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NITRATE REDUCTION PATHWAY IN *SHEWANELLA ONEIDENSIS* MR-1

presented by

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NITRATE REDUCTION PATHWAY IN *SHEWANELLA ONEIDENSIS* MR-1

by

Claribel Cruz-García

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ABSTRACT

NITRATE REDUCTION PATHWAY IN *SHEWANELLA ONEIDENSIS* MR-1

by

Claribel Cruz-García

Shewanella oneidensis MR-1 is a gram-negative bacterium with an extraordinary metabolic versatility in anion reduction, including the reduction of NO_3^-, Fe(III), U(VI), Mn(IV), Se(VI), Cr(VI). While reduction of nitrate and nitrite has been described for this microorganism, it is not known whether the reduction is by denitrification or dissimilatory nitrate reduction into ammonium (DNRA). By both physiological and genetic evidence, I proved that DNRA is the nitrate reduction pathway in this organism. Using the complete genome sequence of *S. oneidensis* MR-1, I identified a gene encoding a periplasmic nitrate reductase based on its 72% sequence identity with the *napA* gene in *E. coli*. Anaerobic growth of MR-1 on nitrate was abolished in a site directed *napA* mutant I constructed, indicating that NapA is the only nitrate reductase present. The anaerobic expression of the *napA* and *nrfA*, a homolog of the cytochrome b552 nitrite reductase in *E. coli*, increased with increasing nitrate concentration until a plateau was reached at 3 mM KNO_3. This indicates that these genes are not repressed by increasing concentrations of nitrate.

The reduction of nitrate generates intermediates that can be toxic to the microorganism. To determine the genetic response of MR-1 to high concentrations of nitrate, DNA microarrays were used to obtain a complete gene expression profile of MR-1 at low (1 mM) versus high (40 mM) nitrate concentrations. Genes encoding transporters and efflux pumps were up-regulated, perhaps as a mechanism to export toxic

compounds. In addition, the gene expression profile of MR-1, grown anaerobically with nitrate as the only electron acceptor, suggested that this dissimilatory pathway contributes to N assimilation. Hence the nitrate reduction pathway could serve a dual purpose.

The role of EtrA, a homolog of Fnr (global anaerobic regulator in *E. coli*) was examined using an *etrA* deletion mutant I constructed, *S. oneidensis* Etra7-1. The global transcriptome suggested a starvation response for anaerobic cultures of EtrA7-1 when nitrate was the electron acceptor. Genes involved in the activation and synthesis of the LambdaSo, MuSo1 and MuSo2 prophages of MR-1 were up-regulated, suggesting a phage infection. This could be responsible for the low growth yields observed for EtrA7-1 when compared to the wild type. Starvation is a stress condition that is known to induce viral infections. Even though starvation was not directly targeted for examination, the results in this study suggest that EtrA might play an important role in the survival of MR-1 under starvation. Moreover, the low biomass suggests a greater sensitivity of MR-1 to starvation than the toxicity associated with high nitrate concentrations. Down-regulation of genes involved in the nitrate reduction pathway was also observed for EtrA7-1 relative to the wild type, which suggests a positive regulatory role for this protein in the nitrate reduction pathway of *S. oneidensis* MR-1.

DEDICATION

To my family for all the support, love and encouragement.

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CHAPTER I

INTRODUCTION AND RATIONALE

INTRODUCTION

Nitrogen is one of the crucial elements in the building blocks of life: nucleic acids and proteins. Nitrogen exists in nature in various oxidative states that range from +5 for nitrate (the most oxidized specie) to -3 for ammonium (the most reduced compound). Nitrogen compounds found in the biosphere undergo several transformations, which constitute the biogeochemical nitrogen cycle. These transformations that are predominantly carried out by bacteria and archaea regulate the local and global concentration of each nitrogen form. Thus, these processes are important in maintaining the various nitrogen species in balanced or non-harmful levels. Nitrate is one of the most important nitrogen species in agriculture and in the environment. Due to excessive use of fertilizers, nitrate has become a contaminant in groundwater. Nitrate is also of clinical concern since elevated levels in drinking water have been associated with some forms of cancer and methaemoglobinemia (11, 18, 49).

Nitrate is a reactant in three pathways in bacteria. (i) Assimilatory nitrate reduction is one of the most important biological transformations since it provides a nitrogen source for organism growth. This pathway reduces nitrate to nitrite, and finally to ammonium, which is used for the synthesis of amino acids. (ii) Nitrate respiration uses nitrate as the electron acceptor for the generation of energy from a proton motive force. It is a component in denitrification, which is a respiring process where nitrate is converted sequentially into dinitrogen ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). The electron accepting capacity of nitrate in denitrification is 5 electrons per nitrogen. Nitrogen oxide gases produced as intermediates in this pathway are associated with the green house effect and ozone depletion. In agriculture, this process represents an area of great concern because

nitrogen is the most limiting nutrient in crop cultivation and significant loss of nitrogen fertilizer is attributed to denitrification. On the other hand, bacterial denitrification is used for nitrate removal as part of a wastewater treatment prior to the release in the environment. (iii) Dissimilatory nitrate reduction into ammonium (DNRA) is an important process in some anaerobic environments. Three possible functions have been proposed for DNRA: nitrite detoxification, redox balancing, and energy generation. DNRA is characterized by the reduction of nitrate to nitrite but, instead of being reduced to N_2 as in denitrification, nitrite is reduced directly to ammonium. In contrast to denitrification, DNRA conserves nitrogen, which makes it advantageous. This process is most favorable in anaerobic environments due to the high capacity of nitrate to accept electrons when reduced into ammonium (8 electrons per nitrogen). Due to its importance, nitrate reduction has become the subject of many research studies. However, there are crucial aspects that are yet to be elucidated.

Shewanella oneidensis MR-1

Shewanella oneidensis MR-1 is a Gram negative, facultatively anaerobic, polarly flagellated γ proteobacterium. Formerly known as *Shewanella putrefaciens,* MR-1 was first isolated from the Oneida Lake, NY, USA from which its name was acquired. This bacterium has been found in soil, sediments, the water column and clinical environments (19, 51). Bacteria from this genus have been studied for decades, but due to recent findings, which identify *Shewanella oneidensis* MR-1's potential metal bioremediation, there has been increasing interest to better understand the metabolic capabilities of this organism (20, 47, 51).

Unlike most isolated bacteria, MR-1 is a metal-ion reducer and has the advantage of utilizing a wide array of compounds as electron acceptors including oxygen, nitrate, nitrite, fumarate, thiosulfate, elemental sulfur, dimethyl sulfoxide, trimethylamine N-oxide, iron oxide, manganese oxide, chromium oxide and uranium oxide (19). The Institute for Genomic Research (TIGR) released the genome sequence of *Shewanella oneidensis* MR-1 (NC004347 whole genome, NC004349 megaplasmid) in 2002. The genome sequence revealed the presence of numerous genes that encode for an array of electron transport systems including up to 39 c-type cytochromes, the greatest number identified in a bacterial genome thus far. Although the genome does not show an extremely high number of metal ion transporters, the large number of cytochrome genes might explain the metabolic versatility of this microorganism. Studies have indicated that MR-1 can utilize nitrate and nitrite as electron acceptors in a two-step reaction coupled to growth where nitrate is reduced to nitrite and nitrite into ammonium (21). In addition, studies have indicated that MR-1 can reduce Cr(IV) by co-metabolism with nitrate reduction, for energy acquisition (25). MR-1 reduces U(VI) and Cr(IV) into the insoluble forms U(IV) and Cr(VI), which are immobile, thus less toxic and bioavailable. Chromium reduction by co-metabolism with nitrate has been observed in *S. oneidensis* MR-1 while reduction of uranium has been associated with nitrite reduction in other members of the genus (52). Even though ammonium has been detected as a product of nitrate reduction in MR-1, there is a controversy about whether denitrification is taking place in the cell (14, 21). According to the genome sequence of *Shewanella oneidensis* MR-1, the genes necessary to carry out this process are absent; however, those required for DNRA are present (TABLE 1.1).

TABLE 1.1. Genes involved in nitrate reduction in *E. coli* and their homologues in *Shewanella oneidensis* MR-1.

Escherichia coli	Shewanella oneidensis *MR-1*	COG Annotation
napFDAGHBC	*napDAGHB, napF, cymA (napC homologue)*	Periplasmic nitrate reductase
narGHJI	----------	Membrane-bound nitrate reductase
narZYWX	----------	Membrane-bound nitrate reductase
narL, narX	----------	Two-component regulatory system nitrate sensitive
narP, narQ	*narP, narQ*	Two-component regulatory system nitrate sensitive
nrfABCDEFG	*nrfA, nrfF, nrfDCG*	Periplasmic nitrite reductase
nirB	----------	Membrane-bound nitrite reductase
fnr	*etrA*	Oxygen sensing regulator

Dashed lines represent no homologues found in MR-1.

Genes and proteins involved in bacterial nitrate reduction

Most of the genetic and biochemical studies on nitrate respiration and DNRA have been done in *E. coli* (26, 39, 45, 46). Two types of bacterial nitrate reductases have been described: assimilatory nitrate reductases (Nas) and dissimilatory nitrate reductases (Nap and Nar).

Assimilatory nitrate reductases have been found in phototrophic and heterotrophic bacteria. They are comprised of two types, ferredoxin- or flavodoxin-dependent. These enzymes reduce nitrate to nitrite, which is then further reduced into ammonium and incorporated into cell material. Genes involved in assimilatory nitrate reduction are found in the same operon as those involved in assimilatory nitrite reduction. Different nomenclature has been given to homologous genes of these enzymes in different bacteria but the *nas* term is more commonly used and some authors believe it is more appropriate (26, 38).

In contrast to the assimilatory nitrate reductases, many types of dissimilatory nitrate reductases have been observed in bacteria and archaea. A single organism may possess more than one type (e.g. *E. coli* expresses three dissimilatory nitrate reductases) (5, 10, 32, 45). These enzymes can be distinguished not only by their structure and gene sequence but also by their biochemical properties (5, 26, 39, 46). There are two types of dissimilatory nitrate reductases: the membrane-bound respiratory (Nar) and the periplasmic (Nap) nitrate reductases. In *E. coli* the membrane-bound nitrate reductases can be further divided into NarZ and NarA. NarZ, encoded by *nar*ZYWV genes, is constitutively expressed in the cell at low levels under aerobic or anaerobic conditions (5, 26). NarA, encoded by *nar*GHIJ genes, and in contrast to NarZ, is only expressed under

anaerobic conditions. The activity of NarA represents 90% of the total activity of the membrane-bound nitrate reductases in *E. coli* K12 (26).

The bacterial membrane-bound nitrate reductase has three subunits: α (112-140KDa), β (52-64KDa) and γ (19-25KDa). Nar uses NADH as an electron donor, can reduce chlorate and is inhibited by low concentrations of azide (3, 21, 45). This enzyme has been found in microorganisms capable of denitrification and anaerobic respiration (26). This protein is expressed under anoxic conditions. Since it is membrane-bound, it has the ability to generate energy by a proton motive force.

In contrast to Nar, the periplasmic nitrate reductase (Nap) is expressed under aerobic and/or anaerobic conditions and its activity is not affected by low concentrations of azide (3, 45). Several biochemical properties have been used to identify NapA from NarG but the two most common are inability to use NADH as an electron donor and to reduce chlorate. Nap has been purified as a two-subunit enzyme consisting of a molybdopterin-containing catalytic subunit A (~90 KDa) and a [4Fe4S] cluster and a diheme cytochrome c_{552} subunit B (~16KDa). Since Nap cannot generate a proton motive force for energy conservation due to its location (periplasm), different roles have been attributed to this enzyme in different bacteria. Experiments on the Nap systems of *Thiosphaera panthotropha, Rhodobacter capsulatus and Rhodobacter sphaeroides* DSM 158 suggest a redox-balancing role when there is an excess of reducing agents (37, 38, 41). Due to the presence of Nap in most aerobic denitrifiers, a second role has been suggested for Nap and that is as the enzyme responsible for the first step in aerobic denitrification (3, 33). These studies suggest that since Nap is expressed under aerobic and anaerobic conditions, it plays a role in the transition from aerobic to anaerobic

respiration. Once the conditions become more anaerobic, Nar is the primary enzyme and Nap plays a secondary role. However, a study on *Pseudomonas* sp. strain G-179 showed that while this is true for *P. denitrificans* it is not for *Pseudomonas* sp. strain G-179 where Nap is the primary enzyme required for the reduction of nitrate into nitrite in denitrification (3). This investigation suggested that the periplasmic nitrate reduction in this organism generates energy by showing the inability of Nap$^-$ mutants to grow on nitrate. The mechanism of energy generation using Nap in this organism is not clear. *Pseudomonas* sp. strain G-179 was originally classified as a *Pseudomonas* but a phylogenetic analysis using the 16S rDNA sequence of various nitrate reducers revealed a 97% similarity to *Rhyzobium galegae* (3).

In *E. coli,* Nap is encoded by the *napFDAGHBC* operon. Studies in this organism have demonstrated that NapABCD, but not NapFGH, are essential for periplasmic nitrate reduction when glycerol, formate and glucose are used as substrate (32). In an attempt to elucidate the role of NapFGH, mutants defective in either ubiquinol or menaquinol biosynthesis, revealed that NapG and H, but not F, are essential for electron transfer from ubiquinol to NapAB (5). This investigation also proved that NapC has an essential role in electron transfer from both ubiquinol and menaquinol to NapAB. In further investigations, the deletion of either *napG* or *napH* abolished the activity of NapA in cultures of naphtoquinone defective strains of *E. coli* K12 (6). This study indicates that *napG* and *napH* encode an electron transfer complex. The function of this complex seems to be important to maintain a redox-balancing growth.

Several studies in the Nap systems of enteric bacteria have shown expression only under anaerobic conditions (32, 33, 45, 53). This contrast to the biochemical properties

observed for the Nap in some denitrifiers, however this difference has been attributed to the variation in the organization of its operon (45). This group of bacteria, including *E. coli,* is capable of DNRA, but not of denitrification, which suggests a different but unclear role for this system. The genome sequence of various microorganisms reveals a pattern in the genetic organization of the *nap* operon that might determine its physiological role (32, 33). In all organisms, for which the genome sequence has been published, the *nap* operon consists of *napDABC* genes as a common template. In addition to this template, different permutations of other genes have been documented in different types of bacteria. For example, the *nap* operon in denitrifiers consists of five genes *napEDABC*, whilst *E. coli*, which is a non-denitrifier and other ammonifying bacteria do not carry the *napE* gene but contain *napGHF* genes in addition to the *napDABC*. *Pseudomonas* sp. G-179, which utilizes a different denitrification pathway, as described above, possesses a *nap* operon that even when it contains the *napE* gene, is different from that of the denitrifiers (*napEFDABC).* The *nap* operon of *Shewanella oneidensis* MR-1 is different from any *nap* operon described; this strain does not possess *napC* or *napF* (*napDAGHB)* (19). The organization of the *nap* operon of MR-1 is very interesting since the internal genes are arranged as those in the *nap* operon of *E. coli,* however homologues of *napF* and *napC (cymA)* are found in different loci (30). This genetic organization may explain similarities and uniqueness of the nitrate reduction pathway of MR-1 compared to those in *E. coli* and in other bacteria.

Recent experiments on *E. coli* have demonstrated that the *nap* operon is preferentially expressed under low concentrations of nitrate and/or nitrite, whilst the *nar* operon is expressed when the concentrations of nitrate and/or nitrite are high (33, 53). In

an effort to demonstrate whether the Nap system was an energy generating process in *E. coli*, an experiment was performed using NarA$^-$/NarZ$^-$ mutants, thus only Nap was carrying out any nitrate reduction in the cell. Since the mutant grew poorly, a second mutation was performed abolishing NarL (response regulator) activity. The NarL$^-$ mutation resulted in improved growth, which confirms that the poor growth was partly due to repression of *nap* expression by NarL. Another experiment using NarL$^-$ mutants expressing only *nap* as the sole nitrate reductase, demonstrated that Nap activity could be high enough to support anaerobic respiration but that it does not constitute a site to generate a proton motive force (45). This experiment confirmed the observations for the regulation of the expression of *nar* and n*ap* operons. The results demonstrated that NarL and NarP induce the *nap* and *nar* genes in response to NarX and NarQ (12, 13). *E. coli* possesses a nitrate and nitrite two-component regulatory system consisting of NarX, NarQ, NarP and NarL. NarQ and NarX are the two sensor transmitter proteins while NarP and NarL are the two response regulators. It has been shown that NarP and NarL compete for the same DNA-binding site. They both induce *nar* and *nap* but it has been shown that NarL, but not NarP, represses *nap* at high concentrations of nitrate. This has also been observed and studied in depth for the nitrite reductase systems reported in *E. coli* (54).

The reduction of nitrate is followed by the action of a nitrite reductase. *E. coli* has two nitrite reductases encoded by the *nrf*ABCDEFG and the *nir*B operons, which reduce nitrite into ammonium (7, 9, 54). Even though these enzymes catalyze the same reaction they have different roles and biochemical properties. NirB is a cytoplasmic enzyme while NrfA is located in the periplasm. NarL, NarP, NarQ and NarX regulatory systems along

with the regulatory protein Fnr regulate the expression of these two enzymes (31, 48, 54, 57).

In a study of *E. coli,* NrfA was highly expressed under low concentrations of nitrate and/or nitrite while NirB was induced only under high concentrations of nitrate (54). Hence, NirB was proposed to have a role in detoxifying the cell from high levels of nitrite produced by the action of NarG, which is also induced under high concentrations of nitrate. The authors also suggested that NirB might recycle NADH by oxidizing it when there is an excess of reducing equivalents. On the other hand, since NrfA is expressed under low concentrations of nitrate and inhibited at high concentrations, it may work together with Nap using the nitrite that it generates. As previously mentioned, recent investigations suggest a redox-balancing role for NapA in *E. coli* conferred by a NapG/NapH complex (6). It is still not clear whether *E. coli* can generate energy from this process but it has been suggested that Nap and Nrf form a complex where some of their components are membrane-associated enabling the conservation of energy (34).

A model for the regulation of these genes in *E. coli* establishes that at low concentrations of nitrate, NarP and NarL are autophosphorylated, which will allow them to bind the activation sites at positions –79 and –70 of the *nrfA* operon (FIG. 1.1). Fnr will bind to its recognition site in the operon and the expression of *nrf* will be induced. As nitrate reaches a higher concentration, NarL will also bind to –50 and –22 sites, for which the affinity is lower. At site -50 NarL will interfere with Fnr binding and at site –22 it will interfere with RNA polymerase binding to the promoter. These will cause suppression in the expression of *nrf* (45, 53, 54).

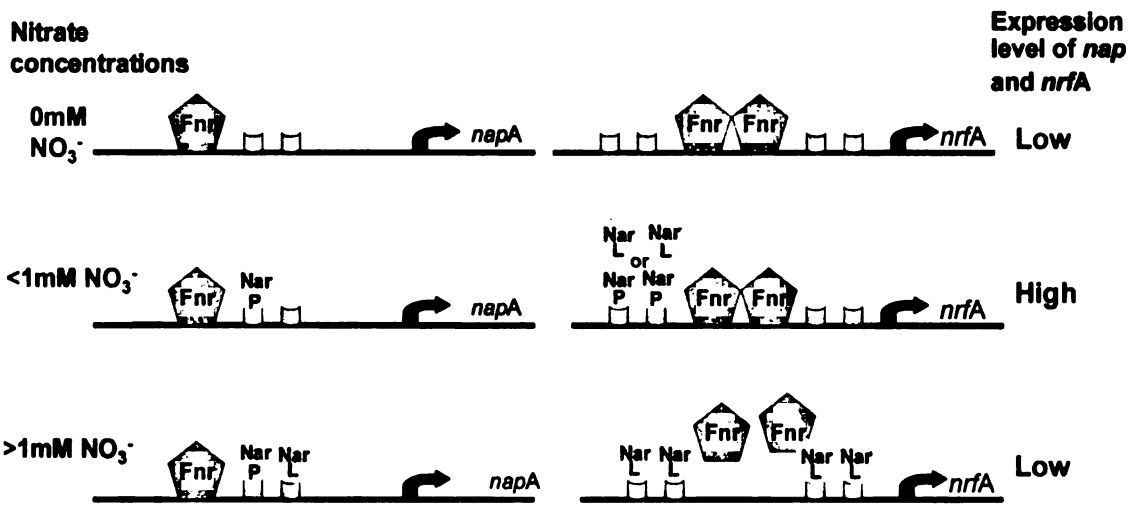

FIG. 1.1. Model proposed for the regulation of the expression of the *nrf* and *nap* genes in *Escherichia coli.* When the concentrations of nitrate are low, the expression level of these genes is induced by the response regulator NarP and the transcription factor Fnr. Once the nitrate concentrations increase, NarL gets activated and blocks the sites for Fnr and NarP, therefore the expression of *napA* and *nrfA* is repressed (Wang and Gunsalus, 2000; Potter et al., 2001).

The benefits of periplasmic nitrate reduction in terms of energy acquisition are not completely clear. Many experiments to elucidate the role and mechanism of this pathway have revealed intriguing results on the physiology of various organisms that keep challenging researchers around the world. The nitrate reduction process in *S. oneidensis* MR-1 seems to be even more interesting, since it is very likely that periplasmic nitrate reduction into ammonium is the primary nitrate reduction pathway used by this organism when nitrate is the only electron acceptor. *S. oneidensis* MR-1 possesses homologues for the genes that have been reported as necessary to carry out only this pathway (TABLE 1.1). Previous studies have suggested that *S. oneidensis* MR-1 is capable of denitrification, however this has been a subject of controversy (14, 21). An experiment with different strains of *Shewanella putrefaciens* (including MR-1) using nitrate and nitrite as sole electron acceptors has primarily detected production of N_2O, and ammonium in low concentrations in the cultures of MR-1 (21). This study also describes the partial purification of a membrane-bound nitrate reductase from MR-1, which the authors claim corresponds to Nar, and a membrane-bound nitrite reductase. These results do not coincide with the genome sequence of *S. oneidensis* MR-1 since only the *nap* and *nrf* operons are present, which encode periplasmic proteins.

The study of the nitrate reduction pathway in *Shewanella oneidensis* MR-1 has been limited to physiological investigations. There has been a lack of detailed genetic studies. Understanding expression of the genes involved in this process in MR-1 is needed to clarify some of the questions regarding the role of the Nap system in this organism. Also, genetic expression analyses can help understand the metabolism of nitrate and its potential to conserve energy for cell survival in other organisms, some of

them pathogens like *Haemophilus influenzae* (34). Since MR-1 is genetically similar to *E. coli,* the models discussed above can be used to help explain what is occurring in MR-1 (27). The evolutionary implications of the absence of the *napF* and *napC* homologues from the *nap* operon of MR-1 might help answer more questions about the pathway.

Several studies mentioned above have been performed using mutants defective in alternative nitrate reduction pathways, which have given rise to important models proposed for this system. Based on these approaches and on the fact that the genome sequence of MR-1 does not show the genes for the NarL/NarX two-component regulatory system, and Nap is the only nitrate reductase encoded, this is an excellent organism to study and to confirm what has been proposed for this system without the need of extensive DNA manipulation. This research focuses on the study of the nitrate reductase(s) involved in the reduction of nitrate and the elucidation of this mechanism in *Shewanella oneidensis* MR-1. This investigation also clarifies some of the contributions of the periplasmic nitrate reduction to the anaerobic metabolism, which is an important process in the global nitrogen cycle.

OBJECTIVES AND EXPERIMENTAL APPROACHES

Objective 1. Determine the growth rate of *Shewanella oneidensis* MR-1 when either nitrate, nitrite or nitrous oxide was used as the only electron acceptor.

This objective will not only provide the optimal concentrations of nitrate and nitrite necessary for growth but will also provide information about the tolerance of this microorganism to the levels of nitrate and nitrite supplied. The growth of *Shewanella oneidensis* MR-1 was examined and compared to that of the positive control, *Pseudomonas stutzeri,* for the reduction of nitrous oxide when the later was the only electron acceptor. Nitrous oxide is one of the intermediates in denitrification and this experiment is positive evidence for this pathway.

Objective 2. Determine the intermediates in the nitrate reduction pathway in *S. oneidensis* MR-1 by their sequential production and consumption.

The growth rate, and the concentration of nitrate, nitrite and ammonium were analyzed over time in anaerobic cultures of *Shewanella oneidensis* MR-1 grown in minimal medium with 2 mM KNO_3 as the only electron acceptor. This experiment also establishes the balance of N-compounds.

Objective 3: Determine the gene expression pattern of *S. oneidensis* MR-1 when grown anaerobically under different concentrations of nitrate.

To quantify *napA*, *nrfA*, *narQ* and *narP* mRNA copy numbers, Quantitative-Real Time-PCR was used (8). The principle of Q-RT-PCR resides in a constant monitoring of the

PCR amplification of the targeted template allowing the quantification of the initial gene copy number. The cautious design of a set of primers, and sometimes of a probe, that anneals between the primers specific for the gene of interest is important for the success of the Q-RT-PCR. The TaqMan technology from Applied Biosystems was used for the Q-RT-PCR analysis. This technology requires the addition of the probe that contains a reporter dye at the 5'end and a quencher at the 3'end (www.appliedbiosystems.com). The amplification of the template starts with the annealing of the primers and the probe to the template following the elongation step by the DNA polymerase. Once the polymerase encounters the probe, it degrades the probe by its 5' nuclease activity. At this point the dye is released and separated from the quencher, which was keeping it from expressing all the fluorescence. Fluorescence increases with the increase in amplification of the template. This fluorescence is then detected by a RT PCR machine through an optic fiber. These data are translated into a value known as the C_T or threshold cycle which is calculated from the curve generated from the fluorescent measurements recorded every few seconds. In order to ensure accuracy of the technique, many safety measures should be taken into consideration. For example, it is important to include a standard and an internal control in the experiment if an absolute quantification is desired. The standard could be a PCR product of the gene of interest in a known concentration. This will allow the extrapolation of the C_T to the actual concentration or copy number of the targeted transcript. The internal control should be a gene that does not change between conditions or treatments, so it can be utilized to account for mechanical errors and for reaction inhibitors. Primers and a probe specific for the gene chosen as control are used to

quantify its transcripts as it is done for the genes of interest. Quality of the samples and the use of quality reagents are also critical for obtaining accurate results.

Objective 4: Analyze the global gene expression profile of *S. oneidensis* MR-1 when grown anaerobically under high and low concentrations of nitrate.

In order to globally analyze the difference in gene expression under a low and a high concentration of nitrate, a *Shewanella oneidensis* MR-1 complete genome DNA microarray was used (17). The spotted DNA microarray contains 4648 unique fragments representing individual open reading frames from *Shewanella oneidensis* MR-1. Total RNA extractions from MR-1 anaerobic cultures grown on 1 mM and 40 mM KNO_3 were labeled with two different fluorescent dyes and hybridized in triplicate on these microarray slides. This experiment examines the genetic or molecular behavior of the cell when the concentrations of nitrate are increased dramatically.

Objective 5: Determine existence of more than one nitrate reductase in MR-1.

The construction of a *nap*$^-$ mutant was performed using the Cre-lox mutagenesis protocol for Gram-negative bacteria (24). Mutagenesis in *Shewanella oneidensis* MR-1 as well as in many other bacteria has been a great challenge (23). This protocol had to be modified in order to overcome some of the obstacles presented when it was applied to MR-1. This protocol consists of four major steps, which will be discussed briefly. The first step is the insertion of the PCR products of the regions flanking the *nap* gene by ligation. This procedure requires the design of two pairs of primers (amplifying a region of 400-600 bp

in length for better efficiency of recombination) for the amplification of the regions flanking the *nap* gene. Each primer is designed so their respective 5' termini posses complementary tags to permit insertion into the suicide vector. This can be accomplished if the tags include an enzyme recognition site (two different tags were used per primer set to allow directional insertion of the fragments). The *napA* flanking regions are then cloned into the suicide vector, which in this case was pCM184. This suicide vector possesses a kanamycin resistance (Kan) cassette flanked by two *loxP* sites and two multiple cloning sites. The flanking regions of the *napA* gene were ligated, flanking the two *loxP* sites. Once the vector is characterized by sequencing, the second step is its electroporation into *E.coli* β2155 competent cells for subsequent transference of the vector into MR-1. This *E. coli* strain is an auxotroph for di-amino-pimelic acid (DAP), which in its absence disables the growth of the bacterium. This is a great advantage because, after the mating *E. coli* β2155 harboring the vector with the flanking regions of the gene of interest and *Shewanella oneidensis* MR-1, they are spread on LB plates with kanamycin but no DAP. This media will allow only the growth of the MR-1 cells that were successfully transformed with the vector. Since this is a suicide vector it is expect to be eliminated as the bacteria duplicates, because it should not replicate in MR-1. Unfortunately, that was not the case and the vector remained in the cell. Hence, another vector, pKNOCK-Gm, known to be a suicide vector in MR-1 was then selected and the fragment including the flanking regions of *napA*, *loxP* sites and the kanamycin cassette from pCM184 were excised by enzymatic digestion and moved into the multiple cloning site of pKNOCK-Gm (as the name implies, it is gentamycin resistant) (1). Then, this vector (pCCG01) was electroporated into *E. coli* β2155 competent cells, from which it

was subsequently transferred by conjugation into MR-1. The MR-1 kanamycin resistant, gentamycin susceptible colonies were then diagnosed for the deletion by PCR. The kanamycin cassette replaces the gene by homologous recombination between the flanking regions in the suicide vector with those in the chromosome. The third step involves the introduction by conjugation of a plasmid encoding the Cre recombinase (pCM157) into the mutated colonies. The Cre recombinase recognizes the *loxP* sites flanking the kanamycin cassette and recombines them, excising the kanamycin cassette and leaving a residual *loxP* sequence. The fourth and last step is the curing of the plasmid. This was achieved by inoculating the positive transformants in liquid media without tetracycline, the antibiotic for which this plasmid confers resistance, and it is transferred until the resistance is lost. Characterization of the mutant was performed by diagnostic PCR and DNA sequencing. Growth on nitrate as the only electron acceptor was tested for the mutant.

Objective 6: Determine the role of EtrA in the nitrate reduction pathway of *Shewanella oneidensis* MR-1.

A *S. oneidensis* MR-1 *etrA* deletion mutant was generated as described above. The growth, nitrate reduction capabilities and global gene expression profile when cultivated with nitrate as the only electron acceptor were analyzed in comparison with those of the wild type. DNA microarray analyses were done as described above. The results obtain from this work will resolve how *S. oneidensis* MR-1 reduces nitrate and provides insight into the global regulatory control of this process in response to nitrate concentrations.

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CHAPTER II

Reduction of Nitrate in *Shewanella oneidensis* MR-1

ABSTRACT

Nitrate is an important environmental resource and can also be a contaminant. Bioremediation of nitrate can be achieved by nitrate reduction to dinitrogen gas, which in nature is mostly performed by denitrifying bacteria. The enzymes and the mechanisms behind nitrate reduction have been the subject of many studies in *E. coli* as well as in denitrifying bacteria. *Shewanella oneidensis* MR-1 is a γ-proteobacterium, which has the ability to utilize a wide variety of electron acceptors including nitrate and nitrite. It has been a subject of controversy as to whether this gram-negative bacterium reduces nitrate by denitrification or by dissimilatory nitrate reduction into ammonium (DNRA). In this study, *Shewanella oneidensis* MR-1 was grown anaerobically using KNO_3 or $NaNO_2$ as the sole electron acceptor, showing a maximum growth yield at 4 mM and 2 mM, respectively. Anaerobic cultures of MR-1 in minimal medium using 20 mM lactate as the electron donor and 2 mM KNO_3 as the electron acceptor showed a sequential reduction of nitrate to nitrite and then to ammonium. Nitrate reduction into ammonium was coupled to growth. When MR-1 was inoculated under anaerobic conditions using N_2O as the only electron acceptor, no growth or gas production was observed. These results establish that *Shewanella oneidensis* MR-1 is not capable of denitrification but instead carries out DNRA.

INTRODUCTION

Shewanella oneidensis MR-1 is a metal ion reducer that has extraordinary versatility in the variety of compounds it can reduce under oxygen-limited conditions. Among the compounds it can utilize, there is oxygen, nitrate, nitrite, fumarate, Mn(III), Fe(III), elemental sulfur, sulfide, thiosulfate, dimethyl sulfoxide (DMSO), trimethylamine *N*-oxide (TMAO), Cr(VI), and U(VI) (9). *S. oneidensis* MR-1 reduces nitrate, generating energy for growth, as it reduces Cr(VI) into its insoluble form, a less harmful form (12). Nitrate, chromium and uranium, all reduced by *S. oneidensis* MR-1, are regulated pollutants of great concern due to their human health implications. All of them have been linked to various forms of cancer (5, 7, 8, 12, 16, 21).

In *S. oneidensis* MR-1 two different pathways have been described for the reduction of nitrate: the dissimilatory nitrate reduction into ammonium (DNRA) and denitrification (6, 10). In denitrification nitrate is sequentially reduced into dinitrogen ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), whilst in DNRA, nitrate is reduced into nitrite and then into ammonium. No bacterium has been reported to carry out both complete pathways (18). In order to determine which of these pathways takes place in the organism, the nitrogen intermediates and products are determined (20). To measure denitrification, acetylene is used since it inhibits the enzyme that catalyzes the reduction of nitrous oxide into dinitrogen (nitrous oxide reductase) causing the accumulation of nitrous oxide which is easily quantified. Dinitrogen production can also be measured but due to its high abundance in the atmosphere it is harder to quantify its production. Accumulation of ammonium is measured in cultures to determine whether DNRA is the nitrate reducing

process. In the studies mentioned above where the reduction of nitrate was measured in cultures of MR-1, ammonium was measured only in one of them (10) and the concentration detected was very low. Each of these previous reports detected nitrous oxide but in very low amounts compared to the concentration of nitrate supplied. Although these studies concluded that denitrification is occurring in MR-1, the data from its genome sequence does not support this conclusion. The complete genome sequence of MR-1 revealed the presence of all the genes necessary to carry out DNRA but key genes for denitrification, such as those for denitrifying nitrite, nitric oxide and nitrous oxide reductases, are not present (9). Preliminary studies in our laboratory have shown that insignificant amounts of N_2O and no N_2 have been detected when *S. oneidensis* MR-1 was cultivated anaerobically with nitrate as the only electron acceptor suggesting that the nitrate reduction pathway in this organism is yet to be resolved.

Nitrate reduction in bacteria has been studied for decades (20). Despite all of the knowledge acquired on this subject, there are still important genetic and biochemical aspects that need clarification. This study focuses on nitrate metabolism of *Shewanella oneidensis* MR-1. MR-1 was cultivated under different growth conditions to determine the optimal concentrations of nitrate and nitrite for growth as well as the intermediates produced from the reduction of nitrate when the later is used as the sole electron acceptor. The inability of *Shewanella oneidensis* MR-1 to use nitrous oxide and the lack of gas production from the reduction of nitrate confirm that denitrification is not taking place in this microorganism and the production of ammonium shows that DNRA is the operative nitrate reduction pathway.

MATERIALS AND METHODS

Growth conditions and bacterial strains. *Shewanella oneidensis* MR-1 (ATCC 700550) was the strain used in this study. *Pseudomonas stutzeri* (provided by Verónica Grüntzig at the Center for Microbial Ecology at Michigan State University) was the bacterium used as a positive control in the nitrous oxide utilization assay. Growth curves were performed in anaerobic modified LML liquid medium (2). This media was supplied with 20 mM lactate as the electron donor, 0.01% of vitamin-free Casamino Acids, and 0.01% of a trace metals solution (11). The medium also contained either 0.5, 1, 2 and 4 mM $NaNO_2$ or 0.5, 1, 3, 4, 10, 40 and 100 mM KNO_3 as electron acceptors. The anaerobic cultivation was performed in 30 ml Balch tubes with 15 ml of medium, which was previously degassed by boiling and purged with helium. The tubes were closed with butyl rubber stoppers to prevent aeration. A vitamin solution (23) was added by injection after autoclaving. These cultures were inoculated by injection of a 1% *Shewanella oneidensis* MR-1 culture that had been grown aerobically for 12 h in LML. This inoculum originated from an overnight starter culture grown in LB medium. Incubation was performed at 30°C without shaking.

Cultures used to determine consumption and production of nitrate, nitrite and ammonium by *Shewanella oneidensis* MR-1 were cultivated in Modified M1 minimal medium (14) without NH_4Cl. The M1 medium was supplemented with 20 mM sodium lactate as the electron donor. HEPES (pH7.2) was added to buffer the medium at a 50 mM final concentration. KNO_3 was added as the electron acceptor at 2 mM final

concentration. Other medium components were prepared as specified above. Negative controls of each growth condition were no inoculation and media without the electron acceptors but inoculated.

Nitrate, nitrite and ammonium analyses. To determine the consumption and production of intermediates during the bacterial reduction of nitrate, three 30 ml Balch tubes containing 15 ml of M1 medium with 2 mM KNO_3 were inoculated as indicated above. These cultures were incubated at 30°C and an OD measurement at 600 nm was taken every 3 to 4 h after an initial 8 h incubation period using a Varian Cary 50 BIO UV-Vis spectrophotometer (Varian, Zug, Switzerland). After the OD measurements were taken, a 2 ml sample was sterile filtered (0.22 μm syringe filter). These samples were analyzed for nitrate and nitrite using a Lachat QuickChem Automated Flow Injection Ion Analyzer following the Copperized Cadmium Reduction Method as in QuickChem Method No. 10-107-04-1-A (Lachat Instruments, 1988) at the Soil Testing Lab at Michigan State University. The ammonium analysis was performed by the salicylate colorimetric method (15).

Cell growth on nitrous oxide as sole electron acceptor. To determine whether or not *Shewanella oneidensis* MR-1 is capable of utilizing nitrous oxide as an electron acceptor, a 1% inoculum from an aerobic culture grown for 12 h was used to inoculate 20 Balch tubes with 10 ml of LML medium prepared anaerobically. Nitrous oxide gas was added to 10 tubes. The remaining tubes with their helium headspace were used as negative controls for anaerobicity, without nitrous oxide or any other electron acceptor. These cultures were incubated at 30°C without shaking for 3 weeks. Each tube had a Durham tube (inverted smaller tube) at the bottom to observe gas production. The same procedure

was performed as a positive control for nitrous oxide utilization, using a *Pseudomonas stutzeri* culture grown for 12 h as an inoculum. Ten tubes without inoculum served as negative controls for gas production. This same experiment was also performed using 4 mM KNO_3 as the only electron acceptor, instead of nitrous oxide, with *S. oneidensis* MR-1 and *P. stutzeri* as inocula.

RESULTS

Effects of increasing concentrations of nitrate or nitrite in the growth of *Shewanella oneidensis* MR-1. When *Shewanella oneidensis* MR-1 was inoculated in anaerobic LML medium in the presence of KNO_3 as the only electron acceptor, the biomass increased with increasing concentrations of nitrate until 4 mM KNO_3, where maximum yield occurred (FIG. 2.1). When the nitrate concentrations were higher than 4 mM, the biomass was dramatically reduced and the maximum yield reached was similar at all concentrations tested up to 100 mM, which was the highest. However, the growth rate in cultures with NO_3^- showed an increase until 2 mM (1 h^{-1}), and decreased at higher concentrations (FIG. 2.3). Similar growth rates were calculated for MR-1 cultures on NO_3^- concentrations higher than 2 mM (0.5 h^{-1}).

When nitrite was supplied as the only electron acceptor, biomass of MR-1 increased with increasing concentrations of $NaNO_2$ until 2 mM, where the maximum cell yield was observed (FIG. 2.2). Higher concentrations of $NaNO_2$ resulted in lower growth yields. The growth rate of MR-1 cultures on NO_2^- decreased with increasing NO_2^- concentrations (FIG. 2.3).

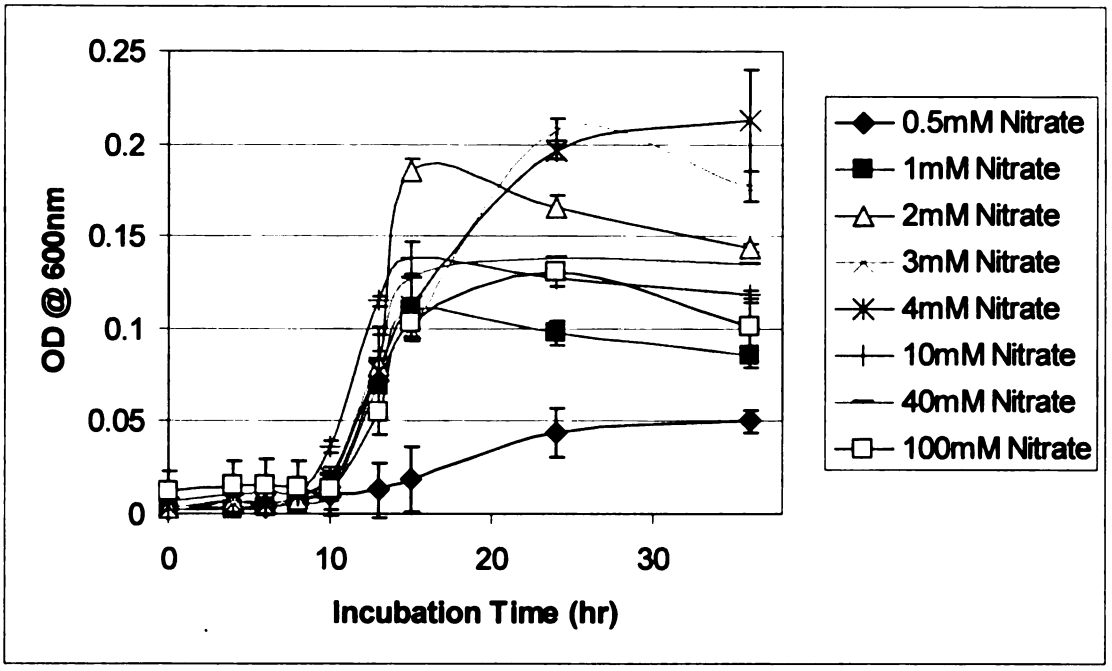

FIG. 2.1. Growth of *Shewanella oniedensis* MR-1 on different concentrations of nitrate. The data are mean and standard deviation of three biological replicates monitored by optical density measurements.

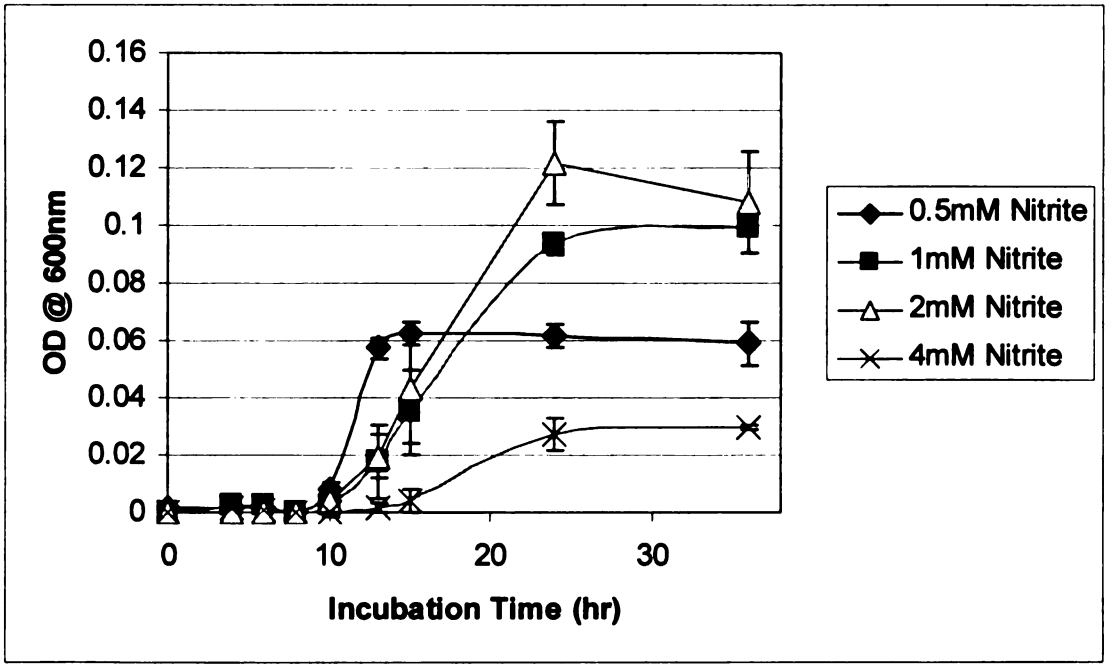

FIG. 2.2. Growth of *Shewanella oneidensis* MR-1 on different concentrations of nitrite. The data are mean and standard deviation of three biological replicates monitored by optical density measurements.

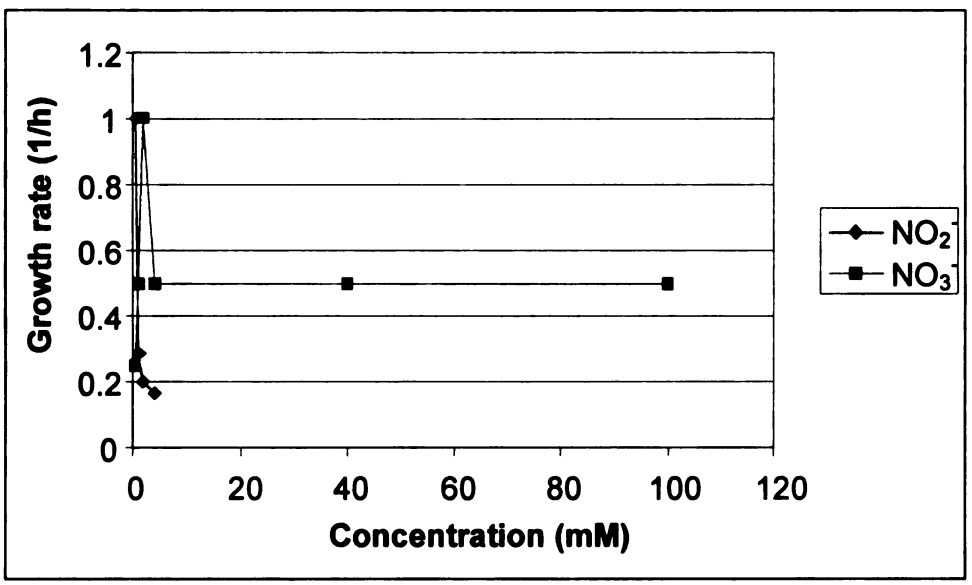

FIG. 2.3. Growth rate of *Shewanella oneidensis* MR-1 in anaerobic cultures at different nitrate and nitrite. The growth rate was calculated from the results of the growth curves shown in FIG 1 and 2. These values were calculated using the average of the optical densities obtained in three biological replicates.

Consumption and production of intermediates in nitrate reduction of *Shewanella oneidensis* MR-1. After 8 h incubation, the culture was in early log phase and nitrate was almost completely depleted and nitrite was produced to a stoichiometric maximum (2 mM)(FIG. 2.4). Ammonium remained at its initial concentration. After 12 h, growth was approximately at mid-log phase and nitrite consumption was commenced along with ammonium accumulation. Nitrate and nitrite were completely consumed and growth reached its maximum at 15 h. At this point ammonium concentration reached 2 mM. After this time the cultures continued to accumulate ammonium to approximately 3 mM

presumably as a result of cell death. The maximum consumption rate of NO_3^- was 0.2 mM/h, whilst for nitrite it was 0.5 mM/h.

Growth of *Shewanella oneidensis* MR-1 when N_2O is the sole electron acceptor.

When *S. oneidensis* MR-1 was incubated with N_2O or NO_3^- as its sole electron acceptor, no N_2 production was observed. In the contrary, those tubes inoculated with *P. stutzeri* showed N_2 production, and growth from both electron acceptors, as expected for the positive control. Growth of MR-1 did occur with NO_3^- but not in N_2O cultures. This study indicates the inability of *Shewanella oneidensis* MR-1 to grow or produce any N_2 gas from the reduction of nitrate or nitrous oxide.

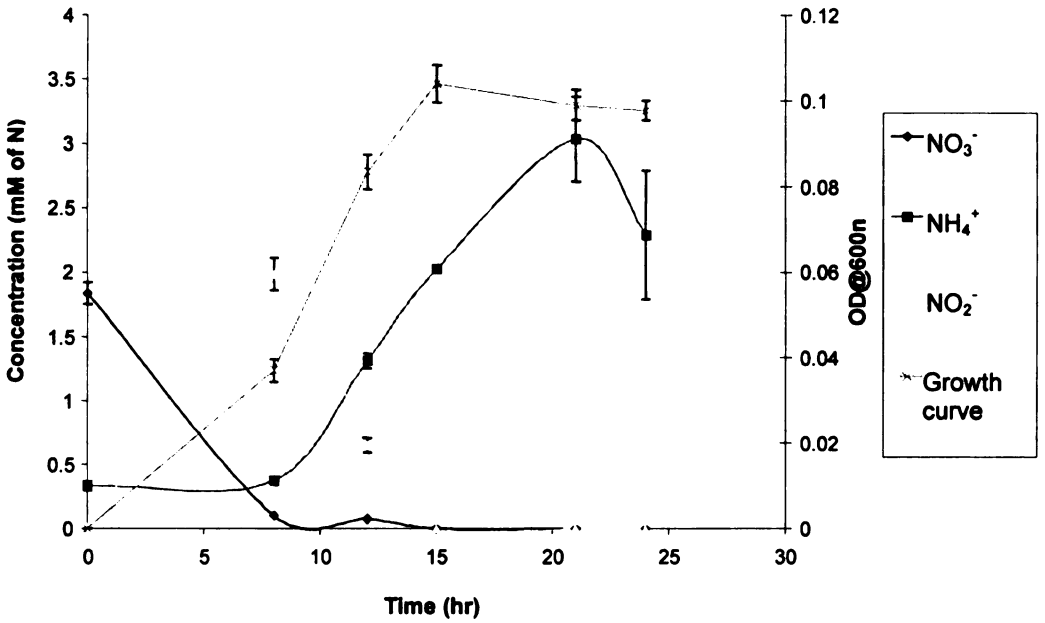

FIG 2.4. Nitrate, nitrite and ammonium concentrations in *Shewanella oneidensis* MR-1 in anaerobic cultures grown in the presence of nitrate as the electron acceptor. OD measurements were obtained at each sampling. These measurements (including the N-species concentrations) are the mean and standard deviation of three biological replicates.

TABLE 2.1. Stoichiometry of nitrogen ions measured in *Shewanella oneidensis* MR-1 anaerobic cultures with 2 mM KNO_3 as the electron acceptor and lactate as the electron donor. The concentrations of each of the N-compounds were an average from three biological replicates and the averages were added for the inorganic nitrogen budget of the cultures.

