

**Table S1.** Strains, plasmids and primers

| Strains primers or plasmids                                   | Relevant genotype, description or sequence   | Reference or source |
|---|--|---------------------|
| <b>Strains</b>  |  |                     |
| <i>E. coli</i> 10-beta  | $\Delta(ara-leu)$ 7697 <i>araD139 fhuA</i> $\Delta lacX74 galK16 galE15 e14-$ $\phi 80dlacZ\Delta M15$ <i>recA1 relA1 endA1 nupG rpsL</i> (Str <sup>R</sup> ) <i>rph spoT1</i> $\Delta(mrr-hsdRMS-mcrBC)$      | New England Biolabs |
| <i>E. coli</i> DH5 $\alpha$                                   | F <sup>-</sup> $\phi 80lacZ\Delta M15 \Delta(lacZYA-argF)U169$ <i>recA1 endA1 hsdR17</i> (r <sub>K</sub> <sup>-</sup> , m <sub>K</sub> <sup>+</sup> ) <i>phoA supE44</i> $\lambda$ - <i>thi-1 gyrA96 relA1</i> | New England Biolabs |
| <i>E. coli</i> BL21 (DE3)                                     | F- <i>ompT hsdS<sub>B</sub></i> (r <sub>B</sub> <sup>-</sup> , m <sub>B</sub> <sup>-</sup> ) <i>gal dcm</i> (DE3)  | Novagen             |
| <i>Hyphomicrobium denitrificans</i> $\Delta tsdA$             | Sm <sup>r</sup> , in-frame deletion of <i>tsdA</i> in <i>H. denitrificans</i> Sm200  | [1]                 |
| <i>Hyphomicrobium denitrificans</i> $\Delta tsdA \Delta shdR$ | Sm <sup>R</sup> , in-frame deletion of <i>shdR</i> (Hden_0682) in <i>H. denitrificans</i> $\Delta tsdA$  | [2]                 |
| <i>Hyphomicrobium denitrificans</i> $\Delta tsdA \Delta soxR$ | Sm <sup>R</sup> , deletion of <i>soxR</i> (Hden_0700) in <i>H. denitrificans</i> $\Delta tsdA$   | This work           |
| <b>Primers</b>  |  |                     |
| EMSA-Fr   | TTCCCGCCCCGCTTGGTTT  | [2]                 |
| EMSA_Fr2_Fr   | TCAGCGCTCGCCTGGAAGTC   | This work           |
| EMSA_Fr3_Rev  | TCTAAGCATCAACATATTCATATCTTTATATATTTTCG   | This work           |
| EMSA-Rev  | AGGAGTTGCATCCAAAAAAGCGTG   | [2]                 |
| EMSA-Hden_0703/04-fw  | GGGTCACCAAATTCTGCAGGTCTC   | This work           |
| EMSA-Hden_0703/04-rev   | ATCACGCCATCTCTCCCGGAA  | This work           |
| EMSA-Hden_0699/0698-fw  | AATTCCACGGCTCCGCC  | This work           |
| EMSA-Hden_0699/0698-rev                                       | TCGACAGCTTGCGGAAATCC   | This work           |
| EMSA-sHdrR-LipS1_F  | TAGAGCGAGTCTTCAGC  | This work           |
| EMSA-sHdrR-LipS1_R  | CGGCCCTCTGAGAAAAG  | This work           |
| EMSA-LipX-DsrE_F  | GACTTCGCCGATCAATCGATC  | This work           |
| EMSA-LipX-DsrE_R  | TGCCACCTCCCCGATATG   | This work           |
| rpoB-denitf   | AGGACGTGTTACCTCGATT  | [3]                 |
| rpoB-denitr   | CGGCTTCGTCAAGTTCTTC  | [3]                 |
| SoxT1A 0681_qPCR-Fr   | CCCAGTGATACGATTGCGA  | This work           |
| SoxT1A 0681_qPCR-Rev  | CTAAAATGCCGCCGGTGATG   | This work           |
| LplA_qPCR-Fr  | GGCCATGATCGATTTGCACC   | This work           |
| LplA_qPCR-Rev   | CGAGATAAATTGCACCGCCG   | This work           |
| sHdrA_qPCR-Fr   | CCGATCACCATTCCGTTCTGA  | This work           |
| sHdrA_qPCR-Rev  | CAATTGTTTCCGGGCCGATC   | This work           |

