**Supplementary Information**

A comparative proteomic analysis of *Desulfovibrio vulgaris* Hildenborough in response to the antimicrobial agent free nitrous acid

Shu-Hong Gao1,5, Jun Yuan Ho1, Lu Fan2, Amanda Nouwens3, Robert D. Hoelzle1,4, Benjamin Schulz3, Jianhua Guo1, Jizhong Zhou5, Zhiguo Yuan1 and Philip L Bond1\*

1Advanced Water Management Centre, The University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia

2 Department of Ocean Science and Engineering, Southern University of Science and Technology, Shenzhen, 518055, China

3 School of Chemistry & Molecular Biosciences, The University of Queensland, St Lucia, Brisbane, QLD 4072, Australia

4 Australian Centre for Ecogenomics, The University of Queensland, St. Lucia, Brisbane, Qld 4072, Australia

5 Institute for Environmental Genomics, Department of Microbiology and Plant Biology, University of Oklahoma, Norman, Oklahoma 73019, USA

\*Corresponding author

Phil L Bond, Level 4 Gehrmann Building, Advanced Water Management Centre, The University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia

Phone: + 61 7 3346 3226

FAX: + 61 7 3365 4726

E-mail: phil.bond@awmc.uq.edu.au

**Material and Methods**

**Culture growth and FNA treatment on *D. vulgaris***

Professor Jizhong Zhou and Dr Aifen Zhou from Institute for Environmental Genomics, University of Oklahoma, offered strain *D. vulgaris* Hildenborough (ATCC 29579, NCBI accession number NC\_002937.3). A defined lactate sulfate medium (LS4D medium) was used to cultivate the culture for all the performed experiments as previously described (Zhou et al., 2012; Zhou et al., 2013). 140 ml LS4D medium was added to the 160 ml serum bottles and then flushed with nitrogen gas for 30 min before capping with butyl rubber stopper for sterilization. Before each inoculation, 0.7 mL titanium-citrate was added to each serum bottle to remove any potential oxygen.1.5 ml culture glycerol stock was inoculated to one serum bottle before incubation at 37°C for 48 h to achieve the early stationary phase of growth (optical density at 600 nm [OD600] 0.9 to 1.0). OD600 of the culturewas adjusted to 0.5 and 10 ml of this culture (standard inoculum) was transferred into 140 ml of autoclaved anaerobic LS4D medium in serum bottles inside the anaerobic chamber. The cultures were further incubated at 30°C without shaking for further use of experiments described below.

We applied different concentrations of nitrite to the early log phase culture (after 26h incubation, OD600 around 0.3) at 30°C to obtain the starting FNA concentration of 0, 1.0, 4.0, and 8.0 μg N/L. The experiment was performed in triplicate for each FNA concentration. FNA levels are determined by measured nitrite concentration, pH and temperature and was calculated based on the equation described before (Anthonisen et al., 1976; Gao et al., 2015) . The cultures were incubated for a further 12 h (a total of 38 h incubation) to conduct proteomics study. Samples from triplicated controls (without FNA addition) and FNA-treated cultures (1.0, 4.0, and 8.0 μg N/L) were taken for total protein extraction at 2, 8, and 12 h after FNA addition. Culture optical density at 600nm (OD600) was monitored with a Cary 50 Bio UV-visible spectro-photometer (Varian, Australia), and pH was determined by a labCHEM-pH benchtop pH-mV temperature meter. Cell viability test with and without FNA was performed as described in the manufacturer’s instruction (Molecular Probes, BacLight bacterial viability kit, catalog no. L7012). The stained cells were then quantified using the FACSAria™ II (BD Biosciences, San Jose, USA) type flow cytometer.

**Protein extraction, quantification, and digestion**

For total protein extraction, 10 ml of the bacterial suspension was centrifuged at 15,000 × g for 5 minutes, supernatant was discarded and pellets were immediately frozen in liquid nitrogen before storing at -80ºC. Protein extractions were performed on these cell pellets. Each pellet was dissolved in 1 ml extraction buffer (77 mg dithiothreitol & 1 tablet of complete Protease Inhibitor (Roche, catalog no.11836153001) in 10 ml B-PER II Bacterial Protein Extraction Reagent (Thermo Scientific, catalog no. 78260) and subjected to three freeze/thaw cycles by placing into liquid nitrogen and thawing at 4 ˚C. We removed the cell debris by centrifuging the pellets at 15,000 × g for 15 min at 4 ˚C. From the supernatant, proteins were precipitated by adding trichloroacetic acid (13% v/v final concentration) (Sigma, catalog no T6399) and incubated overnight at 4 ˚C. Precipitated proteins were collected by centrifugation at 15,000 × g for 15 min at 4 ˚C before washing in cold 80% acetone twice, dried for no more than 5 minutes and resuspended in buffer (8 M urea, 50 mM ammonium bicarbonate). Total resuspended protein was quantified by the 2D Quant kit (GE Healthcare, catalog no GE806483-56).

The protein samples were then reduced and alkylated by adding dithiothreitol (5 mM final concentration) for 30 min at 56 ˚C, cooled to room temperature, then treated with iodoacetamide (25 mM final concentration) and incubated at room temperate in the dark for 30 min. The same volume of dithiolthreitol was added after iodoacetamide alkylation to quench the excess iodoacetamide. Protein samples were diluted with 50 mM ammonium bicarbonate buffer to reduce urea concentration to less than 2 M, and digested for 6 hours with trypsin (Promega, catalogue number V5111) at an enzyme to protein ratio of 1:100 at 37˚C. Following this, the protein samples were digested overnight again with additional trypsin using the same enzyme to protein ratio at 37˚C. C18 Zip-tip (Millipore, ZTC18S096) clean-up was performed on the digested proteins (Kappler・Nouwens AS, 2013). For each sample, 1 µg of the digested protein was used for subsequent SWATH-MS analysis in triplicate. In addition, 5 μg aliquots of each triplicate samples were taken and pooled for mass spectrometry analysis and information dependent analysis (IDA) library construction, which was performed in duplicate.