Incubation Time (h)	NO_3^- (mM)	NO_2^- (mM)	NH_4^+ (mM)	Balance N (mM)
0	1.84	0.00	0.33	2.17
8	0.10	1.98	0.37	2.45
12	0.07	0.65	1.31	2.03
15	0	0.00	2.03	2.03
21	0.00	0.00	3.04	3.04
24	0.00	0.00	2.29	2.29

DISCUSSION

This study clarifies the dissimilatory nitrate reduction pathway of *Shewanella oneidensis* MR-1. Stoichiometric results of inorganic nitrogen species confirmed that nitrate is reduced to ammonium. These experiments showed a sequential reduction of nitrate into nitrite and subsequently into ammonium. The production of the NO_2^- in amounts equivalent to the nitrate supplied indicates that the entire nitrate concentration was converted to first nitrite before been converted to ammonium. This indicates that DNRA is the pathway occurring in *Shewanella oneidensis* MR-1. Also, the inability of MR-1 to reduce N_2O supports DNRA as the nitrate reduction pathway, instead of denitrification.

In this study, neither nitrous oxide nor dinitrogen were measured, but previous experiments performed in our laboratory have demonstrated that when MR-1 was grown using nitrate as the only electron acceptor insignificant amounts of N_2O and no N_2 were detected. Studies on MR-1 that led to the claim of MR-1 as a denitrifier were based on nitrous oxide production but without establishing the stoichiometry of the products (6, 10). Detection and production of nitrous oxide from nitrate has been reported for a number of non-denitrifiers but in all cases this production is less than 30% of the nitrate reduced (3, 4, 19). An investigation to examine the different sources of nitrous oxide in the environment confirmed that all the nitrate-respiring bacteria analyzed, including the *Escherichia* and the *Enterobacter* genera, produced N_2O (3). Moreover, studies in the non-denitrifier *Escherichia coli*, detected N_2O as a product of the reduction of NO_2^- by the nitrate reductase. In this study, reduction of nitrite and production of nitrous oxide decreased significantly in an *E. coli* nitrate reductase mutant. Reduction of nitrite into NH_4^+ and N_2O was observed for the wild type, however no energy was generated when

N_2O was produced. Nitrous oxide production has also been suggested to be the result of an abiotic process, where hydroxylamine (an intermediate in the nitrite reduction into ammonium by NrfA) is converted to nitric oxide and N_2O under specific conditions (4, 24).

Denitrification is defined as the complete reduction of nitrate into dinitrogen (20). In this study, cultures of MR-1 failed to produce gas when grown with NO_3^- or N_2O as electron acceptors. In contrast to cultures with N_2O, growth on nitrate was detected, which indicates that even when the cell had enough energy to grow, the metabolic pathway used to generate the energy was not producing gas intermediates as would occur in denitrification. Moreover, cultures of *P. stutzeri*, a well-characterized denitrifier, which was used as a positive control, produced gas. This indicates that contrary to standard denitrifiers, MR-1 is incapable of carrying out the last step of denitrification. This coincides with the lack of the *nosZ* gene in the genome, which encodes the nitrous oxide reductase described in other bacteria (18).

No organism has been described that can execute the complete pathway of both DNRA and denitrification. *Wolinella succinogenes,* a ε-proteobacterium, has been hypothesized to reduce nitrous oxide to dinitrogen with nitrate as the electron acceptor when either H_2 or formate is supplied as electron donor (18). The genome sequence of *W. succinogenes,* a non-denitrifier that, like MR-1, can reduce nitrite into ammonium, has been recently reported as encoding a unique *nos* cluster lacking some of the genes found in other bacteria but having a novel N_2O reductase gene. This nitrous oxide reductase has been identified as a cytochrome c and the authors hypothesized that it can be part of a novel electron transport when either H_2 or formate is used as the electron donor. Thus,

even when the microorganism cannot carry out denitrification some of its products can still be detected in the cultures. Some of the *nos* genes (*nosLDFYA)* have been described in the genome sequence of MR-1 but there is no homolog for a nitrous oxide reductase. In a study comparing the gene expression profile of MR-1 under aerobic versus anaerobic growth with nitrate as the electron acceptor, the *nos* genes were induced (1). Even though MR-1 does not posses a homolog of this particular *nos* gene, it is possible that MR-1 might be capable of a novel nitrous oxide reduction like *W. succinogenes*, but its conversion to N_2 was not seen under the growth conditions used in this study. However, the studies mentioned previously in which nitrous oxide was detected as the result of nitrate reduction in cultures of MR-1, added H_2 to the medium (6, 10).

Even though the genome of MR-1 codes for some of the proteins involved in denitrification, it does not seem to possess all of the genes required for this process. The genome sequence does not show homologues of *nir* (genes for the reduction of nitrite into nitric oxide), *nor* (genes involved in the reduction of NO into N_2O), and *nosZ* (enzyme required for the reduction of N_2O into N_2). In experiments were the gene expression profile of MR-1 was studied using DNA microarray in cultures grown under anaerobic conditions with nitrate as the sole electron acceptor, significant induction of *napBGHA, nrfA, narQ,* and *cymA* (*napC* homolog, ref. 13) genes was reported (1). All of these genes are required for the DNRA pathway in *E. coli* when the reaction takes place in the periplasm (17, 22). Although this study presents physiological evidence to support that MR-1 is not capable of denitrification, mutational and gene expression analyses is needed to better characterize the nitrate reduction process in this bacterium.

This study confirms the ability of *Shewanella oneidensis* MR-1 to use the DNRA pathway for growth. However, the reduction in biomass and growth rate with increasing concentrations of nitrate and nitrite indicates cytotoxicity at higher concentrations. Growth rate on nitrate decreased at concentrations higher than 2 mM, while the growth rate on nitrite decreased at concentrations higher than 0.5 mM. This indicates a higher cytotoxicity caused by nitrite than nitrate. Also the consumption rate of nitrite was faster than that of nitrate, which might indicate a need of the organism for a faster disposal of the more toxic compound. Increasing concentrations of nitrate become toxic as well but the cell seems to tolerate it better showing a higher and constant growth yield independent of the nitrate concentration up to 100 mM. These results suggests that nitrite causes toxic effects in MR-1 and that MR-1 probably has a mechanism to protect the cell against the nitrite toxicity, perhaps by sensing the concentrations of nitrate and controlling the amount that gets reduced, which will result in lower growth yields that could be kept constant independent of the nitrate concentration. In conclusion, even when the cell is sensitive to low concentrations of nitrite, it can still grow under considerably high concentrations of nitrate and use it for growth.

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CHAPTER III

NapA is the Enzyme Responsible for the Reduction of Nitrate in *Shewanella oneidensis* MR-1

ABSTRACT

NapA is a microbial periplasmic nitrate reductase that is of interest due to a possible relationship between the organization of its operon and the physiological role it plays in the organism. *S. oneidensis* MR-1 has one ORF (SO0848) similar to known nitrate reductases, and it has 72% sequence identity to the *napA* gene of the well-studied *Escherichia coli* K12. A *napA* deletion mutant was unable to grow on nitrate, confirming that NapA is the only functional nitrate reductase in MR-1. MR-1 also possesses a gene that codes for nitrite reductase (SO3980), NrfA. The expression of *nap*A was up-regulated at high (40 mM KNO_3) concentrations of nitrate, which indicates that there is not a repressor regulation system for this operon at high nitrate concentrations as there is in *E. coli*, and is consistent with the absence of an alternative pathway in MR-1. *napA* and *nrfA,* quantified by Q-RT-PCR, were both expressed under aerobic conditions, although reduction of nitrate, was highly inhibited, indicating that the lack of activity under this condition was likely due to oxygen inhibition of the nitrate reductase and not to its transcription.

INTRODUCTION

Nitrate reduction has been extensively studied in many bacteria. In Gram-negative bacteria nitrate reduction is characterized by the location and biochemical properties of their nitrate reductase. This enzyme catalyzes the reduction of nitrate into nitrite, the first step in the nitrate reduction pathway. Two types of enzymes have been described, an assimilatory and a dissimilatory nitrate reductase. The assimilatory nitrate reductase is a membrane-bound protein that catalyzes the conversion of nitrate into ammonium. The ammonium is then incorporated into amino acids and then into cell material. There are two dissimilatory nitrate reductases, classified by their location in the cell: a membrane-bound (Nar) and a periplasmic (Nap) nitrate reductase. Nar is associated with the cytoplasmic membrane and Nap is solubilized in the periplasmic space (23). In most non-denitrifying bacteria these enzymes have been associated with a nitrite reductase. Nar has been associated with the NirB nitrite reductase, which is also located in the cytoplasmic membrane. On the other hand, Nap has been found in association with NrfA, which is a periplasmic nitrite reductase (6, 23). The proximity of these proteins within the cell has been described as energetically advantageous for cell growth. Moreover, a multi-enzyme complex between the Nap and Nrf proteins has been proposed for *E. coli* and other bacteria. This complex might help conserve energy in the cell by avoiding losses of nitrite ensuring high efficiency in its reduction to ammonium (23).

NapA has been identified in different kinds of bacteria including denitrifiers, non-denitrifiers, pathogens and even phototrophic bacteria. Studies in *Shewanella oneidensis* MR-1 have described the capabilities of MR-1 to reduce nitrate (7, 13, 38). A nitrate

reductase was partially purified from membrane extracts of MR-1 suggesting that it is a membrane-associated protein (13). In this same study, a nitrite reductase was also partially purified and it also seemed to be membrane-bound. However, the biochemical and structural properties of the nitrate and nitrite reductases described in that study do not correspond to those previously described for the membrane-bound nitrate and nitrite reductases in other organisms. One of the criteria used to classify the nitrate reductase of MR-1 as membrane-bound was its activity in the membrane fraction of the cell but, in a study of *Pseudomonas* spp. G-179, Nap was found in both the periplasmic and the membrane fractions of the cell (2). This study suggested that Nap might undergo a maturation process where Nap is cleaved from the membrane. Another possibility might be the formation of a protein complex between the soluble subunits and a membrane-bound protein such as NapC, which is essential for the Nap system.

Although these authors found three subunits of the nitrate reductase in MR-1, their molecular weights are not in the range of those previously isolated: α, β and γ were 90, 70 and 55 KDa, respectively for MR-1. In addition, the nitrite reductase detected, contrary to the Nir isolated from denitrifiers, was not periplasmic but membrane-bound. The other biochemical and structural properties observed for the nitrate reductase detected in MR-1 are similar to those observed for NapA in other bacteria (2, 25, 32, 34). Some of these properties include insensitivity towards low concentrations of azide and activity in the presence of oxygen (significantly lower than that under anoxic conditions).

According to the complete genome sequence of MR-1 (11), this microorganism possesses a likely periplasmic nitrate reductase, which shares 72% sequence identity to the NapA of *E. coli*. The *nap* operon has been sequenced in different bacteria and a

relationship between its organization and its physiological role has been proposed (21, 22, 23). In all organisms, for which the genomic sequence has been published, the *nap* operon consists, among others, of *nap*DABC genes in this same order. *S. oneidensis* MR-1 possess *nap*DAGHB, and is the only organism so far which does not have the *nap*C gene in the same gene cluster, although it has a homolog (*cym*A) located in a different loci. There is also a homolog of the *napF* but it is also located in a different loci. MR-1 also possesses the *nrfABCDEF* genes, and the *narP* and *narQ* genes, which code for one of the two-component regulatory systems involved in the regulation of the *nrf* and *nap* operons in *E. coli*. The *cymA* gene encodes a cytochrome c proven to be required for nitrate reduction in MR-1 as well as for its homolog in *E. coli* (18, 19). Studies in a *S. oneidensis* MR-1 *cymA* deletion mutant indicate its requirement not only for nitrate respiration but also for the reduction of nitrite via NrfA, Fe(III), and fumarate and for growth with dimethyl sulfoxide (DMSO) (28, 29). That investigation proved the role of CymA as a common electron supplier for at least five different anaerobic energy-generating processes. DNA microarray data from MR-1 cultures grown under anaerobic conditions with nitrate as the sole electron acceptor demonstrated significant induction of *nrfA, napBGHA, narQ,* and *cymA* genes (3).

In this study the expression of the *napA* and *nrfA* genes was monitored in aerobic and anaerobic cultures of MR-1 under different nitrate concentrations. An increase in the expression of these genes was observed with increasing concentrations of nitrate until it reached a plateau. Also, a global gene expression profile was examined for MR-1 anaerobic cultures grown at a low versus a high concentration of nitrate. A *Shewanella*

oneidensis MR-1 *napA* deletion mutant was generated to test whether this was the enzyme responsible for the nitrate reduction.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains, plasmids, primers and probes used in this study are described in TABLE 3.1. Cultures of *Shewanella oneidensis* MR-1 for DNA microarray and Q-RT-PCR experiments were incubated at 30°C after inoculation in Modified M1 minimal medium (17) with no NH_4Cl to avoid interference with chemical analyses. HEPES (pH 7.2) was added to buffer the medium at a 50 mM final concentration. The medium was supplemented with 20 mM lactate and KNO_3 was added as the electron acceptor at 1 mM and 40 mM final concentration for the DNA microarray experiments. The Q-RT-PCR experiments also included expression analyses of cultures under 0.1 mM, 0.25 mM, 0.5 mM, 3 mM, 10 mM, and 15 mM KNO_3. Aerobic cultures of MR-1 with 3 mM KNO_3 and without it were analyzed using Q-RT-PCR. In order to make the media anoxic, the media were degassed by boiling and purged with helium. The medium (100 ml) was transferred to 250 ml serum bottles and closed with a butyl black stopper. The medium was inoculated by injection with a 1% inoculum from a 12 h aerobic culture in M1 media, which originated from an overnight starting culture in aerobic M1 media as well. The medium was autoclaved and 0.1 ml of Wolfe's vitamin solution (41) was added by injection with a sterile syringe. Incubation was performed at 30°C without shaking. Negative controls for each growth condition were no inoculation and medium without the electron acceptors but inoculated.

Cultures of *Escherichia coli* β2155 (auxotroph of diaminopimelic acid) were grown in Luria-Bertani (LB) medium supplemented with 100 ug/ml of diaminopimelic acid (DAP). These cultures were incubated at 37°C. *Shewanella oneidensis* MR-1 was cultivated in LB media and incubated at 30°C during the mutagenesis process. Antibiotics for *E.coli* were prepared and added as described elsewhere (26). The antibiotics used for the selection of MR-1 positive transformants were added in the following concentrations: 25 ug/ml of kanamycin, 7.5 ug/ml of gentamycin, and 10 ug/ml tetracycline.

Total RNA preparations. To determine gene expression profiles total RNA was extracted from cultures of *S. oneidensis* MR-1 grown in triplicate as described above. Cells were collected at mid-log phase and concentrated by centrifuging at 4°C for 30 min at 7,500 rpm. The pellets were washed with 1 ml of an ice-cold 1X DEPC-treated PBS solution (26). The RNA was extracted with The RNAwiz Solution following the instructions of the manufacturer (Ambion, Inc.). The RNA extraction was followed by an isopropanol precipitation (26) and its resuspention in the RNA storage solution (Ambion, Inc.). The RNA samples used for DNA microarray analyses were treated with RNase-free DNaseI (Roche) to eliminate residual DNA. The samples were purified by phenol, phenol:chloroform (1:1) and chloroform extractions, and stored in ethanol at -80°C until ready for use. The RNA samples used for Q-RT-PCR were DNase treated using the DNA-free Kit (Ambion, Inc.) and purified using the RNeasy Mini Kit (Qiagen). Quality of the RNA was observed using the RNA 6000 Pico LabChip kit and the 2100 Bioanalyzer (Agilent Technologies). The RNA concentration was determined with OD measurements at 260 nm using a Varian Cary 50 BIO UV-Vis spectrophotometer (Varian, Zug, Switzerland).

TABLE 3.1. Bacteria, plasmids, primers and oligonucleotides used in this study.

Strain, plasmid, primer or probe	Description or nucleotide sequence[a,b]	Source, reference or relative position of primer or probe
Bacterial Strains		
E. coli β2155	Diaminopimelic acid auxothroph used for cloning and conjugation	8
S. oneidensis MR-1	Lake Oneida, N.Y., sediment	16
S. oneidensis MR-1 Δ*napA*	*napA* gene deletion derived from MR-1	This study
Plasmids		
pCM157	Broad-host-range *cre* expression vector	14
pCM184	Broad-host-range allelic exchange vector	14
pCCG185	pCM184 with *napA* upstream flank	This study
pCCG186	pCCG185 with *napA* downstream flank	This study
pKNOCK-Gm	Broad-host-range allelic exchange vector	1
pCCG01	pKNOCK-Gm with *napA* flanking regions separated by two *loxP* sites flanking a kanamycin resistance gene	This study
Primers[c]		
napAN Fwd	GCATATGGGCGGCTAATGCT**CAT**AGTGTT	CAT Start codon
napAN Rev	CGAATTCTCTTGCCCCATTCCTCCCT	504nt upstream[d]
napAC Fwd		the start codon
napAC Rev	CGAGCTCCAGACATCGCAGCGTAATCCTT	2452
napAScreen Fwd	GCCGCGGAGTGCCCCGTAAAAGTGATGAA	517nt downstream
napAScreen Rev	AGACATCGCAGCGTAATCTC	stop codon
napAScreenout Fwd	TCCCTCTCCAAAGGGATAGC	499nt downstream
napAScreenout Rev	GTGTCATGCTCTGCGGATT	stop codon
23SRT Fwd	AATGCGCCTGGGATTGAA	484nt upstream
23SRT Rev	TAGCGAAATTCCTTGTCGGG	start codon
23Stemp Rev	GAGACAGCGTGGCCATCATT	728nt downstream
napART Fwd	GTATCAGTTAGCTCAACGCCTC	stop codon
napART Rev	AGAAAGCCCTGTTAACCGTGG	591nt upstream
napAtemp Rev	TCATCCGCAGCAATGGTGT	start codon
nrfART Fwd	GATCGAAGCTACGGTTCTCG	1920
nrfART Rev	GCCACATGTATGCCGTGACT	1985
nrfAtemp Rev	TTTACAGCTCCAGCAAGCCA	2847
	ACGTTTCATACTCGGGATGC	210
		101
		751
		257
		357
		775
Probes		
23SRTProbe	AGTTCCGACCTGCACGAATGGCG	1942
napARTProbe	CTGTATTAAAGGTTACTTCCTGTCGAAAATCATGTACGG	237
nrfARTProbe	CGTAATACCTTGCGTACTGGCGCGC	283

[a] The sequence for the primers is written from the 5'end to the 3'end.

[b] Primers were designed using putative gene sequences of *S. oneidensis* MR-1.

[c] For primer sequences, the restriction sites incorporated are underlined. CATATG, NdeI; GAATTC, EcoRI; GAGCTC, SacI; CCGCGG, SacII.

[d] Even though the *napA* gene is in the opposite direction in the genome, the sequence at the right end of the start codon will be denominated as upstream and the one at the left side of the stop codon as downstream.

Quantitative-Real-Time-PCR (Q-RT-PCR). Total RNA (1 ug) was reverse-transcribed using the Superscript II Kit (Invitrogen) and hexamer primers. cDNA was purified using the Qiagen Purification Kit (Qiagen). The cDNA concentration was determined by spectrophotometry at 260 nm and 700 pg was used as the template for the Q-RT-PCR. Gene specific primers and probes were designed using Primer Express® 1.0 software (Applied Biosystems). BLAST of the sequences against the MR-1 genome was performed to test for specificity. The reaction was carried out with 1X TaqMan® Universal PCR Master Mix (Applied Biosystems) and 500 nM each of, primers and probes. The quantification of *napA* transcripts was done by Q-RT-PCR using the napART Fwd and napART Rev primers, and the napART probe (TABLE 1). In the same manner, *nrfA* transcripts were quantified using the nrfART Fwd and nrfART Rev primers, and the nrfART probe. The reaction was performed using the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems) at the Genomics Technical Support Facility at Michigan State University. A standard calibration curve was prepared with a serial dilution of a PCR product of the putative genes in MR-1 as templates. The PCR product for the *napA* calibration curve was amplified with the napART Fwd and napAtemp Rev. The PCR product for the *nrfA* calibration curve was amplified with nrfART Fwd and nrfAtemp Rev. Primers and a probe specific for the 23S rDNA (23SRTFwd, 23SRTRev and 23SRT probe) were used to quantify the 23S rRNA transcripts. This gene was used as an internal control to normalize the difference in the efficiency of the amplification and technical manipulations (4). A calibration curve was also prepared for the 23S rRNA gene using a PCR product amplified with the 23SRT Fwd and the 23Stemp Rev. The template concentration for this reaction was optimized to

7 pg but the concentration of primers and probe was the same as for the other genes. Triplicates of each sample were run for all the reactions. A negative control (no cDNA added) was run for each of the reactions.

Absolute quantification was calculated by interpolating each sample with their corresponding standard curve.

***napA* deletion mutagenesis.** Molecular procedures such as genomic and plasmid purifications, restriction digestions, sticky ends repair, ligations and electroporations were performed as previously described (26). Primers for PCR reactions (TABLE 3.1) were designed using the Vector NTI® software (InforMax, Inc.) and synthesized at Integrated DNA Technologies (www.idtdna.com).

a) napA allelic exchange vector generation. The MR-1 *napA* flanking regions were cloned into the broad-host-range vector pCM184 for further replacement of the *napA* gene in MR-1 by a kanamycin cassette. This replacement occurred by homologous recombination of the flanking regions in the vector with those in the genome. The kanamycin cassette in pCM184 is flanked by two *loxP* sites, which are in turn flanked by two multiple cloning sites (MCS) (14). The primers napAN Fwd (NdeI) and napAN Rev (EcoRI) were used to amplify the region upstream of the *napA* gene start codon while the napAC Fwd (SacI) and napAC Rev (SacII) amplified the region downstream the stop codon. The product of these reactions was approximately 500 bp each, to ensure high efficiency of recombination (14). Each primer had a restriction site linker at the 5'end for an enzyme that was chosen using the pCM184 MCS as a reference for directional cloning of the fragments. After amplification, the N fragment (upstream) was double digested

with NdeI and EcoRI and the C fragment (downstream) with SacI and SacII. These two fragments were cloned one at a time into the vector (pCM184) that was previously digested with the corresponding restriction enzymes depending on the fragment to be cloned. After the fragment and the vector were digested, these were gel purified using the QIAquick® Gel Extraction Kit (Qiagen). A vector:insert ratio of 1:3 was used for the ligation reaction and 1 ul of this overnight reaction was used to electroporate *E. coli* ß2155 electrocompetent cells. The positive transformants were selected by plating on LB agar supplemented with kanamycin and DAP. The colonies were screened with the primers used to amplify each fragment. Once both fragments were inserted a final PCR screen was performed to check for the correct incorporation of the fragments into the vector generating pCCG186. A set of primers including napAScreen Fwd, which anneals to the inside region of the C fragment and napAScreen Rev that anneals to the inside of the N fragment were used for the screen.

Due to replication of the suicide vector (pCCG186) in MR-1, pCCG186 was digested with EcoRI and SacI to excise the kanamycin cassette with the *loxP* sites and the N and C fragments (a fragment of approximately 2.5 kbp), which were transferred into pKNOCK-Gm (1) for the generation of pCCG01. The pKNOCK-Gm broad-host-range vector has a gentamycin resistance gene and a R6K origin, which needs a π protein for propagation. Since this protein is not present in MR-1 this plasmid made a good suicide vector. To clone the fragment excised from pCCG186 into pKNOCK-Gm, this vector was digested with SmaI, which is a blunt end cutter and the plasmid was dephosphorylated with calf intestinal phosphatase to avoid self-ligation. The sticky ends product of the digestion of the insert with EcoRI and SacI were repaired using T4 DNA

polymerase. The reaction products were gel purified as described above and a vector:insert ratio of 1:16 was used for the ligation reaction. Electroporation and screening of the transformants was performed as described above.

b) *napA* allelic exchange vector transfer into *Shewanella oneidensis* MR-1.

pCCG01 was introduced into MR-1 by conjugation (protocol from Margie Romine, Pacific National Laboratory). The *E. coli* β2155 harboring pCCG01 was inoculated in LB liquid media supplemented with 100 ug/ml of DAP and 50 ug/ml of kanamycin. Overnight cultures of MR-1 and *E. coli* β2155 harboring pCCG01 were mixed in microtubes, the mixtures consisted of 0.5 ml of MR-1 with either 0.5 ml or 1 ml of *E. coli* cultures. Individual controls for each culture were also prepared simultaneously. These cultures were mixed by vortex and concentrated by centrifuging at 4,000 rpm for 2 min at room temperature. The supernatant was discarded and the pellet was gently swirled with the pipette tip in the remaining of the medium (approximately 100 ul). Then, this solution was spotted on an LB plate with DAP and incubated at room temperature for 12 to 16 h. Controls were spotted on individual plates. After incubation, the spotted cultures were scrapped and resuspended in 1 ml of 1X Phosphate-Saline Buffer (PBS) (26). Two 10-fold serial dilutions were prepared and plated on LB agar with 25 ug/ml of kanamycin and no DAP to avoid growth of the *E.coli* β2155, thus only the MR-1 cells that were successfully transformed with pCCG01 were able to grow. To check for the loss of the vector and replacement of the *napA* gene by homologous recombination the colonies obtained were transferred to LB plates with kanamycin and LB plates with kanamycin and gentamycin. Positive MR-1 *napA* deletion mutants were expected to be resistant to kanamycin and susceptible to gentamycin. Those colonies were screened by PCR using

napAScreenout Fwd and napAScreenout Rev which anneal approximately 210 bp downstream and 90 bp upstream from the *napA* flanking regions included in pCCG01, respectively. This screening ensures that the recombination occurred in the targeted area.

c) Removal of kanamycin cassette. The two *loxP* sites flanking the kanamycin cassette are recognition sites for Cre recombinase. This recombinase excises the region inside the *loxP* sites by recombination leaving one of the *loxP* sites. pCM157 is a *cre* gene expression vector (14) which was electroporated into *E.coli* β2155. This vector was transformed into *Shewanella oneidensis* MR-1 ΔnapA Kan^r by conjugation as described above. Colonies susceptible to kanamycin and resistant to tetracycline were selected as positive transformants. These were screened by PCR using napAScreenout primers to confirm the loss of the kanamycin cassette.

d) pCM157 curation from *S. oneidensis* MR-1 Δ*napA*. Cultures of MR-1 Δ*napA* (Tet^r Kan^s) were transferred three times on LB liquid media with no antibiotics and then screened for tetracycline susceptibility on LB agar. This phenotype indicates the loss of the pCM157 plasmid. Colonies were transferred and screened until the correct phenotype was obtained. Colonies susceptible to kanamycin and tetracycline were diagnosed with PCR the napAScreen primers and with primers targeting the inside region of the MR-1 *napA* gene (napART Fwd and napAtemp Rev). DNA sequencing performed at the Genomics Technical Support Facility at Michigan State University confirmed the deletion. The napAScreen Fwd was used to sequence the upstream region from the *loxP* site and the napAScreen Rev for the sequence downstream. These two sequences were assembled into one sequence using Vector NTI Suite 8.0 software.

Growth comparisons of *Shewanella oneidensis* MR-1 wild type and MR-1 Δ*napA*. The cultures of the the wild type and three MR-1 Δ*napA* independent mutants were cultivated aerobically and anaerobically with 3 mM KNO_3 in M1 minimal medium as described above. Growth was monitored constantly by OD measurements at 600nm. Samples from each culture were collected throughout the incubation period for determination of nitrate, nitrite and ammonium concentrations by a Lachat QuickChem Automated Flow Injection Ion Analyzer following the Copperized Cadmium Reduction Method as in QuickChem Method No. 10-107-04-1-A (Lachat Instruments, 1988) at the Soil Testing Lab at Michigan State University. The ammonium analysis was performed by the salicylate colorimetric method (20).

Gene expression profiles of MR-1 growth anaerobically with 1 mM and 40 mM KNO_3. Global gene expression profiles of anaerobic cultures of MR-1 at a low and a high nitrate concentration were compared using DNA microarray technology. A *Shewanella oneidensis* MR-1 complete genome microarray containing a total of 4197 PCR amplicons and 451 oligonucleotides representing individual open reading frames (9) was provided by Liyou Wu and Jizhong Zhou at Oakridge National Laboratory, Oakridge, TN, USA. cDNA preparation and labeling were performed as previously described (27) using a 2:3 ratio of 5-(3-aminoallyl)-dUTP and dTTP. Hybridization and post-hybridization washes were done as described elsewhere (10). Three biological replicates per treatment were used for the hybridization of six microarray slides including technical duplicates (dye-swap). The slides were scanned using an Axon 4000B scanner (Axon Instruments, Inc.). The data analysis was performed using the GeneSpring 6.0 software (Silicon Genetics). The data was normalized per chip and per gene (Lowess Normalization) and the spots

with less than 55% of pixels greater than background plus two standard deviations were eliminated from the analyses (15). The data was filtered using the Benjamini and Hochberg false discovery rate with 95% confidence and only those genes with a >2-fold change in magnitude were considered significant.

RESULTS

Expression analyses of napA and nrfA in cultures of *Shewanella oneidensis* MR-1 by Q-RT-PCR. Liquid samples from the cultures used in the Q-RT-PCR analyses were analyzed to quantify nitrate, nitrite and ammonium concentrations in the medium. Insignificant amounts of nitrite and ammonium were produced in aerobic cultures supplemented with 3 mM KNO_3, and not much nitrate consumption was seen (FIG. 3.1). The anaerobic cultures with 0.1 mM to 1 mM KNO_3 showed disappearance of nitrate and nitrite, although in cultures with the higher initial concentrations, nitrate, nitrite and ammonium remained.

The expression of *napA* in anaerobic cultures of MR-1 increased with increasing concentrations of KNO_3 until 3 mM after which the expression reached a plateau (FIG. 3.2). The expression of *napA* in the MR-1 aerobic cultures with 3 mM KNO_3 and without nitrate did not show a significant difference between each other or when compared with the anaerobic cultures grown on 0.5 mM, 1 mM, 3 mM KNO_3 ($F_{\alpha=0.05}$ 9, 20). The expression of *napA* in anaerobic cultures with 3 mM, 10 mM, 15 mM and 40 mM KNO_3 was not significantly different between each other. However, the difference in its expression in anaerobic cultures on 40 mM KNO_3 when compared to that of aerobic and

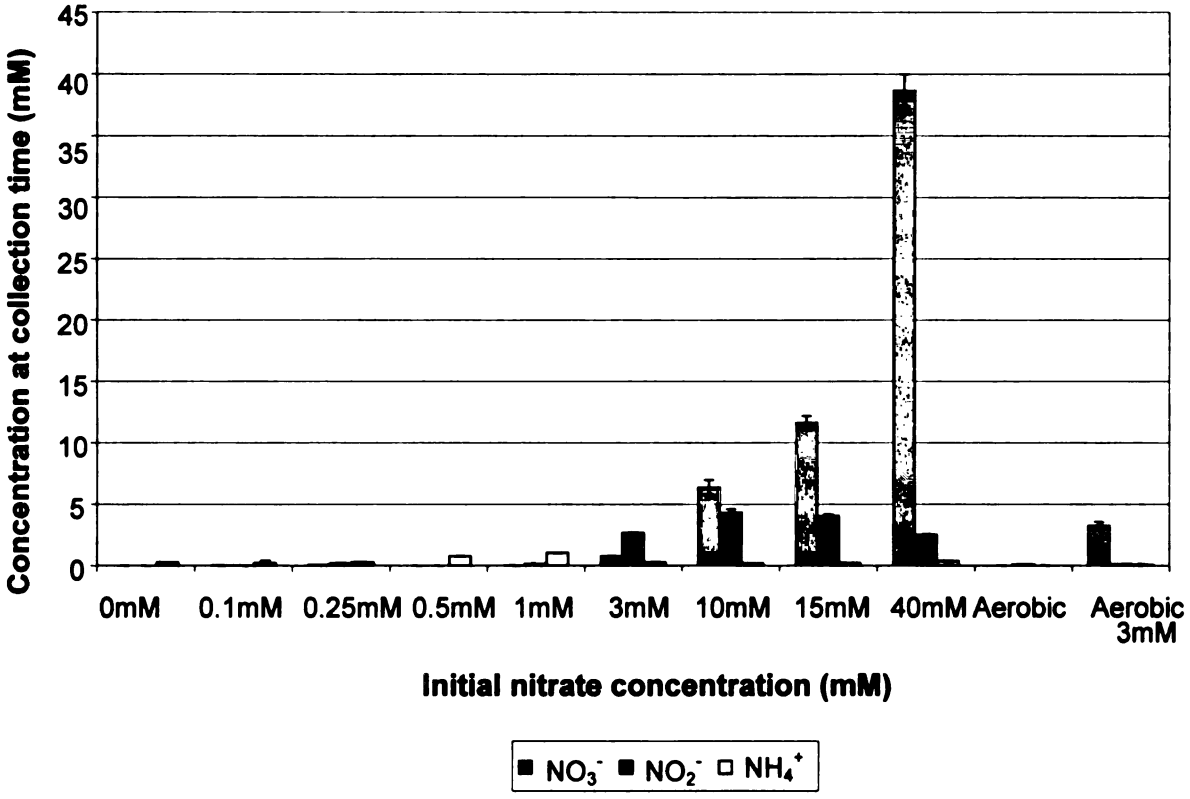

FIG. 3.1. Concentrations of nitrate, nitrite and ammonium in cultures of *Shewanella oneidensis* MR-1 used for Q-RT-PCR analyses at the time the cells were harvested for RNA extraction. Three biological replicates were used for each condition to calculate the mean and the standard deviation.

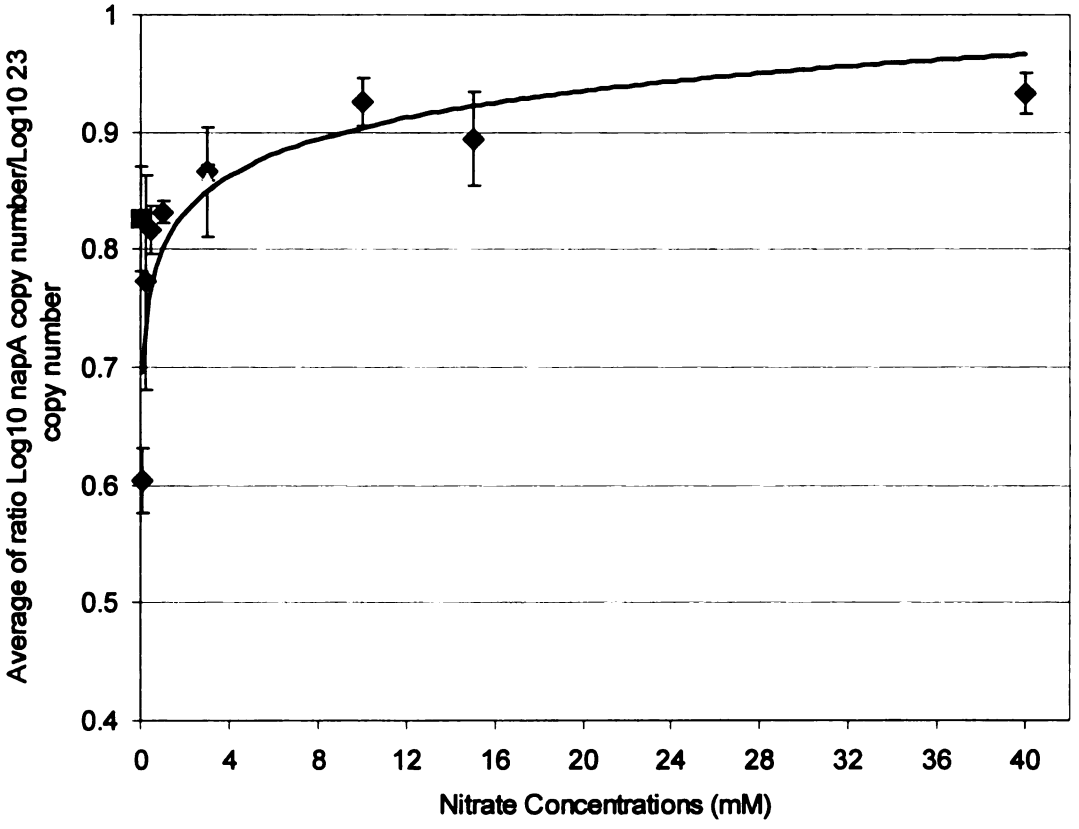

FIG. 3.2. Expression of *napA* in cultures of *Shewanella oneidensis* MR-1 grown at different nitrate concentrations under anaerobic conditions (♦), aerobically with no nitrate (■) and aerobically with 3 mM nitrate ( ). Three biological replicates each done with three analytical replicates were used to calculate the mean and the standard deviation for each growth condition.

anaerobic cultures on 0.1 mM, 0.25 mM, 0.5 mM and 1 mM KNO_3 was statistically significant.

The maximum expression of *nrfA was* reached at 1 mM KNO_3 and higher concentrations showed a constant expression of the gene (FIG. 3.3). The expression of *nrfA* was not significantly different ($F_{\alpha=0.05}$ 9, 20) between anaerobic cultures grown on nitrate at concentrations higher than 0.1 mM KNO_3. The difference in the expression of *nrfA* in the aerobic cultures without nitrate when compared to that of aerobic cultures with 3 mM KNO_3 was statistically significant. The *nrfA* expression in aerobic cultures with and without nitrate was not significantly different when compared to the other anaerobic cultures (except for those at 0.1 mM KNO_3, which was statistically different from all the other conditions tested).

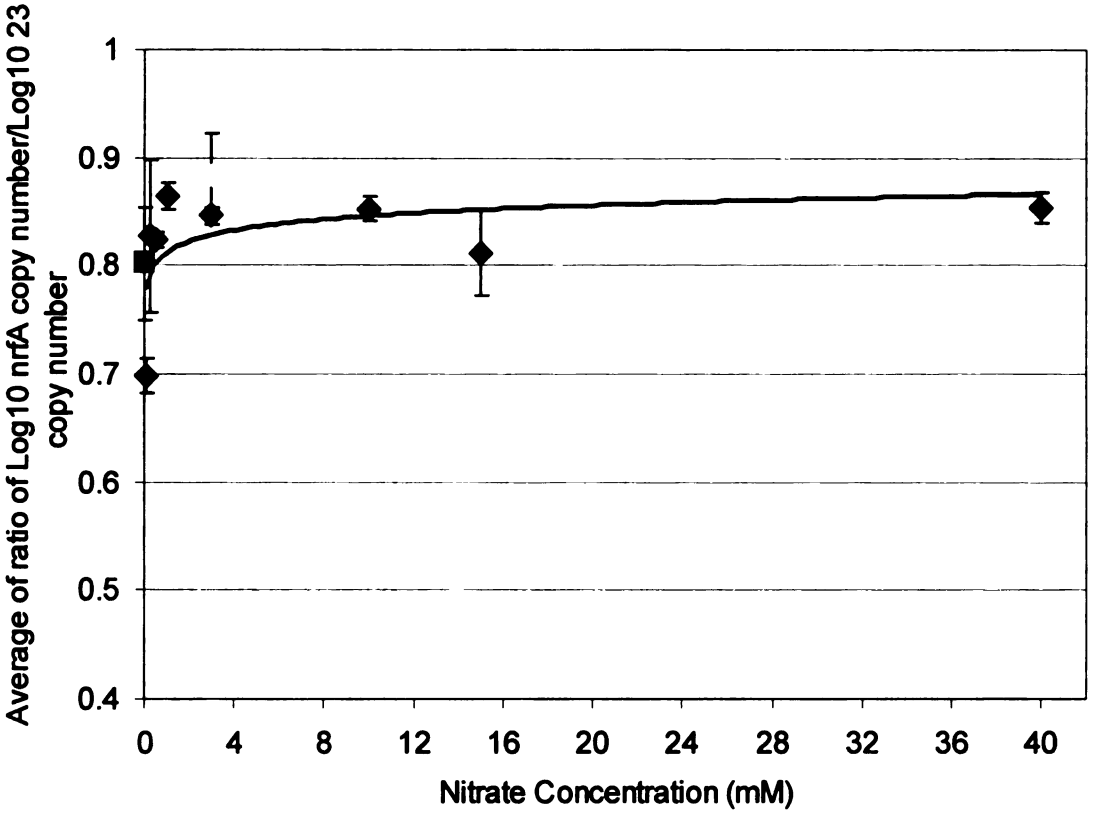

FIG. 3.3. Expression of *nrfA* in cultures of *Shewanella oneidensis* MR-1 grown at different nitrate concentrations under anoxic conditions (♦), aerobically with no nitrate (■) and aerobically with 3 mM nitrate ( ). Three biological replicates each done with three analytical replicates were used to calculate the mean and the standard deviation for each growth condition.

***napA* deletion mutagenesis and complementation analysis.** The *napA* deletion mutants obtained were confirmed by two diagnostic PCR reactions. The first reaction generated a fragment of approximately 1300 bp, which corresponds to the size of the flanking regions and the *loxP* residual left after the recombination by the Cre recombinase (FIG. 3.4). This PCR product was sequenced. A control using the MR-1 wild type genomic DNA as template was used for the same reaction. Since in this case the *napA* gene is present the fragment size is approximately 3800 bp long. This PCR reaction does not only confirm the absence of the gene but also demonstrates the correct location for the incorporation of the construct at the time of the homologous recombination. This is a critical problem for mutagenesis in MR-1, where after the transformation with the allelic exchange vector, the construct often gets integrated somewhere else in the genome. The second diagnostic PCR reaction amplified a region inside the *napA* gene. No amplification was observed in the case of the Δ*napA* mutants as expected if the gene was deleted. A positive control for this reactions was performed using the MR-1 wild type genomic DNA as the template. The fragment for this reaction was approximately 541 bp long.

The PCR products of the mutated region in the Δ*napA* (FIG. 3.4; lanes 1, 2 and 3) were used for DNA sequencing (FIG. 3.5). This sequence includes approximately 500 bp to each side of the deletion and the *loxP* residual (in boldface).

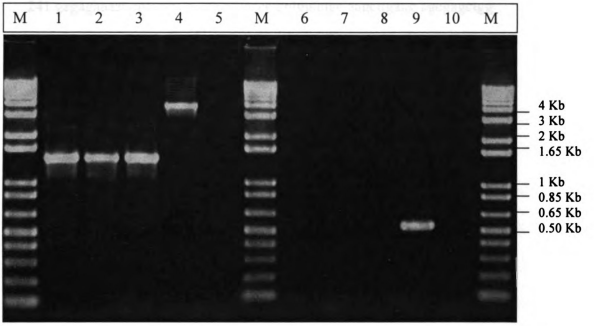

FIG. 3.4. *napA* gene deletion confirmation by PCR. This is a 1% agarose gel 1X TAE which has the PCR reactions to confirm the MR-1 Δ*napA* mutants. Lanes labeled as M are for 1 Kb plus DNA ladder (Invitrogen Life Technologies). Lanes 1-5 correspond to PCR screening with napAScreenout primers. Lanes 6-10 correspond to PCR screening with napART Fwd and napAtemp Rev primers. Sample order: lanes 1 and 6, MR-1 Δ*napA* 22; lanes 2 and 7, MR-1 Δ*napA* 66; lanes 3 and 8, MR-1 Δ*napA* 68; lanes 4 and 8, MR-1 wild type; and lanes 5 and 10 negative control (no DNA).

1 agcatttttc tcatcgatca gtctccaatc cccattttag catcgctaat gtgctcaagt

61 tgaggatcaa gcgcacccga tgggcaggct tttatgcagg gaatatcctc acacatttcg

121 cagggaatgt gtcttgcggt aaagaatggc gtgccagtgg cggcgccatc gaaccaacgt

181 gccagtgtta gcgtgtcgta agggcaagcc tccacacaca aaccgcagcg cacgcaggcc

241 gagagaaaat cgctctcctc aagggcgccc ggtggtctgc aagcttgagg agcgagctgg

301 ccttggcttt ttgccgtagc cgttaagcct aatcccacga gtcccatcac acagccagcc

361 tttgccgtcg tggccaaaaa ctggcgacgg ttgacttgct tggctgtgaa tgcactctta

421 acttgctgac tcaccttaga ctccttaatt gctattaatt gacgccatcg ctatcaaatg

481 aaggcttagg ccttcatcac ttttacgggg cactccgcgg tatcgataag **ctggatccat**

541 **aacttcgtat aatgtatgct atacgaagtt at**gcggccgc catatgggcg gctaatgctc

601 atagtgtttc ctcactcatt ttttctaaca gttcttgttc taggggctcg acttggtggt

661 aaatcaaact ggcggataac acgccggaca gggcattgat ggcttcaaca ttatcgagaa

721 tggccttttg gctatctcct tcgagggtaa taaccaattt accttcgggg gaaatggcgt

781 ggatatcgca gccctttaag gcggttatat cggcctctac ctgttgtaag gcattgggcg

841 cggcatgtac cacgaggctg gtaacatggt attcctgact catagcggtg atccttatct

901 ggagatgcat tcagttgcgt ttgataaatt ttagtacaac taaatgtgga tacctgcgag

961 tctagacctg attaaaaatg tgggtatacc tcacaagagg tattgaaggg gatagtcgat

1021 cgggatcaaa gtttta

FIG. 3.5. DNA sequence of *napA* deletion in MR-1. This sequence was assembled using vector NTI Suite 8.0 software. The assembly included the sequence upstream the *napA* gene and the one downstream using primers napAScreen Fwd and napAScreen Rev, respectively. The sequences underlined represent the sequence of the napAC Rev and napAN Fwd primers. The sequence in boldface corresponds to the *loxP* residual.

Growth of MR-1 wild type versus MR-1 Δ*napA* mutant. Growth under aerobic and anaerobic conditions with nitrate as the electron acceptor was compared in cultures of the wild type versus the Δ*napA* mutant (FIG. 3.6). Based on OD measurements, the aerobic growth was similar in the wild type and in the mutant. However, for anaerobic cultivation with 3 mM nitrate, no growth was detected for the Δ*napA* mutant, contrary to the wild type for which growth was observed. No growth was detected in the anaerobic controls without nitrate.

Analyses of nitrate, nitrite and ammonium were performed in the medium to determine whether the mutant could reduce nitrate (FIG. 3.7). No nitrite was detected in the medium. Low concentrations of ammonium were detected in cultures lacking nitrate as well as in the mutant cultures supplied with nitrate probably generated from cell material. As expected, in the wild type cultures reduction of nitrate and production of nitrite and ammonium was detected (FIG. 3.7).

Due to the length of the *napA* gene (2,484 bp), the construct of an expression vector for the complementation of the ΔnapA mutant was not possible. However, the inability to grow and reduce nitrate when nitrate was the only electron acceptor was observed for three independent MR-1 Δ*napA* deletion mutants. This ensures that no other spontaneous mutation was causing the phenotype. These results are not shown for simplicity of the graphs.

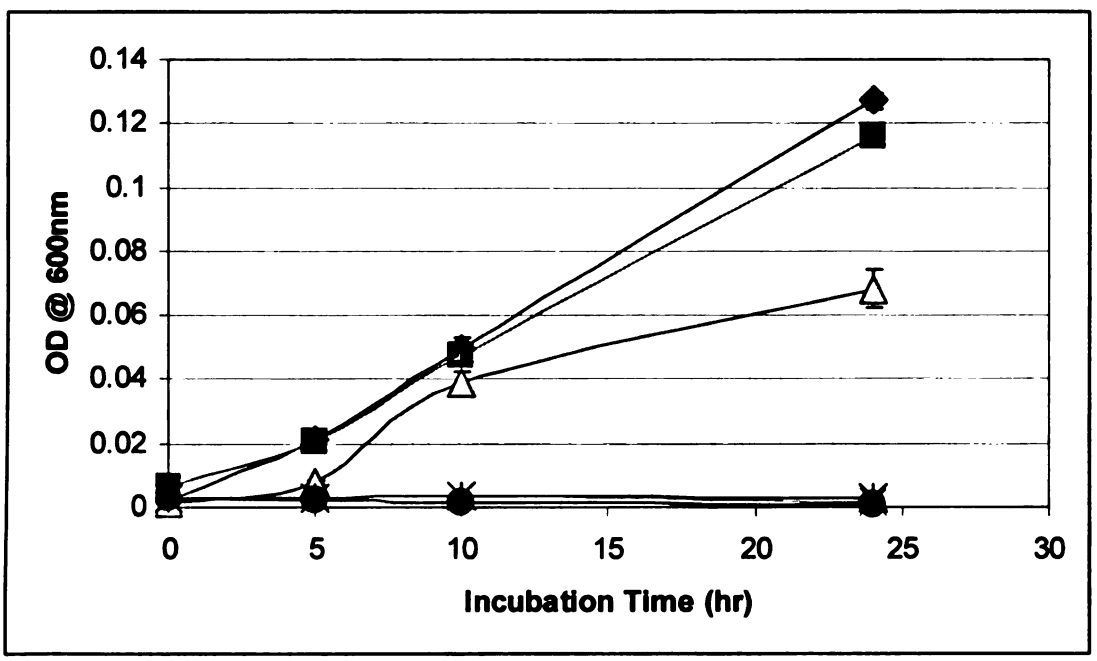

FIG. 3.6. Growth curves for *Shewanella oneidensis* MR-1 wild type grown in M1 medium aerobically (♦), and anaerobically with 3 mM nitrate ( ) and *Shewanella oneidensis* MR-1 Δ*napA* grown aerobically (■) and anaerobically with 3 mM nitrate ( ). Cultures without nitrate inoculated with the wild type (*) and the Δ*napA* mutant (●) were included as negative controls.

