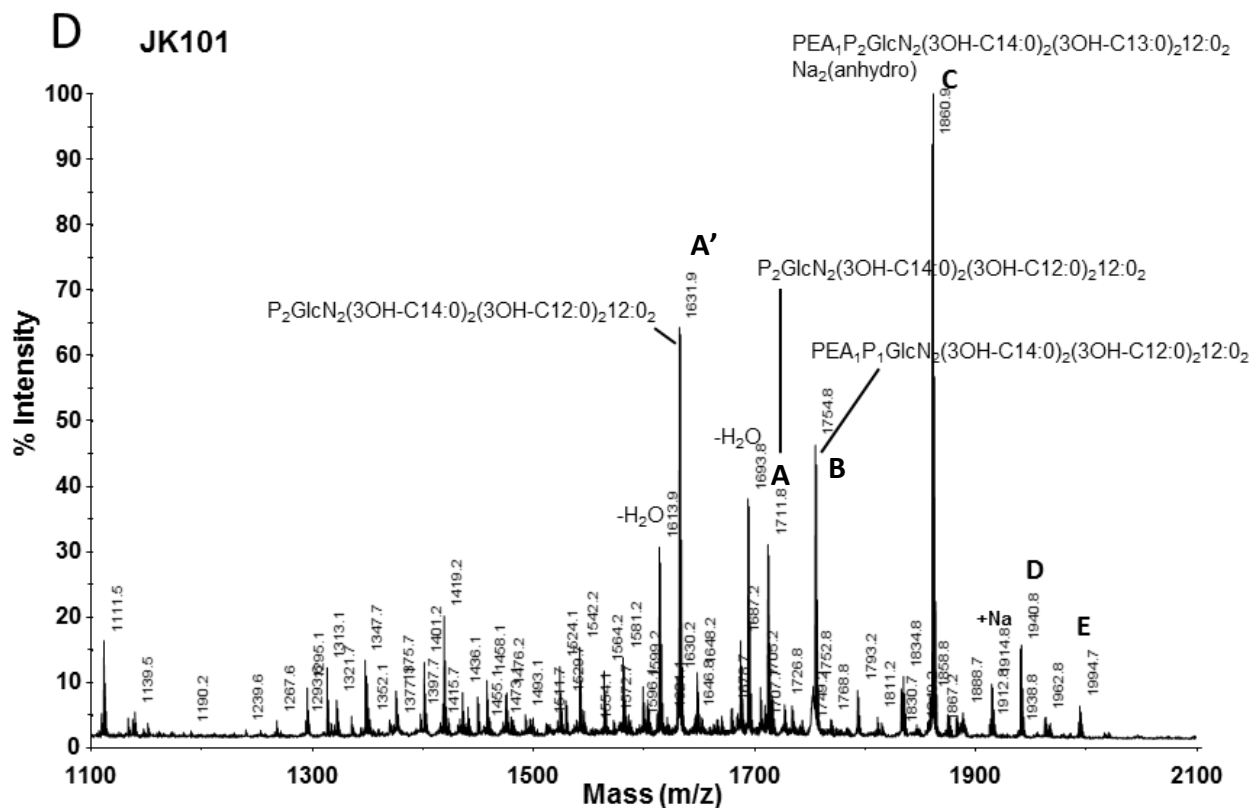
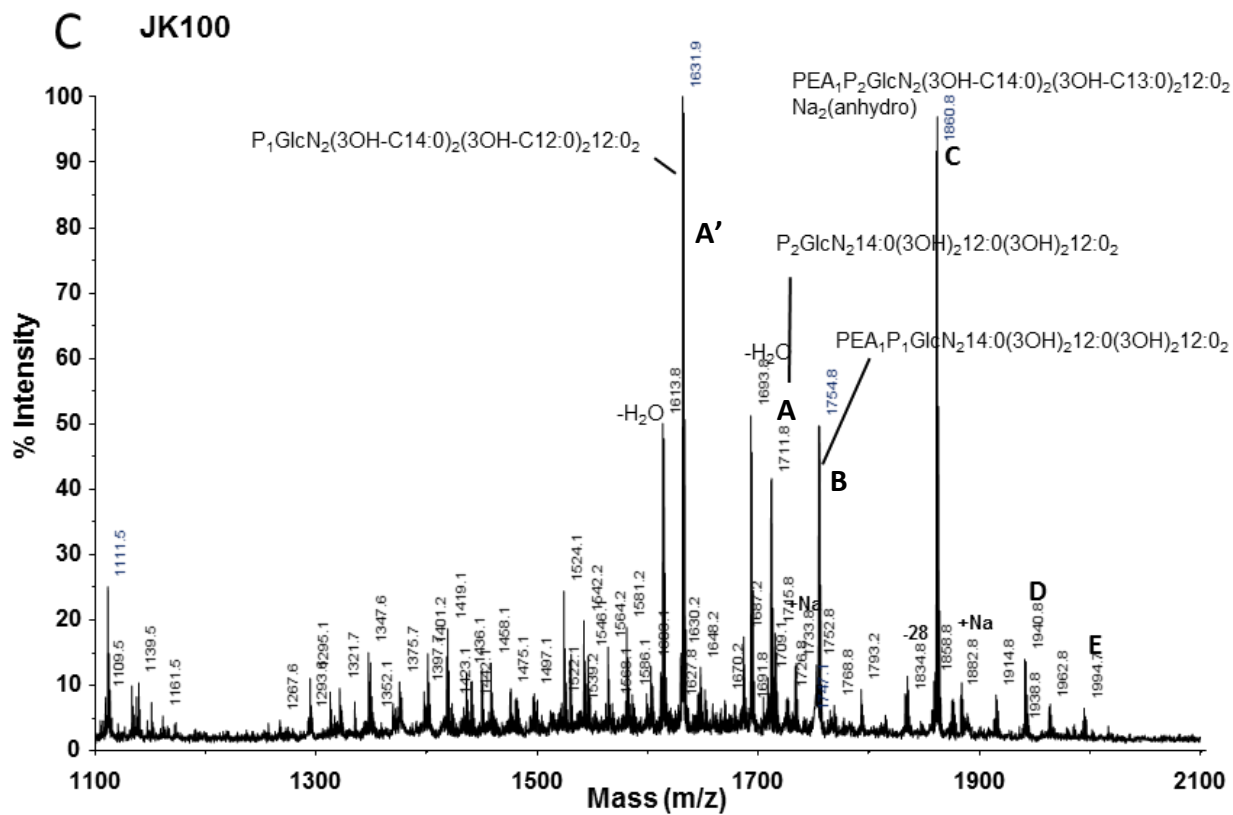


