

This file contains the supplementary data for the article by Argueta-Zepeda et al. **The *Stenotrophomonas maltophilia* MntR miniregulon includes novel extracytoplasmic components and affects replication in *Acanthamoeba castellanii* phagosomes.**
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Table 1. Sm18 significant up and down-regulated genes from 0 μ M to 8 μ M of Mn(II) in minimal medium MOPS with 10 μ M of Iron. Cutoff: adjusted pvalue (padj) < 0.01 and Log₂ fold change (Log₂FC) \geq 1 or \leq -1.

Locus id	Gene name	Log ₂ FC	DE	padj	Gene description
V8P27_004008	mntP	3.4	Up	4.2 e ⁻⁶¹	Manganese efflux pump
V8P27_003797	V8P27_003797	2.0	Up	1.4 e ⁻⁰⁸	Copper chaperone SCO1/SenC
V8P27_003805	V8P27_003805	1.8	UP	1.4 e ⁻⁰⁸	HTH-type transcriptional regulator
V8P27_003804	V8P27_003804	1.7	UP	6.0 e ⁻⁰³	DUF6436 domain-containing protein
V8P27_002360	mntH	1.0	Down	2.0 e ⁻⁰⁵	Mn2+/Fe2+ uptake protein

Table 2. Sm18 significant up and down-regulated genes from 0 μ M to 8 μ M of Mn(II) in minimal medium MOPS without Iron. Cutoff: adjusted pvalue (padj) < 0.01 and Log₂ fold change (Log₂FC) \geq 1 or \leq -1.

Locus id	Gene name	Log ₂ FC	DE	padj	Gene description
V8P27_004008	mntP	4.0	Up	2.2 e ⁻¹³¹	Manganese efflux pump
V8P27_001285	V8P27_001285	2.2	Up	4.0 e ⁻⁰⁴	TonB-dependent receptor
V8P27_004004	V8P27_004004	1.3	Up	1.8 e ⁻²⁰	Major Facilitator Superfamily transporter
V8P27_004005	V8P27_004005	1.3	Up	4.8 e ⁻¹¹	DcaP outer membrane protein
V8P27_001286	V8P27_001286	1.2	Up	3.7 e ⁻⁰⁴	MerC domain-containing protein
V8P27_000822	prpE	1.0	Up	1.3 e ⁻⁰⁹	propionate-CoA ligase
V8P27_002357	V8P27_002357	4.6	Down	8.5e ⁻¹²²	TonB-dependent receptor
V8P27_002358	V8P27_002358	2.8	Down	5.3 e ⁻⁰³	Thioredoxin-fold protein
V8P27_003990	V8P27_003990	2.0	Down	1.0 e ⁻⁰⁵	MFP of RND efflux pump
V8P27_001267	V8P27_001267	1.8	Down	6.5 e ⁻⁰³	Cation Diffusion Facilitator transporter
V8P27_002373	V8P27_002373	1.5	Down	8.4 e ⁻⁰⁹	Small Multidrug Resistance transporter
V8P27_003991	V8P27_003991	1.5	Down	1.8 e ⁻¹⁴	RND efflux pump
V8P27_002360	mntH	1.5	Down	8.2 e ⁻¹⁵	Mn2+/Fe2+ uptake protein
V8P27_003989	V8P27_003989	1.4	Down	4.0 e ⁻⁰⁴	OMF of RND efflux pump
V8P27_001268	nfi	1.3	Down	3.6 e ⁻⁰⁸	Endonuclease V
V8P27_001249	V8P27_001249	1.3	Down	7.1 e ⁻⁰⁷	Uncharacterized protein
V8P27_001248	map	1.2	Down	1.7 e ⁻¹²	Type I methionyl aminopeptidase