**Table S1. pH during the 48 h incubation in the presence of different FNA concentrations.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours | Control |  | 1.0 μg N/L | 4.0 μg N/L | 8.0 μg N/L |
|  | Mean | STDEV | Mean | STDEV | Mean | STDEV | Mean | STDEV |
| 0 | 7.18 | 0.02 | 7.18 | 0.02 | 7.16 | 0.02 | 7.18 | 0.05 |
| 1 | 7.21 | 0.03 | 7.20 | 0.04 | 7.24 | 0.04 | 7.25 | 0.04 |
| 2 | 7.22 | 0.02 | 7.23 | 0.03 | 7.21 | 0.02 | 7.23 | 0.03 |
| 7 | 7.21 | 0.03 | 7.24 | 0.02 | 7.20 | 0.04 | 7.25 | 0.03 |
| 12 | 7.16 | 0.02 | 7.18 | 0.07 | 7.19 | 0.05 | 7.25 | 0.06 |
| 24 | 7.29 | 0.05 | 7.30 | 0.01 | 7.30 | 0.02 | 7.27 | 0.03 |
| 48 | 7.35 | 0.06 | 7.32 | 0.03 | 7.28 | 0.05 | 7.27 | 0.04 |

**Table S2. Protein abundance dynamics, expressed as log2 fold changes in the presence of different FNA concentrations relevant to metabolism**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene ID | Gene name | 1 μg vs Control | 4 μg vs Control | 8 μg vs Control |
| 2h\_LFC | 8h\_LFC | 12h\_LFC | 2h\_LFC | 8h\_LFC | 12h\_LFC | 2h\_LFC | 8h\_LFC | 12h\_LFC |
| **Nitrite reduction** |  |  |  |  |  |  |  |  |  |
| DVU0625 | Cytochrome c-552 nitrite reductase | 0.39 | 0.99 | 0.68 | 0.09 | -0.22 | -0.37 | 0.14 | -0.14 | -0.29 |
| DVU2543 | Hybrid cluster protein | -0.22 | 5.37 | 4.05 | 2.94 | 4.51 | 3.81 | 1.73 | 3.72 | 3.09 |
| DVU2544 | Iron-sulfur cluster-binding protein | -0.23 | 4.60 | 3.93 | 2.43 | 3.20 | 3.66 | 1.35 | 1.93 | 2.61 |
| **Sulfate reduction** |  |  |  |  |  |  |  |  |  |
| DVU0263 | Acidic cytochrome c3 | 0.84 | 0.58 | 0.28 | -0.03 | 0.23 | -0.45 | 0.23 | -0.14 | -0.90 |
| DVU0266 | Uncharacterized protein | 0.81 | -0.21 | -0.30 | -0.03 | -0.50 | -0.68 | 0.07 | -0.38 | -0.70 |
| DVU0402 | Sulfite reductase, dissimilatory-type subunit alpha | 0.56 | 0.27 | 0.04 | -0.04 | 0.19 | 0.14 | -0.08 | 0.07 | 0.16 |
| DVU0403 | Sulfite reductase, dissimilatory-type subunit beta | 0.56 | 0.02 | 0.03 | 0.05 | -0.11 | 0.01 | 0.02 | -0.05 | -0.16 |
| DVU0847 | Adenylylsulphate reductase beta-subunit | 0.21 | 0.27 | -0.03 | 0.31 | 0.89 | 0.41 | 0.27 | 0.58 | 0.46 |
| DVU0846 | Adenylyl-sulphate reductase, alpha subunit | 0.21 | 0.17 | 0.15 | 0.00 | 0.55 | 0.50 | -0.05 | 0.39 | 0.49 |
| DVU0848 | Heterodisulfide reductase, putative | 0.21 | -0.09 | 0.01 | -0.12 | 0.13 | 0.22 | 0.01 | 0.13 | 0.20 |
| DVU0849 | Heterodisulfide reductase, iron-sulfur-binding subunit, putative | 0.21 | -0.13 | 0.04 | 0.25 | 0.24 | 0.51 | 0.11 | -0.06 | 0.55 |
| DVU0850 | Heterodisulfide reductase, transmembrane subunit, putative | 0.20 | -0.35 | 0.10 | -0.36 | -1.65 | -0.54 | 0.34 | -0.80 | -0.98 |
| DVU1287 | Reductase, iron-sulfur binding subunit, putative | 0.09 | 0.22 | -1.61 | -0.56 | NA | 0.31 | -0.73 | -0.21 | 0.78 |
| DVU1295 | Sulfate adenylyltransferase | 0.09 | 0.20 | 0.11 | -0.09 | 0.44 | 0.63 | -0.23 | 0.38 | 0.55 |
| DVU1597 | Sulfite reductase, assimilatory-type | 0.00 | 0.12 | -0.05 | -0.19 | 0.00 | 0.06 | 0.10 | -0.14 | -0.28 |
| **Lactate oxidation** |  |  |  |  |  |  |  |  |  |
| DVU0448 | GDP-mannose 4,6-dehydratase | 0.53 | -0.25 | -0.09 | -0.20 | -0.62 | -0.41 | -0.37 | -0.31 | -0.08 |
| DVU0565 | Glyceraldehyde-3-phosphate dehydrogenase | 0.43 | -0.27 | -0.22 | 0.14 | -0.78 | -1.06 | 0.34 | -0.55 | -0.73 |
| DVU0587 | Formate dehydrogenase, alpha subunit, selenocysteine-containing | 0.42 | -0.13 | 0.27 | 0.21 | -0.26 | -0.08 | 0.47 | 0.05 | 0.22 |
| DVU0588 | Formate dehydrogenase, beta subunit, putative | 0.41 | 0.08 | 0.25 | 0.19 | -0.19 | -0.09 | 0.32 | -0.08 | -0.07 |
| DVU0589 | Molybdopterin-guanine dinucleotide biosynthesis protein B, putative | 0.41 | -0.41 | -0.19 | -0.66 | -1.62 | -0.96 | -0.14 | -1.10 | -1.24 |
| DVU1569 | Pyruvate ferredoxin oxidoreductase, alpha subunit | 0.01 | -0.28 | 0.03 | 0.15 | -1.34 | -0.53 | 0.34 | -0.57 | -0.67 |
