The DmsABC S-oxide reductase is an essential component of a new system of extracellular stress defence in *Haemophilus influenzae*

SUPPLEMENTARY DATA & FIGURES

Marufa Nasreen1, Daniel Ellis1, Jennifer Hosmer1, Ama-Tawiah Essilfie2, Emmanuelle Fantino3, Peter Sly3, Alastair G. McEwan1, Ulrike Kappler1

1School of Chemistry and Molecular Biosciences, Australian Infectious Diseases Research Centre, The University of Queensland, St. Lucia, Qld 4072, Australia

2QIMR Berghofer Medical Research Institute, 300 Herston Road, Herston QLD 4006, Australia

3Child Health Research Centre, 62 Graham St, South Brisbane QLD 410, Australia



**Figure S1:** Comparison of mouse lung infections with Hi2019WT and Hi2019D*dmsA*. **Panel A:** Bacteria colony forming unit (CFU) recovered from mouse Broncho-Alveolar Lavage Fluid (BALF). **Panels B-D:** relative expression of HIF1a, TGFB and BIRC3 in mouse lung tissue during infection with NTHi strains. qPCR data was normalized against expression of ACTB, cDNA was generated using random hexamers. **Panels E:** TNFa levels in mouse BALF determined by ELISA. **Panels F-H:** Neutrophil, macrophage and lymphocyte cell counts (Giemsa stain) in mouse BALF.

Statistical analyses: Panel A: multiple un-paired t-tests, \*\*\*\* p<0.0001; Other Panels: 2-Way ANOVA, Sidaks multiple comparison correction, \* p<0.05, \*\* p<0.01, \*\*\*\* p<0.0001

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**Figure S2:** Intracellular survival of Hi2019 (Panel A) and 86-028NP (Panel B) WT and D*dmsA* strains in bone marrow derived murine macrophages. Following infection (1h), treatment with polymyxin and use of a polymyxin maintenance dose was used to ensure that only intracellular bacteria were present in the assay.

Statistical analyses for intracellular CFU/mL: 2-Way ANOVA, Sidaks’ multiple comparison correction, \*\*\*\* p<0.0001.



**Figure S3:** Transepithelial resistance (TEER) of uninfected NHNE compared to NHNE infected with an equal mixture of Hi2019WT and Hi2019D*dmsA*.



**Figure S4:** Nitrosative stress resistance in *H. influenzae* wild-type and D*dmsA* strains. Experiments were carried out as described in the Methods section, data shown are averages of three technical replicates, the experiment was repeated twice on different days and a single, a representative dataset is shown.

Statistical analysis by 2-Way ANOVA with Tukey’s multi-comparison correction was carried out and revealed that comparisons between strains at a given treatment concentration returned non-significant changes.



**Figure S5:** S-oxide reductase activity in *H influenzae* sulfoxide reductase double mutant strains. Substrates used: Panel A: DMSO, Panel B: L-methionine (S/R) sulfoxide. Data shown are averages and standard deviation of at least three independent enzyme assays.

Statistical testing used 1-Way-ANOVA with Dunnet’s multicomparison correction. \*\*\*\* p<0.0001



**Figure S6:** Infection of 16HBE14 human bronchial epithelial tissue cells with *H. influenzae* Hi2019 S-oxide reductase double mutant strains for 4h or 24 h. Data shown are averages and standard deviation of three biological replicates.

Statistical analyses: 2-Way ANOVA, Sidaks’ multi-comparison correction, \* p<0.05, \*\*p<0.01, \*\*\*\* p<0.0001



**Figure S7:** Activity of purified *H. influenzae* MtsZ with Nicotinamide N-Oxide and Pyrimidine -N-oxide. Kinetic parameters: Nicotinamide-N-oxide: *K*M: 46±8 mM, *k*cat: 93±5 s-1; Pyrimidine-N-oxide: *K*M: 35±8 mM, *k*cat: 33.2±2.7 s-1

