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ISOLATION, MOLECULAR & PHYSIOLOGICAL
CHARACTERIZATION OF SULFATE-REDUCING,
HETEROTROPHIC, DIAZOTROPHS

BY

ANNALIESE KATRIN JONES

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER'S OF SCIENCE
IN
INTEGRATIVE AND EVOLUTIONARY BIOLOGY

UNIVERSITY OF RHODE ISLAND

2015

MASTER OF SCIENCE
OF
ANNALIESE KATRIN JONES

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ABSTRACT

Nitrogen (N_2) fixation is the process by which N_2 gas is converted to biologically reactive ammonia, and is a cellular capability widely distributed amongst prokaryotes. This process is essential for the input of new, reactive N in a variety of environments. Heterotrophic bacterial N fixers residing in estuarine sediments have only recently been acknowledged as important contributors to the overall N budget of these ecosystems and many specifics about their role in estuarine N cycling remain unknown, partly due to a lack of knowledge about their autecology and a lack of cultivated representatives. Nitrogenase reductase (*nifH*) gene composition and prevalence in Narragansett Bay sediments has revealed that two distinct phylogenetic groups dominate N-fixation. Analysis of *nifH* transcripts has revealed one active group to be the *Desulfovibrionaceae*, belonging to the Deltaproteobacteria. We see *nifH* expression from this group across sampling sites and times, despite the fact that Narragansett Bay sediments are replete with combined N, which is thought to inhibit N fixation in the environment. Here we present the genomic and physiological data in relation to N_2 fixation by two heterotrophic members of the *Desulfovibrionaceae*, isolated from sediments of the Narragansett Bay estuary in 2010 and 2011, respectively: *Desulfovibrio* sp. NAR1 and *Desulfovibrio* sp. NAR2. To elucidate how nitrogenase activity in these organisms responds to the presence of different sources of combined N, and to link observed physiology with genomic potential (i.e. gene content), we performed a two-part study that coupled high-throughput genome sequencing and analysis with physiological investigations of growth on different N sources with N fixation rate measurements. The genomes of the two diazotrophic

Narragansett Bay *Desulfovibrio* isolates (NAR1 and NAR2) were sequenced using a high throughput platform, subsequently assembled, annotated, and investigated for genes related to N fixation and overall N metabolism which were then compared across 34 additional *Desulfovibrio* genomes which were publicly available. To link findings at the molecular level with observations at the physiological level, N fixation rates were measured using the acetylene reduction assay (ARA) under conditions free of reactive N, and under the following combined N conditions: 12 mM urea, 12 mM NO_3^- , and 12 mM NH_3 . Both isolates can sustain growth by N_2 fixation in the absence of biologically available N and our data indicate that nitrogenase activity is completely inhibited by the presence of ammonia, yet uninhibited by nitrate and urea, which are other forms of combined N found in Narragansett Bay. This agrees with observations made at the genome level, as neither our isolates nor the other *Desulfovibrio* examined in comparison appeared to have the genetic capability to use NO_3^- or urea catabolically to meet cellular N demands. This study indicates that the *Desulfovibrionaceae* are restricted in terms of the N sources they are capable of using, and that this may be a factor contributing to the observed N fixation by this group in sediments that are not limited for sources of combined N. Genome sequencing also reveals both isolates to be metabolically versatile and unique. The NAR1 isolate possesses genes involved in bacterial mercury methylation, and displays near obligate biofilm formation. Genes were also found in the NAR1 isolate which suggest the involvement of c-di-GMP in cell-to-cell communication and biofilm formation. This is particularly interesting since biofilm formation and quorum sensing is not well characterized among the *Desulfovibrio*, despite biofilm formation being displayed by many members of this

genus. While investigating the role of these organisms as important contributors of fixed N in Narragansett Bay, it was critical that we examine these additional aspects of their metabolism in order to gain a better understanding of controls on growth that may also impact biomass and the ability of these organisms to achieve significant rates of N fixation in the environment.

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PREFACE

This thesis was formatted in accordance with the manuscript format guidelines established by the Graduate School of the University of Rhode Island.

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INTRODUCTION

The Role of Heterotrophic Diazotrophs in Estuarine Sediments

Estuarine sediments, such as those in Narragansett Bay, have historically been shown to be major regions of nitrogen (N) removal via the activity of denitrifying bacteria (Nixon et al. 1996). Denitrification results in the release of nitrogen (N_2) gas and the loss of biologically reactive N. The opposing process of N fixation converts N_2 gas to biologically reactive ammonia, and has been demonstrated to occur in Narragansett Bay sediments by anaerobic diazotrophs (Fulweiler et al. 2013; Brown & Jenkins 2014). Historically, N fixation activities in marine systems have primarily been attributed to cyanobacterial species residing in euphotic regions of the ocean (Karl et al. 2002) and contributions to this process from anaerobic bacteria were not previously documented. Additionally, due to the energetically costly nature of biological N fixation (Stam et al. 1987) and the typically abundant concentrations of combined N in estuarine systems including Narragansett Bay (Rabalais 2002; Pryor et al. 2007; DiMilia et al. 2011), it was previously thought that any heterotrophic N fixation occurring in the sediments was likely an inconsequential process (Howarth et al. 1988). However, a growing body of evidence involving direct observations of N_2 flux across the sediment water interface in a variety of marine, salt marsh, and sea grass systems (Fulweiler et al. 2007; Gardner et al. 2006; Ferguson et al. 2007; Welsh et al. 1996a; McGlathery et al. 2004) have revealed that heterotrophic N-fixation is an important source of biologically reactive N in these systems and has begun to alter the previous conclusions regarding the role of heterotrophic N fixing bacteria.

In Narragansett Bay the most active of these heterotrophic diazotrophs, based on the number of *nifH* (dinitrogenase reductase) transcripts, belong to two distinct phylogenetic groups; members of the *Desulfovibrionaceae* and the *Geobacteraceae* (Fulweiler et al. 2013). In order to gain a better understanding of the ecology of these active N fixers, we attempted to isolate representatives of both phylogenetic groups from Narragansett Bay sediments. We were initially successful in isolating two organisms belonging to the *Desulfovibrionaceae* (Spinette and Jenkins, unpublished data), which are the topic of this thesis. Members of the *Desulfovibrionaceae* are heterotrophic sulfate (SO_4^{2-}) reducers and have been shown to fix N in culture (Sisler & ZoBell, 1951) as well as under a variety of environmental conditions (Welsh et al. 1996a; Bertics et al. 2012; Brown & Jenkins 2014; Fulweiler et al. 2013; Burns et al. 2002). Members of this group hold particular interest within the scientific community for their ability to perform a wide variety of metabolic functions, including; the reduction of sulfate and sulfur to sulfide species, the ability to use recalcitrant carbon sources, the ability to fix N, and the ability to transform certain heavy metal species (Geets et al. 2006; Annachhatre & Suktrakoolvait, 2001; Sisler & ZoBell, 1951; Parks et al. 2013). Additionally, the *Desulfovibrio* impact a variety of industries and applications. In the oil industry they are considered to be a nuisance, contributing to the souring of oil via sulfide production and causing the corrosion of pipelines and wells (Muyzer & Stams, 2008). In wastewater treatment plants, sulfate reducers are frequently employed as a means of removing sulfates and converting hydrogen sulfide species into precipitated heavy metals (Hulshoff et al. 1998). Similarly, the *Desulfovibrio* play an important role in bioremediation by reducing and immobilizing

heavy metals (Martins et al. 2009). Lastly, these organisms hold potential for use as an alternate energy source in biological fuel cells (Gutierrez-Sanchez et al. 2011).

In addition to the properties described above, these organisms are of particular interest regarding their response in environments that experience seasonal hypoxic periods (Ehrlich, 2014). Respiration rates of aerobic bacteria in the water column are high during hypoxia, which depletes the water column of overall O₂, and reduces the amount of O₂ that can penetrate the sediments. Oxygen depleted sediments are an expanded niche for anaerobic bacteria such as sulfate-reducing heterotrophic N-fixing microbes, such as the *Desulfovibrio*. At the same time, hypoxic conditions prevent the primary process of N removal in these sediments, coupled nitrification-denitrification, from occurring. Sulfide, which results from the respiration of sulfate reducers, directly inhibits nitrification (Joye & Hollibaugh 1995). The inhibition of nitrification means that the subsequent process of denitrification cannot take place, and this hinders removal of reactive N from the sediments. Additional N removal reactions such as anaerobic ammonium oxidation are also inhibited (Thamdrup & Dalsgaard, 2002). The increased N-fixation activity by *Desulfovibrio* spp. and impairment of N removal processes during hypoxic conditions could lead to a greater N inventory, increasing eutrophication and exacerbating hypoxic conditions in estuarine systems like Narragansett Bay (Ehrlich, 2014).

However, despite the multiple applications for the *Desulfovibrio* and previous studies documenting their ecological importance and ability to fix N, there is little previous research investigating their N fixation capabilities and overall N metabolism in a physiological or genomic context. This is likely due to the fact that members of

this genus, and other anaerobic heterotrophic diazotrophs, have only recently begun to be appreciated as significant contributors to N fixation in estuarine systems. As a result investigations have not yet moved beyond those regarding their ecological significance and roles in the environment, which have primarily focused on group abundance and dynamics *in situ*, to examinations of *Desulfovibrio* genome content at the individual level in the context of N fixation capabilities and overall N metabolism. In other words, shifting from questions regarding “who, what, and where” to questions regarding “why and how” these organisms fix N in their respective habitats.

Anaerobic heterotrophic diazotrophs, specifically members of the *Desulfovibrionaceae*, present a unique challenge because currently there is no model or set of supporting physiological data that exist which explain why N-fixation activity by these organisms is observed in environments which are not limited for combined N. The paradigm for aerobic N fixing bacteria is that combined N represses N fixation (Mackerras & Smith 1986; Howarth et al. 1988), and this is something that still remains to be investigated in anaerobic diazotrophs. Similarly, there has been very little investigation at the genomic level into the N metabolism of these organisms, their potential ability as a group to assimilate different combined sources of N, or a specific description of controls on their overall N fixation. We hypothesize that members of this genus may not be capable of using certain forms of N in an assimilatory manner and thus would need to continue to fix N under otherwise N replete conditions. This may be an important contributing factor to what has been seen regarding their activity in the environment (Fulweiler et al 2013; Brown & Jenkins 2014; Ehrlich 2014). Because of their N metabolism and with hypoxia expected to

increase due to anthropogenic activities and climate change (Diaz & Rosenberg, 2008; Middleburg and Levin, 2009), we could see an expanded niche and potentially stimulated N fixation by anaerobic heterotrophs.