Supplemental Figures and Tables

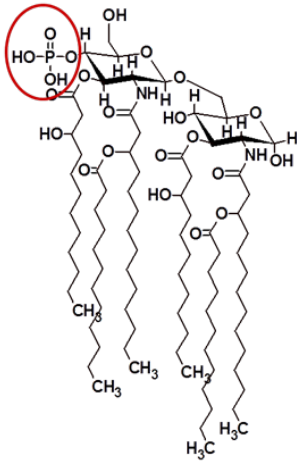
Supplemental Figure S1



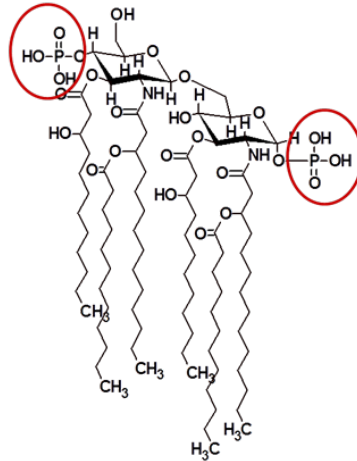


E

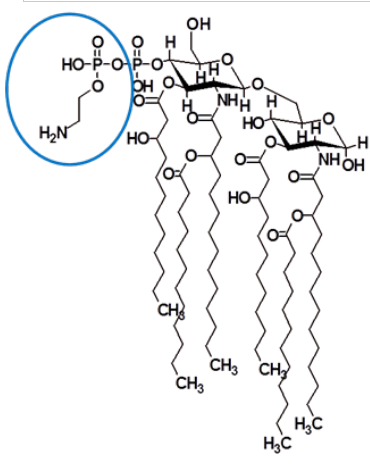
Species A' (1631.8 Da)



Species A (1711.8 Da)