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

|  |  |  |
| --- | --- | --- |
| **Strain** | **Description** | **Source/Ref** |
| *Escherichia coli* DH5α | F– φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1hsdR17(rK–, mK+) phoA supE44λ– thi-1 gyrA96 relA1, cloningstrain | Life Technologies |
| *Haemophilus influenzae* 2019 | Clinical isolate from a chronicobstructive pulmonary diseasepatient.Sequence type 321 | (1) |
| *Haemophilus influenzae* 86-028NP | Clinical isolate from a patientwith otitis media.Sequence type 33 | (2) |
| Hi3 | Clinical isolate, UQ collection | This study |
| Hi2019*ΔdmsA* | Hi2019WT with *dmsA* genedisrupted by the insertion of akanamycin antibiotic resistancecassette(*dmsA*::*kan*) | (3) |
| Hi2019*ΔmsrAB* | Hi2019WT with *msrAB* genedisrupted by the insertion of akanamycin antibiotic resistancecassette(*msrAB::kan*) | (4) |
| Hi2019*ΔmtsZ* | Hi2019WT with *mtsZ* genedisrupted by the insertion of akanamycin antibiotic resistancecassette(*mtsZ::kan*) | (5) |
| 86028*ΔdmsA* | 86028WT with *dmsA* genedisrupted by the insertion of akanamycin antibiotic resistancecassette(*dmsA*:*:kan*) | This study |
| Hi2019*ΔdmsAΔmtsZ* | Hi2019WT with *dmsA* genedisrupted by the insertion of akanamycin antibiotic resistancecassette and with *mtsZ* gene disrupted by the insertion of a tetracycline antibiotic resistance cassette(*dmsA*::*kan* and *mtsZ*:*:tet*) | This study |
| Hi2019*ΔmsrABΔmtsZ* | Hi2019WT with *msrAB* genedisrupted by the insertion of akanamycin antibiotic resistancecassette and with *mtsZ* gene disrupted by the insertion of a tetracycline antibiotic resistance cassette(*msrAB*::*kan* and *mtsZ*::*tet*) | This study |
| Hi2019*ΔdmsAΔmsrAB* | Hi2019WT with *dmsA* genedisrupted by the insertion of akanamycin antibiotic resistancecassette and with *msrAB* gene disrupted by the insertion of a tetracycline antibiotic resistance cassette(*dmsA::kan* and *msrAB*::*tet*) | This study |
| *Actinobacillus pleuropneumoniae* (strain 4074)  | Clinical isolate from a swinewith respiratory disease | ATCC |
| **Plasmid**  | **Description** | **References or Source** |
| pUC4K | Cloning vector used to isolatethe kanamycin resistancecassette. Vector also containsampicillin resistance cassette | (6) |
| pRK415 | Cloning vector used to isolatethe tetracycline resistancecassette. | (7) |
| pGEM-T Easy | Cloning vector | Promega |
| pBluescript II SK+ | Cloning vector | Stratagene |
| pGEM-Hi*dmsA* | pGEM-T Easy derivativecontaining a 1000bp DNAfragment carrying the *dmsA*gene and flanking regions | (3) |
| pGEM-Hi*dmsA::kan* | pGEM-Hi*dmsA* with the dmsAgene disrupted by a kanamycinantibiotic resistance cassette | (3) |
| pGEM-Hi*mtsZ* | pGEM-T Easy derivativecontaining a 1000bp DNAfragment carrying the *mtsZ*gene and flanking regions | (5) |
| pGEM-Hi*mtsZ::kan* | pGEM-Hi*mtsZ* with the *mtsZ*gene disrupted by a kanamycinantibiotic resistance cassette | (5) |
| pBlue-Hi*msrAB* | pBluescript derivativecontaining a 1000bp DNAfragment carrying the *msrAB* gene and flanking regions | (4) |
| pBlue-Hi*msrAB::kan* | pBlue-Hi-msrAB with the *msrAB* gene disrupted by a kanamycin antibiotic resistance cassette | (4) |
| pGEM-Hi*mtsZ::tet*  | pGEM-Hi*mtsZ* with the *mtsZ*gene disrupted by a tetracyclineantibiotic resistance cassette | This study |
|  pBlu-Hi*msrAB::tet* | pGEM-Hi*msrAB* with the *msrAB* gene disrupted by a tetracyclineantibiotic resistance cassette | This study |