| DVU1570 | Pyruvate ferredoxin oxidoreductase, beta subunit | 0.01 | 0.11 | 0.08 | 0.15 | -0.79 | -0.89 | 0.22 | -0.63 | -0.77 |
| DVU2201 | Alcohol dehydrogenase, iron-containing | -0.10 | -0.18 | -0.21 | 0.30 | -0.53 | -0.67 | 0.51 | -0.44 | -0.78 |
| DVU2451 | L-lactate permease family protein | -0.17 | 0.05 | -0.67 | -0.01 | 0.02 | -0.63 | -0.06 | -0.01 | -0.45 |
| DVU2482 | Formate dehydrogenase, alpha subunit, selenocysteine-containing | -0.18 | -0.06 | -0.11 | 0.34 | -0.52 | -0.64 | 0.44 | -0.31 | -0.43 |
| DVU3026 | L-lactate permease family protein | -0.40 | 0.45 | -0.10 | -0.03 | 0.18 | 0.36 | 0.24 | -0.26 | 0.26 |
| DVU3028 | Glycolate oxidase iron-sulfur subunit | -0.41 | -0.43 | -0.24 | 0.43 | -0.42 | -0.17 | 1.21 | -0.49 | -0.23 |
| DVU3027 | Glycolate oxidase, subunit GlcD | -0.41 | -0.03 | -0.10 | 0.24 | -0.04 | -0.05 | -0.76 | -0.43 | -0.22 |
| DVU3025 | Pyruvate-ferredoxin oxidoreductase | -0.40 | 0.06 | -0.11 | 0.11 | 0.12 | -0.16 | 0.17 | 0.17 | -0.18 |
| DVU3029 | Phosphate acetyltransferase | -0.41 | 0.22 | -0.67 | 0.35 | 0.34 | 0.02 | 0.69 | 0.49 | 1.16 |
| DVU3030 | Acetate kinase | -0.42 | -0.01 | -0.13 | -0.03 | 0.09 | 0.13 | -0.15 | 0.10 | 0.26 |
| DVU3032 | Uncharacterized protein | -0.42 | 0.24 | 0.07 | 0.24 | 0.64 | 0.46 | 0.19 | 0.64 | 0.70 |
| DVU3033 | Iron-sulfur cluster-binding protein | -0.46 | 0.21 | 0.14 | 0.64 | 0.87 | 0.86 | 0.79 | 0.75 | 0.80 |
| DVU3348 | 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase, subunit beta | -1.37 | -0.24 | -0.23 | -0.18 | -2.14 | -0.72 | -0.08 | -0.74 | -0.38 |
| DVU3349 | Pyruvate flavodoxin/ferredoxin oxidoreductase, thiamine diP-binding domain protein | -1.46 | -0.26 | -0.25 | -0.34 | -0.57 | -0.30 | -0.23 | -0.43 | -0.20 |
| **ATP synthesis** |  |  |  |  |  |  |  |  |  |
| DVU0777 | ATP synthase subunit alpha | 0.27 | 0.10 | 0.13 | 0.16 | 0.37 | 0.44 | 0.01 | 0.41 | 0.46 |
| DVU0774 | ATP synthase epsilon chain | 0.28 | -0.75 | -1.38 | -1.06 | -0.40 | -1.22 | -0.65 | -0.01 | 0.46 |
| DVU0775 | ATP synthase subunit beta | 0.28 | 0.14 | 0.13 | 0.37 | 0.50 | 0.51 | 0.20 | 0.50 | 0.57 |
| DVU0776 | ATP synthase gamma chain | 0.28 | -0.14 | 0.14 | 0.28 | -0.41 | -0.18 | 0.37 | -0.21 | 0.01 |
| **Electron transfer** |  |  |  |  |  |  |  |  |  |
| DVU0431 | Ech hydrogenase, subunit EchD, putative | 0.53 | -0.37 | -0.57 | 0.06 | -0.87 | NA | -0.27 | -0.03 | 0.14 |
| DVU0535 | Protein DVU0535 (HMC operon ORF 2) | 0.43 | 0.31 | -0.20 | 0.93 | 0.85 | 1.68 | 0.22 | 0.97 | 2.30 |
| DVU0536 | High-molecular-weight cytochrome c | 0.43 | 0.27 | 0.30 | -0.42 | -0.40 | 0.04 | -0.80 | -0.93 | -0.99 |
| DVU2792 | Electron transport complex protein RnfC, putative | -0.27 | -0.68 | -0.23 | -0.16 | -1.02 | -1.10 | -0.61 | -1.25 | -0.70 |
| **Hydrogenases** |  |  |  |  |  |  |  |  |  |
| DVU1769 | Periplasmic [Fe] hydrogenase large subunit | -0.02 | 0.09 | -0.05 | 0.11 | 0.71 | -0.01 | 0.28 | -0.43 | 0.11 |
| DVU1917 | Periplasmic [NiFeSe] hydrogenase, small subunit | -0.05 | 0.13 | -0.05 | 0.11 | -0.08 | -0.16 | 0.04 | 0.07 | -0.11 |
| DVU1918 | Periplasmic [NiFeSe] hydrogenase, large subunit, selenocysteine-containing | -0.05 | 0.23 | 0.29 | -0.01 | 0.05 | 0.19 | 0.00 | 0.06 | 0.03 |
| DVU1921 | Periplasmic [NiFe] hydrogenase small subunit 1 | -0.06 | 0.08 | -0.62 | NA | 0.27 | -0.16 | NA | NA | NA |
| DUV1922 | Periplasmic [NiFe] hydrogenase, large subunit, isozyme 1 | -0.06 | 0.43 | 0.05 | -0.53 | 0.28 | -0.40 | -1.54 | -1.49 | -1.58 |
| DVU2329 | Hydrogenase accessory protein HypB | -0.13 | -0.30 | -0.30 | -0.22 | -0.40 | -0.57 | -0.64 | -0.28 | -0.34 |

Note: The detected proteins with LFC≥0.5 or ≤-0.5 and adjusted q value less than 0.05 are highlighted in red and defined as the changed proteins in response to FNA. All the other proteins with q value larger than 0.05 regardless of LFC are defined as no changed proteins.

