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

SUPPLEMENTARY DATA & FIGURES

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**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 endA1  hsdR17(rK–, mK+) phoA supE44  λ– thi-1 gyrA96 relA1, cloning  strain | Life Technologies |
| *Haemophilus influenzae* 2019 | Clinical isolate from a chronic  obstructive pulmonary disease  patient.  Sequence type 321 | (1) |
| *Haemophilus influenzae* 86-  028NP | Clinical isolate from a patient  with otitis media.  Sequence type 33 | (2) |
| Hi3 | Clinical isolate, UQ collection | This study |
| Hi2019*ΔdmsA* | Hi2019WT with *dmsA* gene  disrupted by the insertion of a  kanamycin antibiotic resistance  cassette  (*dmsA*::*kan*) | (3) |
| Hi2019*ΔmsrAB* | Hi2019WT with *msrAB* gene  disrupted by the insertion of a  kanamycin antibiotic resistance  cassette  (*msrAB::kan*) | (4) |
| Hi2019*ΔmtsZ* | Hi2019WT with *mtsZ* gene  disrupted by the insertion of a  kanamycin antibiotic resistance  cassette  (*mtsZ::kan*) | (5) |
| 86028*ΔdmsA* | 86028WT with *dmsA* gene  disrupted by the insertion of a  kanamycin antibiotic resistance  cassette  (*dmsA*:*:kan*) | This study |
| Hi2019*ΔdmsAΔmtsZ* | Hi2019WT with *dmsA* gene  disrupted by the insertion of a  kanamycin antibiotic resistance  cassette 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* gene  disrupted by the insertion of a  kanamycin antibiotic resistance  cassette 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* gene  disrupted by the insertion of a  kanamycin antibiotic resistance  cassette 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 swine  with respiratory disease | ATCC |
| **Plasmid** | **Description** | **References or Source** |
| pUC4K | Cloning vector used to isolate  the kanamycin resistance  cassette. Vector also contains  ampicillin resistance cassette | (6) |
| pRK415 | Cloning vector used to isolate  the tetracycline resistance  cassette. | (7) |
| pGEM-T Easy | Cloning vector | Promega |
| pBluescript II SK+ | Cloning vector | Stratagene |
| pGEM-Hi*dmsA* | pGEM-T Easy derivative  containing a 1000bp DNA  fragment carrying the *dmsA*  gene and flanking regions | (3) |
| pGEM-Hi*dmsA::kan* | pGEM-Hi*dmsA* with the dmsA  gene disrupted by a kanamycin  antibiotic resistance cassette | (3) |
| pGEM-Hi*mtsZ* | pGEM-T Easy derivative  containing a 1000bp DNA  fragment carrying the *mtsZ*  gene and flanking regions | (5) |
| pGEM-Hi*mtsZ::kan* | pGEM-Hi*mtsZ* with the *mtsZ*  gene disrupted by a kanamycin  antibiotic resistance cassette | (5) |
| pBlue-Hi*msrAB* | pBluescript derivative  containing a 1000bp DNA  fragment 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 tetracycline  antibiotic resistance cassette | This study |
| pBlu-Hi*msrAB::tet* | pGEM-Hi*msrAB* with the *msrAB* gene disrupted by a tetracycline  antibiotic resistance cassette | This study |

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

|  |  |
| --- | --- |
| **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.

|  |  |  |  |
| --- | --- | --- | --- |
|  | *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).