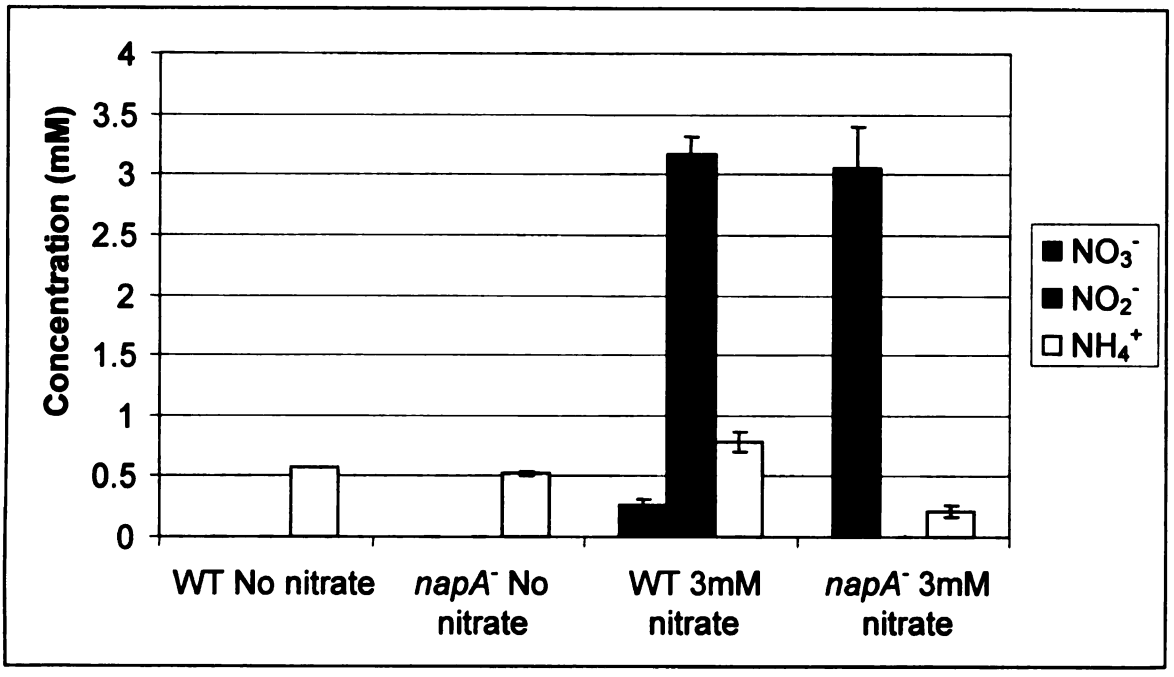

FIG. 3.7. Concentrations of nitrate, nitrite and ammonium in cultures of *Shewanella oneidensis* MR-1 wild type and *Shewanella oneidensis* MR-1 Δ*napA* for growth curve after 24 h incubation period.

Gene expression profiles of MR-1 anaerobic growth on 1 mM and 40 mM KNO_3.

The total number of genes differentially expressed, greater than 2-fold in anaerobic cultures of MR-1 on 1 mM KNO_3 compared to those on 40 mM, was 1082 genes. Of these, 517 were up-regulated and 565 down-regulated at 40 mM relative to 1 mM KNO_3 cultures. The genes up-regulated and down-regulated has been grouped in 20 functional "TIGR Role" categories (FIG. 3.8).The categories with higher percentage of up-regulated as well as the down-regulated genes were the "conserved hypothetical" (22% of the down-regulated and 15.2% of the up-regulated genes) and the "hypothetical proteins" (19.7% of the down-regulated and 12.4% of the up-regulated genes). The other categories with high percentage of up-regulated genes were "Protein synthesis" (11.5%), "Transport binding proteins" (8.85%) and "Energy metabolism" (8.85%)(FIG. 3.8). The up-regulated genes are arranged per category and only those showing a five-fold change or higher were reported in this chapter, except for the genes involved in regulatory functions for which all the genes with a fold change higher than two were included (TABLE 3.2). A complete list of all the genes induced two-fold or higher is provided (SUPPLEMENTAL TABLE B.1). The genes induced in the protein synthesis category include a variety of ribosomal protein genes, different tRNA synthetase genes and other genes involved in translation. Among the transport binding proteins there are a large number of genes encoding ABC transporters such as sulfate, copper and molybdenum ABC transporters. Also, three genes included in this category and annotated as proton/glutamate symporters (SO0157, SO0922 and SO3562) were highly induced. In the energy metabolism category, genes associated with nitrate metabolism such as *hcp, napADG* and *H* were significantly induced. In this category a large number of up-

regulated genes encode cytochromes such as *cydAB, ccoNOPQ, ccmF-1* and SO4047, SO4048 and SO4643. Genes encoding ATP synthases were also induced (*atpIBE* and *F).*

The "purines, pyrimidines, nucleosides and nucleotides" category also comprise a significant percentage of up-regulated genes (4.42%). This group includes *carAB, nrdDG, guaB, apt, udp, upp,* among others. There are also genes involved in amino acid biosynthesis that were significantly induced. Some up-regulated genes included in the "other categories" and in "conserved hypothetical" and "hypothetical" are associated with the LambdaSo phage, which is one of the three phages reported in MR-1 (11). Induction of genes associated with redox response and oxidative stress was also observed (*katG-2, dsbB, uvrA, uvrD* and some co-chaperone genes) (TABLE 3.2, SUPPLEMENTAL TABLE B.1).

The categories with higher percentages of down-regulated genes following the "conserved hypothetical" and the "hypothetical proteins" are the "unknown function" (11.8%), the "regulatory functions" (7.9%) and the "energy metabolism" (6.4%)(FIG.3.8). The down-regulated genes have been grouped per functional "TIGR Role" category and only those down-regulated 5-folds or higher were reported in this chapter, except for those involved in regulatory functions for which all the genes with a fold change higher than two were included (TABLE 3.3). A complete list of all the genes repressed two-fold or higher is provided (SUPPLEMENTAL TABLE B.2). Under the "unknown function" category there are genes that encode hydrolases (e.g. SO0177, SO1585, SO1670, SO2333, SO4039, SO4092), oxydoreductases (SO0900, SO3382, SO2813), domain proteins (e.g. SO0033, SO0296, SO0805, SO0815, SO1208, SO2495, SO2862, SO3489) and AMP binding proteins (SO0075, SO0355, SO1971). Among the

genes that belong to the "regulatory function" there is an array of transcriptional regulators for genes involved in the synthesis of amino acids (*glnB-2, glnD, metJ*, among others) and of other proteins such as flagellin (*flgM)*, prophage LambdaSo Cro/CI family (SO2990) and AraC/XylS family (SO1762, SO3488). Down-regulated genes involved in energy metabolism include genes of the TCA cycle, DMSO anaerobic reduction (*dmaA-1, dmaB-1*) and genes involved in redox response such as thioredoxin (*trxAC)*, glutaredoxin (SO2745) and NADH dehydrogenase I (*nuoA)*. Other genes involved in redox response and oxidative stress in MR-1 and in other bacteria (24) that were down-regulated include *katB, hemH-2, phrB, ahpCF, sodB, ohr* and some genes that encode heat shock proteins (*hslU, grpE, ibpA)*(TABLE 3.3, SUPPLEMENTAL TABLE B.2).

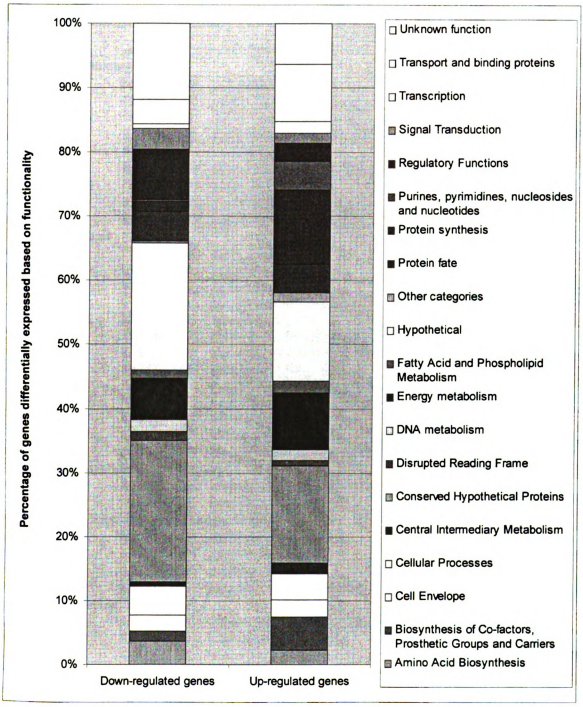

FIG. 3.8. Distribution of differentially expressed genes (> 2-fold change) grouped in 20 functional categories after cultivation on 1 mM (reference) versus 40 mM nitrate concentration. The total of genes down-regulated is 519 and the up-regulated is 571.

TABLE 3.2. Genes induced in anaerobic cultures of MR-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	COG Annotation
1) Transport and Binding Proteins			
SO0827	lldP	22.48 (±11.00)	L-lactate permease
SO4652	sbp	18.15 (± 8.56)	sulfate ABC transporter, periplasmic sulfate-binding protein
SO4654	cysW-2	10.07 (± 2.89)	sulfate ABC transporter, permease protein
SO3553		9.973 (± 2.32)	sulfate permease family protein
SO4150		9.901 (± 2.23)	transporter, putative
SO3599	cysP	9.506 (± 1.97)	sulfate ABC transporter, periplasmic sulfate-binding protein
SO4653	cysT-2	8.15 (± 1.79)	sulfate ABC transporter, permease protein
SO4077		6.736 (± 1.30)	TonB-dependent receptor, putative
SO2857		6.331 (± 1.71)	sodium/solute symporter family protein
2) Energy Metabolism			
SO0849	napD	18.8 (± 9.08)	napD protein
SO1363	hcp	15.57 (± 4.04)	prismane protein
SO1926	gltA	14.24 (± 5.58)	citrate synthase
SO0902	nqrA-1	13.64 (± 5.33)	NADH:ubiquinone oxidoreductase, Na translocating, alpha subunit
SO2136	adhE	12.51 (± 3.13)	aldehyde-alcohol dehydrogenase
SO1364		8.712 (± 2.22)	iron-sulfur cluster-binding protein
SO0848	napA	8.617 (± 4.86)	periplasmic nitrate reductase
SO0903	nqrB-1	6.49 (± 1.93)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB
SO2743	acs	6.439 (± 1.54)	acetyl-coenzyme A synthetase
SO3286	cydA	5.869 (± 1.37)	cytochrome d ubiquinol oxidase, subunit I
SO4509		5.607 (± 2.30)	formate dehydrogenase, alpha subunit
3) Amino Acid Biosynthesis			
SO0277	argF	8.366 (± 1.39)	ornithine carbamoyltransferase
SO2903	cysK	5.94 (± 2.23)	cysteine synthase A
4) Purines, Pyrimidines, nucleosides and nucleotides			
SO1218	deoA	6.847 (± 1.45)	thymidine phosphorylase
SO2791	cdd	5.448 (± 1.39)	cytidine deaminase
SO2403	cmk	5.285 (± 1.39)	cytidylate kinase
SO1301	pyrB	5.165 (± 1.73)	aspartate carbamoyltransferase
5) Regulatory Functions			
SO1415		11.18 (± 3.81)	transcriptional regulator, TetR family
SO3901	icc	7.949 (± 3.12)	lacZ expression regulator
SO3627		6.185 (± 2.77)	transcriptional regulator, TetR family
SO3059		5.778 (± 0.90)	formate hydrogenlyase transcriptional activator, putative
SO2305	lrp	5.163 (± 1.63)	leucine-responsive regulatory protein
SO0843		5.153 (± 1.40)	transcriptional regulator, LysR family
SO1806	pspF	4.98 (± 1.18)	psp operon transcriptional activator
SO1916		2.802 (± 0.34)	transcriptional regulator, LysR family
SO2490		2.714 (± 0.62)	transcriptional regulator, RpiR family
SO3538	hlyU	2.457 (± 0.42)	transcriptional regulator HlyU
SO0393	fis	2.422 (± 0.34)	DNA-binding protein Fis
SO1328		2.3 (± 0.46)	transcriptional regulator, LysR family
SO3874		2.228 (± 0.61)	transcriptional regulator, LysR family
SO1687		2.17 (± 0.57)	transcriptional regulator, MerR family

(Continued)

TABLE 3.2. (Cont'd) Genes induced in anaerobic cultures of MR-1 at 1 mM (reference) versus 40 mM KNO_3

Gene ID	Gene name	Relative expression[a]	COG Annotation
SO2652		2.154 (± 0.30)[b]	prophage MuSo2, transcriptional regulator, Cro/CI family
SO3460		2.135 (± 0.33)	transcriptional regulator, LysR family
6) Protein Synthesis			
SO0230	rpsJ	7.618 (± 2.33)	ribosomal protein S10
SO1855	rmf	6.964 (± 3.00)	ribosome modulation factor
SO0007	rpmH	6.416 (± 2.58)	ribosomal protein L34
SO1288	rpsU	6.221 (± 1.08)	ribosomal protein S21
SO2261		6.027 (± 1.03)	RNA methyltransferase, TrmH family, group 1
SO3940	rplM	5.857 (± 1.86)	ribosomal protein L13
SO1357	rpsP	5.855 (± 1.81)	ribosomal protein S16
SO0241	rplN	5.674 (± 1.32)	ribosomal protein L14
SO1359	trmD	5.544 (± 1.39)	tRNA (guanine-N1)-methyltransferase
SO0231	rplC	5.379 (± 1.44)	ribosomal protein L3
SO2402	rpsA	5.299 (± 2.07)	ribosomal protein S1
SO3939	rpsI	5.279 (± 1.91)	ribosomal protein S9
SO0242	rplX	5.148 (± 0.66)	ribosomal protein L24
7) Protein Fate			
SO2196		8.50 (± 1.45)	LPXTG-site transpeptidase family protein
SO2267	hscB	7.891 (± 3.31)	co-chaperone Hsc20
SO1252		5.58 (± 1.02)	peptidase, U32 family
SO0218	secE	5.48 (± 1.27)	preprotein translocase, SecE subunit
8) Cellular Processes			
SO3245	flgF	9.09 (± 2.30)	flagellar basal-body rod protein FlgF
SO3229	fliE	7.49 (± 1.13)	flagellar hook-basal body complex protein FliE
SO0837		6.70 (± 1.82)	beta-lactamase, putative
SO3250	flgB	5.46 (± 6.74)	flagellar basal-body rod protein FlgB
9) Conserved Hypothetical Proteins			
SO4302		21.56 (±12.00)	conserved hypothetical protein
SO0944		13.57 (± 4.92)	conserved hypothetical protein
SO2821		13.16 (± 6.13)	conserved hypothetical protein
SO3542		12.73 (± 3.19)	conserved hypothetical protein
SO4504		9.54 (± 2.98)	conserved hypothetical protein
SO1287		9.45 (± 3.25)	conserved hypothetical protein
SO4505		8.70 (± 2.79)	conserved hypothetical protein
SO0449		7.53 (± 1.11)	conserved hypothetical protein
SO3891		7.14 (± 4.93)	conserved hypothetical protein
SO3507		6.88 (± 1.14)	conserved hypothetical protein
SO1657		6.68 (± 1.88)	conserved hypothetical protein
SO0324		6.51 (± 1.24)	conserved hypothetical protein
SO4651		6.12 (± 5.86)	conserved hypothetical protein
SO3720		5.29 (± 1.09)	conserved hypothetical protein
SO3085		5.15 (± 2.76)	conserved domain protein
SO4131		5.08 (± 0.95)	conserved hypothetical protein
10) Hypothetical Proteins			
SO0941		8.25 (±2.07)	hypothetical protein

(Continued)

TABLE 3. 2. (Cont'd) Genes induced in anaerobic cultures of MR-1 at 1 mM (reference) versus 40 mM KNO_3

Gene ID	Gene name	Relative expression[a]	COG Annotation
SO4656		7.59 (±2.84)	hypothetical protein
SO0581		7.55 (± 2.36)	hypothetical protein
ORF03631		7.05 (± 2.75)	hypothetical protein
SOA0157		6.87 (± 2.38)	hypothetical protein
SO1947		5.91 (± 1.01)	hypothetical protein
SO4701		5.50 (± 1.27)	hypothetical protein
SO1516		5.35 (± 2.44)	hypothetical protein
SOA0158		5.13 (± 1.51)	hypothetical protein
11) Central Intermediary Metabolism			
SO3738	cysJ	13.93(±3.94)	sulfite reductase (NADPH) flavoprotein alpha-component
SO1871		11.13(±6.92)	S-adenosylmethionine decarboxylase proenzyme, putative
SO3727	cysD	8.33 (±3.00)	sulfate adenylyltransferase, subunit 2
SO3726	cysN	7.3 (± 1.87)	sulfate adenylyltransferase, subunit 1
SO3737	cysI	7.01 (±1.53)	sulfite reductase (NADPH) hemoprotein beta-component

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures of MR-1 grown at 40 mM KNO_3 to that of the anaerobically grown at 1 mM KNO_3 (reference).
[b]The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

TABLE 3.3. Genes repressed in anaerobic cultures of MR-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	COG Annotation
1) Energy Metabolism			
SO0274	ppc	0.17(±0.04)[b]	phosphoenolpyruvate carboxylase
SO0452	trxC	0.11 (± 0.05)	thioredoxin 2
SO0406	trxA	0.09 (± 0.05)	thioredoxin 1
SO3683		0.07 (± 0.01)	coniferyl aldehyde dehydrogenase
2) Amino Acid Biosynthesis			
SO3019	trpE	0.19 (± 0.05)	anthranilate synthase component I
SO1268		0.17 (± 0.03)	glutamine synthetase
SO4349	ilvC	0.05 (± 0.02)	ketol-acid reductoisomerase
3) Protein Synthesis			
SO1473	smpB	0.20 (± 0.08)	SsrA-binding protein
SO3403	yfiA-1	0.18 (± 0.05)	ribosomal subunit interface protein
SO1786	glnS	0.13 (± 0.04)	glutaminyl-tRNA synthetase
4) Regulatory Functions			
SO4057	metJ	0.50 (± 0.17)	met repressor
SO2990		0.50 (± 0.14)	prophage LambdaSo, transcriptional regulator, Cro/CI family
SO1393		0.50 (± 0.08)	transcriptional regulator, TetR family
SO3519	glnB-2	0.50 (± 0.12)	nitrogen regulatory protein P-II 1
SO0529	trpI	0.46 (± 0.14)	trpba operon transcriptional activator
SO1259		0.44 (± 0.12)	transcriptional regulator, LysR family
SO0346		0.44 (± 0.17)	transcriptional regulator. GntR family
SO3419	trpR	0.43 (± 0.09)	trp operon repressor
SO1762		0.42 (± 0.07)	transcriptional regulator, AraC/XylS family
SO2493		0.42 (± 0.07)	transcriptional regulator, TetR family
SO3082	sixA	0.41 (± 0.09)	phosphohistidine phosphatase SixA
SO4567		0.41 (± 0.11)	transcriptional regulator, AsnC family
SOA0165		0.40 (± 0.06)	transcriptional regulator, LysR family
SO2455		0.40 (± 0.07)	transcriptional regulator, LysR family
SO0295		0.38 (± 0.07)	transcriptional regulator, LysR family
SO3494		0.38 (± 0.06)	transcriptional regulator, TetR family
SOA0041		0.38 (± 0.03)	transcriptional regulator, PemK family
SO3254	flgM	0.37 (± 0.10)	negative regulator of flagellin synthesis FlgM
SO0402		0.33 (± 0.07)	transcriptional regulator, LysR family
SO0989		0.33(± 0.11)	transcriptional regulator, LysR family
SO1626	glnD	0.29 (± 0.07)	protein-P-II uridylyltransferase
SO0433	rsd	0.28 (± 0.10)	regulator of sigma D
SO1265		0.27 (± 0.09)	transcriptional regulator, putative
SO1603		0.25 (± 0.10)	transcriptional regulator, putative
SO4326		0.24 (± 0.06)	transcriptional regulator, TetR family
SO1343	rseA	0.23 (± 0.05)	sigma-E factor negative regulatory protein
SO2847		0.23 (± 0.11)	transcriptional regulator, LysR family
SO3799	asnC	0.22 (± 0.09)	regulatory protein AsnC
SO2282		0.22 (± 0.03)	transcriptional regulator, GntR family
SO1898		0.20 (± 0.08)	transcriptional regulator, putative
SO1669	tyrR	0.17 (± 0.04)	transcriptional regulatory protein TyrR

(Continued)

TABLE 3.3. (Cont'd) Genes repressed in anaerobic cultures of MR-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	COG Annotation
SO1607		0.16 (± 0.04)	transcriptional regulator, LysR family
SO2046		0.15 (± 0.04)	transcriptional regulator, MarR family
SO3684		0.15 (± 0.02)	transcriptional regulator, TetR family
SO3488		0.14 (± 0.02)	transcriptional regulator, AraC/XylS family
SO3660		0.13 (± 0.02)	sigma-54 dependent transcriptional regulator/sensory box protein
SO4312		0.09 (± 0.06)	adenylate cyclase CyaA, putative
SO0443		0.06 (± 0.03)	transcriptional regulator, MerR family
5) Transport and Binding Proteins			
SO3802		0.20 (± 0.06)	ABC transporter, ATP-binding protein
SO0139	ftn	0.19 (± 0.07)	ferritin
SO4598		0.17 (± 0.07)	heavy metal efflux pump, CzcA family
SO2045		0.11 (± 0.04)	cation efflux family protein
SO0857		0.08 (± 0.03)	ABC transporter, ATP-binding protein
6) Unknown Function			
SO2849		0.20 (± 0.03)	acetyltransferase, GNAT family
SO3715		0.18 (± 0.12)	oxygen-insensitive NAD(P)H nitroreductase
SO0911		0.15 (± 0.14)	ParA family protein, degenerate
SO2228		0.14 (± 0.07)	CBS domain protein
SO1609	syd	0.14 (± 0.16)	syd protein
SO3382		0.12 (± 0.31)	oxidoreductase, short-chain dehydrogenase/reductase family
SO0698	fsxA	0.12 (± 0.19)	fxsA protein
SO3586		0.12 (± 0.04)	glyoxalase family protein
SO2850		0.11 (± 0.05)	acetyltransferase, GNAT family
7) Protein Fate			
SO4699	prlC	0.20 (± 0.05)	oligopeptidase A
SO2447		0.17 (± 0.04)	channel protein, hemolysin III family subfamily
SO3577	clpB	0.12 (± 0.02)	clpB protein
SO1126	dnaK	0.07 (± 0.02)	chaperone protein DnaK
SO2277	ibpA	0.03 (± 0.03)	16 kDa heat shock protein A
8) Cellular Processes			
SO4170		0.18 (± 0.04)	C-factor, putative
SO3585		0.17 (± 0.06)	azoreductase, putative
SO2754	motY	0.17 (± 0.07)	sodium-type flagellar protein MotY, authentic frameshift
SO3349		0.13 (± 0.79)	glutathione peroxidase, putative
SO0956	ahpF	0.09 (± 0.05)	alkyl hydroperoxide reductase, F subunit
SO0958	ahpC	0.08 (± 0.05)	alkyl hydroperoxide reductase, C subunit
SO1158		0.07 (± 0.75)	Dps family protein
SO1070	katB	0.05 (± 0.05)	catalase
9) DNA Metabolism			
SO3866		0.20 (± 0.04)	site-specific recombinase, phage integrase family
SO3384	phrB	0.07 (± 0.05)	deoxyribodipyrimidine photolyase
11) Biosynthesis of co-factors, prothetic groups and carriers			
SO3348	hemH-2	0.05 (± 0.06)	ferrochelatase

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures of MR-1 grown at 40 mM KNO_3 to that of the anaerobically grown at 1 mM KNO_3 (reference).
[b] The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

DISCUSSION

This study demonstrates that NapA is the sole nitrate reductase in *Shewanella oneidensis* MR-1. The gene content derived from the complete genome sequence of MR-1, the gene expression analyses, and the deletion of the *napA* gene are all consistent in establishing that this periplasmic nitrate reductase is in fact responsible for the first step in the nitrate reduction pathway of *Shewanella oneidensis* MR-1. The inability of the MR-1 Δ*napA* mutant to grow when nitrate was the only electron acceptor demonstrated its role as the sole enzyme responsible for the reduction of nitrate into nitrite in this microorganism.

We observed an increase in the expression of *napA* and *nrfA* with increasing concentrations of nitrate until a plateau was reached. This was also confirmed in the DNA microarray analyses where *napADGH* expression was induced at high concentrations of nitrate (40 mM KNO_3). Expression of *nrfA* did not change under these two conditions as was previously observed in the Q-RT-PCR analysis where its expression reached a maximum at 1 mM nitrate and its level remained constant at higher concentrations. These results indicate that MR-1 does not posses an alternative nitrate/nitrite reductase system for high concentrations of nitrate as is observed in *E. coli* (39). Gene expression studies of *napA* and *narG* in *E. coli* have demonstrated an increase in the expression of *napA* but not *narG* when nitrate is present in concentrations below 1 mM and a repression of *napA* and induction of *narG* when nitrate concentrations are higher than 1 mM nitrate (39). This trend was also observed for the nitrite reductases NrfA and NirB (40). In this case *nrfA* is expressed at low concentrations and repressed at high concentrations of nitrate whilst *nirB* is expressed at high concentrations of nitrate.

Moreover, in *E. coli* there are two different two-component regulatory systems for the regulation of the expression of *nap* and *nar* genes, NarP/NarQ and NarL/NarX. NarP and NarQ activate the expression of the *nap* and *nrf* genes when the concentrations of nitrate are below 1 mM (33, 39, 40). When the concentration of nitrate exceeds 1 mM, NarL and NarX repress the *nap* and *nrf* operons and induce the *nar* and *nir* genes. In contrast to *E. coli*, *Shewanella oneidensis* MR-1 possesses only homologs for *narP* and *narQ*, which explains the expression pattern observed for the *nap* and *nrfA* genes in this organism. This is also true for the human pathogen *Haemophilus influenzae,* which colonizes many human body fluids (35). Compared to *H. influenzae, Shewanella oneidensis* MR-1 has been found in more diverse environments such as sediments, water columns as well as an opportunistic pathogen in humans and aquatic animals (11, 37, 38). These environments, including bodily fluids, are scarce in nitrate, therefore it is advantageous for MR-1 and for any other organism that lives in such habitats to posses a nitrate scavenging system to generate energy for growth. This is a good survival strategy for MR-1 since this microorganism seems to prefer microaerophilic conditions. In aerobic conditions MR-1 cultures form clumps of cells that keep the cultures in a microaerophilic space (38). Since NapA has a very high affinity for nitrate and is much more active in anaerobic conditions, its presence in MR-1 is vital.

The gene expression profile of MR-1 growth at 1 mM nitrate when compared to that at 40 mM nitrate changed dramatically. A high percentage of up-regulated genes belong to the "protein synthesis" functional category, while the number of down-regulated genes in this category is considerably low. This might indicate that there is an increase in the expression of genes necessary for the synthesis of the proteins when the

concentrations of nitrate increase. It might also indicate that at higher concentrations the synthesis of more proteins is necessary. "Transport and binding proteins" and "energy metabolism" are also categories with a high percentage of induced genes. These three categories contain genes that are important for growth and energy generation. Induction of these genes represents an increase in metabolic activity in the cell. Many of the genes in the "transport and binding proteins" category belong to the ABC transporter system which has been associated with the transport of nitrate, sulfate, copper and molybdenum that are important molecules involved in cell growth and energy generation. Changes in the expression of these genes in response to nitrate reduction have been reported previously in bacteria, including MR-1 (3, 5). Genes encoding sulfate transporters and enzymes involved in sulfate metabolism were highly induced. This indicates a need for sulfate due to the increase in metabolic activity, especially in protein synthesis. Genes that belong to the "energy metabolism" category that were up-regulated at 40 mM KNO_3 include genes involved in glycolysis, nitrate reduction, electron transport and synthesis of ATP. This up-regulation suggests that an increase in nitrate reduction by NapA promotes growth and energy generation in MR-1. Induction of genes involved in purine, pyrimidine, nucleoside, nucleotide and amino acid biosynthesis was also observed. This not only indicates an increase in metabolic activity but also might suggest nitrate assimilation in MR-1. Nitrate assimilation has been reported in organisms which possess an assimilatory nitrate reductase, which reduces nitrate into ammonium in the cytoplasm. This ammonium is a precursor for amino acid and nucleotide biosynthesis. Enzymes involved in the conversion of ammonium into organic material present in MR-1 include carbamoyl-phosphate and glutamine synthases. Induction of the genes that encode the

small and the large subunit of the carbamoyl-phosphate synthase were observed at 40 mM nitrate. Repression of the gene encoding glutamine synthase and of other genes involved in its transcription activation *glnD, glnB-2, ntrB and ntrC* was observed at high concentrations of nitrate. This repression may be caused by the accumulation of some of the metabolites in the synthesis of amino acids. The transcription of the genes encoding the glutamine synthase is halted at high concentrations of glutamine by feedback inhibition. Glutamine is the product of the ATP-dependent amidation reaction of glutamate, which is catalyzed by glutamine synthase. High concentrations of ammonium also inhibit the activation of this enzyme. Nitrate assimilation is an ATP consuming process, which is carefully regulated to avoid unnecessary energy expenditure. In cultures of MR-1 examine in this study, nitrite gets reduced into ammonium, which accumulates in the medium. Similar to *Rhodobacter capsulatus,* which is capable of assimilating nitrate (5), growth curves of MR-1 in which nitrate, nitrite and ammonium were monitored over time indicated a start of ammonium consumption late in the growth curve. In *Rhodobacter capsulatus*, when hydroxylamine was supplied to the medium, ammonium assimilation did not occur until hydroxylamine was completely reduced and ammonium accumulated in the medium.

The reduction of nitrate produces intermediates that are highly toxic to the cell. Nitrite, nitric oxide, hydroxylamine and even ammonium cause cytotoxic effects in many organisms (5, 30). One of the important queries in MR-1 is, how does this microorganism grow on such high concentrations of nitrate? Nitrite, an oxidative-stress causing agent, is produced following the reduction of nitrate. Nitric oxide and hydroxylamine are intermediates that are generated in the reduction of nitrite into ammonium. These

intermediates are generated within the nitrite reductase and remain trapped during the reaction. Studies in bacteria suggest that when high concentrations of these intermediates are generated they are release from the enzyme (30, 31). However, some bacteria posses an enzyme called prismane that reduces hydroxylamine into ammonium detoxifying and reducing oxidative stress in the cell. This enzyme is encoded by the *hcp* gene (SO1363) in MR-1, which is significantly induced at high concentrations of nitrate. There are also a number of up-regulated genes that have been associated with oxidative stress response in MR-1 as well as in other microorganisms (24). In our study there is a shift in the oxidative stress responses when there is an increase in the concentrations of nitrate. Some genes are down-regulated (*dmaA-1, dmaB-1, trxAC, katB, hemH-2, phrB, ahpCF, sodB, ohr*) and others up-regulated (*katG-2, dsbB, uvrA, uvrD*) in response to a higher nitrate concentration. The products of these genes protect the cell from DNA damage or convert some of the toxic intermediates in less toxic forms. There is also induction of 15 genes that are involved in the activation of prophage LambdaSo, which is one of the three prophages described in MR-1. Activation of LambdaSo has been previously described in MR-1 when subjected to oxidative stress conditions (24). This prophage can cause lysis, affecting cell growth. In cultures of MR-1 where the concentrations of nitrate exceeded 5 mM, growth rate decreased and reached a plateau with increasing nitrate concentrations. The oxidative stress and the activation of this prophage might be causing an inhibitory effect on cell growth.

This study provides a better understanding of the nitrate reduction pathway of MR-1 and also gives a global profile of its genetic expression in response to an increase in nitrate concentration. We conclude that NapA is the sole enzyme responsible for

nitrate reduction in MR-1. Even though NapA is a periplasmic protein, our study suggests that there is energy generation in this process. Since CymA is associated with the membrane and it is required for nitrate and nitrite reduction, it is possible that it can be generating a membrane potential that provides energy for the cell (28, 29). Also, MR-1 has the largest number of cytochromes among bacteria, which play a key role in electron transport in various anaerobic processes. This might also be a very important mechanism in MR-1 to generate energy from many other processes since many of the reductases (including the metal reductases) present are periplasmic. We also suggest that nitrate might be assimilated, however more studies on this subject need to be performed. There are also striking similarities in the genetic machinery and nitrate reduction pathway of MR-1 when compared to some pathogenic bacteria. These make MR-1 a very good model to study and understand the mechanisms of these microorganisms to survive in their natural environments.

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CHAPTER IV

Role of EtrA in the Regulation of the Nitrate Reduction Pathway in *Shewanella oneidensis* MR-1

ABSTRACT

EtrA is an *Escherichia coli* Fnr homolog, which has been identified as a possible global regulator of the anaerobic metabolism of MR-1. EtrA shares 50.8% and 73.6% amino acid sequence identity with the oxygen-sensing regulator in *E. coli,* Fnr, and with the Anr (anaerobic regulator of arginine deaminase and nitrate reductase regulator) protein of *Pseudomonas aeruginosa*, respectively. This similarity suggests an oxygen sensing regulatory role for EtrA in MR-1. Physiological and genetic expression analyses of a *S. oneidensis* MR-1 *etrA* knockout strain (EtrA7-1) indicates a regulatory role of EtrA in the expression of genes likely involved in nitrate reduction, specifically the *nap* genes, *nrfA, cymA* and *hcp,* and in other anaerobic metabolism processes. This was concluded after detecting a significant decrease in the expression of these genes in EtrA7-1 relative to the wild type. However, the nitrate reduction activity was not shut down in the mutant, suggesting the existence of other regulator(s) involved in the regulation of this process. Evidence for negative regulation of putative genes related to aerobic metabolism was also obtained. A starvation genetic response was observed for this mutant and the effects on its growth were examined. A significant decrease in the growth of the mutant was observed when compared to that of the wild type. During this period, the cells entered a state of prophage activation possibly in response to stressful growth conditions (starvation and oxidative stress) as a result of absence of EtrA.

INTRODUCTION

The regulatory mechanisms that control the bacterial anaerobic metabolism have been of interest in *Shewanella oneidensis* MR-1 and other microorganisms (3, 26, 37, 38, 45, 47). In *Escherichia coli*, for example, the transition from aerobic to anaerobic conditions is mainly regulated by Fnr (fumarate-nitrate reduction regulatory protein) and by the two-component regulatory system ArcAB (aerobic respiratory control) (39, 44). This regulation occurs at the transcriptional level. Recently a genetic expression study in *E. coli* K12 indicated that one-third of its 4,290 genes were differentially expressed during aerobic versus anaerobic growth. Among the differentially expressed genes, 712 (49%) genes were directly or indirectly affected by Fnr. Fnr possesses a $[4Fe\text{-}4S]^{2+}$ cluster that acts as a sensory domain for oxygen (8). When oxygen levels increase, a two-step reaction transformation occurs where O_2 reacts with the cluster and transforms it into a $[3Fe\text{-}4S]^{1+}$ cluster. The second step is a non-redox reaction in which the $[3Fe\text{-}4S]^{1+}$ is converted into a $[2Fe\text{-}2S]^{2+}$ cluster. This transformation apparently changes the conformation of the protein impeding its binding to DNA, which in turns affects the transcription of the genes it regulates.

The regulation of the *nap* and *nrf* genes has been studied in detailed in *Escherichia coli* (7, 9, 10, 25, 39, 45). Mutational studies have revealed the direct participation of Fnr and of two additional two-component regulatory systems NarL/NarX and NarP/NarQ in the regulation of the expression of the *nap* and *nrf* genes. In *S. oneidensis* MR-1, there are homologues for *narQ* and *narP* but not for *narX* or *narL*. In *E. coli*, NarP enhances the expression of the *nap* and *nrf* genes, while NarL acts as a negative regulator at high concentrations of nitrate (45).

In contrast to *E. coli*, the regulation of the nitrate reduction pathway of MR-1 has not been studied in depth. Mutational studies in MR-1 have identified two possible regulators for the nitrate reduction pathway, EtrA (electron transport regulator protein) and CRP (cyclic AMP receptor protein) (37, 38). EtrA is an Fnr homolog that shares 50.8% and 73.6% of amino acid sequence identity with Fnr (fumarate-nitrate reduction regulatory protein) in *Escherichia coli* and Anr (anaerobic regulator of arginine deaminase and nitrate reductase) in *Pseudomonas aeruginosa*, respectively. This high degree of similarity suggests a potential for EtrA to regulate metabolic activities by sensing oxygen-limiting conditions. Despite the lack of physiological evidence to support a regulatory role of EtrA in the anaerobic metabolism of MR-1 (26), a genetic expression study using a partial *S. oneidensis* MR-1 DNA microarray suggested an involvement of EtrA in the regulation of proteins associated in aerobic and anaerobic metabolism (3). This study compared the growth of *Shewanella oneidensis* MR-1 DSP10 strain (a rifampicin spontaneous mutant) with a DSP10 *etrA* deletion mutant. The results suggested a negative regulation in the expression of genes related to aerobic metabolism and a positive regulation in the expression of genes associated with anaerobic metabolism by EtrA, as it has been observed for Fnr in E. coli (39).

The study of *Shewanella oneidensis* MR-1 has recently increased due to its potential as a bioremediator (19, 20, 46), which also motivated support for a genomic sequencing effort. Bioremediation is a challenging but cost-effective procedure. A variety of strategies have been developed to enhance the effectiveness of the inocula in the environment and starvation is one of these. It has been observed that cells that have been exposed to long periods of nutrient and energy limitation express proteins that protect

them against stressful growth conditions. Thus, these cells are pre-adapted to survive the harsh conditions in the environment (16). Starvation has been studied in a few species of the *Shewanella* genus (2, 6, 16), however to our knowledge there is not much information regarding the cultivation of *S. oneidensis* MR-1 in starvation conditions.

To demonstrate the function of EtrA, we generated a gene-deletion mutant in a wild type background. Previous experimentation in *etrA* mutant strains are complicated by rifampicin resistance in the host strain which modifies the electron transport function of the cell membrane. In this study, genetic expression of an *etrA* knockout strain (EtrA7-1) under anaerobic conditions with nitrate as the sole electron acceptor was compared with that of the wild type using a complete *S. oneidensis* MR-1 genome array. The genetic expression pattern of the anaerobic growth of the EtrA7-1 was also examined at a high and a low concentration of nitrate. The genetic expression profile of EtrA7-1 indicates a dramatic starvation response at the transcriptional level. The physiological and genetic analyses of EtrA7-1 suggest an involvement of EtrA in the regulation of the expression of the *nap* operon, the *cymA* and the *nrfA* genes. Regulation of other genes associated with energy metabolism is suggested and these results were compared with the previous findings of Beliaev et al., 2002 done in the DSP10 host.

MATERIAL AND METHODS

Bacterial strains and growth conditions. The bacterial strains, plasmids and primers used in this study are presented in TABLE 4.1. Cultures of *Shewanella oneidensis* MR-1, *Shewanella oneidensis* MR-1 Δ*etrA*, *Shewanella oneidensis* MR-1 Δ*etrA* complement

TABLE 4.1. Bacteria, plasmids, and primers used in this study.

Strain, plasmid, primer or probe	Description or nucleotide sequence[a,b]	Source, reference or relative position of primer or probe
Bacterial Strains		
E. coli β2155	Diaminopimelic acid auxothroph used for cloning and conjugation	12
S. oneidensis		
MR-1	Lake Oneida, N.Y., sediment	33
EtrA7-1	*etrA* gene deletion derived from MR-1	This study
EtrA7-1 complement	*etrA* deletion mutant complemented with the *etrA* gene cloned into pCM62	This study
EtrA7-1 with pCM62	*etrA* deletion mutant transformed with the pCM62 as a negative control for complementation	This study
Plasmids		
pCM62	Cloning vector	28
pCM157	Broad-host-range *cre* expression vector	27
pCM184	Broad-host-range allelic exchange vector	27
pCCG195	pCM184 with *etrA* upstream flank	This study
pCCG196	pCCG195 with *etrA* downstream flank	This study
pKNOCK-Gm	Broad-host-range allelic exchange vector	1
pCCG02	pKNOCK-Gm with *etrA* flanking regions separated by two *loxP* sites flanking a kanamycin resistance gene	This study
pCCG02c	pCM62 with MR-1 putative *etrA* gene	This study
Primers[c]		
etrAN Fwd	GCCGCGGTCATGTCGGTTCTCAAGT	CAT Start codon
etrAN Rev	CGAGCTCCGACAGCTATCTGTTAGTCT	503 nt upstream[d] the start codon
etrAC Fwd	CGAATTCAAATCACCGCTTTTAACTTG	493 nt downstream the stop codon
etrAC Rev	GCATATGCCAGATAAATCACACCTTTT	TAA Stop codon
etrAScreenout Fwd	AATTCTTCAGGCATTTGACTCG	1188 nt downstream the stop codon
etrAScreenout Rev	GGCCGTATCTTGAGTTATACCC	559 nt upstream start codon
etrAcomp Fwd	GGATCCAGGTGTGATTTATCTGGCG	TTA Stop codon
etrAcomp Rev	GAATTCCCGACATGACAATAGAGCAGA	ATG Start codon

[a] The sequence for the primers is written from the 5'end to the 3'end.
[b] Primers were designed using putative gene sequences of *S. oneidensis* MR-1.
[c] For primer sequences, the restriction sites incorporated are underlined. CATATG, NdeI; GAATTC, EcoRI; GAGCTC, SacI; CCGCGG, SacII; GGATCC, BamHI.
[d] Even though the *etrA* gene is in the opposite direction in the genome, the sequence at the right end of the start codon will be denominated as upstream and the one at the left side of the stop codon as downstream.

and *Shewanella oneidensis* MR-1 harboring pCM62 (as the negative control for complementation) were incubated at 30°C after inoculation in Modified M1 minimal medium (32) with no NH_4Cl to avoid interference with chemical analyses. HEPES (pH 7.2) was added to buffer the medium at a 50 mM final concentration. The medium was supplemented with 20 mM lactate. KNO_3 was added as the electron acceptor in concentrations specified below. The medium prepared for the RNA extractions was degassed by boiling, purged with helium and transferred to 250 ml serum bottles. The cultures used for growth curve determinations were performed in 30 ml Balch tubes. The serum bottles and tubes were closed with butyl black stoppers to avoid oxygenation of the medium. The medium was autoclaved and 0.1 ml of Wolfe's vitamin solution (52) was added by injection with a sterile syringe. The medium was inoculated by injection with a 1% inoculum from a 12 h aerobic culture in M1 medium, which originated from an overnight starting culture in aerobic M1 medium, as well. Incubation was performed at 30°C without shaking. Negative controls (i) without inoculation and (ii) medium without the electron acceptors but inoculated, were run in parallel with all growth and gene expression experiments.

Cultures of *Escherichia coli* β2155 (auxotroph of diaminopimelic acid) were grown in Luria-Bertani (LB) medium supplemented with 100 ug/ml of diaminopimelic acid (DAP). These cultures were incubated at 37°C. *Shewanella oneidensis* MR-1 was cultivated in LB medium and incubated at 30°C during the mutagenesis process. Antibiotics for *E.coli* were prepared and added as described elsewhere (40). The antibiotics used for the selection of MR-1 positive transformants were added in the

following concentrations: 25 ug/ml of kanamycin, 7.5 ug/ml of gentamycin, and 10 ug/ml tetracycline.

RNA extractions. To compare the gene expression profile of the wild type with that of the EtrA7-1, total RNA was extracted from cultures of *S. oneidensis* MR-1 ($OD_{600\ nm}$ 0.03-0.05) and EtrA7-1 ($OD_{600\ nm}$ 0.012-0.015) grown in triplicate as described above. The wild type was grown at 2 mM KNO_3, while EtrA7-1 was inoculated in 1, 2 and 40 mM KNO_3 media. Cells were collected at mid-log phase and concentrated by centrifuging at 4°C for 30 min at 7,500 rpm. The pellets were washed with 1 ml of an ice-cold 1X DEPC-treated PBS solution (40). The RNA was extracted with The RNAwiz Solution following the instructions of the manufacturer (Ambion, Inc.). The RNA extraction was followed by an isopropanol precipitation (40) and its resuspension in the RNA storage solution (Ambion, Inc.). These samples were treated with RNase-free DNaseI (Roche) to eliminate residual DNA. The samples were purified by phenol, phenol:chloroform (1:1) and chloroform extractions, and stored in ethanol at -80°C until ready for use. Quality of the RNA was observed using the RNA 6000 Pico LabChip kit and the 2100 Bioanalyzer (Agilent Technologies). The RNA concentration was determined with OD measurements at 260 nm using a Varian Cary 50 BIO UV-Vis spectrophotometer (Varian, Zug, Switzerland).

***etrA* deletion mutagenesis.** Molecular procedures such as genomic and plasmid purifications, restriction digestions, sticky ends repair, ligations and electroporations were performed as previously described (40). Primers for PCR reactions (TABLE 4.1) were

designed using the Vector NTI® software (InforMax, Inc.) and synthesized at Integrated DNA Technologies (www.idtdna.com).

a) etrA allelic exchange vector generation. The MR-1 *etrA* flanking regions were cloned into the broad-host-range vector pCM184. This vector was used to replace the *etrA* gene in MR-1 with a kanamycin cassette by homologous recombination. The kanamycin cassette in pCM184 is flanked by two *loxP* sites, which are in turn were flanked by two multiple cloning sites (MCS) (27). The primers etrAN Fwd (SacII) and etrAN Rev (SacI) were used to amplify the region upstream of the *etrA* gene start codon while the etrAC Fwd (EcoRI) and napAC Rev (NdeI) amplified the region downstream of the stop codon. The product of these reactions was approximately 500 bp each, to ensure good efficiency of recombination (27). Each primer had a restriction site linker at the 5'end for an enzyme that was chosen using the pCM184 MCS as a reference for directional cloning of the fragments. These two fragments were cloned individually into pCM184 and transformed into *E. coli* ß2155 electrocompetent cells by electroporation. The positive transformants were selected by inoculating on LB agar supplemented with kanamycin and DAP. The colonies were screened using the primers used to amplify each fragment.

The pCCG196 vector (pCM184 with the *etr*A flanking regions) was replicating independently in MR-1 due to the presence of a ColE1 origin present in pCM184, and therefore in pCCG196. To generate a suicide vector for MR-1, pCCG196 was digested with EcoRI and SacI. This reaction excises the kanamycin cassette with the *loxP* sites and the N and C fragments (a fragment of approximately 2.5 kbp) to further clone it into pKNOCK-Gm (1) for the generation of pCCG02. The pKNOCK-Gm broad-host-range

vector has a gentamycin resistance gene and a R6K origin, which needs a π protein in order to be propagated. Since this protein is not present in MR-1, this plasmid made a good suicide vector. To clone the fragment excised from pCCG186 into pKNOCK-Gm, this vector was digested with SmaI, which is a blunt end cutter and the plasmid was dephosphorylated with calf intestinal phosphatase to avoid self-ligation. The sticky ends product of the digestion of the insert with EcoRI and SacI were repaired using T4 DNA polymerase. These reactions were gel purified and a vector:insert ratio of 1:16 was used for the ligation reaction. Electroporation and screening of the transformants was performed as described above.

b) *etrA* allelic exchange vector transfer into *Shewanella oneidensis* MR-1.

pCCG02 was transformed into MR-1 by conjugation (protocol from Margie Romine, Pacific Northwest National Laboratory). The *E. coli* β2155 harboring pCCG02 was inoculated in LB liquid media supplemented with 100 ug/ml of DAP and 50 ug/ml of kanamycin. Overnight cultures of MR-1 and *E. coli* β2155 harboring pCCG02 were mixed in ratios of 1:2. This mixture was then concentrated by centrifugation and the supernatant was discarded. The conjugation mixtures were spotted on an LB plate with DAP and incubated at room temperature for 12 to 16 h. Controls were spotted on individual plates. After incubation, the spotted cultures were scrapped and resuspended in 1 mL of 1X Phosphate-Saline Buffer (PBS) (40). Two 10-fold serial dilutions were prepared and plated on LB plates with 25 ug/mL of kanamycin and no DAP to avoid growth of the *E.coli* β2155. Selection of positive transformants was performed by screening on LB plates with kanamycin and LB plates with kanamycin and gentamycin. Positive MR-1 *etrA* deletion mutants were expected to be resistant to kanamycin and

susceptible to gentamycin. Colonies with this phenotype were screened by PCR using etrAScreenout Fwd and etrAScreenout Rev which anneal approximately 700 bp downstream and 56 bp upstream from the *etrA* flanking regions included in pCCG02, respectively. This screening ensures that the recombination occurred in the targeted area.

c) Removal of kanamycin cassette. The two *loxP* sites flanking the kanamycin cassette are recognition sites for Cre recombinase. This recombinase excises the region inside the *loxP* sites by recombination leaving one of the *loxP* sites. pCM157 is a *cre* gene expression vector (27) which was electroporated into *E.coli* β2155. This vector was transformed into *Shewanella oneidensis* MR-1 Δ*etrA* Kan^r by conjugation as described above. Colonies susceptible to kanamycin and resistant to tetracycline were selected as positive transformants. These were screened by PCR using etrAScreenout primers to confirm the loss of the kanamycin cassette.

d) pCM157 curation from *S. oneidensis* MR-1 Δ*etrA*. Cultures of MR-1 Δ*etrA* (Tet^r Kan^s) were transferred three times on LB liquid media with no antibiotics and then screened for tetracycline susceptibility on LB agar. This phenotype indicates the loss of the pCM157 plasmid. Colonies were transferred and screened until the correct phenotype was obtained. Colonies susceptible to kanamycin and tetracycline were diagnosed by PCR with the etrAScreenout primers and using primers targeting the inside region of the MR-1 *etrA* gene (etrAcomp Fwd and etrAcomp Rev). DNA sequencing performed at the Genomics Technical Support Facility at Michigan State University confirmed the deletion. The etrAC Fwd was used to sequence the upstream region from the *loxP* site and the etrAN Rev for the sequence downstream. These two sequences were assembled into one sequence using Vector NTI Suite 8.0 software.

Δ*etrA* complement construct. Plasmid pCM62 was used as the vector for the expression of the *etrA* gene in one of the *etrA* knockout strains obtained called EtrA7-1. The *etrA* expression vector was called pCCG02c. The *etrA* gene was amplified from *S. oneidensis* MR-1 genomic DNA using the etrAcomp Fwd and etrAcomp Rev. The amplicon was double digested with BamHI and EcoRI, which were the restriction sites linked to the 5'end of etrAcomp Fwd and etrAcomp Rev, respectively. The pCM62 plasmid was also double digested with BamHI and EcoRI. The products of these digestion reactions were gel purified and the vector:insert ratio was 1:3. Ligation, electroporation into *E.coli* β2155 and conjugation into MR-1 was performed as described above. The vector pCM62 was transferred to MR-1 by conjugation. This strain was used as a control for the complementation analyses to check for any effects caused due to its presence. Antibiotic selection of positive transformants was performed by streaking on LB plates with tetracycline. Tetracycline-resistant colonies were diagnosed by PCR using the etrAcomp primers and subsequently sequenced to verify the deletion.

Growth comparisons of *Shewanella oneidensis* MR-1 and EtrA7-1. Cultures of the wild type, EtrA7-1, EtrA7-1 complement and EtrA7-1 harboring pCM62 were grown anaerobically with 3 mM KNO_3 in M1 minimal medium as described above. Growth was monitored constantly by OD measurements at 600 nm. Samples from each culture were collected throughout the incubation period for determination of nitrate, nitrite and ammonium concentrations by a Lachat QuickChem Automated Flow Injection Ion Analyzer following the Copperized Cadmium Reduction Method as in QuickChem Method No. 10-107-04-1-A (Lachat Instruments, 1988) at the Soil Testing Lab at

Michigan State University. The ammonium analysis was performed by the salicylate colorimetric method (34).

