**Supplemental material**

# The network of chromosomal and plasmid encoded genes for the adaptation of the marine bacterium *Dinoroseobacter shibae* to anaerobic conditions.

Matthias Ebert[1§], Sebastian Laaß[1§], Melanie Burghartz[1], Jörn Petersen[2], Sebastian Koßmehl[3], Lars Wöhlbrand[3], Ralf Rabus[3], Christoph Wittmann[4], Petra Tielen[1#], Dieter Jahn[1]

[1]Institute for Microbiology, Technische Universität Braunschweig, D-38106 Braunschweig, Germany

[2]Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7 B, D-38124 Braunschweig, Germany

[3]Institut für Chemie und Biologie des Meeres, Carl von Ossietzky Universität Oldenburg, Carl-von-Ossietzky Str. 9-11, D-26111 Oldenburg

[4]Institute of Biochemical Engineering, Technische Universität Braunschweig, D-38106 Braunschweig, Germany

[§]Authors contributed equally.

**Contents:**

4. Supplemental Figures

Figure S1

**Establishment of a mariner-based transposon system for *D. shibae***

In the initial step of our investigations it was necessary to define suitable parameters for the transfer of a transposon into the genome of *D. shibae*. First, the plasmid pBT20 encoding a mariner transposon was chosen (Fig. 1). It further harbors the C9 mariner transposase necessary for random recombination and transposon integration. The plasmid contains two origins of replication. The *oriT* from the broad host range plasmid pRK2 allows for conjugation into Gram-negative bacteria (1). With its origin of replication R6Kγ (IncX) the plasmid pBT20 can only be replicated in strains expressing *pir* (2). In *pir*-negative recipients like *D. shibae* the plasmid should behave like a suicide vector mediating plasmid loss and selection for integrated transposons as observed for other bacteria (1, 3). A corresponding procedure was established and systematically optimized to finally obtained 9.0 x $10^3$ transposon mutants/per 2.5 x $10^8$ bacteria. Precultures of *D. shibae* DFL12$^T$ (acceptor strain) and *E. coli* ST18 (donor strain) were incubated under indicated different growth conditions. In the exponential growth phase, co-cultures of both strains were prepared in a volume ratio of 10:1 of donor to acceptor strain. For this purpose *D. shibae* and *E. coli* ST18 cell cultures were mixed and sedimented together for 4 min (4000 x g). The supernatant was discarded and the cell material resuspended in hMB medium. Spots of the co-cultures were incubated for conjugation on hMB-agar plate supplemented with 50 µg/ml aminolevulinic acid for 24 h at 30 °C. The *D. shibae* DFL12$^T$ transposon-mutants were subsequently selected on hMB-agar plates supplemented with 80 µg/ml gentamycin. For this purpose, serial dilutions of the conjugation cultures of $10^{-1}$-$10^{-7}$ were plated to maximize the efficiency. Single transposon mutants were isolated and preserved.

**Localization of the loci of transposon insertion in the DNA of *D. shibae***

For the localization of the integrated transposons in the chromosome or the plasmids of *D. shibae* an arbitrary PCR approach was used (1). This method allows the amplification of DNA

fragments consisting of 250 bp of the transposon DNA and a random length part from the genome or plasmids of *D. shibae* (Fig. 1a). The insertion loci were finally identified via DNA sequence determination of the arbitrary PCR products. Randomly selected mutants showed PCR products from 270 bp up to 2000 bp (Fig. 1b). The obtained DNA sequences for the various transposon integration sites indicated that no hot spots of integration were present.

**Selection of transposon mutants with an anaerobic growth phenotype**

The isolated 4500 *D. shibae* transposon mutants were screened for growth defects under anaerobic, denitrifying conditions. For 1580 out of 4500 tested mutants, the loci of integration were identified by DNA sequencing (Table S1, Supplemental Material). Residual transposon mutants are currently under investigation. Overall, 718 out of 1000 transposon mutants were in different genes. Assuming the presence of approximately 12% essential genes (4), which do not result in viable transposon mutants upon mutation, a saturation of 82% of the genome can be deducted. From the 4500 tested mutant strains 53 showed a decreased or even loss of growth under tested anaerobic conditions (Table 1). This suggests that approximately 1% of the genome of *D. shibae* was found essential or important for anaerobic growth. Besides insertion into the chromosome multiple insertions into the pDSHI01, pDSHI02, pDSHI03 and pDSHI04 were found. In total, *D. shibae* contains 5 plasmids, which are the 191 kb plasmid pDSHI01, the 153 kb plasmid pDSHI02, the 126 kb plasmid pDSHI03, the 86 kb plasmid pDSHI04 and the 72 kb plasmid pDSHI05 (5). In figure 1 of the results section the random distribution of transposon integration sites resulting in anaerobic growth defects of corresponding strains is shown. Three genes were hit twice by the transposon – *napA* encoding the nitrate reductase, the promotor region of the transposase gene Dshi_3356 and the plasmid encoded gene Dshi_3678/3874/4083. For eight plasmid encoded genes the final identification of the loci of integration failed, because they exists as multiple copies on the

various plasmids of *D. shibae*. (5). Interestingly, no transposon insertion was found for plasmid pDSHI05 indicating that genes encoded by this plasmid do not play a role in anaerobic growth. As expected, accumulation of transposon integration sites was observed between region 3142258 to 3767137 bp of the *D. shibae* chromosome (Fig. 1). This region contains several known genes and operons involved in anaerobic metabolism (5).

**Genes essential for aerobic and anaerobic growth of *D. shibae***

Table 1 provides an overview over the functional categories for the genes for which the 53 transposon mutants were isolated. These categories are described and discussed in the results section and here in the Supplemental Material. Only 5 of the obtained mutants showed a decreased growth under aerobic conditions indicating a general role for the *D. shibae* life cycle. For example, Dshi_1643 encoding RecA, which is essential for the repair and maintenance of DNA, was disrupted by the transposon close to the end of the gene. Partial activity might be retained. Nevertheless, the significant decrease of growth under aerobic conditions and complete loss of growth under anaerobic conditions showed the general importance of this gene for *D. shibae*. Accordingly, when the *recA* gene was deleted in *E. coli* a growth rate decrease by 50 % was observed under anaerobic conditions compared to the wildtype (6).

A similar phenotype was observed for disrupted gene Dshi_1932, encoding a putative glutathione-S-transferase. Glutathione S-transferase catalyzes the conjugation of the reduced tripeptide glutathione via the sulfhydryl groups to electrophilic substrates. In eukaryotes substrates are made soluble and accessible to detoxification this way. Multiple families of the enzyme catalyzing a broad variety of reactions were described (7). In contrast glutathione and the glutathione-S-transferase possess various controversially discussed functions in bacteria (8). They are involved in the protection against oxidative stress, in the maturation of proteins,

106 in the metabolism of aromatic compounds and in antibiotic resistance (9). Interestingly, the
107 enzyme was also found in the periplasm contributing to redox processes, including disulfide
108 bond formation (see results and below) and cytochrome c formation (10). Our findings
109 underscore the importance of the enzyme for *D. shibae*, especially for anaerobic growth.
110
111 **Transcriptome and proteome of aerobically and anaerobically grown *D. shibae***
112 To identify anaerobically induced genes, transcriptome and proteome analyses were
113 performed. For this purpose *D. shibae* DFL12$^T$ was cultivated in a chemostat using salt water
114 medium (SWM) supplemented with 25 mM nitrate to sustain denitrification when oxygen
115 became limited. After 20 h *D. shibae* reached the steady state with a constant cell density of
116 $OD_{578}$ = 0.5 +/- 0.04. After 40 h of growth the aeration was switched off and the oxygen
117 concentration in the culture decreased within 20 min to 0.05 µM indicating microaerobic to
118 anaerobic conditions. The corresponding transcriptome dataset revealed 474 genes which
119 were differentially expressed by at least 1.74-fold when denitrifying and aerobic growth
120 conditions were compared. Out of the 474 genes 207 were found induced and 267 showed a
121 decreased expression. Proteome profiles were obtained via shot-gun analyses or inspection of
122 the membrane protein-enriched fraction (both by means of nanoLC-ESI MS). The analyses
123 detected 878 different proteins in the whole cell shot-gun approach and 1215 different
124 proteins in the membrane fraction, which covered nearly 25 % of the predicted proteins of *D.*
125 *shibae*. Results were compared with the results of the transposon mutagenesis (Table S2).
126
127 **General aspects of cytochrome *c* and disulfide bond formation**
128 Disulfide bond formation in bacteria occurs in the periplasm. Corresponding target proteins
129 are secreted via the Sec-system into the periplasm. The disulfide bridges are then formed by
130 the disulfide bond protein A (DsbA). Recycling of DsbA is performed by the inner membrane
131 protein DsbB, which in turn transfers the electrons to various aerobic and anaerobic electron

transport chains of energy generation (11). DsbC (Dshi_3082) is a protein disulfide isomerase, which corrects DsbA misfolds or introduces non conservative disulfides in periplasmic and outer membrane proteins like LptD (12-15). To fullfill its task DsbC is kept reduced in the periplasm by DsbD, an integral membrane protein of the inner membrane. Our study identified chromosomal *dsbC* and a plasmid encoded *dsbD* gene as essential for anaerobic growth. Both DsbC and DsbD were linked to cytochrome *c* biosynthesis and anaerobic metabolism before (16-18). In *E. coli* DsbA, DsbB and DsbD were found essential for anaerobic nitrate and nitrite respiration (17). In *Xanthomonas oryzae* DsbC was found essential for a pathogenesis in rice (19). In *Xylella*, DsbC is required for biofilm formation (20).