Thesis Motivation and Outline

Members of the *Desulfovibrio* were found to be one of the primary bacterial groups responsible for driving N-fixation activity in Narragansett Bay (Brown, 2013; Fulweiler et al. 2013). Previously, sulfate-reducing bacteria (SRB) as a group have been found to fix N in culture (Sisler & ZoBell, 1951) and drive N fixation in a variety of sediments that were not found to be limited for sources of combined N, such as those colonized by *Zostera* spp. (McGlathery et al. 1998; Welsh et al. 1996b), hypoxic sediments from Eckernförde Bay, Germany (Bertics et al. 2012), and anoxic sediments from Chesapeake Bay, USA (Burns et al. 2002).

Heterotrophic N fixers, such as members of the *Desulfovibrio* spp., have only recently been appreciated as important contributors to the overall N budget of estuarine ecosystems. Presently, the majority of these environmentally active microbes have not been isolated in culture and lack complete genome sequence information. Although there are cultured representatives of the *Desulfovibrionaceae*, there remain relatively few members of the *Desulfovibrio* genus with completely sequenced and annotated genomes; there are currently 2 deep sea species, 1 groundwater species, 1 terrestrial species, a handful of estuarine species, and a similarly few extremophilic representatives with completed genomes (Ji et al. 2013; Pedersen et al., 2014; Hauser et al. 2011; Brown et al. 2011; Brown et al. 2011; Nakazawa et al. 2009; Heidelberg et al. 2004; Pradel et al. 2013). Of those representatives with completed genomes there is

still much yet to be discerned regarding their metabolism, nutrient cycling properties, and responses or adaptations to different environmental stressors, particularly from a genomic perspective and more specifically as those factors pertain to N fixation.

There is a similar lack of information regarding N fixation rate data among the *Desulfovibrio*. Although there are previous studies examining N fixation rates for heterotrophic diazotrophs in sediments using the acetylene reduction assay (McGlathery *et al.* 1998; Welsh *et al.* 1996b), these studies have used environmental samples and thus are examining the overall N fixation ability of a mixed microbial consortia and are not able to establish cell-specific rates of N₂ fixation, or a connection between those rates and the responsible cells. This lack of evidence is most likely due to the fact many of these organisms remain uncultured, and where there are cultivated representatives the potential contributions to heterotrophic N fixation by those specific organisms remain largely disregarded.

The lack of cultivated *Desulfovibrio* representatives and corresponding analysis of their genomic capabilities in regards to N fixation and metabolism, combined with a lack of organism specific physiological data regarding N fixation rates and rate responses to environmental N conditions, have provided motivation for the current study. The genomes of two *Desulfovibrio* spp. isolates, *Desulfovibrio* sp. NAR1 and *Desulfovibrio* sp. NAR2 (Spinette & Jenkins, unpublished data), were sequenced, annotated, and analyzed, both independently and comparatively, in reference to other members of this genus, with particular focus on genomic potential involving N fixation and overall N metabolism. Physiological experiments targeting N fixation rates and rate responses to different combined N sources were also performed for the

Desulfovibrio isolate NAR1 using the acetylene reduction assay (Capone, 1993). This study seeks to begin closing the gap between observed environmental activities and a comprehensive genomic view of environmentally relevant members of the *Desulfovibrionaceae*.

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Genome comparison and molecular characterization of two novel diazotrophs from
Narragansett Bay, RI

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Abstract:

The ability to reduce atmospheric nitrogen (N_2) to ammonia, known as N_2 fixation or diazotrophy, is a metabolic capability widely distributed amongst prokaryotes and it is essential for the input of new, reactive N to a variety of environments. Recently, heterotrophic bacterial N fixers residing in estuarine sediments have been acknowledged as important contributors to the overall N budget of these ecosystems. However, many specifics about their role in estuarine N cycling remain unknown, partly due to a lack of understanding regarding their autecology and a lack of cultivated representatives. Nitrogenase reductase (*nifH*) gene composition and prevalence in Narragansett Bay has revealed that two distinct phylogenetic groups dominate N-fixation in these sediments. Analysis of *nifH* transcripts has revealed one of these groups to be members of the *Desulfovibrionaceae*, belonging to the Deltaproteobacteria. To examine how nitrogenase activity in these organisms responds to the presence of different sources of combined N, and to link observed physiology with predicted capability based on gene content, we performed a two-part study that coupled high-throughput genome sequencing and comparative analysis to N fixation rate measurements. Here we present the genomic and physiological data in relation to N_2 fixation by two members of the *Desulfovibrionaceae*, isolated from sediments of the Narragansett Bay estuary in 2010 and 2011, respectively: *Desulfovibrio* sp. NAR1 and *Desulfovibrio* sp. NAR2. Genome sequencing revealed both isolates to be metabolically versatile and unique, and both isolates are capable of sustaining growth by N_2 fixation in the absence of reactive N. Our N fixation rate data indicate that nitrogenase activity is completely inhibited by the presence of ammonia, yet

uninhibited by nitrate and urea. This in turn agrees with observations made at the genome level, as both isolates and the additional *Desulfovibrio* genomes examined in comparison appear to lack the genetic capability to use nitrate or urea catabolically in order to meet cellular N requirements. This study indicates that the *Desulfovibrionaceae* may be limited in terms of the N sources they are capable of using catabolically, which could be a factor contributing to the observed N fixation by this group in sediments that are replete for sources of combined N, such as those of the Narragansett Bay estuary.

Introduction:

Estuarine sediments typically exhibit a nitrogen (N) cycle that is dominated by processes of N removal, such as coupled nitrification and denitrification (1). The opposing process of N₂ fixation by prokaryotic organisms, known as diazotrophs, is the primary source of reactive N to the world's oceans and consequently acts as a control on both the N budget and primary production in many marine ecosystems (2-3).

Historically, N fixation has been a process primarily attributed to cyanobacterial species residing in the water column, although genetic potential to fix N is widely distributed amongst prokaryotes, including members of bacteria and archaea (4). Due to the energetically unfavorable nature of biological N fixation and typically abundant concentrations of combined N found in estuarine systems, heterotrophic N fixation was previously thought to be an inconsequential process in these environments (5). However, recent research involving direct observations of N₂ flux across the sediment-water interface in a variety of marine, salt marsh, and sea

grass systems support that heterotrophic N fixation is an important source of reactive N in these systems, and has begun to alter the historically held conclusions regarding the role of heterotrophic diazotrophs (6–10). Additionally, significant N fixation has been documented in waters where cyanobacteria are not believed to be present or active (11–13).

Previous investigation of heterotrophic N fixation in Narragansett Bay, RI, has revealed that most active N fixing organisms belong to two distinct phylogenetic groups, one of which consists of members of the *Desulfovibrionaceae* (14). Members of the *Desulfovibrio* spp. (*Desulfovibrionaceae*) are heterotrophic sulfate (SO_4^{2-}) reducers and have been shown to fix N in culture (15). Members of this genus are also noted for their ability to perform a wide variety of metabolic functions, including the reduction of sulfate to sulfur and sulfide species, the ability to utilize recalcitrant carbon sources, the ability to fix N, and the ability to transform certain metal species (15–18). Due to their wide variety of metabolic functions the *Desulfovibrio* are important in a number of industries and applications. They are considered a nuisance to the oil industry and can contribute to the souring of oil via the production of sulfides and corrosion of pipelines and wells (19), they are used in wastewater treatment plants as a way to remove sulfates and convert hydrogen sulfide byproducts into precipitated heavy metals (20), they play an important role in bioremediation by reducing and immobilizing heavy metals (21), and they hold potential for use as an alternate energy source in biological fuel cells (22).

Additionally, it has been known for some time that many sulfate reducing bacteria (SRB), particularly members of the *Desulfovibrio*, have the genetic potential

to fix N (23). These organisms have been shown to fix N in a laboratory setting (15, 24) and in a variety of habitats including coral reefs, photosynthetic microbial mats, mangrove sediments, sea grass rhizospheres (25–27), shallow estuarine sediments (6, 14, 28), bioturbated sediments (29), and salt marshes (9). There is further evidence supporting that these organisms play a critical role in supplying fixed N to their environment, particularly in anaerobic or anoxic sediments (14, 30) and benthic sediments which are N deficient (31–33). This mounting evidence has led to the ecological significance of the SRB, in terms of N fixation, being more widely accepted and acknowledged.

However, the majority of investigations involving these diazotrophs have focused on their ecological significance and role in the environment, primarily community composition, and group behavior and dynamics *in situ*. There have been no examinations to date of *Desulfovibrio* genome content in the context of nitrogen fixation capabilities and overall nitrogen metabolism, despite the fact that multiple complete genomes for diazotrophic *Desulfovibrio* exist (34–39). There could be several reasons for this; one potential reason is that as bacterial genomes have become easier and more cost effective to sequence, genome data is now being produced faster than it can be thoroughly analyzed. If this is the cause then it is likely that as analysis catches up with high-throughput sequencing technologies, thorough investigations of *Desulfovibrio* genomes in the context of their N fixation capabilities will begin to emerge. Another possible explanation is that because these diazotrophic SRB have only recently begun to be more widely accepted among the ecological community as important contributors of fixed N to their environments, investigations have not yet

shifted from answering broader questions regarding the ecological significance and role of these diazotrophs in the environment, to more targeted examinations of genome content at the individual level in the context of N fixation capabilities and overall N metabolism.

Of the cultivated *Desulfovibrio* species there remain relatively few environmental representatives with complete (closed and annotated), well-studied genomes. There are currently 2 deep-sea species, 2 freshwater species, a handful of estuarine species, and similarly few extremophilic representatives with complete genomes (34–42). Of the representatives with completed genomes there is still much yet to be discovered regarding their metabolism, nutrient cycling properties, and responses or adaptations to different environmental conditions and stressors, particularly from a molecular perspective as it pertains to the role of these organisms in N cycling.

There is a similar lack of information regarding N fixation rate data among the *Desulfovibrio*. Although there are previous studies examining N fixation rates for heterotrophic diazotrophs in sediments using the acetylene reduction assay (McGlathery *et al.* 1998; Welsh *et al.* 1996b), these studies have used environmental samples and thus are examining the overall N fixation ability of a mixed microbial consortia and are not able to establish cell-specific rates of N₂ fixation, or a connection between those rates and the responsible cells. This lack of evidence is most likely due to the fact many of these organisms remain uncultured, and where there are cultivated representatives the potential contributions to heterotrophic N fixation by those specific organisms remain largely disregarded.

Whatever the underlying cause, there is an observable disconnect between the acknowledged role of the SRB, specifically the *Desulfovibrio*, as N fixers in the environment and our understanding of them at the individual genomic level as it pertains to their documented role as diazotrophs. The data presented here seeks to address this disconnect and begin closing the gap between observed environmental activities and a comprehensive molecular view of ecologically relevant members of the *Desulfovibrionaceae*.