Table 3. Strains used and constructed in this study

Strain name	Key strain	Reference/source
DH5 α	Standard <i>Escherichia coli</i> cloning strain. F– endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20	Laboratory strain collection
S17.1	Standard <i>Escherichia coli</i> conjugative donor strain. recA, thiE1, pro-82, endA, hadR17. RP4-2(Km::Tn7, Tc::Mu1)	(1)
Sm18	<i>Stenotrophomonas maltophilia</i> strain Sm18. Environmental isolate from Cuernavaca, Morelos, México.	(2)
Neff	<i>Acanthamoeba castellanii</i> strain Neff. Environmental isolate from USA	ATCC 30010
Sm18 Δ 02357	Deletion mutant of the ORF 02357 from <i>Stenotrophomonas maltophilia</i> Strain Sm18	This work
Sm18 Δ 02358	Deletion mutant of the ORF 02358 from <i>Stenotrophomonas maltophilia</i> Strain Sm18	This work
Sm18 Δ mntH	Deletion mutant of the ORF mntH from <i>Stenotrophomonas maltophilia</i> Strain Sm18	This work
Sm18 Δ mntP	Deletion mutant of the ORF mntP from <i>Stenotrophomonas maltophilia</i> Strain Sm18	This work
Sm18 Δ mntR	Deletion mutant of the ORF mntR from <i>Stenotrophomonas maltophilia</i> Strain Sm18	This work
Sm18/332	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the empty vector pSEVA332	This work
Sm18 Δ mntH/332	Sm18 Δ mntH deletion mutant transformed with the empty vector pSEVA332	This work
Sm18 Δ mntH/332::mntH	Sm18 Δ mntH deletion mutant transformed with the vector pSEVA332::mntH	This work
Sm18 Δ mntP/332	Sm18 Δ mntP deletion mutant transformed with the empty vector pSEVA332	This work
Sm18 Δ mntP/332::mntP	Sm18 Δ mntP deletion mutant transformed with the vector pSEVA332::mntP	This work
Sm18::mTn7TC1_Pc_mScarlet-I	<i>Stenotrophomonas maltophilia</i> strain Sm18 derivative tagged with mScarlet-I expressed from the strong constitutive Pc promoter.	(3)
Sm18 Δ 02357::mTn7TC1_Pc_mScarlet-I	Derivative of the Sm18 Δ 02357 deletion mutant, tagged with mScarlet-I and expressed under the strong constitutive Pc promoter	This work

Sm18Δ02358::mTn7TC1_Pc_mScarlet-I	Derivative of the Sm18Δ02358 deletion mutant, tagged with mScarlet-I and expressed under the strong constitutive Pc promoter	This work
Sm18ΔmntH::mTn7TC1_Pc_mScarlet-I	Derivative of the Sm18ΔmntH deletion mutant, tagged with mScarlet-I and expressed under the strong constitutive Pc promoter	This work
Sm18ΔmntP::mTn7TC1_Pc_mScarlet-I	Derivative of the Sm18ΔmntP deletion mutant, tagged with mScarlet-I and expressed under the strong constitutive Pc promoter	This work
Sm18ΔmntR::mTn7TC1_Pc_mScarlet-I	Derivative of the Sm18ΔmntR deletion mutant, tagged with mScarlet-I and expressed under the strong constitutive Pc promoter	This work
Sm18VIM02357	Interrupted mutant of the ORF 02357 from <i>Stenotrophomonas maltophilia</i> Strain Sm18	This work
Sm18VIMmntH	Interrupted mutant of the ORF mntH from <i>Stenotrophomonas maltophilia</i> Strain Sm18	This work
Sm18VIMmntP	Interrupted mutant of the ORF mntP from <i>Stenotrophomonas maltophilia</i> Strain Sm18	This work
Sm18VIMmntH::mTn7TC1_Pc_mScarlet-I	Derivative of the Sm18VIMmntH interrupted mutant, tagged with mScarlet-I and expressed under the strong constitutive Pc promoter	This work
Sm18/327	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the empty expression vector pSEVA327	This work
Sm18/327-pr_02357-02358	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the expression vector pSEVA327-pr_02357-02358	This work
Sm18/327-pr_mntH	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the expression vector pSEVA327-pr_mntH	This work
Sm18/327-pr_mntP	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the expression vector pSEVA327-pr_mntP	This work
Sm18/337R	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the empty expression vector pSEVA337R	This work
Sm18/337R-pr_02357-02358	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the expression vector pSEVA337R-pr_02357-02358	This work
Sm18/337R-pr_mntH	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the expression vector pSEVA337R-pr_mntH	This work
Sm18/337R-pr_mntP	<i>Stenotrophomonas maltophilia</i> strain Sm18 transformed with the expression vector pSEVA337R-pr_mntP	This work

