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# The nature of electron acceptor (MnIV/NO<sub>3</sub>) triggers differential expression of genes associated with stress and ammonium limitation responses in *Shewanella* algae C6G3

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2 **The nature of electron acceptor (MnIV/NO<sub>3</sub>) triggers differential expression of genes associated**  
3 **with stress and ammonium limitation responses in *Shewanella algae* C6G3**

4  
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7  
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13 **Abstract-**

14 *Shewanella algae* C6G3 can reduce dissimilatively nitrate into ammonium and manganese-oxide  
15 (MnIV) into MnII. It has the unusual ability to produce anaerobically nitrite from ammonium in the  
16 presence of MnIV. To gain insight into their metabolic capabilities, global mRNA expression patterns  
17 were investigated by RNA-seq and qRT-PCR in cells growing with lactate and ammonium as carbon  
18 and nitrogen sources and with either MnIV or nitrate as electron acceptors. Gene exhibiting higher  
19 expression levels in the presence of MnIV belonged to functional categories of carbohydrate, coenzyme,  
20 lipid metabolisms and inorganic ion transport. Comparative transcriptomic pattern between MnIV and  
21 NO<sub>3</sub> revealed that the strain presented an ammonium limitation status with MnIV, despite the presence  
22 of non-limiting concentration of ammonium under both culture conditions. In addition, in presence of  
23 MnIV, *nrB/nrtC* regulators, ammonium channel, nitrogen regulatory protein P-II, glutamine synthetase  
24 and asparagine synthetase glutamine dependent genes were over-represented. Under nitrate condition,  
25 the expression of genes involved in the synthesis of several amino acids was increased. Finally,  
26 expression level of genes associated with the general stress response was also amplified and among  
27 them, *katE*, a putative catalase/peroxidase present on several *Shewanella* genomes, was highly expressed  
28 with a relative median value higher in MnIV condition.

29  
30 *Key words: Shewanella algae, Manganese-oxide, nitrate, nitrogen-limitation, transcriptome, catalase*

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34

## 36 1. Introduction

37 The genus *Shewanella* widely distributed in marine and freshwater environments is recognized for its  
38 metabolic versatility toward broad range of electron acceptors, including oxygen (O<sub>2</sub>), nitrate (NO<sub>3</sub><sup>-</sup>),  
39 nitrite (NO<sub>2</sub><sup>-</sup>), fumarate, ferric iron, manganese-oxide (MnO<sub>2</sub> or MnIV), thiosulfate, elemental sulfur,  
40 trimethylamine N-oxide (TMAO), dimethyl sulfoxide (DMSO), and anthraquinone-2,6-disulfonate  
41 (AQDS) (Beliaev *et al.*, 2005, Fredrickson *et al.*, 2008). This metabolic versatility provides high  
42 competitiveness to these species, especially in stratified environments with electron acceptor gradients.  
43 The marine sediments are examples where dissolved oxygen concentration decreases with depth  
44 following a vertical slope. Oxygen is substituted by alternative electron acceptors leading to their vertical  
45 zonation comprising from surface to deep, NO<sub>2/3</sub><sup>-</sup>, Mn(III/IV), Fe(III), SO<sub>4</sub><sup>2-</sup>, CO<sub>2</sub><sup>-</sup> (Emerson & Hedges,  
46 2006, Hansel, 2017). The metabolic versatility of *Shewanella* species confers them an important role in  
47 the turnover of organic matter and in the carbon cycle, coupled to anaerobic respiration with electron  
48 acceptors, such as FeIII, MnIV and NO<sub>2/3</sub><sup>-</sup> (Coates *et al.*, 1998).

49 In marine sediments, the concentration of ammonium is generally much higher than that of NO<sub>2/3</sub>.  
50 These nitrogen oxyanions are mainly produced from ammonium by nitrification in the overlying aerobic  
51 zone and can diffuse to the anaerobic area where they fuel many dissimilatory reduction pathways (e. g.  
52 denitrification, dissimilatory reduction of nitrate into ammonium or Anammox) leading or not to a  
53 nitrogen lost for the system such as gaseous production (Bonin *et al.*, 1998). Many studies have  
54 highlighted the strong coupling between aerobic ammonium oxidation and anaerobic NO<sub>3</sub><sup>-</sup> or NO<sub>2</sub><sup>-</sup>  
55 reduction communities (Fernandes *et al.*, 2016). The study of organisms and mechanisms leading to  
56 NO<sub>2/3</sub><sup>-</sup> production is therefore essential for the comprehension of the functioning of the anaerobic part  
57 of sediments. Several studies have demonstrated the stimulation of activities associated with nitrogen  
58 cycle in anaerobic marine sediments in the presence of MnIV, supporting the hypothesis of the  
59 occurrence of a manganese-oxide (MnIV) dependent anaerobic NO<sub>2/3</sub><sup>-</sup> production pattern (Luther *et al.*,  
60 1997, Anschutz *et al.*, 2005, Javanaud *et al.*, 2011, Fernandes *et al.*, 2015). The anaerobic ammonium  
61 oxidation seems to be favored with a 2-4 fold NH<sub>4</sub>/MnIV concentration ratio and is concomitant to  
62 higher ammonium consumption (Lin & Taillefert, 2014).

63 During the analysis of the anaerobic NO<sub>2/3</sub><sup>-</sup> peak observed in some marine sediments, a  
64 *Shewanella algae* C6G3 strain was isolated and characterized from an intertidal zone (Javanaud *et al.*,  
65 2011, Aigle *et al.*, 2015, Aigle *et al.*, 2017). *S. algae* is an ubiquitous species of coastal marine  
66 environments (Caccavo *et al.*, 1992, Beleneva *et al.*, 2009) and the strain C6G3 can perform the  
67 dissimilatory reduction of nitrate into ammonium and MnIV reduction. It displays the additional ability  
68 to produce anaerobically biotic NO<sub>2</sub><sup>-</sup> with ammonium as the sole nitrogen source and MnIV as the sole

69 electron acceptor. Neither ammonia monooxygenase nor hydroxylamine oxidase gene, known in aerobic  
70 nitrifiers for oxidation of ammonium into nitrite or nitrate were detected within C6G3 genome (Aigle et  
71 al., 2017). In addition, no genes were identified coding for either hydrazine synthase, hydrazine  
72 dehydrogenase or hydroxylamine oxidase involved in Anammox in which ammonium is used as electron  
73 donor and nitrite as electron acceptor in anaerobiosis and generating N<sub>2</sub>. Among the genus *Shewanella*,  
74 many studies have focused on the *S. oneidensis* MR-1, the most-described strain capable of dissimilatory  
75 metabolism of MnIV. The mechanism of extracellular electron exchange has been particularly well  
76 detailed (Richardson *et al.*, 2012, Shi *et al.*, 2012, White *et al.*, 2016). Using microarrays, specific  
77 expression profiles have been determined after short time incubations with individual non-metal electron  
78 acceptors resulting in the identification of unique groups of nitrate-, thiosulfate- and TMAO-induced  
79 genes (Beliaev *et al.*, 2005).

80 Several differences between *S. algae* C6G3 and *S. oneidensis* genomes have been characterized  
81 so far, especially concerning N-cycle and MnIV reduction. Indeed, in *S. algae*, additional genes are  
82 present such as a second *nap* operon (encoding a nitrate reductase), *nrfA* (encoding a second nitrite  
83 reductase) and additional genes in the MTR pathway (*mtrH*) (Aigle et al., 2017). Furthermore, in contrast  
84 to *S. algae* C6G3 and ATCC5119 strains, no anaerobic biotic NO<sub>2/3</sub> production was detected in *S.*  
85 *oneidensis* with ammonium as the sole nitrogen source and MnIV as the sole electron acceptor (Aigle et  
86 al., 2017). To understand the impact of MnIV and NO<sub>3</sub><sup>-</sup> on the metabolism of *S. algae* C6G3,  
87 transcriptomic profiles obtained in the presence of these electron acceptors were compared. Several  
88 putative overexpressed pathways were further investigated along growth by qRT-PCR. Due to the  
89 capability of the strain to oxidize ammonium in nitrite in anaerobiosis, these analyses were focused on  
90 NH<sub>4</sub> metabolism and stress response.

91

## 92 **Materials and Methods**

### 93 **Growth conditions**

94 *Shewanella algae* C6G3 was grown anaerobically at 28 °C in artificial sea water (Baumann *et al.*, 1971)  
95 with DL-lactate (25 mM), K<sub>2</sub>HPO<sub>4</sub> (0.43 mM), FeSO<sub>4</sub> (13,2 μM), NH<sub>4</sub>Cl (20 mM) and trace mineral  
96 element solution and vitamin (Balch *et al.*, 1979). Nitrate (3 mM) or manganese oxides (1 mM) were  
97 added as sole source of electron acceptor. Manganese oxide was synthesized following the protocol of  
98 Laha (Laha & Luthy, 1990). Cells were grown under strict anaerobic conditions with a 100% N<sub>2</sub> gas  
99 head space. Cultures dedicated to RNA-seq, expression profiling, or physiology were made in duplicate.  
100 Nitrate, nitrite, MnII and 16S rDNA were measured as previously described (Aigle et al., 2017)

## 101 **Transcriptome analysis**

102

103 For RNA-seq, cells from two independent cultures for each growth condition at three quarters of their  
104 exponential phase of growth were pelleted and washed twice with RNAprotect® Bacteria Reagent  
105 (Qiagen): Tris-NaCl buffer (0.05 M Tris; 0.15 M NaCl; pH 7.8) 2:1 and rinsed with Tris-NaCl buffer.  
106 After lysis in RLT buffer of RNeasy® kit (Qiagen), total RNA was extracted with phenol: chloroform:  
107 isoamyl alcohol 125:24:1 pH 4.5, gently mixed and centrifuged 15 min at 12.000 g at 4 °C, and then  
108 with chloroform: isoamyl alcohol 24:1, gently mixed and centrifuged 15 min at 12.000 g at 4 °C. Total  
109 RNA samples were purified using RNeasy® Mini QIAcube Kit (Qiagen) and DNA contamination was  
110 removed using the TURBO DNA-free™ kit (Ambion® by Life Technologies™) according to the  
111 manufacturer's instructions.

112 After RNA quality check, (Agilent 2100 Bioanalyzer Santa Clara, CA, USA) and quantification  
113 (Nanodrop 2000c Thermo Scientific), the absence of DNA contamination was verified by PCR  
114 following the protocol of Aigle *et al.* (2017). The Transcriptomic and Genomic Marseille-Luminy  
115 (TGML) platform performed further sample treatments (Amrani *et al.*, 2014). Templates sequencing  
116 was performed with semiconductor sequencing chips 318 on the Ion Torrent PGM (Life Technology).  
117 Despite the lower number of reads generated by this technology, it was chosen because at that time it  
118 allowed to sequence longer reads than Illumina. The RNA-seq data performed in duplicated for each  
119 culture condition have been deposited on NCBI GEO platform (accession number GSE109146).

120

121 RNA data were analyzed as previously described (Amrani *et al.*, 2014). Briefly, the R package DESeq  
122 was used to analyze the differential expression of genes between the different culture conditions (Anders  
123 & Huber, 2010). P-values were calculated and adjusted for multiple testing using the false discovery rate  
124 controlling procedure (Benjamini & Hochberg, 1995). An adjusted P-value <0.05 was considered  
125 statistically significant. The Kegg Orthology identifiers of genes and the KEGG pathways assignment  
126 were performed on *S. algae* C6G3 genome using KAAS tool (KEGG Automatic Annotation Server,  
127 GenomeNet).