In this study, we report the nitrogenase activity (NA) of two anaerobic, heterotrophic, diazotrophs (isolated from Narragansett Bay, RI sediments) under different combined N sources (ammonia, nitrate, and urea). We then present a comparative analysis of genomic traits related to observed NA and combined N utilization in these organisms in the context of other members of the *Desulfovibrio* genus.

Materials and Methods:

The environmental *Desulfovibrio* sp. examined here were isolated from sulfate-reducing, diazotrophic enrichment cultures inoculated with estuarine sediment gathered at a site off the northern tip of Jamestown, RI (41° 35.292'N 71° 22.748'W) (Spinette and Jenkins, unpublished data). Enrichment cultures were established using anaerobic culture methods described by Hungate (43), and individual species of sulfate reducing bacteria were isolated on roll tubes. NAR1 was isolated from sediment slurry gathered in June 2010 spanning a depth of 0 to 2 cm and NAR2 was isolated from sediment slurry gathered in October of 2010 spanning a depth of 0 to 2 cm.

Growth and Glycerol Stock Preparation of *Desulfovibrio* sp. NAR1

Desulfovibrio sp. NAR1 was grown in several media, all of which were variations on the NBSO media (.2 μ m filtered Narragansett Bay seawater, 60 mM lactate, 1.5 mM sodium thioglycolate, .45 mM sodium sulfide, 1 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, 1 mg/L resazurin as a redox indicator, and 300 mM NaHCO_3) in gas tight Hungate tubes with $\text{N}_2:\text{CO}_2$ gasses (80:20%) in the headspace. All cultures were grown at 21°C. Regular NBSO was used as the primary media for maintaining the isolate in culture, with the richer NBSOY (NBSO + .05% yeast extract) medium used occasionally. Media formulations used in the acetylene reduction assay were NBSO, NBSO + 12 mM NH_3 , NBSO + 12 mM NO_3^- , and NBSO + 12 mM urea. Culture biomass was estimated by calculating the number of genomes present in each culture from the total DNA extracted (for calculations used see Supplemental Figure S1). DNA was extracted from cultures grown on glass beads using the Qiagen DNeasy Plant Mini kit (Qiagen, Gaithersburg, MD, USA) with ~.30 g of glass beads as starting material, and a modified protocol (see Supplemental Information for complete extraction protocol) to lyse cells, the resulting cell lysate was then used in a phenol/chloroform extraction followed by an ethanol precipitation. DNA that was extracted for purposes other than genome sequencing was quantified using a Qubit fluorometer with the Quant-iT high sensitivity assay kit (Life Technologies, Grand Island, NY). Glycerol stocks of *Desulfovibrio* sp. NAR1 were made following a general protocol (see Supplemental Protocol 2), and cultures of NAR1 were successfully recovered from those stocks.

Acetylene Reduction Assay

The NA of *Desulfovibrio* sp. NAR1 was measured under various combined N conditions. 10 mL cultures of NAR1 containing 2 g of 1 mm diameter glass beads in carbonate buffered NBSO, NBSO + 12 mM NH_3 , NBSO + 12 mM NO_3^- , NBSO + 12 mM urea were prepared in either duplicate or triplicate and NA was assessed using the acetylene reduction assay (ARA) and methods described previously by Capone (44). Acetylene was generated in house by reacting calcium carbide with water and was collected in a 1 L Supel-Inert film gas sampling bag. Acetylene was added to the culture tubes to a final concentration of 10 to 20% of the total headspace. A Shimadzu GC8 gas chromatograph (Shimadzu Corporation, Kyoto, Japan), with a 2.5 m long stainless steel column containing Haysep T packing 80/100 mesh, was used to measure ethylene production in all ARAs. The injector and column were set to 130°C and 100°C, respectively. Gas samples of 100 μL were taken from the tube's headspace with a gas tight syringe and immediately injected into the gas chromatograph. Ethylene production was measured over the course of 24 hours. Samples were usually measured 1, 3, 6 and occasionally 24 hours after acetylene was added. Cultures that were part of the same assay were inoculated at the same time using the same parent culture. A set of multiple potential parent cultures was established for each set of experimental tubes; NA was measured in the parent tubes to ensure that the inoculum for the experimental cultures was actively fixing N at the time of inoculation, once NA was established in one member of the parent culture series the next un-tampered parent culture was used to inoculate the experimental culture series. A series of potential parent cultures was necessary, rather than measuring the NA of one culture

and using that same culture as a parent inoculum, to mitigate the negative effects of long term acetylene exposure on the growth of NAR1. Cultures were also no longer sterile after being used in the acetylene reduction assay (ARA), and so could not themselves be used as parents. Additionally, measurement of NA in the parent culture was a critical means of determining when the culture was ready to be used as inoculum, since cell enumeration of the NAR1 isolate remains challenging, and as of yet no hard correlation between culture age and NA has been established.

After establishing a baseline NA using N limited cultures (Fig. 7), ethylene production for cultures of NAR1 grown under different combined N treatments was measured (Fig. 9). NA was measured on separate, triplicate sets of 10mL carbonate-buffered NBSO cultures containing 2g of 1mm diameter glass beads with the addition of one of the following: 12 mM NO_3^- (nitrate set), 12 mM NH_3 (ammonia set), 12 mM urea (urea set). A control set of tubes was grown under previously discussed N limited conditions. The measurements taken on Day 8 and Day 10 utilized triplicate cultures for all combined N treatments and the N limited control, an additional control only time-point with culture samples in duplicate was measured on Day 14 to ensure that NA was decreasing and that peak NA had been observed at the time combined N treatments were being examined. Biomass measurements for all samples used were obtained by extracting DNA from each tube, and then calculating the total number of genomes present based on the amount of DNA extracted. This data is presented in Figure 8. Biomass estimates were then used to normalize ethylene production rate to cell abundance.

Genome sequencing, assembly, annotation, and gene comparison

Desulfovibrio sp. NAR1 was grown under anaerobic conditions in carbonate buffered NBSOY media (filtered Narragansett Bay seawater, 60 mM lactate, 1.5 mM sodium thioglycolate, 450 uM sodium sulfide, 1 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, 0.05% wt/vol yeast extract, 1 mg/L resazurin as a redox indicator, and 300 mM NaHCO_3) with a 20:80% $\text{CO}_2:\text{N}_2$ gas mixture in the headspace, until an optical density of 0.2-0.3 was reached. NAR1 will only exhibit planktonic growth when grown in NBSOY media, and the absence of biofilm was necessary for successful nucleic acid extractions. Once the desired OD had been reached, culture samples were harvested by filtering 2 mL of culture onto 0.2 um polyester filter membranes (Millipore, Darmstadt, Germany). Filters were then either placed into sterile 2 mL screw-cap microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA) tubes and flash frozen using liquid nitrogen for later analysis, or immediately used in DNA extractions. NAR2 cultures were grown under anaerobic conditions in carbonate buffered NBSO media (filtered Narragansett Bay seawater, 60 mM lactate, 1.5 mM sodium thioglycolate, 450 uM sodium sulfide, 1 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, 1 mg/L resazurin as a redox indicator, and 300 mM NaHCO_3) + 12 mM NH_3 with a 20:80% $\text{CO}_2:\text{N}_2$ gas mixture in the headspace, until an optical density of 0.2-0.3 was reached. Cells were harvested in the same manner as NAR1.

Genomic DNA was extracted from polyethylene filters using the Qiagen DNeasy Plant Mini kit (Qiagen, Gaithersburg, MD, USA) with a modified protocol (see supplemental data for complete extraction protocol) to lyse cells, and the resulting cell lysate was then used in a phenol/chloroform extraction followed by an ethanol precipitation. Multiple DNA extracts were pooled together during the ethanol

precipitation step to increase the DNA concentration in the final extract. DNA was re-suspended in 10mM Tris-HCl. DNA quality and quantity was assessed using the Thermo Scientific Nanodrop 2000c, and a Qubit fluorometer was used with the Quant-iT broad range dsDNA assay kit (Life Technologies, Grand Island, NY) as an additional means of quantifying DNA and affirming the concentration reported by the Nanodrop. To verify the purity of the DNA extracts the 16s rDNA and *nifH* genes were amplified using previously described PCR primers and protocols (14, 45). Sanger sequencing of cloned PCR products was performed at the University of Rhode Island Genome and Sequencing Center (GSC). Both genes were analyzed using at least 10 clones, and all clones for each gene were required to perfectly match and align before the isolate culture was considered pure. Genomic DNA from NAR1 and NAR2 was sheared using a Covaris S220 high performance ultra sonicator (Covaris, Woburn, MA, USA) and a paired end (PE) sequencing library (~700 bp inserts) was constructed using the Apollo 324 robot (WaferGen Biosystems, Fremont, CA, USA) and the Illumina Nextera kit chemistry (Epicentre Technologies, Madison, WI, USA). The sequence libraries for both isolates were then multiplexed and run on the Illumina MiSeq instrument according to manufacturers specifications. Preparation of libraries and sequencing of genomes for both isolates was done by the staff of the URI GSC. Resulting read data for both isolates was trimmed using Trimmomatic (46) to remove adapters and low quality bases. Trimmomatic was used in paired-end (PE) mode using the following parameters and settings: -phred33 (use phred 33 scores), ILLUMINACLIP (cut adapters and other Illumina specific sequences from the read) with a seed mismatch of 3, palindrome clip threshold of 25, simple clip threshold of 9,

minimum adapter length of 4, and the keep both reads parameter set to “true”, LEADING (remove low quality bases from start of read) with a minimum quality score threshold of 15, TRAILING (remove low quality bases from end of read) with a minimum quality score threshold of 15, SLIDINGWINDOW (use a region of bases to assess quality) with a window length of 100 bp and a minimum quality score threshold of 15, MAXINFO (perform adaptive quality trim) with a target length of 100 bp and strictness setting of .8, CROP (removes bases from end of read, user specifies number of bases to keep) set to 240 bp, HEADCROP (removes bases from start of read, user specifies number of bases to remove) set to 10 bp, and MINLEN (removes reads that fall below specified minimum length) set to 100 bp. Genome sequences were assembled using SPAdes de-novo assembler version 3.0.0 (47) with the pre-selected k-mer size range for MiSeq 250bp reads (k= 21, 33, 55, 77, 99, 127) and the --careful (read correcting) parameter turned on. The quality of genome assemblies for both isolates was assessed using QUAST (48), the complementary assessment software to the SPAdes assembler. Genome assemblies were further assessed for quality using the specialized assembly evaluation software REAPR (49) as a final step before annotation. Both genomes were annotated using the Integrated Microbial Genomes Expert Review (IMG/ER) (50) pipeline. Genomes were also annotated using Rapid Annotation using Subsystem Technology (RAST) as a point of comparison to IMG/ER, however, IMG/ER annotations were ultimately accepted for the final draft and used in comparative analysis. It is important to note that annotations generated by both annotators agreed across most major metabolic pathways. A separate set of open reading frames (ORFs) was also called for both isolates using command-line Prodigal

(51) as a means to check the ORF calling of both automatic annotators. Some genes, particularly those of importance to this study (e.g. *nif*), were annotated manually using Geneious version 6.1.6. The gene content of both environmental *Desulfovibrio* isolates in comparison to 34 additional *Desulfovibrio* genomes was examined using the Function Analysis feature in the IMG/ER (50) tool set. Results of the analysis in IMG/ER were confirmed visually by examining the sequence and annotating the gene or genes in question using Geneious version 6.1.6, and by TBLASTN.