**Table S3. Proteins abundance dynamics see above in the presence of different FNA concentrations relevant to protein synthesis and amino acid metabolism**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene ID | Gene name | 1 μg vs Control | 4 μg vs Control | 8 μg vs Control |
| 2h\_LFC | 8h\_LFC | 12h\_LFC | 2h\_LFC | 8h\_LFC | 12h\_LFC | 2h\_LFC | 8h\_LFC | 12h\_LFC |
| DVU0504 | 30S ribosomal protein S15 | 0.44 | -2.00 | 2.34 | -0.10 | -0.08 | 2.35 | -0.15 | 0.40 | 2.78 |
| DVU0835 | 50S ribosomal protein L19 | 0.22 | -0.44 | -1.08 | 0.03 | -1.62 | -1.77 | 0.16 | -0.67 | -0.89 |
| DVU0837 | Ribosome maturation factor RimM | 0.22 | 0.57 | -0.05 | -0.24 | 1.09 | 0.51 | -0.48 | 0.86 | 0.20 |
| DVU0839 | 30S ribosomal protein S16 | 0.22 | 0.08 | 0.01 | -0.30 | 0.06 | -0.56 | -0.16 | 0.00 | 0.03 |
| DVU0870 | Ribosome-recycling factor (RRF)  | 0.19 | -0.02 | 0.10 | -0.62 | 0.76 | -0.17 | -0.20 | 1.00 | 0.46 |
| DVU0874 | 30S ribosomal protein S2 | 0.19 | -0.25 | 0.46 | 0.99 | 0.32 | 1.25 | 0.67 | 0.08 | 1.42 |
| DVU0927 | 50S ribosomal protein L21 | 0.18 | 0.98 | 0.25 | 0.11 | 1.12 | 0.86 | 0.42 | 1.11 | 1.18 |
| DVU0956 | 30S ribosomal protein S6 | 0.17 | 0.15 | 0.09 | -0.43 | 0.81 | 0.93 | 0.75 | 0.65 | 1.32 |
| DVU0957 | 30S ribosomal protein S18 | 0.17 | 0.13 | -0.30 | 0.54 | 1.00 | 0.49 | 0.25 | 0.83 | 0.85 |
| DVU0958 | 50S ribosomal protein L9 | 0.17 | 0.51 | -0.17 | 0.07 | 1.39 | 0.80 | 1.08 | 0.75 | 0.86 |
| DVU1209 | 50S ribosomal protein L32 | 0.12 | 1.13 | 0.80 | -0.71 | 2.02 | 2.33 | 0.37 | 1.57 | 2.42 |
| DVU1211 | 50S ribosomal protein L28 | 0.12 | 0.87 | 1.05 | 0.70 | 0.81 | 1.04 | 0.93 | 0.45 | 0.72 |
| DVU1298 | 30S ribosomal protein S12 | 0.09 | -0.17 | -0.74 | -0.61 | -0.41 | 0.06 | 0.26 | -0.31 | -0.29 |
| DVU1299 | 30S ribosomal protein S7 | 0.09 | -0.35 | 0.04 | 0.04 | -0.90 | 0.18 | 0.36 | -0.26 | -0.08 |
| DVU1302 | 30S ribosomal protein S10 | 0.08 | 0.17 | 0.68 | 0.05 | -0.24 | 0.54 | 0.14 | 0.31 | 0.57 |
| DVU1303 | 50S ribosomal protein L3 | 0.08 | 0.42 | 0.28 | -0.03 | 0.30 | 0.67 | -0.06 | -0.04 | 0.57 |
| DVU1304 | 50S ribosomal protein L4 | 0.08 | -0.25 | 0.78 | -0.98 | -0.58 | 0.23 | 0.39 | 0.33 | 1.86 |
| DVU1305 | 50S ribosomal protein L23 | 0.08 | -0.25 | 0.60 | 0.11 | -0.73 | 1.17 | 1.60 | 0.24 | 0.78 |
| DVU1306 | 50S ribosomal protein L2 | 0.08 | 0.28 | 0.17 | 0.12 | 0.23 | 0.65 | 0.24 | 0.28 | 0.61 |
| DVU1307 | 30S ribosomal protein S19 | 0.08 | 0.15 | -0.05 | 0.09 | -0.27 | -0.17 | 0.13 | -0.17 | -0.27 |
| DVU1308 | 50S ribosomal protein L22 | 0.08 | 1.32 | 0.26 | -0.32 | 0.35 | 0.50 | -0.19 | 0.04 | 1.01 |
| DVU1309 | 30S ribosomal protein S3 | 0.07 | 0.97 | 0.53 | -0.03 | 1.88 | 1.33 | -2.14 | 1.28 | 1.54 |
| DVU1310 | 50S ribosomal protein L16 | 0.07 | -0.42 | -0.36 | -1.24 | 0.35 | 1.02 | -0.07 | -0.23 | 1.94 |
| DVU1312 | 30S ribosomal protein S17 | 0.07 | 0.50 | -1.40 | -0.08 | -1.77 | -0.13 | 0.23 | -0.40 | -0.05 |
| DVU1313 | 50S ribosomal protein L14 | 0.07 | 0.17 | 0.30 | -0.43 | 0.37 | -0.05 | 0.47 | 0.18 | 0.01 |
| DVU1315 | 50S ribosomal protein L5 | 0.07 | -0.18 | -0.48 | -0.94 | 0.95 | 0.49 | 1.69 | 0.73 | 1.11 |