During cytochrome *c* maturation iron protoporphyrin IX is exported via the Ccm-system through the cytoplasmic membrane into the periplasm and covalently linked via disulfide bridges to an apoprotein (21). Three different machineries for cytochrome *c* formation (system I, II, III) are known. In *D. shibae* most likely analogously to *Rhodobacter capsulatus* (22) the apocytochrome protein gets translocated into periplasm via the Sec-system. Subsequently, DsbA/DsbB, most likely in cooperation with DsbC/DsbD catalyze the thio-oxireduction of the apocytochrome *c*. The Ccm machinery (CcmABCDEFGH) transports, chaperones, delivers and ligates the heme to the apocytochrome *c* (23). In this context, a *ccmH* (Dshi_3777/4053) mutant abolished anaerobic growth of *D. shibae*. The CcmH protein is part of the CcmFHI heme ligation complex which forms the stereo-specific thioether bonds between the vinyl groups of the Fe-protoporphyrin IX and the thiol group of the apocytochrome *c* (24, 25). Interestingly, mutations of cysteine residues in the active site of *E. coli* CcmH inactivated the protein under aerobic but not anaerobic conditions (26). Clearly, different modes of cytochrome *c* generation exist for anaerobic and aerobic growth conditions.

157 *D. shibae* possesses *fixNOQP*- and *cycHJKL*-operons encoding $cbb_3$-type cytochrome
158 oxidases. $Cbb_3$-type cytochrome oxidases have a high $O_2$-affinity and are important for energy
159 generation under microaerobic conditions (27). In *P. aeruginosa* it was shown that all of the
160 here outlined cytochrome oxidases are essential for anaerobic growth. The isoforms fulfilled
161 specialized roles under different oxygen tensions. They cannot complement for each other
162 (28). However, in *D. shibae* mutation of *fixP* did not lead to an anaerobic phenotype. As
163 expected the mutant with defect in the aerobic $caa_3$-type cytochrome *c* oxidase subunit II
164 encoding gene Dshi_1140 was found without anaerobic growth phenotype. Accordingly, the
165 expression of this gene was found decreased under anaerobic conditions (Table S2).
166
167 **Electron donating systems for respiratory energy generation under anaerobic conditions**
168 Membrane localized NADH dehydrogenases serve as primary dehydrogenase of many
169 electron transport chains. Different types of NADH dehydrogenases are known including type
170 I proton translocating (Nuo), type II-non-ion translocating (Ndh) and type III-$Na^+$-
171 translocating. *D. shibae* possesses genes for all three types of NADH dehydrogenases. The
172 operon encoding the main proton-translocating NADH dehydrogenase I
173 *nuoABCDEFGHIJKLMN* is encoded by the genes Dshi_1307-Dshi_1329. A mutant defect in
174 *nuoM* (Dshi_1328) showed no anaerobic growth defect (Table S1) indicating a minor role of
175 the proton-translocation NADH dehydrogenase Nuo for anaerobic energy generation.
176 Accordingly, the transcription of *nuoM* was found uneffected by the switch from aerobic to
177 anaerobic conditions (Table S2). The genes Dshi_1390 and Dshi_3590 encode two potential
178 type II NADH dehydrogenases (Ndh). Expression of both genes was not influenced by
179 oxygen tension. The *mnhABCDEFG* operon encodes the potential type III NADH
180 dehydrogenase (Dshi_0728-Dshi_0734). The expression of the *mnhABCDEFG*-operon was
181 found slightly decreased under conditions of oxygen depletion (Table S2). A second type III
182 NADH dehydrogenase operon *phaACDEF* is located on the plasmid pDSHI02. The gene

183 *phaA* (Dshi_3805) was found essential for the anaerobic growth of *D. shibae* (Table 1). The
184 gene is part of the *phaACDEF* operon (Dshi_3805-Dshi_3810) encoding a $Na^+$-translocating
185 type III NADH dehydrogenase (NADH:quinone oxidoreductase). In accordance with its
186 function the expression of the *phaACDEF* gene cluster was found enhanced under conditions
187 of oxygen depletion. Currently, almost nothing is known about the function of the Pha-type
188 NADH dehydrogenase. However, its essential anaerobic functions raises the question for the
189 importance of $Na^+$-dependent processes under anaerobic conditions in *D. shibae*.
190 Mutations in other established primary dehydrogenases like glucose dehydrogenase (*gcd*,
191 Dshi_0476), formate dehydrogenase (*fdnG*, Dshi_0504, *fdhD*, Dshi_3282) and lactate
192 dehydrogenase (*lldD2*, Dshi_0948) did not yield an anaerobic phenotype (Table S2).
193 Consequently these primary dehydrogenases are not essential for anaerobic growth.
194 A mutant carrying a defect in gene Dshi_0323, annotated to encode ferredoxin, with amino
195 acid sequence homology to NapF was found unable to grow under oxygen limitation (Table
196 1). Ferredoxins (EC 1.7.7.2) are iron-sulfur proteins that mediate electron transfer for a wide
197 range of metabolic reactions. In several bacteria reduced ferredoxins function as electron
198 donors during the reduction of nitrate to nitrite (29) or for the assimilatory reduction of nitrate
199 to ammonium (30, 31). In agreement, only nitrate reduction was found essential for anaerobic
200 growth. Here we add a $Na^+$- translocating NADH dehydrogenase and a NapF-like ferredoxin
201 to the essential components of the anaerobic electron transport chains.
202
203 **Adaptation of the central metabolism to anaerobic growth**
204 The Dshi_1134 gene encodes 3-oxoacid CoA-transferase subunit B, which catalyzes the
205 reaction of 3-oxoacids with succinyl-CoA to obtain activated 3-oxoacyl-CoA. Its exact
206 function for anaerobic growth remains to be determined.
207 The two $C_4$-dicarboxylate transporter genes *dctM1* (Dshi_1037) and *dctM3* (Dshi_3395) and a
208 TRAP transporter solute receptor (Dshi_1195) were inactivated during transposon

mutagenesis, causing an anaerobic growth phenotype. The first two genes are forming potential operons with *dctP*- and *dctQ*-type genes. Clearly, two tripartite ATP-independent periplasmic (TRAP) transporters for the uptake of succinate, the major carbon source of the used SWM medium, were hit (32). However, both potential operons were not found oxygen tension-regulated. Corresponding proteins were detected in our proteome analysis for aerobic and anaerobic growth conditions. In *P. aeruginosa dctPQM* was subject to catabolite repression via the *crc*-system (33). Their contribution to the anaerobic metabolism of *D. shibae* remains to be determined. The transposon mutant of Dshi_3708 hit a gene encoding an AraC, helix-turn-helix domain containing, transcriptional regulator. The gene is localized downstream of the *dctPQM*-operon encoded by Dshi_3711-3709. It was proposed that these transporters are high-affinity, $Na^+$-dependent unidirectional secondary transporters (34, 35), again pointing towards an important function of $Na^+$-dependent transport processes for anaerobic growth.

Surprisingly, only few enzymes of the central metabolism were found essential for anaerobic growth. Many enzymes of the citric cycle and fermentation can be eliminated by transposon mutagenesis without major consequences for aerobic or anaerobic growth. Other enzymes might be important for both life styles and therefore not be traceable via the mutagenesis approach. Only one of the pyruvate dehydrogenases was found essential for anaerobic growth. Again, evidence for the importance of $Na^+$ gradient-dependent processes was obtained.

**Proteases are involved in anaerobic growth of *D. shibae***

Four protease encoding genes for aminopeptidase N (Dshi_1223), DNA binding, ATP-depending protease Lon (Dshi_1777), the potential AAA-ATPase subunit of a Clp-type protease (Dshi_1883) and a hemolysin type RTX (repeats-in-toxin) protein (Dshi_3872) were identified with the transposon mutagenesis approach to be essential for anaerobic denitrifying growth (Table 1).