Gene expression analyses of EtrA7-1. A *Shewanella oneidensis* MR-1 complete genome microarray containing a total of 4197 PCR amplicons and 451 oligonucleotides representing individual open reading frames (15) was used to examine the global genetic expression of EtrA7-1 under different growth conditions. Gene expression profiles of anaerobic cultures of the wild type *S. oneidensis* MR-1 and EtrA7-1 grown at 2 mM nitrate were compared. Also, the gene expression pattern of MR-1 Δ*etrA* grown at a low (1 mM KNO_3) concentration of nitrate was compared to that at a high (40 mM KNO_3) concentration. cDNA preparation and labeling were performed as previously described (41) using a 2:3 ratio of 5-(3-aminoallyl)-dUTP and dTTP. Hybridization and post-hybridization washes were done as described elsewhere (18). Three biological replicates per treatment were used for the hybridization of six microarray slides including technical duplicates (dye-swap) per experiment. The slides were scanned using an Axon 4000B scanner (Axon Instruments, Inc.). The data analysis was performed using the GeneSpring 6.0 software (Silicon Genetics). The data was normalized per chip and per gene (Lowess Normalization) and the spots with less than 55% of pixels greater than background plus two standard deviations were eliminated from the analyses (30). The data was filtered using the Benjamini and Hochberg false discovery rate with 95% confidence and only those genes with a >2-fold change in magnitude were considered significant.

RESULTS

***etrA* deletion mutagenesis and physiology of the MR-1 Δ*etrA* mutant.** Six MR-1 Δ*etrA* deletion mutants were diagnosed by two different PCR reactions (FIG. 4.1). The primers used for the first reaction targeted the inside region of the gene and as expected, no amplification was observed when using the DNA from the MR-1 *etrA* deletion mutants as a template (FIG. 4.1; lanes 1-6). In lane 8, where the DNA of the wild type was the template, there was amplification of a band of approximately 750bp (expected size for the PCR product using the etrAcomp primers). The second diagnostic reaction included the etrAScreenout primers that should generate PCR products of approximately 1.75 kb and 2.5 kb when the DNA of the MR-1 Δ*etrA* and the wild type are used as the templates, respectively. This is shown in lanes 9-14 for the MR-1 Δ*etrA* mutants and lane 16 for the wild type. The product of this last reaction was used as the template for sequencing using the etrAC Fwd and the etrAN Rev primers (FIG. 4.2). This sequence shows the replacement of the gene with the *loxP* site residual and confirms the complete deletion of the *etrA* gene from *S. oneidensis* MR-1 genome.

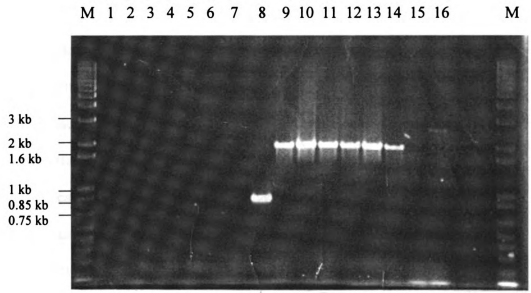

FIG. 4.1. *etrA* gene deletion confirmation by PCR. This is a 1% agarose gel 1X TAE which has the PCR reactions to confirm the MR-1 Δ*etrA* mutants. Lanes labeled as M are for 1 Kb plus DNA ladder (Invitrogen Life Technologies). Lanes 1-8 correspond to PCR screening with the etrAcomp primers. Lanes 9-16 correspond to PCR screening with the etrAScreenout primers. Sample order: lanes 1 and 9, EtrA7-1; lanes 2 and 10, EtrA14-1; lanes 3 and 11, EtrA15-1; lanes 4 and 12, EtrA15-2; lanes 5 and 13, EtrA55-3; lanes 6 and 14, EtrA55-7; lanes 7 and 15, negative controls (no DNA); lanes 8 and 16, MR-1 wild type.

1 caatcgcatg gttaacaatg ctttcaaacg gacgattatg ccaaatgact tgagtatcaa

61 tgttgagtcc taggtgattg taggggggcga gtagatcttg gatccacgcc acacgctgat

121 caatcacacc ttggcgcatc gcttcgcgct cttggctcga taaaattgaa gtcatttcat

181 aggagaagtc aaatattgag agaaacacag tcacatgggc gttactttta ctggccaagg

241 taacagctcg ggcgagagca acctgatttt ctgtcgtggg atcgacaact accagtattt

301 tttgataatc cttcatagca tgttccttta gtcgtaggct catgtttatc atgagccttt

361 ggcaattagc tgtattgttc tagatcaaaa ctcttttcaa aaccgatgcc tagcgtaaag

421 cataaacgct aaaaggtgtg atttatctgg catatggcgg **ccgcataact tcgtatagca**

481 **tacattatac gaagttat**gg atccagctta tcgataccgc ggtcatgtcg gttctcaagt

541 taatccactg cagccatgtt aaaccaattc attcgcttgg gctagtttag ctgcgacagg

601 gcgatatata aagtttggcc accaaagacg attaatagta agcccactca ataatctaac

661 ggtttttgt tgtacccaat tggctaaacg ctttgctgcc acacgccagc actaagtagc

721 gccgggagag tccctaaacc aaaggcgagc ataatcaagg cgccttggct ggcagaacct

781 gccgccacag accaagttaa ggtgctatat accagtccac agggcagcca tccccatatc

841 aatccagcgg tgatggcttg cattggcgtg gtgatcggca caagacgctg ggctatgggt

901 tttaaataac gccacaacac ttggccgagg cgttcaattt gtactattcc gacccaaatt

961 ttagcaatgt ataaacctgt cgcgatcatc atgat

FIG. 4.2. DNA sequence of the *etrA* deletion in MR-1. This sequence was assembled using vector NTI Suite 8.0 software. The assembly included the sequence upstream the *etrA* gene and the one downstream using primers etrAScreenout Fwd and etrAScreenout Rev, respectively. The sequences underlined represent the stop (left side) and the start codon sequences of the *etrA* gene . The sequence in boldface corresponds to the *loxP* residual.

Growth comparisons of *Shewanella oneidensis* MR-1 and EtrA7-1. A growth curve comparing the wild type with EtrA7-1 (the MR-1 Δ*etrA* mutant chosen at random) was conducted (FIG. 4.3). The growth curves of EtrA7-1 complemented with the *etrA* gene (this complement harbors pCCG02c) and the EtrA7-1 harboring the pCM62 were also included. The growth of EtrA7-1 is approximately 20% of that of the wild type. The EtrA7-1 complement grew slower than the wild type but after 23 h of incubation it reached an optical density similar to that of the wild type. The EtrA7-1 harboring the pCM62 was used to account for any differences observed due to the presence of the vector and not caused by the complementation. There are no differences between the growth of the mutant and this control indicating that the presence of pCM62 in the mutant does not affect its growth. After 10 h incubation, all samples show reduction of nitrate (FIG. 4.4). The MR-1 Δ*etrA* complement samples present less accumulation of nitrite when compared to the wild type, which explains the delay in growth observed. This is reasonable since the gene has not been transcribed using its natural promoter, which can cause a delay in expression and differences in protein concentration. However, samples taken after 23 h incubation showed concentrations in the complement similar to those in the wild type (FIG. 4.5). EtrA7-1 and the control harboring pCM62 grew similarly. In both cultures, there is reduction of most of the nitrate and nitrite accumulation. Ammonium production is limited. In these cases, nitrate reduction hardly improved growth (FIG.4. 3).

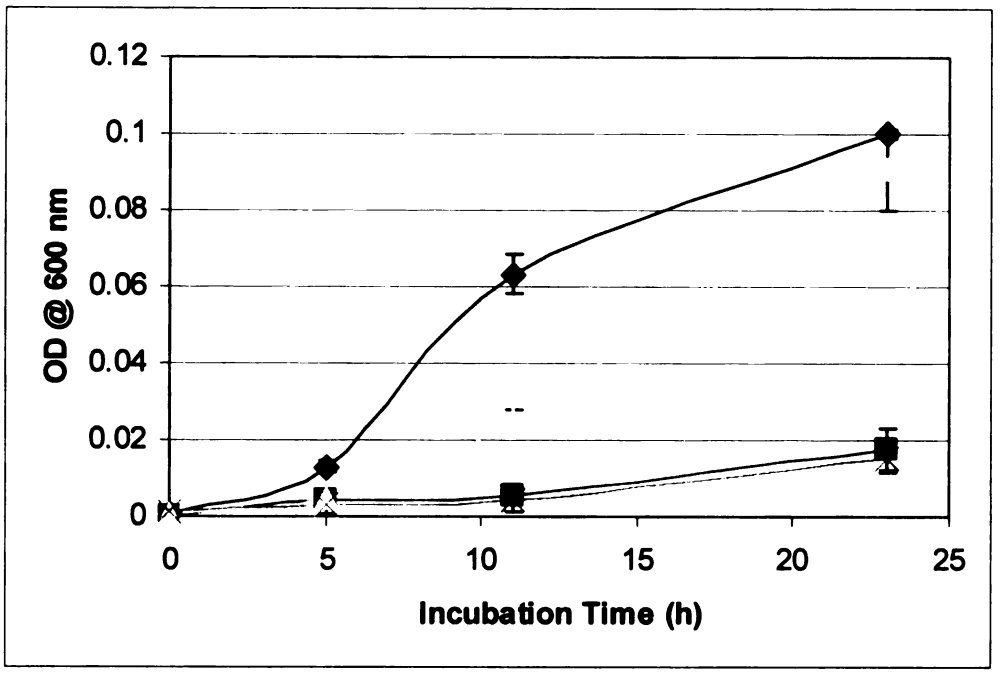

FIG. 4.3. Growth of *Shewanella oneidensis* MR-1 wild type (♦), EtrA7-1 (■), EtrA7-1 complement ( ),and EtrA7-1 harboring pCM62 ( ) under anaerobic conditions with 3 mM KNO_3. Each time point is an average of three biological replicates.

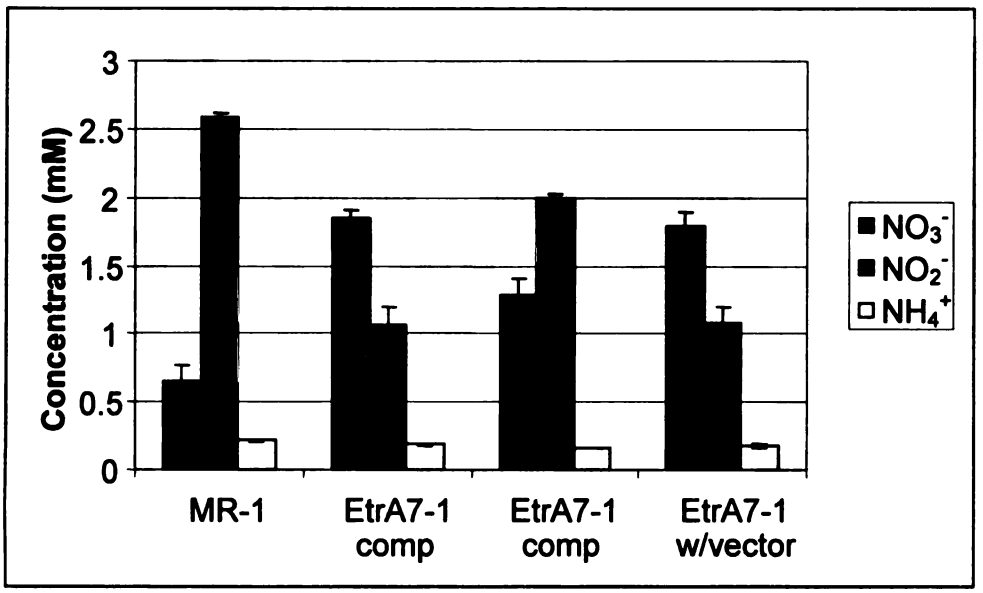

FIG. 4.4. Concentrations of nitrate, nitrite and ammonium in cultures of *Shewanella oneidensis* MR-1 wild type , EtrA 7-1, Etra7-1 complement (harboring pCCG02c) and EtrA7-1 harboring pCM62 during growth curve after 10 h incubation period. Each measurement is an average of three biological replicates.

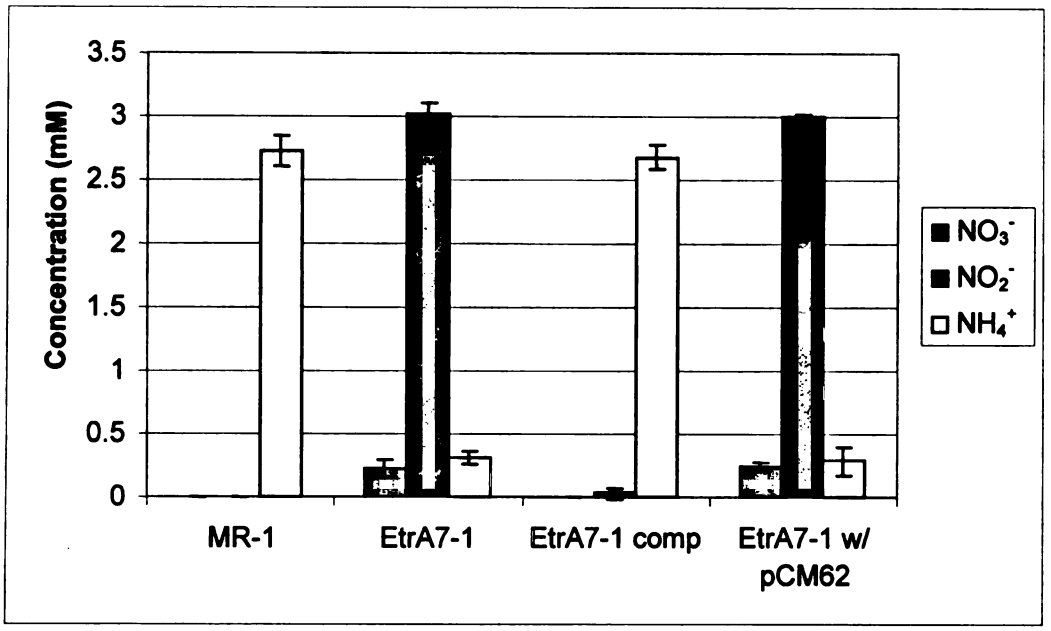

FIG. 4.5. Concentrations of nitrate, nitrite and ammonium in cultures of *Shewanella oneidensis* MR-1 wild type, EtrA 7-1, Etra7-1 complement (harboring pCCG02c) and EtrA7-1 harboring pCM62 during growth curve after 23 h incubation period. Each measurement is an average of three biological replicates.

Gene expression profile of wild type versus EtrA7-1. The global expression pattern of EtrA7-1 was compared to that of the wild type when grown anaerobically with 2 mM KNO_3. Out of 627 differentially expressed genes in EtrA7-1 relative to the wild type, there are 302 up-regulated and 325 down-regulated genes. The differentially expressed genes were classified in 20 functional "TIGR Role" categories (FIG. 4.6). "Conserved hypothetical proteins" was the predominant category in both up-regulated (17.8%) and down-regulated genes (15.9%). Other categories under the up-regulated genes include "hypothetical" (14.2%), "protein synthesis" (9.9%), "energy metabolism" (8.6%) and "unknown function" (7.6%). Many of the up-regulated genes that are grouped in the "energy metabolism" category include genes that encode a formate dehydrogenase (SO4509-4511), a cytochrome c oxidase *ccoPQN* (SO2361-2362, SO2364), NADH:ubiquinone oxidoreductases *nqrA*-2, *nqrB*-2, *nqrC*-2, *nqrD*-2, *nqrE*-2, *nqrF*-2 (SO1103-1108), genes of proteins involved in gluconeogenesis like *pckA* (SO0162) and in glycogen synthesis like *glgX, glgC and glgA* (SO1495, SO1498-1499). In this group there was also activation of the succinate dehydrogenase gene *sdhC* (SO1927), the succinyl-CoA synthase operon *sucABCD* (SO1930-1933) and the acetate CoA-transferase, synthase (SO1891-1892) (TABLE 4.2). A complete list of all the genes induced two-fold or higher is provided (SUPPLEMENTAL TABLE B.3).

The category "transport and binding proteins" contained 6.3% of the up-regulated genes. In this category there are genes encoding heavy metal efflux pumps and systems (SO0520, SO4597-4598, SOA0153), and ABC transporters (SO1690), especially those specific for phosphate transport (SO1560, SO1723-1724), *pstB-1* (SO1725), *pstB-2*

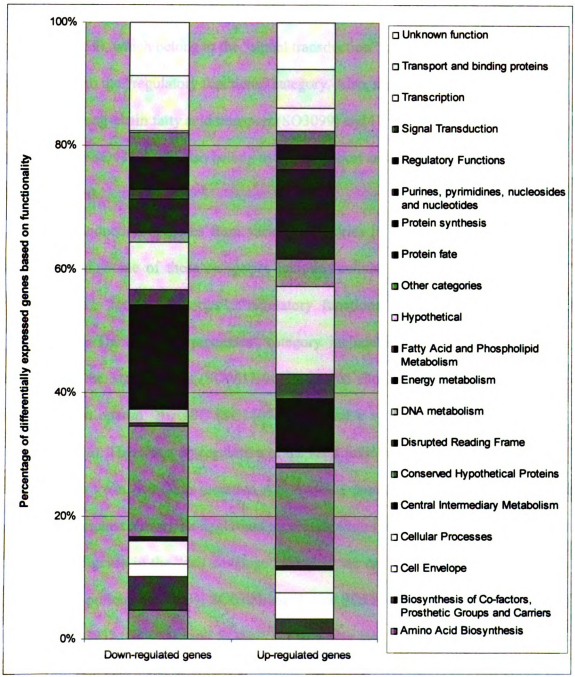

FIG. 4.6. Distribution of differentially expressed genes (> 2-fold change) grouped in 20 functional categories in anaerobic cultures of EtrA7-1 with 2 mM KNO_3 as the sole electron acceptor relative to the wild type (reference strain). The total of genes down-regulated is 325 and the up-regulated is 302.

(SO4289) and *pstA* (SO4290). There was up-regulation of genes encoding the *pho* regulon, which regulates these phosphate transporters genes, such as *phoB* (SO1558), *phoR* (SO1559), which belong to the "signal transduction" category and *phoU* (SO1726) that belongs to the "regulatory functions" category. Also, the genes encoding proteins involved in long-chain fatty acid transport (SO3099) and HlyD family secretion proteins (SO1925 and SO4319) that also belong to the "transport and binding proteins" category were activated.

The induction of genes from various categories in response to stress conditions was observed. Some of these categories include "cellular processes", "cell envelope", "protein fate", "other categories", "regulatory functions", "transcription" and "DNA metabolism". The "cellular processes" category include a stringent starvation protein encoded by the *sspAB* genes (SO0611-0612), a cold shock protein (SO1648), a phage shock protein operon *pspABC* (SO1807-1809), and a virulence regulator encoded by *bipA* (SO4408). There was up-regulation of the RTX toxin operon (SO4317-4320), which codes for the toxin, the toxin secretion ATP-binding protein, the HlyD family secretion protein (mentioned above), an agglutination protein (*aggA*) and an OmpA family protein. Up-regulated genes in the "cell envelope" category are mostly involved in the synthesis of structural proteins (SO0004, SO0300, *dacA*-1 or SO1164, SO1166, *rodA* or SO1167, SO1245, SO3933, SO4321, and SO4377). There was also induction of genes encoding transferases (SO3172 and SO3176) as well as the *mrdA* gene (SO1168) that codes for a penicillin-binding protein 2. In the "fatty acid and phospholipid metabolism" induction of genes *fabF*-1 (SO2774), *fabD* (SO2777), *fabH-1* (SO2778), *fabB* (SO3072), *fabG*-2 (SO4382), and *fabF*-2 (SO4383) involved in the synthesis of membrane components was

detected. In the "protein fate" category there was up-regulation of genes *lepB* (SO1347), *dsdB* (SO1887) and of the *hslO* gene that code for a chaperone (SO0163) and the export protein genes *secD*-1 and *secF*-1(SO1193-1194). Other up-regulated genes involved in stress response include *rpoD* (SO1284), *era* (SO1349), *recO* (SO1350), *cinA* (SO0272) and a gene that encodes a site-specific recombinase (SOA0086). Another stress response observed was the induction of genes involved in the activation of the MR-1 prophages. There was induction of 25 genes of the LambdaSo phage (SO2940-2974) and 2 of the late genes of the MuSo1 (SO0674-0675) and MuSo2 (SO2684-2685) phages. There is induction of host genes that are also required for activation of the LambdaSo phage such as *nusA* (SO0219) and *nusG* (SO0219).

The down-regulated genes show a different pattern (FIG. 6). "Energy metabolism" associated proteins are the second largest category (16.9%) of down-regulated genes. In this category there are down-regulated genes involved in anaerobic metabolism such as the *napBHGAD* operon (SO0845-0849), the *cymA* (SO4591) and the *nrfA* (SO3980) genes, the fumarate reductase genes *frdAB* (SO0398-0399), the *nqrB*-1, *C*-1, *D*-1, *E*-1 and *F*-1 operon (SO0903-0907), the *nuoECDB* operon (SO1018-1020), the *cydAB* genes (SO3285-3286), the outer membrane protein genes *mtrAB* (SO1776-1777) and *omcAB* (SO1778-1779), the prismane protein *hcp* gene (SO1363), and the alcohol dehydrogenease genes *adhB* (SO1490), and *adhE* (SO2136)(TABLE 4.3). The genes that encode the anaerobic dimethyl sulfoxide reductase *dmaA*-1 (SO1429) and *dmsB*-1 (SO1430) as well as genes of a quinine-reactive Ni/Fe hydrogenase the *hydC, hyaB,* and *hoxK* (SO2097-2099) are down-regulated. Other down-regulated genes in this category ("energy metabolism") include some electron transfer flavoproteins (*etfA* or SO3144,

SO4453), a formate dehydrogenase (SO4513 and SO4515), as well as genes involved in metabolism of carbon containing compounds such as the *pflAB* (SO2912-2913), *ackA* (SO2915), *pta* (SO2916) and the *ppc* (SO0274) genes. Another category with a high percentage of down-regulated genes is the "transport and binding proteins" which contain genes that code for ABC transporters (*cydCD* or SO3285-3286), *nosF* or SO0487, SO0821, SO4446-4448), TonB-dependent receptors (*nosA* or SO0630), and two L-lactate permeases (*lldP*, SO1522). Genes that encode secretion proteins such as the HlyD family secretion protein (SO0820, SO3483), as well as efflux proteins (SO0822, SO2045, SO4475) and transporters such as the formate transporter (SO2911), an ammonium transporter (SO3820), and an outer membrane porin (SO3896) were also down-regulated. In the "regulatory functions" category there is down-regulation of *etrA* (as expected since it was deleted), and repression of the LambdaSo phage transcriptional regulator of the Cro/CI family (SO2990) was also apparent (ratio of 0.43). A complete list of all the genes repressed two-fold or higher is provided (SUPPLEMENTAL TABLE B.4).

TABLE 4.2. Genes induced in anaerobic cultures of EtrA7-1 relative to the wild type (reference strain).

Gene ID	Gene name	Relative expression[a]	COG Annotation
1) Energy metabolism			
SO0162	pckA	2.21 (± 0.48)[b]	phosphoenolpyruvate carboxykinase (ATP)
SO0747	fpr	2.17 (± 1.01)	ferredoxin--NADP reductase
SO1103	nqrA-2	2.25 (± 0.54)	NADH:ubiquinone oxidoreductase, Na translocating, alpha subunit
SO1104	nqrB-2	2.70 (± 1.01)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB
SO1105	nqrC-2	3.15 (± .080)	NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit
SO1106	nqrD-2	4.65 (± 2.07)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrD
SO1107	nqrE-2	3.63 (± 1.61)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE
SO1108	nqrF-2	4.21 (± 2.05)	NADH:ubiquinone oxidoreductase, Na translocating, beta subunit
SO1495	glgX	2.03 (± 0.50)	glycogen operon protein
SO1498	glgC	6.86 (± 4.90)	glucose-1-phosphate adenylyltransferase
SO1499	glgA	5.42 (± 5.26)	glycogen synthase
SO1891		3.77 (± 1.80)	3-oxoadipate CoA-succinyl transferase, beta subunit
SO1892	atoD	3.21 (± 2.14)	acetate CoA-transferase, subunit A
SO1927	sdhC	2.47 (± 1.26)	succinate dehydrogenase, cytochrome b556 subunit
SO1930	sucA	3.02 (± 1.22)	2-oxoglutarate dehydrogenase, E1 component
SO1931	sucB	3.60 (± 1.58)	2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase
SO1932	sucC	3.29 (± 0.98)	succinyl-CoA synthase, beta subunit
SO1933	sucD	3.28 (± 1.24)	succinyl-CoA synthase, alpha subunit
SO2361	ccoP	2.30 (± 0.92)	cytochrome c oxidase, cbb3-type, subunit III
SO2362	ccoQ	3.44 (± 1.16)	cytochrome c oxidase, cbb3-type, CcoQ subunit
SO2364	ccoN	2.76 (± 1.07)	cytochrome c oxidase, cbb3-type, subunit I
SO4509		2.33 (± 0.56)	formate dehydrogenase, alpha subunit
SO4510	fdhB-1	4.03 (± 1.57)	formate dehydrogenase, iron-sulfur subunit
SO4511		2.53 (± 0.31)	formate dehydrogenase, C subunit, putative
2) Transport and binding proteins			
SO1723		7.86 (± 3.75)	phosphate ABC transporter, permease protein, putative
SO4319		6.21 (± 1.58)	HlyD family secretion protein
SO1724		4.75 (± 2.78)	phosphate ABC transporter, permease protein, putative
SO1560		4.51 (± 3.45)	phosphate-binding protein
SO1725	pstB-1	3.56 (± 2.74)	phosphate ABC transporter, ATP-binding protein
SO2750	tolR	2.95 (± 1.06)	tolr protein
SO4598		2.82 (± 1.85)	heavy metal efflux pump, CzcA family
SO1925		2.68 (± 1.43)	HlyD family secretion protein
SO4597		2.47 (± 1.55)	heavy metal efflux system protein, putative
SOA0153		2.20 (± 0.65)	heavy metal efflux pump, CzcA family
SO0520		2.08 (± 0.34)	heavy metal efflux pump, CzcA family
SO4289	pstB-2	2.08 (± 1.02)	phosphate ABC transporter, ATP-binding protein
SO4290	pstA	2.04 (± 1.06)	phosphate ABC transporter, permease protein

(Continued)

TABLE 4.2. (Cont'd) Genes induced in anaerobic cultures with nitrate of EtrA7-1 relative to the wild type (reference strain).

Gene ID	Gene name	Relative expression[a]	COG Annotation
3) Cellular processes			
SO0611	sspA	2.60 (± 0.58)	stringent starvation protein a
SO0612	sspB	2.09 (± 0.32)	stringent starvation protein b
SO1648		2.46 (± 1.75)	cold shock domain family protein
SO1807	pspA	3.11 (± 1.64)	phage shock protein A
SO1808	pspB	3.49 (± 0.42)	phage shock protein B
SO1809	pspC	3.45 (± 1.84)	phage shock protein C
SO2355		2.30 (± 0.31)	universal stress protein family
SO3582		2.95 (± 0.52)	methyl-accepting chemotaxis protein
SO4317		2.55 (± 0.63)	RTX toxin, putative
SO4320	aggA	7.18 (± 2.36)	agglutination protein
SO4408	bipA	2.45 (± 0.38)	virulence regulator BipA
4) Cell envelope			
SO4321		7.10 (± 1.77)	OmpA family protein
SO1245		5.26 (± 0.92)	membrane protein, putative
SO1164	dacA-1	2.67 (± 0.69)	D-alanyl-D-alanine carboxypeptidase
SO1166		2.03 (± 0.92)	membrane-bound lytic transglycosylase, putative
SO1167	rodA	2.93 (± 0.69)	rod shape-determining protein RodA
SO1168	mrdA	2.94 (± 1.02)	penicillin-binding protein 2
5) Fatty acid and phodpholipid metabolism			
SO2774	fabF-1	3.07 (± 0.27)	3-oxoacyl-(acyl-carrier-protein) synthase II
SO2777	fabD	3.35 (± 0.61)	malonyl CoA-acyl carrier protein transacylase
SO2778	fabH-1	2.14 (± 0.29)	3-oxoacyl-(acyl-carrier-protein) synthase III
SO3072	fabB	2.44 (± 0.88)	3-oxoacyl-(acyl-carrier-protein) synthase I
SO4382	fabG-2	2.13 (± 0.51)	3-oxoacyl-(acyl-carrier-protein) reductase
SO4383	fabF-2	2.09 (± 0.99)	3-oxoacyl-(acyl-carrier-protein) synthase II
6) Protein Fate			
SO1193	secD-1	6.40 (± 2.28)	protein-export membrane protein SecD
SO1194	secF-1	6.25 (± 0.91)	protein-export membrane protein SecF
SO2964		6.17 (± 0.85)	ClpP protease family protein[c]
SO2887	dsbB	6.14 (± 3.76)	disulfide bond formation protein b
SO0218	secE	3.31 (± 1.04)	preprotein translocase, SecE subunit
SO0163	hslO	2.82 (± 0.45)	chaperonin HslO
7) Biosynthesis of co-factors, prosthetic groups and carriers			
SO1109	apbE	6.44 (± 2.17)	thiamin biosynthesis lipoprotein ApbE
8) Signal transduction			
SO1558	phoB	5.22 (± 2.59)	phosphate regulon response regulator PhoB
SO1559	phoR	3.68 (± 1.86)	phosphate regulon sensor protein PhoR
9) Regulatory functions			
SO1726	phoU	2.59 (± 1.77)	phosphate transport system regulatory protein PhoU
SO1349	Era	2.70 (± 0.51)	GTP-binding protein Era
SO4312		2.11 (± 0.69)	adenylate cyclase CyaA, putative

(Continued)

TABLE 4.2. (Cont'd) Genes induced in anaerobic cultures with nitrate of EtrA7-1 relative to the wild type (reference strain).

Gene ID	Gene name	Relative expression[a]	COG Annotation
10) Transcription			
SO0219	nusG	2.09 (± 0.64)	transcription antitermination protein NusG
SO1203	nusA	2.50 (± 0.36)	N utilization substance protein A
SO1284	rpoD	2.70 (± 1.22)	RNA polymerase sigma-70 factor
SO1348	rnc	3.02 (± 0.88)	ribonuclease III
11) DNA metabolism			
SO1350	recO	5.00 (± 1.76)	DNA repair protein RecO
SOA0086		3.00 (± 0.94)	site-specific recombinase, resolvase family
SO0272	cinA	2.11 (± 0.77)	competence/damage-inducible protein CinA
12) Conserved hypothetical and hypothetical proteins			
SO4322		7.24 (± 2.46)	conserved hypothetical protein
SO0005		7.00 (± 3.12)	conserved hypothetical protein TIGR00278
SO2967		6.01 (± 0.69)	conserved hypothetical protein
SO2972		5.31 (± 1.67)	hypothetical protein
13) Other cathegories[c]			
SO0674		1.89 (± 0.92)	prophage MuSo1, protein Gp32, putative
SO0675		2.06 (± 0.82)	prophage MuSo1, major head subunit, putative
SO2684		2.54 (± 0.30)	prophage MuSo2, protein Gp32, putative
SO2685		2.23 (± 0.63)	prophage MuSo2, major head subunit, putative
SO2940		3.58 (± 1.10)	prophage LambdaSo, host specificity protein J, putative
SO2941		2.70 (± 0.72)	prophage LambdaSo, tail assembly protein I
SO2948		2.53 (± 0.44)	prophage LambdaSo, tail assembly protein K, putative
SO2953	H	3.61 (± 1.02)	prophage LambdaSo, tail length tape meausure protein
SO2956		2.67 (± 1.47)	prophage LambdaSo, major tail protein V, putative
SO2963		4.57 (± 0.65)	prophage LambdaSo, major capsid protein, HK97 family
SO2965		7.02 (± 0.93)	prophage LambdaSo, portal protein, HK97 family
SO2969		5.60 (± 1.27)	prophage LambdaSo, holin, putative
SO2973		4.43 (± 0.93)	prophage LambdaSo, lysozyme, putative

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures with 2 mM KNO_3 of EtrA7-1 to that of MR-1 (reference).

[b]The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

[c]These genes are of prophage origin.

TABLE 4.3. Genes repressed in anaerobic cultures with nitrate of EtrA7-1 relative to the wild type (reference strain).

Gene ID	Gene name	Relative expression[a]	COG Annotation
1) Energy Metabolism			
SO0398	frdA	0.30 (±0.16)[b]	fumarate reductase flavoprotein subunit
SO0399	frdB	0.39 (± 0.06)	fumarate reductase iron-sulfur protein
SO0274	ppc	0.48 (± 0.19)	phosphoenolpyruvate carboxylase
SO0845	napB	0.15 (± 0.04)	cytochrome c-type protein NapB
SO0846	napH	0.18 (± 0.11)	iron-sulfur cluster-binding protein napH
SO0847	napG	0.14 (± 0.07)	iron-sulfur cluster-binding protein NapG
SO0848	napA	0.18 (± 0.13)	periplasmic nitrate reductase
SO0849	napD	0.30 (± 0.04)	napD protein
SO0903	nqrB-1	0.34 (± 0.15)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB
SO0904	nqrC-1	0.28 (± 0.09)	NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit
SO0905	nqrD-1	0.27 (± 0.14)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrD
SO0906	nqrE-1	0.23 (± 0.07)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE
SO0907	nqrF-1	0.23 (± 0.08)	NADH:ubiquinone oxidoreductase, Na translocating, beta subunit
SO0970		0.31 (± 0.17)	fumarate reductase flavoprotein subunit precursor
SO1018	nuoE	0.44 (± 0.17)	NADH dehydrogenase I, E subunit
SO1019	nuoCD	0.35 (± 0.13)	NADH dehydrogenase I, C/D subunits
SO1020	nuoB	0.40 (± 0.10)	NADH dehydrogenase I, B subunit
SO1363	hcp	0.13 (± 0.08)	prismane protein
SO1364		0.12 (± 0.07)	iron-sulfur cluster-binding protein
SO1429	dmaA-1	0.43 (± 0.09)	anaerobic dimethyl sulfoxide reductase, A subunit
SO1430	dmsB-1	0.29 (± 0.04)	anaerobic dimethyl sulfoxide reductase, B subunit
SO1490	adhB	0.28 (± 0.12)	alcohol dehydrogenase II
SO1776	mtrB	0.22 (± 0.04)	outer membrane protein precursor MtrB
SO1777	mtrA	0.25 (± 0.06)	decaheme cytochrome c MtrA
SO1778	omcB	0.30 (± 0.09)	decaheme cytochrome c
SO1779	omcA	0.30 (± 0.05)	decaheme cytochrome c
SO2097	hydC	0.07 (± 0.04)	quinone-reactive Ni/Fe hydrogenase, cytochrome b subunit
SO2098	hyaB	0.11 (± 0.10)	quinone-reactive Ni/Fe hydrogenase, large subunit
SO2099	hoxK	0.07 (± 0.11)	quinone-reactive Ni/Fe hydrogenase, small subunit precursor
SO2136	adhE	0.40 (± 0.10)	aldehyde-alcohol dehydrogenase
SO2727		0.32 (± 0.23)	cytochrome c3
SO2912	pflB	0.18 (± 0.11)	formate acetyltransferase
SO2913	pflA	0.20 (± 0.13)	pyruvate formate-lyase 1 activating enzyme
SO2915	ackA	0.23 (±0.16)	acetate kinase
SO2916	pta	0.23 (± 0.14)	phosphate acetyltransferase
SO3117		0.41 (± 0.11)	thioredoxin, putative
SO3144	etfA	0.36 (± 0.13)	electron transfer flavoprotein, alpha subunit
SO3285	cydB	0.21 (± 0.06)	cytochrome d ubiquinol oxidase, subunit II
SO3286	cydA	0.22 (± 0.10)	cytochrome d ubiquinol oxidase, subunit I
SO3980	nrfA	0.18 (± 0.06)	cytochrome c552 nitrite reductase
SO4453		0.40 (± 0.13)	electron transfer flavoprotein-ubiquinone oxidoreductase, putative

(Continued)

TABLE 4.3. (Cont'd) Genes repressed in anaerobic cultures with nitrate EtrA7-1 relative to the wild type (reference strain).

Gene ID	Gene name	Relative expression[a]	COG Annotation
SO4513		0.06 (± 0.02)	formate dehydrogenase, alpha subunit
SO4515		0.07 (± 0.01)	formate dehydrogenase, C subunit, putative
SO4591	cymA	0.39 (± 0.27)	tetraheme cytochrome c
2) Transport and binding proteins			
SO0487	nosF	0.28 (± 0.05)	copper ABC transporter, ATP-binding protein
SO0630	nosA	0.30 (± 0.06)	TonB-dependent receptor
SO0820		0.20 (± 0.05)	HlyD family secretion protein
SO0821		0.14 (± 0.05)	ABC transporter, ATP-binding/permease protein
SO0822		0.10 (± 0.04)	outer membrane efflux family protein
SO0827	lldP	0.31 (± 0.07)	L-lactate permease
SO1522		0.47 (± 0.07)	L-lactate permease, putative
SO2045		0.45 (± 0.08)	cation efflux family protein
SO2911		0.40 (± 0.20)	formate transporter, putative
SO3483		0.22 (± 0.08)	HlyD family secretion protein
SO3779	cydC	0.44 (± 0.12)	ABC transporter, ATP-binding protein CydC
SO3780	cydD	0.32 (± 0.08)	ABC transporter, ATP-binding protein CydD
SO3820		0.16 (± 0.26)	ammonium transporter, degenerate
SO3896		0.26 (± 0.17)	outer membrane porin, putative
SO4446		0.24 (± 0.12)	molybdenum ABC transporter, ATP-binding protein
SO4447		0.32 (± 0.20)	molybdenum ABC transporter, permease protein
SO4448		0.35 (± 0.17)	molybdenum ABC transporter, periplasmic molybdenum-binding protein
SO4475		0.30 (± 0.10)	cation efflux family protein
ORF3506		0.19 (± 0.06)	ammonium transporter (tpt)
3) Regulatory functions			
SO2356	etrA	0.05 (± 0.01)	electron transport regulator A
SO2990		0.43 (± 0.16)	prophage LambdaSo, transcriptional regulator, Cro/CI family
SO4603	lexA	0.47 (± 0.12)	LexA repressor
4) Cellular processes			
SO4226	ftsL	0.48 (± 0.06)	cell division protein FtsL
SO4299	cat	0.50 (± 0.06)	chloramphenicol acetyltransferase
SO4405	katG-2	0.31 (± 0.09)	catalase/peroxidase HPI
5) Signal transduction			
SO4477	cpxR	0.32 (± 0.10)	transcriptional regulatory protein CpxR
SO4478	cpxA	0.34 (± 0.15)	sensor protein CpxA
SO4633	ompR	0.38 (± 0.19)	transcriptional regulatory protein OmpR
SO4634	envZ	0.33 (± 0.20)	osmolarity sensor protein EnvZ

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures with 2 mM KNO_3 of EtrA7-1 to that of MR-1 (reference).
[b]The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

Comparison of the gene expression profile of anaerobic cultures of EtrA7-1 grown at 1 mM KNO_3 and at 40 mM KNO_3. A total of 358 genes were differentially expressed when anaerobic growth of EtrA7-1 at a low nitrate concentration was compared to that at a high concentration. This total was divided in two groups, 154 genes up-regulated (TABLE 4.4) and 204 genes down-regulated (TABLE 4.5). A complete list of all the genes induced and repressed two-fold or higher is provided (SUPPLEMENTAL TABLES B.5 and B.6). Among the up-regulated genes there are genes involved in energy metabolism, specifically in the regeneration of acetyl CoA, the pyruvate dehydrogenase multi-enzyme complex E1, E2 and E3 (SO0424-0426). In addition, the formate acetyltransferase operon (SO2912-2916) is up-regulated, which is involved in the conversion of acetyl CoA and formate into CoA and pyruvate. There is also induction of genes involved in carbohydrate metabolism such as *ppc* (SO0274), *tkt* (SO0930), *malQ* (SO1493), *glgB* (SO1494), *glgA* (SO1499), *eda* (SO2486), *edd* (SO2487), *pgl* (SO2488), *zwf* (SO2489), and *glmS* (SO4741) genes. Also genes involved in anaerobic metabolism are up-regulated such as the *nqrB-1, C-1, D-1, F-1* operon (SO0903-0907), and the genes encoding cytochromes *scyA* (SO0264), and *cydB* (SO3285). There is also activation of genes involved in amino acid scavenging and biosynthesis such as *glnB*-1, *glnA*, *ntrB*, and *ntrC*. Induction of the *amt* (SO0760) and *tpt* gene (ORF03506), which encode two ammonium transporters, was detected.

The down-regulated genes include ABC transporters (SO0821, S01042-1044, and SO1959), genes involved in oxidative stress response *katG*-1 (SO0725), *katB* (SO1070), *dnaK* (SO1126), and *dmaA*-1 (SO1429) and the phage shock protein genes *pspABC* (SO1807-1809). Also, the *ilvADMGC* operon (SO4344-4349) is highly down-regulated.

TABLE 4.4. Genes induced in anaerobic cultures of EtrA7-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	COG Annotation
1) Energy metabolism			
SO0264	scyA	2.35 (± 0.74)[b]	cytochrome c
SO0274	ppc	4.17 (± 1.61)	phosphoenolpyruvate carboxylase
SO0424	aceE	2.72 (± 0.88)	pyruvate dehydrogenase complex E1, pyruvate dehydrogenase
SO0425	aceF	2.68 (± 0.44)	pyruvate dehydrogenase complex E2, dihydrolipoamide acetyltransferase
SO0426	lpdA	1.99 (± 0.34)	pyruvate dehydrogenase complex E3, lipoamide dehydrogenase
SO0903	nqrB-1	2.05 (± 0.31)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB
SO0904	nqrC-1	2.39 (± 1.05)	NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit
SO0906	nqrE-1	2.61 (± 1.29)	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE
SO0907	nqrF-1	2.62 (± 1.06)	NADH:ubiquinone oxidoreductase, Na translocating, beta subunit
SO1493	malQ	6.56 (± 8.84)	4-alpha-glucanotransferase
SO1494	glgB	6.28 (± 8.81)	1,4-alpha-glucan branching enzyme
SO1499	glgA	6.54 (± 9.60)	glycogen synthase
SO2486	eda	3.45 (± 0.96)	2-deydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate aldolase
SO2487	edd	4.01 (± 0.77)	6-phosphogluconate dehydratase
SO2488	pgl	3.30 (± 1.00)	6-phosphogluconolactonase
SO2489	zwf	2.60 (± 0.44)	glucose-6-phosphate 1-dehydrogenase
SO2912	pflB	2.59 (± 0.80)	formate acetyltransferase
SO2913	pflA	3.14 (± 1.01)	pyruvate formate-lyase 1 activating enzyme
SO2915	ackA	3.28 (± 1.35)	acetate kinase
SO2916	pta	2.36 (± 1.12)	phosphate acetyltransferase
SO3285	cydB	2.68 (± 1.41)	cytochrome d ubiquinol oxidase, subunit II
SO4509		2.69 (± 1.19)	formate dehydrogenase, alpha subunit
SO4511		2.45 (± 1.49)	formate dehydrogenase, C subunit, putative
SO4741	glmS	3.44 (± 0.87)	glucosamine--fructose-6-phosphate aminotransferase
2) Amino acid biosynthesis			
SO4410	glnA	6.18 (± 3.91)	glutamine synthetase, type I
SO1121	proB	2.26 (± 0.57)	glutamate 5-kinase
SO1122	proA	2.50 (± 0.42)	gamma-glutamyl phosphate reductase
3) Regulatory functions			
SO0761	glnB-1	3.37 (± 3.05)	nitrogen regulatory protein P-II 1
4) Signal transduction			
SO4471	ntrB	2.89 (± 1.40)	nitrogen regulation protein
SO4472	ntrC	2.76 (± 1.37)	nitrogen regulation protein NR(I)
5) Transport and binding proteins			
ORF03506		3.38 (± 1.44)	ammonium transporter (tpt)
SO0760	amt	7.79 (± 6.39)	ammonium transporter

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures of EtrA7-1 grown at 40 mM KNO_3 to that of the anaerobically grown at 1 mM KNO_3 (reference).
[b] The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

TABLE 4.5. Genes repressed in anaerobic cultures of EtrA7-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	COG Annotation
1) Energy Metabolism			
SO1427		0.35(± 0.12)[b]	decaheme cytochrome c
SO1429	dmaA-1	0.47 (± 0.24)	anaerobic dimethyl sulfoxide reductase, A subunit
SO4513		0.26 (± 0.34)	formate dehydrogenase, alpha subunit
SO4515		0.29 (± 0.26)	formate dehydrogenase, C subunit, putative
2) Cellular processes			
SO0725	katG-1	0.24 (± 0.10)	catalase/peroxidase HPI
SO1070	katB	0.22 (± 0.21)	catalase
SO1807	pspA	0.18 (± 0.07)	phage shock protein A
SO1808	pspB	0.12 (± 0.05)	phage shock protein B
SO1809	pspC	0.14 (± 0.05)	phage shock protein C
3) Cell envelope			
SO0150		0.28 (± 0.11)	lipoprotein, putative
SO2194		0.02 (± 0.00)	OmpA family protein
SO4334		0.03 (± 0.01)	inner membrane protein, putative
4) Transport and binding proteins			
SO0519		0.34 (± 0.10)	cation efflux protein, putative
SO0822		0.52 (± 0.22)	outer membrane efflux family protein
SO1042		0.42 (± 0.09)	amino acid ABC transporter, ATP-binding protein
SO1043		0.37 (± 0.07)	amino acid ABC transporter, permease protein
SO1044		0.20 (± 0.07)	amino acid ABC transporter, periplasmic amino acid-binding protein
SO1557		0.30 (± 0.09)	outer membrane porin, putative
SO1560		0.10 (± 0.04)	phosphate-binding protein
SO1689		0.32 (± 0.21)	cation transport ATPase, E1-E2 family
SO1723		0.12 (± 0.05)	phosphate ABC transporter, permease protein, putative
SO1724		0.45 (± 0.14)	phosphate ABC transporter, permease protein, putative
SO1925		0.17 (± 0.08)	HlyD family secretion protein
5) Protein fate			
SO1126	dnaK	0.47 (± 0.26)	chaperone protein DnaK
6) Amino acid biosynthesis			
SO4344	ilvA	0.16 (± 0.05)	threonine dehydratase
SO4345	ilvD	0.23 (± 0.05)	dihydroxy-acid dehydratase
SO4346	ilvM	0.26 (± 0.09)	acetolactate synthase II, small subunit
SO4347	ilvG	0.27 (± 0.08)	acetolactate synthase II, large subunit
SO4349	ilvC	0.18 (± 0.10)	ketol-acid reductoisomerase
7) Signal Transduction			
SO1558	phoB	0.11 (± 0.04)	phosphate regulon response regulator PhoB
SO1945	phoQ	0.41 (± 0.10)	sensor protein PhoQ
SO1946	phoP	0.34 (± 0.10)	transcriptional regulatory protein PhoP
SO4477	cpxR	0.33 (± 0.12)	transcriptional regulatory protein CpxR
SO4478	cpxA	0.49 (± 0.17)	sensor protein CpxA
8) Regulatory proteins			
SO1937	fur	0.38 (± 1.14)	ferric uptake regulation protein

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures of EtrA7-1 grown at 40 mM KNO_3 to that of the anaerobically grown at 1 mM KNO_3 (reference).
[b] The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

DISCUSSION

This study shows a genetic stress response caused by the deletion of the *etrA* gene in *Shewanella oneidensis* MR-1. The MR-1 Δ*etrA* mutant (EtrA7-1) was confirmed using PCR diagnostic techniques, DNA sequencing and expression analysis. As it has been observed previously for various *etrA* mutants in MR-1, EtrA7-1 retained its ability to reduce nitrate, however, the anaerobic growth when nitrate was the only electron acceptor was significantly lower than that of the wild type.

The inability of EtrA7-1 to grow despite its ability to reduce nitrate could be due to stress factors caused or enhanced by the mutation. The genetic expression pattern of EtrA7-1 when compared to that of the wild type after anaerobic cultivation with nitrate revealed the expressin of various genes that have been previously reported to respond to stressful conditions (i.e. starvation). Among these genes there is up-regulation of the *sspAB* genes that encode the stringent starvation protein. This protein in *E. coli* is highly expressed during nutrient starvation conditions and the SspA protein has been found to be required for the transcription of bacterial phage late genes (17). Up-regulation of the *pspABC* operon was also detected. These genes encode the phage shock protein, which in *E. coli* is secreted to the periplasm and maintains the proton motive force under stress conditions provoked by filamentous phage infection (23). There was induction of a virulence factor (*bipA*) found in various *E. coli* enteric pathogens. This protein is a chaperone and it has been associated with rearrangements in the cytoskeleton of the infected host cells, in regulation of cell motility by flagellae and in the regulation of the expression of capsular genes (29). In addition, there is up-regulation of the genes involved in activation of the *S. oneidensis* MR-1 prophage LambdaSo as well as two

genes that encode late genes of MuSo1 and MuSo2, suggesting activation of their lytic cycle. There is also induction of bacterial genes (*nusA* and *nusG*) that are required to stabilize the Lambda protein antitermination complex in *E. coli* (4, 48) as well as a membrane-bound lytic transglycosylase (SO1166) that has the potential to help the process of lyses. Conversely, there is repression of the LambdaSo transcription regulator Cro/CI family, which represses the transcription of the Lambda genes in *E. coli* (49). This expression pattern supports the activation of the lytic cycle of these phages. Induction of these prophages by stressful conditions, specifically irradiation exposure, has been previously reported in *S. oneidensis* MR-1 (36). In this study induction of the early genes was observed for cells collected after a short incubation period of 5-20 min, whilst activation of late genes was observed when cells were incubated for a longer time (60 min). I also reported previously induction of some of these prophage genes in response to accumulation of nitrite and of other probable intermediaries of the nitrate reduction pathway, which could cause oxidative stress and DNA damage to the cell (Chapter 3). Moreover, it is known that starvation can activate the lytic cycle of prophages in other bacteria (49).