128 For the kinetic of gene expression, RNA was extracted along growth (4 or 5 time points spread from  
129 early exponential to early stationary phase of growth) from two independent cultures for each growth  
130 condition. Reverse transcriptase reactions with random primers, cDNA quantification by qPCR and data  
131 analysis were performed as previously described (Aigle *et al.*, 2017). Primers used for qPCR are listed  
132 in Table S1. Row data of target gene expression per ng of total RNA are presented in Table S2. Two  
133 technical qPCR quantifications were performed for each RNA extraction. From a previous study, we

134 have noticed a lower quality of RNA from MnIV grown cells due to splitting of RNA by MnII. The  
135 quantification of the expression of the house-keeping gene (*rpoD*) encoding the sigma<sup>70</sup> subunit of the  
136 RNA polymerase was used to normalize gene expression in order to avoid putative bias from RNA  
137 extraction and reverse transcription efficiency. The median value for *rpoD*/ng of total RNA from  
138 duplicate MnIV or NO<sub>3</sub> grown cells at all the time points were 1.49 ·10<sup>3</sup> and 5.47 ·10<sup>3</sup> cDNA/ng RNA,  
139 respectively. No clear tendency was seen according to either condition or phase of growth (Fig S1). All  
140 cDNA quantifications by qPCR were done on the same reverse transcription.

141

## 142 **Results and discussion**

143 RNA-seq assays generated 1,725,084 high-quality reads for the four tested samples (two per culture  
144 conditions). Between 83 to 95% of the high-quality reads were mapped to *S. algae* C6G3 genome (Table  
145 S3). Correlation matrix between RNA-seq normalized expression levels of each independent culture  
146 obtained with DESeq (Anders & Huber, 2010) indicated that the similarity between the 2 biological  
147 replicates was above 90% (Fig.S2).

148 This preliminary study allowed to identify 148 genes as differentially expressed (adjusted p-value  
149 <0.05), 67 and 81 were over-represented under MnIV or nitrate conditions, respectively (Fig S3, S4,  
150 Table S4). Most of significant differentially expressed genes belonged to COG categories of energy  
151 production and conversion (C), amino acid transport and metabolism (E), carbohydrate transport and  
152 metabolism (G) , co-enzyme metabolism (I), lipid metabolism (L), inorganic ion transport and  
153 metabolism (P), posttranslational modification, protein turnover and chaperones (O) (Fig. 1).

154 Under both growth conditions, lactate was the only carbon source available and was partially oxidized  
155 into acetate that accumulated in the medium as for many species of its genus (Fig. 2) (Aigle *et al.*, 2015)  
156 (Tang *et al.*, 2007, Lian *et al.*, 2016). *S. algae* C6G3 genome contains five formate dehydrogenase gene  
157 clusters (Aigle *et al.*, 2015), among them four, i.e. *fdnGHI-fdhC*, *fdhA1B1C1*, *fdhA2B2C2*, *hyaAB*, are  
158 also present in *S. oneidensis* MR-1, while the fifth, i.e. *fdoGHI* is present in *E. coli* and in *Sinorhizobium*  
159 *meliloti* (Heidelberg *et al.*, 2002, Wang & Gunsalus, 2003, Pickering & Oresnik, 2008). Under nitrate  
160 condition, two formate dehydrogenase clusters (*fdnGHI-fdhD* and *fdoGHI* gene clusters) were over-  
161 represented in RNA seq compared to MnIV condition (log<sub>2</sub> fold change MnIV/NO<sub>3</sub> between -3.7 and -  
162 6.1; and -3.6 and 5.6, respectively Table S4)). Regulation of *fdnGHI* genes by nitrate was previously  
163 demonstrated in *E. coli* (Wang & Gunsalus, 2003) and *S. oneidensis* MR-1 (Beliaev *et al.*, 2005) whereas  
164 *fdoGHI* was shown to encode a formate dehydrogenase complex NAD<sup>+</sup> independent (Pickering &  
165 Oresnik, 2008). Considering its over-representation under nitrate condition observed in our study,  
166 *fdoGHI* is probably implicated in the process of nitrate reduction. The *fdh* gene cluster was over-

167 represented in the presence of MnIV (genes *fdhA* and *fdhC*, log<sub>2</sub> fold change MnIV/NO<sub>3</sub> between 1.7  
168 and 2.33, Table S4). Under this growth condition, it has been proposed that nitrate and nitrite are  
169 produced from ammonium (Aigle et al 2017 and Fig.2). Consequently, the dehydrogenase encoded by  
170 the *fdh* cluster could be involved in the process of either NO<sub>2/3</sub><sup>-</sup> production or MnIV reduction.

171 Nitrate and nitrite reduction is a stepwise process during *S. algae* C6G3 anaerobic growth with nitrate  
172 as electron acceptor (Fig. 2). Dissimilatory nitrate reduction into ammonium is catalyzed by two  
173 enzymes, the nitrate reductase (*napA*) oxidizing nitrate to nitrite, and the nitrite reductase formate  
174 dehydrogenase (*nrfA*) that oxidizes nitrite to ammonium (Simon, 2002, Simpson *et al.*, 2010, Aigle *et*  
175 *al.*, 2015). As the RNA-seq was performed at the ¾ of the exponential phase of growth when all nitrate  
176 is reduced to nitrite, an over-representation of the *nrfA* gene in RNA seq was expected (log<sub>2</sub> fold change  
177 MnIV/NO<sub>3</sub> of -2.5, Table S4) in agreement with our previous results obtained by qRT-PCR (Aigle et al.,  
178 2017). The similar expression level of the metal transfer reduction operon (*mtrABCDEF*) between both  
179 growth conditions is also in agreement with previous observations (Beliaev *et al.*, 2005, Aigle *et al.*,  
180 2017).

181

182 The over-representation in RNA seq of genes responsible for ammonium assimilation under N-limiting  
183 condition, when MnIV was the electron acceptor, is the most striking observation of the present study  
184 (Fig. 3, Table S4). In liquid medium, ammonium (NH<sub>4</sub><sup>+</sup>) concentration is in equilibrium with ammonia  
185 (NH<sub>3</sub>) according to acid base equilibrium constant (K<sub>a</sub>). Regardless the electron acceptor, the culture  
186 medium contained at the beginning of the growth 20 mM of NH<sub>4</sub>Cl. According to the pK<sub>a</sub> of NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub>  
187 (9.23) and the pH of the medium (7.8), the NH<sub>3</sub> concentration is estimated at ~1.2 mM. While ammonia  
188 gas can passively diffuse through the cellular membrane, NH<sub>4</sub><sup>+</sup> needs to be absorbed through an  
189 ammonium channel encoded by the *amtB* gene. This gene is expressed only under drastic ammonium  
190 limitation (van Heeswijk *et al.*, 2013). In this study, despite NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> availability in the cultures, the  
191 NH<sub>4</sub><sup>+</sup> uptake pathway genes were over-represented in the presence of MnIV compared to that of nitrate.  
192 Moreover, genes coding for the NRI/NRII-two component system nitrogen regulator (*ntrB/nrtC* also  
193 called *glnG/glnL*, log<sub>2</sub> fold change MnIV/NO<sub>3</sub> of 2.9 and 3.5), the ammonium channel (*amtB*, log<sub>2</sub> fold  
194 change MnIV/NO<sub>3</sub> of 6.1), the nitrogen regulatory protein P-II (*glnK*, log<sub>2</sub> fold change MnIV/NO<sub>3</sub> of  
195 5.5), the glutamine synthetase (*glnA*, log<sub>2</sub> fold change MnIV/NO<sub>3</sub> of 2.7) and the asparagine synthetase  
196 glutamine dependent (*asnB*, log<sub>2</sub> fold change MnIV/NO<sub>3</sub> of 6.5) were over-represented similarly (Table  
197 S4). Entry of ammonium has been convincingly shown to occur through ammonium channels that are  
198 blocked by the nitrogen regulatory protein P-II, encoded by the *glnK* gene. The association of AmtB/PII  
199 complex is controlled by the UTase enzyme, sensitive to the glutamine product of the glutamine



200 synthetase encoded by the *glnA*, to 2-oxoglutarate concentration (an intermediate of the Krebs cycle and  
201 an indicator of nitrogen status) and to MgII and ADP/ATP ratio (van Heeswijk *et al.*, 2013).

202 Under nitrogen limitation, GlnA catalyzes the formation of glutamine from glutamate and ammonia. The  
203 produced glutamine is the substrate of glutamate synthase (GOGAT), which catalyzes the conversion of  
204 2-oxoglutarate and glutamine into two molecules of glutamate. In order to maintain balanced  
205 metabolism, the cell must coordinate the assimilation of nitrogen with the assimilation of carbon and  
206 other essential nutrients. This coordination is carried out in part by a signal transduction system, which  
207 measures signals of carbon and nitrogen status and regulates the activity of GlnA, and by the  
208 transcription of nitrogen-regulated (Ntr) genes, whose products facilitate the use of poor nitrogen sources  
209 (van Heeswijk *et al.*, 2013).

210 *S. algae* C6G3 genes organization is similar to what is known for *E. coli* where *glnA* is part of the *glnALG*  
211 or *glnA-ntrBC* operon whereas *amtB* and *glnK* are located in close vicinity (data not shown). The kinetic  
212 of the expression of these two operons was followed by qRT-PCR targeting one of their genes (*glnA* and  
213 *amtB*) at 4 or 5 time points in duplicate-cultures with MnIV or NO<sub>3</sub> as electron acceptors. The median  
214 value of *glnA* expression level of all time points of both duplicate cultures was higher under MnIV than  
215 under NO<sub>3</sub> condition, whether it is expressed in absolute quantification (5.98 10<sup>4</sup> vs 0.89 10<sup>4</sup> of *glnA*  
216 cDNA/ng RNA, Table S2) or relative to *rpoD* (46.1 vs 2.9 ). This over-expression was observed for all  
217 time points of the cultures confirming our RNA-seq results (Fig. 3). The *rpoD*-relative expression level  
218 of *amtB* was lower than that of *glnA* but presented also a higher median under MnIV (1.7) than under  
219 nitrate (0.6) conditions. Its expression in both culture duplicates was higher under MnIV condition than  
220 under NO<sub>3</sub> for three of the four time points of the cultures (Fig. 3). Since the expression of these two  
221 genes is induced by low intracellular NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> concentration, this observation suggests that this strain  
222 senses nitrogen limitation in the presence of MnIV throughout growth. It is known that 2-oxoglutarate  
223 can stimulate ammonium assimilation by dissociation of the Amt-GlnK complex (van Heeswijk *et al.*,  
224 2013). Interestingly, the increased expression of the gene coding for isocitrate dehydrogenase (IDH),  
225 leading to 2-oxoglutarate production, was also confirmed by qRT-PCR in the early exponential phase of  
226 growth suggesting a probable production of 2-oxoglutarate, preventing the closure of ammonium  
227 channel in the presence of MnIV (Fig.3).

228 Several explanations could be proposed for the surprising difference of nitrogen status (limiting vs non-  
229 limiting available nitrogen) between cultures grown with either nitrate or MnIV as electron acceptors.  
230 The limitation by the initial concentration of ammonium concentration (20 mM) can be excluded since  
231 its concentration was identical in both culture media and the overexpression of *glnA* and *amtB* occurred  
232 from the early exponential phase of growth. Furthermore, several genes responsible for amino acid  
233 synthesis such as valine/isoleucine (*ilvC*), methionine /threonine (*metB*) or cysteine (*cysK* and *cysN*)

234 were under-expressed under MnIV condition (Fig. 3). The ammonium need for the general biomass  
235 development is probably much lower in presence of MnIV since the cell density at the end of the culture  
236 was 28 fold lower than that obtained with nitrate. (Fig.2). Ammonium could be destined to anabolism  
237 of specific compounds as suggested by the synthesis of higher number of cytochromes coupled to the  
238 overproduction of some of them in MnIV grown cells (Fig. S4) and by the overexpression of genes  
239 included in carbohydrate, inorganic ion transport and metabolism; coenzyme and lipid, metabolism COG  
240 categories (Fig.1). Among these categories, seven genes involved in the synthesis of storage products  
241 such as glycogen were found (*glgA*, *glgB*, *glgC*, *glgP*, *pgl*, *malQ*, log2 fold change MnIV/NO<sub>3</sub> between  
242 2.4 and 5.5, Table S4). The expression of one of them, *glgB* was confirmed by qRT-PCR for two time  
243 points of growth under MnIV condition (Fig. 3). Glycogen has been shown to accumulate in large  
244 amount within cells when growth is limited by a deficiency of some factors other than the carbon and  
245 energy source (Wilkinson, 1963). Other hypotheses could be formulated to explain the expression of  
246 genes induced by NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> limitation: (i), an abiotic reaction involving Mn(II) and scavenging part of  
247 available ammonia such as  $Mn^{2+} + 2NH_3 + 2H_2O \leftrightarrow Mn(OH)_2 + 2NH_4^+$ ; (ii) an additional need of  
248 ammonium to partially scavenge the oxides generated from MnIV (MnO<sub>2</sub>) reduction into MnII,  
249 concomitant to nitrite formation with an unresolved mechanism. Nitrite consumption is visible between  
250 20 and 40 h in culture grown with MnIV (Fig.2). Due to the presence of *nrfA* on *S. algae* genome and  
251 its expression under this condition (Aigle *et al.*, 2017), the formed nitrite could be further reduced by  
252 DNRA into ammonium; (iii) the last one could be the combination of several causes. Further  
253 investigations are needed to validate one or several of these hypotheses.