Phylogenetic Analysis and Evolutionary Gene Networks

20 different vertically transferred marker genes from both of our *Desulfovibrio* isolates and 34 additional *Desulfovibrio* representatives were selected and used as input into a multigene phylogenetic tree. A list of genes can be found in Supplemental Table S4 and a specific list of additional genomes can be found in Supplemental Table S3. The genomes selected for use in this comparison are all complete or well-annotated draft *Desulfovibrio* genomes available in GenBank or IMG databases at the time of this study. The 20 marker genes were extracted from the complete genome sequences or assemblies of all 36 representatives using Geneious v. 6.16. The marker genes were then all aligned separately using MAFFT version 7 with the G-INS-i setting (52), and resulting alignments were concatenated using SequenceMatrix (53). jModelTest (54) was used to assess the best-fit model of nucleotide substitution for each gene alignment. A multi-gene phylogenetic tree (Fig. 1) was constructed from the concatenated gene alignment using Bayesian phylogenetic inference software MrBayes version 3.2.1 (55), with the best-fit model predictions from jModelTest used

as additional input to inform the tree-building software. The program was run for 1000000 generations, which was sufficient for convergence to be reached.

Desulfovibrio sp. NAR1 and *Desulfovibrio* sp. NAR2, their three closest relatives (*D. piezophilus*, *D. aespoeensis* str. Aspo-2, *D. desulfuricans* str. ND132 for NAR1; and *D. acrylicus*, *D. desulfuricans* subsp. aestuarii, *D. alaskensis* str. G20 for NAR2), and *D. vulgaris* str. Hildenborough were chosen for inclusion in evolutionary gene network analysis. The base evolutionary gene network was constructed using EGN (56) , with amino acid multi-FASTA files of all proteins for all included organisms as input. The local alignment software used was for the creation of input files was BLAST, with a minimum e-value setting of 1e-05. The “quicker” algorithm was selected for creating edge files, with an e-value threshold of 1e-05, a hit identity threshold of 65%, a minimum hit length requirement of at least 70% of the smallest homolog, a minimum hit length of 75 amino acids, the best reciprocal threshold of the best e-value was set to 5%, and the minimum match coverage threshold of the sequence length was set to 65%. The resulting base evolutionary gene network was then filtered using a python script (written by Jim Grandpre and Scott Grandpre), to reveal networks that contained only NAR1 and NAR2 (Fig. 3.3), only NAR2 (Fig. 3.4), and only NAR1 (Fig. 3.2).

Whole Genome Alignment

Mauve version 2.3.1 (57) was used to compare FASTA sequences of *Desulfovibrio* sp. NAR1 to the complete genome sequence of *Desulfovibrio piezophilus*, and *Desulfovibrio* sp. NAR2 to the complete genome sequence of *Desulfovibrio alaskensis* str. G20. The contigs of each environmental isolate were

reordered relative to the genome of their respective reference, the final alignment (Fig. 2) shows the reordered draft genomes aligned to their references. Aligning of reordered genomes aided in viewing the regions (local collinear blocks, or LCBs) of draft genomes that were presumably homologous and internally free from genomic rearrangements relative to the parts of the complete genome of their respective references. The reordered draft genomes are also important for any future work involving genome gap closure or additional genome comparisons.

Results and discussion:

Growth and acetylene reduction of *D. sp. NAR1* under N limited conditions

Cultures of NAR1 were maintained under anaerobic conditions on N limited sulfate reducing media NBSO, either with or without glass beads as a biofilm substrate. Under N limiting conditions NAR1 exhibited biofilm growth, making cell enumeration very challenging. Initial attempts to assess growth of the NAR1 isolate using spectrophotometry failed because the isolate grows as a biofilm under almost all conditions; the only condition under which it will not form a biofilm is when it is grown with NBSOY and there is no physical surface present that the isolate can easily adhere to (such as sediment, glass beads, diatomaceous earth, etc.). Cultures of NAR1 grown with NBSOY and no additional physical surface were planktonic and could be measured using spectrophotometry. However, since our assessment of NA in this isolate involved media formulations other than NBSOY that also contained glass beads, spectrophotometry was not a viable option for assessing growth in those cultures. Other methods, such as flow cytometry and qPCR were attempted as a means to measure the growth of NAR1. Flow cytometry was not a practical option as it was

only possible with planktonic cells (grown in rich media), and even non-biofilm cells produced enough exopolymeric substances (EPS) to cause cells to stick to the inside of the injection line and interfere with the functioning of the instrument. Quantitative PCR attempts also failed, most likely due to the EPS produced by this organism. DNA extracts from older cultures did not amplify as well as DNA extracted from younger cultures, suggesting an inhibition of the DNA polymerase enzyme. As DNA concentration was not very high in older cultures (≤ 10 ng/uL), and DNA of comparatively lower concentrations from younger cultures amplified, it is unlikely that DNA concentration is the cause of the failed amplification in DNA samples taken from older cultures. However, it is likely that older cultures have more EPS present, supported by the obvious biofilm formation seen in cultures that are ≥ 2 weeks old. Protein contamination in DNA extracts, most likely due to EPS and biofilm material, was also an issue that was encountered in gathering DNA for genome sequencing, and was resolved by ethanol precipitation and pooling of multiple samples to purify and concentrate the DNA. However, because the goal of DNA extractions from experimental tubes used in the ARA was to enumerate cells in a specific culture, pooling of multiple replicates would defeat the purpose, and the amount of DNA extracted from a single culture was low enough that additional ethanol precipitation to purify DNA and remove EPS would likely cause most of the sample to be lost. For these reasons, qPCR was not a practical method for quantifying biomass in this study. Biomass estimates were ultimately made by extracting DNA from pure cultures grown under N limited conditions, and subsequently using the DNA yield to calculate the total number of genomes present in the culture volume, which was used as

representation of cell abundance. Figure 6 shows the log growth of NAR1 under N limited conditions. This organism exhibits very slow growth and very little change in overall biomass when grown under N limited conditions. However, slow growth is not unusual for environmental bacteria such as members of the *Desulfovibrio* (58), and it is likely that there is some loss of DNA during the extraction process, which would result in a lower reported cell count.

Ethylene production by NAR1 (Fig. 7) was first measured on N limited cultures over a period of ~14 days prior to examining the effects of combined nitrogen on NA in the NAR1 isolate. Previous pilot experiments had shown that no NA occurred before the third day after inoculation, so these time points were not included in the study. Figure 7 shows NA for the NAR1 isolate for the same time period in which growth was measured (Fig. 6). These measurements indicate that peak NA in N limited cultures of NAR1 occurs early on in the growth of the organism, late lag or early log phase, which is a trend that had been observed in previous assays (data not shown). Peak NA is within range of that described for other bacterial isolates (59). The observed high activity on Day 5, when the culture is young and the cell count is lower, may be due to the N demands of the organisms as they prepare to enter log phase. It is unclear at this time whether biofilm formation plays a role in increased NA or overall N demand. Since *Desulfovibrio* biofilms are known to be composed primarily of protein (60, 61), this remains a possibility and should be considered in future investigations. Further studies would be needed in order to elucidate what specific factors contribute to timing of peak NA in this isolate.

Growth and acetylene reduction of NAR1 under differing combined N conditions

After establishing a baseline NA using N limited cultures (see previous section), ethylene production for cultures of NAR1 grown under different combined N treatments was measured using the ARA (Fig. 9). NA was not observed with the ammonia treatment at either time-point and this agrees with what is known regarding nitrogenase gene regulation in response to ammonium availability for aerobic N fixers (62), although the inhibition of NA by ammonia has not previously been established for sulfate-reducing diazotrophs, or other anaerobic N fixers. No negative effect on NA was observed with either the nitrate treatment or the urea treatment, which is not in agreement with the previously held idea that NA is inhibited by other sources of combined nitrogen (63). The fact that neither nitrate nor urea have an inhibitory effect on NA also agrees with our genomic analysis of this isolate, since there is nothing in the genome of NAR1 to suggest that this organism can use either nitrate or ammonia as a source of N. Rates of ethylene production for the nitrate treatment are very similar to those of the N limited control, while the rates for the urea treatment are slightly higher for both time-points. This increase in NA with the presence of urea has been observed in previous assays with this isolate, and the cause is currently unknown. As the organism lacks any evidence of a urease or a urea amidolyase, it is unlikely that urea is being broken down into any intermediate that could be affecting N fixation, or utilized as a carbon source, which could affect growth. The lack of effect on growth is further demonstrated by examining cell abundance for the urea treatment at both time-points (Fig. 8), which is very similar to that of the N limited control.

The total cell abundance data corresponding to the ARA experiment does not follow what would be expected for the typical logarithmic growth of bacteria. There could be several reasons for this, the most likely cause having to do with how the replicates are set up and inoculated. Since NAR1 grows in a biofilm, it is very difficult to control how many cells are contained in the inoculum, even when the same volume of culture is used for every inoculation. Because it is impossible to ensure a homogenous suspension of cells with a biofilm-forming organism, it is likely that one tube may be inoculated with a large segment of dislodged biofilm while another tube may receive fewer cells in comparison. Currently, our methods do not allow us to break up the biofilm into segments that can be accurately quantified and then used as inoculum, or to force NAR1 to grow planktonically under N limited conditions. Keeping in mind that replicates in this experiment are separate cultures and that Fig. 8 shows the total number of cells present in these cultures measured at the respective time-point, instead of the same culture with change in cell abundance quantified over time, it is plausible that the lower cell count on day 10 for all treatments except nitrate is due to fewer cells being present in the inoculum used for those cultures compared to the day 8 cultures. Possibly inflated ethylene production rates due to discrepancies in culture density are corrected for by normalizing ethylene production rates to cell abundance.