| DVU1317 | 30S ribosomal protein S8 | 0.07 | 0.33 | 0.16 | -0.17 | 0.65 | 0.82 | 0.41 | 0.99 | 0.67 |
| DVU1318 | 50S ribosomal protein L6 | 0.07 | 0.72 | 1.71 | -0.49 | 0.29 | 0.53 | 0.05 | -1.21 | 0.89 |
| DVU1319 | 50S ribosomal protein L18 | 0.06 | 0.26 | 0.12 | -0.28 | 0.20 | 1.10 | 0.25 | 0.43 | 1.05 |
| DVU1320 | 30S ribosomal protein S5 | 0.06 | -0.31 | 0.53 | -1.02 | -0.40 | 0.03 | -0.81 | -0.05 | 0.20 |
| DVU1322 | 50S ribosomal protein L15 | 0.06 | 0.57 | 0.22 | -0.20 | 0.67 | 0.75 | -0.12 | 0.45 | 0.74 |
| DVU1326  | 30S ribosomal protein S13 | 0.06 | 0.22 | -0.16 | 0.16 | 0.04 | 0.00 | 0.42 | 0.19 | 0.08 |
| DVU1327 | 30S ribosomal protein S11 | 0.06 | 0.68 | 0.73 | -0.20 | 0.02 | 0.84 | 0.35 | -0.38 | 0.30 |
| DVU1328 | 30S ribosomal protein S4 | 0.06 | 0.35 | 0.10 | -0.47 | 0.83 | 0.98 | 0.50 | 0.90 | 1.07 |
| DVU1330 | 50S ribosomal protein L17 | 0.05 | 0.25 | 0.13 | -0.01 | 0.34 | 0.88 | 0.60 | 0.40 | 1.18 |
| DVU1429 | Ribosome-binding ATPase YchF | 0.03 | 0.74 | 0.04 | 0.08 | 0.76 | 0.53 | 0.37 | -0.50 | 0.22 |
| DVU1469 | Ribosomal protein S1, putative | 0.02 | 0.08 | -0.03 | 0.11 | -0.19 | -0.45 | -0.01 | -0.17 | -0.46 |
| DVU1574 | 50S ribosomal protein L25  | 0.01 | -0.14 | 0.21 | 0.00 | 0.28 | 0.10 | 0.27 | -0.88 | 0.16 |
| DVU1618 | Ribosomal silencing factor RsfS | 0.00 | 0.13 | -0.82 | -1.11 | -0.08 | 0.14 | -0.72 | 0.41 | 0.98 |
| DVU1792 | 30S ribosomal protein S21 | -0.02 | 1.27 | 0.19 | 0.76 | 1.35 | -0.76 | 1.89 | -0.03 | 1.02 |
| DVU1896 | 30S ribosomal protein S20 | -0.04 | 0.74 | 0.96 | -0.34 | 0.51 | 1.11 | -0.10 | 0.43 | 1.27 |
| DVU2339 | Ribosomal protein L11 methyltransferase, putative | -0.14 | -0.29 | -0.19 | 0.69 | 0.15 | 0.50 | 0.35 | -0.18 | 0.59 |
| DVU2512 | Ribosomal RNA small subunit methyltransferase H  | -0.20 | NA | -0.37 | 2.53 | NA | 0.41 | 1.80 | NA | 0.03 |
| DVU2518 | 50S ribosomal protein L13 | -0.20 | 1.43 | 0.32 | -0.27 | 1.17 | 0.59 | 0.51 | 1.61 | 0.81 |
| DVU2519 | 30S ribosomal protein S9 | -0.20 | 0.11 | -0.02 | -0.10 | -0.07 | 0.23 | 0.01 | 0.07 | 0.24 |
| DVU2535 | 50S ribosomal protein L20 | -0.21 | 0.55 | -0.20 | 0.51 | -1.12 | -1.03 | 0.27 | -1.18 | -0.37 |
| DVU2536 | 50S ribosomal protein L35 | -0.21 | -0.51 | -1.00 | -0.69 | -0.62 | -0.03 | -0.24 | -0.20 | 0.41 |
| DVU2921 | 50S ribosomal protein L33 | -0.31 | 0.13 | -2.32 | -1.30 | 0.33 | -0.01 | 0.77 | -0.23 | -0.65 |
| DVU2924 | 50S ribosomal protein L11 | -0.32 | 0.85 | -0.63 | 0.04 | 0.45 | 0.71 | 0.36 | 0.46 | 0.56 |
| DVU2925 | 50S ribosomal protein L1 | -0.32 | 0.63 | 0.16 | -0.48 | 1.22 | 1.18 | 0.50 | 1.24 | 0.53 |
| DVU2926 | 50S ribosomal protein L10 | -0.32 | -0.11 | -0.28 | -0.96 | -0.61 | -0.57 | -1.43 | -2.16 | -1.80 |
| DVU2927 | 50S ribosomal protein L7/L12 | -0.33 | 0.16 | -0.16 | -0.01 | 0.15 | 0.04 | -0.11 | 0.44 | 0.06 |
| DVU2981 | 2-isopropylmalate synthase | -0.37 | 0.17 | 0.22 | 0.04 | 0.43 | 0.65 | -0.11 | 0.32 | 0.33 |
| DVU3150 | Ribosomal protein S1 | -0.61 | 0.07 | 0.07 | 0.04 | 0.14 | 0.10 | -0.13 | 0.16 | 0.02 |
| DVU3168 | Glutamate-1-semialdehyde 2,1-aminomutase | -0.63 | 0.06 | 0.01 | 0.27 | 0.65 | 0.65 | -0.05 | 0.63 | 0.55 |