The induction of these genes suggests that the lytic cycle of these phages is compromising the survival of this organism under starvation conditions created by the absence of *etrA*. The EtrA protein is a global regulator that activates the expression of various genes involved in metabolism when oxygen is not present. In this study the growth conditions were optimal for normal growth, however, despite the availability of sufficient nutrient concentrations in the growth medium the bacterium was not able to use them, generating a starvation response. Another piece of evidence for the "internal

starvation condition" is the significant induction observed for the *pho* genes. The *pho* regulon (*phoBRU*), which has been very well studied in *E. coli,* activates and induces the expression of other genes when the cell experiences phosphate starvation (21, 50, 51). In *E. coli,* this regulator system induces the expression of genes that encode transport proteins for inorganic phosphate and other phosphorus sources, and of genes involved in phosphorus utilization. In this study there is induction of a variety of genes involved in the transport and metabolism of phosphate indicating phosphate starvation conditions in EtrA7-1. There are also genes induced such as *era*, which regulates the TCA cycle and responds to starvation (34), and *recO* that is involved in repair of DNA damage (36).

Among the physical starvation responses described in bacteria, there is the reduction of cellular size and biofilm dissolution. Cell size reduction has been observed for some bacteria of the *Shewanella* genus (2, 6, 16). This particular response has been of interest in the study of *S. algae* since it has been observed that when bacteria are inoculated in contaminated sites, its transport is limited to the surroundings of the injection wells and very low bacterial numbers travel downstream the plume (6). Thus, if cells are cultivated in starvation conditions, the culture is more resistant to the harsh environmental conditions and the size of the cells is small enough enabling the inocula to penetrate deeper and travel further. The results presented herein show a significant percentage of genes up-regulated that belong to the "cell envelope" and to the "fatty acid and phospholipid metabolism" categories. These categories include genes involved in cell membrane composition, and shape determination (*rodA*), which can be involved in rearranging the cell membrane composition and in reducing the cellular size, which in

turn conserves energy. A rearrangement in the cell membrane composition can also increase the membrane permeability to the substrates needed.

Detachment of bacteria has been observed for biofilms exposed to long periods of starvation (47). In the EtrA7-1 cultures examined there is down-regulation of genes associated with biofilm formation such as the *cpxAB* genes that in *E. coli* are described as part of a signal transduction pathway for the adherence process (13). In addition to these proteins, *E. coli* possesses a second signal transduction pathway, the EnvZ-OmpR two-component system, which operates the same process. In EtrA7-1 these genes (*envZ* and *ompR*) are down-regulated as well. Repression of these genes in the mutant suggests an impediment to biofilm formation, which represents a starvation response. However, in studies of MR-1 induction of biofilm detachment was associated with oxygen limiting conditions (47). To determine the regulators involved in this process, genes encoding possible regulators such as *etrA, crp,* and *arcA* were deleted. The MR-1 Δ*etrA* mutant was reported to be defective in its detachment response (47) but to a lesser extent when compared to the other regulators examined (Crp and ArcA). The authors suggested an EtrA involvement in the regulation of biofilm detachment in MR-1, however a direct linkage of these regulators with detachment of biofilms could not be concluded. In EtrA7-1, high induction levels of a gene annotated as aggA were detected. This gene encodes an agglutination protein, which is involved in the fimbrial biogenesis system of pathogenic gram-negative bacteria (22). The fimbriae mediate the aggregation of cells adhered to the epithelial host cells. In MR-1, this agglutination protein has been associated with biofilm formation since it was the most up-regulated protein in MR-1 biofilm forming cells (11). Therefore, our results suggest an involvement of EtrA in the

repression of *aggA* during anaerobic conditions, which might trigger an activation of detachment in MR-1. Conversely, the repression of the *cpxAB* genes might be due to starvation conditions and not to regulation by EtrA, however the lack of these transcripts can induced detachment in the mutant. This response might counteract the effects of AggA in adherence, which might explain the variable detachment phenotype observed for the MR-1 Δ*etrA* mutant by Thormann et al. (47).

Accumulation of nitrite is another stressful situation observed in the cultures of EtrA7-1. Nitrite can trigger a stress response since, as previously discussed, high concentrations are toxic to the cell. This type of stress can be responsible for the up-regulation of genes that help alleviate the damage associated with it. Among these genes, there is *hslO* that encodes a chaperone that assist the cells in the folding of proteins and repair, and *rpoD,* which codes for the sigma factor 70 that regulates many of these genes including those regulated by the *pho* regulon (24). There are also genes that encode export proteins and efflux systems that can help the cell in detoxification. It is also important to mention that other genes that encode for proteins that protect the cell against oxidative damage are down-regulated such as the *uvrC, recC, hemB-1, hemB-2, hemH-1, katG-2, parE* and *lexA* (36). The cause of down-regulation in these genes is unclear.

Induction of various genes that are involved in carbon metabolism was detected. This suggests a regulatory role of EtrA in their expression. Some of these genes are involved in the TCA cycle and in some aerobic metabolism processes. In *E. coli*, Fnr not only induces the expression of genes necessary for anaerobic metabolism but also represses genes involved in the TCA cycle and some other genes involved in aerobic metabolism (39). Since EtrA is structurally similar to Fnr and since it has been associated

with oxygen sensing and regulation of anaerobic metabolism it is possible that EtrA is repressing the expression of some of these genes in MR-1 in the absence of oxygen. These results were observed previously in experiments performed in an MR-1 *etrA* mutant (3). In addition, some of these genes and even a higher number of up-regulated genes in this category were observed when the growth of the mutant (EtrA7-1) was compared under two nitrate concentrations (1 mM versus 40 mM KNO_3). Various global regulators control the expression of these genes, thus this regulation cannot be expected to be an ON/OFF expression regulation. Therefore, if there is an increase in the induction factor it is reasonable to expect an increase in the genetic expression response. In this case, at higher nitrate concentrations there is an increase in the expression of these genes.

Down-regulation in the expression of some genes in EtrA7-1 in response to anaerobic conditions on nitrate might represent an involvement of EtrA in their regulation. Among these, the ones of greater interest in this study were those involved in the nitrate reduction pathway. Those include the genes in the *nap* operon (*napBHGAD*), the *cymA* gene (*napC* homolog), and the *nrfA* gene, which encodes the cytochrome c552 nitrite reductase. Down-regulation of other genes that has been associated with the nitrate reduction pathway includes the *hcp* gene, which encodes the prismane protein associated in other bacteria with the reduction of hydroxylamine (5, 14). Among the down-regulated genes, there are genes that encode proteins necessary for anaerobic processes other than nitrate reduction. These genes include the fumarate reductase (*frdAB*), the anaerobic dimethyl sulfoxide reductase genes *dmaA*-1 and *dmsB*-1 and the quinone—reactive Ni/Fe hydrogenase genes the *hydC, hyaB,* and *hoxK*. These genes have been considered candidates for EtrA regulation (3, 26, 37, 38), and these results are consistent with those

observed by Beliaev, et al. (3). Moreover, the latter identified possible recognition sites of EtrA for some of these genes including *napDAHGB, nrfA, frdAB, hcp*, and *hydC*.

The genetic expression differences observed for EtrA7-1 when its growth was compared anaerobically at a low versus a high nitrate concentration showed the down-regulation of genes that are oxidative stress inducible as well as down-regulation of the *pspABC* operon (mentioned previously). In addition, down-regulation in the expression of the *pho* genes (*phoBPQ)* was detected. Up-regulation was observed for genes involved in the transport and metabolism of carbohydrate such as the TCA cycle as mentioned previously. In addition, there was induction of genes involved in ammonium assimilation (*glnB-1, glnA,* and *ntrBC*) as well as two ammonium transporters, *amt* (SO0760) and tpt (ORF03506) and the glutamate kinase *proAB* (SO1121-1122). These genes are activated in amino acid starvation conditions. This ammonium starvation condition might be created by an imbalance in the utilization of the carbon source versus the low levels of ammonium produced. The reduction of nitrite into ammonium occurs very slowly in the mutant due to the low levels of nitrite reductase. This creates a deficiency of ammonium and subsequently of amino acids, which in turn activate the expression of these genes. The same occurs with the activation of the *pho* genes. Since the reduction of nitrite into ammonium, which is the one that gives more energy to the cell (compare with the reduction of nitrate into nitrite), is slowed down to a minimum, a deficiency of phosphate (ATP) is generated. Moreover, genes involved in storage of carbohydrates such as the glycogen synthase gene *glgAC* (SO1498-1499) and *glgX* (SO1495) are up-regulated. This was also observed when the genetic expression pattern of the mutant was compared to that of the wild type. These genes are activated in response to high concentrations of

glucose indicating an excess of carbon in the cell relative to the concentration of ammonium, which was limited in EtrA7-1.

EtrA is a global regulator that might be acting in cooperation with other proteins to control various anaerobic metabolism processes in MR-1 (3, 26, 38). Therefore, the expression of these genes cannot be expected to be under an "all or none" regulation mechanism but rather, it is regulated in a gradual fashion that depends on many factors that accumulate to increase or decrease its expression. In this study there is a decrease in the rate of nitrate and nitrite reduction. Nevertheless, the activity is not halted indicating that even when EtrA is not present there are other regulators stimulating the expression of the genes involved in this pathway such as the genes that encode the only nitrate reductase in MR-1, NapA. In *E. coli* the *nap* and *nrf* genes are positively regulated by Fnr and NarP. MR-1 possesses the genes for a homolog of the two-component regulatory system in E. coli NarQ/NarP (SO3981-3982). This can explain the decrease in the expression of these genes rather than a complete shut down. A double mutant defective in NarP and EtrA will determine whether these regulators act jointly to control the expression of these genes.

A positive regulatory role of EtrA in the expression of the *napBGHAD* genes, the *nrfA* and the *cymA* genes is suggested. In studies where the *cymA* gene was deleted in S. *oneidensis* MR-1, researchers observed that nitrate reduction, among other anaerobic processes, was abolished indicating a requirement for *cymA* in this process (42, 43). CymA is a homologue of the NapC (one of the components of the NapABC multi-enzyme complex) of *E. coli.* This protein, CymA, has been suggested to be part of the electron transport complex of the nitrate reduction pathway in MR-1 (31, 42, 43).

Even though, this study was not designed to investigate starvation in MR-1, the deletion of the *etrA* gene in this organism stimulated a massive starvation response. However, the results obtained suggest that even when MR-1 can activate genes in response to starvation to increase its survival, the activation of the lytic cycle of three prophages may provoke an aggressive infection. This multiple infection compromises the cell survival efforts under these circumstances. It is important to point out though that these results would not be expected to reflect the starvation response of MR-1 under normal starvation conditions, since this was a product of a primary perturbation (*etrA* gene deletion). This investigation not only helps to understand the regulatory systems present in MR-1 but also its protection mechanisms and the obstacles these might experience to reduce the chances of survive under stressful conditions.

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CHAPTER V

Summary and Future Research

SUMMARY

The goal of this study was to clarify and examine the nitrate reduction pathway in *Shewanella oneidensis* MR-1. This was performed by combining classical microbiology approaches with innovative molecular biotechnology. Physiological and stoichiometric analyses indicated that nitrate is reduced to nitrite and nitrite is completely reduced to ammonium. This concludes that DNRA is the nitrate reduction pathway in MR-1 and not denitrification. MR-1 demonstrated greater cytotoxicity to nitrite as compared to nitrate. Even though nitrate concentrations higher than 2 mM were proven to be toxic, the growth rate of MR-1 remained steady beyond 2 mM. Gene expression analyses in MR-1 indicated an oxidative stress response to high nitrate concentrations. Also, induction of prophage related genes was observed. Oxidative stress and activation of the prophage lytic cycle are potential causes of growth inhibition at high nitrate concentrations. On the other hand, up-regulation of genes encoding transport and efflux systems as well as enzymes and proteins that metabolize toxic intermediates was observed. These proteins and other stress response chaperones protect the cell against oxidative damage and help in its survival and its internal stability. This in turns might decrease the expression of the prophage related genes giving the bacterium a chance to live in these environmental conditions. In MR-1, tolerance to nitrite is also obtained via a higher rate of consumption of nitrite as compared to the reduction of nitrate. This was observed in physiological experiments and could be inferred from the *nrfA* expression analyses. The transcription of the *nrfA* gene reached a plateau at a lower nitrate concentration as compared to *napA*, which might be a strategy in MR-1 to immediately reduce the nitrite in the cell for detoxification purposes.

Expression studies for *napA* and *nrfA* genes demonstrated that increasing nitrate concentrations do not cause repression in their expression as occurs in *E. coli.* This implies that there is no alternative mechanism for the reduction of nitrate in MR-1. Further experiments where the *napA* gene was completely deleted from the genome of MR-1 concluded that NapA is the only nitrate reductase and therefore responsible for nitrate reduction pathway in MR-1. In addition, gene expression studies in MR-1 where nitrate was supplied as the only electron acceptor and ammonium was not added suggested nitrate assimilation in MR-1. Since nitrate assimilation was beyond the scope of this study, this needs further investigation.

Regulation of the nitrate reduction pathway was examined by mutational analyses. Studies with a MR-1 Δ*etrA* deletion mutant indicated a decrease in biomass and in the rate of nitrate reduction when compared to the wild type. Since reduction of nitrate was not abolished, this suggests a partial but not absolute regulatory role of EtrA in the nitrate reduction pathway of MR-1. This has been observed in *E. coli* where Fnr and the two-component regulatory system NarP/NarQ regulate the expression of *napA* and *nrfA*. As stated in Chapter III, MR-1 possesses homologues for the *narP* and *narQ* genes, which increases the chances in MR-1 to have a similar regulatory mechanism controlling this pathway.

A starvation stress response was suggested by the gene expression analyses of the *etrA* mutant. In addition, induction of the prophage related genes was detected. However, in this case, contrary to the induction observed in response to a high nitrate concentration, the up-regulation of the prophage related genes was massive including genes for all three prophages described in MR-1. This implies a more aggressive phage infection which

could have caused the low biomass observed in MR-1 Δ*etrA* deletion mutant. This indicates that starvation stress is more detrimental to MR-1 than the oxidative stress caused by high concentrations of nitrate. The cell response is similar to that observed for MR-1 after exposure to radiation (6). This is the first time starvation has been examined in MR-1.

This study not only shed light to the nitrate reduction pathway of this organism but it also advances the understanding of the internal response to stress conditions and its degree of tolerance. MR-1 has the potential to be an excellent bioremediator with a very unique anaerobic metabolism. However, its sensitivity to environmental stress might limit its performance in the field. Understanding its biology and its genetic machinery will help increase its chances as a bioremediator. This work also demonstrates the potential of the microarray technology in the formulation of hypotheses and as a screening method to identified and examine genes of unknown function that can help us explain some of the cellular processes.

FUTURE RESEARCH

Future studies in the area should focus on the examination of nitrate assimilation in MR-1. Assimilation of nitrate is defined as the reduction of nitrate to ammonium, which is subsequently incorporated into cell material for the synthesis of nucleic acids and proteins (4). This process does not occur in all bacteria although it is widely spread among different species. Since NapA is the only nitrate reductase in *Shewanella oneidensis* MR-1, nitrate assimilation will be a product of the activity of NapA. The aim

of this study was not to evaluate nitrate assimilation, thus this was not examined. However, if this process indeed takes place in MR-1, this will be the first time a role in nitrate assimilation is attributed to NapA. This will denote that MR-1 is even more powerful than it is known and other bacteria that posses NapA and similar genetic capabilities might also be able to assimilate nitrate. Nitrate assimilation could be address via nitrogen isotopic fractionation (3).

Starvation in *Shewanella oneidensis* MR-1 has not been directly investigated. More studies to better understand its effects in MR-1 will help elucidate ways to counteract some of the obstacles it might encountered as bioremediator. Starvation is a critical stress often experienced by bacteria when applied as a bioremediator in contaminated sites. Tolerance and survival mechanisms to this kind of stress increase the potential of a bacterium as a bioremediator. MR-1 has been proven to grow and effectively consumed nitrate at high concentrations (this study) as well as other harmful compounds (8), however, its survival is threatened by other type of stresses such as radiation exposure and possibly starvation (6). These types of stresses are well known to cause activation of the lytic cycle of prophages in MR-1 and in other bacteria (9), which compromise its survival. This is a significant obstacle in the effective performance of MR-1 as a bioremediator. However, bacteria that posses the metabolic machinery but that as MR-1 are susceptible to other stresses could be genetically manipulated to improve their capabilities in such harsh conditions. Therefore, studies to examine the regulation of the transcription of the prophage related genes in MR-1 are crucial to improve its performance. Mutation analyses to investigate the regulation of genes such as the LambdaSo prophage Cro/CI repressor family should be undertaken. A homologue of this

gene in E. coli is known to repress the expression of the prophage genes required to initialize activation of the Lambda prophage lytic cycle in *E. coli.* Also, point mutation of enzyme recognition sites and deletion of key genes will help in the development of a free-phage strain.

Unfortunately, little is known about the prophage related genes in MR-1. Therefore, research should focus in studying the biology and more specific, its genome. The majority of these genes are conserved hypothetical or hypothetical proteins. Once the function of these genes is characterized, genetic engineering approaches can be undertaken to repress transcription. The same is true for many of the genes differentially expressed in response to high concentrations of nitrate. A dramatic genetic expression difference was observed when the growth of MR-1 was compared on a low versus a high nitrate concentration. Many of these genes belong to the conserved hypothetical and hypothetical proteins category, which have not been characterized (its function is unknown). The elucidation of the role of these genes in the cell will explain the tolerance of MR-1 to some of this toxic metabolites and its unique metabolic versatility.

This study also identified genes that are directly or indirectly regulated by EtrA. Several studies have attempted to elucidate the role of EtrA in MR-1, however, since it does not act alone, its study is more complicated (1, 5, 7). Therefore, none of these studies prove direct regulation of EtrA on some of these genes. This can be achieved by cloning the promoter region of the candidate genes into a *lacZ* expression vector (2). This vector can be transformed in MR-1 wild type and in the MR-1 *etrA-* mutant. Expression of *lacZ* can then be compared to determine whether or not EtrA will bind the promoter affecting the transcription of this gene. An increase in the concentration of *lacZ*

transcripts in the strain lacking EtrA as compared to the wild type will indicate a repression role by EtrA for the gene regulated by the operon under examination. Whilst, increasing concentration of *lacZ* transcripts in the wild type as compared to the *etrA* mutant will represent an induction role by EtrA. Also, generation of double mutants defective in EtrA and NarP will determined the regulatory mechanism of the expression of the genes associated to the nitrate reduction pathway.

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APPENDIX A

Shewanella oneidensis MR-1 deletion mutants

Additional MR-1 deletion mutants

I encountered several obstacles in the generation of the deletion mutants presented in this work but fortunately I was able to solve them. Due to time limitations, I could not complete the final steps of construction of the ones described in this appendix. These mutations were generated as described in Chapters III and IV and the primer sequences used are provided (TABLE A.1).

Some of the difficulties encountered included non-specific insertion of the construct in the genome, replication of the suicide vector in MR-1 and contamination of the mutant with the wild type. To select the colonies with the insertion in the correct location in the genome, primers for PCR targeting the sequences upstream and downstream of the construct were designed. The size of the product of this reaction will indicate whether or not the construct replaced the gene of interest. To solve the replication of the suicide vector in MR-1 it was necessary to transfer the constructs to a plasmid that possessed a different origin. MR-1 can replicate plasmids that possess origins from pUC plasmids, which were present in the pCM184. The constructs were transferred to the pKNOCK-Gm plasmid as described in Chapters III and IV, which has an R6K origin that requires a π protein that is not present in MR-1. This vector worked as a suicide vector in MR-1. Once the constructs were introduced by conjugation in MR-1, candidate colonies for the deletion were screened by PCR. The PCR reaction revealed two fragments, one that showed removal of the gene and a band of the size of the wild type phenotype. Furthermore, sequencing attempts showed mixed product. These results indicated that the mutant colony was contaminated with the wild type. This could be due to the excessive production of exopolysaccharide (EPS), which has been described in

MR-1 as a protection against oxygen toxicity. The EPS protects the wild type from the antibiotic selection and it makes its elimination difficult. After many, many attempts to dilute out the wild type by serial liquid growth and plating, I could not succeed in recovering only the mutant. The mutants presented in this appendix were left at this point (TABLE A7.1). To solve this, these cultures need to be transfer three times on LB agar and screened by PCR. If no clean colonies (free of wild type) are selected, a colony needs to be transfer to LB broth, incubated overnight and then transfer three times on LB agar. These steps need to be repeated until a clean mutant can be selected. To prevent further undesirable mutations it is important that the colonies are transfer only three times on agar and then in LB broth. One idea to reduce EPS production might be to grow the mutant under microaerophilic conditions.

These mutants are located in the -80°C freezer in Rm545 of the Plant and Soil Sciences Building. The box is labeled as *Shewanella oneidensis* MR-1 deletion mutants.

SUPPLEMENTAL TABLE A.1. *Shewanella oneidensis* MR-1 deletion mutants and primer sequences.

	Primers used for construct	
MR-1 Mutant (Gene ID)	**N fragment forward primer**	**N fragment reverse primer**
MR-1 Δ*narP* (SO3982)	CGAATTCAGATTAACCATCAAGGAACC[a]	GCATATGATGTGTAGGCTCCTGTTG
MR-1 Δ*narQ* (SO3981)	CGAATTCATACATGTGGCCACGAGG	GCATATGTATGTCGAATACCTTGTG
MR-1 Δ*nrfA* (SO3980)	GCCGCGGCATCTCCAGCGCCAAAGT	CGAGCTCGCGTGTTTTCAAACTCGG
MR-1 Δ*napA*/Δ*nrfA* (SO0848)/(SO3980)	GCCGCGGCATCTCCAGCGCCAAAGT[b]	CGAGCTCGCGTGTTTTCAAACTCGG[b]
	C fragment forward primer	**C fragment reverse primer**
MR-1 Δ*narP* (SO3982)	GCCGCGGAATTAATGCTTAATACGTTAAC	CGAGCTCTCAAGCAAGTTATGATGAA
MR-1 Δ*narQ* (SO3981)	GCCGCGGGGGTAAACCTTATTCAGTT	CGAGCTCCTATTGCTTAAGCCTTCC
MR-1 Δ*nrfA* (SO3980)	CGAATTCGGCTTTTCTGCCGCGTTATCGT	GCATATGGTAATCATCCTTGAAGAT
MR-1 Δ*napA*/Δ*nrfA* (SO0848)/(SO3980)	CGAATTCGGCTTTTCTGCCGCGTTATCGT[b]	GCATATGGTAATCATCCTTGAAGAT[b]

[a] Primer sequences are written from the 5'end to the 3' end. Underlined sequences represent restriction enzymes recognition sites: GAATTC, EcoRI; CATATG, NdeI; GAATTC, SacI; CCGCGG, SacII.

[b] The MR-1 Δ*napA*/Δ*nrfA* double mutant was generated by deleting the *nrfA* gene in the MR-1 Δ*napA* deletion mutant reported in ChapterIII.

APPENDIX B

Supplemental Tables

SUPPLEMENTAL TABLE B.1. Genes induced in anaerobic cultures of MR-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	STD[b]	COG Annotation	Possible Function
SO0827	lldP	22.48	11.00	L-lactate permease	Transport and binding proteins
SO4302		21.56	12.00	conserved hypothetical protein	Hypothetical proteins
SO2194		21.33	6.85	OmpA family protein	Cell envelope
SO0849	napD	18.8	9.08	napD protein	Energy metabolism
SO2195		18.56	5.41	inter-alpha-trypsin inhibitor domain protein	Unknown function
SO4652	sbp	18.15	8.56	sulfate ABC transporter, periplasmic sulfate-binding protein	Transport and binding proteins
SO2263		17.26	10.84	Rrf2 family protein	Unknown function
SO1363	hcp	15.57	4.04	prismane protein	Energy metabolism
SO1926	gltA	14.24	5.58	citrate synthase	Energy metabolism
SO3738	cysJ	13.93	3.94	sulfite reductase (NADPH) flavoprotein alpha-component	Central intermediary metabolism
SO0902	nqrA-1	13.64	5.33	NADH:ubiquinone oxidoreductase, Na translocating, alpha subunit	Energy metabolism
SO0944		13.57	4.92	conserved hypothetical protein	Hypothetical proteins
SO2821		13.16	6.13	conserved hypothetical protein	Hypothetical proteins
SO3542		12.73	3.19	conserved hypothetical protein	Hypothetical proteins
SO2136	adhE	12.51	3.13	aldehyde-alcohol dehydrogenase	Energy metabolism
SO1646		12.05	2.97	GGDEF family protein	Unknown function
SO3728	cobA	11.88	3.58	uroporphyrin-III C-methyltransferase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1415		11.18	3.81	transcriptional regulator, TetR family	Regulatory functions
SO1871		11.13	6.92	S-adenosylmethionine decarboxylase proenzyme, putative	Central intermediary metabolism
SO0827	lldP	22.48	11.00	L-lactate permease	Transport and binding proteins
SO2264	iscS	10.95	2.15	cysteine desulfurase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4654	cysW-2	10.07	2.89	sulfate ABC transporter, permease protein	Transport and binding proteins
SO3553		9.97	2.32	sulfate permease family protein	Transport and binding proteins
SO4150		9.90	2.23	transporter, putative	Transport and binding proteins
SO4504		9.54	2.98	conserved hypothetical protein	Hypothetical proteins
SO1287		9.45	3.25	conserved hypothetical protein	Hypothetical proteins
SO0435	hemE	9.15	3.06	uroporphyrinogen decarboxylase	Biosynthesis of cofactors, prosthetic groups, and carriers

SO3245	flgF	9.09	2.30	flagellar basal-body rod protein FlgF	Cellular processes
SO4730	hemN	9.00	3.51	oxygen-independent coproporphyrinogen III oxidase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1364		8.71	2.22	iron-sulfur cluster-binding protein	Energy metabolism
SO4505		8.70	2.79	conserved hypothetical protein	Hypothetical proteins
SO0848	napA	8.62	4.86	periplasmic nitrate reductase	Energy metabolism
SO4334		8.61	3.64	inner membrane protein, putative	Cell envelope
SO2196		8.50	1.45	LPXTG-site transpeptidase family protein	Protein fate
SO2266		8.44	3.23	HesB/YadR/YfhF family protein	Unknown function
SO0277	argF	8.37	1.39	ornithine carbamoyltransferase	Amino acid biosynthesis
SO3727	cysD	8.33	3.00	sulfate adenylyltransferase, subunit 2	Central intermediary metabolism
SO2265		8.32	2.53	NifU family protein	Unknown function
SO0941		8.25	2.09	hypothetical protein	
SO4653	cysT-2	8.15	1.79	sulfate ABC transporter, permease protein	Transport and binding proteins
SO3901	icc	7.95	3.12	lacZ expression regulator	Regulatory functions
SO2267	hscB	7.89	3.31	co-chaperone Hsc20	Protein fate
SO2820		7.77	2.21	transposase, IS110 family, degenerate	Disrupted reading frame
SO0230	rpsJ	7.62	2.33	ribosomal protein S10	Protein synthesis
SO4656		7.59	2.84	hypothetical protein	
SO0581		7.55	2.36	hypothetical protein	
SO0449		7.53	1.11	conserved hypothetical protein	Hypothetical proteins
SO3229	fliE	7.49	1.13	flagellar hook-basal body complex protein FliE	Cellular processes
SO3726	cysN	7.3	1.87	sulfate adenylyltransferase, subunit 1	Central intermediary metabolism
SO3891		7.14	4.93	conserved hypothetical protein	Hypothetical proteins
ORF03631		7.05	2.75	hypothetical protein	
SO3737	cysI	7.01	1.53	sulfite reductase (NADPH) hemoprotein beta-component (cysI)	Central intermediary metabolism
SO0325		7.01	1.23	dsrE-related protein	Unknown function
SO1855	rmf	6.96	3.00	ribosome modulation factor	Protein synthesis
SO3507		6.88	1.14	conserved hypothetical protein	Hypothetical proteins
SOA0157		6.87	2.38	hypothetical protein	
SO1218	deoA	6.85	1.45	thymidine phosphorylase	Purines, pyrimidines, nucleosides, and nucleotides
SO2260	suhB	6.79	1.78	extragenic suppressor protein SuhB	Unknown function

SO4077		6.74	1.30	TonB-dependent receptor, putative	Transport and binding proteins
SO0837		6.70	1.82	beta-lactamase, putative	Cellular processes
SO1657		6.68	1.88	conserved hypothetical protein	Hypothetical proteins
SO0324		6.51	1.24	conserved hypothetical protein	Hypothetical proteins
SO0903	nqrB-1	6.49	1.93	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB	Energy metabolism
SO2743	acs	6.44	1.54	acetyl-coenzyme A synthetase	Energy metabolism
SO0007	rpmH	6.42	2.58	ribosomal protein L34	Protein synthesis
SO2857		6.33	1.71	sodium/solute symporter family protein	Transport and binding proteins
SO1911		6.27	2.38	oxidoreductase, short chain dehydrogenase/reductase family	Unknown function
SO1288	rpsU	6.22	1.08	ribosomal protein S21	Protein synthesis
SO3627		6.19	2.77	transcriptional regulator, TetR family	Regulatory functions
SO4651		6.12	5.86	conserved hypothetical protein	Hypothetical proteins
SO1300	hemL	6.05	1.36	glutamate-1-semialdehyde-2,1-aminomutase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2261		6.03	1.03	RNA methyltransferase, TrmH family, group 1	Protein synthesis
SO0940		5.95	1.28	transcriptional regulator-related protein	Unknown function
SO2903	cysK	5.94	2.23	cysteine synthase A	Amino acid biosynthesis
SO2193		5.93	3.22	DNA-binding response regulator	Signal transduction
SO1947		5.91	1.01	hypothetical protein	
SO3286	cydA	5.87	1.37	cytochrome d ubiquinol oxidase, subunit I	Energy metabolism
SO3940	rplM	5.86	1.86	ribosomal protein L13	Protein synthesis
SO1357	rpsP	5.86	1.81	ribosomal protein S16	Protein synthesis
SO4619	yhgI	5.80	1.31	yhgI protein	Unknown function
SO3059		5.78	0.90	formate hydrogenlyase transcriptional activator, putative	Regulatory functions
SO0241	rplN	5.67	1.32	ribosomal protein L14	Protein synthesis
SO1658		5.63	1.16	hypothetical protein	
SO4509		5.61	2.30	formate dehydrogenase, alpha subunit	Energy metabolism
SO0142	ribB	5.59	1.64	3,4-dihydroxy-2-butanone 4-phosphate synthase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1252		5.58	1.02	peptidase, U32 family	Protein fate

SO1359	trmD	5.54	1.39	tRNA (guanine-N1)-methyltransferase	Protein synthesis
SO4701		5.50	1.27	hypothetical protein	
SO0218	secE	5.48	1.27	preprotein translocase, SecE subunit	Protein fate
SO3250	flgB	5.46	6.74	flagellar basal-body rod protein FlgB	Cellular processes
SO1358	rimM	5.45	1.73	16S rRNA processing protein RimM	Transcription
SO2791	cdd	5.45	1.39	cytidine deaminase	Purines, pyrimidines, nucleosides, and nucleotides
SO0006	rnpA	5.43	1.76	ribonuclease P protein component	Transcription
SO0231	rplC	5.38	1.44	ribosomal protein L3	Protein synthesis
SO1516		5.35	2.44	hypothetical protein	
SO2402	rpsA	5.30	2.07	ribosomal protein S1	Protein synthesis
SO3720		5.29	1.09	conserved hypothetical protein	Hypothetical proteins
SO2403	cmk	5.29	1.39	cytidylate kinase	Purines, pyrimidines, nucleosides, and nucleotides
SO3939	rpsI	5.28	1.91	ribosomal protein S9	Protein synthesis
SO1301	pyrB	5.17	1.73	aspartate carbamoyltransferase	Purines, pyrimidines, nucleosides, and nucleotides
SO2305	lrp	5.16	1.63	leucine-responsive regulatory protein	Regulatory functions
SO0843		5.15	1.40	transcriptional regulator, LysR family	Regulatory functions
SO3085		5.15	2.76	conserved domain protein	Hypothetical proteins
SO0242	rplX	5.15	0.66	ribosomal protein L24	Protein synthesis
SOA0158		5.13	1.51	hypothetical protein	
SO4131		5.08	0.95	conserved hypothetical protein	Hypothetical proteins
SO0243	rplE	5.05	1.83	ribosomal protein L5	Protein synthesis
SO2192		4.99	3.03	sensor histidine kinase	Signal transduction
SO1806	pspF	4.98	1.18	psp operon transcriptional activator	Regulatory functions
SO0439		4.93	1.20	hypothetical protein	
SO2427		4.89	0.59	TonB-dependent receptor, putative	Transport and binding proteins
SO2632		4.88	1.24	hypothetical protein	
SO2785	rne	4.88	1.03	ribonuclease E	Transcription
SO4708		4.81	1.04	conserved hypothetical protein	Hypothetical proteins
SO0975		4.80	0.68	hypothetical protein	
SO0276	argB	4.80	0.62	acetylglutamate kinase	Amino acid biosynthesis
SO3047		4.77	0.98	conserved hypothetical protein	Hypothetical proteins
SO4510	fdhB-1	4.75	2.08	formate dehydrogenase, iron-sulfur subunit	Energy metabolism
SO2362	ccoQ	4.74	0.75	cytochrome c oxidase, cbb3-type, CcoQ subunit	Energy metabolism

SO4195	ccoO	4.74	1.11	PAP2 family protein	Unknown function
SO2363		4.73	0.69	cytochrome c oxidase, cbb3-type, subunit II	Energy metabolism
SO1560		4.70	0.78	phosphate-binding protein	Transport and binding proteins
SO2962		4.69	0.02	hypothetical protein	SO2962
SO3739		4.64	0.81	hypothetical protein	SO3739
SO0438		4.63	1.56	oxidoreductase, short chain dehydrogenase/reductase family	Unknown function
SO4034	deaD	4.62	1.42	ATP-dependent RNA helicase DeaD	Transcription
SO2268	hscA	4.60	1.24	chaperone protein HscA	Protein fate
SO2792		4.60	1.12	conserved hypothetical protein	Hypothetical proteins
SO3888		4.59	1.17	conserved hypothetical protein	Hypothetical proteins
SO4506		4.55	1.08	iron-sulfur cluster-binding protein	Energy metabolism
SO0279	argH	4.53	0.64	argininosuccinate lyase	Amino acid biosynthesis
SOA0058		4.51	1.02	hypothetical protein	
SO3417	slyD	4.50	1.12	peptidyl-prolyl cis-trans isomerase SlyD	Protein fate
SO0448		4.46	2.64	hypothetical protein	
SO4405	katG-2	4.44	1.06	catalase/peroxidase HPI	Cellular processes
SO4700		4.43	1.02	hypothetical protein	
SO0232	rplD	4.41	1.04	ribosomal protein L4	Protein synthesis
SO3629		4.40	0.85	expression activator-related protein	Unknown function
SO1360	rplS	4.38	0.95	ribosomal protein L19	Protein synthesis
SO1758		4.35	0.64	conserved hypothetical protein	Hypothetical proteins
SO4151		4.32	0.74	polysaccharide deacetylase family protein	Energy metabolism
SO3725		4.31	1.47	hypothetical protein	
SO4306	xerC	4.29	1.70	integrase/recombinase XerC	DNA metabolism
SO3395		4.28	1.25	hypothetical protein	
SO3652	rplU	4.27	1.60	ribosomal protein L21	Protein synthesis
SO2432		4.26	1.02	conserved hypothetical protein TIGR01033	Hypothetical proteins
SO0157		4.23	0.92	proton/glutamate symporter	Transport and binding proteins
SO0220	rplK	4.23	1.25	ribosomal protein L11	Protein synthesis
SO2963		4.22	0.95	prophage LambdaSo, major capsid protein, HK97 family	Other categories
SO3258		4.22	2.08	conserved hypothetical protein	Hypothetical proteins
SO0748		4.21	0.85	hypothetical protein	

SO0219	nusG	4.20	1.33	transcription antitermination protein NusG	Transcription
SO1673		4.19	0.67	outer membrane protein OmpW, putative	Cell envelope
SO4066		4.18	0.51	phosphoribosylaminoimidazole-succinocarboxamide synthase, putative	Purines, pyrimidines, nucleosides, and nucleotides
SO3046		4.16	0.80	methyl-accepting chemotaxis protein, authentic point mutation	Cellular processes
SO3024	trpA	4.16	1.73	tryptophan synthase, alpha subunit	Amino acid biosynthesis
SO1680		4.15	0.94	enoyl-CoA hydratase/isomerase family protein	Fatty acid and phospholipid metabolism
SO3834	hemA	4.15	0.96	glutamyl-tRNA reductase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2972		4.15	0.54	hypothetical protein	
SO2361	ccoP	4.13	0.45	cytochrome c oxidase, cbb3-type, subunit III	Energy metabolism
SO3554	purE	4.13	0.73	phosphoribosylaminoimidazole carboxylase, catalytic subunit	Purines, pyrimidines, nucleosides, and nucleotides
SO2806		4.13	0.74	conserved hypothetical protein	Hypothetical proteins
SO0858		4.12	1.03	sodium:alanine symporter family protein	Transport and binding proteins
SO3724		4.12	3.77	hypothetical protein	
SO4511		4.10	1.51	formate dehydrogenase, C subunit, putative	Energy metabolism
SO2876		4.10	0.70	hypothetical protein	
SO4650		4.08	0.74	conserved hypothetical protein	Hypothetical proteins
SO1219	deoB	4.07	0.39	phosphopentomutase	Purines, pyrimidines, nucleosides, and nucleotides
SO1628		4.06	0.98	hypothetical protein	
SO3736	cysH	4.06	0.59	phosphoadenosine phosphosulfate reductase	Central intermediary metabolism
SO1169		4.06	0.67	conserved hypothetical protein TIGR00246	Hypothetical proteins
SO2137		4.06	0.80	conserved hypothetical protein TIGR00427	Hypothetical proteins
SO0847	napG	4.05	1.28	iron-sulfur cluster-binding protein NapG	Energy metabolism
SO1049		4.05	1.09	acetyltransferase, GNAT family	Unknown function
SO2085	pheS	4.02	0.50	phenylalanyl-tRNA synthetase, alpha subunit	Protein synthesis
SO0811		4.01	3.25	inosine-uridine preferring nucleoside hydrolase family protein	Purines, pyrimidines, nucleosides, and nucleotides
SO0244	rpsN	4	0.74	ribosomal protein S14	Protein synthesis
SO0221	rplA	3.99	1.05	ribosomal protein L1	Protein synthesis
SO1181	miaB	3.98	0.47	tRNA-i(6)A37 modification enzyme MiaB	Hypothetical proteins
SO1277		3.97	0.83	proton-dependent oligopeptide transporter (POT) family protein	Transport and binding proteins

SO4408	bipA	3.96	1.11	virulence regulator BipA	Cellular processes
SO0245	rpsH	3.96	0.72	ribosomal protein S8	Protein synthesis
SO0233	rplW	3.95	1.03	ribosomal protein L23	Protein synthesis
SO3896		3.92	0.67	outer membrane porin, putative	Transport and binding proteins
SO4204	tatC	3.92	0.88	Sec-independent periplasmic protein translocation protein TatC	Protein fate
SO1789		3.92	0.40	conserved hypothetical protein	Hypothetical proteins
SO4655	cysA-2	3.91	0.69	sulfate ABC transporter, ATP-binding protein	Transport and binding proteins
SO2832		3.90	0.90	conserved hypothetical protein	Hypothetical proteins
SO4157		3.87	1.15	DNA-binding response regulator	Signal transduction
SO4720		3.87	1.46	ABC transporter, permease protein	Transport and binding proteins
SO3833	prfA	3.86	1.03	peptide chain release factor 1	Protein synthesis
SO4133	udp	3.85	1.06	uridine phosphorylase	Purines, pyrimidines, nucleosides, and nucleotides
SO0719		3.84	0.75	TonB-dependent receptor, putative	Transport and binding proteins
SO0919		3.84	0.50	serine transporter, putative	Transport and binding proteins
SO2587	hemB-1	3.83	1.05	delta-aminolevulinic acid dehydratase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1416		3.83	0.85	DNA-binding response regulator	Signal transduction
SO0728		3.82	0.88	conserved hypothetical protein	Hypothetical proteins
SO3113	tgt	3.81	0.55	queuine tRNA-ribosyltransferase	Protein synthesis
SO3000		3.77	1.10	conserved hypothetical protein	Hypothetical proteins
SO1788	miaE	3.77	0.70	tRNA-(MS[2]IO[6]A)-hydroxylase	Protein synthesis
SO4754	atpI	3.77	1.14	ATP synthase protein I	Energy metabolism
ORF04429		3.72	0.44	methyl-accepting chemotaxis protein	
SO0715		3.69	1.41	oxidoreductase, molybdopterin-binding	Unknown function
SO4452	moaA	3.68	1.56	molybdenum cofactor biosynthesis protein A	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4480	aldA	3.66	0.53	aldehyde dehydrogenase	Energy metabolism
SO4520		3.63	0.57	oxygen-independent coproporphyrinogen III oxidase, putative	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1576		3.62	0.65	glutathione S-transferase family protein	Central intermediary metabolism
SO3651	rpmA	3.62	1.44	ribosomal protein L27	Protein synthesis
SO1128		3.61	1.58	hypothetical protein	
SO1075		3.59	0.68	conserved hypothetical protein	Hypothetical proteins
SO3404		3.57	0.55	methyl-accepting chemotaxis protein	Cellular processes
SOA0156		3.56	1.09	hypothetical protein	

SO4155		3.56	0.86	sensor histidine kinase	Signal transduction
SO4753	atpB	3.55	0.75	ATP synthase F0, A subunit	Energy metabolism
SO1173		3.53	0.61	rare lipoprotein B	Cell envelope
SO2592	pyrD	3.53	0.44	dihydroorotate dehydrogenase	Purines, pyrimidines, nucleosides, and nucleotides
SO2964		3.51	0.94	ClpP protease family protein	Protein fate
SO0331		3.51	0.78	conserved hypothetical protein	Hypothetical proteins
SO3562		3.50	0.69	proton/glutamate symporter, putative	Transport and binding proteins
SO3723	cysC	3.45	1.11	adenylylsulfate kinase	Central intermediary metabolism
SO3986	lysC	3.45	0.68	aspartokinase III, lysine-sensitive	Amino acid biosynthesis
SOA0086		3.44	0.49	site-specific recombinase, resolvase family	DNA metabolism
SO2834	nrdD	3.44	1.17	anaerobic ribonucleoside-triphosphate reductase	Purines, pyrimidines, nucleosides, and nucleotides
SO1170		3.44	0.65	iojap domain protein	Unknown function
SO2112	rplY	3.44	1.19	ribosomal protein L25	Protein synthesis
SO2947		3.43	1.10	hypothetical protein	
SO0403		3.42	0.57	hypothetical protein	
SO3023	trpB	3.42	0.56	tryptophan synthase, beta subunit	Amino acid biosynthesis
SO2965		3.41	0.99	prophage LambdaSo, portal protein, HK97 family	Other categories
SO4451	moaC	3.41	0.47	molybdenum cofactor biosynthesis protein C	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1124		3.41	1.21	conserved hypothetical protein TIGR00011	Hypothetical proteins
SO3900		3.40	0.79	conserved hypothetical protein	Hypothetical proteins
SO4508		3.40	1.48	hypothetical protein	
SO4136		3.39	0.48	decarboxylase, pyridoxal-dependent	Unknown function
SO0870	panB	3.36	0.83	3-methyl-2-oxobutanoate hydroxymethyltransferase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1517		3.36	1.00	hypothetical protein	
SO3231	flrB	3.35	0.68	flagellar regulatory protein B	Signal transduction
SO2185	ppx	3.34	0.54	exopolyphosphatase	Central intermediary metabolism
SO0246	rplF	3.34	0.61	ribosomal protein L6	Protein synthesis
SO0222	rplJ	3.34	0.91	ribosomal protein L10	Protein synthesis
SO2364	ccoN	3.33	0.74	cytochrome c oxidase, cbb3-type, subunit I	Energy metabolism
SO4623		3.33	1.13	DNA-binding response regulator	Signal transduction
SO2905		3.32	0.44	O-methyltransferase, putative	Cellular processes

SO2831	ribA	3.31	0.61	GTP cyclohydrolase II	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4138		3.28	0.90	conserved hypothetical protein	Hypothetical proteins
SO1207	rpsO	3.28	0.66	ribosomal protein S15	Protein synthesis
SO1665	galU	3.28	0.68	UTP-glucose-1-phosphate uridylyltransferase	Cell envelope
SO3249	flgC	3.28	0.30	flagellar basal-body rod protein FlgC	Cellular processes
SO0486	nosD	3.27	0.71	copper ABC transporter, periplasmic copper-binding protein	Transport and binding proteins
SO1912	tesB	3.25	0.26	acyl-CoA thioesterase II	Fatty acid and phospholipid metabolism
SO2781		3.24	0.96	conserved hypothetical protein	Hypothetical proteins
SO0227	rpsG	3.23	0.87	ribosomal protein S7	Protein synthesis
SO2779	plsX	3.21	0.58	fatty acid/phospholipid synthesis protein PlsX	Fatty acid and phospholipid metabolism
SO3112	yajC	3.21	0.37	preprotein translocase, YajC subunit	Protein fate
ORF02714		3.21	0.60	phosphate binding protein	
SO2304	ald	3.20	0.70	alanine dehydrogenase, authentic point mutation	Energy metabolism
SO0228	fusA-1	3.19	0.75	translation elongation factor G	Protein synthesis
SO2633	trmU	3.19	0.55	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	Protein synthesis
SO2956		3.19	0.77	prophage LambdaSo, major tail protein V, putative	Other categories
SO0505		3.18	1.08	conserved domain protein	Hypothetical proteins
SO4053		3.17	0.63	methyl-accepting chemotaxis protein	Cellular processes
SO2099	hoxK	3.16	0.70	quinone-reactive Ni/Fe hydrogenase, small subunit precursor	Energy metabolism
SO4303		3.15	0.38	hypothetical protein	
SO1942		3.14	0.45	HDIG domain protein	Unknown function
SO1941	corA	3.14	0.86	magnesium and cobalt transport protein CorA	Transport and binding proteins
SO0150		3.13	0.37	lipoprotein, putative	Cell envelope
SO0935	nhaD	3.13	0.72	Na+/H+ antiporter	Transport and binding proteins
SO2846		3.12	0.92	conserved hypothetical protein	Hypothetical proteins
SO1168	mrdA	3.11	0.69	penicillin-binding protein 2	Cell envelope
SO0527		3.11	0.43	conserved hypothetical protein	Hypothetical proteins
SO4503		3.11	0.43	formate dehydrogenase accessory protein FdhD, putative	Energy metabolism
SO2299	thrS	3.11	0.69	threonyl-tRNA synthetase	Protein synthesis

SO4203	tatB	3.09	0.64	Sec-independent protein translocase protein TatB	Protein fate
SO1247		3.09	0.61	hypothetical protein	
SO2433	aspS	3.08	1.30	aspartyl-tRNA synthetase	Protein synthesis
SO4512		3.07	0.47	conserved hypothetical protein	Hypothetical proteins
SO2065	tyrP	3.07	0.78	tyrosine-specific transport protein	Transport and binding proteins
SO2912	pflB	3.06	0.88	formate acetyltransferase	Energy metabolism
SO2290		3.05	0.76	rhodanese domain protein	Unknown function
SO4422		3.05	0.43	siderophore receptor, putative, degenerate	Disrupted reading frame
SO0005		3.05	0.43	conserved hypothetical protein TIGR00278	Hypothetical proteins
SO3293	guaB	3.04	0.97	inosine-5-monophosphate dehydrogenase	Purines, pyrimidines, nucleosides, and nucleotides
SO0015	glyQ	3.04	0.30	glycyl-tRNA synthetase, alpha subunit	Protein synthesis
SO1251		3.03	0.71	ferredoxin, 4Fe-4S	Energy metabolism
SO0846	napH	3.03	1.09	iron-sulfur cluster-binding protein napH	Energy metabolism
SO0265		3.02	0.66	conserved hypothetical protein	Hypothetical proteins
SO0872	pcnB	3.02	0.67	polyA polymerase	Transcription
SO4123	argS	3.02	0.55	arginyl-tRNA synthetase	Protein synthesis
SO4466		3.01	1.02	methyl-accepting chemotaxis protein	Cellular processes
SO0028	trkH-2	3.00	0.44	potassium uptake protein TrkH	Transport and binding proteins
SO0558	smtA	3	0.74	smtA protein	Unknown function
SO3695	pyrC	2.99	0.49	dihydroorotase, homodimeric type	Purines, pyrimidines, nucleosides, and nucleotides
SO3837	prsA	2.99	0.78	ribose-phosphate pyrophosphokinase	Purines, pyrimidines, nucleosides, and nucleotides
SO2269		2.96	0.99	ferredoxin, 2Fe-2S	Energy metabolism
SO1783	feoA	2.96	0.50	ferrous iron transport protein A	Transport and binding proteins
SO0630	nosA	2.96	2.18	TonB-dependent receptor	Transport and binding proteins
SO2401	ihfB	2.94	0.57	integration host factor, beta subunit	DNA metabolism
SO4026		2.94	1.18	hypothetical protein	
SO1413		2.94	1.09	tetraheme cytochrome c, putative	Energy metabolism
SO1944		2.93	0.51	hypothetical protein	
SO0226	rpsL	2.92	0.95	ribosomal protein S12	Protein synthesis
SO1220		2.92	1.03	hypothetical protein	
SO1915		2.91	0.29	serine protease, subtilase family	Protein fate
SO0189		2.90	0.67	fibronectin type III domain protein	Unknown function
SO1141	carA	2.90	0.29	carbamoyl-phosphate synthase, small subunit	Purines, pyrimidines, nucleosides, and nucleotides