Genome sequencing outputs, assembly and annotation

Genome sequencing using the Illumina MiSeq platform was carried out for *Desulfovibrio* sp. NAR1 and *Desulfovibrio* sp. NAR2. The number of paired-end, 250bp reads obtained for each isolate was more than 16 million. Phred per-base

quality scores of ≥ 10 were reported for NAR1 and NAR2 raw reads, which represent inferred base call accuracy of at least 90% (64, 65), for all reads. Ambiguous N bases were not detected in either raw data set. Although these values indicate that the probability of an incorrect base call was minimal for the raw sequences, both data sets were trimmed prior to being used in *de novo* assemblies. The number of paired-end reads remaining for each isolate after trimming was more than 13 million, which represents a range between 700x and 900x expected genome coverage for each isolate. Phred per-base quality scores of ≥ 30 were reported for NAR1 and NAR2 trimmed reads, which in turn represent inferred base call accuracy of at least 99.9% for all reads. Ambiguous N bases were not detected in either trimmed data set. The average read length after trimming was 200bp and 210bp for NAR1 and NAR2, respectively. These resulting quality values represent an improvement over the raw data sets and indicate that the probability of an incorrect base call is minimal for these sequences, at this point both trimmed data sets were considered to be appropriate for generating *de novo* assemblies.

Statistics for the *de novo* assemblies of *D. sp.* NAR1 and *D. sp.* NAR2 are shown in Table 1 (Table 1). The NAR1 genome data was assembled first, and used to establish a pipeline for working with the NAR2 data. Both isolate data sets were trimmed and assembled using identical parameters, using all reads in their respective sets. Individual contig coverage values were averaged for both assemblies, and are reported in Table 1. Fold coverage for individual contigs for NAR1 and NAR2 ranged from 339x-909x, and 705x-1,876x, respectively (Supplemental Tables S1 and S2). Comparisons of assembly statistics such as N_{50} , N_{75} , number of contigs, and longest

contigs for these isolates compared to other published draft genomes indicate that good-quality assemblies have been achieved for both isolates (66, 67). Results of assessments with assembly evaluation software REAPR (49) also supported that high-quality draft genomes had been generated, finding 95.33% of assembled genome bases to be error free for NAR1 and 99.36% for NAR2. The total number of nucleotides for NAR1 was near to the completely sequenced genomes of *Desulfovibrio desulfuricans* subsp. *aestuarii* (draft) and *Desulfovibrio piezophilus* (Table 2) (38, 68), which are both ~3.6Mb. The total number of nucleotides for NAR2 was near to the completely sequenced genome of *Desulfovibrio vulgaris* str. Miyazaki F (Table 2) (68), which is ~4.0Mb. The G+C contents of both isolates are near to those of other *Desulfovibrio*, which are between 45 and 67% (34–42, 50). Contigs from both isolate assemblies were ultimately reordered using Mauve alignments (57), as shown in Figure 2. Genomes were annotated with IMG/ER, RAST (50, 69) and manually using BLAST tools (70) into protein coding genes (PCGs), RNA coding genes (RCGs) and hypothetical PCGs (Table 2). The majority of PCGs in both isolates, ~80%, have function assignments.

Comparison of isolate genomes using IMG/ER and RAST

A combination of IMG/ER, RAST, and manual annotation using BLAST tools (50, 69, 70) was used to annotate both isolate draft genomes. IMG/ER was used as the primary annotator and comparison tool, RAST was used sparingly, and manual annotation was used primarily where automatic annotations seemed questionable, were missing, or when genes were of special interest to this study.

Total coding bases were split into PCGs, RCGs, and hypothetical PCGs. This data, along with the breakdown of RCGs into those accounting for rRNA, tRNA, and other RNA genes, is shown in Table 2 along with the same data for two closely related *Desulfovibrio* species for each isolate; *D. desulfuricans* str. ND132 and *D. piezophilus* for NAR1, and *D. desulfuricans* subsp. *aestuarii* and *D. alaskensis* str. G20 for NAR2. The more phylogenetically distant representative, *D. vulgaris* str. Miyazaki F, was also included as an additional point of comparison. Tabulated attributes are similar overall between isolates and the previously sequenced genomes listed. However, the NAR1 genome is note-worthy with respect to the two annotated clustered regularly interspaced short palindromic repeat regions (CRISPRs), where 3 of the other genomes included in Table 2 have only one CRISPR region and 3 do not have any. CRISPR-Cas systems play a role in adaptive immunity against phages and other invading genetic elements and are present in approximately 40% of sequenced eubacteria genomes and 90% of archaea genomes (71, 72). It is interesting to note that NAR2 lacks a CRISPR-Cas system, particularly since these organisms were isolated from the same site and would presumably have been exposed to similar phage attacks and the same foreign DNA in the environment.

Isolate PCGs with KEGG, COG, Pfam and TIGRfam (73–76) annotations in IMG/ER were compared across subcategories in terms of number of genes contained in each subcategory and the percentage of total PCGs with pathway association that the gene number represents. The percentage is not particularly meaningful on its own because it is taken from the total number of genes with pathway association, which can vary depending on which database is being considered, and it is not a

representation of total PCGs. Even so, the percentage of genes represented in each of the various subcategories remained relatively consistent between the two isolates. So from a broad, overall standpoint, the two isolates look similar in terms of gene content with function assignments. However, not all possible metabolic subcategories are represented, and details regarding gene content rather than overall gene number cannot be assessed in this manner.

The RAST database uses SEED (69) as the core means of annotation and subsystem assignment, which IMG/ER does not, so RAST was used as an additional point of comparison for isolate annotations made by IMG/ER. Comparison of subsystem gene content for both isolates in RAST revealed the NAR1 isolate to have a relatively large number of genes involved in cell wall and capsule formation; 134 genes compared to NAR2, which has 81 genes with that subsystem assignment. Of the 136 genes involved in cell wall and capsule formation in NAR1, 57 are involved in capsular and extracellular polysaccharide production and an additional 47 genes are involved in cell wall and capsule formation with no subcategory. The NAR2 isolate, comparatively, has 35 genes involved in extra cellular polysaccharide production and only 15 genes are involved in cell wall and capsule formation with no subcategory. This difference is in agreement with the observed exudate and biofilm production in NAR1, which is not observed at all in the NAR2 isolate. The number of genes assigned to motility and chemotaxis subsystems is also of interest; the NAR1 isolate has 136 genes assigned to this subcategory, with 100 associated with flagellar motility and 36 being assigned to bacterial chemotaxis. NAR2, comparatively, has 94 genes assigned to motility and chemotaxis, with all of these genes being associated with

flagellar motility. This difference between the two isolates in terms of genes involved in chemotaxis could also be related to the observed biofilm formation in NAR1, and could be part of the pathway that signals biofilm formation. As this pathway is not yet described among the *Desulfovibrio*, these genes should be considered as targets for future studies involving biofilm formation in this genus.

Both isolates are notable in regards to the number of genes assigned to virulence, disease, and defense subcategories. The NAR1 isolate has 61 genes assigned to this subsystem, and the NAR2 isolate has a comparative 70 genes. Within this subsystem, both isolates have genes involved in copper homeostasis, mercury, cobalt, zinc, and cadmium resistance. The NAR1 isolate has a gene encoding MliC, a membrane bound inhibitor of c-type lysozyme. This may partially explain why the NAR1 isolate was difficult to lyse enzymatically. The NAR2 isolate does not have this gene, which agrees with the fact that no difficulties with enzymatic lyses were noted for this isolate. The NAR2 isolate has genes encoding proteins involved in multidrug-resistance (MDR) efflux pumps, and is predicted to possess a functional multidrug and toxic-compound extrusion (MATE) family MDR efflux pump (77). The NAR1 isolate has genes encoding proteins involved in arsenic resistance (*ars*, *arr*, and ACR3), NAR2 does not have any of these genes present. The *Desulfovibrio* genus is often regarded for its resistance to heavy metals and for the ability to transform certain toxic heavy metal species (18, 21), so the presence of genes involved in this sort of metabolism in both isolates can be expected. And while it may not be surprising that both isolates possess these sorts of resistances, it does uniquely equip them for survival in Narragansett Bay sediments, as relatively high levels of Cr, Cu, Ni, Pb, and

Zn have been found in Bay sediments (78). Additionally, Narragansett Bay sediments have been shown to be contaminated with Hg, MeHg (79), and arsenic (80).

Also within the virulence, disease, and defense subcategory, both isolates have predicted beta-lactamases. Antibiotic resistant bacteria have been found widely in aquatic environments (81, 82), including urban river sediments (83), and *Desulfovibrio* have been shown to contain beta-lactamases, with certain species having implications in human *Desulfovibrio* infections (84). So although the presence of predicted beta-lactamases in these isolates may not be surprising in regards to environment or genus assignment, it should still be noted and considered as a possible target for future physiological studies, especially since the potential human pathogenicity of these isolates remains unknown. The NAR1 isolate in particular should be considered, as it contains additional putative genes for colicin V production and fosfomycin resistance. The presence of these genes has been observed and annotated in other *Desulfovibrio*, but there has yet to be physiological confirmation of colicin V production in this genus, while fosfomycin resistance has already been observed in some *Desulfovibrio* species with clinical relevance (85).

Whole genome comparison and alignments

The draft genomes of isolates *Desulfovibrio* sp. NAR1 and *Desulfovibrio* sp. NAR2 were compared to all complete or well-annotated draft *Desulfovibrio* genomes available in GenBank or IMG databases (50, 86) at the time of this study (for a list of genomes see Supplemental Table S3), which was a total of 34 additional genomes. A multi-gene phylogenetic tree (Figure 1) for all 36 *Desulfovibrio* representatives was constructed using 20 different vertically transferred genes (Table S4), to firmly

establish the phylogenetic relationship among the genomes being examined and to provide a more comprehensive assessment and point of reference in regards to which *Desulfovibrio* representatives were most closely related to environmental isolates NAR1 and NAR2. This kind of assessment had been made prior solely on the basis of 16s, *nifH*, and *dsrAB* genes. The two environmental isolates do not cluster close to one another within this group; the NAR1 isolate clusters most closely with *D. piezophilus*, *D. desulfuricans* str. ND132, and *D. aespoeensis* str. Aspo-2, while NAR2 clusters most closely with *D. acrylicus*, *D. desulfuricans* subsp. *aestuarii*, and *D. alaskensis* strains. These results agree for the most part with what had been seen previously with the three-gene assessment, however *D. alaskensis* strains lack *nif*, so a relationship based on N fixing genes could not be examined.

From the *Desulfovibrio* genome phylogeny shown in Fig. 1, the closest relative to each isolate with a complete, gapless genome was selected as a reference for whole genome comparison, alignment, and contig re-ordering using Mauve version 2.3.1 (57). Results of this alignment can be seen in Figure 2. Each local collinear block (LCB) contains a colored similarity profile of the local sequence, with the height of the colored profile corresponding to the average degree of sequence conservation in that section. Areas that are white, which are seen most frequently in the NAR2 alignment, suggest that this genome contains a significant amount of sequence variability compared with *D. alaskensis* str. G20, which is not surprising as G20 is only 89% similar to NAR2 at the 16s level. The more closely related organisms to NAR2 did not have closed genomes and so could not be used as references in this assessment. The NAR1 alignment contains less white space than the NAR2 alignment,

which agrees with the higher level of sequence similarity, 95%, between NAR1 and *D. piezophilus* at the 16s level. In both alignments note the “X” pattern formed by connected LCBs, this typically occurs at the origin of replication in aligned genomes. The number, arrangement, and heights of similarity profiles in the whole genome alignments of both environmental isolates are indicative of organisms related at the genus level, but not at the species or strain level.