Note: The detected proteins with LFC≥0.5 or ≤-0.5 and adjusted q value less than 0.05 are highlighted in red and defined as the changed proteins in response to FNA. All the other proteins with q value larger than 0.05 regardless of LFC are defined as no changed proteins.

**Table S4. Proteins abundance dynamics in the presence of different FNA concentrations relevant to oxidative stress**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene ID | Gene name | 1 μg vs Control | 4 μg vs Control | 8 μg vs Control |
| 2h\_LFC | 8h\_LFC | 12h\_LFC | 2h\_LFC | 8h\_LFC | 12h\_LFC | 2h\_LFC | 8h\_LFC | 12h\_LFC |
|  |   |  |  |   |  |  |   |  |  |   |
| DVU0019 | Nigerythrin | 2.32 | 0.21 | 0.27 | 0.21 | 0.23 | 0.23 | -0.1 | -0.26 | -0.32 |
| DVU0264 | Ferredoxin, 4Fe-4S, putative | 0.84 | -0.18 | -0.19 | -0.09 | -0.13 | -0.27 | -0.03 | -0.11 | -0.32 |
| DVU0273 | Uncharacterized protein | 0.8 | 0.03 | 0.26 | -0.41 | -1.1 | -0.57 | -0.39 | -0.23 | -0.41 |
| DVU0278 | Glyoxalase family protein | 0.72 | 0.52 | 0.53 | -0.22 | -0.39 | -0.34 | -0.17 | -0.6 | -0.55 |
| DVU0305 | Ferredoxin II | 0.68 | -0.05 | -0.45 | -0.01 | -0.71 | -0.42 | -0.04 | -0.31 | -0.55 |
| DVU0772 | Uncharacterized protein | 0.29 | -1.27 | 0.45 | 0.73 | 2.91 | 3.12 | 0.18 | 1.67 | 2.69 |
| DVU0995 | ThiJ/PfpI family protein | 0.16 | 0.01 | 0.2 | -0.08 | -0.32 | -0.34 | -0.09 | -0.64 | -0.72 |
| DVU1228 | Probable thiol peroxidase | 0.11 | -0.25 | -0.11 | -0.07 | -0.71 | -0.56 | -0.06 | -0.81 | -0.9 |
| DVU1397 | Bacterioferritin  | 0.04 | -0.19 | -0.42 | -0.01 | 0.13 | -0.02 | -0.07 | -0.22 | -0.42 |
| DVU1457 | Thioredoxin reductase, putative | 0.02 | 0.39 | 0.37 | -0.08 | 0.07 | 0.12 | -0.11 | 0.04 | 0.07 |
| DVU1568 | Ferritin  | 0.01 | -0.05 | 0.17 | -0.17 | -1.03 | -0.76 | -0.13 | -1.16 | -0.85 |
| DVU1838 | Thioredoxin-disulfide reductase | -0.03 | 0.2 | -0.39 | 0 | -0.04 | -0.24 | 0.06 | -0.11 | -0.08 |
| DVU1839 | Thioredoxin | -0.03 | 0.08 | 0.02 | 0.07 | -0.04 | 0.05 | 0.13 | 0.03 | 0.14 |
| DVU1984 | Peptide methionine sulfoxide reductase MsrA  | -0.08 | -0.53 | 0.64 | -0.52 | -0.2 | -0.48 | 0.09 | 0.75 | -0.24 |
| DVU2247 | Alkyl hydroperoxide reductase C | -0.12 | 0.31 | 0.2 | -0.19 | 0.73 | 0.85 | -0.42 | 0.59 | 0.72 |
| DVU2318 | Rubrerythrin, putative | -0.13 | -0.45 | -0.34 | -0.25 | -0.23 | -0.12 | -0.32 | -0.51 | -0.34 |
| DVU2410 | Superoxide dismutase, Fe-Mn family | -0.16 | -0.48 | 0.41 | 0.15 | -1.62 | -0.29 | -0.42 | -1.26 | -1.01 |
| DVU2680 | Flavodoxin | -0.25 | 0.98 | 0.71 | 0.13 | 0.84 | 0.52 | -0.33 | 0.26 | 0.17 |
| DVU3049 | Hemerythrin family protein | -0.48 | 0.24 | 0.28 | -0.24 | -0.18 | -0.11 | -1.2 | -0.27 | -0.08 |
| DVU3093 | Rubredoxin | -0.55 | 0.05 | -0.08 | 0.67 | 0.88 | -0.26 | 0 | -0.93 | 0.01 |
| DVU3094 | Rubrerythrin (Rr) | -0.55 | -0.08 | 0.01 | -0.19 | -0.71 | -0.51 | -0.2 | -0.77 | -0.83 |
| DVU3183 | Desulfoferrodoxin (Dfx)  | -0.64 | -0.08 | 0.02 | -0.13 | -0.26 | -0.32 | -0.13 | -0.41 | -0.64 |
| DVU3185 | Rubredoxin-oxygen oxidoreductase | -0.64 | -0.05 | -0.18 | -0.02 | -0.35 | -0.43 | -0.04 | -0.25 | -0.37 |
| DVU3212 | Pyridine nucleotide-disulfide oxidoreductase | -0.73 | 0.25 | 0.16 | 0.33 | 0.63 | 0.81 | 0.21 | 0.82 | 1.01 |
| DVU3276 | Ferredoxin I | -0.98 | -0.93 | -0.77 | 0.15 | -1.33 | -2.01 | -0.29 | -1.7 | -2.2 |
| DVU3282 | ADP-ribosylglycohydrolase family protein | -1.04 | 0.2 | 0.16 | -0.17 | 0.18 | 0.06 | -0.19 | -0.07 | -0.27 |
| DVUA0091 | Catalase  | NA | -0.19 | -0.19 | -0.18 | -0.39 | -0.77 | 0.08 | -0.44 | -1.15 |

Note: The detected proteins with LFC≥0.5 or ≤-0.5 and adjusted q value less than 0.05 are highlighted in red and defined as the changed proteins in response to FNA. All the other proteins with q value larger than 0.05 regardless of LFC are defined as no changed proteins.