The gene Dshi_1223 encodes the aminopeptidase PepN, which is involved in the degradation of the intracellular peptides generated by protein breakdown during the normal growth or in response to nutrient starvation and temperature stress (36, 37). PepN is also a negative regulator of sodium-salicylate-induced stress (37). Interestingly, *Vibrio fisheri* aminopeptidase N is essential for the colonization of the squid *Euprymna scolopes* (38). Analogously, *D. shibae* colonizes dinoflagellates.

The gene Dshi_1883 encodes a putative ClpA/ClpB family protein. This type of ATP-dependent proteases plays a major role in the degradation of misfolded proteins and is induced in response to heat shock and many other stress signals (39-41). The gene Dshi_1777 encodes the Lon protease. Lon proteases degrade short-lived regulatory and abnormal proteins in presence of ATP (39). Lon proteases negatively control the acid resistance genes of *E. coli* (42), bacterial communication via quorum sensing (43, 44), motility via flagellar structures (45, 46), the SOS response to DNA damage (47, 48) and heme biosynthesis (49). The mutation of *lon* confers reduced pathogenicity/virulence to many bacteria, for example via regulation of type III secretion system (50, 51). Lon proteases activity is controlled by phospholipids, especially cardiolipin (52). Finally, Lon protease is essential for the anaerobic survival of *E. coli* during glucose starvation (53).

The gene Dshi_3872 encodes a hemolysin-type calcium-binding region protein belonging to the RTX exoprotein family with similarity to the peptidase serralysin (54). These proteins are exported across the bacterial envelope via a type I secretion system and contain glycine and aspartate rich repeats for $Ca^{2+}$ binding. The target of this protein, maybe the dinoflagellate, remains to be determined. A hemolysin-type transporter is cotranscribed with Dshi_3873. The clear measurable decrease of growth of these mutants under anaerobic conditions compared to the wildtype indicated an important regulatory and restructuring effect of these proteases for the adaptation to anaerobiosis.

**Peptide transport is necessary for anaerobic growth**

The gene Dshi_3796 encodes the ATP-binding protein OppD of an oligopeptide ABC transporter. The gene is obviously necessary for anaerobic growth of *D. shibae*. The *oppD* gene was not found differently expressed between aerobic and anaerobic conditions (Table 1 and 2). Clearly, no peptides or amino acids are provided by the SWM medium. The Opp transporter was shown to be involved in the transport of small external signal peptides involved in cell-cell communication and intracellular regulation in *Enterococcus faecalis* and other Gram-positive bacteria (55). The use of signal peptides in Gram-negative bacteria is currently discussed. Their essential function to anaerobic growth in *D. shibae* remains to be determined.

**Restructuring of the cell envelope during anaerobic growth**

Several genes encoding enzymes involved in the restructuring of the cytoplasmic membrane, the cell wall and the lipopolysaccharide layer (LPS) of the outer membrane were found essential for anaerobic growth. The *alkB2* gene (Dshi_0027) encodes an alkane-1-monooxygenase. It is an integral membrane di-iron enzyme which oxidases $C_{12}$ up to $C_{20}$ *n*-alkanes using molecular oxygen. Acyl lipid desaturases are members of this enzyme family. Differential gene expression for *alkB1* and *alkB2* genes from *P. aeruginosa* in dependence of the growth phase was described (56).

The *fadK* gene (Dshi_3403) encodes a short chain acyl-CoA synthetase, which activates fatty acids via the ATP-dependent formation of a fatty acyl-CoA. The acyl-CoAs are central intermediates for fatty acid transport, β-oxidation or phospholipid biosynthesis. The *E. coli fadK* is maximally expressed under anaerobic conditions and repressed in the presence of oxygen (57). However, the expression of its *D. shibae fadK* counterpart was not respondinig to oxygen tension. One general explanation of our observations is the proven existence of two

distinct pathways for the β-oxidation of lipids under aerobic and anaerobic conditions in bacteria.

The gene Dshi_0808 encodes MipA, a scaffolding protein essential to murein biosynthesis (58). Several proteins of cell wall formation are coordinated in their function via MipA interaction. The MipA protein was found essential for biofilm formation in *E. coli* and *Salmonella* (59, 60). The expression of the *mipA* gene in *E. coli* responded to the glucose concentration in the medium (61). Interestingly, the *mipA* gene of *E. coli* is regulated by NO via a cascade including the NO sensor NsrR, the alternative sigma factor E and the small RNA RybB (62) indicating its essential function in nitrate respiration. However, no differential *mipA* expression was observed for *D. shibae*.

The gene Dshi_1766 encodes a LysM domain protein. The LysM domain is a binding motif for peptidoglycan (63). Obviously, this protein involved in the cell wall metabolism is essential for anaerobically growing *D. shibae*.

Finally, the glycosyl transferse gene Dshi_3576 is forming at its genomic locus an operon with various genes involved in LPS biosynthesis. Changes in the LPS structure in response to anaerobic growth have been reported before (64, 65).

Obviously, the cell envelope composed of the inner membrane, the cell wall and the outer membrane with its LPS have to adapt to anaerobic growth conditions. Most likely we identified some of the essential players involved in these processes.

**Cation efflux proteins sustain anaerobic growth delay**

Transition metals are known to cause toxicity under anoxic conditions (66). The gene Dshi_3624 encodes the cation efflux system CzcD. It was found induced under anaerobic conditions in *P. stutzeri* (67). It is known that transition metals interfere with the anaerobic metabolism and must be exported.

**The flagellar hook-length control protein FliK and nitrogen regulation**

The Dshi_3364 gene encodes the flagellar hook-length control protein FliK. Besides its function in flagellar biosynthesis, the gene was important for biofilm development in *Shewanella oneidensis* (68). For *Campylobacter jejuni* modulation of the sigma 54-dependent regulon by the *fliK* locus was observed, including the regulation of the *nuo* genes for NADH dehydrogenase (69). Similar observations were made for *Heliobacter pylori*. Here, the FliK influenced the transcription of genes for a ferredoxin and thioredoxin (70). Obviously, the FliK protein mediates multiple regulatory functions besides its structural function during flagella formation. One of these functions is essential for anaerobic growth of *D. shibae* under anaerobic conditions.

## Supplemental References


1. **O'Toole GA, Pratt LA, Watnick PI, Newman DK, Weaver VB, Kolter R.** 1999. Genetic approaches to study of biofilms. Methods Enzymol. **310:**91-109.
2. **Matsumoto-Mashimo C, Guerout AM, Mazel D.** 2004. A new family of conditional replicating plasmids and their cognate *Escherichia coli* host strains. Res. Microbiol. **155:**455-461.
3. **Herrero M, de Lorenzo V, Timmis KN.** 1990. Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram-negative bacteria. J. Bacteriol. **172:**6557-6567.
4. **Jacobs MA, Alwood A, Thaipisuttikul I, Spencer D, Haugen E, Ernst S, Will O, Kaul R, Raymond C, Levy R, Chun-Rong L, Guenthner D, Bovee D, Olson MV, Manoil C.** 2003. Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U S A. **100:**14339-14344.
5. **Wagner-Döbler I, Ballhausen B, Berger M, Brinkhoff T, Buchholz I, Bunk B, Cypionka H, Daniel R, Drepper T, Gerdts G, Hahnke S, Han C, Jahn D, Kalhoefer D, Kiss H, Klenk HP, Kyrpides N, Liebl W, Liesegang H, Meincke L, Pati A, Petersen J, Piekarski T, Pommerenke C, Pradella S, Pukall R, Rabus R, Stackebrandt E, Thole S, Thompson L, Tielen P, Tomasch J, von Jan M, Wanphrut N, Wichels A, Zech H, Simon M.** 2010. The complete genome sequence of the algal symbiont *Dinoroseobacter shibae*: a hitchhiker's guide to life in the sea. ISME J. **4:**61-77.
6. **Kouzminova EA, Rotman E, Macomber L, Zhang J, Kuzminov A.** 2004. RecA-dependent mutants in *Escherichia coli* reveal strategies to avoid chromosomal fragmentation. Proc. Natl. Acad. Sci. U S A. **101:**16262-16267.
7. **Board PG, Menon D.** 2013. Glutathione transferases, regulators of cellular metabolism and physiology. Biochim. Biophys. Acta. **1830:**3267-3288.
8. **Vuilleumier S.** 1997. Bacterial glutathione S-transferases: what are they good for? J. Bacteriol. **179:**1431-1441.
9. **Allocati N, Federici L, Masulli M, Di Ilio C.** 2012. Distribution of glutathione transferases in Gram-positive bacteria and Archaea. Biochimie **94:**588-596.
10. **Eser M, Masip L, Kadokura H, Georgiou G, Beckwith J.** 2009. Disulfide bond formation by exported glutaredoxin indicates glutathione's presence in the *E. coli* periplasm. Proc. Natl. Acad. Sci. U S A. **106:**1572-1577.
11. **Depuydt M, Messens J, Collet JF.** 2011. How proteins form disulfide bonds. Antioxid. Redox. Signal. **15:**49-66.
12. **Berkmen M, Boyd D, Beckwith J.** 2005. The nonconsecutive disulfide bond of *Escherichia coli* phytase (AppA) renders it dependent on the protein-disulfide isomerase, DsbC. J. Biol. Chem. **280:**11387-11394.
13. **Hiniker A, Bardwell JC.** 2004. In vivo substrate specificity of periplasmic disulfide oxidoreductases. J. Biol. Chem. **279:**12967-12973.
14. **Vertommen D, Depuydt M, Pan J, Leverrier P, Knoops L, Szikora JP, Messens J, Bardwell JC, Collet JF.** 2008. The disulphide isomerase DsbC cooperates with the oxidase DsbA in a DsbD-independent manner. Mol. Microbiol. **67:**336-349.
15. **Denoncin K, Vertommen D, Paek E, Collet JF.** 2010. The protein-disulfide isomerase DsbC cooperates with SurA and DsbA in the assembly of the essential beta-barrel protein LptD. J. Biol. Chem. **285:**29425-29433.