Gene network analysis

Evolutionary gene networks (87) were used to compare the genomes of the two environmental isolates, three of their closest relatives, and *D. vulgaris* Hildenborough, whose genome has been well studied and serves as an additional point of comparison. The genomes of *D. piezophilus*, *D. desulfuricans* str. ND132, and *D. aespoeensis* str. Aspo-2 were included as part of the NAR1 cluster, and the genomes of *D. acrylicus*, *D. desulfuricans* subsp. *aestuarii* and *D. alaskensis* str. G20 were included as part of the NAR2 cluster. The initial gene network was run using parameters discussed previously in the Methods section, and results were subsequently filtered to select networks that consisted of only NAR1 and NAR2 (Fig 3.2), only NAR2 (Fig 3.4), and only NAR1 (Fig. 3.3). Selected connected components involving N fixation proteins from the un-filtered network are shown in Fig 3.1.

The un-filtered network consisted of approximately 6,000 connected components, primarily housekeeping proteins, proteins involved in basal metabolic activities, and numerous hypothetical proteins. Connected components that were of particular interest in the unfiltered network were those involving the N fixation (*nif*) genes, as shown in Fig 3.4. The NifH network (Fig. 3.1 A) consists of all

representatives included in this analysis that are capable of fixing N, the shape of the network indicates how similar the NifH sequences are between these representatives at the amino-acid level, with an edge (black line) connecting every node (circle) to every other node in the network. The NifB network (Fig. 3.1 B) consists of all included representatives capable of fixing N except *D. vulgaris*. This network is particularly interesting in that we can see sequences from each of the environmental isolates clustering distinctly with sequences from their closest relatives, the warmer colored circles being NAR1 and associated relatives, and the cooler colored circles being NAR2 and associated relatives. The NifK protein network (Fig. 3.1 C) shows similar relationships to those seen with the NifH network, except that *D. vulgaris* is once again absent. The NifD network was identical to the NifK network, and not shown.

The network filtered for connected components containing only NAR1 and NAR2 consisted of a total of 9 connected components. These were proteins involved in choline/glycine/betaine transport (Fig 3.2 A), metal dependent hydrolases of the beta-lactamase superfamily (Fig. 3.2 B), glyoxalase family proteins-glyoxalase I (Fig. 3.2 C), xanthine dehydrogenases catalases/peroxidases, permeases of drug metabolite transporters (DMT), periplasmic aromatic aldehyde oxidoreductases, and hypothetical membrane proteins. Because these two isolates do not share a close phylogenetic relationship within the *Desulfovibrio* considered here; as indicated by separate clustering in Fig. 1, and only 87% 16s sequence similarity between the two isolates, these networks could indicate proteins that confer specific benefits for survival in Narragansett Bay sediments. The fact that some of these genes (e.g. beta-lactamases, DMTs) are involved in bacterial defense, bacterial detoxification (glyoxalase family

proteins) or as of yet have an un-described function but are exclusively shared between these two isolates, support this hypothesis. Because of this potential connection these proteins should be considered as targets for future investigations, especially those involving transcriptomic or gene expression analysis.

The filtered network containing only NAR1 had approximately 650 connected components (see Appendix A for a list of corresponding proteins), the majority of these connected components consisted of proteins involved in signal transduction and amino acid transport, with a smaller portion of the overall networks consisting of proteins involved in bacterial defense. Components of particular interest are shown in Fig 3.3, which include a putative sensory box/GGDEF family protein network (Fig 3.3 A), a putative diguanylate cyclase and receptor proteins network (Fig 3.3 B), and a periplasmic binding and signal transduction proteins network (Fig 3.3 C), as they have implications in biofilm formation in NAR1. These proteins and their potential role in this isolate's biofilm production will be discussed further in a later section. Additional proteins found to be unique to NAR1 primarily had to do with signal transduction and amino acid transport, which is suggestive of involvement in biofilm formation and exudate production. The fact that these proteins network exclusively from any predicted proteins in NAR2 agrees with what we have observed at the physiological level, with biofilm and exudate production being restricted to NAR1 and not observed at all in NAR2.

There were only 2 connected components that consisted of just NAR2 (Fig 3.4), non-specific predicted membrane proteins (Fig. 3.4 A), and bacteriophage head to tail connecting proteins (Fig. 3.4 B). The two non-specific predicted membrane

proteins (Dn2DRAFT 02896, Dn2DRAFT 02903) share the highest degree of BLAST homology using BLASTX with a hypothetical protein in *Thiocapsa marina*, a purple sulfur bacterium. Dn2DRAFT 02896 shares 59% amino acid identity across 97% of the query, and Dn2DRAFT 02903 shares 60% amino acid identity across 98% of the query with a hypothetical protein (Seq ID: ref|WP_007193013.1) in *T. marina*. All BLAST results with a high enough degree of amino acid similarity to be of interest ($\geq 60\%$ sequence similarity) were to other hypothetical proteins, primarily from beta-proteobacteria, and so did not reveal any insight as to the possible function of this protein in NAR2.

Nitrogen fixation

Of the 34 representatives used in comparison to the environmental isolates, only 7 representatives lack the *nif* operon (Table 3). Only one representative, *Desulfovibrio* sp. U5L has genes for an alternate Fe-Fe nitrogenase. Both environmental isolates NAR1 and NAR2 have a full *nif* operon (Fig. 4), with the arrangement of their N fixation gene cluster being similar to those of their closest N-fixing relatives. There is also supporting physiological evidence that both isolates fix N. The presence of an iron-molybdenum nitrogenase appears to be a shared characteristic for this representative group of *Desulfovibrio*.

In addition to the examination of N fixation, an analysis of metabolism of other N substrates (ammonia, nitrate, and urea) was performed and discussed in the following sections. The analysis of additional aspects of N metabolism in these isolates is critical to improving our understanding of why *D. sp.* NAR1 and *D. sp.* NAR2 exhibit the N fixation behavior we have observed in Narragansett Bay, as an

inability to use other sources of N that are commonly found in the environment could account for a continued need to fix N. To do that it is important for us to be able to take the physiology discussed in the previous sections, and connect it with related functional gene content, which is discussed below.

Urea metabolism

Currently, the most attention regarding bacterial urea metabolism is given to organisms that make up mammalian gut consortia and intestinal human pathogens, and little focus has been placed on the urea metabolism of environmental representatives like the *Desulfovibrio*, or on sulfate-reducing bacteria in general. However, there have been some examinations of the uptake and metabolism of urea by environmental bacteria and phytoplankton (88, 89). These studies have shown that rates of bacterial urea uptake in the environment are highly variable, genes for urea transport and catabolism are not wide-spread amongst bacteria, and that other forms of N are generally preferred to urea, which could be due to the fact that urea catabolism is an energetically expensive process. Although some members of the *Desulfovibrio* are known to have urea transporters and ureases, little is known about the fate of urea once it enters a *Desulfovibrio* cell.

There exist at least four families of transporters that facilitate selective permeation of urea: an ATP-dependent ABC type urea transporter (90), an ion motive force-dependent urea transporter (91), an acid-activated urea channel that belongs to the urea/amide channel family (92), and the urea transporter (UT) family, this last type being the most widely distributed family. UT members are found in bacteria, fungi, insects and vertebrates (91, 93–96). In many bacteria and eukaryotes, urea in the cell

can be broken down to ammonia and CO₂ by a urease. Some bacteria and eukaryotes also use urea amidolyases (UALase) to decompose urea (88). A crystalline structure for a UT family urea transporter from *Desulfovibrio vulgaris* Hildenborough is available and its activity and mechanism of action have been proven in vivo (97). However, subsequent urea metabolism after transport has not been thoroughly investigated.

In this study, an examination using IMG/ER, with visual sequence confirmation in Geneious, and further confirmation using tblastn revealed that of the 34 additional *Desulfovibrio* genomes considered here, only 2 representatives have a complete set of genes necessary for urea transport and catabolism (*D. desulfuricans* subsp. *desulfuricans* and *D. fructosivorans*), the remaining organisms lack either a transporter, a urease, or all genes involved in urea transport and catabolism (Table 3).

D. sp. NAR1 and *D. sp. NAR2* both lacked genes for all proteins discussed above according to an examination in IMG/ER and visual examination of annotations in Geneious. A tblastn search for all genes previously discussed and genes encoding an alternate catabolic enzyme, a urea amidolyase, was performed for both isolates. No homologs for proteins involved in urea transport or catabolism from *D. desulfuricans* subsp. *desulfuricans* were found in NAR1, however, a homolog of the amino acid/amide transport permease protein UrtC was found in NAR2. The NAR2 amino acid sequence shared 44% homology across 227 amino acid residues to UrtC from *D. desulfuricans* subsp. *desulfuricans* (locus tag: G449DRAFT_2704). The gene encoding the UrtC homolog in NAR2 is flanked by other genes involved in amino acid transport, specifically branched chain amino acid transport. Since UrtC belongs to a

larger family of amino acid/amide transporters, it is probable that the UrtC homolog in NAR2 has a role in amino acid transport and that the blast similarity to the urea transporter is due to the structural similarity of the substrates that the two enzymes bind. There were no homologs in NAR2 for the other *D. desulfuricans* subsp. *desulfuricans* proteins involved in urea transport and catabolism, including the urease. No homologs for the UT type urea transporter from *D. vulgaris* Hildenborough or amidolyase from *Rhizobium* sp. was found in either isolate.

Based on these findings, it can be concluded that the *D. sp.* NAR1 draft genome does not contain genes for any of the described enzymatic machinery necessary for urea transport or catabolism. The *D. sp.* NAR2 draft genome does contain a possible urea transporter, but there is no evidence supporting that this isolate has an ability to use urea catabolically. These findings in the genome data agree with what has been observed at the physiological level for both isolates, to the extent that both isolates fix N even in the presence of urea, which supports the conclusion that they cannot catabolize urea. However, the NAR1 isolate has exhibited increased NA in the presence of urea, which would seem to indicate that the isolate has some means of sensing its presence. It would appear that any possible method NAR1 could be employing to sense urea and/or transport it into the cell is not a part of described urea metabolism or transport in bacteria. Since urea metabolism in environmental representatives is currently not very well described, further physiological and molecular investigations are needed to elucidate the mechanism and response seen in NAR1. The fact that neither isolate is predicted to be capable of catabolizing urea does

not make them unique amongst the *Desulfovibrio*, or amongst the eubacteria in general.

