Supporting Information

Rho and riboswitch-dependent regulations of *mntP* expression evade manganese and membrane toxicities

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Running title: Rho suppresses *mntP* expression

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Supporting information text

Construction of *E. coli* strains containing *mntP*-FLAG genotype

To generate *E. coli* strains with *mntP*-FLAG in the chromosome, we designed a synthetic DNA fragment containing homology 1 and homology 2 sites at the 3'- end of the *mntP* ORF from BiotechDesk, India. The homology-1 site, the 113-nucleotide long sequence from the 3'-end coding sequence of the *mntP* gene, was followed by a FLAG epitope coding sequence, Kan^R cassette of pKD4 vector, and homology-2 site. The synthetic DNA fragment was transformed in the WT and ΔRS strains and screened for kanamycin resistance. Finally, the Kan^R was removed by the FLP-recombinase system, as described (43). The Figure S4 shows the different sequence elements of *mntP*-FLAG synthetic DNA

Supplementary Figures

Figure S1.

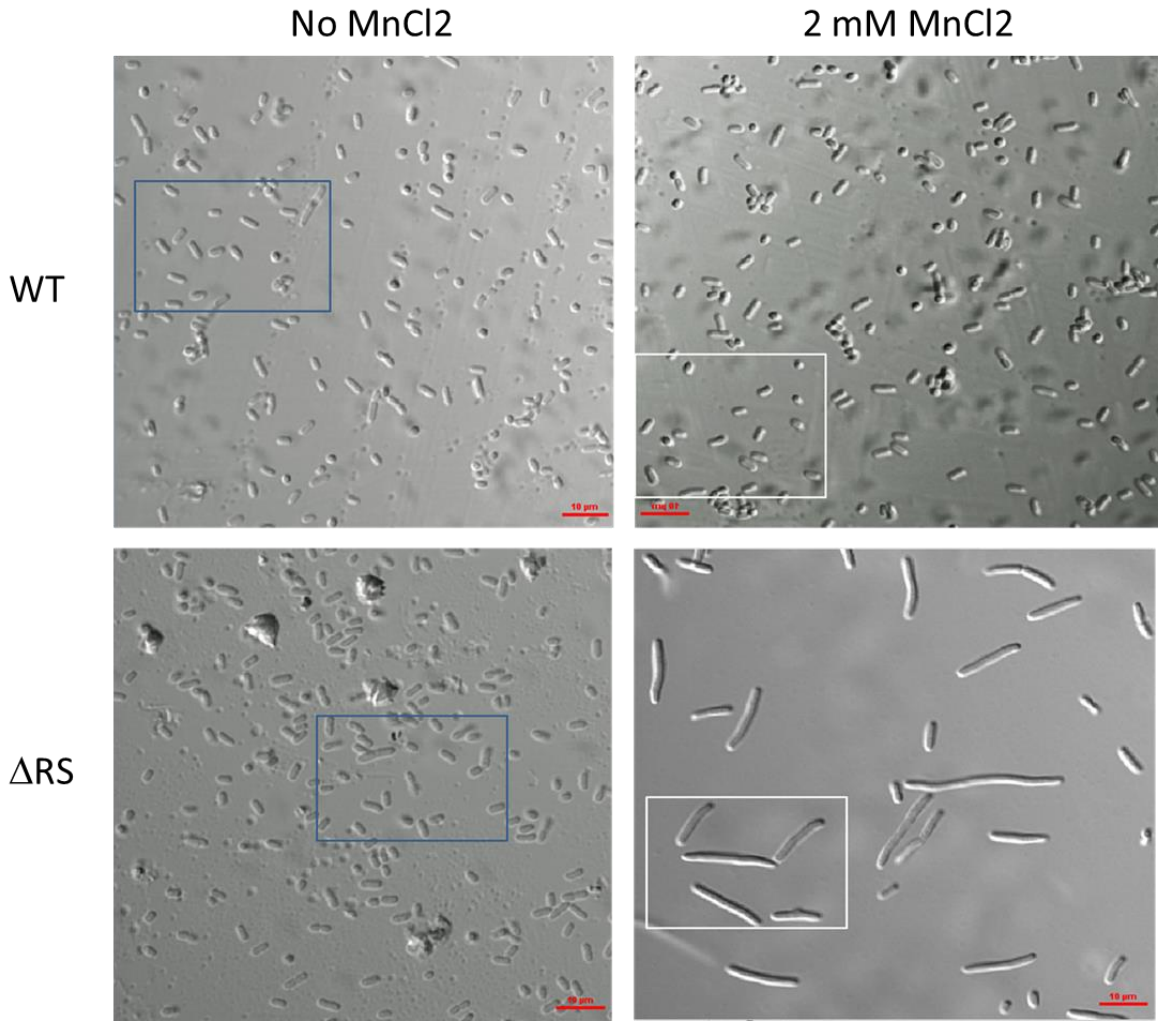

Figure S1. The cell morphologies judged under a confocal microscope when the WT and ΔRS strains were treated with Mn. The rectangular boxes in the panels are presented in Figure 4C.

Figure S2.

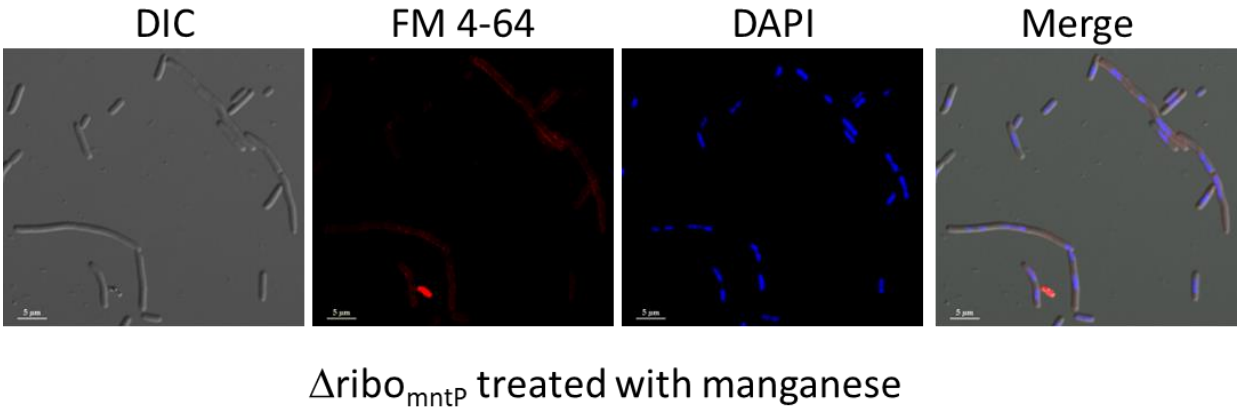

Figure S2. Mn-treated Δ*RS* cells were stained with FM-4-64 and DAPI to show that the filamentous cells had no septum, and discrete chromosomal DNA units were arranged throughout the filamented cells.

Figure S3.