16. **Stirnimann CU, Rozhkova A, Grauschopf U, Grutter MG, Glockshuber R, Capitani G.** 2005. Structural basis and kinetics of DsbD-dependent cytochrome *c* maturation. Structure **13:**985-993.
17. **Metheringham R, Tyson KL, Crooke H, Missiakas D, Raina S, Cole JA.** 1996. Effects of mutations in genes for proteins involved in disulphide bond formation in the periplasm on the activities of anaerobically induced electron transfer chains in *Escherichia coli* K12. Mol. Gen. Genet. **253:**95-102.
18. **Kumar P, Sannigrahi S, Scoullar J, Kahler CM, Tzeng YL.** 2011. Characterization of DsbD in *Neisseria meningitidis*. Mol. Microbiol. **79:**1557-1573.
19. **Guo W, Cui YP, Li YR, Che YZ, Yuan L, Zou LF, Zou HS, Chen GY.** 2012. Identification of seven *Xanthomonas oryzae* pv. oryzicola genes potentially involved in pathogenesis in rice. Microbiology **158:**505-518.
20. **Santos CA, Toledo MA, Trivella DB, Beloti LL, Schneider DR, Saraiva AM, Crucello A, Azzoni AR, Souza AA, Aparicio R, Souza AP.** 2012. Functional and structural studies of the disulfide isomerase DsbC from the plant pathogen *Xylella fastidiosa* reveals a redox-dependent oligomeric modulation in vitro. FEBS J. **279:**3828-3843.
21. **Sanders C, Turkarslan S, Lee DW, Daldal F.** 2010. Cytochrome *c* biogenesis: the Ccm system. Trends Microbiol. **18:**266-274.
22. **Ekici S, Pawlik G, Lohmeyer E, Koch HG, Daldal F.** 2012. Biogenesis of *cbb*(3)-type cytochrome *c* oxidase in *Rhodobacter capsulatus*. Biochim. Biophys. Acta. **1817:**898-910.
23. **Richard-Fogal CL, San Francisco B, Frawley ER, Kranz RG.** 2012. Thiol redox requirements and substrate specificities of recombinant cytochrome *c* assembly systems II and III. Biochim. Biophys. Acta. **1817:**911-919.
24. **Verissimo AF, Yang H, Wu X, Sanders C, Daldal F.** 2011. CcmI subunit of CcmFHI heme ligation complex functions as an apocytochrome *c* chaperone during c-type cytochrome maturation. J. Biol. Chem. **286:**40452-40463.
25. **Richard-Fogal CL, Frawley ER, Bonner ER, Zhu H, San Francisco B, Kranz RG.** 2009. A conserved haem redox and trafficking pathway for cofactor attachment. Embo J. **28:**2349-2359.
26. **Robertson IB, Stevens JM, Ferguson SJ.** 2008. Dispensable residues in the active site of the cytochrome *c* biogenesis protein CcmH. FEBS Lett. **582:**3067-3072.
27. **Williams HD, Zlosnik JE, Ryall B.** 2007. Oxygen, cyanide and energy generation in the cystic fibrosis pathogen *Pseudomonas aeruginosa*. Adv. Microb. Physiol. **52:**1-71.
28. **Comolli JC, Donohue TJ.** 2004. Differences in two *Pseudomonas aeruginosa cbb*3 cytochrome oxidases. Mol. Microbiol. **51:**1193-1203.
29. **Moreno-Vivian C, Cabello P, Martinez-Luque M, Blasco R, Castillo F.** 1999. Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. J. Bacteriol. **181:**6573-6584.
30. **Jepson BJ, Anderson LJ, Rubio LM, Taylor CJ, Butler CS, Flores E, Herrero A, Butt JN, Richardson DJ.** 2004. Tuning a nitrate reductase for function. The first spectropotentiometric characterization of a bacterial assimilatory nitrate reductase reveals novel redox properties. J. Biol. Chem. **279:**32212-32218.
31. **Fujinaga K, Taniguchi Y, Sun Y, Katayama S, Minami J, Matsushita O, Okabe A.** 1999. Analysis of genes involved in nitrate reduction in *Clostridium perfringens.* Microbiology **145 ( Pt 12):**3377-3387.
32. **Forward JA, Behrendt MC, Wyborn NR, Cross R, Kelly DJ.** 1997. TRAP transporters: a new family of periplasmic solute transport systems encoded by the dctPQM genes of *Rhodobacter capsulatus* and by homologs in diverse gram-negative bacteria. J. Bacteriol. **179:**5482-5493.

33. **Valentini M, Lapouge K.** 2012. Catabolite repression in *Pseudomonas aeruginosa* PAO1 regulates the uptake of C(4) -dicarboxylates depending on succinate concentration. Environ. Microbiol.
34. **Mulligan C, Fischer M, Thomas GH.** 2011. Tripartite ATP-independent periplasmic (TRAP) transporters in bacteria and archaea. FEMS Microbiol. Rev. **35:**68-86.
35. **Mulligan C, Geertsma ER, Severi E, Kelly DJ, Poolman B, Thomas GH.** 2009. The substrate-binding protein imposes directionality on an electrochemical sodium gradient-driven TRAP transporter. Proc. Natl. Acad. Sci. U S A. **106:**1778-1783.
36. **Bhosale M, Kumar A, Das M, Bhaskarla C, Agarwal V, Nandi D.** 2013. Catalytic activity of Peptidase N is required for adaptation of *Escherichia coli* to nutritional downshift and high temperature stress. Microbiol. Res. **168:**56-64.
37. **Chandu D, Nandi D.** 2003. PepN is the major aminopeptidase in *Escherichia coli*: insights on substrate specificity and role during sodium-salicylate-induced stress. Microbiology **149:**3437-3447.
38. **Fidopiastis PM, Rader BA, Gerling DG, Gutierrez NA, Watkins KH, Frey MW, Nyholm SV, Whistler CA.** 2012. Characterization of a *Vibrio fischeri* aminopeptidase and evidence for its influence on an early stage of squid colonization. J. Bacteriol. **194:**3995-4002.
39. **Fu GK, Smith MJ, Markovitz DM.** 1997. Bacterial protease Lon is a site-specific DNA-binding protein. J. Biol. Chem. **272:**534-538.
40. **Van Melderen L, Aertsen A.** 2009. Regulation and quality control by Lon-dependent proteolysis. Res. Microbiol. **160:**645-651.
41. **Gottesman S.** 2003. Proteolysis in bacterial regulatory circuits. Annu. Rev. Cell. Dev. Biol. **19:**565-587.
42. **Heuveling J, Possling A, Hengge R.** 2008. A role for Lon protease in the control of the acid resistance genes of *Escherichia coli*. Mol. Microbiol. **69:**534-547.
43. **Bertani I, Rampioni G, Leoni L, Venturi V.** 2007. The *Pseudomonas putida* Lon protease is involved in N-acyl homoserine lactone quorum sensing regulation. BMC Microbiol. **7:**71.
44. **Takaya A, Tabuchi F, Tsuchiya H, Isogai E, Yamamoto T.** 2008. Negative regulation of quorum-sensing systems in *Pseudomonas aeruginosa* by ATP-dependent Lon protease. J. Bacteriol. **190:**4181-4188.
45. **Claret L, Hughes C.** 2000. Rapid turnover of FlhD and FlhC, the flagellar regulon transcriptional activator proteins, during *Proteus* swarming. J. Bacteriol. **182:**833-836.
46. **Tomoyasu T, Takaya A, Isogai E, Yamamoto T.** 2003. Turnover of FlhD and FlhC, master regulator proteins for *Salmonella flagellum* biogenesis, by the ATP-dependent ClpXP protease. Mol. Microbiol. **48:**443-452.
47. **Mizusawa S, Gottesman S.** 1983. Protein degradation in *Escherichia coli*: the *lon* gene controls the stability of SulA protein. Proc. Natl. Acad. Sci. U S A. **80:**358-362.
48. **Goodman MF.** 2000. Coping with replication 'train wrecks' in *Escherichia coli* using Pol V, Pol II and RecA proteins. Trends Biochem. Sci. **25:**189-195.
49. **Wang L, Elliott M, Elliott T.** 1999. Conditional stability of the HemA protein (glutamyl-tRNA reductase) regulates heme biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **181:**1211-1219.
50. **Butler SM, Festa RA, Pearce MJ, Darwin KH.** 2006. Self-compartmentalized bacterial proteases and pathogenesis. Mol. Microbiol. **60:**553-562.
51. **Losada LC, Hutcheson SW.** 2005. Type III secretion chaperones of *Pseudomonas syringae* protect effectors from Lon-associated degradation. Mol. Microbiol. **55:**941-953.