254 Nitrate (NO<sub>3</sub>) dissimilation may produce reactive oxygen/nitrogen species (RONS). For example, *E. coli*  
255 cells, grown anaerobically with nitrate as terminal electron acceptor, generate significant NO on adding  
256 nitrite. The periplasmic cytochrome *c* nitrite reductase (Nrf) was shown, by comparing Nrf<sup>+</sup> and Nrf<sup>-</sup>  
257 mutants, to be largely responsible for NO generation (Corker & Poole, 2003). From micro-array,  
258 upregulation of a large number of stress responses genes under both nitrate and metal-reducing  
259 conditions have been shown in *S. oneidensis* (Beliaev *et al.*, 2005). In *S. algae* C6G3, several genes of  
260 stress responses were expressed similarly under nitrate compared to MnIV condition, indicating an  
261 analogous stress status. However several other genes (*clpA*, *clpB*, *katE*, *groEL*, *dnaK*, *rpoS*, *katE*, Fig.2  
262 and Table S4) known to be involved in the general stress response, are apparently over-represented at  
263 least in one conditions as suggested by our RNA-seq preliminary results. The time course of their  
264 expressions along growth were investigated by qRT-PCR. Overall, the median expression levels of genes  
265 *dnaK*, *groEL* encoding chaperones, *rpoS* encoding the general stress response sigma factor, and *clpB*  
266 encoding a protease machinery component were higher under nitrate than MnIV condition whether they  
267 were expressed either as absolute quantification (*dnaK* mRNA abundance : 5.68 10<sup>4</sup> vs 3.08 10<sup>3</sup>  
268 cDNA/ng RNA; *groEL* mRNA abundance: 7.8 10<sup>3</sup> vs 2.93 10<sup>3</sup> cDNA/ng RNA; *rpoS* mRNA abundance:

269 2.77 10<sup>4</sup> vs 3.07 10<sup>3</sup> cDNA/ng RNA; *clpB* mRNA abundance: 3.89 10<sup>3</sup> vs 5.16 10<sup>2</sup> cDNA/ng RNA,  
270 Table S 2), or relative to *rpoD* (*dnaK*:4.3 vs 2.5; *groEL*: 1.5 vs 1.12; *rpoS* : 5.4 vs 2.1, *clpB* : 0.38 vs  
271 0.31). However, their temporal expressions differed. Considering *rpoS*, its relative expression level was  
272 higher in the presence of nitrate than of MnIV for all the phases of growth (early expo 2, exp, early stat  
273 1, Fig.4). In contrast, the expression of *dnaK*, *clpB* and *groEL* depended on the phase and was  
274 overexpressed under MnIV condition in early exponential phase of growth whereas an opposite tendency  
275 was seen under NO<sub>3</sub> condition in exponential phase of growth concomitant with the nitrite peak (Fig. 2,  
276 4).

277 Interestingly, under manganese condition, *S. algae* C6G3 transcriptome analysis underlined the strong  
278 over-representation (log<sub>2</sub> fold change of 4.7, Table S3) of one out of the four catalase/peroxidase genes  
279 present in its genome (SA002\_03500, corresponding to *KatE* found in several *Shewanella* species, Table  
280 S5). Among the stress genes identified by RNA-seq, *katE* relative expression level was higher  
281 particularly under MnIV condition, as shown by the median *rpoD*-relative expression of *katE* reaching  
282 21.3 and 6.7 under MnIV or NO<sub>3</sub> conditions, respectively. At a lesser extent, the preferential expression  
283 under MnIV condition presented the same tendency for the absolute quantification (*katE* mRNA  
284 abundance 3.41 10<sup>4</sup> vs 3.36 10<sup>4</sup> cDNA/ng RNA). The over-representation of *katE* in the presence of  
285 MnIV was confirmed by qRT-PCR in early exponential and stationary phase of growth (Fig. 4),  
286 suggesting a specific response to MnIV or to a metabolic byproduct of the strain under these growth  
287 conditions.

#### 288 4. Conclusion

289 Our aim was to better understand the metabolism of *S. algae* when it encounters different electron  
290 acceptors such as MnIV and nitrate and the strategies the bacterium develops to adapt its energetic  
291 pathways. A transcriptomic analysis revealed major differences in the expression of genes involved in  
292 central metabolism such as amino acid synthesis and ammonium assimilation linked to the use of  
293 different electron acceptors. Furthermore, *katE*, a gene encoding for a putative catalase/peroxidase, was  
294 highly expressed in both conditions but with a greater relative median value under MnIV condition  
295 suggesting an important role.

296

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301

- 303 Aigle A, Michotey V & Bonin P (2015) Draft-genome sequence of *Shewanella* algae strain C6G3. *Stand*  
304 *Genomic Sci* **10**.
- 305 Aigle A, Bonin P, Iobbi-Nivol C, Mejean V & Michotey V (2017) Physiological and transcriptional approaches  
306 reveal connection between nitrogen and manganese cycles in *Shewanella* algae C6G3. *Scientific Reports* **7**:  
307 44725.
- 308 Amrani A, Bergon A, Holota H, Tamburini C, Garel M, Ollivier B, Imbert J, Dolla A & Pradel N (2014)  
309 Transcriptomics reveal several gene expression patterns in the piezophile *Desulfovibrio hydrothermalis* in  
310 response to hydrostatic pressure. *PLoS One* **9**: e106831.
- 311 Anders S & Huber W (2010) Differential expression analysis for sequence count data. *Genome Biol* **11**: R106.
- 312 Anschutz P, Dedieu K, Desmazes F & Chaillou G (2005) Speciation, oxidation state, and reactivity of  
313 particulate manganese in marine sediments. *Chem Geol* **218**: 265-279.
- 314 Balch WE, Fox GE, Magrum LJ, Woese CR & Wolfe RS (1979) Methanogens - Re-Evaluation of a Unique  
315 Biological Group. *Microbiol Rev* **43**: 260-296.
- 316 Baumann P, Baumann L & Mandel M (1971) Taxonomy of marine bacteria: the genus *Beneckeia*. *J Bacteriol*  
317 **107**: 268-294.
- 318 Beleneva IA, Magarlamov TY, Eliseikina MG & Zhukova NV (2009) Biochemical and pathogenic properties of  
319 the natural isolate of *Shewanella* algae from Peter the great bay, sea of Japan. *J Invertebr Pathol* **102**: 250-255.
- 320 Beliaev AS, Klingeman DM, Klappenbach JA, Wu L, Romine MF, Tiedje JM, Nealson KH, Fredrickson JK &  
321 Zhou J (2005) Global transcriptome analysis of *Shewanella oneidensis* MR-1 exposed to different terminal  
322 electron acceptors. *J Bacteriol* **187**: 7138-7145.
- 323 Benjamini Y & Hochberg Y (1995) Controlling the false discovery rate-a practical and powerful approach to  
324 multiple testing. *J Roy Stat Soc B Met* **57**: 289-300.
- 325 Bonin P, Omnes P & Chalamet A (1998) Simultaneous occurrence of denitrification and nitrate ammonification  
326 in sediments of the french mediterranean coast. *Hydrobiologia* **389**: 169-182.
- 327 Caccavo F, Blakemore RP & Lovley DR (1992) Isolation and characterisation of a Fe(III)-reducing and  
328 Mn(IV)-reducing microorganisms from Great Bay, New Hampshire. *Abstr Pap Am Chem S* **203**: 1236GEOC.
- 329 Coates JD, Councell T, Ellis DJ & Lovley DR (1998) Carbohydrate oxidation coupled to Fe(III) reduction, a  
330 novel form of anaerobic metabolism. *Anaerobe* **4**: 277-282.
- 331 Corker H & Poole RK (2003) Nitric oxide formation by *Escherichia coli*. Dependence on nitrite reductase, the  
332 NO-sensing regulator Fnr, and flavohemoglobin Hmp. *J Biol Chem* **278**: 31584-31592.
- 333 Emerson S & Hedges J (2006) *6.11 Sediment Diagenesis and Benthic Flux*. Elsevier, Netherlands.
- 334 Fernandes SO, Javanaud C, Michotey VD, Guasco S, Anschutz P & Bonin P (2016) Coupling of bacterial  
335 nitrification with denitrification and anammox supports N removal in intertidal sediments (Arcachon Bay,  
336 France). *Estuar Coast Shelf S* **179**: 39-50.
- 337 Fernandes SO, Javanaud C, Aigle A, Michotey VD, Guasco S, Deborde J, Deflandre B, Anschutz P & Bonin PC  
338 (2015) Anaerobic nitrification-denitrification mediated by Mn-oxides in meso-tidal sediments: Implications for  
339 N<sub>2</sub> and N<sub>2</sub>O production. *Journal of Marine Systems* **144**: 1-8.
- 340 Fredrickson JK, Romine MF, Beliaev AS, *et al.* (2008) Towards environmental systems biology of *Shewanella*.  
341 *Nat Rev Microbiol* **6**: 592-603.
- 342 Hansel CM (2017) Manganese in Marine Microbiology. *Adv Microb Physiol* **70**: 37-83.
- 343 Heidelberg JF, Paulsen IT, Nelson KE, *et al.* (2002) Genome sequence of the dissimilatory metal ion-reducing  
344 bacterium *Shewanella oneidensis*. *Nat Biotechnol* **20**: 1118-1123.
- 345 Javanaud C, Michotey V, Guasco S, Garcia N, Anschutz P, Canton M & Bonin P (2011) Anaerobic ammonium  
346 oxidation mediated by Mn-oxides: from sediment to strain level. *Res Microbiol* **162**: 848-857.
- 347 Laha S & Luthy RG (1990) Oxidation of aniline and other primary aromatic amines by Manganese-dioxide.  
348 *Environ Sci Technol* **24**: 363-373.
- 349 Lian Y, Yang Y, Guo J, Wang Y, Li X, Fang Y, Gan L & Xu M (2016) Electron acceptor redox potential  
350 globally regulates transcriptomic profiling in *Shewanella decolorationis* S12. *Sci Rep* **6**: 31143.
- 351 Lin H & Taillefert M (2014) Key geochemical factors regulating Mn(IV)-catalyzed anaerobic nitrification in  
352 coastal marine sediments. *Geochim Cosmochim Acta* **133**: 17-33.
- 353 Luther GW, Sundby B, Lewis BL, Brendel PJ & Silverberg N (1997) Interactions of manganese with the  
354 nitrogen cycle: Alternative pathways to dinitrogen. *Geochimica Et Cosmochimica Acta* **61**: 4043-4052.
- 355 Pickering BS & Oresnik IJ (2008) Formate-dependent autotrophic growth in *Sinorhizobium meliloti*. *J Bacteriol*  
356 **190**: 6409-6418.