|   |  |           |
|---|--|-----------|
| sHdrB2_qPCR-Fr                                    | GACGTGGCCTACTATTCCGG   | This work |
| sHdrB2_qPCR-Rev                                   | CCGCGACGACAGATAGGTTT   | This work |
| LbpA2_qPCR-Fr                                     | GGTTCCAAGAGCAGCCTGAT   | This work |
| LbpA2_qPCR-Rev                                    | TCGTTGATCTCCAGAACCGC   | This work |
| SoxXA_qPCR-Fr                                     | CGGCGCTCATTACCTATCTC   | This work |
| SoxXA_qPCR-Rev                                    | TCGGGGTGTCTTTTTCAGTC   | This work |
| TusA_qPCR-Fr                                      | TCTGACAGTTGATGCCAAGG   | This work |
| TusA_qPCR-Rev                                     | CGTTTCCTCATGTTCAAGCA   | This work |
| CytP450_qPCR-Fr                                   | CAATACGGTTCTCGGACGTT   | This work |
| CytP450_qPCR-Rev                                  | CATTTCGTTTCCTGACGAGGT  | This work |
| SoxT1B (0699)_qPCR-Fr                             | GCCGCCGTCTCAGTAAATAA   | This work |
| SoxT1B (0699)_qPCR-Rev                            | AGCAGAAGACGGCAGATGAT   | This work |
| SoxR_qPCR-Fr                                      | TGAAGCGGACGAGGAAGTAT   | This work |
| SoxR_qPCR-Rev                                     | GAGACTGTGGGCTGGTTGAT   | This work |
| sHdrR_qPCR-Fr                                     | TTAGGAAGTCCGCATCGTCT   | This work |
| sHdrR_qPCR-Rev                                    | GCACTCGTTGCGCAATAATA   | This work |
| SoxY_qPCR-Fr                                      | GTTCAGCTTGCGGACTTTTC   | This work |
| SoxY_qPCR-Rev                                     | GCCAATCGTCACCTTCACTT   | This work |
| P1 fwd up hden_0700                               | TATA <b>CTGCAGG</b> ATCAAGGACGTGGTGGCG (PstI)  | This work |
| P2 rev up hden_0700                               | CTCTCTATCGTTTGCGGCTCCATTCCCTATCCCTCGGTCGC  | This work |
| P3 fwd down hden_0700                             | GCGCACCGAGGGATAGGAATGGAGCCGCAAACGATAGAGAG  | This work |
| P4 rev down hden_0700                             | GTACT <b>CTAGA</b> ACGAACGCTGCCAGAAGCCC (XbaI)   | This work |
| pET22 SoxR-Strep fw                               | TATA <b>CATATG</b> TGGAGCCACCCGCAGTTTCGAGAAAGCTAGCTCGGGCATCTTGCCAAAC (NdeI)                            | This work |
| pET22 SoxR-Strep rev                              | TGCT <b>AAGCTT</b> CTATCGTTTGCGGCTCGGTT (HindIII)  | This work |
| SoxR C(50)S_fwd                                   | CTGATCCTCTCCCTGCTCGCTG   | This work |
| SoxR C(50)S_rev                                   | CAGGCGGGATTCTGTGAGC  | This work |
| SoxR C(116)S_fwd                                  | GATAAGTTTTCCCGCGAGGAAC   | This work |
| SoxR C(116)S_rev                                  | GTAGATGGCGCCGATGAA   | This work |
| <b>Plasmids</b>                                   |  |           |
| pET-22b(+)  | Ap <sup>r</sup>  | Novagen   |
| pET-22b-SoxR-N-Strep                              | Ap <sup>r</sup> , NdeI/HindIII fragment of amplified SoxR in Nde/HindIII of pET                        | This work |
| pET-22b-SoxR C <sup>50</sup> S                    | Ap <sup>r</sup> , pET-22b-SoxR-N-Strep with a Cys <sup>50</sup> Ser exchange                           | This work |
| pET-22b-SoxR C <sup>116</sup> S                   | Ap <sup>r</sup> , pET-22b-SoxR-N-Strep with a Cys <sup>116</sup> Ser exchange                          | This work |
| pET-22b-SoxR C <sup>50</sup> S C <sup>116</sup> S | Ap <sup>r</sup> , pET-22b-SoxR-N-Strep with Cys <sup>50</sup> Ser and Cys <sup>116</sup> Ser exchanges | This work |

|                                       |   |           |
|---------------------------------------|---|-----------|
| pk18 <i>mobsacB</i> -Tc               | Km <sup>r</sup> , Tc <sup>r</sup> pHP45ΩTc tetracycline cassette inserted into pk18 <i>mobsacB</i> using SmaI   | [2]       |
| pk18 <i>mobsacB</i> _Tc_Δ <i>soxR</i> | Km <sup>r</sup> , Tc <sup>r</sup> , 1.04 kb SOE PCR fragment implementing deletion of nucleotides 4 to 362 of <i>soxR</i> cloned into pk18 <i>mobsacB</i> -Tc using PstI and XbaI restriction sites | This work |

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## References

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2. Li, J.; Koch, J.; Flegler, W.; Garcia Ruiz, L.; Hager, N.; Ballas, A.; Tanabe, T.S.; Dahl, C. A metabolic puzzle: consumption of C<sub>1</sub> compounds and thiosulfate in *Hyphomicrobium denitrificans* X<sup>T</sup>. *Biochim. Biophys. Acta - Bioenergetics* **2022**, *1864*, 148932.
3. Martineau, C.; Mauffrey, F.; Villemur, R. Comparative analysis of denitrifying activities of *Hyphomicrobium nitratorans*, *Hyphomicrobium denitrificans*, and *Hyphomicrobium zavarzinii*. *Appl. Environ. Microbiol.* **2015**, *81*, 5003-5014.