**Table S5. Proteins abundance dynamics in the presence of different FNA concentrations relevant to others**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene ID | Gene name | 1 μg vs Control | 4 μg vs Control | 8 μg vs Control |
| 2h\_LFC | 8h\_LFC | 12h\_LFC | 2h\_LFC | 8h\_LFC | 12h\_LFC | 2h\_LFC | 8h\_LFC | 12h\_LFC |
| DVU0004 | DNA gyrase subunit A  | 3.71 | 0.22 | 0.19 | 0.2 | 0 | -0.64 | -0.19 | -0.52 | -0.54 |
| DVU0007 | Asparagine--tRNA ligase  | 2.98 | -0.2 | -0.21 | -0.13 | -0.54 | -0.58 | 0.03 | -0.29 | -0.53 |
| DVU0034 | DSBA-like thioredoxin domain protein | 1.91 | -0.39 | 0.08 | -0.1 | -1.12 | -0.5 | 0.11 | -0.58 | -0.41 |
| DVU0042 | RNA methyltransferase | 1.91 | 0.82 | -0.16 | 0.53 | 1.29 | 0.77 | -0.12 | -0.05 | 0.55 |
| DVU0060 | Efflux transporter, RND family, MFP subunit | 1.82 | -0.3 | -0.16 | -0.4 | -0.79 | -0.61 | -0.17 | -0.4 | -0.54 |
| DVU0261 | Universal stress protein family | 0.85 | -0.27 | -0.19 | -0.04 | -0.62 | -0.68 | 0.14 | -0.42 | -0.6 |
| DVU0319 | NAD-dependent epimerase/dehydratase family protein | 0.66 | -0.19 | 0.01 | 0.35 | -0.14 | -0.34 | 0.27 | -0.59 | -0.43 |
| DVU0353 | Alcohol dehydrogenase, iron-containing | 0.61 | 0.04 | -0.02 | -0.08 | -0.24 | -0.2 | -0.08 | -0.34 | -0.54 |
| DVU0410 | Uncharacterized protein | 0.55 | -0.24 | -0.03 | -0.18 | -0.64 | -0.76 | -0.27 | -0.34 | -0.67 |
| DVU0417 | Arginine decarboxylase  | 0.54 | -0.33 | -0.45 | -0.38 | -0.97 | -0.84 | -0.14 | -0.75 | -0.65 |
| DVU0418 | Saccharopine dehydrogenase  | 0.54 | -0.18 | -0.39 | -0.14 | -0.47 | -0.56 | -0.14 | -0.76 | -1.32 |
| DVU0419 | Carboxynorspermidine/carboxyspermidine decarboxylase  | 0.54 | -0.68 | -0.27 | -0.2 | -0.87 | -0.67 | -0.49 | -1.92 | -0.93 |
| DVU0423 | Universal stress protein family | 0.53 | 0.21 | 0.17 | 0.55 | 0.42 | 0.42 | 0.03 | 0.18 | 0.11 |
| DVU0664 | Cysteine desulfurase  | 0.37 | -0.11 | -0.15 | -0.23 | -0.2 | -0.27 | -0.23 | -0.19 | -0.52 |
| DVU0671 | Uncharacterized protein | 0.36 | 0.05 | 0.02 | 0.09 | 0.4 | 0.57 | 0.11 | 0.49 | 0.59 |
| DVU0811 | Chaperone protein DnaK (HSP70) | 0.25 | 0.24 | -0.11 | -0.14 | -0.32 | -0.37 | -0.13 | -0.25 | -0.68 |
| DVU0854 | NirD protein, putative | 0.2 | -0.41 | -0.17 | -0.07 | -0.75 | -0.65 | 0.07 | -0.57 | -0.43 |
| DVU0857 | Radical SAM domain protein | 0.19 | -0.21 | 0 | -0.29 | -0.31 | -0.14 | -0.35 | -0.64 | -0.45 |
| DVU1030 | Universal stress protein | 0.15 | 0.2 | 0.08 | 0.42 | 0.75 | 0.84 | 0.03 | 0.57 | 0.8 |
| DVU1176 | Uncharacterized protein | 0.13 | -0.08 | 0 | 0.02 | -0.52 | -0.33 | -0.08 | -0.36 | -0.35 |
| DVU1241 | Uncharacterized protein | 0.11 | 5.48 | 4.26 | 3.63 | 5.1 | 4.43 | 3.28 | 4.99 | 4.13 |
| DVU1257 | RNA-binding protein | 0.1 | 0.01 | -0.15 | -0.44 | -1.04 | -0.95 | -0.53 | -0.65 | -0.74 |
| DVU1260 | Outer membrane protein P1, putative | 0.1 | 0.11 | 0.14 | 0.16 | 0.4 | 0.43 | 0.01 | 0.53 | 0.63 |
| DVU1343 | Cation ABC transporter, periplasmc-binding protein | 0.05 | 0.36 | 0.28 | 0.21 | 1.16 | 1.1 | 0.15 | 1.06 | 0.89 |
| DVU1382 | HesB family selenoprotein | 0.04 | 0.14 | -0.07 | -0.2 | -0.18 | -0.45 | -0.16 | -0.13 | -0.6 |
| DVU1612 | ACT domain protein | 0 | 0.2 | 0.09 | 0.28 | 0.53 | 0.7 | 0.23 | 0.44 | 0.84 |
| DVU1864 | DNA-binding protein HU, beta subunit, putative | -0.04 | 1.37 | 1.5 | 0.17 | 1.43 | 1.82 | -0.54 | 1.49 | 2.56 |
| DVU1878 | Threonine aldolase, low-specificity | -0.04 | -0.16 | -0.08 | -0.12 | -0.6 | -0.61 | -0.52 | -0.71 | -0.68 |
| DVU1937 | Phosphonate ABC transporter, periplasmic phosphonate-binding protein, putative | -0.06 | -0.07 | 0.01 | 0.01 | -0.22 | -0.38 | 0.11 | -0.29 | -0.84 |
| DVU1951 | Indolepyruvate oxidoreductase subunit IorA  | -0.07 | -0.3 | -0.29 | -0.6 | -0.38 | -0.12 | -0.85 | -0.38 | -0.23 |
| DVU2093 | ThiH protein | -0.09 | -0.18 | -0.12 | -0.21 | -1.88 | -1.23 | 0.17 | -0.79 | -1.13 |
| DVU2105 | Uncharacterized protein | -0.09 | -0.34 | -0.4 | -0.56 | -1.02 | -0.76 | -0.89 | -1.51 | -1.62 |
| DVU2108 | MTH1175-like domain family protein | -0.1 | -0.28 | -0.2 | -0.02 | -0.6 | -1.03 | 0.07 | -0.81 | -1.78 |
| DVU2347 | Acetylornithine aminotransferase  | -0.14 | -0.05 | 0.03 | 0.01 | -0.85 | -1.06 | -0.18 | -0.99 | -1.07 |
| DVU2422 | Nitroreductase family protein | -0.16 | -0.44 | -0.21 | -0.03 | -0.69 | -0.87 | 0.18 | -0.56 | -0.88 |
| DVU2427 | Uncharacterized protein | -0.16 | 0.03 | -0.03 | 0.32 | 0.2 | -0.55 | 0.2 | -0.06 | -0.62 |
| DVU2441 | Heat shock protein, Hsp20 family | -0.17 | 0.04 | -0.1 | 0.6 | 0.89 | 0.8 | 0.5 | 1 | 0.89 |
| DVU2569 | Peptidyl-prolyl cis-trans isomerase  | -0.23 | -0.15 | -0.21 | -0.28 | -0.49 | -0.78 | -0.4 | -0.44 | -0.61 |
| DVU2590 | Sensory box protein | -0.24 | -0.13 | -0.12 | -0.09 | -0.29 | -0.67 | -0.09 | -0.2 | -0.51 |
| DVU2770 | Response regulator | -0.26 | -0.01 | -0.11 | 0.63 | -0.11 | -0.16 | 0.7 | -0.12 | -0.11 |
| DVU2929 | DNA-directed RNA polymerase subunit beta | -0.34 | -0.18 | 0.14 | -0.42 | -0.52 | -0.32 | -0.58 | -0.46 | -0.89 |
| DVU3055 | Ribonuclease, Rne/Rng family  | -0.51 | -0.05 | -0.33 | 0.26 | -1.77 | -0.42 | 0.44 | -0.51 | -0.81 |
| DVU3065 | AMP-binding enzyme family protein | -0.53 | -0.25 | -0.26 | -0.26 | -0.21 | -0.27 | -0.47 | -0.33 | -0.53 |
| DVU3181 | Phosphoribosylformylglycinamidine synthase subunit PurL | -0.64 | 0.02 | -0.02 | -0.06 | -0.07 | -0.16 | -0.2 | -0.2 | -1.21 |
| DVU3199 | Nucleoid-associated protein DVU3199 | -0.7 | 0.45 | 1.19 | 0.42 | 0.58 | 0.68 | 0.29 | 0.63 | 0.62 |
| DVU3228 | Chemotaxis protein CheY | -0.76 | -0.16 | 0.07 | -0.14 | -0.52 | -0.47 | 0.43 | -1.01 | -0.24 |
| DVU3319 | Bifunctional protein PutA | -1.22 | -0.24 | -0.08 | -0.07 | -0.75 | -0.74 | 0.11 | -0.62 | -0.88 |
| DVU4006 | hypothetical protein  | NA | 0.34 | 0.15 | 0.36 | 0.8 | 0.57 | 0.03 | 0.65 | 0.48 |
| DVUA0132 | CRISPR-associated protein, TM1801 family | 4.94 | 0.04 | 0.11 | -0.35 | -0.8 | -0.49 | 0.05 | -0.59 | -1.19 |
| DVUA0146 | Uncharacterized protein | 3.96 | -1.19 | 0.08 | -0.86 | -0.38 | 0.56 | -0.83 | -1.96 | -1.52 |
| DVUA202  | hypothetical protein  | NA | -0.2 | -0.08 | -0.08 | -0.64 | -0.86 | 0.08 | -0.75 | -0.99 |