357 Richardson DJ, Butt JN, Fredrickson JK, *et al.* (2012) The 'porin-cytochrome' model for microbe-to-mineral  
358 electron transfer. *Mol Microbiol* **85**: 201-212.  
359 Shi L, Rosso KM, Clarke TA, Richardson DJ, Zachara JM & Fredrickson JK (2012) Molecular Underpinnings  
360 of Fe(III) Oxide Reduction by *Shewanella Oneidensis* MR-1. *Front Microbiol* **3**: 50.  
361 Simon J (2002) Enzymology and bioenergetics of respiratory nitrite ammonification. *FEMS Microbiol Rev* **26**:  
362 285-309.  
363 Simpson PJ, Richardson DJ & Codd R (2010) The periplasmic nitrate reductase in *Shewanella*: the resolution,  
364 distribution and functional implications of two NAP isoforms, NapEDABC and NapDAGHB. *Microbiology*  
365 **156**: 302-312.  
366 Tang YJ, Meadows AL, Kirby J & Keasling JD (2007) Anaerobic central metabolic pathways in *Shewanella*  
367 *oneidensis* MR-1 reinterpreted in the light of isotopic metabolite labeling. *J Bacteriol* **189**: 894-901.  
368 van Heeswijk WC, Westerhoff HV & Boogerd FC (2013) Nitrogen assimilation in *Escherichia coli*: putting  
369 molecular data into a systems perspective. *Microbiol Mol Biol Rev* **77**: 628-695.  
370 Wang H & Gunsalus RP (2003) Coordinate regulation of the *Escherichia coli* formate dehydrogenase fdnGHI  
371 and fdhF genes in response to nitrate, nitrite, and formate: roles for NarL and NarP. *J Bacteriol* **185**: 5076-5085.  
372 White GF, Edwards MJ, Gomez-Perez L, Richardson DJ, Butt JN & Clarke TA (2016) Mechanisms of Bacterial  
373 Extracellular Electron Exchange. *Adv Microb Physiol* **68**: 87-138.  
374 Wilkinson JF (1963) Carbon and energy storage in bacteria. *J Gen Microbiol* **32**: 171-176.

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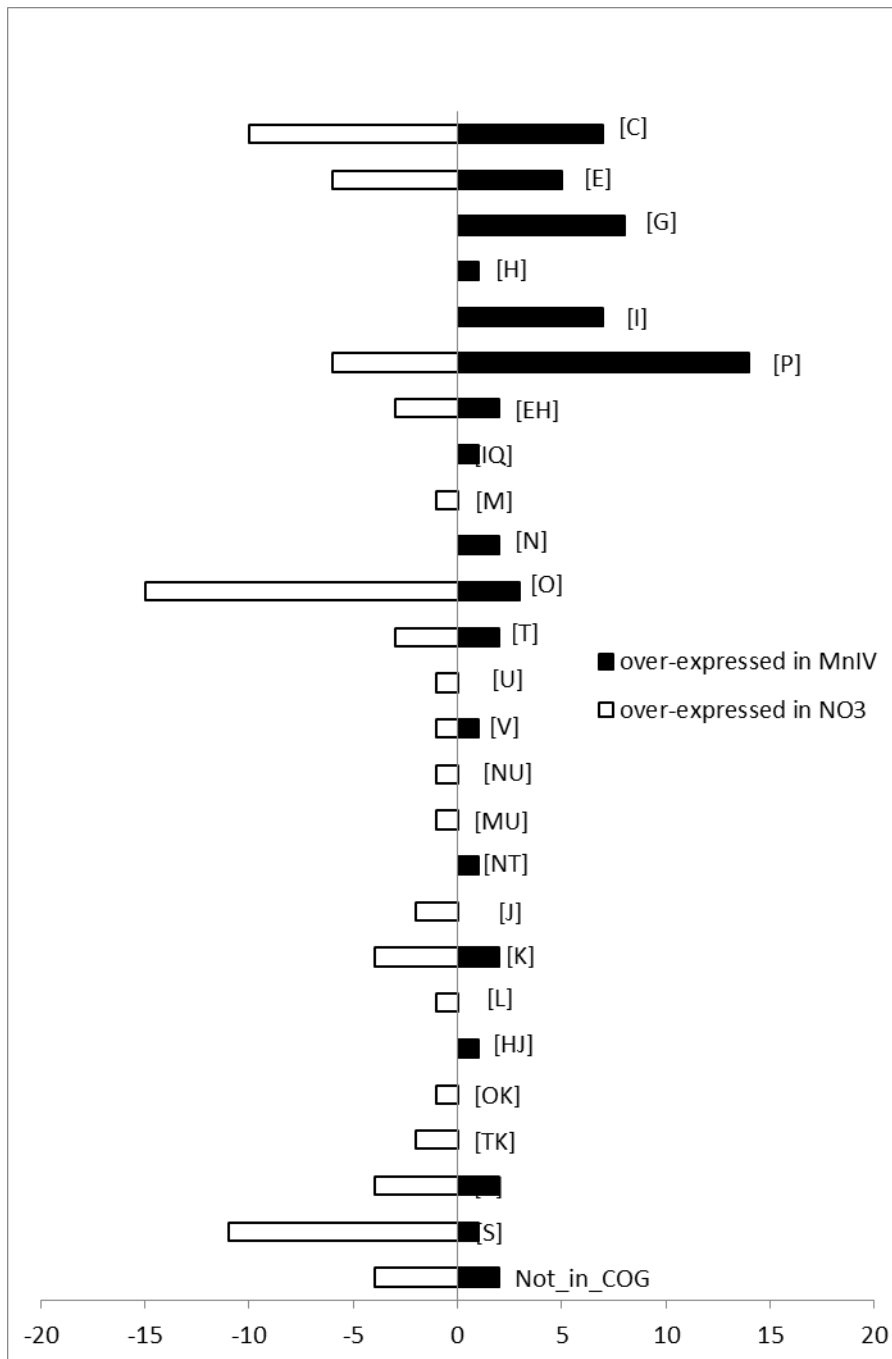


Fig.1. Over-expressed genes in manganese oxide or nitrate condition based on genes belonging to a COG categories (adjusted p-value <0.05). C, energy production and conversion; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; G, carbohydrate transport and metabolism; H, coenzyme metabolism; I, lipid metabolism; J, translation, ribosomal structure, and biogenesis; K, transcription; L, DNA replication, recombination, and repair; M, cell envelope biogenesis, outer membrane; N, cell motility and secretion; O, posttranslational modification, protein turnover, and chaperones; P, inorganic ion transport and metabolism; Q, secondary metabolite biosynthesis, transport, and catabolism; R, general function prediction only; S, function unknown; T, signal transduction mechanisms; V, defense mechanisms

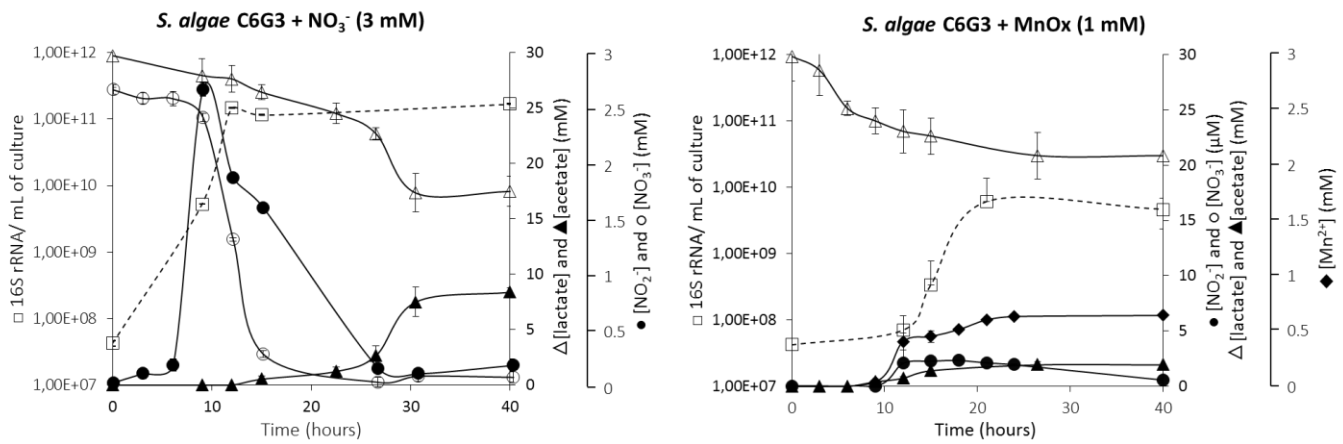


Fig. 2: Kinetic of growth of *S. algae* C6G3 in anaerobiosis and in the presence of nitrate or Mn(IV) as electron acceptor in minimal medium with lactate as electron donor. Bars represent the standard deviation.

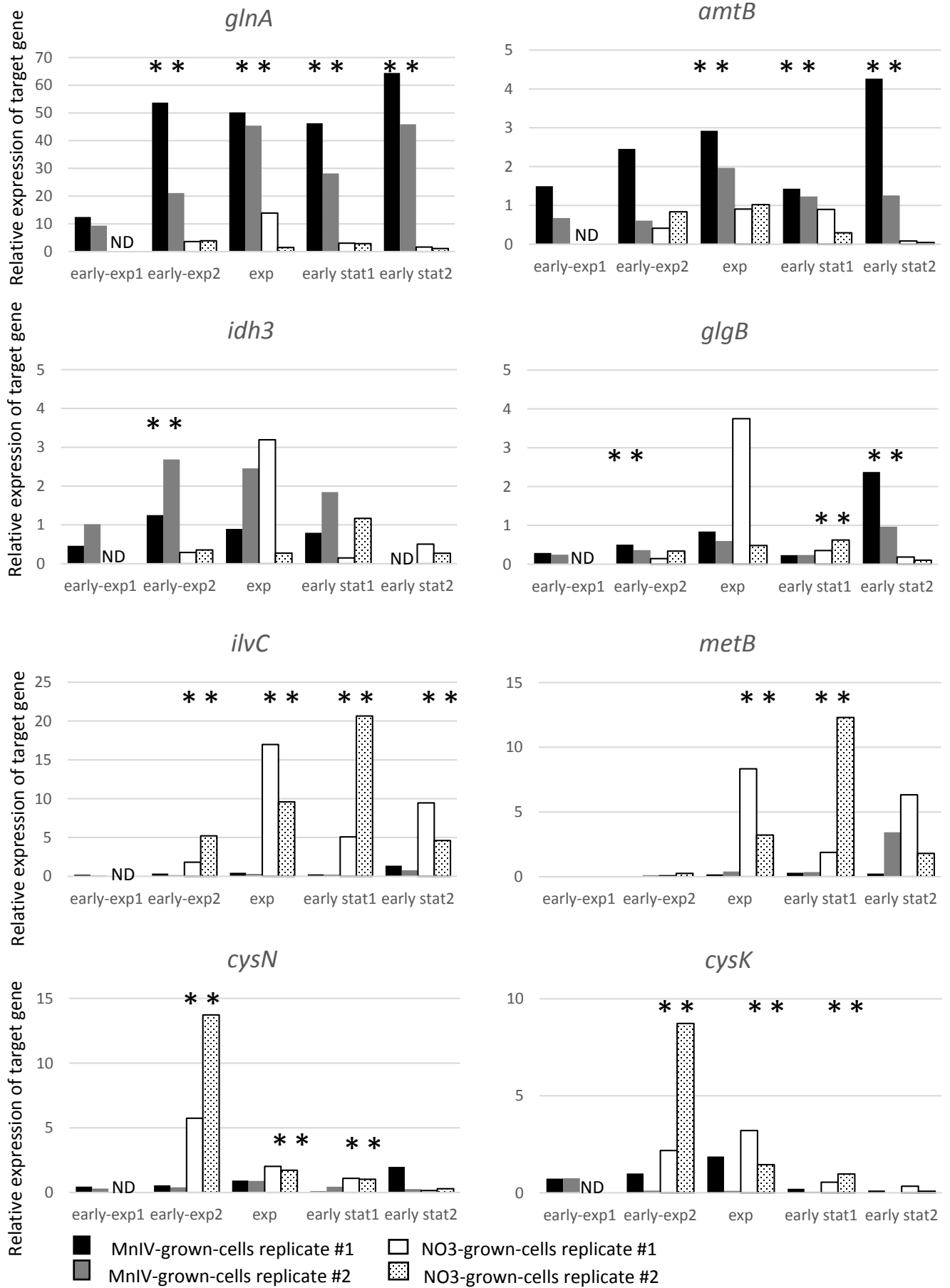


Fig.3. Gene expression level relative to *rpoD* of ammonium, sulfate assimilation and aa synthesis pathway along anaerobic bacterial growth with nitrate or manganese-oxide as the electron acceptor. *amtB*: ammonium transporteur, *glnA*: glutamine synthesis, *IDH*: 2 oxoglutarate synthesis, *cysN*: sulfate assimilation pathway, *cysK*: cystein synthesis, *ilvC*: valine synthesis pathway, *metB*: methionine synthesis. \*denotes difference in expression level between condition. ND: Not determined