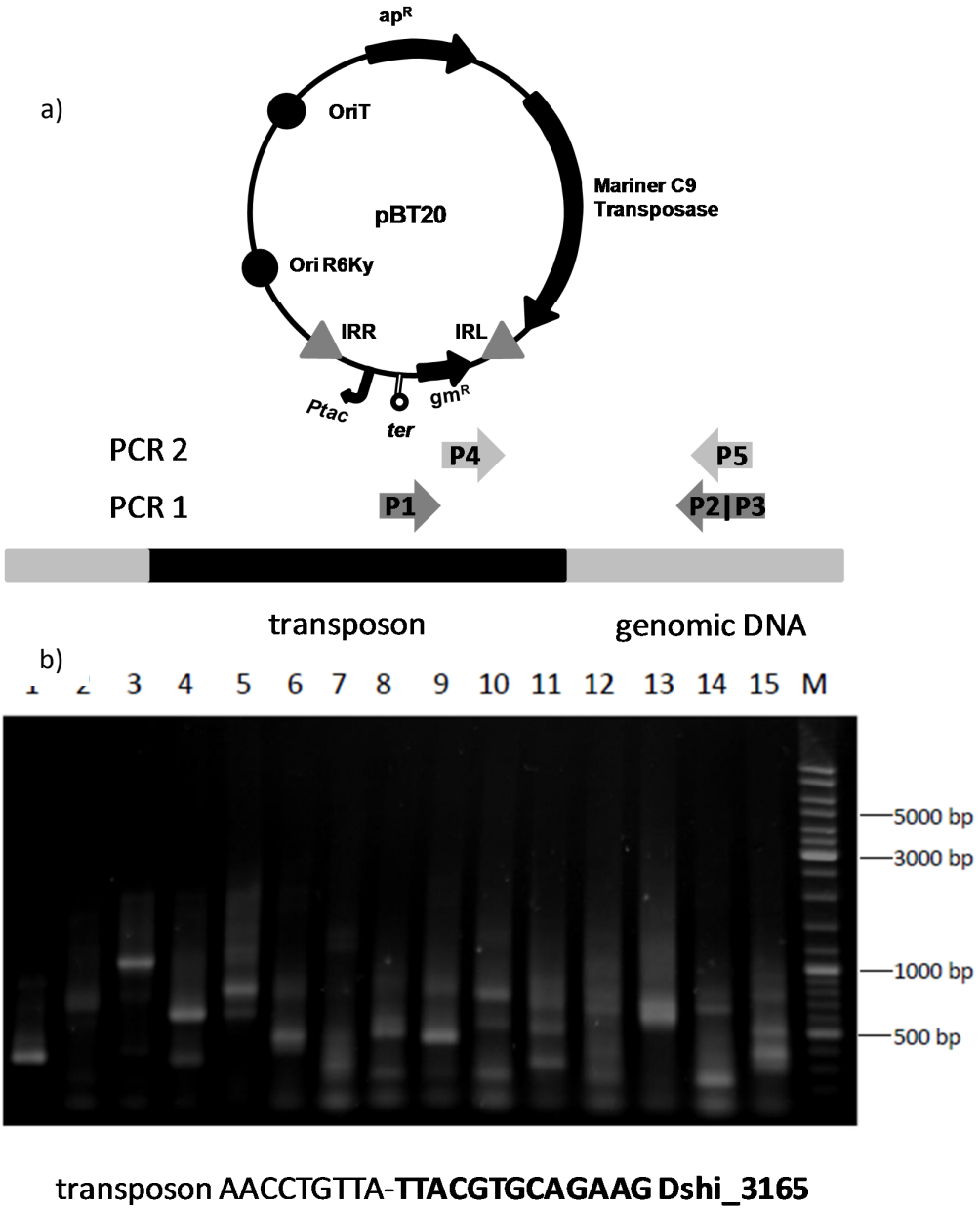
52. **Minami N, Yasuda T, Ishii Y, Fujimori K, Amano F.** 2011. Regulatory role of cardiolipin in the activity of an ATP-dependent protease, Lon, from *Escherichia coli*. J. Biochem. **149:**519-527.
53. **Luo S, McNeill M, Myers TG, Hohman RJ, Levine RL.** 2008. Lon protease promotes survival of *Escherichia coli* during anaerobic glucose starvation. Arch. Microbiol. **189:**181-185.
54. **Linhartova I, Bumba L, Masin J, Basler M, Osicka R, Kamanova J, Prochazkova K, Adkins I, Hejnova-Holubova J, Sadilkova L, Morova J, Sebo P.** 2010. RTX proteins: a highly diverse family secreted by a common mechanism. FEMS Microbiol. Rev. **34:**1076-1112.
55. **Lazazzera BA.** 2001. The intracellular function of extracellular signaling peptides. Peptides **22:**1519-1527.
56. **Marin MM, Yuste L, Rojo F.** 2003. Differential expression of the components of the two alkane hydroxylases from *Pseudomonas aeruginosa*. J. Bacteriol. **185:**3232-3237.
57. **Morgan-Kiss RM, Cronan JE.** 2004. The *Escherichia coli fadK* (*ydiD*) gene encodes an anerobically regulated short chain acyl-CoA synthetase. J. Biol. Chem. **279:**37324-37333.
58. **Vollmer W, von Rechenberg M, Holtje JV.** 1999. Demonstration of molecular interactions between the murein polymerase PBP1B, the lytic transglycosylase MltA, and the scaffolding protein MipA of *Escherichia coli*. J. Biol. Chem. **274:**6726-6734.
59. **Rivas L, Fegan N, Dykes GA.** 2008. Expression and putative roles in attachment of outer membrane proteins of *Escherichia coli* O157 from planktonic and sessile culture. Foodborne Pathog. Dis. **5:**155-164.
60. **Giaouris E, Samoilis G, Chorianopoulos N, Ercolini D, Nychas GJ.** 2013. Differential protein expression patterns between planktonic and biofilm cells of *Salmonella enterica* serovar Enteritidis PT4 on stainless steel surface. Int. J. Food. Microbiol. **162:**105-113.
61. **Yang JN, Wang C, Guo C, Peng XX, Li H.** 2011. Outer membrane proteome and its regulation networks in response to glucose concentration changes in *Escherichia coli*. Mol. Biosyst. **7:**3087-3093.
62. **Thompson KM, Rhodius VA, Gottesman S.** 2007. SigmaE regulates and is regulated by a small RNA in *Escherichia coli*. J. Bacteriol. **189:**4243-4256.
63. **Buist G, Steen A, Kok J, Kuipers OP.** 2008. LysM, a widely distributed protein motif for binding to (peptido)glycans. Mol. Microbiol. **68:**838-847.
64. **Landini P, Zehnder AJ.** 2002. The global regulatory hns gene negatively affects adhesion to solid surfaces by anaerobically grown *Escherichia coli* by modulating expression of flagellar genes and lipopolysaccharide production. J. Bacteriol. **184:**1522-1529.
65. **Davies RL, Parton R, Coote JG, Gibbs HA, Freer JH.** 1992. Outer-membrane protein and lipopolysaccharide variation in *Pasteurella haemolytica* serotype A1 under different growth conditions. J. Gen. Microbiol. **138:**909-922.
66. **Bird LJ, Coleman ML, Newman DK.** 2013. Iron and copper act synergistically to delay anaerobic growth in bacteria. Appl. Environ. Microbiol.
67. **Yang Y, Zhu H, Colvin VL, Alvarez PJ.** 2011. Cellular and transcriptional response of *Pseudomonas stutzeri* to quantum dots under aerobic and denitrifying conditions. Environ. Sci. Technol. **45:**4988-4994.
68. **Thormann KM, Saville RM, Shukla S, Pelletier DA, Spormann AM.** 2004. Initial Phases of biofilm formation in *Shewanella oneidensis* MR-1. J. Bacteriol. **186:**8096-8104.
69. **Kamal N, Dorrell N, Jagannathan A, Turner SM, Constantinidou C, Studholme DJ, Marsden G, Hinds J, Laing KG, Wren BW, Penn CW.** 2007. Deletion of a

previously uncharacterized flagellar-hook-length control gene fliK modulates the sigma54-dependent regulon in *Campylobacter jejuni*. Microbiology **153:**3099-3111.