Note: The detected proteins with LFC≥0.5 or ≤-0.5 and adjusted q value less than 0.05 are highlighted in red and defined as the changed proteins in response to FNA. All the other proteins with q value larger than 0.05 regardless of LFC are defined as no changed proteins.

**Figure S1.** Growth profiles of *Desulfovibrio vulgaris* batch cultures in the presence of different starting FNA concentrations (μg N/L). FNA was added at time 0 h. The control culture has no FNA addition. Error bars show the standard deviations of the results from triplicate cultures. The key shows the FNA starting concentrations. Statistics and significant difference was performed by Student t test on each time point between treatment and control samples where the mark of \* stands for the significant difference between control and treatment samples. (\* represents p < 0.05 while \*\* represents p < 0.001).



**Figure S2.** Live cell numbers per microliter with four different FNA concentrations (0, 1.0, 4.0, and 8.0 μg N/L) over incubation. Statistics and significant difference was performed by Student t test on each time point between treatment and control samples where the mark of \* stands for the significant difference between control and treatment samples. ( \* represents p < 0.05 while \*\* represents p < 0.001).



**Figure S3**. Signal intensities for all the samples after LC MS/MS measurement.



**Figure S4.** FNA levels in the presence of different FNA concentration over incubation. Statistics and significant difference was performed by Student t test on each time point between treatment and control samples where the mark of \* stands for the significant difference between control and treatment samples. (\* represents p < 0.05 while \*\* represents p < 0.001).



**References**

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B., Srinath, E.G., 1976. Inhibition of nitrification by ammonia and nitrous acid. Water Pollut Control Fed. 48, 835-852.

Gao, S.H., Fan, L., Yuan, Z., Bond, P.L., 2015. The concentration-determined and population-specific antimicrobial effects of free nitrous acid on *Pseudomonas aeruginosa* PAO1. Appl Microbiol Biotechnol. 99, 2305-2312.

Kappler, U., Nouwens, A.S., 2013. The molybdoproteome of *Starkeya novella* - insights into the diversity and functions of molybdenum containing proteins in response to changing growth conditions. Metallomics. 5, 325-334.

Zhou, A., Baidoo, E., He, Z., Mukhopadhyay, A., Baumohl, J.K., Benke, P., Joachimiak, M.P., Xie, M., Song, R., Arkin, A.P., Hazen, T.C., Keasling, J.D., Wall, J.D., Stahl, D.A., Zhou, J., 2013. Characterization of NaCl tolerance in *Desulfovibrio vulgaris* Hildenborough through experimental evolution. ISME J. 7, 1790-1802.

Zhou, A.F., Chen, Y.Y.I., Zane, G.M., He, Z.L., Hemme, C.L., Joachimiak, M.P., Baumohl, J.K., He, Q., Fields, M.W., Arkin, A.P., Wall, J.D., Hazen, T.C., Zhou, J.Z., 2012. Functional characterization of crp/fnr-type global transcriptional regulators in *Desulfovibrio vulgaris* Hildenborough. Appl Environ Microbiol. 78, 1168-1177.