SO0500		2.89	0.95	methyl-accepting chemotaxis protein	Cellular processes
SO4722	mobA	2.89	0.71	molybdopterin-guanine dinucleotide biosynthesis protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1042		2.89	0.32	amino acid ABC transporter, ATP-binding protein	Transport and binding proteins
SO4047		2.86	0.37	cytochrome c family protein	Energy metabolism
SO2803		2.86	1.56	DNA internalization-related competence protein ComEC/Rec2	Cellular processes
SO3929	priB	2.85	1.66	primosomal replication protein n	DNA metabolism
SO0768		2.85	0.89	conserved hypothetical protein	Hypothetical proteins
SO2879	uraA	2.85	1.31	uracil permease	Transport and binding proteins
SO1883		2.85	1.18	acetyltransferase, GNAT family	Unknown function
SO2634		2.85	0.36	conserved hypothetical protein	Hypothetical proteins
SO2610		2.84	0.69	hydrolase, TatD family	Unknown function
SO0595		2.84	0.69	hypothetical protein	
SO4030	uvrA	2.83	0.61	excinuclease ABC, A subunit	DNA metabolism
SO4200		2.83	0.38	conserved hypothetical protein	Hypothetical proteins
SO0045		2.83	0.84	Rrf2 family protein	Unknown function
SO0165	gspC	2.82	0.43	general secretion pathway protein C	Protein fate
SO4447		2.81	0.61	molybdenum ABC transporter, permease protein	Transport and binding proteins
SO2759	upp	2.81	0.33	uracil phosphoribosyltransferase	Purines, pyrimidines, nucleosides, and nucleotides
SO1916		2.80	0.34	transcriptional regulator, LysR family	Regulatory functions
SO3590		2.80	1.15	hypothetical protein	
SO1142	carB	2.79	1.09	carbamoyl-phosphate synthase, large subunit	Purines, pyrimidines, nucleosides, and nucleotides
SO3307		2.79	1.69	hypothetical protein	
SO2913	pflA	2.79	0.95	pyruvate formate-lyase 1 activating enzyme	Energy metabolism
SO0842	fusA-2	2.79	0.87	translation elongation factor G	Protein synthesis
SO4202	tatA	2.78	0.34	Sec-independent protein translocase protein TatA	Protein fate
SO4719		2.78	0.61	conserved hypothetical protein	Hypothetical proteins
SO3930	rpsF	2.78	0.42	ribosomal protein S6	Protein synthesis
SO0447		2.78	0.65	hypothetical protein	
SO1989	cheV-1	2.77	0.74	chemotaxis protein CheV	Cellular processes

SO4752	atpE	2.77	0.73	ATP synthase F0, C subunit	Energy metabolism
ORF02653		2.77	0.40	processing protease, putative	
SO4538		2.76	0.43	peptidase, M16 family, degenerate	Disrupted reading frame
SO0394		2.75	0.72	conserved hypothetical protein	Hypothetical proteins
SO2724		2.75	0.54	protease, putative	Protein fate
SO2451		2.74	0.47	conserved hypothetical protein	Hypothetical proteins
SO0223	rplL	2.74	0.70	ribosomal protein L7/L12	Protein synthesis
SO3928	rpsR	2.73	0.73	ribosomal protein S18	Protein synthesis
SO2129		2.72	0.64	transposase, putative	Other categories
SO4507		2.72	0.59	TorA specific chaperone, putative	Energy metabolism
SO4313	hemC	2.72	0.40	porphobilinogen deaminase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2490		2.71	0.62	transcriptional regulator, RpiR family	Regulatory functions
SO0247	rplR	2.71	0.31	ribosomal protein L18	Protein synthesis
SO1211	prfC	2.70	0.50	peptide chain release factor 3, authentic frameshift	Protein synthesis
SO4124		2.70	0.90	cell division protein FtsN, putative	Cellular processes
SO0396	frdC	2.70	0.23	fumarate reductase cytochrome B subunit	Energy metabolism
SO2840		2.70	0.91	conserved hypothetical protein	Hypothetical proteins
SO3111	secD-2	2.70	0.59	protein-export membrane protein SecD	Protein fate
SO0249	rpmD	2.70	0.91	ribosomal protein L30	Protein synthesis
SO0234	rplB	2.69	0.62	ribosomal protein L2	Protein synthesis
SO2858		2.68	1.32	conserved hypothetical protein	Hypothetical proteins
SO2223		2.68	1.81	peptidase, putative	Protein fate
SO3722		2.68	0.71	conserved hypothetical protein	Hypothetical proteins
SO3751		2.68	2.58	hypothetical protein	
SO4199	ubiE	2.67	0.67	ubiquinone/menaquinone biosynthesis methlytransferase UbiE	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4304		2.67	1.12	conserved hypothetical protein	Hypothetical proteins
SO1640	fabZ	2.66	0.24	(3R)-hydroxymyristoyl-(acyl-carrier-protein) dehydratase	Fatty acid and phospholipid metabolism
SO4314	hemD	2.66	0.43	uroporphyrinogen-III synthase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1490	adhB	2.64	0.85	alcohol dehydrogenase II	Energy metabolism
SO4335		2.64	0.81	phosphatidylglycerophosphatase B, putative	Fatty acid and phospholipid metabolism
SO2176		2.63	1.16	conserved hypothetical protein	Hypothetical proteins

SO1696		2.63	1.31	hypothetical protein	
SO2636		2.63	0.50	conserved hypothetical protein	Hypothetical proteins
SO2635	purB	2.62	0.41	adenylosuccinate lyase	Purines, pyrimidines, nucleosides, and nucleotides
SO0251	secY	2.62	0.57	preprotein translocase, SecY subunit	Protein fate
SO2043		2.62	0.31	conserved hypothetical protein	Hypothetical proteins
SO0729		2.62	1.98	hypothetical protein	
SO2469		2.62	0.82	conserved hypothetical protein	Unknown function
SO0526		2.61	1.19	acetyltransferase, GNAT family	Unknown function
SO1683		2.61	0.32	3-oxoacyl-(acyl-carrier-protein) reductase, putative	Fatty acid and phospholipid metabolism
SO3892		2.61	0.61	hypothetical protein	
SO3719		2.6	1.03	hypothetical protein	
SO2780	rpmF	2.60	1.55	ribosomal protein L32	Protein synthesis
SO3285	cydB	2.60	0.56	cytochrome d ubiquinol oxidase, subunit II	Energy metabolism
SO0014	glyS	2.60	0.40	glycyl-tRNA synthetase, beta subunit	Protein synthesis
SO2833	nrdG	2.58	0.06	anaerobic ribonucleoside-triphosphate reductase activating protein	Purines, pyrimidines, nucleosides, and nucleotides
SO0224	rpoB	2.57	0.81	DNA-directed RNA polymerase, beta subunit	Transcription
ORF00995		2.57	0.51	hypothetical protein	
SO2328	efp	2.57	0.66	translation elongation factor P	Protein synthesis
SO2969		2.57	0.61	prophage LambdaSo, holin, putative	Other categories
SO0027		2.56	0.62	protoporphyrinogen oxidase, putative	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4721		2.56	0.70	ABC transporter, ATP-binding protein	Transport and binding proteins
SO3600	cysT-1	2.56	0.49	sulfate ABC transporter, permease protein	Transport and binding proteins
SO3068	truA	2.56	0.31	tRNA pseudouridine synthase A	Protein synthesis
SO1641	lpxA	2.56	0.68	acyl-(acyl-carrier-protein)--UDP-N-acetylglucosamine O-acyltransferase	Cell envelope
SO3008		2.56	0.48	hypothetical protein	
SO2461		2.55	0.84	surface protein, putative, truncation	Disrupted reading frame
SO3931		2.55	0.53	conserved hypothetical protein	Hypothetical proteins
SO3902		2.54	0.77	conserved hypothetical protein	Hypothetical proteins
SO3009		2.53	1.58	hypothetical protein	
SO1934		2.51	1.03	hypothetical protein	
SO2411	gyrA	2.51	1.32	DNA gyrase, A subunit	DNA metabolism

SO2394	dacB	2.50	0.28	penicillin-binding protein 4	Cell envelope
SO0266	ccmF-1	2.50	0.42	cytochrome c-type biogenesis protein CcmF	Energy metabolism
SO0807	hpt-1	2.50	0.73	hypoxanthine-guanine phosphoribosyltransferase	Purines, pyrimidines, nucleosides, and nucleotides
SO0008	dnaA	2.49	0.27	chromosomal replication initiator protein DnaA	DNA metabolism
SO0248	rpsE	2.48	0.56	ribosomal protein S5	Protein synthesis
SO3237		2.48	0.90	flagellin	Cellular processes
SO3733		2.47	0.61	hypothetical protein	
SO2973		2.47	0.95	prophage LambdaSo, lysozyme, putative	Other categories
SO4072	yliG	2.47	0.24	MiaB-like putative RNA modifying enzyme YliG	Hypothetical proteins
SO4417	dcuB	2.47	0.67	anaerobic C4-dicarboxylate membrane transporter	Transport and binding proteins
SO0090		2.46	0.78	hypothetical protein	
SO4450	moaD	2.46	0.57	molybdenum cofactor biosynthesis protein D	Biosynthesis of cofactors, prosthetic groups, and carriers
SO3538	hlyU	2.46	0.42	transcriptional regulator HlyU	Regulatory functions
SO0922		2.46	0.37	proton/glutamate symporter	Transport and binding proteins
SO1526	ispA	2.45	0.40	geranyltranstransferase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2092	hypC	2.45	0.83	hydrogenase assembly chaperone hypC/hupF	Protein fate
SO3244	flgG	2.44	0.44	flagellar basal-body rod protein FlgG	Cellular processes
SO1790	ppiB-1	2.44	0.26	peptidyl-prolyl cis-trans isomerase B	Protein fate
SO2805		2.43	0.43	conserved hypothetical protein	Hypothetical proteins
SO1518		2.43	0.95	conserved hypothetical protein	Hypothetical proteins
SO1682	mmsB	2.43	0.55	3-hydroxyisobutyrate dehydrogenase	Energy metabolism
SO4622		2.43	0.69	sensor histidine kinase	Signal transduction
SO2179		2.42	0.38	hypothetical protein	
SO0393	fis	2.42	0.34	DNA-binding protein Fis	Regulatory functions
SO0869	panC	2.40	0.74	pantoate--beta-alanine ligase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0098	hutH	2.40	0.38	histidine ammonia-lyase	Energy metabolism
SO3927	rplI	2.40	0.28	ribosomal protein L9	Protein synthesis
SO0152		2.39	0.49	conserved hypothetical protein	Hypothetical proteins
SO3411		2.39	0.34	protease, putative	Protein fate
SO3214		2.39	0.50	hypothetical protein	

SO2684		2.39	0.82	prophage MuSo2, protein Gp32, putative	Other categories
SO4412		2.38	0.62	conserved domain protein	Hypothetical proteins
SO4385		2.38	0.42	von Willebrand factor type A domain protein	Unknown function
SO1527	xseB	2.38	0.56	exodeoxyribonuclease VII, small subunit	DNA metabolism
SO2778	fabH-1	2.38	0.45	3-oxoacyl-(acyl-carrier-protein) synthase III	Fatty acid and phospholipid metabolism
SO0514		2.37	0.37	hypothetical protein	
SO3740	pntA	2.37	0.60	NAD(P) transhydrogenase, alpha subunit	Energy metabolism
SO2332		2.36	0.42	conserved hypothetical protein	Hypothetical proteins
SO4246	rpmG	2.36	0.67	ribosomal protein L33	Protein synthesis
SO4205		2.36	0.36	hypothetical protein	
SO4255	pyrE	2.35	0.38	orotate phosphoribosyltransferase	Purines, pyrimidines, nucleosides, and nucleotides
SO0250	rplO	2.35	0.33	ribosomal protein L15	Protein synthesis
SO4048		2.35	0.31	cytochrome c family protein	Energy metabolism
SO2926		2.33	0.47	ABC transporter, permease, putative	Transport and binding proteins
SO1221	deoD-2	2.33	0.40	purine nucleoside phosphorylase	Purines, pyrimidines, nucleosides, and nucleotides
SO0515		2.33	0.91	hypothetical protein	
SO4194		2.33	0.76	conserved hypothetical protein	Hypothetical proteins
SO3014		2.33	0.24	conserved hypothetical protein	Hypothetical proteins
SO0553		2.33	0.71	hypothetical protein	
ORF04321		2.32	0.43	glutamyl-tRNA synthetase (gltX)	
SO2750	tolR	2.32	0.46	tolr protein	Transport and binding proteins
SO3699		2.32	0.30	hypothetical protein	
SO0885		2.32	0.17	ABC transporter, ATP-binding protein	Transport and binding proteins
SOA0126		2.32	0.31	transposase, IS110 family, degenerate	Other categories
SO2295		2.32	0.21	MATE efflux family protein	Transport and binding proteins
SO4254	folE	2.31	0.53	GTP cyclohydrolase I	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0567	plsC	2.31	0.23	1-acyl-sn-glycerol-3-phosphate acyltransferase	Fatty acid and phospholipid metabolism
SO2502		2.31	0.63	hypothetical protein	
SO2008		2.30	0.77	conserved hypothetical protein	Hypothetical proteins
SO1328		2.3	0.46	transcriptional regulator, LysR family	Regulatory functions
SO2302	rplT	2.3	0.73	ribosomal protein L20	Protein synthesis
SO4446		2.30	0.48	molybdenum ABC transporter, ATP-binding protein	Transport and binding proteins

SO3565	cpdB	2.30	0.41	2,3-cyclic-nucleotide 2-phosphodiesterase	Purines, pyrimidines, nucleosides, and nucleotides
SO4413		2.30	0.44	conserved hypothetical protein	Hypothetical proteins
SO4628		2.30	0.35	sulfatase	Unknown function
SO1867		2.29	0.53	conserved hypothetical protein	Hypothetical proteins
SO1882		2.28	0.86	AcrB/AcrD/AcrF family protein	Transport and binding proteins
SO1202		2.28	0.51	conserved hypothetical protein	Hypothetical proteins
SO3657		2.28	0.33	transporter, LysE family	Transport and binding proteins
SO2884		2.28	0.68	conserved hypothetical protein	Hypothetical proteins
SOA0007		2.28	0.54	hypothetical protein	
SO2390	pssA	2.27	0.39	CDP-diacylglycerol--serine O-phosphatidyltransferase	Fatty acid and phospholipid metabolism
SO4502		2.26	0.43	conserved domain protein	Hypothetical proteins
SO0741	ggt-1	2.25	0.43	gamma-glutamyltranspeptidase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1522		2.25	0.47	L-lactate permease, putative	Transport and binding proteins
SO4713		2.25	0.56	menaquinone-specific isochorismate synthase, putative	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1639	lpxD	2.25	0.33	UDP-3-O-(3-hydroxymyristoyl) glucosamine n-acyltransferase	Cell envelope
SO3694		2.25	0.33	conserved hypothetical protein	Hypothetical proteins
SO0467	uvrD	2.25	0.32	DNA helicase II	DNA metabolism
SO3106	aprE	2.24	0.79	cold-active serine alkaline protease	Protein fate
SO3832		2.23	0.74	hemK family protein	Unknown function
SO4353		2.23	0.32	hypothetical protein	
SO3874		2.23	0.61	transcriptional regulator, LysR family	Regulatory functions
SO3401		2.22	0.47	conserved hypothetical protein TIGR01033	Hypothetical proteins
SO2993		2.22	0.36	prophage LambdaSo, type II DNA modification methyltransferase, putative, truncation	DNA metabolism
SO1303		2.22	0.34	hypothetical protein	
SO2830		2.22	0.47	hypothetical protein	
SO3001		2.21	1.23	hypothetical protein	
SO2012	apt	2.21	0.28	adenine phosphoribosyltransferase	Purines, pyrimidines, nucleosides, and nucleotides
SO1777	mtrA	2.21	0.35	decaheme cytochrome c MtrA	Energy metabolism
SO4201	aarF	2.21	0.17	ubiquinone biosynthesis protein AarF	Biosynthesis of cofactors, prosthetic groups, and carriers

SO3067	folC	2.21	0.35	FolC bifunctional protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0360	rpoZ	2.20	0.58	DNA-directed RNA polymerase, omega subunit	Transcription
SO4134		2.20	0.93	conserved hypothetical protein	Hypothetical proteins
SO1910		2.20	0.47	1,4-dihydroxy-2-naphthoate octaprenyltransferase, putative	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2218	asnS	2.19	0.64	asparaginyl-tRNA synthetase	Protein synthesis
SO3230	flrC	2.19	0.30	flagellar regulatory protein C	Signal transduction
SO3613	purT	2.19	0.48	phosphoribosylglycinamide formyltransferase	Purines, pyrimidines, nucleosides, and nucleotides
SO0301		2.19	0.27	conserved hypothetical protein TIGR00096	Hypothetical proteins
SO2251		2.19	0.40	conserved hypothetical protein	Hypothetical proteins
SO4296		2.18	0.54	NupC family protein	Transport and binding proteins
SO0287	aroB	2.18	0.22	3-dehydroquinate synthase	Amino acid biosynthesis
SO4418		2.17	0.56	trypanothione synthetase domain protein	Unknown function
SO1642	lpxB	2.17	0.40	lipid A disaccharide synthase	Cell envelope
SO1687		2.17	0.57	transcriptional regulator, MerR family	Regulatory functions
SO2883		2.17	0.79	conserved hypothetical protein	Hypothetical proteins
ORF04796		2.17	0.51	component of sodium-driven polar flagellar motor (motY)	
SO1361	aroF	2.17	0.40	phospho-2-dehydro-3-deoxyheptonate aldolase, tyr-sensitive	Amino acid biosynthesis
SO2467		2.17	0.51	adhesion-related protein	Unknown function
SO0904	nqrC-1	2.16	0.50	NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit	Energy metabolism
SO1053		2.16	0.48	conserved hypothetical protein	Hypothetical proteins
SO4448		2.16	0.26	molybdenum ABC transporter, periplasmic molybdenum-binding protein	Transport and binding proteins
SO0235	rpsS	2.16	0.99	ribosomal protein S19	Protein synthesis
SO2098	hyaB	2.15	0.72	quinone-reactive Ni/Fe hydrogenase, large subunit	Energy metabolism
SO2652		2.15	0.30	prophage MuSo2, transcriptional regulator, Cro/CI family	Regulatory functions
SO1769		2.15	0.41	glutamate decarboxylase, putative	Central intermediary metabolism
SO1648		2.15	0.62	cold shock domain family protein	Cellular processes
SO2755	rnt	2.14	0.59	ribonuclease T	Transcription

SO1223	serB	2.14	0.76	phosphoserine phosphatase	Amino acid biosynthesis
SO3460		2.14	0.33	transcriptional regulator, LysR family	Regulatory functions
SO0046		2.13	0.60	conserved hypothetical protein	Hypothetical proteins
SO0288		2.13	0.30	damX domain protein	Unknown function
SO3154	proS	2.13	0.38	prolyl-tRNA synthetase	Protein synthesis
SO0330		2.13	0.48	conserved hypothetical protein	Hypothetical proteins
SO1802	sapD	2.13	0.39	peptide ABC transporter, ATP-binding protein	Transport and binding proteins
SO0564		2.13	0.24	conserved hypothetical protein	Hypothetical proteins
SO1793	tig	2.12	0.29	trigger factor	Protein fate
SO1637		2.12	0.45	bacterial surface antigen	Cell envelope
SO0286	aroK	2.12	0.46	shikimate kinase	Amino acid biosynthesis
SO1315	tyrS	2.10	0.45	tyrosyl-tRNA synthetase	Protein synthesis
SO3954		2.10	0.31	ABC transporter, ATP-binding protein, putative	Transport and binding proteins
SO3768		2.09	0.34	ion transporter	Transport and binding proteins
SO2842		2.09	0.64	peptidase, M23/M37 family	Protein fate
ORF02163		2.09	0.27	transposase for insertion sequence element is904 (orfB)	
SO2060		2.09	1.59	hypothetical protein	
SO0590	psd	2.09	0.29	phosphatidylserine decarboxylase, authentic frameshift	
ORF00969		2.08	0.41	hypothetical protein	
SO3142	dcp-1	2.08	0.66	peptidyl-dipeptidase Dcp	Protein fate
SO2262	cysE	2.08	0.27	serine acetyltransferase	Amino acid biosynthesis
SO1643	rnhB	2.08	0.41	ribonuclease HII	Transcription
SO4751	atpF	2.08	0.61	ATP synthase F0, B subunit	Energy metabolism
SO3253		2.07	0.49	flagellar basal-body P-ring formation protein FlgA, putative	Cellular processes
SO0359	spoT	2.07	0.61	guanosine-3,5-bis(diphosphate) 3-pyrophosphohydrolase	Cellular processes
SO3601	cysW-1	2.07	0.48	sulfate ABC transporter, permease protein	Transport and binding proteins
SO4178		2.07	0.32	conserved hypothetical protein	Hypothetical proteins
SO0252	rpmJ	2.06	0.88	ribosomal protein L36	Protein synthesis

SO1544		2.06	0.58	hypothetical protein	
SO3025		2.06	0.24	conserved hypothetical protein	Hypothetical proteins
SO4247	rpmB	2.06	0.90	ribosomal protein L28	Protein synthesis
SO0207	murI	2.06	0.29	glutamate racemase	Cell envelope
SO0487	nosF	2.05	0.31	copper ABC transporter, ATP-binding protein	Transport and binding proteins
SO3533	ribF	2.05	0.43	riboflavin biosynthesis protein RibF	Biosynthesis of cofactors, prosthetic groups, and carriers
SO3025		2.06	0.24	conserved hypothetical protein	Hypothetical proteins
SO4247	rpmB	2.06	0.90	ribosomal protein L28	Protein synthesis
SO0207	murI	2.06	0.29	glutamate racemase	Cell envelope
SO0487	nosF	2.05	0.31	copper ABC transporter, ATP-binding protein	Transport and binding proteins
SO3533	ribF	2.05	0.43	riboflavin biosynthesis protein RibF	Biosynthesis of cofactors, prosthetic groups, and carriers
SO3025		2.06	0.24	conserved hypothetical protein	Hypothetical proteins
SO4247	rpmB	2.06	0.90	ribosomal protein L28	Protein synthesis
SO0207	murI	2.06	0.29	glutamate racemase	Cell envelope
SO0487	nosF	2.05	0.31	copper ABC transporter, ATP-binding protein	Transport and binding proteins
SO3533	ribF	2.05	0.43	riboflavin biosynthesis protein RibF	Biosynthesis of cofactors, prosthetic groups, and carriers
SO3025		2.06	0.24	conserved hypothetical protein	Hypothetical proteins
SO4247	rpmB	2.06	0.90	ribosomal protein L28	Protein synthesis
SO0207	murI	2.06	0.29	glutamate racemase	Cell envelope
SO0487	nosF	2.05	0.31	copper ABC transporter, ATP-binding protein	Transport and binding proteins
SO3533	ribF	2.05	0.43	riboflavin biosynthesis protein RibF	Biosynthesis of cofactors, prosthetic groups, and carriers
SO3025		2.06	0.24	conserved hypothetical protein	Hypothetical proteins
SO4247	rpmB	2.06	0.90	ribosomal protein L28	Protein synthesis
SO0207	murI	2.06	0.29	glutamate racemase	Cell envelope
SO0487	nosF	2.05	0.31	copper ABC transporter, ATP-binding protein	Transport and binding proteins
SO3533	ribF	2.05	0.43	riboflavin biosynthesis protein RibF	Biosynthesis of cofactors, prosthetic groups, and carriers
SOA0117		2.05	0.51	hypothetical protein	
SO1184	pth	2.05	0.39	peptidyl-tRNA hydrolase	Protein synthesis
SO3750		2.05	0.29	peptidase, M16 family, authentic frameshift	Protein fate
SO4755		2.04	0.40	ParB family protein	Cellular processes
SO2491	pykA	2.04	0.36	pyruvate kinase II	Energy metabolism
SO3282		2.04	0.42	methyl-accepting chemotaxis protein	Cellular processes
SO1389		2.03	0.64	ROK family protein	Unknown function

SO1791	cysS	2.03	0.63	cysteinyl-tRNA synthetase	Protein synthesis
SO1107	nqrE-2	2.03	0.30	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE	Energy metabolism
SO1304		2.03	0.30	HesB/YadR/YfhF family protein	Unknown function
SO2998		2.03	0.42	hypothetical protein	
SO0632	hrpB	2.02	0.88	ATP-dependent helicase HrpB	Unknown function
SO1035	cobT	2.02	0.39	nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1052	pit	2.01	0.51	low-affinity inorganic phosphate transporter	Transport and binding proteins
SO3524	pilE	2.01	0.17	type IV pilus biogenesis protein PilE	Cell envelope
SO2856		2.01	1.00	CBS domain protein	Unknown function
SO4613		2.01	0.25	cytochrome oxidase assembly protein, putative	Energy metabolism
SO3022	trpC/F	2.00	0.71	indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase	Amino acid biosynthesis
SO1940		2.00	0.25	hypothetical protein	
SO2887	dsbB	2.00	0.65	disulfide bond formation protein b	Protein fate
SO2301	rpmI	2.00	0.63	ribosomal protein L35	Protein synthesis

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures of MR-1 grown at 40 mM KNO_3 to that of the anaerobically grown at 1 mM KNO_3 (reference).

[b] The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

SUPPLEMENTAL TABLE B.2. Genes repressed in anaerobic cultures of MR-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	STD[b]	COG Annotation	Possible Function
SO2277	ibpA	0.03	0.03	16 kDa heat shock protein A	Protein fate
SO3817	panE	0.03	0.05	2-dehydropantoate 2-reductase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1590		0.04	0.02	hypothetical protein	
SO0367		0.04	0.05	conserved hypothetical protein	Hypothetical proteins
SO4349	ilvC	0.04	0.02	ketol-acid reductoisomerase	Amino acid biosynthesis
SO1592		0.05	0.03	hypothetical protein	
SO1070	katB	0.05	0.05	catalase	Cellular processes
SO3381		0.05	0.06	conserved hypothetical protein	Hypothetical proteins
SO3348	hemH-2	0.05	0.06	ferrochelatase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0443		0.06	0.03	transcriptional regulator, MerR family	Regulatory functions
SO1126	dnaK	0.07	0.02	chaperone protein DnaK	Protein fate
SO4169		0.07	0.10	conserved hypothetical protein	Hypothetical proteins
SO3683		0.07	0.01	coniferyl aldehyde dehydrogenase	Energy metabolism
SO1158		0.07	0.75	Dps family protein	Cellular processes
SO3384	phrB	0.07	0.05	deoxyribodipyrimidine photolyase	DNA metabolism
SO0958	ahpC	0.08	0.05	alkyl hydroperoxide reductase, C subunit	Cellular processes
SO1589		0.08	0.03	hypothetical protein	
SO3787		0.08	0.02	hypothetical protein	
SO3343		0.08	0.03	conserved hypothetical protein	Hypothetical proteins
SO0886		0.08	0.05	conserved hypothetical protein	Hypothetical proteins
SO3385		0.08	0.34	transcriptional regulator, MerR family	Regulatory functions
SO3101		0.08	0.03	conserved hypothetical protein	Hypothetical proteins
SO1588		0.08	0.03	hypothetical protein	
SO0857		0.08	0.03	ABC transporter, ATP-binding protein	Transport and binding proteins
SO4473		0.09	0.06	outer membrane protein, putative	Cell envelope
SO0406	trxA	0.09	0.05	thioredoxin 1	Energy metabolism
SO0771		0.09	0.03	conserved hypothetical protein	Hypothetical proteins

SO0956	ahpF	0.09	0.05	alkyl hydroperoxide reductase, F subunit	Hypothetical proteins
SO4312		0.09	0.06	adenylate cyclase CyaA, putative	Cellular processes
SO0753		0.09	0.05	hypothetical protein	Regulatory functions
SO1274		0.09	0.03	conserved hypothetical protein	
SO1667		0.10	0.01	pterin-4-alpha-carbinolamine dehydratase	Hypothetical proteins
SO0452	trxC	0.11	0.05	thioredoxin 2	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2772		0.11	0.06	cytosolic long-chain acyl-CoA thioester hydrolase family protein	Energy metabolism
SO2045		0.11	0.04	cation efflux family protein	Fatty acid and phospholipid metabolism
SO2850		0.11	0.05	acetyltransferase, GNAT family	Transport and binding proteins
SO2773		0.11	0.20	hypothetical protein	Unknown function
SO3586		0.12	0.04	glyoxalase family protein	
SO3577	clpB	0.12	0.02	clpB protein	Unknown function
SO0957		0.12	0.07	hypothetical protein	Protein fate
SO4348		0.12	0.05	hypothetical protein	
SO4740		0.12	0.05	conserved hypothetical protein	
SO0698	fsxA	0.12	0.19	fxsA protein	Hypothetical proteins
SO0444		0.12	0.03	hypothetical protein	Unknown function
SO1264		0.12	0.04	conserved hypothetical protein	
SO3786		0.12	0.02	hypothetical protein	Hypothetical proteins
SO1899		0.12	0.04	conserved hypothetical protein	
SO3382		0.12	0.31	oxidoreductase, short-chain dehydrogenase/reductase family	Hypothetical proteins
SO3660		0.13	0.02	sigma-54 dependent transcriptional regulator/sensory box protein	Unknown function
SO3349		0.13	0.79	glutathione peroxidase, putative	Regulatory functions
SO1786	glnS	0.13	0.04	glutaminyl-tRNA synthetase	Cellular processes
SO0554		0.13	0.07	hypothetical protein	Protein synthesis
SO0322		0.14	0.06	hypothetical protein	
SO3488		0.14	0.02	transcriptional regulator, AraC/XylS family	
SO1609	syd	0.14	0.16	syd protein	Regulatory functions
SO2228		0.14	0.07	CBS domain protein	Unknown function
SO4280		0.14	0.04	hypothetical protein	Unknown function

SO2863		0.14	0.03	conserved hypothetical protein	Hypothetical proteins
SO0911		0.14	0.14	ParA family protein, degenerate	Unknown function
SO3684		0.15	0.02	transcriptional regulator, TetR family	Regulatory functions
SO2046		0.15	0.04	transcriptional regulator, MarR family	Regulatory functions
SO4647		0.15	0.04	DNA-binding response regulator	Signal transduction
SO3383		0.16	0.70	transcriptional regulator-related protein	Unknown function
SO1607		0.16	0.04	transcriptional regulator, LysR family	Regulatory functions
SO1267		0.16	0.03	conserved hypothetical protein	Hypothetical proteins
SO1268		0.17	0.03	glutamine synthetase	Amino acid biosynthesis
SO2447		0.17	0.04	channel protein, hemolysin III family subfamily	Protein fate
SO1669	tyrR	0.17	0.04	transcriptional regulatory protein TyrR	Regulatory functions
SO2754	motY	0.17	0.07	sodium-type flagellar protein MotY, authentic frameshift	Cellular processes
SO4126		0.17	0.06	hypothetical protein	
SO4070		0.17	0.05	conserved hypothetical protein	Hypothetical proteins
SO0274	ppc	0.17	0.04	phosphoenolpyruvate carboxylase	Energy metabolism
SO4598		0.17	0.05	heavy metal efflux pump, CzcA family	Transport and binding proteins
SO3585		0.17	0.06	azoreductase, putative	Cellular processes
SOA0062		0.17	0.05	hypothetical protein	
SO4170		0.18	0.04	C-factor, putative	Cellular processes
ORF02463		0.18	0.07	hypothetical protein	
SO3403	yfiA-1	0.18	0.05	ribosomal subunit interface protein	Protein synthesis
SO3715		0.18	0.12	oxygen-insensitive NAD(P)H nitroreductase	Unknown function
SO3019	trpE	0.18	0.05	anthranilate synthase component I	Amino acid biosynthesis
SO0552		0.19	0.04	hypothetical protein	
SO2938		0.19	0.04	hypothetical protein	
SO4429		0.19	0.07	hypothetical protein	
SO0139	ftn	0.19	0.07	ferritin	Transport and binding proteins
SO2922		0.19	0.03	conserved hypothetical protein	Hypothetical proteins
SO3802		0.20	0.05	ABC transporter, ATP-binding protein	Transport and binding proteins
SO1473	smpB	0.20	0.08	SsrA-binding protein	Protein synthesis
SO4699	prlC	0.20	0.05	oligopeptidase A	Protein fate
SO2849		0.20	0.03	acetyltransferase, GNAT family	Unknown function

SOA0132		0.20	0.03	conserved hypothetical protein	Hypothetical proteins
SO1227		0.20	0.05	conserved hypothetical protein	Hypothetical proteins
SO1888		0.20	0.05	conserved hypothetical protein	Hypothetical proteins
SO1898		0.20	0.08	transcriptional regulator, putative	Regulatory functions
SO0895		0.20	0.04	pirin family protein	Unknown function
SO0900		0.20	0.09	oxidoreductase, aldo/keto reductase family	Unknown function
SO0524		0.20	0.06	HlyD family secretion protein	Transport and binding proteins
SO3866		0.20	0.04	site-specific recombinase, phage integrase family	DNA metabolism
SO3380		0.21	0.07	conserved hypothetical protein	Hypothetical proteins
SO0112		0.21	0.04	conserved hypothetical protein	Hypothetical proteins
SO0334		0.21	0.05	conserved hypothetical protein	Hypothetical proteins
SO0052	secB	0.21	0.07	protein-export protein SecB	Protein fate
SO3089		0.21	0.05	fatty oxidation complex, beta subunit	Fatty acid and phospholipid metabolism
SO3626		0.21	0.04	hypothetical protein	
SO3658		0.21	0.06	hypothetical protein	
SO4162	hslV	0.22	0.20	ATP-dependent protease HslV	Protein fate
SO0051		0.22	0.10	hypothetical protein	
SO4281		0.22	0.09	potassium uptake protein KtrA, putative	Transport and binding proteins
SO1524	grpE	0.22	0.06	heat shock protein GrpE	Protein fate
SO1196	rrmJ	0.22	0.02	ribosomal RNA large subunit methyltransferase J	Protein synthesis
SO2282		0.22	0.03	transcriptional regulator, GntR family	Regulatory functions
SO1987		0.22	0.07	ATP-dependent protease La (LON) domain protein	Protein fate
SO3799	asnC	0.22	0.09	regulatory protein AsnC	Regulatory functions
SO4327		0.22	0.05	HlyD family secretion domain protein	Unknown function
SO3410		0.22	0.08	hypothetical protein	
SO2847		0.23	0.11	transcriptional regulator, LysR family	Regulatory functions
SOA0153		0.23	0.05	heavy metal efflux pump, CzcA family	Transport and binding proteins
SO1324	gltD	0.23	0.03	glutamate synthase, small subunit	Amino acid biosynthesis
SO1343	rseA	0.23	0.05	sigma-E factor negative regulatory protein	Regulatory functions
SO3847		0.23	0.05	conserved hypothetical protein	Hypothetical proteins
SO2541		0.23	0.04	response regulator	Signal transduction

SO1021	nuoA	0.23	0.15	NADH dehydrogenase I, A subunit	Energy metabolism
SO0101	fdnG	0.23	0.13	formate dehydrogenase, nitrate inducible, alpha subunit, selenocysteine-containing	Energy metabolism
SO3496		0.23	0.04	aldehyde dehydrogenase	Energy metabolism
SO1064		0.23	0.05	conserved hypothetical protein	Hypothetical proteins
SO4462		0.24	0.06	hypothetical protein	
SO4738		0.24	0.04	hypothetical protein	
SO2975		0.24	0.06	hypothetical protein	
SO1748		0.24	0.04	hypothetical protein	
SO4646		0.24	0.07	hypothetical protein	
SO1276	gabT	0.24	0.06	4-aminobutyrate aminotransferase	Central intermediary metabolism
SO4292	pstS	0.24	0.04	phosphate ABC transporter, periplasmic phosphate-binding protein	Transport and binding proteins
SO3391		0.24	0.07	ATP-dependent protease, putative	Protein fate
SO4326		0.24	0.06	transcriptional regulator, TetR family	Regulatory functions
SO3276		0.24	0.03	hypothetical protein	
ORF00299		0.24	0.04	hypothetical protein	
SO1603		0.25	0.10	transcriptional regulator, putative	Regulatory functions
SO3102		0.25	0.04	AcrA/AcrE family protein	Unknown function
SOA0072		0.25	0.05	conserved hypothetical protein	Hypothetical proteins
SO0022	pepQ	0.25	0.06	prolidase	Protein fate
SO4365		0.25	0.02	hypothetical protein	
SO0088		0.25	0.07	hypothetical protein	
SO1392		0.25	0.05	conserved hypothetical protein	Hypothetical proteins
SO0335		0.26	0.05	conserved hypothetical protein	Hypothetical proteins
SOA0003		0.26	0.05	type II restriction endonuclease, putative	DNA metabolism
SO4583	rpoH	0.26	0.10	RNA polymerase sigma-32 factor	Transcription
SO0576		0.26	0.08	PhoH family protein	Unknown function
SO1877	bcp	0.26	0.14	bacterioferritin comigratory protein	Unknown function
SO2014		0.26	0.07	conserved hypothetical protein TIGR00103	Hypothetical proteins
SO2645		0.26	0.03	conserved hypothetical protein	Hypothetical proteins
SO3392		0.26	0.07	oxidoreductase, FMN-binding	Unknown function
SO4168		0.26	0.12	hypothetical protein	
SO4322		0.26	0.08	conserved hypothetical protein	Hypothetical proteins

SO4054	metF	0.26	0.04	5,10-methylenetetrahydrofolate reductase	Amino acid biosynthesis
SO1430	dmsB-1	0.26	0.04	anaerobic dimethyl sulfoxide reductase, B subunit	Energy metabolism
SO2484		0.27	0.07	conserved hypothetical protein	Hypothetical proteins
SOA0095		0.27	0.05	partitioning protein A	Other categories
SO1471		0.27	0.08	site-specific recombinase, phage integrase family	DNA metabolism
SO3581		0.27	0.02	hypothetical protein	
SO1265		0.27	0.09	transcriptional regulator, putative	Regulatory functions
SO2737	bioD	0.27	0.07	dethiobiotin synthase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4606		0.27	0.04	cytochrome c oxidase, subunit II	Energy metabolism
SO3277		0.27	0.05	transcriptional regulator, TetR family	Regulatory functions
SO2672		0.27	0.09	conserved hypothetical protein	Hypothetical proteins
SO2899	cysZ	0.27	0.04	cysZ protein	Transport and binding proteins
SO3552		0.28	0.04	von Willebrand factor type A domain protein	Unknown function
SO2921	folX	0.28	0.06	D-erythro-7,8-dihydroneopterin triphosphate epimerase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0573		0.28	0.03	hypothetical protein	
SO0551		0.28	0.10	conserved hypothetical protein	Hypothetical proteins
SO0433	rsd	0.28	0.10	regulator of sigma D	Regulatory functions
SO2178	ccpA	0.28	0.06	cytochrome c551 peroxidase	Cellular processes
SO2312		0.28	0.06	serine protease inhibitor, ecotin	Protein fate
SO4444		0.28	0.07	capsular synthesis regulator component B, putative	Signal transduction
SOA0057		0.28	0.18	hypothetical protein	
SO0868		0.28	0.10	hypothetical protein	
SO0355		0.28	0.04	AMP-binding protein	Unknown function
SO1626	glnD	0.29	0.07	protein-P-II uridylyltransferase	Regulatory functions
SO4471	ntrB	0.29	0.05	nitrogen regulation protein	Signal transduction
SO1597		0.29	0.13	conserved hypothetical protein	Hypothetical proteins
SO1538		0.29	0.04	isocitrate dehydrogenase, NAD-dependent	Energy metabolism
SO3810		0.29	0.03	OmpA-like transmembrane domain protein	Unknown function
SO0124		0.29	0.03	hypothetical protein	
SO3268		0.29	0.04	alpha amylase domain protein	Unknown function

SO0178		0.29	0.12	hypothetical protein	
SO2470		0.29	0.10	conserved hypothetical protein	Hypothetical proteins
SO1246		0.29	0.04	hypothetical protein	
SO4428		0.29	0.07	DNA-binding response regulator	Signal transduction
SO4741	glmS	0.30	0.05	glucosamine--fructose-6-phosphate aminotransferase (isomerizing)	Central intermediary metabolism
SO0617	argD	0.30	0.05	acetylornithine aminotransferase	Amino acid biosynthesis
SO3497		0.30	0.08	aminotransferase, class III	Unknown function
SO2375		0.30	0.06	membrane protein, putative	Cell envelope
SO0084		0.30	0.05	HAD-superfamily hydrolase, subfamily IA, variant 1 family protein	Unknown function
SO3150		0.30	0.06	lipoprotein, putative	Cell envelope
SO3274		0.30	0.09	conserved hypothetical protein	Hypothetical proteins
SO2763		0.30	0.05	conserved hypothetical protein	Hypothetical proteins
SO4341		0.31	0.08	hypothetical protein	
SO1984		0.31	0.15	hypothetical protein	
SO2892		0.31	0.23	hypothetical protein	
SO3994		0.31	0.05	hypothetical protein	
SO0653		0.31	0.04	hypothetical protein	
SO1532		0.31	0.09	hypothetical protein	
SO2248	sdaA	0.31	0.06	L-serine dehydratase 1	Energy metabolism
SO1431		0.31	0.08	conserved hypothetical protein	Hypothetical proteins
SO3801		0.31	0.10	ABC transporter, permease protein	Transport and binding proteins
SO1385		0.31	0.05	methyl-accepting chemotaxis protein	Cellular processes
SOA0023	higA	0.32	0.10	proteic killer suppressor protein	Other categories
SO4161		0.32	0.12	hypothetical protein	
SO1237		0.32	0.06	acetoin utilization protein AcuB, putative	Energy metabolism
SO2745		0.32	0.26	glutaredoxin	Energy metabolism
SO3855	sfcA	0.32	0.07	malate oxidoreductase	Energy metabolism
SO0989		0.33	0.11	transcriptional regulator, LysR family	Regulatory functions
SO0543		0.33	0.30	hypothetical protein	
SO3414	thrB	0.33	0.07	homoserine kinase	Amino acid biosynthesis
SO0402		0.33	0.07	transcriptional regulator, LysR family	Regulatory functions
SO0541		0.33	0.04	metallo-beta-lactamase family protein	Unknown function

SO0183		0.33	0.05	acetyltransferase, GNAT family	Unknown function
SO0388		0.33	0.07	site-specific recombinase, phage integrase family	DNA metabolism
SO4407		0.33	0.04	GGDEF family protein	Unknown function
SO2881	sodB	0.33	0.06	superoxide dismutase, Fe	Cellular processes
SO2893		0.33	0.08	conserved hypothetical protein	Hypothetical proteins
SO3413	thrC	0.33	0.04	threonine synthase	Amino acid biosynthesis
SO4271		0.34	0.15	site-specific recombinase, phage integrase family, authentic frameshift	DNA metabolism
SO4470		0.34	0.12	conserved hypothetical protein	Hypothetical proteins
SO1957		0.34	0.07	conserved hypothetical protein	Hypothetical proteins
SO3808		0.34	0.04	conserved hypothetical protein	Hypothetical proteins
SO3409		0.34	0.06	OsmC/Ohr family protein	Unknown function
SO4163	hslU	0.34	0.10	heat shock protein HslVU, ATPase subunit HslU	Cellular processes
SO0789		0.34	0.05	conserved hypothetical protein	Hypothetical proteins
SO2924		0.34	0.07	signal peptidase I family protein	Protein fate
SOA0032		0.34	0.06	conserved hypothetical protein	Hypothetical proteins
SO1429	dmaA-1	0.34	0.05	anaerobic dimethyl sulfoxide reductase, A subunit	Energy metabolism
SO4597		0.34	0.10	heavy metal efflux system protein, putative	Transport and binding proteins
SO4288		0.34	0.05	exopolysaccharide synthesis protein, putative	Unknown function
SO1999		0.34	0.06	phospholipase/carboxylesterase family protein	Unknown function
SO3758		0.34	0.03	conserved hypothetical protein	Hypothetical proteins
ORF02277		0.34	0.04	formate dehydrogenase-o, major subunit (fdnG)""	
SO0035	smg	0.34	0.07	smg protein	Unknown function
SO0770	mdh	0.35	0.08	malate dehydrogenase	Energy metabolism
SO0971		0.35	0.09	hypothetical protein	
SO3072	fabB	0.35	0.03	3-oxoacyl-(acyl-carrier-protein) synthase I	Fatty acid and phospholipid metabolism
SO3498		0.35	0.04	methylmalonic acid semialdehyde dehydrogenase, interruption	Disrupted reading frame
SO0114		0.35	0.04	conserved hypothetical protein	Hypothetical proteins
SO2048		0.35	0.10	membrane protein, putative	Cell envelope
SO3145	etfB	0.35	0.05	electron transfer flavoprotein, beta subunit	Energy metabolism

SO1983		0.35	0.09	hypothetical protein	
SO4346	ilvM	0.35	0.12	acetolactate synthase II, small subunit	Amino acid biosynthesis
SOA0055		0.36	0.09	hypothetical protein	
SO1432		0.36	0.06	hypothetical protein	
SO1342	rpoE	0.36	0.06	RNA polymerase sigma-24 factor	Transcription
SO3333		0.36	0.04	transporter, putative	Transport and binding proteins
SO1960		0.36	0.07	conserved hypothetical protein	Hypothetical proteins
SO2794		0.36	0.07	conserved hypothetical protein	Hypothetical proteins
SO2546		0.36	0.08	conserved hypothetical protein	Hypothetical proteins
SO0783		0.36	0.06	hypothetical protein	
SO3973		0.36	0.09	RIO1/ZK632.3/MJ0444 family, putative	Unknown function
SO4725		0.36	0.09	conserved hypothetical protein	Hypothetical proteins
SO4328		0.36	0.09	AcrB/AcrD/AcrF family protein, authentic frameshift	Cellular processes
SO3020	trpG	0.36	0.10	glutamine amido-transferase	Amino acid biosynthesis
SO4558		0.36	0.06	hypothetical protein	
SO0512	aroQ	0.37	0.04	3-dehydroquinate dehydratase, type II	Amino acid biosynthesis
SO0616		0.37	0.05	esterase, putative	Central intermediary metabolism
SO4344	ilvA	0.37	0.06	threonine dehydratase	Amino acid biosynthesis
SO1225		0.37	0.10	conserved hypothetical protein	Hypothetical proteins
SO3254	flgM	0.37	0.10	negative regulator of flagellin synthesis FlgM	Regulatory functions
SO1956		0.37	0.14	conserved hypothetical protein	Hypothetical proteins
SO3815		0.37	0.15	conserved hypothetical protein	Hypothetical proteins
SO4679		0.37	0.06	glycosyl transferase, group 1 family protein	Cell envelope
SO4191		0.37	0.11	DedA family protein	Unknown function
SO0758		0.37	0.09	hypothetical protein	
SO2230		0.38	0.06	hypothetical protein	
SOA0041		0.38	0.06	transcriptional regulator, PemK family	Regulatory functions
SO0694	galK	0.38	0.05	galactokinase	Energy metabolism
SO3494		0.38	0.03	transcriptional regulator, TetR family	Regulatory functions
SO0307		0.38	0.15	hypothetical protein	
SO4535		0.38	0.21	hypothetical protein	
SO3262	ilvB	0.38	0.05	acetolactate synthase isozyme I, large subunit	Amino acid biosynthesis
SO4492		0.38	0.10	conserved hypothetical protein	Hypothetical proteins

SO3716		0.38	0.12	DnaJ domain protein	Protein fate
SO0194		0.38	0.05	acyltransferase family protein	Unknown function
SO4644		0.38	0.08	hypothetical protein	
SO1635	dxr	0.38	0.14	1-deoxy-D-xylulose 5-phosphate reductoisomerase	Biosynthesis of cofactors, prosthetic groups, and carriers
SOA0056		0.38	0.13	hypothetical protein	
SO0295		0.38	0.07	transcriptional regulator, LysR family	Regulatory functions
SO1890		0.38	0.16	conserved hypothetical protein	Hypothetical proteins
SO4258		0.38	0.04	site-specific recombinase, phage integrase family	DNA metabolism
SO4078	pmbA	0.38	0.09	pmba protein	Cellular processes
SO4453		0.38	0.06	electron transfer flavoprotein-ubiquinone oxidoreductase, putative	Energy metabolism
SO1317		0.38	0.07	hypothetical protein	
SO1513		0.38	0.08	hypothetical protein	
SO1390		0.39	0.08	peptidyl-prolyl cis-trans isomerase, FKBP-type	Protein fate
SO1224		0.39	0.08	conserved hypothetical protein	Hypothetical proteins
SO4090		0.39	0.06	outer membrane efflux protein	Transport and binding proteins
SO1961	maa	0.39	0.06	maltose O-acetyltransferase	Cellular processes
SO1101	luxS	0.39	0.22	autoinducer-2 production protein LuxS	Cellular processes
SO2917		0.39	0.11	hypothetical protein	
SO1197	ftsH	0.39	0.07	cell division protein FtsH	Cellular processes
SO0726		0.39	0.06	ABC transporter, ATP-binding protein	Transport and binding proteins
SO3261		0.39	0.05	polysaccharide biosynthesis related-protein	Unknown function
SO2764		0.39	0.10	nagD protein, degenerate	Disrupted reading frame
SO0304		0.39	0.05	conserved hypothetical protein	Hypothetical proteins
SO3823		0.39	0.15	ISSo5, transposase, interruption	Disrupted reading frame
SO0113		0.39	0.05	conserved hypothetical protein	Hypothetical proteins
SO2413	ubiG	0.40	0.07	3-demethylubiquinone-9 3-methyltransferase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0854		0.40	0.08	type IV pilin, putative	Cell envelope
SO2455		0.40	0.07	transcriptional regulator, LysR family	Regulatory functions
SO3584		0.40	0.11	conserved hypothetical protein	Hypothetical proteins
SO1557		0.40	0.12	outer membrane porin, putative	Transport and binding proteins

SO3073		0.40	0.05	conserved hypothetical protein	Hypothetical proteins
SO0618	astA	0.40	0.06	arginine N-succinyltransferase	Energy metabolism
SO1814		0.40	0.10	hypothetical protein	
SOA0165		0.40	0.06	transcriptional regulator, LysR family	Regulatory functions
SO0345	prpB	0.40	0.08	methylisocitrate lyase	Energy metabolism
SO2201		0.40	0.05	conserved hypothetical protein	Hypothetical proteins
SO4710		0.40	0.05	hypothetical protein	
SO4148		0.40	0.09	HlyD family secretion protein	Transport and binding proteins
SO0542		0.40	0.13	conserved hypothetical protein	Hypothetical proteins
SO3305		0.40	0.11	DNA-binding response regulator, LuxR family	Signal transduction
SO0637		0.41	0.13	conserved hypothetical protein	Hypothetical proteins
SO4567		0.41	0.11	transcriptional regulator, AsnC family	Regulatory functions
SO0132		0.41	0.07	flagellar protein FliL, putative	Cellular processes
SO2404	aroA	0.41	0.04	3-phosphoshikimate 1-carboxyvinyltransferase	Amino acid biosynthesis
SO3838		0.41	0.06	methyl-accepting chemotaxis protein	Cellular processes
SO1222		0.41	0.12	hypothetical protein	
SO3082	sixA	0.41	0.09	phosphohistidine phosphatase SixA	Regulatory functions
SO4055	metL	0.41	0.08	aspartokinase II/homoserine dehydrogenase, methionine-sensitive	Amino acid biosynthesis
SO2637		0.41	0.10	hypothetical protein	
SO0622		0.41	0.07	DNA-binding response regulator	Signal transduction
SO1608		0.41	0.08	conserved hypothetical protein	Hypothetical proteins
SO4089		0.41	0.07	HlyD family secretion protein	Transport and binding proteins
SO3791		0.41	0.09	renal dipeptidase family protein	Protein fate
SO3831		0.41	0.05	acetyltransferase, GNAT family	Unknown function
SO4472	ntrC	0.41	0.08	nitrogen regulation protein NR(I)	Signal transduction
SO0199		0.41	0.07	hypothetical protein	
SO1254		0.41	0.07	conserved hypothetical protein	Hypothetical proteins
SO2217	ddlA	0.41	0.08	D-alanine--D-alanine ligase	Cell envelope
SO0960		0.42	0.11	conserved hypothetical protein	Hypothetical proteins
SO1152		0.42	0.18	hypothetical protein	
SO0625		0.42	0.08	conserved domain protein	Hypothetical proteins