70. **Douillard FP, Ryan KA, Hinds J, O'Toole PW.** 2009. Effect of FliK mutation on the transcriptional activity of the {sigma}54 sigma factor RpoN in *Helicobacter pylori*. Microbiology **155:**1901-1911.

**Fig. S1: Experimental approach of transposon mutagenesis and insertion site localization.** The structure of the employed mariner transposon carrying plasmid pBT20 is depicted (a). Below the strategy of arbritary PCR approach is shown (b). Arrows represent the annealing location and orientation of the used primers. The first PCR-round contained primers 1, 2 and 3. The second PCR-round included primers 4 and 5 (modified after O'Toole *et al.*, 1999). Analysis of arbitrary PCR products. Obtained PCR products were separated by agarose gel electrophoreses and visualized by ethidium bromid staining. Shown are the used marker and lanes 1 to 15 display the PCR products from different *D. shibae* DFL12$^{T}$ transposon mutants. For more information also see the Supplemental Material Table: S1

536 **Fig. S1**




transposon AACCTGTTA-**TTACGTGCAGAAG Dshi_3165**

537

538

539

540

541

542

543 Table S2: Transcriptional profiling of gene classes including essential genes for anaerobic
544 growth. Maximal fold change of gene expression within 2 h of oxygen depletion and presence
545 of cytoplasmic and membrane proteins under anaerobic, denitrifying conditions. (o) not
546 detected, (+) detected

| Locus tag | Gene name | Function of the gene product | Fold change/Detection | | |
|---|---|---|---|---|---|
| | | | Transcript | Protein | |
| | | | anaerob | aerob | anaerob |
| **Denitrification** | | | | | |
| Dshi_3161 | *napC* | NapC/NirT cytochrome *c* domain-containing protein | 2.9 | o | + |
| Dshi_3162 | *napB* | Nitrate reductase cytochrome *c*-type subunit (NapB) | 2.5 | o | + |
| Dshi_3163 | *napH* | Quinol dehydrogenase membrane component | 3.2 | o | o |
| Dshi_3164 | *napG* | NapG family ferredoxin-type protein | 3.0 | o | o |
| Dshi_3165 | *napA* | Nitrate reductase catalytic subunit | 4.4 | + | + |
| Dshi_3166 | *napD* | NapD family protein | 3.6 | o | o |
| Dshi_3167 | *napF* | Ferredoxin-type protein NapF | 1.4 | o | o |
| Dshi_1667 | *nasD* | Nitrite reductase (NAD(P)H), large subunit | 1.2 | o | o |
| Dshi_1668 | *nasE* | Nitrite reductase (NAD(P)H), small subunit | 1.1 | o | o |
| Dshi_1669 | *nasA* | Molybdopterin oxidoreductase | -1.0 | o | o |
| Dshi_1670 | | Hypothetical protein | 1.0 | o | o |
| Dshi_1671 | *cysG* | Uroporphyrin-III C-methyltransferase | 1.1 | o | o |
| Dshi_0323 | | Ferredoxin | -1.3 | o | o |
| Dshi_0540 | | NnrU family protein | 1.8 | + | + |
| Dshi_3180 | *nirS* | Nitrite reductase precursor | 31.6 | o | + |
| Dshi_3181 | *nosR2* | Nitrous oxide reductase regulatory protein NosR | 13.0 | o | + |
| Dshi_3192 | | Hypothetical protein | 14.2 | o | o |
| Dshi_3195 | *nosD* | Nitrous oxide maturation protein | 12.8 | o | o |
| **Molybdopterin biosynthesis** | | | | | |
| Dshi_2974 | *moeB* | Molybdopterin biosynthesis protein | 1.1 | o | + |
| Dshi_3089 | *moaB* | Molybdopterin binding domain | -1.1 | + | + |
| Dshi_1290 | *moaA* | Molybdenum cofactor biosynthesis protein A | -1.4 | + | + |
| Dshi_3482 | *modB* | Molybdenum transport system permease protein ModB | -1.1 | o | o |

| | | | | | |
|---|---|---|---|---|---|
| Dshi_3483 | *modC* | Molybdenum import ATP-binding protein ModC | -1.1 | o | o |
| **Cytochromes *c* and its biosynthesis** | | | | | |
| Dshi_0508 | | Cytochrome *c* class I | -1.2 | o | o |
| Dshi_1932 | | Glutathione S-transferase | -1.8 | o | o |
| Dshi_2081 | | Putative cytochrome *c* | -2.5 | o | o |
| Dshi_2868 | | Cytochrome *c* | -1.6 | o | o |
| Dshi_3082 | *dsbC* | Putative *C*-type cytochrome biosynthesis protein | -1.2 | o | o |
| Dshi_3407 | *dsbD* | Thiol:disulfide interchange protein DsbD | 1.4 | + | + |
| Dshi_3606<br>Dshi_3944 | | Cytochrome *c* biogenesis protein transmembrane region | -1.0 | o | o |
| Dshi_3620 | *dsbA I* | DSBA oxidoreductase | -1.5 | + | + |
| Dshi_3621 | *dsbB* | Disulfide bond formation protein | -1.2 | o | o |
| Dshi_3775 | *ccmF* | Cytochrome *c*-type biogenesis protein CcmF | -1.2 | o | + |
| Dshi_3776 | *ccmG* | Periplasmic protein thiol disulphide oxidoreductase DsbE | 1.0 | + | + |
| Dshi_3777<br>Dshi_4053 | *ccmH* | Cytochrome *c* biogenesis protein | -1.1 | + | + |
| Dshi_3778 | *ccmI* | TPR repeat-containing protein | -1.1 | + | + |
| Dshi_3779 | *dsbD* | Thiol:disulfide interchange protein DsbD precursor | 1.3 | o | o |
| Dshi_3887 | *cccA* | Cytochrome *c* class I | 1.2 | o | o |
| Dshi_3894 | | Cytochrome *c* | 1.2 | o | o |
| **Anaerobic electron transport chain** | | | | | |
| Dshi_0451 | *pqqB* | Coenzyme PQQ biosynthesis protein B | -1.6 | o | o |
| Dshi_0452 | *pqqC* | Coenzyme PQQ biosynthesis protein C | -1.6 | o | o |
| Dshi_0473 | | Alcohol dehydrogenase class III/S-(hydroxymethyl)glutathione dehydrogenase | -1.5 | o | o |
| Dshi_0476 | *gcd* | Quinoprotein glucose dehydrogenase | -2.1 | + | + |
| Dshi_0504 | *fdnG* | Formate dehydrogenase alpha chain | -1.9 | + | + |
| Dshi_0664 | *fixP* | Cytochrome c oxidase | 5.7 | + | + |
| Dshi_0728 | *mnhA* | NADH dehydrogenase (quinone) | -1.1 | o | o |
| Dshi_0729 | *mnhB* | $Na^+/H^+$ antiporter MnhB subunit-related protein | -1.1 | o | o |
| Dshi_0730 | *mnhC* | Putative monovalent cation/$H^+$ antiporter subunit C | -1.1 | o | o |
| Dshi_0731 | *mnhD* | NADH dehydrogenase (quinone) | -1.1 | o | o |
| Dshi_0732 | *mnhE* | Cation antiporter | -1.1 | o | o |
| Dshi_0733 | *mnhF* | Multiple resistance and pH regulation | -1.2 | o | o |