Sm18ΔmntR/327	Derivative of the Sm18ΔmntR deletion mutant transformed with the empty expression vector pSEVA327	This work
Sm18ΔmntR/327-pr_02357-02358	Derivative of the Sm18ΔmntR deletion mutant transformed with the expression vector pSEVA327-pr_02357-02358	This work
Sm18ΔmntR/327-pr_mntH	Derivative of the Sm18ΔmntR deletion mutant transformed with the expression vector pSEVA327-pr_mntH	This work
Sm18ΔmntR/327-pr_mntP	Derivative of the Sm18ΔmntR deletion mutant transformed with the expression vector pSEVA327-pr_mntP	This work

Table 4: Vectors used and constructed in this study

Plasmid name	Plasmid features	Reference/source
pUC18T_mTn7TC1_Pc_mScarlet-I	pUC18T_mTn7TC1_Pr_mScarlet-I derivative for chromosomal labeling of bacteria with constitutive mScarlet-I expression driven from the strong Pc promoter; 5,893 bp.	(3)
pTNS2	R6K-based plasmid (ApR) encoding the TnsABCD Tn7 transposase expression genes; 9,615 bp.	AddGene #64968
pEX18Tc	Transmissible plasmid containing OriT, for site-targeted mutagenesis in Gram negative bacteria; 6349 bp.	(4)
Sm18Δ02357_pEX18Tc	pEX18Tc derivative with homologous DNA genetic fragments flanking the ORF 02357 of Sm18; 7424 bp.	This work
Sm18Δ02358_pEX18Tc	pEX18Tc derivative with homologous DNA genetic fragments flanking the ORF 02358 of Sm18; 7343 bp.	This work
Sm18ΔmntH_pEX18Tc	pEX18Tc derivative with homologous DNA genetic fragments flanking the ORF <i>mntH</i> of Sm18; 7424 bp.	This work
Sm18ΔmntP_pEX18Tc	pEX18Tc derivative with homologous DNA genetic fragments flanking the ORF <i>mntP</i> of Sm18; 7375 bp.	This work
Sm18ΔmntR_pEX18Tc	pEX18Tc derivative with homologous DNA genetic fragments flanking the ORF <i>mntR</i> of Sm18; 7340 bp.	This work
pEX18TcΔSacB7_PEM7	pEX18Tc derivative with expression of GFP under control of constitutive promoter PEM7 and deletion of the gen SacB; 6031 pb.	Laboratory constructions collection
Sm18VIM02357_pEX18TcΔSacB7_PEM7	pEX18TcΔSacB7_PEM7 derivative with homologous fragment of the ORF 02357 of Sm18; 6404 bp.	This work
Sm18VIMmntH_pEX18TcΔSacB7_PEM7	pEX18TcΔSacB7_PEM7 derivative with homologous fragment of the ORF <i>mntH</i> of Sm18; 6380 bp.	This work

Sm18VIMmntP_pEX18TcΔSacB7_PEM7	pEX18TcΔSacB7_PEM7 derivative with homologous fragment of the ORF <i>mntP</i> of Sm18; 6360 bp.	This work
pSEVA332	Empty vector (chloramphenicol resistance , ori pBBR1, cargo lacZα-pUC19); 3417 bp.	(5)
pSEVA332:: <i>mntH</i>	pSEVA332 digested with PacI and SpeI to replace the lacZα-pUC19 cargo with <i>mntH</i> gene; 4452 bp.	This work
pSEVA332:: <i>mntP</i>	pSEVA332 digested with PacI and SpeI to replace the lacZα-pUC19 with <i>mntP</i> gene; 4077 bp.	This work
pSEVA327	Empty expression vector (chloramphenicol resistance, ori RK2, cargo GFP); 4408 bp.	(5)
pSEVA327-pr_02357-02358	pSEVA327 containing the promoter region of the ORF 02357-02358; 4796 bp.	This work
pSEVA327-pr_ <i>mntH</i>	pSEVA327 containing the promoter region of the ORF <i>mntH</i> ; 4721 bp.	This work
pSEVA327-pr_ <i>mntP</i>	pSEVA327 containing the promoter region of the ORF <i>mntP</i> ; 4976 bp.	This work
pSEVA337R	Empty expression vector (chloramphenicol resistance, ori pBBR1, cargo mCherry); 3661 bp.	(5)
pSEVA337R-pr_02357-02358	pSEVA337R containing the promoter region of the ORF 02357-02358; 4049 bp.	This work
pSEVA337R-pr_ <i>mntH</i>	pSEVA337R containing the promoter region of the ORF <i>mntH</i> ; 3974 bp.	This work
pSEVA337R-pr_ <i>mntP</i>	pSEVA337R containing the promoter region of the ORF <i>mntP</i> ; 4229 bp.	This work