**Table S2: Oligonucleotide primers used in this study.**

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| --- |
| **Primer for pGEM-Hi*dmsA*::kan** |
| HI\_dmsA F | CTACAAACGTTCCACTTGAAC |
| HI\_dmsA R | ATGAGTAACTTTAATCAAATAAGT |
| pUC4K\_PCR F | GTTGGGTAACGCCAGGGTTTTCC |
| pUC4K\_PCR\_R | TCCGGCTCGTATGTTGTGTGGAA |
| **Primer for pGEM-Hi*mtsZ:*:tet** |
| torZ\_extF  | TTA CGC CAC CTG TTT AGG  |
| torZ\_intR  | AAA AGG ATC CGG GGC AAA AAC ATG GTT G  |
| torZ\_intF  | AAA AGG ATC CCG CTT TGC CTG ATG GAC T  |
| torZ\_extR  | ATG AAA AAG AAT AAC GTA AA  |
| pRKtetAR\_F\_Bam | AAAAGGATCCACGCTAGGGCAGGGCATGAAA |
| pRKtetAR\_R\_Bam | AAAAGGATCCGTCCTGCTCGTGATCGGGA |
| **Primer for pBlu-Hi*msrAB*:tet** |
| Hi\_msrA\_XbaI\_P1 F  | AAAATCTAGATGCAAAAGCGTTTAGGCTGAATGC |
| HI\_msrA\_BamHI\_B1\_R | AAAAGGATCCACTCGCCCAGCTTCAAACCAAATA |
| HI\_msrA\_BamHI\_B2\_F  | AAAAGGATCCGCTTGTCCGATCACCGCTTTATCT |
| HI\_msrA\_Pst\_P4\_R  | AAAACTGCAGGATGTGGGCGTTAAGGCTGGTTTA |
| **Primer for RT-PCR** |
| HI\_QP0\_dmsA\_R | CGAACCTGATGATCAAGATTATATG |
| HI\_QP0\_dmsA\_R | AGTAAACTGTGGTAGCCGTTG |
| HI\_QP0\_gyrA\_F | TTGGGCGTGCATTACCTGACGTT |
| HI\_QP0\_gyrA\_R | CCCACAACACGCGCTGATTTTAC |
| Ap Qp gyrA F | AACCGGATCGATATTGGCTAACGC |
| Ap Qp gyrA R | TTCGTCAAACACCGCCGTGAA |
| Ap Qp dmsA F | CCGATTTTAACCGGCAATGTAGGG |
| Ap Qp dmsA R | TGGGATACTTGCTTGCACCGGG |
| Mm-QP-ACTB-F | CTGCGTCTGGACCTGGC |
| Mm-QP-ACTB-R | CTTCTCTTTGATGTCACGCACGAT |
| Mm-QP-BIRC3-F | CTGTGTCAGAAAGGAGTCTGGCT |
| Mm-QP-BIRC3-R | CCATGGGACTGTCCCCTTG |
| Mm-QP-Hif1alpha-F | GCTGGCTCCCTATATCCCAATG |
| Mm-QP-Hif1alpha-R | TGCTGGAACCCAGTAACTGTGC |
| Mm-QP-IL1beta-F | GCTTCAAATCTCGCAGCAGC |
| Mm-QP-IL1beta-R | TCCTCATCCTGGAAGGTCCAC |
| Mm-QP-TNFalpha-F | TGAGCACTGAAAGCATGATCCG |
| Mm-QP-TNFalpha-R | CGATCAGGAAGGAGAAGAGGCTG |
| Mm-QP-IL6-F | GACTTCCATCCAGTTGCCTT |
| Mm-QP-IL6-R | GGTATAGACAGGTCTGTTGG |
| Mm-QP-TGFβ-F | AGAGAAGAACTGCTGTGTGCG |
| Mm-QP-TGFβ-R | ATATAGGGGCAGGGTCCCAG |

**Table S3:**  Growth rates of Hi2019 wildtype and S-/N-oxide reductase double mutant strains under aerobic, microaerobic and anaerobic conditions.