| | | | | | |
|---|---|---|---|---|---|
| | | protein F | | | |
| Dshi_0734 | *mnhG* | Monovalent cation/proton antiporter, MnhG subunit | -1.0 | o | o |
| Dshi_0948 | *lldD2* | L-lactate dehydrogenase | 1.2 | + | o |
| Dshi_1140 | | Cytochrome c oxidase subunit II | -4.2 | + | + |
| Dshi_1144 | *ctaE* | Cytochrome c oxidase subunit II | -2.9 | o | o |
| Dshi_1281 | *fdhB* | Respiratory-chain NADH dehydrogenase domain | 1.2 | o | o |
| Dshi_1282 | *fdhA* | Complex with *fdhB*, Molybdopterin oxidoreductase, contains Fe-S cluster | 1.0 | o | o |
| Dshi_1328 | *nuoM* | Proton-translocation NADH-quinone oxidoreductase | 1.3 | + | + |
| Dshi_1954 | | putative short-chain dehydrogenase | -1.1 | + | o |
| Dshi_2278 | *dmsA* | Dimethyl sulfoxide reductase precurser | 11.7 | o | o |
| Dshi_3324 | *dld2* | D-lactate dehydrogenase | 1.0 | o | o |
| Dshi_3590 | | Ubiquinone dependent NADH dehydrogenase | 2.1 | + | + |
| Dshi_3805 | *phaA* | NADH dehydrogenase | 2.0 | + | + |
| Dshi_3806 | *phaC* | Putative monovalent cation/$H^+$ antiporter subunit | 1.2 | + | + |
| Dshi_3807 | *phaD* | Putative monovalent cation/$H^+$ antiporter subunit | 1.2 | + | + |
| Dshi_3808 | *phaE* | Putative monovalent cation/$H^+$ antiporter subunit | 1.1 | o | o |
| Dshi_3809 | *phaF* | Multiple resistance and pH regulation protein | 1.3 | o | + |
| Dshi_3810 | *phaG* | Monovalent cation/proton antiporter, PhaG subunit | 1.3 | o | o |
| **Photosynthesis** | | | | | |
| Dshi_3524 | *pufM* | Reaction center protein M chain | -1.1 | + | + |
| Dshi_3525 | *pufC* | Photosynthetic reaction center cytochrome c subunit precurser | -1.1 | + | + |
| Dshi_3544 | *acsF* | Aerobic magnesium-protoporphirin IX monomethyl ester | 1.0 | + | + |
| **Fermentation** | | | | | |
| Dshi_0432 | *arcA* | Arginine deminase | 2.5 | o | o |
| Dshi_1825 | *pta1* | Phosphate acetyl transferase | 1.0 | o | o |
| Dshi_3553 | *Acs* | Acetyl-coenzym A synthetase | 1.8 | + | + |
| **Central metabolism** | | | | | |
| Dshi_0535 | *pdhB1* | Pyruvate dehydrogenase E1 component subunit beta | -1.5 | o | + |
| Dshi_1227 | *glcB* | Malate synthase G | -2.1 | o | o |
| Dshi_1966 | *lpdA* | Dihydrolipoyl dehydrogenase | 1.3 | + | + |

| | | | | | |
|---|---|---|---|---|---|
| Dshi_1968 | *aceE* | Pyruvate dehydrogenase E1 component | 3.0 | o | + |
| Dshi_2158 | *pdhA1* | Pyruvate dehydrogenase (acetyl-transferring) | -1.1 | + | + |
| Dshi_2159 | *pdhB2* | Pyruvate dehydrogenase subunit beta | -1.9 | + | + |
| Dshi_2160 | *pdhC1* | Branched-chain alpha-keto acid dehydrogenase subunit E2 | -2.4 | + | + |
| Dshi_2485 | *pyc* | Pyruvate carboxylase | -1.1 | + | + |
| Dshi_2490 | *citE1* | Citrate lyase | -1.7 | o | o |
| Dshi_2491 | *sucC2* | Succinyl-CoA synthetase | -2.3 | o | o |
| Dshi_2876 | *mdh* | Malate dehydrogenase | 1.1 | + | + |
| **Potential $Na^+$-dependent membrane processes** | | | | | |
| Dshi_0699 | *dctM1* | TRAP dicarboxylate transporter, DctM subunit | -1.1 | o | o |
| Dshi_0743 | *mcsS* | MscS mechanosensitive ion channel | 1.1 | o | o |
| Dshi_1035 | *dctP* | TRAP dicarboxylate transporter- DctP subunit | -1.9 | o | o |
| Dshi_1036 | *dctQ* | Tripartite ATP-independent periplasmic transporter DctQ | -1.6 | + | + |
| Dshi_1037 | *dctM1* | TRAP dicarboxylate transporter, DctM subunit | -1.4 | o | o |
| Dshi_1709 | *mscS* | MscS mechanosensitive ion channel | -1.4 | + | + |
| Dshi_1816 | *mscS* | MscS mechanosensitive ion channel | 1.1 | o | + |
| Dshi_2998 | *mscS* | MscS mechanosensitive ion channel | -1.3 | + | + |
| Dshi_3326 | *dctP3* | TRAP dicarboxylate transporter- DctP subunit | -2.1 | + | + |
| Dshi_3328 | *dctM4* | TRAP dicarboxylate transporter, DctM subunit | -1.4 | o | o |
| Dshi_3395 | *dctM3* | TRAP C4-dicarboxylate transport system permease DctM subunit | -1.3 | o | o |
| Dshi_3396 | *dctQ* | Tripartite ATP-independent periplasmic transporter DctQ | -1.6 | o | + |
| Dshi_3397 | *dctP* | TRAP dicarboxylate transporter- DctP subunit | -1.8 | + | + |
| Dshi_3905 | *mscS* | MscS mechanosensitive ion channel | 1.2 | + | o |
| Dshi_4182 | *mscS* | MscS mechanosensitive ion channel | 1.1 | o | o |
| **Peptidases and peptide transporters** | | | | | |
| Dshi_0655 | *oppA* | ABC peptide transporter | -1.1 | + | + |
| Dshi_0658 | *oppDF* | ABC peptide transporter | -1.2 | + | + |
| Dshi_0841 | | Hypothetical protein | -1.3 | o | o |
| Dshi_1223 | *pepN* | Aminopeptidase N | 1.1 | o | o |
| Dshi_1777 | | ATP-dependent protease | -1.0 | + | + |
| Dshi_1883 | | Putative ClpA/ClpB family protein | -1.4 | + | + |
| Dshi_3624 | | Co/Zn/Cd efflux system component | 1.1 | o | o |

| | | | | | |
|---|---|---|---|---|---|
| Dshi_3962 | | | | | |
| Dshi_3626<br>Dshi_3964 | | Co/Zn/Cd resistance protein | -1.0 | o | o |
| Dshi_3625<br>Dshi_3963 | | Hypothetical protein | -1.1 | o | o |
| Dshi_3796 | *oppD* | ABC transporter (importer)<br>ATP-binding protein | -1.1 | o | o |
| **Phages, transposons, insertion elements, DNA restructuring enzymes** | | | | | |
| Dshi_1643 | *recA* | Bacterial DNA recombination | -1.1 | + | + |
| Dshi_2174 | | Putative phage capsid protein | 1.0 | + | + |
| Dshi_2177 | | Phage portal protein, HK97 family | 1.2 | o | o |
| Dshi_3758<br>Dshi_4034 | | Transposase | -1.1 | o | o |
| Dshi_3679<br>Dshi_3875<br>Dshi_4082 | | Integrase catalytic region (transposase) | 1.2 | o | o |
| Dshi_3678<br>Dshi_3874<br>Dshi_4083 | | ATP-binding protein,<br>putative transposase | 1.1 | o | o |
| Dshi_3678<br>Dshi_3874<br>Dshi_4083 | | ATP-binding protein,<br>putative transposase | 1.1 | o | o |
| Dshi_4023 | *repA* | Regulator protein RepA | -1.4 | + | + |
| **Cell envelope** | | | | | |
| Dshi_0027 | *alkB1* | Fatty acid desaturase | -1.0 | o | o |
| Dshi_0543 | | $Na^+/P_i$-cotransporter | 3.5 | o | o |
| Dshi_0750 | | Conserved hypothetical protein | -1.4 | + | + |
| Dshi_0808 | *mipA* | Membrane bound transglycosylase and penicillin-binding protein | 1.0 | + | + |
| Dshi_1134 | | 3-Oxo acid-CoA-transferase (B subunit) | -1.7 | + | + |
| Dshi_1277 | | Hypothetical protein | -1.0 | o | o |
| Dshi_1766 | *lysM* | Pepidoglycan-binging protein LysM | 1.0 | + | + |
| Dshi_2238 | | Periplasmic binding protein/LacI transcriptional regulator | -1.0 | o | o |
| Dshi_2312 | | Type I restriction-modification system (R subunit) | 1.2 | o | o |
| Dshi_2726 | | Hypothetical protein | 1.0 | o | o |
| Dshi_3168 | *apbE* | ApbE family lipoprotein | 23.5 | o | o |
| Dshi_3673 | | Hypothetical protein | 1.2 | o | o |
| Dshi_3708 | | AraC-like ligand binding domain | -1.1 | o | o |
| Dshi_3872 | | Hemolysin-type calcium-binding protein | 1.1 | o | o |

