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Deposited in DRO:

06 March 2019

Version of attached file:

Accepted Version

Peer-review status of attached file:

Peer-reviewed

Citation for published item:

Turner, Andrew G. and Djoko, Karrera Y. and Ong, Cheryl-lynn Y. and Barnett, Timothy C. and Walker, Mark J. and McEwan, Alastair G. (2019) 'Group A Streptococcus co-ordinates manganese import and iron efflux in response to hydrogen peroxide stress.', *Biochemical journal*, 476 (3). pp. 595-611.

Further information on publisher's website:

<https://doi.org/10.1042/BCJ20180902>

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Table S1

Bacterial strains	Description	Reference
<u><i>Escherichia coli</i></u>		
Top10	<i>E. coli</i> laboratory cloning strain	Invitrogen
Top10+pBAD- <i>sodA</i> -TEV-His	<i>E. coli</i> strain for expression of rSodA	This study
<u><i>Streptococcus pyogenes</i></u>		
5448	<i>S. pyogenes</i> invasive M1T1 strain	(1)
5448Δ <i>pmtA</i>	<i>S. pyogenes</i> 5448Δ <i>pmtA</i> :: <i>aphA</i> -3 deletion mutant	(2)
5448Δ <i>pmtA</i> :: <i>pmtA</i>	<i>S. pyogenes</i> 5448 <i>pmtA</i> complemented strain	(2)
5448Δ <i>perR</i>	<i>S. pyogenes</i> 5448Δ <i>perR</i> :: <i>aad9</i> deletion mutant	(3)
5448Δ <i>perR</i> :: <i>perR</i>	<i>S. pyogenes</i> 5448Δ <i>perR</i> complemented strain	(3)
5448Δ <i>mtsR</i>	<i>S. pyogenes</i> 5448Δ <i>mtsR</i> :: <i>aad9</i> deletion mutant	This study
5448Δ <i>mtsR</i> :: <i>mtsR</i>	<i>S. pyogenes</i> 5448Δ <i>mtsR</i> complemented strain	This study
5448Δ <i>mtsABC</i>	<i>S. pyogenes</i> 5448Δ <i>mtsABC</i> :: <i>aphA</i> -3	This study
5448Δ <i>mtsABC</i> :: <i>mtsABC</i>	<i>S. pyogenes</i> 5448Δ <i>mtsABC</i> complemented strain	This study
5448Δ <i>sodA</i>	<i>S. pyogenes</i> 5448Δ <i>sodA</i> :: <i>aphA</i> -3 deletion mutant	This study
5448Δ <i>sodA</i> :: <i>sodA</i>	<i>S. pyogenes</i> 5448Δ <i>sodA</i> complemented strain	This study
Plasmids		
pJRS233	Temperature sensitive shuttle plasmid::Em ^R	(4)
pLZ12-TS	Temperature sensitive shuttle plasmid::Spec ^R	This study
pUC4QKm2	Template for Km ^R	(5)
pUCSpec	Template for Spec ^R	(6)
pBAD-Myc-His-A	Template plasmid for generation of pBAD-TEV-His	This study
pBAD-TEV-His		
pBAD- <i>sodA</i> -TEV-His	pBAD-TEV-His vector with <i>sodA</i> inserted at <i>Nco</i> I and <i>Apa</i> I site	This study
pJRS233- <i>mtsR</i> -KO	pJRS233 + <i>mtsR</i> knockout construct inserted at the <i>Xho</i> I and <i>Bam</i> HI site	This study
pJRS233- <i>mtsR</i> -comp	pJRS233 + <i>mtsR</i> complement construct inserted at the <i>Xho</i> I and <i>Bam</i> HI site	This study
pJRS233- <i>mtsABC</i> -KO	pJRS233 + <i>mtsABC</i> knockout construct inserted at the <i>Xho</i> I and <i>Pst</i> I site	This study
pJRS233- <i>mtsABC</i> -comp	pJRS233 + <i>mtsABC</i> complement construct inserted at the <i>Xho</i> I and <i>Pst</i> I site	This study
pLZ12-TS- <i>sodA</i> -KO	pLZ12-TS + <i>sodA</i> KO construct	This study
pJRS233- <i>sodA</i> -comp	pJRS233 + <i>sodA</i> complement construct	This study