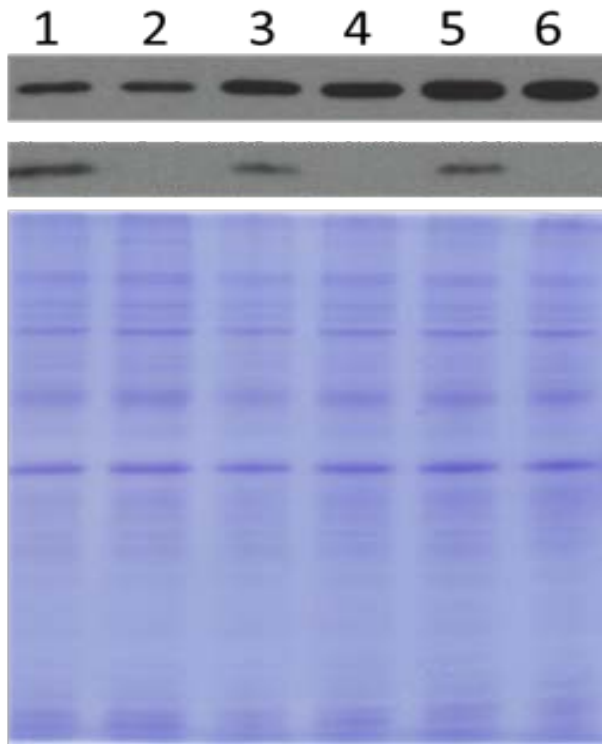


Species B (1754.8 Da)



Supplemental Figure S1: Loss of MisR does not impact decoration of gonococcal lipid A with phosphoethanolamine (PEA) as determined by MALDI-TOF MS analysis. Panel A) FA19 WT lipid A. Panel B) FA19 *lptA::spc* lipid A [this mutant is not decorated with lipid A; (3)]. Note that high molecular weight species C, D, and E (structures under investigation) were also absent from the FA19 *lptA::spc* lipid A spectra, and may require LptA function as a prerequisite step in order to be produced. We propose species C is the dehydrated, disodiated form of $\text{PEA}_1\text{P}_2\text{GlcN}_2(3\text{OH-C14:0})_2(3\text{OH-C13:0})_2\text{12:0}_2$; i.e. 1878.8 Da– 18 Da. Panel C) JK100 (FA19 *misR::kan*) lipid A. Panel D) JK101 (FA19 *misR::kan* pGCC4-*misR*) lipid A. Panel E) Chemical structures of gonococcal lipid A with (cationic; blue circle) and without (anionic; red circles) decoration of the 4' phosphate moiety with phosphoethanolamine.

Supplemental Figure S2



Supplemental Figure S2: MisR does not impact expression of the MtrCDE efflux pump. (Top) Western blot showing levels of the MtrE outer membrane channel protein of the MtrCDE efflux pump in gonococci with and without MisR. (Bottom) Coomassie staining of an identical SDS-PAGE gel run in parallel shows equal loading of all lanes. Lane 1: FA19 (WT), lane 2: JK100 (FA19 *misR::kan*), lane 3: JF1 (FA19 Δ *mtrR*), lane 4: JK200 (JF1 *misR::kan*), lane 5: KH15 (FA19 *mtr-79*), lane 6: JK300 (KH15 *misR::kan*).