549 **Table S3:** Comparison of the growth phenotype, fold change of gene expression after 30 min
550 of oxygen depletion and the presence of cytoplasmic and membrane proteins under anaerobic,
551 denitrifying conditions. In the table (2) stands for normal growth, (1) for decreased growth, (-)
552 for no growth, (o) for not detected, (+) for detected
553

| Locus Tag | Gene name | Function of gene product | Transposon insertion | Growth phenotype | Fold change Transcript anaerob | Presence Protein anaerob |
|---|---|---|---|---|---|---|
| Dshi_3180 | *nirS* | Nitrite reductase precursor | + | 2 | 31.6 | + |
| Dshi_3192 | | Hypothetical protein | + | 2 | 14.2 | o |
| Dshi_3195 | *nosD* | Nitrous oxide maturation protein | + | 2 | 12.8 | o |
| Dshi_2278 | *dmsA1* | Dimethyl sulfoxide reductase precursor | + | 2 | 11.7 | o |
| Dshi_0542 | | Phosphate transporter | + | 2 | 8.3 | o |
| Dshi_3173 | *nirJ* | Putative nitrite reductase heme biosynthesis J protein | + | 2 | 6.6 | o |
| Dshi_2304 | | Putative regulator of cell morphogenesis and NO signaling | + | nD | 6.2 | + |
| Dshi_0664 | *fixP* | Cytochrome *c* oxidase, *cbb3*-type, subunit III | + | 2 | 5.7 | + |
| Dshi_3165 | *napA* | Nitrate reductase catalytic subunit | + | - | 4.4 | + |
| Dshi_0543 | | $Na^+/P_i$-cotransporter | + | - | 3.5 | o |
| Dshi_3152 | | Protein of unknown function DUF1445 | + | 1 | 3.5 | o |
| Dshi_1449 | | TonB-dependent receptor | + | nD | 3.3 | o |
| Dshi_3163 | *napH* | Ferredoxin-type protein NapH | + | 2 | 3.2 | o |
| Dshi_2233 | *phbC* | Poly-beta-hydroxybutyrate polymerase | + | 2 | 3.1 | + |
| Dshi_3558 | | Hypothetical protein | + | 2 | 3.1 | + |
| Dshi_1968 | *aceE* | Pyruvate dehydrogenase subunit E1 | + | 2 | 3.0 | + |
| Dshi_3066 | *atoB* | Acetyl-CoA acetyltransferase | + | 2 | 2.9 | + |
| Dshi_2363 | *ureE* | UreE urease accessory domain-containing protein | + | 2 | 2.7 | o |
| Dshi_0432 | *arcA* | Arginine deiminase | + | 2 | 2.5 | o |
| Dshi_3590 | | NADH dehydrogenase (ubiquinone) | + | 2 | 2.1 | + |
| Dshi_0563 | *irpA* | Iron-regulated protein | + | 2 | 2.1 | + |
| Dshi_2052 | | Hypothetical protein | + | 2 | 2.1 | o |
| Dshi_2966 | *panB* | 3-Methyl-2-oxobutanoate hydroxymethyltransferase | + | 2 | 2.1 | o |
| Dshi_0426 | | Hypothetical protein | + | 2 | 2.1 | o |
| Dshi_3805 | *phaA* | NADH dehydrogenase | + | - | 2.0 | + |
| Dshi_3249 | *fliE* | Flagellar hook-basal body protein FliE | + | 2 | 1.9 | o |
| Dshi_2965 | | Hypothetical protein | + | 2 | 1.8 | o |
| Dshi_3553 | *acs* | Acetate--CoA ligase | + | 2 | 1.8 | + |
| Dshi_0540 | | NnrU family protein | + | 2 | 1.8 | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| Dshi_1399 | *acsA* | Acetate--CoA ligase | + | 2 | 1.8 | + |
| Dshi_0628 | | Extracellular solute-binding protein | + | 2 | -1.8 | + |
| Dshi_3447 | *secB* | Preprotein translocase subunit SecB | + | 2 | -1.8 | + |
| Dshi_2633 | *aspS* | Aspartyl-tRNA synthetase | + | 2 | -1.8 | + |
| Dshi_0008 | | Hypothetical protein | + | 2 | -1.8 | o |
| Dshi_1650 | | Binding-protein-dependent transport systems inner membrane component | + | nD | -1.8 | + |
| Dshi_0319 | *bztB* | Polar amino acid ABC transporter, inner membrane subunit | + | 2 | -1.8 | + |
| Dshi_3397 | *dctP* | TRAP dicarboxylate transporter- DctP subunit | + | 2 | -1.8 | o |
| Dshi_0113 | *ahpC* | Redoxin domain-containing protein | + | 2 | -1.9 | + |
| Dshi_1374 | | NMT1/THI5-like domain-containing protein | + | 2 | -1.9 | + |
| Dshi_0485 | | Hypothetical protein | + | 2 | -1.9 | o |
| Dshi_3981<br>Dshi_3647 | *virB5* | Conjugal transfer protein TrbG/VirB9/CagX | + | 2 | -1.9 | + |
| Dshi_0504 | *fdnG* | Molybdopterin oxidoreductase | + | 2 | -1.9 | + |
| Dshi_2159 | *pdhB* | Pyruvate dehydrogenase subunit beta | + | - | -1.9 | + |
| Dshi_0484 | | YVTN beta-propeller repeat-containing protein | + | 2 | -2.0 | o |
| Dshi_0125 | *fadH* | NADH:flavin oxidoreductase/NADH oxidase | + | 2 | -2.0 | + |
| Dshi_3977 | *virB3* | Hypothetical protein | + | 2 | -2.0 | o |
| Dshi_0389 | *rbsC* | Monosaccharide-transporting ATPase | + | nD | -2.0 | + |
| Dshi_0839 | | Acyl-CoA dehydrogenase domain-containing protein | + | 2 | -2.0 | o |
| Dshi_3976 | *virB2* | CagE TrbE VirB component of type IV transporter system | + | 2 | -2.0 | + |
| Dshi_3111 | *aslA* | Sulfatase | + | 2 | -2.1 | + |
| Dshi_1404 | | Branched-chain amino acid ABC transporter, periplasmic binding protein, putative | + | 2 | -2.1 | o |
| Dshi_1227 | *glcB* | Malate synthase G | + | 2 | -2.1 | o |
| Dshi_1397 | | Phospholipase/lecithinase/ hemolysin-like protein | + | 2 | -2.1 | o |
| Dshi_0373 | *fadD* | Long-chain-fatty-acid--CoA ligase | + | 2 | -2.1 | + |
| Dshi_3326 | *dctP* | TRAP dicarboxylate transporter, DctP subunit | + | 2 | -2.1 | o |
| Dshi_3547 | *cycA* | Cytochrome *c* class I | + | nD | -2.1 | + |
| Dshi_2342 | | TRAP-type mannitol/chloroaromatic compound transport system small permease component-like protein | + | 2 | -2.1 | + |
| Dshi_2964 | | Basic membrane lipoprotein | + | 2 | -2.1 | + |
| Dshi_0476 | *gcd* | Pyrrolo-quinoline quinone | + | 2 | -2.1 | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| Dshi_0506 | *fdnI* | Formate dehydrogenase, gamma subunit | + | 2 | -2.2 | o |
| Dshi_2661 | | Carbon monoxide dehydrogenase subunit G | + | 2 | -2.2 | + |
| Dshi_2842 | | Basic membrane lipoprotein | + | 2 | -2.2 | + |
| Dshi_2617 | | Hypothetical protein | + | 2 | -2.2 | o |
| Dshi_2491 | *sucC* | Succinyl-CoA synthetase subunit beta | + | 2 | -2.3 | o |
| Dshi_2000 | | D-xylose ABC transporter, periplasmic substrate-binding protein | + | 2 | -2.3 | + |
| Dshi_0531 | *araF* | ABC sugar transporter, periplasmic ligand binding protein | + | 2 | -2.4 | + |
| Dshi_0974 | | Extracellular solute-binding protein | + | 2 | -2.4 | + |
| Dshi_3877<br>Dshi_3681 | *thiC* | Thiamine biosynthesis protein ThiC | + | 2 | -2.4 | + |
| Dshi_1652 | | Extracellular solute-binding protein | + | 2 | -2.4 | + |
| Dshi_0378 | | Hypothetical protein | + | 2 | -2.5 | + |
| Dshi_2081 | | Cytochrome *c* class I | + | 2 | -2.5 | o |
| Dshi_1590 | | Membrane protein involved in aromatic hydrocarbon degradation | + | 2 | -2.7 | + |
| Dshi_0872 | | Extracellular solute-binding protein | + | 2 | -2.8 | + |
| Dshi_1144 | *ctaE* | Cytochrome *c* oxidase subunit III | + | 2 | -2.9 | o |
| Dshi_2659 | *coxL* | Aldehyde oxidase and xanthine dehydrogenase molybdopterin binding | + | 2 | -3.0 | + |
| Dshi_0390 | *rbsB* | Periplasmic binding protein/LacI transcriptional regulator | + | 2 | -3.1 | + |
| Dshi_0547 | *ugpB* | Extracellular solute-binding protein | + | nD | -4.2 | + |
| Dshi_1140 | *ctaC* | Cytochrome *c* oxidase subunit II | + | 2 | -4.2 | + |
| Dshi_1195 | | TRAP transporter solute receptor TAXI family protein | + | - | -4.8 | + |
| Dshi_1194 | | TRAP transporter, 4TM/12TM fusion protein | + | 2 | -5.1 | + |

554