Table S2

Primer	Sequence (5'-3')	
<i>Deletion mutation and reverse complementation constructs</i>		
pBAD-site-F	GGGAATTCGAAGCTTGGGCCCCGAGGATCTGTACTTTCAGAGCGTCGACCATCATCATCATCA	
pBAD-site-R	ATATGGTACCAGCTGCAGATCTCGA	
sodA-prot-F	CCCGGG CCATGGC TATTATTTTACCAGAACTTCC	<i>NcoI</i>
sodA-prot-R	CGCGCG GGGCC TTTAGCGGCTTGGTAAAGTTCT	<i>ApaI</i>
sodA-KO-1	<u>TGGTGTTTTATGGCTCTCTTGGTCGTCAGACTGATGGGCCCTGATGCGGCTAGAG</u> ATTTGATTCATGATT	<i>pLZ-5'</i>
sodA-KO -2	<u>CAAAGTTGGCGTATAACATACGCGTATGGAAGTTCTGG</u>	<i>aphA-3 5'</i>
sodA-KO -3	<u>TACTGGATGAATTGTTTTAGACTTTACCAAGCCGCTAAATAAGAAAAGG</u>	<i>aphA-3 3'</i>
sodA-KO -4	<u>TACGAACGGCACAGATGGTCATAACCTGAAGGAAGATCTCTGCATCCATAACA</u> GCTTGCTTAGC	<i>pLZ-3'</i>
sodA-comp-F	<u>CGGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATTGATGCGGCTAGAG</u> ATTTGATTCATGATT	<i>pJRS-5'</i>
sodA-comp-R	<u>CTCTAGAACTAGTGGATCCCCCGGGCTGCAGGAATTCGATTCTGCATCCATAAC</u> AGCTTGCTTAGC	<i>pJRS-3'</i>
pJRS233-F	ATCGAATTCCTGCAGCCCCG	
pJRS233-R	ATCAAGCTTATCGATACCGTCGACC	
km-sodA-F	TACCAGAACTTCCATACGCGTATGTTATACGCCAACTTTGAAAACAACCTTTGAA AAAGC	<i>aphA-3 5'</i>
km-sodA-R	ATTTAGCGGCTTGGTAAAGTCTAAAACAATTCATCCAGTAAAATATAATATTTT ATTTTCTCCCAAT	<i>aphA-3 3'</i>
mtsR-KO-1	CCCGG CTCGAG CCATCAGACACGGCAAAGTA	<i>XhoI</i>
mtsR-KO-2	<u>GATATGATCTTTCATTTCCATAAAACTAAAGTCTTCTTTATTAGGCGTCAT</u>	<i>aad9 5'</i>
mtsR-KO-3	<u>GGAAATATTTCATTCTAATTGGTAATCAGCTTTATGTCACAGCCCTTTAA</u>	<i>aad9 3'</i>
mtsR-KO-4	CGCGCG GGATCC GAATTGCAATCATTGACCAA	<i>BamHI</i>
spec-F	CTTTAGTTTTATGGAAATGAAAGATCATATC	
spec-R	CTGATTACCAATTAGAATGAATATTTCC	
mtsABC-KO-1	CCCGGG CTCGAG TCGTTTGGCCTTTCTCTTATT	<i>XhoI</i>
mtsABC-KO-2	<u>CAAAGTTGGCGTATAACATACAACATCTTCTGGTAATGGTTC</u>	<i>aphA-3 5'</i>
mtsABC-KO-3	<u>TTACTGGATGAATTGTTTTAGTATTGATTACGCCAGCAGCGACA</u>	<i>aphA-3 3'</i>
mtsABC-KO-4	CCCGGG CTGCAG CTTATCAGATAATAGGCTAAATACC	<i>PstI</i>
km-F	TATGTTATACGCCAACTTTG	
km-R	CTAAAACAATTCATCCAGTAA	
pLZ-12-F	AGATCTTCCTTCAGGTTATG	
pLZ-12-R	GGGCCCATCAGTCTGACG	
<i>Gene expression studies</i>		
pmtA-qRT-F	GAAAAGCAAAACCGCCACCT	
pmtA-qRT-R	GGGCACATGGTGAAGCTACT	
sodA-qRT-F	CCTGAACCAAAACGTCCAGT	
sodA-qRT-R	CACCTAAACCATGCGCTTTT	
mtsA-qRT-F	CAAACAATTGATTGCAAAGGATCC	
mtsA-qRT-R	GTCTAGTTTTTCCAATTTAGCCACA	
dpr-qRT-F	ATGCGTGGTCCAGGTTTCTT	
dpr-qRT-R	AATAAGGCGCTCCGCCAATA	
siaA-qRT-F	CACTTCGGTTGCTGTGGTTG	
siaA-qRT-R	ATGGGTAAACCCACACGCTT	
shp-qRT-F	TGGAAGCTTTTCAGGCAGTTGA	
shp-qRT-R	TTCAACAAACATGCTGCCTCG	
shr-qRT-F	GAAAGCAACAAAGACGCGGT	
shr-qRT-R	ACAGTCGATGAGTATCGGCG	
ahpF-qRT-F	TTAATGGCCGAAGTGGAGGC	
ahpF-qRT-R	CCATTTGGCCCCAAGAGCTA	
gyrA-RT-F	GAAGTGATCCCCTGGACCTGA	
gyrA-RT-R	CCCGACCTGTTTGTAGTTGTT