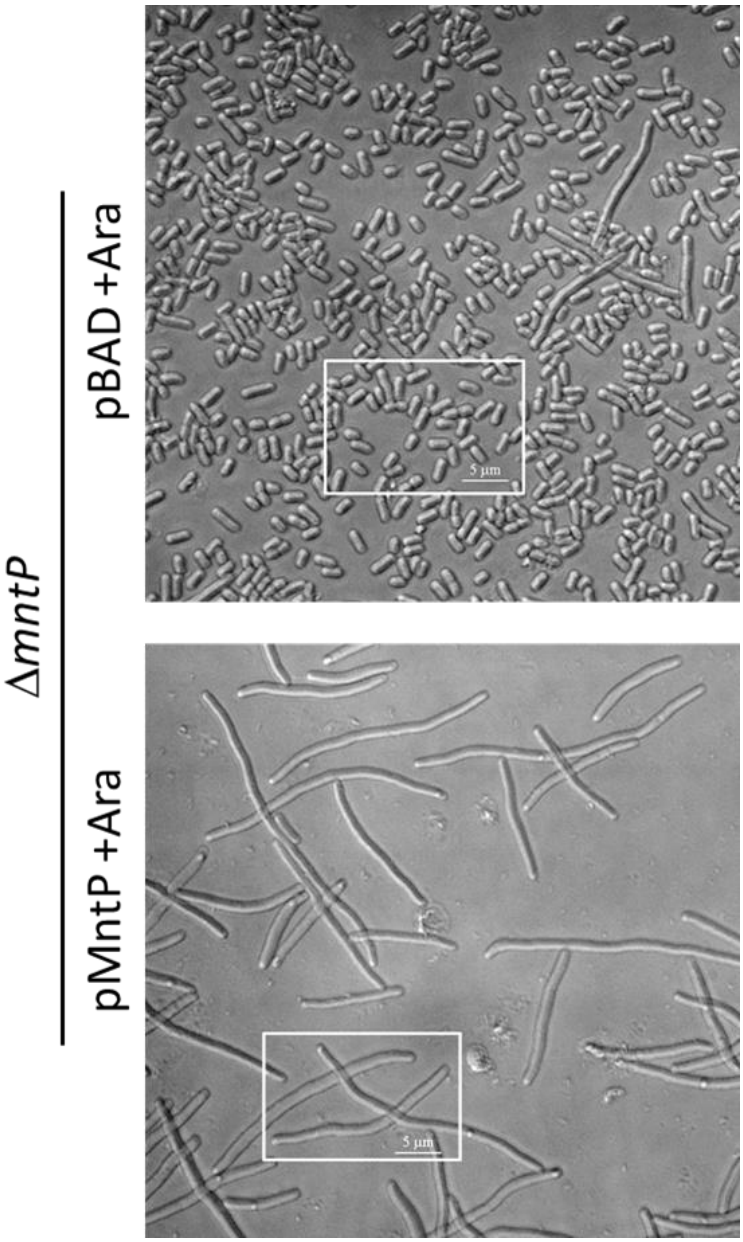

Figure S3. The cell morphologies judged under a confocal microscope with or without MntP overexpression. The rectangular boxes in the panels are presented in Figure 6B.

Figure S4.

A.

GGATGATGGTTGGTCGCTTTATCGGCTCAATTATTGGGAAAAAAGCGGAAATTCTCGGCGGGCTGGTGCTGATCGGCATCGGCGTC
CAGATCCTCTGGACGCACTTCCACGGTACTACTGCTAGCGAGAATTTGTATTTTCAGGGTGAGCTC**GACTACAAAGACCATGACGG**
TGATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGTAGGTGTAGGCTGGAGCTGCTTCGAAGTTCCTATACTTTC
TAGAGAATAGGAACTTCGGAATAGGAACTTCAAGATCCCCTCACGCTGCCGCAAGCACTCAGGGCGCAAGGGCTGCTAAAGGAAGC
GGAACACGTAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAGCTACTGGGCTATCTGGACAAGGGAAAACGCA
AGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGATAGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGA
ATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGC
GCAGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCC
GCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCA
GGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGG
CCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGG
CAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCC
GGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATG
ATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTC
GTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGT
GGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGC
TTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCG
AAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATC
GTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCCAGCTTCAAAAGCGCTCT
GAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTAAGGAGGATATTCATATTTTTTGCTGCCAGTGTTTGCCAG
ACTTCTGGCTTCGCACGCCAGGCAAACGGCGTCATCTGCAATAATGCGACGGCTTCATCACCGCGAAGAC

B.