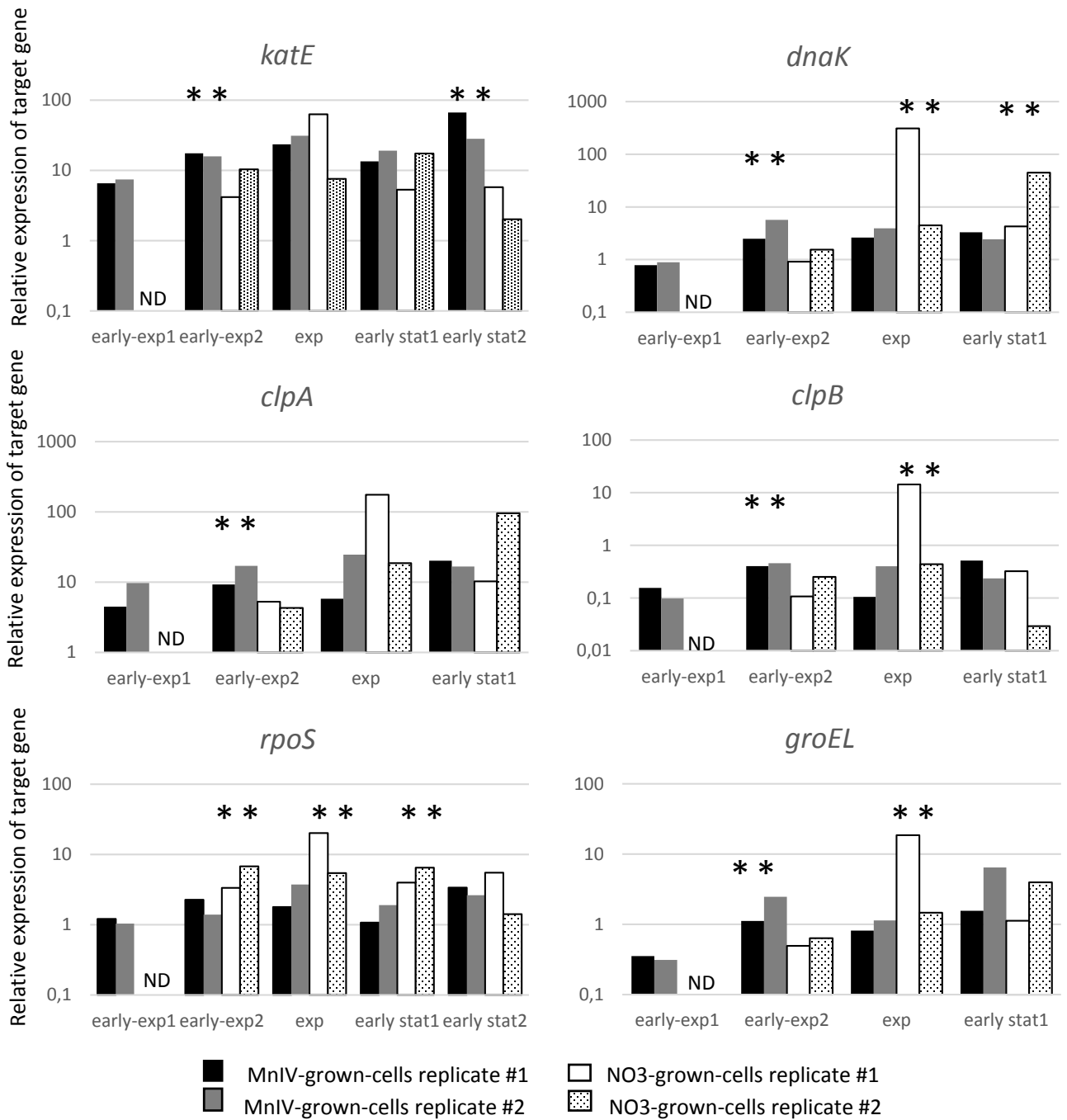


Fig.4. Genes of stress response. Expression level relative to *rpoD*  
 ATP-dependent Clp protease ATP-binding subunit *clpA*; katalase, *katE*; chaperonin GroEL, *groEL*, chaperone protein DnaK, *dnaK*.; \* denotes difference in expression level between conditions. ND: Not determined.

**The nature of electron acceptor (MnIV/NO<sub>3</sub>) triggers differential expression of genes associated with stress and ammonium limitation responses in *Shewanella algae* C6G3**

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Table S1. Target gene characteristics: primer sequence, fragment size, annealing temperature (T<sub>m</sub>) and references.

Primer	Target gene	Primer sequence (5' → 3')	T <sub>m</sub>	Fragment size	Ref.
rpoD-F rpoD-R	<i>rpoD</i>	TAC GCC GAA GGC CTG AAA AT GAT AGA CAG GCC GGT TTC GG	62 °C	90 pb	Aigle et al 2017
cysK-F cysK-R	<i>cysK</i>	AGG CCT GTT GAC CAA AGA CA TCA ACT TGT AAC CTC GGG CG	62 °C	98 pb	This study
katE-F katE-R	<i>katE</i>	TGC AGG AGA GGT TGA TGC AG TGA AGG GTC CAG CAA GGG AT	62 °C	98 pb	This study
glgB-F glgB-R	<i>glgB</i>	GCC GAA CTC TTG GTG CCT TA GTT GAT AGC CCC AGG AAC CG	62 °C	100 pb	This study
glnA-F glnA-R	<i>glnA</i>	ACA GCC ACC TTT ATG CCC AA GCA AAT AGG TTG GTG CCG TC	62 °C	95 pb	This study
amt-F amt-R	<i>amt</i>	GCC AGG TTA ACC CTA TCC CG AGA ACC ACC GTT GAA ACC GA	62 °C	95 pb	This study
cysN-F cysN-R	<i>cysN</i>	TTG GTA TCA GGG CGA AAC CC CTG ACA TAC TGC ACC GGG AA	62 °C	99 pb	This study
dnaK-F dnaK-R	<i>dnaK</i>	TCT TTG GTG ATC AGT GGG GC CAC TGG CTCA CCC AGG AAA T	62 °C	101pb	This study
groEL-F groEL-R	<i>groEL</i>	GGG CGT GAA TGT TCT GGC TA TCT TTG GTG ATC AGT GGG GC	62 °C	102pb	This study
katE-F katE-R	<i>katE</i>	TGC AGG AGA GGT TGAT GCA G TGA AGG GTC CAG CAA GGG A	62 °C	98bp	This study
IDH3-F IDH3-R	<i>IDH3</i>	GAA AGT GGC CCG TGA AGT C GTT TTC CGG GTT CAT CAC CA	60 °C	100pb	This study
clpA-F clpA-R	<i>clpA</i>	AAG GTG GAC ATT CAC GAG CC GGG TGT AAC GCA CTC CAT GA	62 °C	97pb	This study
clpB-F clpB-R	<i>clpB</i>	ATC CTA ACC GGC CCA TAG GT GTG TCG AAC AGG AAC TTG GC	62 °C	97pb	This study
metB-F metB-R	<i>metB</i>	GCC GTG ATT GCC AAA GAT CC GGT CAG GTA GCT GTC GAA GG	62 °C	96 pb	This study
ilvC-F ilvC-R	<i>ilvC</i>	AAT GGC GAC GGT TTG GAG AT CCG ACT TCA CCT CGG CAA TA	62 °C	100 pb	This study

Table S2: Absolute quantification of cDNAs of interest per ng of RNA added in the reverse transcription reactions, in early (1 and 2) exponential, and stationary (1 and 2) phases of growth of *S* algae grown with nitrate or MnIV as electron acceptor. Value for the 2 independent cultures for each condition (MnIV or NO<sub>3</sub>) are shown

	early-exp1	early-exp2	exp	early stat1	early stat2	median value for both duplicate
rpoD-1-ngARN-Mn	6495.24	2299.51	1458.86	7076.27	220.59	1486.81
rpoD-2-ng ARN-Mn	3904.23	1335.19	743.75	1773.24	1514.76	
rpoD-1-ngARN-NO <sub>3</sub>	ND	25336.46	377.95	15699.54	5370.15	5474.30
rpoD-2-ng ARN-NO <sub>3</sub>	ND	5578.45	4790.86	1038.03	8301.43	
katE1-1-ngARN-Mn	42584.54	40288.33	34284.51	95172.72	14713.48	34081.89
KatE-2-ng ARN-Mn	29005.44	21205.85	23227.09	33879.27	42714.03	
katE1-1-ngARN-NO <sub>3</sub>	ND	105862.42	23758.27	83339.97	30979.72	33645.37
KatE-2-ng ARN-NO <sub>3</sub>	ND	57859.39	36311.02	18090.86	16750.27	
glgB-1-ngARN-Mn	1869.37	1154.82	1229.40	1653.14	523.79	839.30
glgB-2-ng ARN-Mn	952.20	483.14	443.34	416.63	1463.71	
glgB1-ngARN-NO <sub>3</sub>	ND	3631.94	1417.17	5511.62	988.66	1646.86
glgB-2-ng ARN-NO <sub>3</sub>	ND	1876.54	2305.59	645.63	856.25	
glnA-1-ngARN-Mn	81049.66	123506.96	73248.25	327575.89	14213.05	59791.98
glnA-2-ng ARN-Mn	36466.36	28197.21	33794.79	50007.44	69576.51	
glnA-1-ngARN-NO <sub>3</sub>	ND	91628.78	5255.36	47389.23	8812.69	8978.83
glnA-2-ng ARN-NO <sub>3</sub>	ND	21747.11	6993.46	2998.06	9144.97	
ilvC-1-ngARN-Mn	1333.69	826.75	678.82	1753.44	301.10	540.71
ilvC2-ng ARN-Mn	424.56	207.25	236.58	402.60	1176.47	
ilvC1-ngARN-NO <sub>3</sub>	ND	46246.53	6414.42	79707.23	50777.09	42142.48
ilvC-2-ng ARN-NO <sub>3</sub>	ND	29050.54	45975.55	21439.82	38309.41	
phoP-1-ngARN-Mn	11049.49	9601.85	5378.20	11879.61	877.62	6855.89
phoP-2-ng ARN-Mn	9094.41	5746.03	5477.82	7965.75	14321.55	
phoP-ngARN-NO <sub>3</sub>	ND	10050.80	4335.99	18318.20	22126.69	8966.92
phoP-2-ng ARN-NO <sub>3</sub>	ND	5716.98	9061.74	8005.89	8872.09	
ropS-1-ngARN-Mn	7834.70	5211.83	2601.29	7562.23	743.03	3074.26
ropS-2-ng ARN-Mn	4050.40	1854.12	2773.34	3375.17	3986.71	
ropS-1-ngARN-NO <sub>3</sub>	ND	84787.33	7623.34	62474.59	29503.58	27731.14
ropS-2-ng ARN-NO <sub>3</sub>	ND	37827.97	25958.70	6715.20	11727.45	
amtB-1-ngARN-Mn	9700.06	5644.67	4260.78	10124.17	940.87	2042.34
amtB-2-ng ARN-Mn	2632.72	811.34	1463.54	2180.80	1903.89	
amtB-1-ngARN-NO <sub>3</sub>	ND	10415.35	342.36	14083.75	434.93	2546.16
amtB-2-ng ARN-NO <sub>3</sub>	ND	4657.40	4886.43	303.82	372.74	
cysK-1-ngARN-Mn	4755.36	2292.21	2730.06	1491.15	27.45	128.43
cysK-2-ng ARN-Mn	2929.67	158.14	79.44	98.71	0.00	
cysK-1-ngARN-NO <sub>3</sub>	ND	55302.10	1213.15	8587.36	1829.35	4399.44