Ammonia metabolism

Ammonium is known to be the most universally utilized source of biologically available N, and is taken up preferentially by estuarine microbes (89). Accordingly, we would expect to find genes for ammonium uptake and incorporation in all *Desulfovibrio* representatives. An examination of environmental isolates and additional representatives revealed that all *Desulfovibrio*, with the exception of *D. cuneatus*, possess at least one copy of the ammonium transporter (*amt*, TIGR accession: TIGR00836) (Table 3). It is possible that because *D. cuneatus* is a draft, the ammonium transporter was either missed in annotation or is missing from the assembly, and that the organism may in fact have the transporter. The majority of representatives have multiple copies of the ammonium transporter, where both NAR1 and NAR2 have a single copy, making them slightly atypical in this regard. There are, however, 4 additional representatives that also have a single copy of the transporter. A BLAST search did not reveal any additional copies of the ammonium transporter in either isolate, but it is possible that both isolates could have an additional copy/copies of the transporter that are missing from the current assemblies, or that they have a different protein acting as ammonium transporter that has not yet had that function formally assigned to it. The ammonium transporter for NAR1 is located on DESnar1_contig6, at 122,261-123,619bp, in the forward direction. It is immediately followed by a copy of N regulatory protein P-II, two hypothetical proteins, and a copy of glutamate synthase ~3.5kb downstream, an arrangement which makes sense in

terms of nitrogen regulation and activity which has been seen in other bacteria (34, 36, 38). In the NAR2 isolate, the ammonium transporter is located on DESnar2_contig2 at 904,931-905,257bp, in the forward direction. It is immediately preceded by a copy of N regulatory protein P-II, and immediately followed by an isocitrate dehydrogenase and a protein disulfide isomerase. NAR2 does have a copy of glutamate synthase, however it is located on a different contig.

Whether ammonia is used directly from the environment or is derived from other N sources, its assimilation involves metabolites. Some metabolites, such as 2-oxoglutarate, signal N sufficiency or deficiency to the regulatory apparatus (98). To confirm the potential for ammonia uptake and incorporation in both isolates, additional proteins involved in the N assimilatory pathway were examined in both isolates and the additional *Desulfovibrio* representatives (Table 3). The N regulatory protein P-II is a 2-oxoglutarate (2OG) sensor, which is involved in the adenylation cascade that regulates the activity and concentration of glutamine synthetase (GS), in response to N source availability (99). The majority of the *Desulfovibrio* genomes examined here, including both environmental isolates, have between 2-4 copies of this protein. NAR1 has 4 copies of the protein, with two copies located side by side in between *nifH* and *nifD* on DESnar1_contig13, and a third copy located near the previously mentioned ammonium transporter. The fourth copy is located on DESnar1_contig1 at 248,250-248,591 in the forward direction. It is surrounded on either side by hypothetical proteins, further upstream are proteins involved in cellular respiration and downstream are proteins involved in the shikimate pathway. NAR2 has 3 copies of the N regulatory protein P-II, with two copies located side by side and

preceded immediately by *nifH* and followed immediately by *nifD*, the same arrangement seen in NAR1. The third copy is located on DESnar2_contig2 and was previously mentioned in relation to the ammonium transporter, which immediately follows this copy of N regulatory protein P-II. The locations of all copies of the P-II protein in both isolates make sense in terms of transcription, regulation, and activity, given the proximity to other genes involved in N metabolism.

Because the majority of prokaryotes possess the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway of assimilation, both environmental isolates were assessed for components of this pathway. The isolates were also assessed for an alternate pathway involving the NADP-linked glutamate dehydrogenase, which catalyzes the amination of 2-oxoglutarate to form glutamate. NAR1 has two copies of glutamine synthetase, the first copy is annotated as being a type III glutamine synthetase, and the second is annotated as a type I glutamine synthetase, both of which have been found previously in prokaryotes (100, 101). NAR1 also has all subunits for the NADPH type GOGAT and an NAD-specific glutamate dehydrogenase. NAR2 has a typical prokaryotic type 1 glutamine synthetase, all subunits of the NADPH GOGAT, and a glutamate/leucine dehydrogenase.

Both environmental isolates appear to have a complete GS/GOGAT system of ammonia assimilation, as well as a glutamate dehydrogenase. They both possess the critically important N regulatory protein P-II, with copies of this gene found at genomic locations that make sense in terms of N sensing and regulation. This provides evidence that both isolates have a predicted means of sensing ammonia in the cell, a means of assimilating it, and a means of signaling the regulation of other genes

involved in N metabolism under differing N conditions. These findings support our physiological observations, in that neither isolate exhibited NA in the presence of ammonia.

Nitrate metabolism

No member of the *Desulfovibrio* examined here, including the environmental isolates, has a predicted means of assimilating nitrate (Nas-type nitrate reductases) (Table 3). However, some *Desulfovibrio* species have previously been shown to reduce nitrate or nitrite and use it as a terminal electron acceptor (102, 103), and a sufficient number of the *Desulfovibrio* genomes examined here appear to have the genetic potential for dissimilatory or respiratory nitrate/nitrite reduction. However, nitrate and nitrite reductases share a significant amount of amino acid sequence similarity with sulfate and sulfite reductases, which poses a challenge to automatic gene annotators and increases the likelihood of incorrect annotations, especially when the genomes of sulfate-reducing organisms (which are known to have sulfate and sulfite reductases) are being examined. It is therefore important to approach assertions of nitrate and nitrite reductases in these organisms that are solely based on computational analyses with caution. Additional evidence at the physiological level for organisms predicted to have the ability to reduce nitrate or nitrite would be needed to confirm the computational assertion. Currently the only member of the representatives examined here to have a confirmed, active, respiratory (Nap) type nitrate reductase is *Desulfovibrio desulfuricans* subsp. *desulfuricans* str. ATCC27774 (102, 104). Both environmental isolates have a putative NapC-type respiratory nitrate reductase subunit and NAR2 has a predicted NorZ apoprotein nitric oxide reductase.

The lack of genes involved in nitrate assimilation or assimilatory nitrate reduction appears to be typical regarding the *Desulfovibrio*, and the presence of genes involved in dissimilatory or respiratory nitrate reduction seems to be variable among the group. The lack of genetic potential for utilizing nitrate in an assimilatory manner in both isolates supports what we have seen at the physiological level, as NA in both isolates was unaffected by the presence of nitrate.

Carbon metabolism

Both isolates have all proteins necessary for glycolysis and gluconeogenesis. It does appear that both isolates have an incomplete TCA cycle, although this is not unusual amongst the *Desulfovibrio* (105, 106). Additionally, both isolates have genes encoding an L-lactate transporter and an L-lactate dehydrogenase, which supports the observed growth of both isolates on lactate. The presence of a complete glycolytic/gluconeogenic pathway further supports both assemblies as being comprehensive, well annotated drafts.

Biofilm formation

The NAR1 isolate has observed biofilm formation, except under conditions where a rich media (NBSO + .05% lysed yeast cell extract) is used and no additional surface for cellular attachment is present (e.g. glass beads, sediment, etc.). This has made it a very challenging organism to work with. Many *Desulfovibrio* are known to form biofilms, including the well-studied *Desulfovibrio vulgaris* Hildenborough (107). However, the genes responsible for biofilm formation in these organisms have not been conclusively identified or well-studied, and cell-to-cell communication and

quorum sensing pathways involved in biofilm formation for these organisms remains unclear.

An examination of NAR1 gene annotations in IMG/ER and Geneious revealed no genes belonging to the well described *lux* family of quorum sensing genes (108, 109), these results were then confirmed using a tblastn analysis with the assembled NAR1 contigs as the database and Lux proteins from *Vibrio fischeri* as the queries. Putative genes involved in biofilm formation in NAR1 were ultimately discovered using a filtered evolutionary gene network (87) (Fig. 3.3). The majority of these genes belong to a family of diguanylate cyclases with GGDEF (110) domains, and c-di-GMP receptor domain proteins.

Cyclic di-GMP (c-di-GMP) is a bacterial second messenger that is widely utilized by bacteria, with more than 80% of sequenced bacteria predicted to use this signal (111, 112). C-di-GMP controls a variety of phenotypes, including biofilm formation, motility, and virulence in multiple bacteria (111, 113, 114). The fact that the predicted diguanylate cyclases in NAR1 did not network with proteins from any other closely related *Desulfovibrio* representatives support the hypothesis that these proteins may serve a unique function in NAR1. Given the documented role of diguanylate cyclases and c-di-GMP in biofilm formation, and that NAR1 is unique in its near obligate biofilm lifestyle when compared with NAR2 and other representatives of the *Desulfovibrio*, it is possible that the unique function served by these proteins is coordination of biofilm formation in NAR1. These genes and the proteins they code for should be further investigated to confirm any role in biofilm formation in this isolate. A separate examination of c-di-GMP levels in NAR1 and other biofilm-

forming *Desulfovibrio* during biofilm growth and during planktonic growth should be considered in order to elucidate the role of c-di-GMP as a signaling molecule for biofilm formation in these organisms.

Mercury methylation

Mercury (Hg) is a pervasive global pollutant known to found in Narragansett Bay sediments (79); in its methylated form (CH_3Hg^+), it bioaccumulates and is highly toxic to humans and other organisms (115). Unlike inorganic forms of Hg, which originate from atmospheric deposition and point discharge, CH_3Hg^+ is generated in the environment by microorganisms. Hg methylation is largely restricted to the proteobacteria and primarily to anaerobic organisms (116). Sulfate-reducing bacteria, such as the *Desulfovibrio*, are the main producers of CH_3Hg^+ (117, 118), although iron-reducing bacteria and methanogens can also be involved (119, 120).

The genetic basis for bacterial mercury methylation was recently described by Parks, et al. (18). Because some of the closest relatives to NAR1 are confirmed Hg methylators and because Narragansett Bay sediments are known to contain Hg, the draft genomes of both environmental isolates were searched for *hgcA* and *hgcB*, the genes required for bacterial Hg methylation (18). The amino acid sequences for both Hg methylation proteins from *D. desulfuricans* str. ND132 were used as queries to search the draft genomes of NAR1 and NAR2 using tblastn. No homologs for these proteins were found in the genome of NAR2, however homologs were found in the genome of NAR1 (Fig. 5). A homolog for HgcA was found on DESnar1_contig14 at 52,665-53,621 base pairs in the forward direction with 63% sequence similarity to HgcA from *D. desulfuricans* str. ND132, and a homolog for HgcB was found on

DESnar1_contig14 at 53,697-53,984 base pairs in the forward direction with 64.6% sequence similarity to HgcB from *D. desulfuricans* str. ND132. The alignment of both proteins found in NAR1 against other confirmed (black arrowhead) and predicted Hg-methylators belonging to the *Desulfovibrio* is shown in Fig. 5. The level of amino acid sequence similarity between NAR1 and the other Hg-methylators combined with the fact that the proteins in NAR1 all have highly conserved domains required for the two previously described Hgc proteins to function in Hg methylation (18), provide evidence for NAR1 having the genetic capability to methylate Hg. Further examination at the physiological level would be needed in order to confirm Hg methylation in this isolate and to establish Hg methylation rates. Hg methylation potential in NAR1 and similar bacteria residing in Narragansett Bay sediments is an important consideration, as CH_3Hg^+ production has the potential to negatively impact other organisms living in the bay.