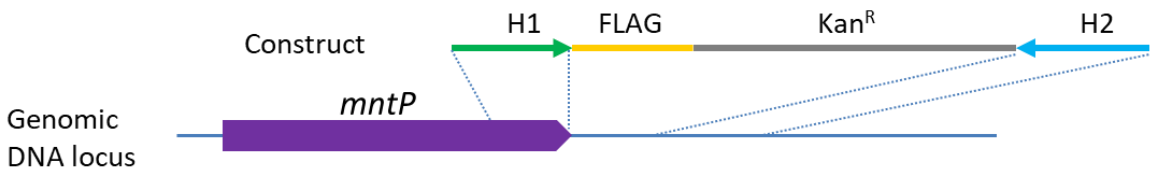

Figure S4. A. The synthetic DNA sequence showing green highlighted Homology 1 site taken from the 3'-end of *mntP ORF*, yellow highlighted FLAG epitope-containing coding sequence, grey highlighted Kan^R cassette flanked by FRT sites from pKD4 vector, cyan highlighted Homology 2 site taken from the noncoding DNA downstream of the *mntP ORF*. **B.** The schematic indicates the synthetic DNA construct and it different parts. The integration sites of the synthetic DNA in the genomic DNA are marked.

Table S1. List of oligonucleotides used in this study

Oligonucleotides	Sequence	Description
T7A1_F	CACTGAGGTACCTCCAGATCCCG AAAATTTATCAA	T7A1 promoter Forward primer With *KpnI* site.
DD6	GAACTCCAGCGGTTGCTCCCTCT CGA	T7A1 promoter Reverse primer
AK 1 mntP_T7A1	AGCAACCGCTGGAGTTCGCCCTC ATTTGGGGAGTAG	*mntP* riboswitch Forward primer with T7A1-reverse primer overhang.
D175	AGCAACCGCTGGAGTTCCCGA TTTCCAGATTCCG	*mntP* riboswitch Forward primer with T7A1-reverse primer overhang
mn70rev	GTCAGTGAATTCTTAGTGGTT CCATTCAAGGAC	Reverse primer with *EcoRI* restriction site
RiboS1	TATATTCTCAAAATATGTTAA GGTTGCGCCCTCATTTGGGGA GTAGCCGTGTAGGCTGGAGCT GCTTCG	Forward primer for *mntP* riboswitch deletion
RiboS3	CATCCATCGACATACCAAACG CAAGAAGAACAGTAGCAGTG ATATTCATATGAATATCCTCC TTAG	Reverse primer for *mntP* riboswitch deletion.
D174	GCTTAGGCTATATTACCTC	Forward primer to check riboswitch deletion by PCR and sequencing
*mntP*F	ATCACGGCTTGTTGTTCATG	Forward primer for checking *mntP* ORF integrity by sequencing
*mntP*R	CAGAAGTCTGGCAAACACTG	Reverse primer for checking *mntP* ORF integrity by sequencing
T7A1P	TCCAGATCCCGAAAATTTATC AA	T7A1 promoter forward PCR primer for in vitro transcription template T1 and T2 preparation

DD144	CAGGGTTTCGACGGCAGCAA AAATAAGGCCGGTTC	Reverse PCR primer for in vitro transcription template T1 preparation
D176	CATGACAATGTCCTGACC	Reverse PCR primer for in vitro transcription template T2 preparation
GK1	AATATCACTGCTACTG	*mntP* ORF forward primer to clone in pBAD vector
GK2	ACAAGTAAGCTTTAACCGTGG AAGTGCGTC	*mntP* ORF reverse primer with stop codon to clone in pBAD vector
DD149	GATTGCCACCAGCCTGGCTGC CATGGCTGTGG	SDM primer 1 to generate pMntP^{D118A} vector from pBAD-*mntP*
DD150	CCACAGCCATGGCAGCCAGG CTGGTGGCAATC	SDM primer 2 to generate pMntP^{D118A} vector from pBAD-*mntP*
KK2	AGCAACCGCTGGAGTTCGTCG AACGTCAACTTAC	*trp t'* forward primer with DD6 overhangs
KK3	GTATGGAATTCCCCGTTGCGT TGCATTGTTTC	*trp t'* reverse primer with *EcoRI* site
mntPF-RT:	CGTCGACACGGTTTCTG	*mntP* forward primer for qPCR
mntPR-RT:	AGCGACCAACCATCATCCCT	*mntP* reverse primer for qPCR
betBF	AACTTCTTCAGCTCCGGTCA	*betB* forward primer for qPCR
betBR	GCCGAAGTTAGTTTGCGGAT	*betB* reverse primer for qPCR