Table 5: Primers synthesized in this study for constructing vectors

Primer name	Primer sequence (5' to 3')	Restriction sites	Reference/source
Sm18_D02361_Fragment1.F	cgttgtaaaacgacggccagtgccatgtttggccagagc	None	This work
Sm18_D02361_Fragment1.R	tcgcagtgcttcacatgaaggcgtcgtcct	None	This work
Sm18_D02361_Fragment2.F	gacgccttcattgtgaagcactgcgagcgc	None	This work
Sm18_D02361_Fragment2.R	tctagagtcgacctgcaggcatgcatttcgcgaccc	None	This work
Sm18_D02362_Fragment1.F	tgtaaaacgacggccagtgccaaaaccaacctcggtgg	None	This work
Sm18_D02362_Fragment1.R	cgcgaccctaccaacagcgtcgcagt	None	This work
Sm18_D02362_Frag	gagcgtgttggtagggtcgcgcaa	None	This work

ment2.F			
Sm18_D02362_Frag ment2.R	agagtcgacctgcaggcatgcatcgctgaaaagcaccccctt	None	This work
Sm18_DmntH_amp1. FOR	taaaacgacggccagtgccagcgcacgaacgcttgaaca agg	None	This work
Sm18_DmntH_amp1. REV	gcatcaccccatggggccgaatatagctcc	None	This work
Sm18_DmntH_amp2. FOR	gccccatggggtgatgccagatccaggg	None	This work
Sm18_DmntH_amp2. REV	ggaaacagctatgaccatgattacgtacttccgcagcgactat ccgcc	None	This work
Sm18DmntP_PCR1.F OR	cgttgtaaaacgacggccagtgccacgcttacgacctcaacg tct	None	This work
Sm18DmntP_PCR1.R EV	cgcggaagcgttaaatgggggacatggacagc	None	This work
Sm18DmntP_PCR2.F OR	atgtccccatttaacgcttccgcgattacgt	None	This work
Sm18DmntP_PCR2.R EV	ggaaacagctatgaccatgattacgcgatgttctcgatcattct gccg	None	This work
Sm18_DmntR_Fragm ent1.F	tgtaaaacgacggccagtgccaaacagcgctcgctgcgacc ga	None	This work
Sm18_DmntR_Fragm ent1.R	gcgcaggcgtgtagcgggtggcgccgg	None	This work
Sm18_DmntR_Fragm ent2.F	cgccaccgctacacgcctgcgcctg	None	This work
Sm18_DmntR_Fragm ent2.R	agagtcgacctgcaggcatgcaatcacttcggccaggtcgca ggcgatgatcg	None	This work
Sm18_VIM_02361.F	aaaaaGAATTCcacaccctcttccatcgg	EcoRI	This work
Sm18_VIM_02361.R	aaaAAGCTTcttgaccacttcgatgcgg	HindIII	This work
Sm18-VIM-mntH.F	aaaaAAGCTTctacatgatctcggtcggct	HindIII	This work
Sm18-VIM-mntH.R	aaaaGGATCCgaagatcaccatcagcagcg	BamHI	This work
Sm18-VIM-mntp.F1	aaaaAAGCTTccccatttcgatcctcctga	HindIII	This work
Sm18-VIM-mntP.R1	aaaaGGATCCcgccgatatgcacatccatg	BamHI	This work
Sm18_mntHcompl.f	aaaaTTAATTAatagtctccaccagctccatc	PacI	This work
Sm18_mntHcompl.R	aaaaACTAGTaaggcatccacgcatggcg	SpeI	This work
Sm18_mntPcompl.F1	aaaaTTAATTAagcttacgacctcaacgtc	PacI	This work
Sm18_mntPcompl.R	aaaaACTAGTatcgcgtgctgaccaagcc	SpeI	This work