Supplemental Table S1

SUPPLEMENTAL TABLE S1 Primers used in this study

Primer name	Sequence	Purpose
aspC1	5'-GCCGGATGCGTCTTTGTAC-3'	Confirmation of pGCC4 constructs
KanB	5'-CGGAAGGAATGTCTCCTGCTAAG-3'	<i>aphA-3</i> cassette internal primer (forward)
KanD	5'-CGCAGAAGGCAATGTCATACCAC-3'	<i>aphA-3</i> cassette internal primer (reverse)
lacZrev	5'-ACGACGACAGTATCGGCCTCAGG-3'	Confirmation of pLES94 fusion constructs
lctP	5'-GCGCGATCGGTGCGTTCT-3'	Confirmation of pGCC4 constructs
5'lptA-Z ^a	5'-TTGGATCCTCCTTTTCAATCCGGGCGTGATGC-3'	Construction of pLES94- <i>lptA</i> (forward)
3'lptA-Z ^{a,b}	5'-TTGGATCCTT/TAT/CATTTGGAATGTGTCGG-3'	Construction of pLES94- <i>lptA</i> (reverse)
misR1	5'-CCATGAGCCGCGTATTACTC-3'	Confirmation of the <i>misR/misS</i> mutants
MisRrev	5'-CGTCGATACTGCGGTCGAAT-3'	Confirmation of <i>misR::kan</i> mutation (reverse primer; anneals to a sequence deleted in the mutant)
misR3PacI*	5'-CAAACAATTAATTAACGCCACGCCCG-3'	Construction of pGCC4- <i>misR</i> (forward)
misR4PmeI*	5'-GTTGGAACAGTTTAAACTGCCCG-3'	Construction of pGCC4- <i>misR</i> (reverse)
MisSR	5'-GCGGACGGTATGTGCGTGA-3'	Confirmation of the <i>misR/misS</i> mutants
misSPacI*	5'-CGCGGCTTAATTAACCTGTT-3'	Construction of pGCC4- <i>misS</i>
misSPmeI*	5'-CGGATCGGTTTAAACCGGCATGAT-3'	Construction of pGCC4- <i>misS</i>
mtrC_promR	5'-CATCGCCTTAGAAGCATAAAAAGCC-3'	Amplification of the <i>mtrR-mtrCDE</i> intergenic region
mtrRJK1	5'-CATCAGGTGTTCTTTGGTTTTCAA-3'	Amplification of the <i>mtrR-mtrCDE</i> intergenic region
16Smai-RTF	5'-CCATCGGTATTCTCCACATCTCT-3'	qRT-PCR (reference gene)
16Smai-RTR	5'-CGTAGGGTGCGAGCGTTAATC-3'	qRT-PCR (reference gene)
bfrA_qRT_F	5'-TTCATCCGACGCATCCTGAT-3'	qRT-PCR
bfrA_qRT_R	5'-TTGAGCCTCTTCGCACAGTT-3'	qRT-PCR
bfrB_qRT_F	5'-TTGGTTATCCGCGAGCTGAA-3'	qRT-PCR

bfrB_qRT_R	5'-CAAATCGTCGGCAGCTTTCA-3'	qRT-PCR
clpB_qRT_F	5'-TCCAACAAGCCCTTGCAGAA-3'	qRT-PCR
clpB_qRT_R	5'-GCTGCTGCAAACGCTGTTTA-3'	qRT-PCR
dnaK_qRT_F	5'-ATGGCTCTGCAACGTCTGAA-3'	qRT-PCR
dnaK_qRT_R	5'-CGAATTTGGCGCGGGTAATT-3'	qRT-PCR
dsbD_qRT_F	5'-CGCAGGTTGCCTTTCCTTATG-3'	qRT-PCR
dsbD_qRT_R	5'-TCGGTTTGCGGATGGTAAGT-3'	qRT-PCR
grpE_qRT_F	5'-AAATGTGGAGGCGGTGGAAA-3'	qRT-PCR
grpE_qRT_R	5'-GCGCAGTTGTTTCGCTTTCA-3'	qRT-PCR
htpX_qRT_F	5'-TCGGCTTCACTGGTTCGATT-3'	qRT-PCR
htpX_qRT_R	5'-TTGGGCTTCGACAGTGTTC-3'	qRT-PCR
nadC_qRT_F	5'-TATTTCCGCCGTCGTCTGTT-3'	qRT-PCR
nadC_qRT_R	5'-CGAGAATCGGCACCATTAATGC -3'	qRT-PCR
tatC_qRT_F	5'-AAAGTTACCCTGATGGCGGC-3'	qRT-PCR
tatC_qRT_R	5'-GGCAAATGCCATGCCGATGAA-3'	qRT-PCR

^aThe engineered BamHI cleavage sites are underlined.

^bThe first two codons, Met-Ile, and partial third codon of the *lptA* ORF [as described for NMB1638 in (1, 2)] are shown in boldface.

^cThe indicated restriction endonuclease cut sites are underlined.

Supplemental Table S2 (A and B)

SUPPLEMENTAL TABLE S2A Selected MisR-activated genes from RNA-Seq (≥ 2 -fold cutoff, Bonferroni-corrected p value ≤ 0.05)