Figure S1 - Viability of GAS strains following hydrogen peroxide challenge. Strains 5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC*::*mtsABC*, 5448Δ*pmtA*, 5448Δ*pmtA*::*pmtA*, 5448Δ*sodA* and 5448Δ*sodA*::*sodA* were grown in THY to mid exponential phase (OD₆₀₀ = 0.6) and challenged with either H₂O, 1 mM H₂O₂ or 4 mM H₂O₂ and cell viability assessed by plating on THY agar after 30 min challenge.

Figure S2 - Growth curve analysis of 5448 WT, 5448 Δ *mtsABC* and 5448 Δ *mtsABC::mtsABC* in the presence of Fe(II) or Zn(II).

Growth curve analysis of 5448 WT, 5448 Δ *mtsABC* and 5448 Δ *mtsABC::mtsABC* in the presence of Fe(II) or Zn(II). Overnight cultures of 5448 WT (black circles), 5448 Δ *mtsABC* (open circles) and 5448 Δ *mtsABC::mtsABC* (grey circles) were diluted to OD₆₀₀ = 0.05 into THY broth alone or THY supplemented with 20 μ M, 100 μ M, 0.5 mM or 1 mM Fe(II) or Zn(II). Growth was monitored at 37°C by optical density recording at 595 nm (OD₅₉₅). Graphs represent mean $\pm$ standard deviation of 3 independent biological replicates

Figure S3 - Gene expression analysis of MtsR-regulated genes in 5448 WT, 5448 Δ *mtsR* and 5448 Δ *mtsR*::*mtsR*

5448 WT, 5448 Δ *mtsR*, and 5448 Δ *mtsR*::*mtsR* were grown at 37°C to mid-exponential phase ($OD_{600} = 0.6-0.8$) in either THY alone, THY + 2 mM Fe(II) or 0.5 mM Mn(II) and gene expression of *shp* (A), *shr* (B), *siaA* (C), and *pmtA* (D) analysed by qPCR using *gyrA* as the reference gene. Data represents the mean $\pm$ standard deviation of 3 independent biological

replicates (2-way ANOVA used comparing all to control of that strain ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

Figure S4 - Gene expression analysis of PerR-regulated genes in 5448 WT, 5448Δ*perR* and 5448Δ*perR*::*perR* at various phases of growth

5448 WT, 5448Δ*perR*, and 5448Δ*perR*::*perR* were grown in THY at 37°C and harvested at OD₆₀₀ = 0.3 (early), OD₆₀₀ = 0.6 (mid), or OD₆₀₀ = 0.8 (late) -exponential phase. Gene expression of *pmtA* (A), *ahpC* (B) and *dpr* (C) was analysed by qPCR using *gyrA* as the reference gene. Data represents the mean ± standard deviation of 3 independent biological replicates (2-way ANOVA used comparing all to control of that strain * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Figure S4D in main manuscript.