cysK-2-ng ARN-NO3	ND	48680.86	6969.53	1008.51	685.09		
cysN-1-ng ARN-Mn	2968.42	1269.33	1341.75	581.76	436.18		622.88
cysN-2-ng ARN-Mn	1197.14	541.64	664.01	798.02	392.81		
cysN-1-ng ARN-NO3	ND	145449.12	762.61	17252.64	836.04		5339.74
cysN-2-ng ARN-NO3	ND	76586.95	8237.84	1063.39	2441.64		
impG-1-ng ARN-Mn	2887.61	468.74	266.81	831.90	129.82		129.06
impG-2-ng ARN-Mn	923.80	128.31	67.08	117.67	118.80		
impG-1-ng ARN-NO3	ND	4091.03	327.16	467.03	185.81		264.18
impG-2-ng ARN-NO3	ND	2022.83	136.06	201.20	91.28		
metB-1-ng ARN-Mn	39.54	111.58	262.75	2143.65	53.48		279.38
metB-2-ng ARN-Mn	41.13	161.79	296.01	632.11	5204.83		
metB-1-ng ARN-NO3	ND	1743.54	3151.11	29435.18	33988.97		13859.03
metB-2-ng ARN-NO3	ND	1426.87	15428.42	12767.69	14950.37		
aceA-1-ng ARN-Mn	404.67	335.09	365.81	529.71			437.08
aceA-2-ng ARN-Mn	1841.14	501.77	499.46	374.71			
aceA-1-ng ARN-NO3	ND	14060.91	528.61	9030.90	7836.71		2653.92
aceA-2-ng ARN-NO3	ND	3162.80	2145.04	1016.46	1035.42		
aceB-1-ng ARN-Mn	15.22	4.01	7.66	8.14	ND		7.90
aceB-2-ng ARN-Mn	46.05	0.00	10.34	8.78	ND		
aceB-1-ng ARN-NO3	ND	320.46	102.63	0.00	205.21		102.21
aceB-2-ng ARN-NO3	ND	101.79	144.74	0.00	57.12		
clpA-1-ng ARN-Mn	29156.47	21295.70	8491.87	143010.12	ND		22054.73
clpA-2-ng ARN-Mn	37951.11	22813.76	18346.82	29660.86	ND		
clpA-1-ng ARN-NO3	ND	133583.58	66358.74	161244.98	269627.47		116544.63
clpA-2-ng ARN-NO3	ND	24002.63	89376.14	99505.68	162236.66		
clpB-1-ng ARN-Mn	1011.61	928.19	153.06	3645.04	ND		516.07
clpB-2-ng ARN-Mn	382.51	612.56	298.30	419.58	ND		
clpB-1-ng ARN-NO3	ND	2724.05	5430.77	5062.44	5151.05		3893.24
clpB-2-ng ARN-NO3	ND	1404.70	2104.60	30.41	9947.10		
dnaK-1-ng ARN-Mn	5108.80	5723.75	3815.04	23368.02	ND		5020.10
dnaK-2-ng ARN-Mn	3481.55	7568.59	2916.27	4316.46	ND		
dnaK-1-ng ARN-NO3	ND	23202.46	116840.11	67171.24	162488.17		56752.60
dnaK-2-ng ARN-NO3	ND	8632.92	21428.57	46333.96	221854.30		
IDH3-1-ng ARN-Mn	2994.22	2876.79	1306.72	5645.21	ND		3075.27
IDH3-2-ng ARN-Mn	3971.72	3587.42	1827.55	3273.74	ND		
IDH3-1-ng ARN-NO3	ND	7361.28	1206.87	2328.58	2710.93		2101.60
IDH3-2-ng ARN-NO3	ND	1970.10	1301.59	1212.98	2233.09		
groEL-1-ng ARN-Mn	2293.31	2564.04	1184.43	11006.34	ND		2926.32
groEL-2-ng ARN-Mn	1213.05	3288.60	842.86	11436.53	ND		
groEL-1-ng ARN-NO3	ND	12510.74	7024.71	17601.16	8583.02		7803.86
groEL-2-ng ARN-NO3	ND	3529.53	7011.11	4100.57	31333.75		

Table S3. Summary statistics of RNA-seq runs

TGML_ID	Sample_ID	Bases	≥ Q20	Reads	Reads Mapped	% Mapping
581	NO3_1_C6G3	63,727,883	55,673,431	469,736	390,727	83.18
582	NO3_2_C6G3	81,989,316	72,118,405	563,025	509,908	90.57
583	MnOx_1_C6G3	58,840,970	51,594,018	411,316	384,501	93.48
584	MnOx_2_C6G3	26,980,852	24,279,815	281,007	267,923	95.34

Table S4. List of differentially expressed genes under MnOx conditions as NO<sub>3</sub><sup>-</sup> with an adjusted p-value <0.05. The genes discussed in the article are indicated in bold.

Locus_tag JGI SA002_	Product_name	Code COG	COG	KEEG Orthology	E.C. number	Log2 fold change MnOx vs. NO3-
<b>00257</b>	<b>Malate synthase, aceB, glcB</b>	<b>C</b>	<b>2225</b>	<b>K01638</b>	<b>2.3.3.9</b>	<b>3.50</b>
00704	Sodium pump decarboxylases, oadG (TC:3.B.1)	C	3630	K01573	4.1.1.3	2.97
00705	Oxaloacetate decarboxylase, oadA (00705+00706)	C	5016	K01571	4.1.1.3	2.40 2.99
00708	Sodium ion-translocating decarboxylase, oadB (TC:3.B.1)	C	1883	K01572	4.1.1.3	3.14
<b>05135</b>	<b>Anaerobic dehydrogenases, fdhA (TC:5.A.3)</b>	<b>C</b>	<b>0243</b>	<b>K00123</b>	<b>1.2.1.2</b>	<b>1.74</b>
<b>05137</b>	<b>Formate dehydrogenase, fdhC</b>	<b>C</b>	<b>2864</b>	<b>K00127</b>	<b>1.2.1.2</b>	<b>2.33</b>
00475	L-lactate dehydrogenase, lldE	C	2048	K18928		-1.67
03282	Acetate kinase, ackA	C	0282	K00925	2.7.2.1	-1.92
<b>03376</b>	<b>Formate dehydrogenase, nitrate inducible, fdnG (TC:5.A.3) (03376+03377+03378)</b>	<b>C</b>	<b>0243</b>	<b>K00123</b>	<b>1.2.1.2</b>	<b>-2.83</b> <b>-3.19</b> <b>-3.40</b>
<b>03379</b>	<b>Formate dehydrogenase fdnH</b>	<b>C</b>	<b>0437</b>	<b>K00124</b>		<b>-3.70</b>
<b>03380</b>	<b>Formate dehydrogenase, fdnI</b>	<b>C</b>	<b>2864</b>	<b>K00127</b>		<b>-4.25</b>
<b>03386</b>	<b>Formate dehydrogenase, fdhD</b>	<b>C</b>	<b>1526</b>	<b>K02379</b>		<b>-6.11</b>
<b>05459</b>	<b>Formate dehydrogenase, fdoI</b>	<b>C</b>	<b>2864</b>	<b>K00127</b>		<b>-5.63</b>
<b>05460</b>	<b>Formate dehydrogenase, fdoH</b>	<b>C</b>	<b>0437</b>	<b>K00124</b>		<b>-4.21</b>
<b>05461</b>	<b>Formate dehydrogenase, fdoG, fdfH (05461+05462) (TC :5.A.3)</b>	<b>C</b>	<b>0243</b>	<b>K00123</b>	<b>1.2.1.2</b>	<b>-3.62</b>
<b>02930</b>	<b>Glutamine synthetase type I, glnA</b>	<b>E</b>	<b>0174</b>	<b>K01915</b>	<b>6.3.1.2</b>	<b>2.68</b>
<b>04432</b>	<b>Nitrogen regulatory protein P-II family, glnK</b>	<b>E</b>	<b>0347</b>	<b>K04752</b>		<b>5.52</b>
<b>03298</b>	<b>Cysteine synthase A, cysK</b>	<b>E</b>	<b>0031</b>	<b>K01738</b>	<b>2.5.1.47</b>	<b>2.55</b>
<b>02212</b>	<b>Sulfate adenylyltransferase, cysD</b>	<b>EH</b>	<b>0175</b>	<b>K00957</b>	<b>2.7.7.4</b>	<b>3.72</b>
<b>02218</b>	<b>Phosphoadenylyl-sulfate reductase thioredoxin-dependent, cysH</b>	<b>EH</b>	<b>0175</b>	<b>K00390</b>	<b>1.8.4.8</b>	<b>3.86</b>
<b>05500</b>	<b>Asparagine synthase, asnB</b>	<b>E</b>	<b>0367</b>	<b>K01953</b>	<b>6.3.5.4</b>	<b>6.57</b>

03348	Cystathionine gamma-synthase, metB	E	0626	K01739	2.5.1.48	-3.21
03349	Aspartate kinase, metL	E	0527	K12525	1.1.1.3	-3.75
03351	5,10-methylenetetrahydrofolate reductase (NAD(P)), metF	E	0685	K00297	1.5.1.20	-3.83
05416	5-methyltetrahydrofolate--homocysteine methyltransferase, metH (05414+05415+05416+05417)	E	1410	K00548	2.1.1.13	-3.53
03382	Selenocysteine synthase, selA (3382+3383)	E	1921	K01042	2.9.1.1	-5.14 -2.85
03737	Aspartate kinase, thrA	E	0527	K12524	1.1.1.3	1.89
02783	Ketol-acid reductoisomerase, ilvC (02783+02784)	EH	0059	K00053	1.1.1.86	-3.93 -4.59
04508	Acetolactate synthase, ilvB, ilvG, ilvI	EH	0028	K01652	2.2.1.6	-1.99
01676	6-phosphogluconolactonase, pgl (01676+01677)	G	0363	K01057	3.1.1.31	2.38
01732	4-alpha-glucanotransferase, malQ (01732+01733)	G	1640	K01196	2.4.1.25	5.54 4.75
01734	Alpha-1,4-glucan:alpha-1,4-glucan 6-glycosyltransferase, glgB	G	0296	K00700	2.4.1.18	4.23
01735	Glycogen debranching enzyme	G	1523	K01196	3.2.1.33	3.66
01738	Glycogen/starch/alpha-glucan phosphorylases, glgP	G	0058	K00688	2.4.1.1	2.65
01739	Glucose-1-phosphate adenylyltransferase, glgC	G	0448	K00975	2.7.7.27	3.22
01742	Glycogen synthase, glgA	G	0297	K00703	2.4.1.21	2.49
02214	Uroporphyrinogen-III C-methyltransferase, cobA (02213+02214)	H	0007	K02303	2.1.1.107	4.04
02545	Alpha-L-glutamate ligases, rimK	HJ	0189	K05844	6.3.2.-	4.58
00520	Acyl-CoA hydrolase	I	1607	K01073	3.1.2.20	4.40
01239	Acyl-CoA dehydrogenases, fadE	I	1960	K06445	1.3.99.2	2.32
01583	Acetyl-CoA dehydrogenase, fadE (01583+1584)	I	1960	K06445	1.3.99.3	2.06 1.81
05346	3-hydroxyacyl-CoA dehydrogenase, fadB	I	1250	K01825	4.2.1.17/ 5.3.3.8	1.77
05347	Fatty oxidation complex, beta subunit, fadA (05347+05348+05349)	I	0183	K00632	2.3.1.16	2.03 2.58
04827	Acyl-CoA synthetases (TC:4.C.1), fadD	IQ	0318	K01897	6.2.1.3	1.80
03384	Selenocysteine-specific elongation factor, selB	J	3276			-4.25
05662	LSU ribosomal protein L29P, rpmC	J	0255	K02904		-2.00
04899	'Cold-shock' DNA-binding domain, cspA	K	1278	K03704		3.07
04948	RNA polymerase, sigma 38 subunit, rpoS	K	0568			1.94
00591	Zn(II)-responsive transcriptional regulator, zntR	K	0789	K13638		-3.55
01336	Transcriptional regulator, MarR family	K	1846			-3.32
01904	Transcriptional regulator, LysR family, metR	K	0583	K03576		-2.70
03691	Transcriptional regulator, LysR family, nhaR	K	0583	K03717		-1.97
00380	AAA domain, sbcC	L	0419	K03546		-2.28
02357	Outer membrane protein (porin)	M	3203			-1.84
02631	Outer membrane protein TolC, TC:1.B.17	MU	1538			-4.46