Sm18_pr_02357.F	aaaaaGAATTCtaccgcaaccatggagcc	EcoRI	This work
Sm18_pr_02357.R	aaaAAGCTTctgactttcacctgcacgc	HindIII	This work
Sm18-pr-mntH-p3.f	atatGGATCCtagtcctccaccagctccatc	BamHI	This work
Sm18-pr-mntH-p3.R	atatAAGCTTaaccaccagtgacccttgctg	HindIII	This work
Sm18-pr-mntP.F1	atataGAATTCgcttacgacctaacgctc	EcoRI	This work
Sm18-pr-mntP.R1	atatGGATCCgatcaggaggatcgaaatg	BamHI	This work

TABLE 6: Primers used and synthesized in this study to verifying constructions or mutants

Primer name	Primer sequence (5' to 3')	Mutant/Constructions	Reference/ source
F24	cgccagggttttccagtcacgac	Forward primer for confirming constructs or merodiploids strains in plasmids pEXTc18 and constructs in pEXTc18ΔSacB7_PEM7	(6)
R24	agcggataacaatttcacacagga	Reverse primer for confirming constructs in plasmids pEXTc18, insertional mutations in pEXTc18ΔSacB7_PEM7 and Forward primer for confirming transcriptional fusions in pSEVA327 and pSEVA 337R	(6)
pBBR1_VER.F	cggccatcgccacatatcc	Forward primer for confirming constructs in plasmid pSEVA332	This work
Sm18_D02361_VER.F	atgttctgatcccgtttcg	Forward primer for confirming deletion mutant Sm18Δ02357	This work
Sm18_D02361_VER.R	tgacgtacaccacttcggtatc	Reverse primer for confirming deletion mutant Sm18Δ02357	This work
Sm18_D02362_VER.F	gctcgtggtacgtcaacctg	Forward primer for confirming deletion mutant Sm18Δ02358	This work
Sm18_D02362_VER.R	gtcgagctgatctcggaacct	Reverse primer for confirming deletion mutant Sm18Δ02358	This work
Sm18_DmntH_VER.F	catagtctccaccagctcc	Forward primer for confirming deletion mutant Sm18ΔmntH	This work
Sm18_DmntH_VER.R	tgaggcgttcttcaggagag	Reverse primer for confirming deletion mutant Sm18ΔmntH	This work
Sm18_DmntP_VER.F	gctatacaaaccagccctgc	Forward primer for confirming deletion mutant Sm18ΔmntP	This work
Sm18_DmntP_VER.R	tggatcaggcgttgagagaaa	Reverse primer for confirming deletion mutant Sm18ΔmntP	This work
Sm18_DmntR_VER.F	agcctggatgagagcgagag	Forward primer for confirming deletion mutant Sm18ΔmntR	This work

Sm18_DmntR_VER.R	ttcatggggccgaatatagc	Reverse primer for confirming deletion mutant Sm18ΔmntR	This work
Sm18_M02362_VER.R	tccaggctgacgtacaccac	Reverse primer for confirming merodiploids Sm18::pEX18Tc-02358	This work
Sm18_MmntR_VER.R	agaccgtggagcgattcct	Reverse primer for confirming merodiploids Sm18::pEX18Tc-mntR	This work
Steno_glmS_down.F (SmaI_glmS_down_15 49F)	gacatgccggtggtggtgat cg	Forward primer for confirming insertion of pUC18T_mTn7TC1_Pc_mScarlet-I in Sm18 Strains	(3)
pTn7.R	cacagcataactggactgat ttc	Reverse primer for confirming insertion of pUC18T_mTn7TC1_Pc_mScarlet-I in Sm18 Strains	(3)

Note: Sm18::pEX18Tc-mntH merodiploid was confirm by F24 and Sm18_Pr_02361.R primers.

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