SO2485		0.42	0.06	deoxyguanosinetriphosphate triphosphohydrolase, putative	Purines, pyrimidines, nucleosides, and nucleotides
SO2366		0.42	0.08	response regulator	Signal transduction
SO0695		0.42	0.08	glutathione-regulated potassium-efflux system protein KefC, putative	Transport and binding proteins
SO2493		0.42	0.08	transcriptional regulator, TetR family	Regulatory functions
SO2650		0.42	0.09	conserved hypothetical protein	Hypothetical proteins
SO2648		0.42	0.13	DNA-binding response regulator, LuxR family	Signal transduction
SO0368		0.42	0.16	helicase	Unknown function
SO2104		0.42	0.15	DNA-binding response regulator YgiX, putative	Signal transduction
SO0197		0.42	0.04	fatty acid desaturase, family 1	Fatty acid and phospholipid metabolism
SO1440		0.42	0.08	conserved hypothetical protein	Hypothetical proteins
SO2862		0.42	0.07	HDIG domain protein	Unknown function
SO3347		0.42	0.25	conserved hypothetical protein TIGR00250	Hypothetical proteins
SO1261		0.42	0.28	mercaptopyruvate sulfurtransferase	Central intermediary metabolism
SO2547		0.42	0.08	response regulator	Signal transduction
SO1762		0.42	0.07	transcriptional regulator, AraC/XylS family	Regulatory functions
SO1971		0.42	0.05	AMP-binding family protein	Unknown function
SO2209		0.42	0.13	hypothetical protein	
SO4003		0.43	0.06	response regulator	Signal transduction
SO1439		0.43	0.16	site-specific recombinase, phage integrase family, interruption-N	Disrupted reading frame
SO3415	thrA	0.43	0.06	aspartokinase I/homoserine dehydrogenase, threonine-sensitive	Amino acid biosynthesis
SO3571		0.43	0.07	conserved hypothetical protein	Hypothetical proteins
SO1979		0.43	0.12	MutT/nudix family protein	DNA metabolism
SO3355		0.43	0.11	conserved hypothetical protein	Hypothetical proteins
SO2239		0.43	0.08	conserved hypothetical protein	Hypothetical proteins
SO3278		0.43	0.12	conserved hypothetical protein	Hypothetical proteins
SO0177		0.43	0.05	HAD-superfamily hydrolase, subfamily IA, variant 3 protein family	Unknown function
SO2471	dapE	0.43	0.06	succinyl-diaminopimelate desuccinylase	Amino acid biosynthesis
SO3419	trpR	0.43	0.07	trp operon repressor	Regulatory functions

SO1897	ivd	0.43	0.06	isovaleryl-CoA dehydrogenase	Energy metabolism
SO2005		0.43	0.11	dksA-type zinc finger protein	Unknown function
SO0095	hutI	0.43	0.05	imidazolonepropionase	Energy metabolism
SO4687	ugd	0.43	0.07	UDP-glucose 6-dehydrogenase	Cell envelope
SO1306		0.43	0.13	conserved hypothetical protein	Hypothetical proteins
SO0635	ppiC-1	0.43	0.38	peptidyl-prolyl cis-trans isomerase C	Protein fate
SO0373		0.43	0.10	site-specific recombinase, phage integrase family, truncation	Disrupted reading frame
SO3344		0.43	0.11	hypothetical protein	
SO4242		0.43	0.17	conserved hypothetical protein	Hypothetical proteins
SO0823		0.43	0.07	conserved hypothetical protein	Hypothetical proteins
SO0297		0.43	0.06	lipoprotein, putative	Cell envelope
SO1151		0.43	0.06	conserved hypothetical protein	Hypothetical proteins
SO4347	ilvG	0.44	0.06	acetolactate synthase II, large subunit	Amino acid biosynthesis
SO3083		0.44	0.12	peptidase, M16 family	Protein fate
SO3628		0.44	0.10	glycerate dehydrogenase, degenerate	Disrupted reading frame
SO1627	map	0.44	0.07	methionine aminopeptidase, type I	Protein fate
SO3679		0.44	0.04	conserved hypothetical protein	Hypothetical proteins
SO0346		0.44	0.09	transcriptional regulator. GntR family	Regulatory functions
SO3841		0.44	0.18	hypothetical protein	
SO1094		0.44	0.07	conserved hypothetical protein	Hypothetical proteins
SO0815		0.44	0.06	TonB-dependent receptor C-terminal domain protein	Unknown function
SO4189		0.44	0.19	conserved hypothetical protein	Hypothetical proteins
SO0306		0.44	0.10	hypothetical protein	
SO1599		0.44	0.23	beta-ketoacyl synthase	Fatty acid and phospholipid metabolism
SO0964		0.44	0.14	conserved hypothetical protein	Hypothetical proteins
SO0755		0.44	0.10	hypothetical protein	
SO0352		0.44	0.08	sensor histidine kinase, putative	Signal transduction
SO3848		0.44	0.06	hypothetical protein	
SO1259		0.44	0.17	transcriptional regulator, LysR family	Regulatory functions
SO4310		0.44	0.09	hypothetical protein	
SO1260		0.44	0.10	conserved hypothetical protein	Hypothetical proteins
SO4311	cyaY	0.44	0.13	cyay protein	Unknown function

SO3088		0.45	0.11	fatty oxidation complex, alpha subunit	Fatty acid and phospholipid metabolism
SO2827		0.45	0.08	conserved hypothetical protein	Hypothetical proteins
SO1062	def-2	0.45	0.09	polypeptide deformylase	Protein fate
SO2540		0.45	0.06	response regulator	Signal transduction
SO0934		0.45	0.07	conserved hypothetical protein	Hypothetical proteins
SO1233	torC	0.45	0.16	tetraheme cytochrome c	Energy metabolism
SO2064		0.45	0.12	conserved domain protein	Hypothetical proteins
SO3418		0.45	0.12	hypothetical protein	
SO3796		0.45	0.08	conserved hypothetical protein	Hypothetical proteins
ORF02915		0.45	0.18	multidrug resistance protein, putative""	Hypothetical proteins
SO0501		0.45	0.07	conserved hypothetical protein	
SO1489		0.45	0.13	hypothetical protein	Transcription
SO3551		0.45	0.07	RNA polymerase sigma-70 factor, ECF subfamily	Disrupted reading frame
SO1799		0.45	0.10	molybdenum-pterin-binding-protein, truncation	Hypothetical proteins
SO2039		0.45	0.19	conserved domain protein	Cell envelope
SO2799		0.46	0.05	lipoprotein, putative	Signal transduction
SO4477	cpxR	0.46	0.08	transcriptional regulatory protein CpxR	Hypothetical proteins
ORF02531		0.46	0.16	hypothetical protein	
SO1331		0.46	0.08	MutT/nudix family protein	DNA metabolism
SO2446		0.46	0.09	hypothetical protein	
SO3942		0.46	0.07	serine protease, HtrA/DegQ/DegS family	Protein fate
SO2495		0.46	0.10	Smr domain protein	Unknown function
SO4283		0.46	0.09	apbE family protein	Cell envelope
SO3583	rsuA-1	0.46	0.09	ribosomal small subunit pseudouridine synthase A	Protein synthesis
SO0296		0.46	0.18	integral membrane domain protein	Unknown function
SO3550		0.46	0.08	hypothetical protein	
SO2063		0.46	0.05	hypothetical protein	
SO1629	rpsB	0.46	0.06	ribosomal protein S2	Protein synthesis
SO2813		0.46	0.06	oxidoreductase, short chain dehydrogenase/reductase family	Unknown function
SO3767		0.46	0.21	hypothetical protein	

SO0529	trpI	0.46	0.12	trpba operon transcriptional activator	Regulatory functions
SO4092		0.46	0.08	hydrolase, carbon-nitrogen family	Unknown function
SO4152		0.46	0.13	hypothetical protein	Hypothetical proteins
SO3794		0.47	0.13	conserved domain protein	Hypothetical proteins
SO4465		0.47	0.11	conserved domain protein	Hypothetical proteins
SO1998		0.47	0.07	conserved hypothetical protein	Energy metabolism
SO2347	gapA-3	0.47	0.11	glyceraldehyde 3-phosphate dehydrogenase	
SO4013		0.47	0.06	hypothetical protein	Cell envelope
SO0999	pbpG	0.47	0.14	D-alanyl-D-alanine endopeptidase	Hypothetical proteins
SO2542		0.47	0.10	conserved hypothetical protein	Hypothetical proteins
SO0555		0.47	0.20	conserved hypothetical protein	
SO1262		0.47	0.06	hypothetical protein	Cellular processes
SO2898		0.47	0.21	SMC family protein	Energy metabolism
SO0878	prkB	0.47	0.07	phosphoribulokinase	Unknown function
SO2153	moxR	0.47	0.08	MoxR protein	Unknown function
SO4039		0.47	0.08	hydrolase, haloacid dehalogenase-like family	Hypothetical proteins
SO0775		0.47	0.13	conserved hypothetical protein	Hypothetical proteins
SO2062		0.47	0.06	conserved hypothetical protein	
SO0147		0.47	0.11	hypothetical protein	Hypothetical proteins
SO3997		0.47	0.10	conserved hypothetical protein TIGR00645	
SO1700		0.47	0.12	hypothetical protein	Energy metabolism
SO0053	gpsA	0.47	0.10	glycerol-3-phosphate dehydrogenase (NAD(P)+)	Hypothetical proteins
SO2769		0.47	0.11	conserved hypothetical protein	Hypothetical proteins
SO1585		0.47	0.13	fumarylacetoacetate hydrolase family protein	Unknown function
SO2533		0.47	0.05	conserved hypothetical protein	Hypothetical proteins
SO0389		0.47	0.11	hypothetical protein	
SO2355		0.47	0.07	universal stress protein family	Cellular processes
SOA0137		0.48	0.11	conserved domain protein	Hypothetical proteins
SO2851		0.48	0.25	histidinol phosphatase domain protein	Unknown function
SO1764		0.48	0.16	conserved hypothetical protein	Hypothetical proteins
SO4515		0.48	0.07	formate dehydrogenase, C subunit, putative	Energy metabolism
SO1441		0.48	0.10	hypothetical protein	
SO0615		0.48	0.11	hypothetical protein	

SO1375		0.48	0.11	carboxypeptidase	Protein fate
SO3354	proC	0.48	0.09	pyrroline-5-carboxylate reductase	Amino acid biosynthesis
SO2622		0.48	0.10	conserved hypothetical protein	Hypothetical proteins
SO1853		0.48	0.10	ABC transporter, ATP-binding protein	Transport and binding proteins
SO4672	glpE	0.48	0.08	glpE protein	Unknown function
SO0856		0.48	0.18	conserved hypothetical protein	Hypothetical proteins
SO4726	mutM	0.48	0.06	formamidopyrimidine-DNA glycosylase	DNA metabolism
SO0805		0.48	0.07	CBS domain protein	Unknown function
SO4732		0.48	0.11	conserved hypothetical protein	Hypothetical proteins
SO4355		0.48	0.21	hypothetical protein	
SO0795		0.48	0.06	conserved hypothetical protein	Hypothetical proteins
SO1256		0.48	0.08	conserved hypothetical protein	Hypothetical proteins
SO0273		0.48	0.08	conserved hypothetical protein	Hypothetical proteins
SO3746	htrB	0.48	0.08	lipid A biosynthesis lauroyl acyltransferase	Cellular processes
SO2333		0.48	0.12	hydrolase, alpha/beta fold family	Unknown function
SO4727		0.48	0.10	conserved hypothetical protein	Hypothetical proteins
SO0700		0.49	0.36	hypothetical protein	
SO0083		0.49	0.08	carboxymuconolactone decarboxylase family protein	Energy metabolism
SOA0122		0.49	0.11	conserved hypothetical protein	Hypothetical proteins
SO3489		0.49	0.05	GGDEF domain protein	Unknown function
SO2414		0.49	0.10	phosphoglycolate phosphatase, putative	Energy metabolism
SO2728	htpX	0.49	0.11	peptidase HtpX	Protein fate
SO0602	miaA	0.49	0.18	tRNA delta(2)-isopentenylpyrophosphate transferase	Protein synthesis
SO3080		0.49	0.09	hemK family protein	Unknown function
SO0699		0.49	0.17	hypothetical protein	
SO3907		0.49	0.12	conserved hypothetical protein	Hypothetical proteins
SO1159		0.49	0.13	hypothetical protein	
SO0075		0.49	0.14	AMP-binding family protein	Unknown function
SO2249		0.49	0.07	hypothetical protein	
SO4665		0.49	0.09	hypothetical protein	
SO4315	hemX	0.49	0.08	hemX protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1208		0.49	0.09	GGDEF domain protein	Unknown function

SO1572		0.49	0.14	hypothetical protein	
SO3785		0.49	0.27	hypothetical protein	
SO0034		0.49	0.09	DNA processing protein DprA, putative	Cellular processes
SO1666	phhA	0.49	0.14	phenylalanine-4-hydroxylase	Energy metabolism
SO1160	rimI	0.49	0.13	ribosomal-protein-alanine acetyltransferase	Protein synthesis
SO4118		0.49	0.10	malate oxidoreductase, putative	Energy metabolism
SO3519	glnB-2	0.49	0.14	nitrogen regulatory protein P-II 1	Regulatory functions
SO0033		0.49	0.08	LysM domain protein	Unknown function
SO3163		0.50	0.07	lipoprotein	Cell envelope
SO4648		0.50	0.06	sensor histidine kinase	Signal transduction
SO3766		0.50	0.13	hypothetical protein	
SO3356		0.50	0.13	conserved hypothetical protein TIGR00251	Hypothetical proteins
SO1393		0.50	0.12	transcriptional regulator, TetR family	Regulatory functions
SO2990		0.50	0.08	prophage LambdaSo, transcriptional regulator, Cro/CI family	Regulatory functions
SO1496		0.50	0.09	glycogen phosphorylase family protein	Energy metabolism
SO0026		0.50	0.14	transcriptional regulator, ArsR family	Regulatory functions
SO4057	metJ	0.50	0.17	met repressor	Regulatory functions
SO3256		0.50	0.11	conserved hypothetical protein	Hypothetical proteins
SO0182		0.50	0.11	hypothetical protein	
SO0568		0.50	0.10	conserved hypothetical protein	Hypothetical proteins

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures of MR-1 grown at 40 mM KNO_3 to that of the anaerobically grown at 1 mM KNO_3 (reference).

[b] The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

SUPPLEMENTAL TABLE B.3. Genes induced in anaerobic cultures of EtrA7-1 relative to the wild type (reference strain).

Gene ID	Gene name	Relative expression[a]	STD[b]	COG Annotation	Possible Function
SO0466		1.97	0.96	hypothetical protein	
SO2501		1.98	0.45	radical activating enzyme	Protein fate
SO2715		1.98	0.58	TonB-dependent receptor	Transport and binding proteins
SO0874	dksA	1.98	1.12	DnaK suppressor protein	DNA metabolism
SO0232	rplD	2.00	0.62	ribosomal protein L4	Protein synthesis
SO4427		2.01	0.48	sensor histidine kinase	Signal transduction
SO3176		2.01	0.88	glycosyl transferase, group 1 family protein	Cell envelope
SO4136		2.01	0.60	decarboxylase, pyridoxal-dependent	Unknown function
SO1202		2.02	0.44	conserved hypothetical protein	Hypothetical proteins
SO1495	glgX	2.03	0.50	glycogen operon protein	Energy metabolism
SO1166		2.03	0.92	membrane-bound lytic transglycosylase, putative	Cell envelope
SO0491		2.03	0.27	peptidase, M13 family	Protein fate
SO2261		2.04	0.50	RNA methyltransferase, TrmH family, group 1	Protein synthesis
SO1276	gabT	2.04	0.88	4-aminobutyrate aminotransferase	Central intermediary metabolism
SO4290	pstA	2.04	1.07	phosphate ABC transporter, permease protein	Transport and binding proteins
SO2687		2.04	0.57	hypothetical protein	
SO1265		2.04	0.87	transcriptional regulator, putative	Regulatory functions
SO3748		2.05	0.48	LysM domain protein	Unknown function
SO2780	rpmF	2.05	1.44	ribosomal protein L32	Protein synthesis
SO0675		2.06	0.82	prophage MuSo1, major head subunit, putative	Other categories
SO1682	mmsB	2.06	0.91	3-hydroxyisobutyrate dehydrogenase	Energy metabolism
SO4371		2.06	0.93	conserved hypothetical protein	Hypothetical proteins
SO0255	rpsD	2.07	0.75	ribosomal protein S4	Protein synthesis
SO1358	rimM	2.08	0.38	16S rRNA processing protein RimM	Transcription
SO4186		2.08	0.34	hypothetical protein	
SO4289	pstB-2	2.08	1.02	phosphate ABC transporter, ATP-binding protein	Transport and binding proteins

SO1854		2.08	0.52	hypothetical protein	
SO0520		2.08	0.34	heavy metal efflux pump, CzcA family	Transport and binding proteins
SO0612	sspB	2.09	0.33	stringent starvation protein b	Cellular processes
SO0219	nusG	2.09	0.64	transcription antitermination protein NusG	Transcription
SO4383	fabF-2	2.09	0.99	3-oxoacyl-(acyl-carrier-protein) synthase II	Fatty acid and phospholipid metabolism
SO2974		2.10	0.82	hypothetical protein	
SO4372		2.10	0.59	thioester dehydrase family protein	Fatty acid and phospholipid metabolism
SO3177		2.10	0.79	formyl transferase domain protein	Unknown function
SO3933		2.11	0.76	membrane protein, putative	Cell envelope
SO4312		2.11	0.69	adenylate cyclase CyaA, putative	Regulatory functions
SO4008		2.11	0.35	hypothetical protein	
SO0272	cinA	2.11	0.77	competence/damage-inducible protein CinA	DNA metabolism
SO1635	dxr	2.12	0.57	1-deoxy-D-xylulose 5-phosphate reductoisomerase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0442	purH	2.12	0.38	phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	Purines, pyrimidines, nucleosides, and nucleotides
SO0590	psd	2.12	0.59	phosphatidylserine decarboxylase, authentic frameshift	Fatty acid and phospholipid metabolism
SO1337		2.13	0.70	hypothetical protein	
SO0361	gmk	2.13	0.44	guanylate kinase	Purines, pyrimidines, nucleosides, and nucleotides
SO4382	fabG-2	2.13	0.51	3-oxoacyl-(acyl-carrier-protein) reductase	Fatty acid and phospholipid metabolism
SO2300	infC	2.14	0.99	translation initiation factor IF-3	Protein synthesis
SO2002		2.14	0.85	hypothetical protein	
SO2778	fabH-1	2.14	0.29	3-oxoacyl-(acyl-carrier-protein) synthase III	Fatty acid and phospholipid metabolism
SO0560	fhs	2.14	0.46	formate--tetrahydrofolate ligase	Central intermediary metabolism
SO1170		2.14	1.00	iojap domain protein	Unknown function
SO4561		2.14	0.29	conserved hypothetical protein	Hypothetical proteins
SO3293	guaB	2.15	0.55	inosine-5-monophosphate dehydrogenase	Purines, pyrimidines, nucleosides, and nucleotides
SO0233	rplW	2.15	0.67	ribosomal protein L23	Protein synthesis
SO0610	petC	2.16	1.17	ubiquinol-cytochrome c reductase, cytochrome c1	Energy metabolism
SO3747		2.16	0.58	sodium/hydrogen exchanger family/TrkA domain protein	Transport and binding proteins
SO2012	apt	2.17	0.36	adenine phosphoribosyltransferase	Purines, pyrimidines, nucleosides, and nucleotides
SO0747	fpr	2.17	1.02	ferredoxin--NADP reductase	Energy metabolism

SO4487		2.17	0.48	DNA-binding response regulator	Signal transduction
SO0246	rplF	2.18	0.38	ribosomal protein L6	Protein synthesis
SO1619		2.18	0.86	conserved hypothetical protein	Hypothetical proteins
SO1536		2.18	0.90	conserved hypothetical protein	Hypothetical proteins
SO3928	rpsR	2.18	0.65	ribosomal protein S18	Protein synthesis
SO3178		2.19	0.93	hypothetical protein	
SO0258		2.19	0.90	hypothetical protein	
SO1360	rplS	2.19	0.25	ribosomal protein L19	Protein synthesis
SO0250	rplO	2.19	0.57	ribosomal protein L15	Protein synthesis
SOA0153		2.20	0.65	heavy metal efflux pump, CzcA family	Transport and binding proteins
SO1831		2.20	0.54	conserved hypothetical protein	Hypothetical proteins
SO2954		2.20	1.32	hypothetical protein	
SO0311		2.21	0.50	conserved hypothetical protein	Hypothetical proteins
SO0286	aroK	2.21	0.48	shikimate kinase	Amino acid biosynthesis
SO0162	pckA	2.21	0.48	phosphoenolpyruvate carboxykinase (ATP)	Energy metabolism
SO3308	engA	2.22	0.64	GTP-binding protein EngA	Unknown function
SO3829		2.23	0.27	conserved hypothetical protein	Hypothetical proteins
SO2685		2.23	0.64	prophage MuSo2, major head subunit, putative	Other categories
SO1163		2.24	0.61	conserved hypothetical protein	Hypothetical proteins
SO1103	nqrA-2	2.25	0.54	NADH:ubiquinone oxidoreductase, Na translocating, alpha subunit	Energy metabolism
SO3985		2.26	0.61	conserved hypothetical protein	Hypothetical proteins
SO4508		2.26	1.01	hypothetical protein	
SO3649		2.26	1.09	GTP-binding protein, GTP1/Obg family	Unknown function
SO2378		2.27	0.54	conserved hypothetical protein	Hypothetical proteins
SO0037		2.27	0.38	Sua5/YciO/YrdC/YwlC family protein	Unknown function
SO2112	rplY	2.28	1.56	ribosomal protein L25	Protein synthesis
SO1634	cdsA	2.29	0.49	phosphatidate cytidylyltransferase	Fatty acid and phospholipid metabolism
SO0249	rpmD	2.29	0.84	ribosomal protein L30	Protein synthesis
SO1424		2.30	0.53	hypothetical protein	
SO2355		2.30	0.31	universal stress protein family	Cellular processes
SO2361	ccoP	2.30	0.92	cytochrome c oxidase, cbb3-type, subunit III	Energy metabolism
SO3172		2.31	0.65	galactosyl transferase	Cell envelope
SO4381		2.31	0.98	thioester dehydrase family protein	Unknown function

SO4213		2.33	1.07	conserved hypothetical protein	Hypothetical proteins
SO0718		2.33	1.13	conserved hypothetical protein	Hypothetical proteins
SO4509		2.33	0.56	formate dehydrogenase, alpha subunit	Energy metabolism
SO0245	rpsH	2.35	0.46	ribosomal protein S8	Protein synthesis
SO0394		2.35	0.60	conserved hypothetical protein	Hypothetical proteins
SO4120	rpmE	2.35	0.60	ribosomal protein L31	Protein synthesis
SO2006		2.36	0.50	NifR3/Smm1 family protein	Unknown function
SO1128		2.36	0.93	hypothetical protein	
SO4376		2.36	0.94	hypothetical protein	
SO3909		2.37	1.11	conserved hypothetical protein	Hypothetical proteins
SO0225	rpoC	2.37	1.24	DNA-directed RNA polymerase, beta subunit	Transcription
SO4343		2.38	1.78	aminotransferase, class V	Unknown function
SO4728		2.38	0.83	conserved hypothetical protein	Hypothetical proteins
SO4596		2.39	1.35	copper-transporting ATPase domain protein	Unknown function
SO2951		2.41	0.77	hypothetical protein	
SO1357	rpsP	2.41	0.96	ribosomal protein S16	Protein synthesis
SO0715		2.42	1.74	oxidoreductase, molybdopterin-binding	Unknown function
SO2786		2.43	0.99	sulfate permease family protein, truncation	Disrupted reading frame
SO3072	fabB	2.44	0.88	3-oxoacyl-(acyl-carrier-protein) synthase I	Fatty acid and phospholipid metabolism
SO3650		2.44	0.26	hypothetical protein	
SO2610		2.44	0.62	hydrolase, TatD family	Unknown function
SO4408	bipA	2.45	0.38	virulence regulator BipA	Cellular processes
SO1648		2.46	1.75	cold shock domain family protein	Cellular processes
SO0197		2.47	0.95	fatty acid desaturase, family 1	Fatty acid and phospholipid metabolism
SO4597		2.47	1.56	heavy metal efflux system protein, putative	Transport and binding proteins
SO1927	sdhC	2.47	1.27	succinate dehydrogenase, cytochrome b556 subunit	Energy metabolism
SO0241	rplN	2.48	0.49	ribosomal protein L14	Protein synthesis
SO2944		2.49	1.25	hypothetical protein	
SO1982		2.49	1.06	hypothetical protein	
SO1203	nusA	2.50	0.36	N utilization substance protein A	Transcription
SO4034	deaD	2.51	0.36	ATP-dependent RNA helicase DeaD	Transcription
SO0337		2.51	0.50	conserved hypothetical protein	Hypothetical proteins
SO1466		2.51	0.37	hypothetical protein	

SO2948		2.53	0.44	prophage LambdaSo, tail assembly protein K, putative	Other categories
SO1924		2.53	1.27	AcrB/AcrD/AcrF family protein	Transport and binding proteins
SO4511		2.53	0.32	formate dehydrogenase, C subunit, putative	Energy metabolism
SO2684		2.54	0.30	prophage MuSo2, protein Gp32, putative	Other categories
SO0531		2.54	1.22	hypothetical protein	
SO3783		2.55	0.94	ATP-dependent RNA helicase, DEAD box family	Transcription
SO4317		2.55	0.63	RTX toxin, putative	Cellular processes
SO4377		2.56	0.89	membrane protein, putative	Cell envelope
SO2889		2.57	0.53	sensory box histidine kinase	Signal transduction
SO4344	ilvA	2.57	0.59	threonine dehydratase	Amino acid biosynthesis
SO3395		2.58	1.20	hypothetical protein	
SO0591		2.58	0.90	conserved hypothetical protein TIGR00157	Hypothetical proteins
SO1726	phoU	2.59	1.77	phosphate transport system regulatory protein PhoU	Regulatory functions
SO1636		2.60	0.28	membrane-associated zinc metalloprotease, putative	Protein fate
SO0611	sspA	2.60	0.58	stringent starvation protein a	Cellular processes
SO0244	rpsN	2.61	0.45	ribosomal protein S14	Protein synthesis
SO0567	plsC	2.63	0.55	1-acyl-sn-glycerol-3-phosphate acyltransferase	Fatty acid and phospholipid metabolism
SO2636		2.63	0.56	conserved hypothetical protein	Hypothetical proteins
SO3179		2.63	1.66	lipopolysaccharide biosynthesis polymerase, putative	Cell envelope
SO4246	rpmG	2.63	1.54	ribosomal protein L33	Protein synthesis
SO2961		2.64	0.57	conserved hypothetical protein	Hypothetical proteins
SO0393	fis	2.64	1.03	DNA-binding protein Fis	Regulatory functions
SO0196	selD	2.64	0.57	selenide, water dikinase	Protein synthesis
SO1632	frr	2.64	0.46	ribosome recycling factor	Protein synthesis
SO0230	rpsJ	2.65	0.43	ribosomal protein S10	Protein synthesis
SO4378		2.65	1.51	FAD-binding protein	Unknown function
SO4490		2.66	0.97	hypothetical protein	
SO2956		2.67	1.47	prophage LambdaSo, major tail protein V, putative	Other categories
SO1164	dacA-1	2.67	0.69	D-alanyl-D-alanine carboxypeptidase	Cell envelope
SO1925		2.68	1.43	HlyD family secretion protein	Transport and binding proteins

SO3315		2.68	0.46	conserved hypothetical protein TIGR00048	Hypothetical proteins
SO1284	rpoD	2.70	1.23	RNA polymerase sigma-70 factor	Transcription
SO1349	era	2.70	0.51	GTP-binding protein Era	Regulatory functions
SO3645		2.70	0.27	hypothetical protein	
SOA0058		2.70	0.74	hypothetical protein	
SO1104	nqrB-2	2.70	1.02	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB	Energy metabolism
SO2941		2.70	0.72	prophage LambdaSo, tail assembly protein I	Other categories
SO3652	rplU	2.71	1.04	ribosomal protein L21	Protein synthesis
SO2199		2.71	0.58	hypothetical protein	
SO4011		2.75	1.58	conserved hypothetical protein	Hypothetical proteins
SO1286	dnaG	2.75	1.06	DNA primase	DNA metabolism
SO2364	ccoN	2.76	1.08	cytochrome c oxidase, cbb3-type, subunit I	Energy metabolism
SO3830		2.76	0.89	conserved hypothetical protein	Hypothetical proteins
SO4254	folE	2.77	0.55	GTP cyclohydrolase I	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4380		2.77	1.20	3-oxoacyl-(acyl-carrier-protein) synthase II, putative	Fatty acid and phospholipid metabolism
SO1633	uppS	2.81	0.59	undecaprenyl diphosphate synthase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4598		2.82	1.86	heavy metal efflux pump, CzcA family	Transport and binding proteins
SO0163	hslO	2.82	0.45	chaperonin HslO	Protein fate
SO1207	rpsO	2.83	1.53	ribosomal protein S15	Protein synthesis
SO3986	lysC	2.86	1.00	aspartokinase III, lysine-sensitive	Amino acid biosynthesis
SO4384		2.87	0.75	hypothetical protein	
SO1686		2.88	0.37	prolyl oligopeptidase family protein	Protein fate
SO2946		2.88	0.95	hypothetical protein	
SO0559		2.89	1.10	MaoC domain protein	Unknown function
SO0003	trmE	2.89	1.59	tRNA modification GTPase TrmE	Protein synthesis
SO0242	rplX	2.89	0.59	ribosomal protein L24	Protein synthesis
SO1628		2.93	1.17	hypothetical protein	
SO1167	rodA	2.93	0.69	rod shape-determining protein RodA	Cell envelope
SO4425		2.94	1.65	GGDEF family protein	Unknown function
SO1168	mrdA	2.94	1.02	penicillin-binding protein 2	Cell envelope
SO2750	tolR	2.95	1.07	tolr protein	Transport and binding proteins

SO3582		2.95	0.52	methyl-accepting chemotaxis protein	Cellular processes
SO0516		2.96	1.18	hypothetical protein	
SO1631	pyrH	2.98	0.72	uridylate kinase	Purines, pyrimidines, nucleosides, and nucleotides
SO2736		2.98	2.04	conserved hypothetical protein	Hypothetical proteins
SO0148		2.99	0.74	hypothetical protein	
SOA0086		3.00	0.94	site-specific recombinase, resolvase family	DNA metabolism
SO1690		3.01	0.94	ABC transporter, ATP-binding protein	Transport and binding proteins
SO1348	rnc	3.02	0.89	ribonuclease III	Transcription
SO1930	sucA	3.02	1.22	2-oxoglutarate dehydrogenase, E1 component	Energy metabolism
SO2426		3.06	1.47	DNA-binding response regulator	Signal transduction
SO2774	fabF-1	3.07	0.27	3-oxoacyl-(acyl-carrier-protein) synthase II	Fatty acid and phospholipid metabolism
SO2924		3.07	2.51	signal peptidase I family protein	Protein fate
SO0336		3.10	1.63	conserved hypothetical protein	Hypothetical proteins
SO2352		3.11	0.93	bax protein, putative	Unknown function
SO1807	pspA	3.11	1.64	phage shock protein A	Cellular processes
SO2945		3.14	0.89	hypothetical protein	
SO1105	nqrC-2	3.15	0.80	NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit	Energy metabolism
SO3770		3.16	1.58	conserved hypothetical protein TIGR00153	Hypothetical proteins
SO2702		3.16	0.69	conserved hypothetical protein	Hypothetical proteins
SO1476	smpA	3.17	2.09	small protein A	Unknown function
SO1617		3.17	0.77	conserved hypothetical protein	Hypothetical proteins
SO1206	truB	3.17	0.61	tRNA pseudouridine synthase B	Protein synthesis
SO1892	atoD	3.21	2.14	acetate CoA-transferase, subunit A	Energy metabolism
SO2260	suhB	3.26	1.81	extragenic suppressor protein SuhB	Unknown function
SO3929	priB	3.27	1.07	primosomal replication protein n	DNA metabolism
SO4488		3.27	1.61	sensor histidine kinase	Signal transduction
SO1933	sucD	3.28	1.24	succinyl-CoA synthase, alpha subunit	Energy metabolism
SO2354		3.29	0.69	conserved hypothetical protein	Hypothetical proteins
SO1932	sucC	3.29	0.98	succinyl-CoA synthase, beta subunit	Energy metabolism
SO4210		3.30	1.90	hypothetical protein	
SO0218	secE	3.31	1.04	preprotein translocase, SecE subunit	Protein fate
SO1618		3.34	0.97	conserved hypothetical protein	Hypothetical proteins
SO2777	fabD	3.35	0.61	malonyl CoA-acyl carrier protein transacylase	Fatty acid and phospholipid metabolism

SO1169		3.37	0.26	conserved hypothetical protein TIGR00246	Hypothetical proteins
SO2198		3.38	1.65	conserved hypothetical protein	Hypothetical proteins
SO2362	ccoQ	3.44	1.16	cytochrome c oxidase, cbb3-type, CcoQ subunit	Energy metabolism
SO1809	pspC	3.45	1.84	phage shock protein C	Cellular processes
SO1347	lepB	3.47	0.36	signal peptidase I	Protein fate
SO1808	pspB	3.49	1.42	phage shock protein B	Cellular processes
SO2960		3.51	0.63	conserved hypothetical protein	Hypothetical proteins
SO3934		3.53	1.47	RNA methyltransferase, TrmH family, group 3	Protein synthesis
SO1725	pstB-1	3.56	2.75	phosphate ABC transporter, ATP-binding protein	Transport and binding proteins
SO2971		3.56	0.85	conserved hypothetical protein	Hypothetical proteins
SO2940		3.58	1.09	prophage LambdaSo, host specificity protein J, putative	Other categories
SO1931	sucB	3.60	1.59	2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase	Energy metabolism
SO2953	H	3.61	1.02	prophage LambdaSo, tail length tape meausure protein	Other categories
SO1288	rpsU	3.63	2.82	ribosomal protein S21	Protein synthesis
SO1107	nqrE-2	3.63	1.62	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE	Energy metabolism
SO1559	phoR	3.68	1.86	phosphate regulon sensor protein PhoR	Signal transduction
SO3364		3.68	1.63	conserved hypothetical protein	Hypothetical proteins
SO2957		3.69	1.27	conserved hypothetical protein	Hypothetical proteins
SO3590		3.71	1.75	hypothetical protein	
SO4489		3.76	0.91	acetyltransferase, GNAT family	Unknown function
SO0110		3.77	1.56	conserved hypothetical protein	Hypothetical proteins
SO1891		3.77	1.80	3-oxoadipate CoA-succinyl transferase, beta subunit	Energy metabolism
SO2955		3.81	0.84	conserved hypothetical protein	Hypothetical proteins
SO0252	rpmJ	3.82	1.59	ribosomal protein L36	Protein synthesis
SO1383		3.84	1.44	ATP-dependent RNA helicase, DEAD box family	Transcription
SO4729		3.84	1.91	conserved hypothetical protein	Hypothetical proteins
SO0004		3.88	0.64	inner membrane protein, 60 kDa	Cell envelope
SO2958		3.90	1.30	conserved hypothetical protein	Hypothetical proteins

SO0300		3.93	1.82	lipoprotein, putative	Cell envelope
SO1351	pdxJ	3.96	0.75	pyridoxal phosphate biosynthetic protein PdxJ	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4510	fdhB-1	4.03	1.57	formate dehydrogenase, iron-sulfur subunit	Energy metabolism
SO1984		4.04	1.43	hypothetical protein	
SO0748		4.05	1.45	hypothetical protein	
SO3646	folA	4.06	1.47	dihydrofolate reductase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2110		4.13	1.52	conserved hypothetical protein	Hypothetical proteins
SO3648		4.14	0.80	conserved hypothetical protein	Hypothetical proteins
SO4318	rtxB	4.16	1.31	toxin secretion ATP-binding protein	Protein fate
SO3912		4.20	0.99	TIM-barrel protein, yjbN family	Unknown function
SO1108	nqrF-2	4.21	2.05	NADH:ubiquinone oxidoreductase, Na translocating, beta subunit	Energy metabolism
SO1205	rbfA	4.34	1.57	ribosome-binding factor A	Transcription
SO2968		4.38	1.13	conserved hypothetical protein	Hypothetical proteins
SO4626	bioH	4.42	1.43	bioH protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2973		4.43	0.93	prophage LambdaSo, lysozyme, putative	Other categories
SO3099		4.43	1.24	long-chain fatty acid transport protein, putative	Transport and binding proteins
SO3647		4.50	2.19	conserved hypothetical protein	Hypothetical proteins
SO1560		4.51	3.46	phosphate-binding protein	Transport and binding proteins
SO2963		4.57	0.65	prophage LambdaSo, major capsid protein, HK97 family	Other categories
SO2888		4.64	1.96	hypothetical protein	
SO1106	nqrD-2	4.65	2.07	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrD	Energy metabolism
SO1724		4.75	2.78	phosphate ABC transporter, permease protein	Transport and binding proteins
SO2962		4.86	0.97	hypothetical protein	
SO2970		4.92	1.54	hypothetical protein	
SO4035		4.96	1.23	hypothetical protein	
SO1983		4.96	1.49	hypothetical protein	
SO1350	recO	5.00	1.77	DNA repair protein RecO	DNA metabolism
SO0007	rpmH	5.03	2.90	ribosomal protein L34	Protein synthesis
SO0768		5.06	2.34	conserved hypothetical protein	Hypothetical proteins
SO1558	phoB	5.22	2.59	phosphate regulon response regulator PhoB	Signal transduction

SO4628		5.25	1.66	sulfatase	Unknown function
SO1245		5.26	0.92	membrane protein, putative	Cell envelope
SO2972		5.31	1.67	hypothetical protein	
SO1499	glgA	5.42	5.26	glycogen synthase	Energy metabolism
SO0769	argR	5.58	2.90	arginine repressor	Regulatory functions
SO2969		5.60	1.27	prophage LambdaSo, holin, putative	Other categories
SO2967		6.01	0.69	conserved hypothetical protein	Hypothetical proteins
SO2887	dsbB	6.14	3.76	disulfide bond formation protein b	Protein fate
SO2964		6.17	0.95	ClpP protease family protein	Protein fate
SO4319		6.21	1.58	HlyD family secretion protein	Transport and binding proteins
SO1194	secF-1	6.25	0.91	protein-export membrane protein SecF	Protein fate
SO1193	secD-1	6.40	2.28	protein-export membrane protein SecD	Protein fate
SO1109	apbE	6.44	2.17	thiamin biosynthesis lipoprotein ApbE	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0006	rnpA	6.77	2.78	ribonuclease P protein component	Transcription
SO1498	glgC	6.86	4.90	glucose-1-phosphate adenylyltransferase	Energy metabolism
SO4323		6.91	2.29	GGDEF domain protein	Unknown function
SO0005		7.00	3.12	conserved hypothetical protein TIGR00278	Hypothetical proteins
SO2965		7.02	0.93	prophage LambdaSo, portal protein, HK97family	Other categories
SO4321		7.10	1.77	OmpA family protein	Cell envelope
SO4320	aggA	7.18	2.36	agglutination protein	Cellular processes
SO4322		7.24	2.46	conserved hypothetical protein	Hypothetical proteins
SO1723		7.86	3.76	phosphate ABC transporter, permease protein	Transport and binding proteins

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures with 2 mM KNO_3 of EtrA7-1 to that of MR-1 (reference).
[b]The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

SUPPLEMENTAL TABLE B.4. Genes repressed in anaerobic cultures with nitrate of EtrA7-1 relative to the wild type (reference strain).

Gene ID	Gene name	Relative expression[a]	STD[b]	COG Annotation	Possible Function
SOA0004		0.02	0.02	type II DNA modification methyltransferase	Energy metabolism
SOA0003		0.03	0.01	type II restriction endonuclease, putative	Other categories
SOA0001		0.03	0.01	replication protein RepA	Unknown function
SO2356	etrA	0.05	0.01	electron transport regulator A	Unknown function
SO0314	speF	0.06	0.06	ornithine decarboxylase, inducible	Hypothetical proteins
SO4513		0.06	0.02	formate dehydrogenase, alpha subunit	Transport and binding proteins
SO4515		0.07	0.01	formate dehydrogenase, C subunit, putative	
SO2099	hoxK	0.07	0.11	quinone-reactive Ni/Fe hydrogenase, small subunit precursor	Energy metabolism
SOA0172		0.07	0.03	site-specific recombinase, resolvase family	Amino acid biosynthesis
SO2097	hydC	0.07	0.04	quinone-reactive Ni/Fe hydrogenase, cytochrome b subunit	Energy metabolism
SOA0061		0.08	0.02	parA protein, putative	Amino acid biosynthesis
SO2492		0.09	0.04	oxidoreductase, acyl-CoA dehydrogenase family	
SOA0060		0.10	0.02	acetyltransferase, GNAT family	Energy metabolism
SO4512		0.10	0.02	conserved hypothetical protein	Fatty acid and phospholipid metabolism
SO0822		0.10	0.04	outer membrane efflux family protein	Energy metabolism
SO2095		0.11	0.09	hypothetical protein	Amino acid biosynthesis
SO2098	hyaB	0.11	0.10	quinone-reactive Ni/Fe hydrogenase, large subunit	Energy metabolism
SO3021	trpD	0.12	0.04	anthranilate phosphoribosyltransferase	Other categories
SO1364		0.12	0.07	iron-sulfur cluster-binding protein	Unknown function
SO3020	trpG	0.12	0.04	glutamine amido-transferase	Unknown function
SO1365		0.12	0.05	hypothetical protein	Hypothetical proteins
SO1363	hcp	0.13	0.08	prismane protein	Transport and binding proteins
SO2395		0.13	0.03	acyl-CoA dehydrogenase family protein	
SO0847	napG	0.14	0.08	iron-sulfur cluster-binding protein NapG	Energy metabolism
SO3022	trpC/F	0.14	0.06	indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase	Amino acid biosynthesis

SO0821		0.14	0.05	ABC transporter, ATP-binding/permease protein	Transport and binding proteins
SO2493		0.14	0.03	transcriptional regulator, TetR family	Regulatory functions
SO2820		0.14	0.04	transposase, IS110 family, degenerate	Disrupted reading frame
SO4476		0.14	0.10	spheroplast protein y precursor, putative	Unknown function
SO2570		0.15	0.06	lipoprotein, putative	Cell envelope
SO0020	fadA	0.15	0.05	fatty oxidation complex, beta subunit	Fatty acid and phospholipid metabolism
SO1519		0.15	0.06	iron-sulfur cluster-binding protein	Energy metabolism
SO0845	napB	0.15	0.04	cytochrome c-type protein NapB	Energy metabolism
SO3820		0.16	0.26	ammonium transporter, degenerate	Transport and binding proteins
SO0021	fadB	0.16	0.04	fatty oxidation complex, alpha subunit	Fatty acid and phospholipid metabolism
SO3484		0.16	0.07	AcrB/AcrD/AcrF family protein	Transport and binding proteins
SO1366		0.16	0.02	sodium/hydrogen exchanger family protein	Transport and binding proteins
SO3019	trpE	0.17	0.10	anthranilate synthase component I	Amino acid biosynthesis
SO2536		0.17	0.06	oxidoreductase, acyl-CoA dehydrogenase family	Unknown function
SO2419		0.17	0.05	2,4-dienoyl-CoA reductase, putative	Fatty acid and phospholipid metabolism
SO1517		0.18	0.11	hypothetical protein	
SO3980		0.18	0.06	cytochrome c552 nitrite reductase	Energy metabolism
SO2912	pflB	0.18	0.11	formate acetyltransferase	Energy metabolism
SO0846	napH	0.18	0.11	iron-sulfur cluster-binding protein napH	Energy metabolism
SO0848	napA	0.18	0.13	periplasmic nitrate reductase	Energy metabolism
SO0935	nhaD	0.18	0.11	Na+/H+ antiporter	Transport and binding proteins
SO2094	hypF	0.19	0.09	hydrogenase maturation protein HypF	Protein fate
SO2228		0.19	0.03	CBS domain protein	Unknown function
ORF03506		0.19	0.06	ammonium transporter (tpt)	Hypothetical proteins
SO3891		0.20	0.11	conserved hypothetical protein	
SO4474		0.20	0.03	hypothetical protein	Energy metabolism
SO2913	pflA	0.20	0.13	pyruvate formate-lyase 1 activating enzyme	Transport and binding proteins
SO0820		0.20	0.05	HlyD family secretion protein	Hypothetical proteins
SO1518		0.21	0.07	conserved hypothetical protein	
SO0403		0.21	0.06	hypothetical protein	Energy metabolism
SO3285	cydB	0.21	0.06	cytochrome d ubiquinol oxidase, subunit II	Hypothetical proteins

ORF02653		0.21	0.09	processing protease, putative	Hypothetical proteins
SO2821		0.21	0.16	conserved hypothetical protein	Energy metabolism
SO1776	mtrB	0.22	0.04	outer membrane protein precursor MtrB	Energy metabolism
SO3286	cydA	0.22	0.10	cytochrome d ubiquinol oxidase, subunit I	Regulatory functions
SO0936		0.22	0.06	transcriptional regulator	Amino acid biosynthesis
SO3023	trpB	0.22	0.05	tryptophan synthase, beta subunit	Hypothetical proteins
SO2914		0.22	0.13	conserved hypothetical protein	Transport and binding proteins
SO3483		0.22	0.08	HlyD family secretion protein	Hypothetical proteins
SO3284		0.22	0.05	conserved hypothetical protein	Energy metabolism
SO0906	nqrE-1	0.23	0.07	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE	Energy metabolism
SO0907	nqrF-1	0.23	0.09	NADH:ubiquinone oxidoreductase, Na translocating, beta subunit	Unknown function
SO3120		0.23	0.13	oxidoreductase, Gfo/Idh/MocA family	Energy metabolism
SO2916	pta	0.23	0.14	phosphate acetyltransferase	Cell envelope
SO1048		0.23	0.02	membrane protein, putative	Energy metabolism
SO2915	ackA	0.24	0.16	acetate kinase	Energy metabolism
SO4404		0.24	0.10	iron-sulfur cluster-binding protein	Hypothetical proteins
SO4714		0.24	0.04	conserved hypothetical protein	Cellular processes
SO2178	ccpA	0.24	0.10	cytochrome c551 peroxidase	Transport and binding proteins
SO4446		0.24	0.12	molybdenum ABC transporter, ATP-binding protein	Energy metabolism
SO1777	mtrA	0.25	0.06	decaheme cytochrome c MtrA	Unknown function
SO1911		0.25	0.17	oxidoreductase, short chain dehydrogenase/reductase family	
SO0404		0.25	0.07	hypothetical protein	
SO1432		0.25	0.07	hypothetical protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0741	ggt-1	0.25	0.11	gamma-glutamyltranspeptidase	Hypothetical proteins
SO1520		0.25	0.07	conserved hypothetical protein	Protein fate
SO3411		0.25	0.15	protease, putative	Hypothetical proteins
SO4302		0.25	0.14	conserved hypothetical protein	Hypothetical proteins
SO3149		0.26	0.11	conserved hypothetical protein	Amino acid biosynthesis
SO3024	trpA	0.26	0.08	tryptophan synthase, alpha subunit	Transport and binding proteins

SO1882		0.26	0.12	AcrB/AcrD/AcrF family protein	Protein fate
SO3564	dcp-2	0.26	0.17	peptidyl-dipeptidase Dcp	Hypothetical proteins
SO2806		0.26	0.03	conserved hypothetical protein	Transport and binding proteins
SO3896		0.26	0.17	outer membrane porin, putative	Fatty acid and phospholipid metabolism
SO3089		0.26	0.05	fatty oxidation complex, beta subunit	Transport and binding proteins
SO2886	nhaB	0.27	0.08	Na+/H+ antiporter	Transport and binding proteins
SO3553		0.27	0.14	sulfate permease family protein	Energy metabolism
SO0905	nqrD-1	0.27	0.14	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrD	Hypothetical proteins
SO1075		0.27	0.14	conserved hypothetical protein	Hypothetical proteins
SO1047		0.27	0.04	conserved hypothetical protein	Disrupted reading frame
SO4538		0.28	0.15	peptidase, M16 family, degenerate	Signal transduction
SO2192		0.28	0.19	sensor histidine kinase	Regulatory functions
SO0490		0.28	0.14	transcriptional regulator	Purines, pyrimidines, nucleosides, and nucleotides
SO2759	upp	0.28	0.14	uracil phosphoribosyltransferase	Energy metabolism
SO0904	nqrC-1	0.28	0.09	NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit	Hypothetical proteins
SO1673		0.28	0.08	outer membrane protein OmpW, putative	Cell envelope
SO1490	adhB	0.28	0.12	alcohol dehydrogenase II	Energy metabolism
SO0487	nosF	0.28	0.06	copper ABC transporter, ATP-binding protein	Transport and binding proteins
SOA0157		0.29	0.11	hypothetical protein	
SO1861	uvrC	0.29	0.06	excinuclease ABC, C subunit	DNA metabolism
SO1430	dmsB-1	0.29	0.04	anaerobic dimethyl sulfoxide reductase, B subunit	Energy metabolism
SO0439		0.29	0.07	hypothetical protein	
SO3148		0.29	0.11	conserved hypothetical protein	Hypothetical proteins
SO0837		0.29	0.11	beta-lactamase, putative	Cellular processes
SO2280		0.29	0.12	bicyclomycin resistance protein	Transport and binding proteins
SO0429		0.29	0.13	peptidase, M13 family	Protein fate
SO4724		0.30	0.07	molybdenum cofactor biosynthesis protein A, putative	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0630	nosA	0.30	0.06	TonB-dependent receptor	Transport and binding proteins
SO1779	omcA	0.30	0.05	decaheme cytochrome c	Energy metabolism