Gene	FA 1090 designation	Protein function	Envelope-associated	Protein quality control	Redox
NGO0179	NGO0179	putative membrane protein	x		
<i>tatC</i>	NGO0181	Sec-independent protein translocase component	x	x	
<i>tatB</i>	NGO0182	Sec-independent protein translocase component	x	x	
<i>htpX</i>	NGO0399	cell envelope zinc-metalloprotease	x	x	
<i>clpA</i>	NGO0408	ATP-binding subunit of the Clp protease		x	
NGO0420	NGO0420	cell wall associated protein	x		
<i>nrdAⁱ</i>	NGO0614	ribonucleotide reductase component			x
<i>nrdB^j</i>	NGO0615	ribonucleoside reductase component			x
NGO0618	NGO0618	hypothetical membrane protein	x		
<i>bfrA^a</i>	NGO0794	bacterioferritin A			x
<i>bfrB^a</i>	NGO0795	bacterioferritin B			x
<i>dsbD</i>	NGO0978	thiol:disulfide interchange protein	x	x	
<i>laz^a</i>	NGO0994	azurin; copper-binding protein	x		x
NGO1190	NGO1190	putative cell wall-associated hydrolase	x		
NGO1215	NGO1215	putative copper binding protein	x		
<i>arsB/nhaD</i>	NGO1411	anion permease	x		
<i>macB</i>	NGO1439	ATP-binding fusion component of the MacAB efflux pump	x		
<i>macA</i>	NGO1440	membrane fusion component of the MacAB efflux pump	x		
<i>Dca</i>	NGO1540	membrane-associated sulfatase	x		
<i>ftsL</i>	NGO1543	cell division protein	x		
<i>ompA</i>	NGO1559	outer membrane protein A	x		
<i>xthA/Nexoⁱ</i>	NGO1561	DNA exonuclease III			x
<i>L7/L12</i>	NGO1852	ribosomal protein	x		
NGO1981 ^g	NGO1981	putative adhesin component	x		x

SUPPLEMENTAL TABLE S2B Selected MisR-repressed genes from RNA-Seq (≥ 2 -fold cutoff, Bonferroni-corrected p value ≤ 0.05)

Gene	FA 1090 designation	Protein function	Envelope-associated	Protein quality control	Redox
<i>grx3^{a,c}</i>	NGO0114	glutaredoxin 3		x	x
<i>recN^a</i>	NGO0318	DNA repair protein			x
<i>ppiB^{f,g}</i>	NGO0376	peptidyl-prolyl cis-trans isomerase B		x	x
<i>nadC</i>	NGO0377	sodium/dicarboxylate symporter	x		
<i>trxI^{a,c,h}</i>	NGO0652	thioredoxin 1		x	x
<i>lon^g</i>	NGO0775	serine protease		x	x
<i>csgG</i>	NGO0834	putative curli assembly/transport component	x		
<i>opcA</i>	NGO0868	adhesion and invasion outer membrane protein	x		
<i>sdhA^{a,e}</i>	NGO0921	succinate dehydrogenase/fumarate reductase	x		x
<i>sdhD^{a,e}</i>	NGO0922	succinate:quinone oxidoreductase (a.k.a. succinate dehydrogenase)	x		x
<i>clpB^g</i>	NGO1046	molecular chaperone		x	x
NGO1245	NGO1245	putative ABC-type multidrug transport system, ATPase and permease components	x		
NGO1361	NGO1361	truncated putative L-lactate permease	x		
NGO1362	NGO1362	putative TonB-dependent iron siderophore receptor (truncated)	x		
<i>mafA2</i>	NGO1393	putative adhesin	x		
<i>grpE^g</i>	NGO1422	adenine nucleotide exchange factor of DnaK		x	x
<i>dnaK^g</i>	NGO1429	Hsp70 chaperone		x	x
<i>potD</i>	NGO1494	spermidine/putrescine-binding periplasmic protein	x		
<i>tbpA^g</i>	NGO1495	transferrin binding protein TonB-dependent transporter	x		x
<i>tbpB^g</i>	NGO1496	transferrin-binding protein	x		x
NGO1552	NGO1552	conserved sodium/proline symporter	x		
NGO1581	NGO1581	putative phosphate/sulfate transporter permease	x		
<i>mafA3</i>	NGO1584	putative adhesin	x		
<i>prlC</i>	NGO1770	oligopeptidase A		x	
NGO1980	NGO1980	malate:quinone oxidoreductase	x		x
<i>thiO</i>	NGO2008	Putative glycine oxidase			x
<i>cytX</i>	NGO2009	putative hydroxymethylpyrimidine transporter	x		
<i>glnQ</i>	NGO2013	ATP-binding component of the periplasmic glutamine permease	x		
NGO2014	NGO2014	predicted periplasmic substrate binding protein for a cysteine ABC transporter	x		
<i>tnaB</i>	NGO2073	tryptophan permease	x		
<i>ppk2^b</i>	NGO2113	putative polyphosphate kinase 2			x

^a(4), ^b(5), ^c(6), ^d(7), ^e(8), ^f(9), ^g(10), ^h(11), ⁱ(12), ^j(13)

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