00350	Flagellin FlaG, flaG	N	1334	K06603		1.92
00351	Flagellin, fliC	N	1344	K02406		2.22
00148	Methyl-accepting chemotaxis protein, mcp	NT	0840	K03406		2.83
00338	Flagellar biosynthesis/type III secretory pathway protein, fliH (TC:3.A.6)	NU	1317	K02411		-1.91
00485	Membrane-bound serine protease, nfeD (00483+00484+00485)	O	1030	K07403		4.90
00748	Subtilisin-like serine proteases	O	1404			3.88
<b>04897</b>	<b>ATP-dependent Clp protease ATP-binding subunit ClpA, clpA</b>	<b>O</b>	<b>0542</b>	<b>K03694</b>	<b>3.4.21.92</b>	<b>1.59</b>
00037	lon ATP-dependent Lon protease 00036+00037	O	0466	K01338	3.4.21.53	-2.03
00466	Molecular chaperone GrpE (heat shock protein), grpE	O	0576	K03687		-1.85
00886	Molecular chaperone, HSP90, htpG (00886+00887)	O	0326	K04079		-2.86
<b>01859</b>	<b>ATP-dependent Clp protease, clpB (01858+01859)</b>	<b>O</b>	<b>0542</b>	<b>K03695</b>		<b>-2.58</b>
01989	Subtilisin-like serine proteases (01989+01990+01991)	O	1404			-3.37
02096	GlyGly-CTERM domain (serine protease) (02096+02097+02098)	O	1404			-2.99
02252	AAA domain (Cdc48 subfamily), protein VasG (TC:3.A.23.1)	O	0542	K11907		-4.77
02658	Threonine peptidase, hslV	O	5405	K01419	3.4.25.2	-2.86
02659	ATP-dependent protease HslVU, hslU	O	1220	K03667		-2.13
03381	Formate dehydrogenase accessory protein, fdhE	O	3058	K02380		-4.11
03391	Predicted redox protein, regulator of disulfide bond formation	O	0425			-5.55
<b>03418</b>	<b>Chaperonin GroEL, groEL</b>	<b>O</b>	<b>0459</b>	<b>K04077</b>		<b>-3.07</b>
<b>04171</b>	<b>Chaperone protein DnaK, dnaK (TC:1.A.33)</b>	<b>O</b>	<b>0443</b>	<b>K04043</b>		<b>-2.73</b>
04497	Molecular chaperone, ibpA 04497+04498	O	0071	K04080		-3.13
05458	Formate dehydrogenase maturation protein, fdhE	O	3058	K02380		-5.82
01619	Hydrogenase accessory protein HypB (01619+01620)	OK	0387	K04652		-3.89
01044	TonB dependent receptor/TonB-dependent Receptor Plug Domain (TC:1.B.14)	P	1629	K02014		2.34
<b>02205</b>	<b>Adenylylsulfate kinase, cysC</b>	<b>P</b>	<b>0529</b>	<b>K00860</b>	<b>2.7.1.25</b>	<b>2.41</b>
02208	Di- and tricarboxylate transporters (TC:2.A.47) (02206+02207+02208)	P	0471			4.20 3.18
<b>02209</b>	<b>Sulfate adenylyltransferase, cysN</b>	<b>P</b>	<b>2895</b>	<b>K00956</b>	<b>2.7.7.4</b>	<b>4.32</b> <b>6.07</b>
<b>02219</b>	<b>Nitrite/Sulfite reductase, cysI (02219+02220+02221)</b>	<b>P</b>	<b>0369</b>	<b>K00381</b>	<b>1.8.1.2</b>	<b>4.97</b> <b>4.30</b>
<b>02222</b>	<b>Sulfite reductase (NADPH), cysJ</b>	<b>P</b>	<b>0369</b>	<b>K00380</b>	<b>1.8.1.2</b>	<b>5.60</b>
02606	TonB dependent receptor (TC:1.B.14) (02605+02606)	P	4771	K02014		2.69 3.01
<b>03501</b>	<b>Catalase (03500+03501), katE</b>	<b>P</b>	<b>0753</b>	<b>K03781</b>	<b>1.11.1.6</b>	<b>4.86</b> <b>4.56</b>

03616	Bacterioferritin, bfr	P	2193	K03594		2.12
<b>04433</b>	<b>Ammonium transporter, amtB (TC:1.A.11)</b>	<b>P</b>	<b>0004</b>	<b>K03320</b>		<b>6.09</b>
01335	Cation diffusion facilitator family transporter (TC:2.A.4)	P	1230			-3.64
03292	Outer membrane receptor proteins, mostly Fe transport (TonB) (TC:1.B.14)	P	1629	K02014		-2.41
03997	ATPase, P-type (transporting), HAD superfamily, subfamily IC/heavy metal translocating P-type ATPase (TC:3.A.3) (03996+03997+03998)	P	2217	K01534	3.6.3.3/ 3.6.3.5	-3.45 -2.59
<b>04430</b>	<b>Respiratory nitrite reductase, nrfA</b>	<b>P</b>	<b>3303</b>	<b>K03385</b>	<b>1.7.2.2</b>	<b>-2.49</b>
04805	Outer membrane receptor proteins, mostly Fe transport (TonB) (TC:1.B.14)	P	1629	K02014		-2.29
02934	Predicted permease, DMT superfamily, pecM (TC:2.A.7)	R	5006	K15269		2.92
04332	Protein related to penicillin acylase, pvdQ (04329+04330+04331+04332)	R	2366	K07116		2.58
00592	Predicted permease (TC:9.B.28), putative copper/zinc efflux protein	R	0701	K07089		-2.87
00730	Predicted esterase	R	0400			-1.75
01001	Extracellular nuclease, putative (01000+01001)	R	2374	K7004		-3.00
03387	Predicted transporter component	R	2391	K07112		-4.41
00506	Uncharacterized conserved protein	S	5507			3.15
00252	Uncharacterized protein conserved in bacteria	S	3228	K09933		-3.61
02247	Type VI secretion protein, icmF	S	3523	K11891		-3.34
02248	Type VI secretion-associated protein, impA	S	3515	K11910		-3.88
02255	Type VI secretion protein, impJ/vasE	S	3522	K11893		-3.29
02261	Type VI secretion protein, impG/vasA	S	3519	K11896		-4.17
02262	Type VI secretion system lysozyme-like protein	S	3518	K11905		-3.87
02263	Type VI secretion protein, impC	S	3517	K11900		-4.19
02264	Type VI secretion protein, impB	S	3516	K11901		-4.92
02704	Uncharacterized protein conserved in bacteria (02704+2705)	S	4582			-2.58
03311	Uncharacterized conserved protein (03311+03312)	S	2968	K09807		-2.99 -2.42
<b>02939</b>	<b>Signal transduction histidine kinase, nitrogen specific NRI, ntrB/glnG</b>	<b>T</b>	<b>3852</b>	<b>K07708</b>	<b>2.7.13.3</b>	<b>2.93</b>
<b>02940</b>	<b>Nitrogen metabolism transcriptional regulator NRII, ntrC/glnL</b>	<b>T</b>	<b>2204</b>	<b>K07712</b>		<b>3.51</b>
03696	Negative regulator of sigma E activity, rseA	T	3073	K03597		-2.07
03697	Sigma E regulatory protein, MucB/RseB, rseB (03697+03698)	T	3026	K03598		-2.15 -2.59
02250	Sigma-54 specific transcriptional regulator, vasH	TK	1221	K11908		-5.56
05562	Two component transcriptional regulator, winged helix family, phoP	TK	0745	K07660		-2.07
02258	FHA domain protein, impI, vasC	U	3456	K11894		3.68
00404	Cation/multidrug efflux pump (TC:2.A.6)	V	0841			3.29
02630	Multidrug resistance efflux pump	V	1566			-3.82



00070	Lysine-specific metallo-endopeptidase	K08646	3.4.24.20	2.97
02245	ImpA-related N-terminal, vasL (02245+02246)	K11911		-3.74
02249	Type VI secretion-associated protein, vasI (TC:3.A.23.1)	K11909		-4.31
03106	Conserved hypothetical protein 698 (TC:2.A.98)			2.82
03389	Sulphur transport (03389+03390)	K07112		-3.67
04527	TonB dependent receptor/TonB- dependent Receptor Plug Domain (TC:1.B.14)			-1.90
04506	OmpA family			2.07
05025	hypothetical protein			-2.00
<b>00064</b>	<b>decaheme c-type cytochrome, OmcA/MtrC family, mtrH</b>			<b>-1.90</b>
<b>00068</b>	<b>decaheme c-type cytochrome, DmsE family, mtrA</b>			<b>-2.44</b>
00378	hypothetical protein			-3.96
00475	Cysteine-rich domain			-1.67
00519	hypothetical protein			2.99
03974	Protein of unknown function (DUF1107)			-2.19
01871	hypothetical protein			-3.67
01993	Curlin associated repeat			-2.83
02089	hypothetical protein			-2.07
02232	hypothetical protein			1.99
02543	Conserved hypothetical protein 2001, proteobacterial 02543 +02544			5.77 5.25
02554	hypothetical protein			3.37
02555	N-linked glycosylation glycosyltransferase PglG			4.65
03104	Protein of unknown function (DUF3612)/Helix-turn-helix domain			2.07
05454	Ankyrin repeats (3 copies)			-1.87
04188	hypothetical protein			3.11
03150	Extracellular lipase, Pla-1/cef family 03150 + 03151			2.35 2.45
03389	Sulphur transport			-3.68
03502	Transposase			4.56
04332	Penicillin amidase			2.58

Table 5. Blastn *katE* (SA002\_03500+SA002\_03501) from *Shewanella algae* C6G3 against all *Shewanella* isolate available on JGI (n=62), and fitness BLAST significant results from JGI (Wetmore et al., 2015 and Rubin et al., 2015).