Figure S5 - Gene expression analysis during hydrogen peroxide stress

5448 WT, 5448 Δ *mtsABC*, 5448 Δ *mtsABC::mtsABC*, 5448 Δ *pmtA* and 5448 Δ *pmtA::pmtA* were grown at 37°C to mid-exponential phase (OD_{600} = 0.6-0.8) in THY and challenged with either sterile H₂O, 1 mM H₂O₂ or 4 mM H₂O₂ and gene expression analysed by qPCR using *gyrA* as the reference gene. Data represents the mean $\pm$ standard deviation of 3 independent biological replicates. 2-way ANOVA used comparing strains to 5448 WT of that treatment (* P < 0.05, ** P < 0.001). Figure S5K in main manuscript.

Figure S6 – Gene expression timecourse analysis during hydrogen peroxide stress

5448 WT was grown at 37°C to mid-exponential phase ($OD_{600} = 0.6-0.8$) in THY and challenged with either sterile H₂O (control) (black bars) or 1 mM H₂O₂ (grey bars) and gene expression analysed at $t = 0, 5, 15, 30$ or 60 min by qPCR using *gyrA* as the reference gene. Data represents the mean $\pm$ standard deviation of 3 independent biological replicates. Figure S6D in main manuscript.

Figure S7 - Superoxide dismutase activity

5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC::mtsABC* and 5448Δ*sodA* (A) or 5448 WT, 5448Δ*pmtA*, 5448Δ*pmtA::pmtA* and 5448Δ*sodA* (D) were grown in THY at 37°C to mid-exponential phase (OD₆₀₀ 0.6 – 0.8), challenged with either H₂O, 1 mM H₂O₂ or 4 mM H₂O₂ cells harvested and SOD activity analysed. 5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC::mtsABC* and 5448Δ*sodA* were grown in THY, THY + 40 μM Fe(II), or THY + 40 μM Mn(II) to mid-exponential growth phase (OD₆₀₀ 0.6 – 0.8), cells harvested and SOD activity analysed (B).

5448 WT, 5448 Δ *pmtA*, 5448 Δ *pmtA::pmtA* and 5448 Δ *sodA* were grown in THY, THY + 2 mM Fe(II) or THY + 0.5 mM Mn(II) to mid-exponential growth phase (OD₆₀₀ 0.6 – 0.8), cells harvested and SOD activity analysed (C) (2-way ANOVA of strains compared to 5448 WT of that condition, * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$).

Figure S8 – SOD in-gel activity assay and coomassie gel

5448 WT, 5448Δ*pmtA* and 5448Δ*pmtA::pmtA* were grown in THY, THY + 2 mM Fe(II) or THY + 0.5 mM Mn(II) to mid-exponential growth phase (OD₆₀₀ 0.6 – 0.8), cells harvested and mechanically lysed. 40 µg protein was loaded into native gels and in-gel SOD activity analysed (top) or stained with coomassie for total protein (bottom).

Figure S9 – Gene expression analysis of *sodA*

Gene expression analysis of *sodA*. 5448 WT, 5448Δ*pmtA* and 5448Δ*pmtA*::*pmtA* were grown at 37°C to mid-exponential phase ($OD_{600} = 0.6-0.8$) in either THY alone, THY + 2 mM Fe(II) or 0.5 mM Mn(II) and gene expression of *sodA* analysed by qPCR using *gyrA* as the reference gene. Data represents the mean $\pm$ standard deviation of 3 independent biological replicates.

Figure S10 - Growth of strains in THY for streptonigrin control

5448 WT (black circles), 5448Δ*mtsR* (white circles), 5448Δ*mtsR*::*mtsR* (grey circles), 5448Δ*perR* (red circles) and 5448Δ*perR*::*perR* (blue circles) were diluted to OD₆₀₀ = 0.05 into THY broth. Growth was monitored at 37°C by optical density recording at 595 nm (OD₅₉₅). Graphs represent mean ± standard deviation of 3 independent biological replicates.

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