SO3900		0.30	0.16	conserved hypothetical protein	Hypothetical proteins
SO1778	omcB	0.30	0.09	decaheme cytochrome c	Energy metabolism
SO0849	napD	0.30	0.04	napD protein	Energy metabolism
SO4475		0.30	0.10	cation efflux family protein	Transport and binding proteins
SO0398	frdA	0.30	0.16	fumarate reductase flavoprotein subunit	Energy metabolism
SO3542		0.30	0.24	conserved hypothetical protein	Hypothetical proteins
SO4405	katG-2	0.31	0.09	catalase/peroxidase HPI	Cellular processes
SO0908		0.31	0.13	conserved domain protein	Hypothetical proteins
SO0970		0.31	0.17	fumarate reductase flavoprotein subunit precursor	Energy metabolism
SO0827	lldP	0.31	0.07	L-lactate permease	Transport and binding proteins
SO3684		0.31	0.24	transcriptional regulator, TetR family	Regulatory functions
SO1428		0.31	0.05	outer membrane protein	Cell envelope
SO4451	moaC	0.31	0.08	molybdenum cofactor biosynthesis protein C	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2771	garR	0.31	0.08	2-hydroxy-3-oxopropionate reductase	Energy metabolism
SO4537		0.32	0.19	peptidase, putative	Protein fate
SOA0019		0.32	0.07	TnSon1, resolvase	Other categories
SO4447		0.32	0.20	molybdenum ABC transporter, permease protein	Transport and binding proteins
SO0778		0.32	0.11	oxidoreductase, FAD-binding, UbiH/Coq6 family	Unknown function
SO0581		0.32	0.07	hypothetical protein	
SO3420		0.32	0.14	cytochrome c	Energy metabolism
SO2943		0.32	0.11	conserved domain protein	Hypothetical proteins
SO2195		0.32	0.16	inter-alpha-trypsin inhibitor domain protein	Unknown function
SO3088		0.32	0.18	fatty oxidation complex, alpha subunit	Fatty acid and phospholipid metabolism
SO2727		0.32	0.23	cytochrome c3	Energy metabolism
SO4477	cpxR	0.32	0.10	transcriptional regulatory protein CpxR	Signal transduction
SO2907		0.32	0.13	TonB-dependent receptor domain protein	Unknown function
SO1431		0.32	0.05	conserved hypothetical protein	Hypothetical proteins
SO2420	sppA	0.32	0.05	signal peptide peptidase SppA, 67K type	Protein fate
SO2090	hypE	0.32	0.06	hydrogenase expression/formation protein HypE	Protein fate
SO3780	cydD	0.32	0.08	ABC transporter, ATP-binding protein CydD	Transport and binding proteins

SO2469		0.32	0.06	conserved hypothetical protein	Unknown function
SO1900		0.32	0.08	prpE protein, putative	Fatty acid and phospholipid metabolism
SO2093	hypB	0.33	0.15	hydrogenase accessory protein HypB	Protein fate
SO3678		0.33	0.16	conserved hypothetical protein	Hypothetical proteins
SO4634	envZ	0.33	0.20	osmolarity sensor protein EnvZ	Signal transduction
SO4349	ilvC	0.33	0.22	ketol-acid reductoisomerase	Amino acid biosynthesis
SO1122	proA	0.33	0.08	gamma-glutamyl phosphate reductase	Amino acid biosynthesis
SO3563		0.33	0.11	SM-20 domain protein	Unknown function
SO0941		0.33	0.05	hypothetical protein	Signal transduction
SO4478	cpxA	0.34	0.15	sensor protein CpxA	Hypothetical proteins
SO2815		0.34	0.14	CBS domain protein	Hypothetical proteins
SO3361		0.34	0.18	conserved hypothetical protein	Protein fate
SO0876	pepB	0.34	0.10	peptidase B	Hypothetical proteins
SO0856		0.34	0.11	conserved hypothetical protein	Energy metabolism
SO0903	nqrB-1	0.34	0.15	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB	Regulatory functions
SO1669	tyrR	0.34	0.13	transcriptional regulatory protein TyrR	Regulatory functions
SO3059		0.34	0.18	formate hydrogenlyase transcriptional activator, putative	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4713		0.34	0.14	menaquinone-specific isochorismate synthase, putative	Unknown function
SO4716		0.34	0.09	acetyltransferase, GNAT family	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2567	menG-1	0.34	0.07	S-adenosylmethionine:2-demethylmenaquinone methyltransferase	Regulatory functions
SO3778		0.35	0.08	adenylate cyclase CyaB, putative	Transport and binding proteins
SO4448		0.35	0.17	molybdenum ABC transporter, periplasmic molybdenum-binding protein	Hypothetical proteins
SO0944		0.35	0.07	conserved hypothetical protein	Protein fate
SO1561		0.35	0.22	peptidase, M1 family	Energy metabolism
SO1019	nuoCD	0.35	0.13	NADH dehydrogenase I, C/D subunits	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4449	moaE	0.35	0.20	molybdenum cofactor biosynthesis protein E	Unknown function
SO1881		0.35	0.07	HlyD family-related protein	Energy metabolism
SO3144	etfA	0.36	0.13	electron transfer flavoprotein, alpha subunit	Biosynthesis of cofactors, prosthetic groups, and carriers

SO1037	cobU	0.36	0.05	cobinamide kinase/cobinamide phosphate guanylyltransferase	Unknown function
SO2472		0.36	0.05	D-alanyl-D-alanine carboxypeptidase-related protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4723		0.36	0.06	molybdopterin biosynthesis MoeA protein, putative	Hypothetical proteins
SO0883		0.36	0.06	conserved hypothetical protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2587	hemB-1	0.36	0.20	delta-aminolevulinic acid dehydratase	Protein fate
SO3142	dcp-1	0.36	0.21	peptidyl-dipeptidase Dcp	Amino acid biosynthesis
SO1676	metA	0.36	0.13	homoserine O-succinyltransferase	Unknown function
SO0940		0.36	0.08	transcriptional regulator-related protein	Protein fate
SO3083		0.37	0.15	peptidase, M16 family	Fatty acid and phospholipid metabolism
SO2768		0.37	0.07	acyl-CoA dehydrogenase family protein	
SO0975		0.37	0.06	hypothetical protein	Unknown function
SO0882		0.37	0.12	oxidoreductase, GMC family	Cellular processes
SO1278		0.37	0.09	methyl-accepting chemotaxis protein	Cell envelope
SO2103		0.37	0.14	apbE family protein	Cell envelope
SO2194		0.37	0.23	OmpA family protein	Hypothetical proteins
SO2251		0.37	0.10	conserved hypothetical protein	Hypothetical proteins
SO2796		0.37	0.13	conserved hypothetical protein	Unknown function
SO2473		0.38	0.08	hydrolase, alpha/beta fold family	Hypothetical proteins
SO0154		0.38	0.09	conserved hypothetical protein	Regulatory functions
SO0141		0.38	0.08	sensory box protein	Purines, pyrimidines, nucleosides, and nucleotides
SO1221	deoD-2	0.38	0.26	purine nucleoside phosphorylase	Purines, pyrimidines, nucleosides, and nucleotides
SO2791	cdd	0.38	0.12	cytidine deaminase	Signal transduction
SO4633	ompR	0.38	0.19	transcriptional regulatory protein OmpR	Signal transduction
SO1997		0.38	0.15	peptidase, M1 family	Protein fate
SO1695		0.39	0.32	sensory box/GGDEF family protein	Regulatory functions
SO2109		0.39	0.08	conserved hypothetical protein	Hypothetical proteins
SO0399	frdB	0.39	0.06	fumarate reductase iron-sulfur protein	Energy metabolism
SO0584		0.39	0.11	methyl-accepting chemotaxis protein	Cellular processes
SO2601		0.39	0.09	carboxyl-terminal protease	Protein fate
SO4591	cymA	0.39	0.27	tetraheme cytochrome c	Energy metabolism
ORF04299		0.39	0.10	conserved hypothetical protein	

				oxidoreductase, short chain	
SO0438		0.39	0.26	dehydrogenase/reductase family	Unknown function
SO3677		0.40	0.04	hypothetical protein	
SO2504		0.40	0.06	conserved hypothetical protein	Hypothetical proteins
SO2911		0.40	0.20	formate transporter, putative	Transport and binding proteins
SO1513		0.40	0.04	hypothetical protein	
SO0694	galK	0.40	0.10	galactokinase	Energy metabolism
ORF04298		0.40	0.04	conserved hypothetical protein	
SO3121		0.40	0.20	conserved hypothetical protein	Hypothetical proteins
SO2538		0.40	0.11	response regulator	Signal transduction
SO2883		0.40	0.13	conserved hypothetical protein	Hypothetical proteins
SO3151		0.40	0.08	conserved hypothetical protein	Hypothetical proteins
SO1020	nuoB	0.40	0.10	NADH dehydrogenase I, B subunit	Energy metabolism
SO4453		0.40	0.13	electron transfer flavoprotein-ubiquinone oxidoreductase, putative	Energy metabolism
SO2136	adhE	0.40	0.10	aldehyde-alcohol dehydrogenase	Energy metabolism
SO0765		0.41	0.05	threonine efflux protein, putative	Transport and binding proteins
SO2823		0.41	0.03	response regulator	Signal transduction
SO0142	ribB	0.41	0.11	3,4-dihydroxy-2-butanone 4-phosphate synthase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4001		0.41	0.08	chemotaxis protein CheY/response regulator receiver domain protein	Cellular processes
SO3117		0.41	0.11	thioredoxin, putative	Energy metabolism
SO4400		0.41	0.12	proline iminopeptidase, putative	Protein fate
SO2214	gluP	0.41	0.10	glucose/galactose transporter, authentic frameshift	Transport and binding proteins
SO4385		0.41	0.11	von Willebrand factor type A domain protein	Unknown function
SO2019	hemH-1	0.41	0.26	ferrochelatase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0350		0.41	0.08	hypothetical protein	
SO1860		0.41	0.10	DNA-binding response regulator, LuxRfamily	Signal transduction
SO2569		0.41	0.19	hypothetical protein	
SO0582	tpm	0.42	0.05	thiopurine S-methyltransferase	Cellular processes
SO2043		0.42	0.10	conserved hypothetical protein	Hypothetical proteins
SO3152		0.42	0.04	acetyltransferase, CysE/LacA/LpxA/NodL family	Unknown function

SO3389		0.42	0.11	sensory box protein	Regulatory functions
SO2366		0.42	0.22	response regulator	Signal transduction
SO2042		0.42	0.10	conserved hypothetical protein	Hypothetical proteins
SO2264	iscS	0.42	0.27	cysteine desulfurase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0488	nosY	0.42	0.20	copper ABC transporter, permease protein	Central intermediary metabolism
SO1036	cobS	0.42	0.05	cobalamin 5-phosphate synthase	Biosynthesis of cofactors, prosthetic groups, and carriers
ORF04429		0.42	0.13	methyl-accepting chemotaxis protein	
SO1408		0.42	0.14	helicase, putative	Unknown function
SO3119		0.43	0.22	hypothetical protein	
SO1121	proB	0.43	0.12	glutamate 5-kinase	Amino acid biosynthesis
SO2497		0.43	0.13	conserved hypothetical protein	Hypothetical proteins
SO0706		0.43	0.05	conserved hypothetical protein	Hypothetical proteins
SO2191	metC	0.43	0.11	cystathionine beta-lyase	Amino acid biosynthesis
SO2539		0.43	0.10	response regulator	Signal transduction
SO2990		0.43	0.16	prophage LambdaSo, transcriptional regulator, Cro/CI family	Regulatory functions
SO1429	dmaA-1	0.43	0.09	anaerobic dimethyl sulfoxide reductase, A subunit	Energy metabolism
SO3993		0.43	0.31	hypothetical protein	
SO2213		0.43	0.10	oligo-1,6-glucosidase	Energy metabolism
SO4208	hemB-2	0.44	0.15	delta-aminolevulinic acid dehydratase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1899		0.44	0.12	conserved hypothetical protein	Hypothetical proteins
SO1875		0.44	0.07	ISSo4, transposase	Other categories
SO0351		0.44	0.08	DNA-binding response regulator, LuxR family	Signal transduction
SO0708		0.44	0.06	transposase, mutator family	Other categories
SO3025		0.44	0.14	conserved hypothetical protein	Hypothetical proteins
SO4038		0.44	0.24	hypothetical protein	
SO3779	cydC	0.44	0.12	ABC transporter, ATP-binding protein CydC	Transport and binding proteins
SOA0158		0.44	0.18	hypothetical protein	
SO3069		0.44	0.14	conserved hypothetical protein	Hypothetical proteins
SO1018	nuoE	0.44	0.17	NADH dehydrogenase I, E subunit	Energy metabolism
SO3095		0.44	0.07	conserved hypothetical protein	Hypothetical proteins

SO2017		0.45	0.26	conserved hypothetical protein	Hypothetical proteins
SO2905		0.45	0.10	O-methyltransferase, putative	Cellular processes
SO0859		0.45	0.15	sensory box histidine kinase/response regulator	Signal transduction
SO4347	ilvG	0.45	0.20	acetolactate synthase II, large subunit	Amino acid biosynthesis
SO2471	dapE	0.45	0.09	succinyl-diaminopimelate desuccinylase	Amino acid biosynthesis
SO3901	icc	0.45	0.26	lacZ expression regulator	Regulatory functions
SO2114		0.45	0.11	conserved hypothetical protein	Hypothetical proteins
SO0884		0.45	0.08	conserved hypothetical protein	Hypothetical proteins
SO4055	metL	0.45	0.10	aspartokinase II/homoserine dehydrogenase, methionine-sensitive	Amino acid biosynthesis
SO1664	galE	0.45	0.15	UDP-glucose 4-epimerase	Energy metabolism
SO1277		0.45	0.29	proton-dependent oligopeptide transporter (POT) family protein	Transport and binding proteins
SO1888		0.45	0.10	conserved hypothetical protein	Hypothetical proteins
SO2045		0.45	0.08	cation efflux family protein	Transport and binding proteins
SO3094		0.46	0.17	TPR domain protein	Unknown function
SO3898		0.46	0.10	L-sorbosone dehydrogenase, putative	Energy metabolism
SO0839		0.46	0.11	transcriptional regulator, LysR family	Regulatory functions
SO2153	moxR	0.46	0.07	MoxR protein	Unknown function
SO2350	aspC-1	0.46	0.13	aspartate aminotransferase	Amino acid biosynthesis
SO4002		0.46	0.11	sensory transduction histidine kinase	Signal transduction
SO1457		0.46	0.09	type I restriction-modification system, M subunit, putative	DNA metabolism
SO2046		0.46	0.11	transcriptional regulator, MarR family	Regulatory functions
ORF04361		0.46	0.13	proline permease (putP)	Protein fate
SO2196		0.46	0.24	LPXTG-site transpeptidase family protein	
SO3375		0.46	0.14	hypothetical protein	Protein fate
SO2016	htpG	0.46	0.17	heat shock protein HtpG	Biosynthesis of cofactors, prosthetic groups, and carriers
SO0777	ubiH	0.46	0.11	2-octaprenyl-6-methoxyphenol hydroxylase	Regulatory functions
SO4468		0.46	0.13	transcriptional regulator, TetR family	Regulatory functions
SO4603	lexA	0.47	0.12	LexA repressor	Cellular processes
SOA0106		0.47	0.13	methyl-accepting chemotaxis protein	Unknown function
SO2566	asmA	0.47	0.12	asmA protein	Purines, pyrimidines, nucleosides, and nucleotides

				deoxyguanosinetriphosphate	
SO2485		0.47	0.12	triphosphohydrolase, putative	Transport and binding proteins
SO2899	cysZ	0.47	0.13	cysZ protein	Unknown function
SO1049		0.47	0.13	acetyltransferase, GNAT family	Hypothetical proteins
SO0119		0.47	0.11	conserved hypothetical protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4722	mobA	0.47	0.07	molybdopterin-guanine dinucleotide biosynthesis protein	
SO0458		0.47	0.24	hypothetical protein	Transport and binding proteins
SO1522		0.47	0.07	L-lactate permease, putative	Hypothetical proteins
SO3888		0.47	0.16	conserved hypothetical protein	Energy metabolism
SO0274	ppc	0.48	0.19	phosphoenolpyruvate carboxylase	Hypothetical proteins
SO2792		0.48	0.10	conserved hypothetical protein	Cellular processes
SO4226	ftsL	0.48	0.06	cell division protein FtsL	Hypothetical proteins
SO2542		0.48	0.08	conserved hypothetical protein	Hypothetical proteins
SO2882		0.48	0.14	conserved hypothetical protein	
SO1135		0.48	0.10	hypothetical protein	DNA metabolism
SO3899	parE	0.48	0.08	DNA topoisomerase IV, B subunit	Hypothetical proteins
SOA0123		0.48	0.18	conserved domain protein	Cell envelope
SO3270		0.49	0.09	polysaccharide biosynthesis protein	Hypothetical proteins
SO0449		0.49	0.19	conserved hypothetical protein	Hypothetical proteins
SO0324		0.49	0.22	conserved hypothetical protein	DNA metabolism
SO2149	recC	0.49	0.06	exodeoxyribonuclease V, gamma subunit	Hypothetical proteins
SO3776		0.49	0.05	conserved hypothetical protein	Cellular processes
SO4299	cat	0.50	0.06	chloramphenicol acetyltransferase	Energy metabolism
SO0095	hutI	0.50	0.14	imidazolonepropionase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1038	cobQ	0.50	0.19	cobyric acid synthase CobQ	
SO2639		0.50	0.17	hypothetical protein	Cellular processes
SO2898		0.50	0.09	SMC family protein	Protein fate
SO3391		0.50	0.09	ATP-dependent protease, putative	Protein fate
SO0541		0.50	0.14	metallo-beta-lactamase family protein	Unknown function
SO3096		0.50	0.14	RNA polymerase sigma-70 factor, ECF subfamily	Transcription
SO1218	deoA	0.51	0.22	thymidine phosphorylase	Purines, pyrimidines, nucleosides, and nucleotides
SO0269		0.51	0.27	thioredoxin, putative	Energy metabolism

SO1539		0.51	0.12	conserved hypothetical protein	Hypothetical proteins
SO3741	pntB	0.51	0.19	NAD(P) transhydrogenase, beta subunit	Energy metabolism
SO3108		0.51	0.09	siroheme synthase, N-terminal component, putative	Hypothetical proteins
SO4205		0.52	0.28	hypothetical protein	
SO3792		0.52	0.18	conserved hypothetical protein	Hypothetical proteins
SO4452	moaA	0.53	0.21	molybdenum cofactor biosynthesis protein A	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4207		0.54	0.26	GGDEF domain protein	Unknown function
SO4559		0.54	0.29	conserved domain protein	Hypothetical proteins

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures with 2 mM KNO_3 of EtrA7-1 to that of MR-1 (reference).
[b]The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

SUPPLEMENTAL TABLE B.5. Genes induced in anaerobic cultures of EtrA7-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	STD[b]	COG Annotation	Possible Function
SO4714		1.84	0.61	conserved hypothetical protein	Hypothetical proteins
SO1258		1.86	0.64	adenylosuccinate synthetase, putative	Purines, pyrimidines, nucleosides, and nucleotides
SO4150		1.89	0.41	transporter, putative	Transport and binding proteins
SO1821		1.90	0.33	outer membrane porin, putative	Transport and binding proteins
SO2525		1.93	0.58	ABC transporter, ATP-binding protein	Transport and binding proteins
SO1178	corC	1.95	0.31	magnesium and cobalt efflux protein CorC	Transport and binding proteins
SO2350	aspC-1	1.99	0.35	aspartate aminotransferase	Amino acid biosynthesis
SO2796		1.99	0.46	conserved hypothetical protein	Hypothetical proteins
SO0426	lpdA	1.99	0.34	pyruvate dehydrogenase complex, E3 component, lipoamide dehydrogenase	Energy metabolism
SO4503		1.99	0.26	formate dehydrogenase accessory protein FdhD, putative	Energy metabolism
SO1750		2.00	0.31	ABC transporter, ATP-binding protein	Transport and binding proteins
SO4133	udp	2.03	0.31	uridine phosphorylase	Purines, pyrimidines, nucleosides, and nucleotides
SO4723		2.03	0.73	molybdopterin biosynthesis MoeA protein, putative	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4400		2.03	0.55	proline iminopeptidase, putative	Protein fate
SO3708		2.04	0.51	membrane protein, putative	Cell envelope
SO0903	nqrB-1	2.05	0.31	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB	Energy metabolism
SO1172	holA	2.05	0.72	DNA polymerase III, delta subunit	DNA metabolism
SO0441	purD	2.05	0.44	phosphoribosylamine--glycine ligase	Purines, pyrimidines, nucleosides, and nucleotides
SO2218	asnS	2.08	0.52	asparaginyl-tRNA synthetase	Protein synthesis
SO2737	bioD	2.08	0.34	dethiobiotin synthase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2739	bioF	2.12	0.29	8-amino-7-oxononanoate synthase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO4716		2.13	0.45	acetyltransferase, GNAT family	Unknown function
SO0883		2.13	0.36	conserved hypothetical protein	Hypothetical proteins
SO1500		2.13	0.51	sensory box protein	Regulatory functions
SO3993		2.14	0.74	hypothetical protein	
SO4422		2.17	0.48	siderophore receptor, putative, degenerate	Disrupted reading frame

SO4201	aarF	2.17	0.30	ubiquinone biosynthesis protein AarF	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1932	sucC	2.18	0.94	succinyl-CoA synthase, beta subunit	Energy metabolism
SO2907		2.18	0.54	TonB-dependent receptor domain protein	Unknown function
SO3898		2.18	0.33	L-sorbosone dehydrogenase, putative	Energy metabolism
SO2738	bioC	2.18	0.35	biotin synthesis protein BioC	Biosynthesis of cofactors, prosthetic groups, and carriers
SO3409		2.18	0.70	OsmC/Ohr family protein	Unknown function
SO2417		2.19	0.70	iron-sulfur cluster-binding protein	Energy metabolism
SO3901	icc	2.20	0.43	lacZ expression regulator	Regulatory functions
SO1270		2.21	0.69	polyamine ABC transporter, periplasmic polyamine-binding protein	Transport and binding proteins
SO1864		2.21	0.66	hypothetical protein	
SO4742		2.22	0.48	transcriptional regulator, DeoR family	Regulatory functions
SO2472		2.22	0.30	D-alanyl-D-alanine carboxypeptidase-related protein	Unknown function
SO0855		2.23	0.31	conserved hypothetical protein	Hypothetical proteins
SO4502		2.23	0.57	conserved domain protein	Hypothetical proteins
SOA0058		2.24	1.04	hypothetical protein	
SO3900		2.25	0.43	conserved hypothetical protein	Hypothetical proteins
SO0546	rimK-1	2.25	0.79	ribosomal protein S6 modification protein	Protein synthesis
SO3740	pntA	2.25	0.72	NAD(P) transhydrogenase, alpha subunit	Energy metabolism
SO0778		2.25	0.60	oxidoreductase, FAD-binding, UbiH/Coq6 family	Unknown function
SO1121	proB	2.27	0.57	glutamate 5-kinase	Amino acid biosynthesis
ORF02515		2.30	0.49	Bacterial extracellular solute-binding proteins, family 3, putative	
SO4209		2.31	0.78	conserved hypothetical protein	Hypothetical proteins
SO2423		2.32	0.92	hypothetical protein	
SO3540		2.32	0.93	conserved hypothetical protein	Hypothetical proteins
SO1828	tonB2	2.33	0.60	TonB2 protein	Transport and binding proteins
SO1825		2.34	0.35	MotA/TolQ/ExbB proton channel family protein	Transport and binding proteins
SO2473		2.34	0.62	hydrolase, alpha/beta fold family	Unknown function
SO0264	scyA	2.35	0.74	cytochrome c	Energy metabolism

SO1861	uvrC	2.35	0.31	excinuclease ABC, C subunit	DNA metabolism
SO3737	cysI	2.36	1.09	sulfite reductase (NADPH) hemoprotein beta-component (cysI)	Central intermediary metabolism
SO4504		2.36	0.56	conserved hypothetical protein	Hypothetical proteins
SO2916	pta	2.36	1.12	phosphate acetyltransferase	Energy metabolism
SO0930	tkt	2.36	0.58	transketolase	Energy metabolism
SO4200		2.37	0.36	conserved hypothetical protein	Hypothetical proteins
SO4506		2.38	0.48	iron-sulfur cluster-binding protein	Energy metabolism
SO1075		2.38	0.60	conserved hypothetical protein	Hypothetical proteins
SO0904	nqrC-1	2.39	1.05	NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit	Energy metabolism
SO1827	exbD2	2.39	0.48	TonB system transport protein ExbD2	Transport and binding proteins
SO1522		2.40	0.41	L-lactate permease, putative	Transport and binding proteins
SO2424		2.42	0.64	zinc carboxypeptidase domain protein	Unknown function
SO4204	tatC	2.42	0.48	Sec-independent periplasmic protein translocation protein TatC	Protein fate
SO4328		2.42	0.33	AcrB/AcrD/AcrF family protein, authentic frameshift	Cellular processes
SO0344	prpC	2.43	0.33	methylcitrate synthase	Energy metabolism
SO4505		2.43	0.37	conserved hypothetical protein	Hypothetical proteins
SO1095		2.44	0.71	O-acetylhomoserine (thiol)-lyase, putative	Amino acid biosynthesis
SO4511		2.45	1.49	formate dehydrogenase, C subunit, putative	Energy metabolism
SO1824		2.46	0.45	conserved hypothetical protein	Hypothetical proteins
SO4507		2.46	0.58	TorA specific chaperone, putative	Energy metabolism
SO1517		2.48	0.75	hypothetical protein	
SO4205		2.49	0.48	hypothetical protein	
SO1122	proA	2.50	0.42	gamma-glutamyl phosphate reductase	Amino acid biosynthesis
ORF02714		2.53	0.51	phosphate binding protein	
SO1520		2.53	0.47	conserved hypothetical protein	Hypothetical proteins
SO0403		2.54	1.03	hypothetical protein	
SO2544		2.56	0.91	sensor histidine kinase/response regulator	Signal transduction
SO1518		2.56	0.50	conserved hypothetical protein	Hypothetical proteins
SO2912	pflB	2.59	0.80	formate acetyltransferase	Energy metabolism
SO2489	zwf	2.60	0.44	glucose-6-phosphate 1-dehydrogenase	Energy metabolism

SO0906	nqrE-1	2.61	1.29	NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE	Energy metabolism
SO0907	nqrF-1	2.62	1.06	NADH:ubiquinone oxidoreductase, Na translocating, beta subunit	Energy metabolism
SO4066		2.62	0.33	phosphoribosylaminoimidazole-succinocarboxamide synthase, putative	Purines, pyrimidines, nucleosides, and nucleotides
SO1826	exbB2	2.63	0.47	TonB system transport protein ExbB2	Transport and binding proteins
SO0758		2.64	0.97	hypothetical protein	
SO3741	pntB	2.65	0.82	NAD(P) transhydrogenase, beta subunit	Energy metabolism
SO1784	feoB	2.65	0.51	ferrous iron transport protein B	Transport and binding proteins
SO4210		2.65	0.59	hypothetical protein	
SO1521		2.66	0.85	iron-sulfur cluster-binding protein	Energy metabolism
SO4655	cysA-2	2.68	0.63	sulfate ABC transporter, ATP-binding protein	Transport and binding proteins
SO0425	aceF	2.68	0.44	pyruvate dehydrogenase complex, E2 component, dihydrolipoamide acetyltransferase	Energy metabolism
SO3285	cydB	2.68	1.41	cytochrome d ubiquinol oxidase, subunit II	Energy metabolism
SO4509		2.69	1.19	formate dehydrogenase, alpha subunit	Energy metabolism
SO0343	acnA	2.71	0.68	aconitate hydratase 1	Energy metabolism
SO2304	ald	2.71	0.48	alanine dehydrogenase, authentic point mutation	Energy metabolism
SO4508		2.71	1.28	hypothetical protein	
SO0424	aceE	2.72	0.88	pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase	Energy metabolism
SO3407		2.73	1.03	conserved hypothetical protein	Hypothetical proteins
SO1273		2.74	1.02	polyamine ABC transporter, permease protein	Transport and binding proteins
SO0757		2.74	0.74	TPR domain protein	Unknown function
SO1519		2.75	0.68	iron-sulfur cluster-binding protein	Energy metabolism
SO4472	ntrC	2.76	1.37	nitrogen regulation protein NR(I)	Signal transduction
SO3722		2.77	0.93	conserved hypothetical protein	Hypothetical proteins
SO3914		2.79	1.96	TonB-dependent receptor, putative	Transport and binding proteins
SO3736	cysH	2.79	0.79	phosphoadenosine phosphosulfate reductase	Central intermediary metabolism
SO3896		2.80	0.85	outer membrane porin, putative	Transport and binding proteins
SO0827	lldP	2.80	1.10	L-lactate permease	Transport and binding proteins

SO0450		2.84	0.48	major facilitator family protein	Transport and binding proteins
SO4656		2.86	1.06	hypothetical protein	
SO4471	ntrB	2.89	1.40	nitrogen regulation protein	Signal transduction
SO4206		2.89	0.46	hydrolase, TatD family	Unknown function
SO0718		2.96	0.83	conserved hypothetical protein	Hypothetical proteins
SO4722	mobA	2.96	0.28	molybdopterin-guanine dinucleotide biosynthesis protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1271		3.00	0.54	polyamine ABC transporter, ATP-binding protein	Transport and binding proteins
SO0782		3.03	0.83	hypothetical protein	
SO1272		3.06	0.75	polyamine ABC transporter, permease protein	Transport and binding proteins
SO2913	pflA	3.14	1.01	pyruvate formate-lyase 1 activating enzyme	Energy metabolism
SO4721		3.14	0.36	ABC transporter, ATP-binding protein	Transport and binding proteins
SO4055	metL	3.14	0.81	aspartokinase II/homoserine dehydrogenase, methionine-sensitive	Amino acid biosynthesis
SO0862	serA	3.16	0.68	D-3-phosphoglycerate dehydrogenase	Amino acid biosynthesis
SO3973		3.16	2.28	RIO1/ZK632.3/MJ0444 family, putative	Unknown function
ORF02801		3.20	1.28	ferric aerobactin receptor, putative""	
SO0273		3.25	1.91	conserved hypothetical protein	Hypothetical proteins
SO3820		3.26	1.77	ammonium transporter, degenerate	Transport and binding proteins
SO2915	ackA	3.28	1.36	acetate kinase	Energy metabolism
SO2488	pgl	3.30	1.01	6-phosphogluconolactonase	Energy metabolism
SO4724		3.33	0.47	molybdenum cofactor biosynthesis protein A, putative	Biosynthesis of cofactors, prosthetic groups, and carriers
SO2914		3.34	0.77	conserved hypothetical protein	Hypothetical proteins
SO0761	glnB-1	3.37	3.05	nitrogen regulatory protein P-II 1	Regulatory functions
ORF03506		3.38	1.44	ammonium transporter (tpt)	Transport and binding proteins
SO4720		3.42	0.43	ABC transporter, permease protein	Central intermediary metabolism
SO4741	glmS	3.44	0.87	glucosamine--fructose-6-phosphate aminotransferase (isomerizing)	Energy metabolism
SO2486	eda	3.45	0.96	2-deydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate aldolase	Energy metabolism
SO0716		3.61	1.22	monoheme cytochrome c, putative	Energy metabolism
SO0717		3.66	1.05	monoheme cytochrome c	Hypothetical proteins
SO4719		3.88	1.14	conserved hypothetical protein	Hypothetical proteins

SO0449		3.88	1.91	conserved hypothetical protein	Transport and binding proteins
SO4652	sbp	3.93	0.61	sulfate ABC transporter, periplasmic sulfate-binding protein	Fatty acid and phospholipid metabolism
SO1486		3.96	2.18	cytosolic long-chain acyl-CoA thioester hydrolase family protein	Energy metabolism
SO2487	edd	4.01	0.77	6-phosphogluconate dehydratase	Energy metabolism
SO0274	ppc	4.17	1.61	phosphoenolpyruvate carboxylase	Unknown function
SO0715		4.36	0.65	oxidoreductase, molybdopterin-binding	Transport and binding proteins
SO2427		4.53	1.33	TonB-dependent receptor, putative	Amino acid biosynthesis
SO4410	glnA	6.18	3.91	glutamine synthetase, type I	Energy metabolism
SO1494	glgB	6.28	8.81	1,4-alpha-glucan branching enzyme	Energy metabolism
SO1499	glgA	6.54	9.60	glycogen synthase	Energy metabolism
SO1493	malQ	6.56	8.84	4-alpha-glucanotransferase	Hypothetical proteins
SO0547		7.45	5.54	conserved hypothetical protein	Transport and binding proteins
SO0760	amt	7.79	6.40	ammonium transporter	Transport and binding proteins

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures of EtrA7-1 grown at 40 mM KNO_3 to that of the anaerobically grown at 1 mM KNO_3 (reference).

[b] The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.

SUPPLEMENTAL TABLE B.6. Genes repressed in anaerobic cultures of EtrA7-1 at 1 mM (reference) versus 40 mM KNO_3.

Gene ID	Gene name	Relative expression[a]	STD[b]	COG Annotation	Possible Function
SO2194		0.02	0.00	OmpA family protein	Cell envelope
SO2195		0.02	0.01	inter-alpha-trypsin inhibitor domain protein	Unknown function
SO4645		0.03	0.01	hypothetical protein	
SO0837		0.03	0.01	beta-lactamase, putative	Cellular processes
SO2196		0.03	0.01	LPXTG-site transpeptidase family protein	Protein fate
SO4334		0.03	0.01	inner membrane protein, putative	Cell envelope
SO2193		0.04	0.03	DNA-binding response regulator	Signal transduction
SO4335		0.05	0.02	phosphatidylglycerophosphatase B, putative	Fatty acid and phospholipid metabolism
SO3846		0.06	0.01	conserved hypothetical protein	Hypothetical proteins
SO1970		0.07	0.02	hypothetical protein	
SO0839		0.08	0.01	transcriptional regulator, LysR family	Regulatory functions
SO1976		0.08	0.03	hydrolase, alpha/beta fold family	Unknown function
SO1924		0.09	0.02	AcrB/AcrD/AcrF family protein	Transport and binding proteins
SO1560		0.10	0.04	phosphate-binding protein	Transport and binding proteins
SO1558	phoB	0.11	0.04	phosphate regulon response regulator PhoB	Signal transduction
SO0838		0.11	0.03	hypothetical protein	
SO1158		0.11	0.03	Dps family protein	Cellular processes
SO2263		0.11	0.05	Rrf2 family protein	Unknown function
SO2192		0.11	0.04	sensor histidine kinase	Signal transduction
SO1723		0.12	0.06	phosphate ABC transporter, permease protein, putative	Transport and binding proteins
SO1808	pspB	0.12	0.06	phage shock protein B	Cellular processes
SO4646		0.13	0.02	hypothetical protein	
SO4647		0.13	0.03	DNA-binding response regulator	Signal transduction
SO2197		0.13	0.02	GGDEF family protein	Unknown function
SO4052		0.14	0.09	transcriptional regulator, MarR family	Regulatory functions
SO1809	pspC	0.14	0.05	phage shock protein C	Cellular processes
SO3585		0.14	0.05	azoreductase, putative	Cellular processes

SOA0164		0.14	0.05	iron-containing alcohol dehydrogenase	Unknown function
SO4337		0.15	0.02	transcriptional regulator, AsnC family	Regulatory functions
SO4344	ilvA	0.16	0.06	threonine dehydratase	Amino acid biosynthesis
SO3841		0.16	0.02	hypothetical protein	
SO3586		0.17	0.07	glyoxalase family protein	Unknown function
SO2264	iscS	0.17	0.11	cysteine desulfurase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1925		0.17	0.08	HlyD family secretion protein	Transport and binding proteins
SO3364		0.17	0.06	conserved hypothetical protein	Hypothetical proteins
SO3840		0.17	0.03	RNA polymerase sigma-70 factor, ECF subfamily	Transcription
SO3909		0.18	0.05	conserved hypothetical protein	Hypothetical proteins
SO4349	ilvC	0.18	0.11	ketol-acid reductoisomerase	Amino acid biosynthesis
SO1807	pspA	0.18	0.07	phage shock protein A	Cellular processes
SO2191	metC	0.19	0.04	cystathionine beta-lyase	Amino acid biosynthesis
SO4051		0.19	0.05	HlyD family secretion protein	Transport and binding proteins
SO1044		0.20	0.07	amino acid ABC transporter, periplasmic amino acid-binding protein	Transport and binding proteins
SO3942		0.20	0.03	serine protease, HtrA/DegQ/DegS family	Protein fate
SO3552		0.21	0.07	von Willebrand factor type A domain protein	Unknown function
SO4428		0.21	0.05	DNA-binding response regulator	Signal transduction
SO1971		0.21	0.05	AMP-binding family protein	Unknown function
SO1070	katB	0.22	0.21	catalase	Cellular processes
SO4429		0.22	0.03	hypothetical protein	
SO0443		0.23	0.06	transcriptional regulator, MerR family	Regulatory functions
SO1047		0.23	0.07	conserved hypothetical protein	Hypothetical proteins
SO4345	ilvD	0.23	0.05	dihydroxy-acid dehydratase	Amino acid biosynthesis
SO0552		0.23	0.04	hypothetical protein	
SO0725	katG-1	0.24	0.10	catalase/peroxidase HPI	Cellular processes
SO1923		0.24	0.04	AcrB/AcrD/AcrF family protein	Transport and binding proteins
SO3587		0.24	0.11	hypothetical protein	
SO3412		0.25	0.09	extracellular solute-binding proteins, family 3 protein	Transport and binding proteins
SO0112		0.26	0.07	conserved hypothetical protein	Hypothetical proteins

SO4346	ilvM	0.26	0.09	acetolactate synthase II, small subunit	Amino acid biosynthesis
SO4513		0.26	0.34	formate dehydrogenase, alpha subunit	Energy metabolism
SO2265		0.27	0.19	NifU family protein	Unknown function
SO3908		0.27	0.07	enoyl-CoA hydratase/isomerase family protein	Fatty acid and phospholipid metabolism
SO0769	argR	0.27	0.12	arginine repressor	Regulatory functions
SO4347	ilvG	0.27	0.08	acetolactate synthase II, large subunit	Amino acid biosynthesis
SO0946		0.27	0.09	conserved hypothetical protein	Hypothetical proteins
SO1983		0.28	0.09	hypothetical protein	
SO3410		0.28	0.04	hypothetical protein	
SO3774		0.28	0.05	proline dehydrogenase/delta-1-pyrroline-5-carboxylate dehydrogenase, putative	Energy metabolism
SO3588		0.28	0.12	gpr1/fun34/yaaH family protein	Cell envelope
SO0150		0.28	0.11	lipoprotein, putative	Cell envelope
SO1984		0.28	0.09	hypothetical protein	
SO0554		0.29	0.07	hypothetical protein	
SO4515		0.29	0.26	formate dehydrogenase, C subunit, putative	Energy metabolism
SOA0161		0.29	0.09	zinc-binding dehydrogenase	Unknown function
SO4649		0.30	0.09	conserved hypothetical protein	Hypothetical proteins
SO1557		0.30	0.09	outer membrane porin, putative	Transport and binding proteins
SO4292	pstS	0.30	0.10	phosphate ABC transporter, periplasmic phosphate-binding protein	Transport and binding proteins
SOA0160		0.30	0.06	esterase, putative	Unknown function
SO3823		0.31	0.10	ISSo5, transposase, interruption	Disrupted reading frame
SO4427		0.31	0.05	sensor histidine kinase	Signal transduction
SO3422	yfiA-2	0.31	0.12	ribosomal subunit interface protein	Protein synthesis
SO1682	mmsB	0.31	0.18	3-hydroxyisobutyrate dehydrogenase	Energy metabolism
SO1428		0.31	0.19	outer membrane protein	Cell envelope
SO1810		0.31	0.06	conserved hypothetical protein	Hypothetical proteins
SO0551		0.32	0.09	conserved hypothetical protein	Hypothetical proteins
SO0276	argB	0.32	0.15	acetylglutamate kinase	Amino acid biosynthesis
SO1689		0.32	0.21	cation transport ATPase, E1-E2 family	Transport and binding proteins
SO1681		0.32	0.05	enoyl-CoA hydratase/isomerase family protein	Fatty acid and phospholipid metabolism

SOA0060		0.32	0.14	acetyltransferase, GNAT family	Unknown function
SO1048		0.33	0.07	membrane protein, putative	Cell envelope
SO4477	cpxR	0.33	0.12	transcriptional regulatory protein CpxR	Signal transduction
SO4473		0.33	0.06	outer membrane protein, putative	Cell envelope
SO3560		0.33	0.13	peptidase, M16 family	Protein fate
SO0923		0.33	0.09	conserved hypothetical protein	Hypothetical proteins
SO2924		0.33	0.17	signal peptidase I family protein	Protein fate
SO3101		0.33	0.11	conserved hypothetical protein	Hypothetical proteins
SO0519		0.34	0.10	cation efflux protein, putative	Transport and binding proteins
SO4664		0.34	0.07	conserved hypothetical protein	Hypothetical proteins
SO1946	phoP	0.34	0.10	transcriptional regulatory protein PhoP	Signal transduction
SO0753		0.34	0.09	hypothetical protein	
SOA0061		0.35	0.29	parA protein, putative	Other categories
SO1892	atoD	0.35	0.05	acetate CoA-transferase, subunit A	Energy metabolism
SO0444		0.35	0.11	hypothetical protein	
SO1049		0.35	0.06	acetyltransferase, GNAT family	Unknown function
SO1893	mvaB	0.35	0.05	hydroxymethylglutaryl-CoA lyase	Central intermediary metabolism
SO3770		0.35	0.10	conserved hypothetical protein TIGR00153	Hypothetical proteins
SO1894		0.35	0.07	acetyl-CoA carboxylase, biotin carboxylase, putative	Fatty acid and phospholipid metabolism
SO1427		0.35	0.12	decaheme cytochrome c	Energy metabolism
SO0516		0.36	0.13	hypothetical protein	
SO2228		0.36	0.14	CBS domain protein	Unknown function
SO3392		0.36	0.08	oxidoreductase, FMN-binding	Unknown function
SO4626	bioH	0.36	0.25	bioH protein	Biosynthesis of cofactors, prosthetic groups, and carriers
SO1680		0.37	0.10	enoyl-CoA hydratase/isomerase family protein	Fatty acid and phospholipid metabolism
SO3414	thrB	0.37	0.16	homoserine kinase	Amino acid biosynthesis
SO1787		0.37	0.17	conserved hypothetical protein	Hypothetical proteins
ORF03269		0.37	0.10	hypothetical protein	Hypothetical proteins
SO2672		0.37	0.06	conserved hypothetical protein	Energy metabolism
SO1891		0.37	0.07	3-oxoadipate CoA-succinyl transferase, beta subunit	Signal transduction
SO4648		0.37	0.06	sensor histidine kinase	Unknown function

SO2266		0.37	0.29	HesB/YadR/YfhF family protein	Transport and binding proteins
SO1043		0.37	0.07	amino acid ABC transporter, permease protein	Transcription
SO4583	rpoH	0.38	0.16	RNA polymerase sigma-32 factor	Hypothetical proteins
SO2893		0.38	0.07	conserved hypothetical protein	
SO0322		0.38	0.10	hypothetical protein	Hypothetical proteins
SO3343		0.38	0.11	conserved hypothetical protein	Hypothetical proteins
SO1064		0.38	0.08	conserved hypothetical protein	Hypothetical proteins
SO0768		0.38	0.07	conserved hypothetical protein	Transcription
SO1205	rbfA	0.38	0.11	ribosome-binding factor A	Unknown function
SO4343		0.38	0.16	aminotransferase, class V	Regulatory functions
SO1937	fur	0.38	0.14	ferric uptake regulation protein	Amino acid biosynthesis
SO3413	thrC	0.38	0.18	threonine synthase	Unknown function
SO4476		0.39	0.16	spheroplast protein y precursor, putative	
SO1982		0.39	0.20	hypothetical protein	Hypothetical proteins
SO4145		0.39	0.09	conserved hypothetical protein	Hypothetical proteins
SO3842		0.40	0.06	conserved hypothetical protein	Protein fate
SO3659		0.40	0.10	thiol:disulfide interchange protein, putative	
SO4348		0.40	0.22	hypothetical protein	Transport and binding proteins
SO1959		0.40	0.10	ABC transporter, periplasmic substrate-binding protein, putative	Fatty acid and phospholipid metabolism
SO0840		0.41	0.11	acetyl-CoA carboxylase multifunctional enzyme accADC, carboxyl transferase subunit alpha/carboxyl transferase subunit beta/biotin carboxylase	Fatty acid and phospholipid metabolism
SO1895		0.41	0.10	enoyl-CoA hydratase/isomerase family protein	Signal transduction
SO1945	phoQ	0.41	0.10	sensor protein PhoQ	Disrupted reading frame
SOA0163		0.41	0.09	conserved hypothetical protein, degenerate	Hypothetical proteins
SO0113		0.41	0.05	conserved hypothetical protein	Regulatory functions
SO3059		0.41	0.19	formate hydrogenlyase transcriptional activator, putative	Hypothetical proteins
SO4490		0.42	0.11	hypothetical protein	
SO2597		0.42	0.08	conserved hypothetical protein	Hypothetical proteins
SO1691	blc	0.42	0.33	lipoprotein Blc	Cell envelope

SO1326		0.42	0.15	conserved hypothetical protein	Hypothetical proteins
SO1042		0.42	0.09	amino acid ABC transporter, ATP-binding protein	Transport and binding proteins
SO4592		0.42	0.23	hypothetical protein	
SO4036		0.43	0.16	hypothetical protein	
SO2312		0.43	0.09	serine protease inhibitor, ecotin	Protein fate
SO3102		0.43	0.11	AcrA/AcrE family protein	Unknown function
SO3577	clpB	0.43	0.11	clpB protein	Protein fate
SO1072		0.43	0.09	chitin-binding protein, putative	Transport and binding proteins
SO0083		0.43	0.18	carboxymuconolactone decarboxylase family protein	Energy metabolism
SO1206	truB	0.43	0.06	tRNA pseudouridine synthase B	Protein synthesis
SO4558		0.44	0.16	hypothetical protein	
SO1679		0.44	0.13	acyl-CoA dehydrogenase family protein	Fatty acid and phospholipid metabolism
SO0821		0.44	0.19	ABC transporter, ATP-binding/permease protein	Transport and binding proteins
SO3461		0.44	0.09	transporter, putative	Transport and binding proteins
SO2446		0.44	0.12	hypothetical protein	
SO2713		0.44	0.10	transporter, putative	Transport and binding proteins
SO4593		0.44	0.26	hypothetical protein	
SO4619	yhgI	0.44	0.10	yhgI protein	Unknown function
SO4342		0.44	0.26	hypothetical protein	
SOA0109		0.44	0.12	hypothetical protein	
SO3584		0.45	0.10	conserved hypothetical protein	Hypothetical proteins
SO4302		0.45	0.04	conserved hypothetical protein	Hypothetical proteins
SO3063		0.45	0.10	sodium:alanine symporter family protein	Transport and binding proteins
SO2201		0.45	0.12	conserved hypothetical protein	Hypothetical proteins
SO2891		0.45	0.08	conserved hypothetical protein	Hypothetical proteins
SO0335		0.45	0.28	conserved hypothetical protein	Hypothetical proteins
SO1724		0.45	0.14	phosphate ABC transporter, permease protein	Transport and binding proteins
SO4426		0.46	0.10	RNA pseudouridylate synthase family protein	Protein synthesis
SO2720		0.46	0.09	conserved hypothetical protein	Hypothetical proteins
SO3415	thrA	0.46	0.23	aspartokinase I/homoserine dehydrogenase, threonine-sensitive	Amino acid biosynthesis

SO3934		0.46	0.07	RNA methyltransferase, TrmH family, group3	Protein synthesis
SO2198		0.46	0.11	conserved hypothetical protein	Hypothetical proteins
SO0977		0.47	0.12	transcriptional regulator, MarR family	Regulatory functions
SO1835		0.47	0.21	conserved hypothetical protein	Hypothetical proteins
SO1429	dmaA-1	0.47	0.24	anaerobic dimethyl sulfoxide reductase, A subunit	Energy metabolism
SO1478		0.47	0.08	methylase, putative	Unknown function
SO3483		0.47	0.14	HlyD family secretion protein	Transport and binding proteins
SO3715		0.47	0.06	oxygen-insensitive NAD(P)H nitroreductase	Unknown function
SO1126	dnaK	0.47	0.26	chaperone protein DnaK	Protein fate
SO1944		0.48	0.13	hypothetical protein	
SO4628		0.48	0.25	sulfatase	Unknown function
SO2719	deoD-3	0.48	0.14	purine nucleoside phosphorylase	Purines, pyrimidines, nucleosides, and nucleotides
SO2596		0.48	0.07	conserved hypothetical protein	Hypothetical proteins
SO3583	rsuA-1	0.48	0.14	ribosomal small subunit pseudouridine synthase A	Protein synthesis
SO1836		0.48	0.15	conserved hypothetical protein	Hypothetical proteins
SO4478	cpxA	0.49	0.17	sensor protein CpxA	Signal transduction
SO4489		0.49	0.15	acetyltransferase, GNAT family	Unknown function
SO4487		0.49	0.08	DNA-binding response regulator	Signal transduction
SO4070		0.50	0.13	conserved hypothetical protein	Hypothetical proteins
SO2001	ushA	0.50	0.08	5-nucleotidase	Purines, pyrimidines, nucleosides, and nucleotides
SO3935	vacB	0.51	0.11	ribonuclease R	Transcription
SOA0059		0.51	0.09	conserved hypothetical protein	Hypothetical proteins
SO3484		0.51	0.21	AcrB/AcrD/AcrF family protein	Transport and binding proteins
SO1204	infB	0.51	0.16	translation initiation factor IF-2	Protein synthesis
SO0822		0.52	0.22	outer membrane efflux family protein	Transport and binding proteins
SO3103		0.52	0.11	AcrB/AcrD/AcrF family protein	Transport and binding proteins
SO2508		0.53	0.14	RnfAE/NqrDE family protein	Energy metabolism
SO2509		0.57	0.17	iron-sulfur cluster-binding protein	Energy metabolism

[a] The relative expression is presented as the ratio of the dye intensity of the anaerobic cultures of EtrA7-1 grown at 40 mM KNO_3 to that of the anaerobically grown at 1 mM KNO_3 (reference).

[b] The standard deviation was calculated from six data points, which included three independent biological samples and two technical samples for each biological sample.