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| --- | --- |
|  | *Growth Rates [h-1]* |
| *Hi2019 strains* | *Aerobic* | *Microaerobic* | *Anaerobic* |
| wildtype | 0.480±0.098 | 0.549±0.045 | 0.397±0.073 |
| D*dmsA*D*msrAB* | 0.350±0.064 | 0.417±0.050 | 0.253±0.018 |
| D*dmsA*D*mtsZ* | 0.410±0.099 | 0.448±0.070 | 0.288±0.071 |
| D*msrAB*D*mtsZ* | 0.349±0.033 | 0.462±0.123 | 0.255±0.025 |

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**Table S2:** Oligonucleotide primers used in this study.

|  |
| --- |
| **Primer for pGEM-Hi*dmsA*::kan construction** |
| HI\_dmsA F | CTACAAACGTTCCACTTGAAC |
| HI\_dmsA R | ATGAGTAACTTTAATCAAATAAGT |
| pUC4K\_PCR F | GTTGGGTAACGCCAGGGTTTTCC |
| pUC4K\_PCR\_R | TCCGGCTCGTATGTTGTGTGGAA |
| **Primer for pGEM-Hi*mtsZ:*:tet construction** |
| torZ\_extF  | TTA CGC CAC CTG TTT AGG  |
| torZ\_intR  | AAA AGG ATC CGG GGC AAA AAC ATG GTT G  |
| torZ\_intF  | AAA AGG ATC CCG CTT TGC CTG ATG GAC T  |
| torZ\_extR  | ATG AAA AAG AAT AAC GTA AA  |
| pRKtetAR\_F\_Bam | AAAAGGATCCACGCTAGGGCAGGGCATGAAA |
| pRKtetAR\_R\_Bam | AAAAGGATCCGTCCTGCTCGTGATCGGGA |
| **Primer for pBlu-Hi*msrAB*:tet construction** |
| Hi\_msrA\_XbaI\_P1 F  | AAAATCTAGATGCAAAAGCGTTTAGGCTGAATGC |
| HI\_msrA\_BamHI\_B1\_R | AAAAGGATCCACTCGCCCAGCTTCAAACCAAATA |
| HI\_msrA\_BamHI\_B2\_F  | AAAAGGATCCGCTTGTCCGATCACCGCTTTATCT |
| HI\_msrA\_Pst\_P4\_R  | AAAACTGCAGGATGTGGGCGTTAAGGCTGGTTTA |
| **Primer for qRT-PCR** |
| HI\_QP0\_dmsA\_R | CGAACCTGATGATCAAGATTATATG |
| HI\_QP0\_dmsA\_R | AGTAAACTGTGGTAGCCGTTG |
| HI\_QP0\_gyrA\_F | TTGGGCGTGCATTACCTGACGTT |
| HI\_QP0\_gyrA\_R | CCCACAACACGCGCTGATTTTAC |
| Ap Qp gyrA F | AACCGGATCGATATTGGCTAACGC |
| Ap Qp gyrA R | TTCGTCAAACACCGCCGTGAA |
| Ap Qp dmsA F | CCGATTTTAACCGGCAATGTAGGG |
| Ap Qp dmsA R | TGGGATACTTGCTTGCACCGGG |
| Mm-QP-ACTB-F | CTGCGTCTGGACCTGGC |
| Mm-QP-ACTB-R | CTTCTCTTTGATGTCACGCACGAT |
| Mm-QP-BIRC3-F | CTGTGTCAGAAAGGAGTCTGGCT |
| Mm-QP-BIRC3-R | CCATGGGACTGTCCCCTTG |
| Mm-QP-Hif1alpha-F | GCTGGCTCCCTATATCCCAATG |
| Mm-QP-Hif1alpha-R | TGCTGGAACCCAGTAACTGTGC |
| Mm-QP-IL1beta-F | GCTTCAAATCTCGCAGCAGC |
| Mm-QP-IL1beta-R | TCCTCATCCTGGAAGGTCCAC |
| Mm-QP-TNFalpha-F | TGAGCACTGAAAGCATGATCCG |
| Mm-QP-TNFalpha-R | CGATCAGGAAGGAGAAGAGGCTG |
| Mm-QP-IL6-F | GACTTCCATCCAGTTGCCTT |
| Mm-QP-IL6-R | GGTATAGACAGGTCTGTTGG |
| Mm-QP-TGFβ-F | AGAGAAGAACTGCTGTGTGCG |
| Mm-QP-TGFβ-R | ATATAGGGGCAGGGTCCCAG |

**Table S3:** Growth rates of S-/N-oxide reductase double mutants Growth rates were derived from data collected on three biological replicates (see Figure 6, panels A-C).