Genome name	Identities
<i>Shewanella algae</i> JCM 21037*	909/953 95%
<i>Shewanella algae</i> NBRC 103173*	909/953 95%

<i>Shewanella haliotis</i> JCM 14758*	908/953 95%
<i>Shewanella sp.</i> JCM 19057	907/953 95%
<i>Shewanella sp.</i> 38A_GOM-205m*	906/953 95%
<i>Shewanella algae</i> MARS 14*	905/953 95%
<i>Shewanella sp.</i> ECSMB14102	896/953 94%
<i>Shewanella chilikensis</i> JC5	878/953 92%
<i>Shewanella algae</i> ACDC*	864/951 91%

\*synteny conserved

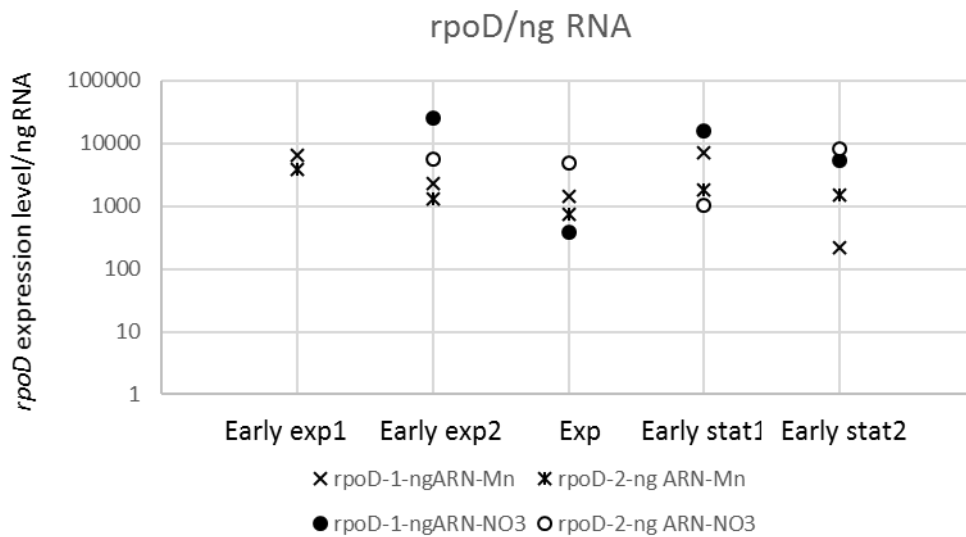


Fig S1. Absolute expression level of *rpoD* coding of the sigma 70 subunit of the RNA polymerase from duplicate cultures with either nitrate (round symbol) or manganese-oxide (cross symbol) as electron acceptor

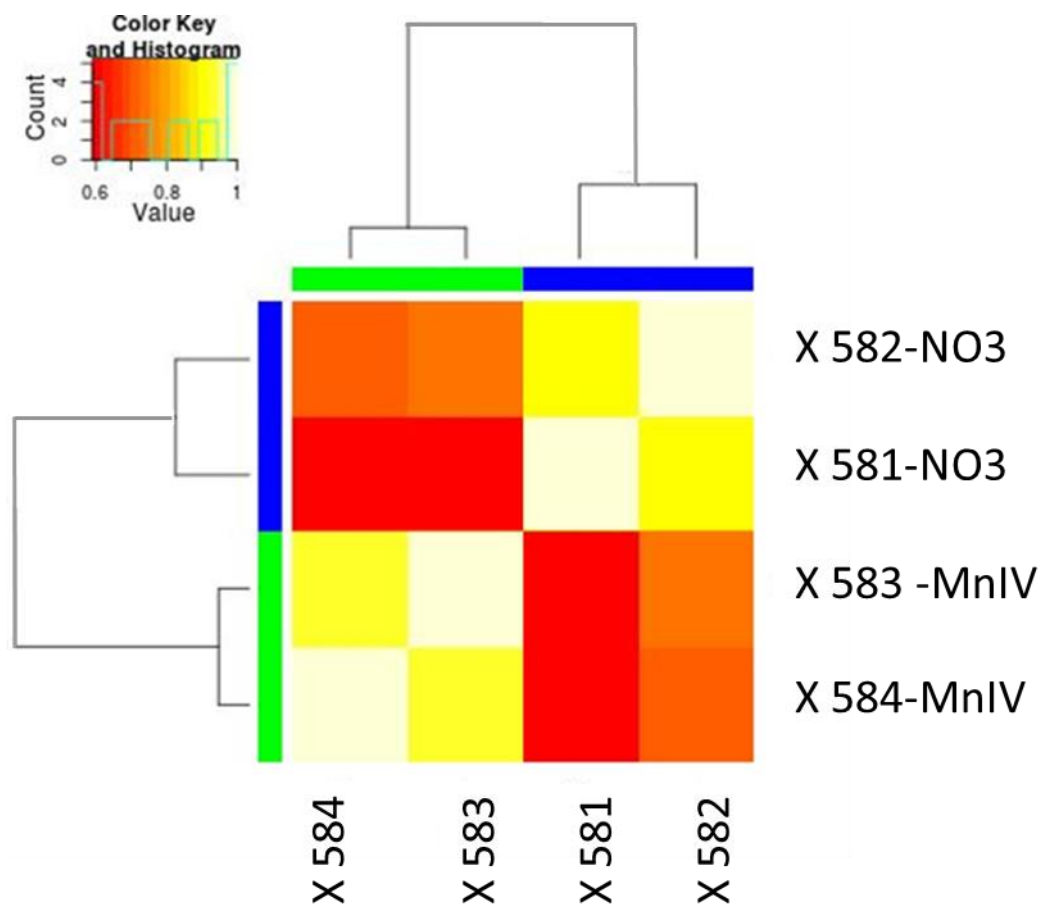


Fig S2. Correlation matrix between RNA-seq normalized expression levels of each independent culture obtained with DESeq.

The R package DESeq was used to analyze the differential expression of genes between the different culture conditions (Anders & Huber, 2010).

Anders S, Huber W (2010) Differential expression analysis for sequence count data. *Genome Biol* 11: R106.

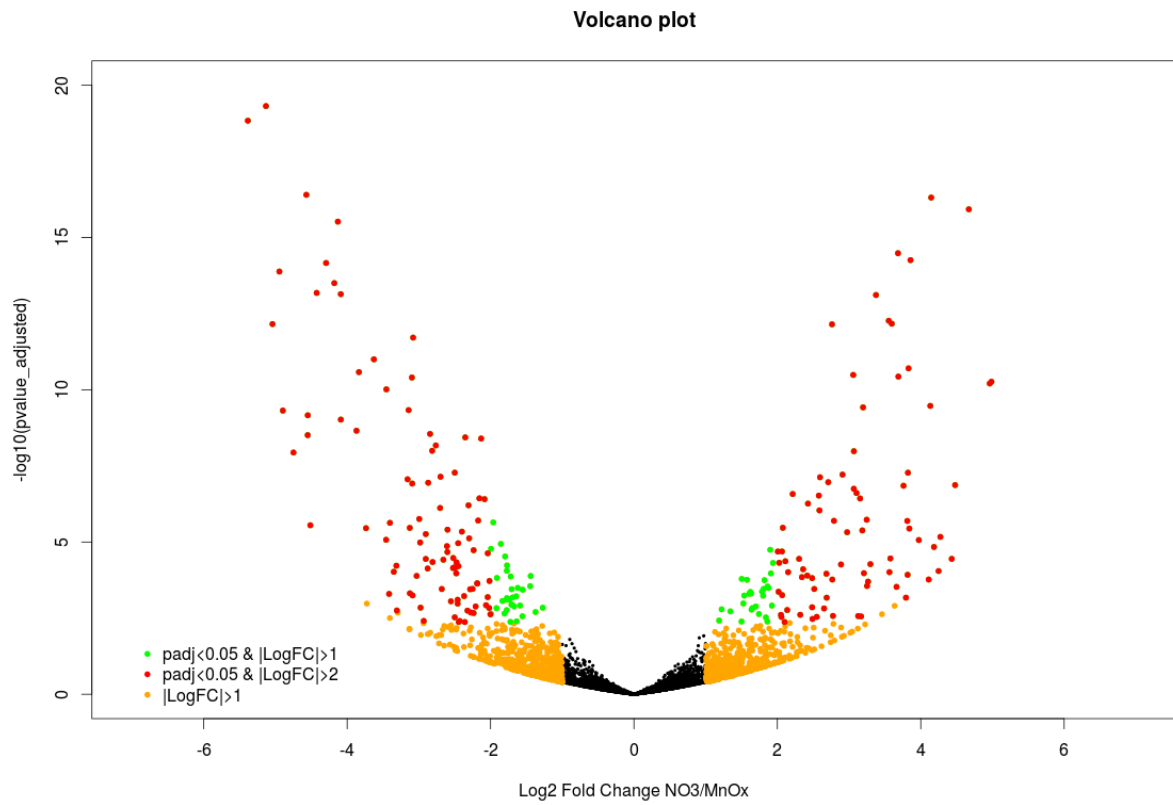


Figure S3. Volcano plot distribution showing transcriptomic data from *S. algae* C6G3 grown on nitrate (NO<sub>3</sub><sup>-</sup>, right part) and manganese oxide (MnIV, left part) as electrons acceptors.

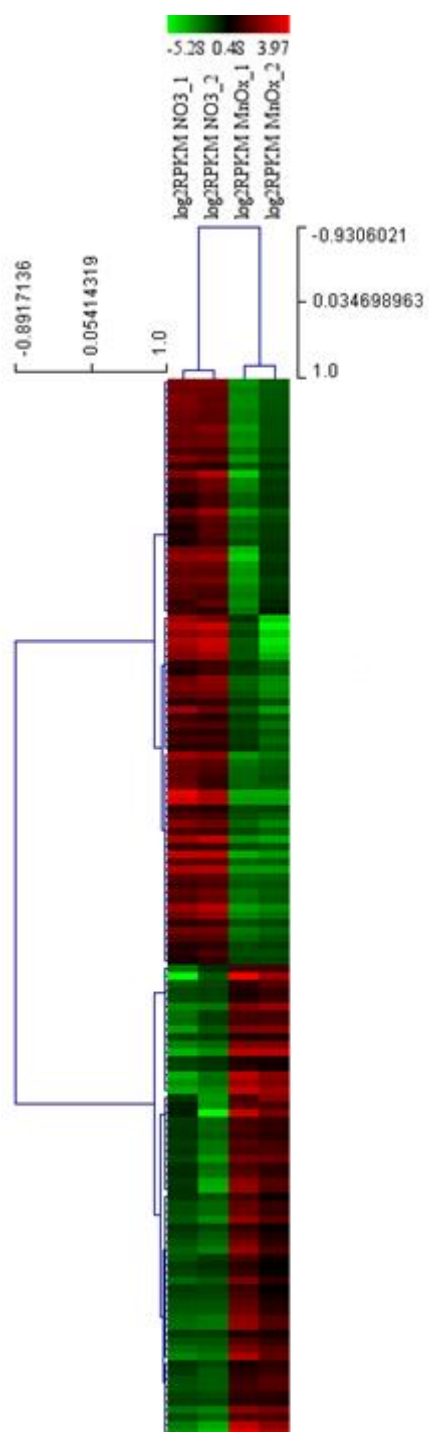


Fig S4: Heat mat of the log<sub>2</sub> RPKM of overexpressed gene among the 4 independent cultures (Two with nitrate as electron acceptor and 2 with MnIV as electron acceptor)

## Material and methods

*c*-type cytochrome detection. Crude extracts (30  $\mu$ g) of *S. algae* grown anaerobically in the presence of either fumarate, Mn or KNO<sub>3</sub> were loaded on 12% SDS-PAGE. After migration, cytochromes were revealed by staining for peroxidase activity of hemes using 3,3',5,5'-tetramethylbenzidine (TMBZ)(Thomas et al 1976) .

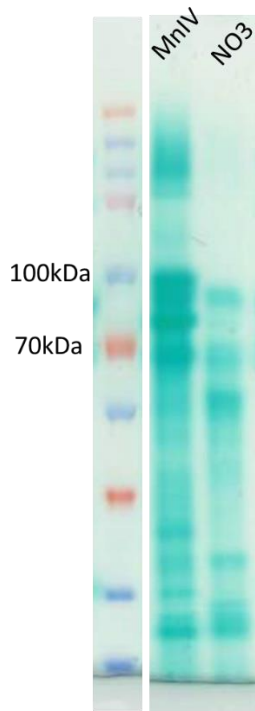


Fig S5. *c*-type cytochrome profile of *S. algae* according to the electron acceptor present in the growth medium.

Crude extract (30 $\mu$ g/ $\mu$ l) of cells grown in the presence of either fumarate, MnIV or NO<sub>3</sub> were submitted to a 12% SDS-PAGE. After electrophoresis, *c*-type cytochromes were visualized by heme staining using TMBZ. M for MW markers

Thomas PE, Lu AY, Ryan D, West SB, Kawalek J, Levin W. Multiple forms of rat liver cytochrome P-450. Immunochemical evidence with antibody against cytochrome P-448. J Biol Chem 1976;251:1385–91.