Conclusions

This study combined high-throughput sequencing, comparative genomics, and physiological N fixation rate approaches to further understand the controls on N fixation and N metabolism in members of the *Desulfovibrionaceae*. Our genomic data and physiological N fixation rate data suggest that environmental isolates *Desulfovibrio* NAR1 and *Desulfovibrio* NAR2 are capable of using ammonia as an N source but are not capable of using nitrate or urea to meet cellular N requirements, although there is a possibility that both isolates may be able to respire nitrate. The majority of additional *Desulfovibrio* genomes examined in comparison to our environmental isolates also showed a lack of genes involved urea and nitrate

catabolism, indicating that an inability to utilize these sources of combined N may be widespread amongst the *Desulfovibrionaceae*. This could in turn provide an explanation for why we see *nifH* expression by this group even in environments that are not limited for combined N, such as sediments of the Narragansett Bay estuary (121, 122). It also suggests that we will continue to see input of fixed N by this group of organisms in Narragansett Bay and other similar estuarine systems, despite the presence of combined N, which could potentially exacerbate eutrophication and linked seasonal hypoxic events in these environments.

The sequenced genomes of both isolates were additionally found to be metabolically versatile and unique compared to one another. Both isolates possessed a significant number of genes involved in bacterial defense and heavy metal resistance, which is not surprising for members of this genus, however, their presence may still provide an advantage for survival in Narragansett Bay sediments. Isolates were also shown to possess different physiological traits that were then reflected at the genome level, such as the near obligate biofilm formation exhibited by NAR1 and the complete lack of biofilm formation seen in NAR2. Results of gene network analysis indicated that cyclic-diguanylate (c-di-GMP) could be an important cell-to-cell signaling molecule involved in biofilm formation in NAR1. This could have implications for future studies involving this isolate or other biofilm forming *Desulfovibrio*, especially since biofilm formation and related cell signaling is not well characterized for members of this genus. NAR1 was also found to have genes involved in bacterial mercury methylation, an environmentally harmful process that is primarily restricted to anaerobic sulfate reducing bacteria (117, 118). Since mercury is

known to be present in Narragansett Bay sediments (79), and changes in Narragansett Bay seem to be shifting towards conditions that favor these sulfate reducers (123), bacterial mercury methylation activity by this diazotrophic community might be an additional aspect to consider in terms of how the biogeochemical roles these organisms play might be linked and how that affects the overall health of Narragansett Bay.

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Figure 2. Whole genome comparisons. Whole genome comparisons of both environmental isolates and their closest relatives using Mauve, version 2.3.1. The pairwise alignment of *Desulfovibrio* sp. NAR1 against *Desulfovibrio piezophilus* (**A**) and *Desulfovibrio* sp. NAR2 against *Desulfovibrio alaskensis* str. G20 (**B**) is shown. The similarly colored blocks are presumably homologous and internally free of rearrangements between genomes. White areas within blocks indicate sequences that were not aligned to the other genome and represent non-homologous regions. Long red lines indicate contig boundaries for draft genomes.

Figure 3.1 Representative protein gene networks consisting of environmental isolates and other *Desulfovibrio* representatives. (A) NifH protein network. (B) NifA protein network. (C) NifK protein network.

Figure 3. Evolutionary gene networks.

Figure 3.2 Representative protein gene networks consisting of exclusively NAR1 and NAR2. (A) Choline/glycine/betaine transporters. (B) Metal dependent hydrolases of the beta-lactamase superfamily. (C) Glyoxalase family proteins-glyoxalase I.

Figure 3.3 Representative protein gene networks consisting of only NAR1. (A) Putative sensory box/GGDEF family proteins-role in signaling and biofilm persistence. (B) Putative diguanylate cyclase and receptor proteins (GGDEF family). (C) Periplasmic binding and signal transduction proteins.

Figure 3.4 All protein gene networks consisting of only NAR2. (A) Non-specific predicted membrane proteins. (B) Bacteriophage head to tail connecting proteins.

Figure 4. N fixation gene clusters. N fixation gene clusters of Narragansett Bay isolates (black arrows) and three closely related *Desulfovibrio* representatives. Color-coded arrows indicate coding sequences (CDS) and their orientation.

Figure 5. Proteins required for Hg methylation. Multiple sequence alignments of 5 *Desulfovibrio* HgcA and HgcB orthologs, including environmental isolate NAR1. Confirmed mercury methylating organisms are indicated with a black arrowhead. Red boxes indicate highly conserved regions previously described by Parks et al. (Parks et al., 2013), including the putative cap helix [consensus sequence motif, N(V/I)WCA(A/G)GK in HgcA, two strictly conserved CX₂CX₂CX₃C motifs characteristic of [4Fe-4S] clusters, and a conserved vicinal pair of cysteines located at the C terminus of HgcB.

Figure 6. Growth of NAR1 under N limitation. Growth of *Desulfovibrio* sp. NAR1 under N limited conditions. Error bars represent one standard deviation from sample mean. For the first 3 time-points, error bars are too small to be seen.

Figure 7. Nitrogenase activity of NAR1 under N limitation.
 Nitrogenase activity of NAR1 grown under N limited conditions.
 Reported as nmols C₂H₄ produced per cell per day, measured using
 the acetylene reduction assay. Error bars represent one standard
 deviation from sample mean.

Figure 8. Cell enumeration of NAR1 cultures grown under different N treatments. Total cell counts as genomes per culture tube for cultures of NAR1 grown with N limited, 12mM ammonia, 12mM nitrate, and 12mM urea treatments. Error bars represent one standard deviation from sample mean.

Figure 9. Nitrogenase activity of NAR1 under different combined N conditions. Nitrogenase activity of NAR1 grown under different combined N conditions: N limited, 12mM ammonia, 12mM nitrate, and 12mM urea. Nitrogenase activity is reported as nmols C₂H₄ produced per cell per day, measured using the acetylene reduction assay. Error bars represent one standard deviation from sample mean.

Assembly	Bases	Contigs	Mean contig length	Longest contig	N50	N75	Average contig coverage	% Reads mapped
<i>Desulfovibrio sp.</i> NAR1	3502190	17	205643	627453	353711	249450	691.6	99.8
<i>Desulfovibrio sp.</i> NAR2	3907388	7	558198	1879556	1122917	1122917	942.1	99.8

Table 1. Assembly statistics. Assembly statistics for the draft genomes of both environmental isolates. “% Reads mapped” refers to the number of reads that mapped back to the final genome assembly.

Organism	Total bases	GC %	Coding bases %	Genes	CRISPR	PCG %	RCG %	rRNA genes %	tRNA genes %	other RNA genes %	Function PCG %	Hypothetical PCG %
<i>D. sp. NAR1 (D)</i>	3508007	55.8	88.64	3279	2	98.08	1.92	0.15	1.56	0.21	80.82	17.26
<i>D. sp. NAR2 (D)</i>	3907388	44.9	83.97	3474	0	96.69	3.31	0.29	2.88	0.14	78.27	18.42
<i>D. desulfuricans</i> ND132	3858983	65.2	88.93	3534	1	98.19	1.81	17	1.56	0.08	79.2	18.99
<i>D. piezophilus</i>	3646098	49.9	89.47	3431	0	97.76	2.24	0.26	1.69	0.29	67.3	30.46
<i>D. desulfuricans</i> subsp. <i>aestuarii</i> (D)	3551364	45.1	85.63	3251	0	96.55	3.45	0.43	2.86	0.15	77.39	19.16
<i>D. alaskensis</i> G20	3730232	57.8	91.13	3874	1	97.68	2.32	0.31	1.7	0.31	59.42	38.26
<i>D. vulgaris</i> Miyazaki F	4040304	67.1	83.35	3281	1	97.68	2.32	0.37	1.95	0	72.66	25.02

Table 2. Comparative genome statistics. Comparative annotated genome statistics for *Desulfovibrio* sp. NAR1, *Desulfovibrio* sp. NAR2, and four of the most closely related *Desulfovibrio* genomes. The genome of *D. vulgaris* Miyazaki F was included as an additional point of reference, as neither isolate was very closely related to it. Isolate genome statistics are indicated by the bold text, and (D) indicates a draft genome.

Organism		D. sp. NAR1	D. sp. NAR2	D. acrylicus	D. aesopensis str. Asp2	D. africanus str. PCS	D. africanus str. Walvis Bay	D. clausenensis str. DSM 16109	D. clausenensis str. G20	D. alkalitolerans	D. aminophilus	D. cf. megneticus	D. cunicatus	D. desulfuricans str. ND132	D. desulfuricans subsp. aestuarii	D. desulfuricans subsp. desulfuricans	D. fructosovorans str. ATCC 27774	D. gages	D. hydrothermalis str. AM13
Predicted nitrogen fixation		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	<i>nifA</i>	2	2	2	1	2	2	-	-	1	2	-	1	-	2	1	2	-	-
	<i>nifB</i>	2	1	1	2	1	2	-	-	-	-	1	-	2	1	1	-	1	2
	<i>nifH</i>	1	1	1	1	1	1	-	-	1	1	-	1	1	1	-	1	3	1
	<i>nifD</i>	1	1	1	1	1	1	-	-	1	1	-	1	1	1	-	1	1	1
	<i>nifK</i>	1	1	1	1	1	1	-	-	1	1	-	1	1	1	-	1	1	1
	<i>nifN</i>	1	1	1	1	1	1	-	-	1	1	-	1	1	1	1	1	1	1
	<i>nifE</i>	1	1	1	1	1	1	-	-	1	1	-	1	1	1	1	1	1	1
	<i>anfD</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>anfK</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>anfG</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Predicted urea transport		-	-	-	-	-	-	-	-	-	✓	-	-	-	-	✓	-	-	✓
	<i>urtA</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
	<i>urtB</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
	<i>urtC</i>	-	1(h)	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
	<i>urtD</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
	<i>urtE</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
	urea transporter <i>utp</i> , Yersinia type	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Predicted urea catabolism		-	-	-	-	-	-	-	-	-	-	-	✓	-	✓	-	-	✓	-
	<i>ureA</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	1	-
	<i>ureB</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	1	-
	<i>ureG</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	1	-
	<i>ureC</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	1	-
Predicted ammonium uptake		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	ammonium transporter <i>amt</i>	1	1	1	2	3	3	2	2	4	2	2	4	-	2	1	2	1	4
Predicted N regulation		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	N regulatory protein P-II	4	3	3	5	4	5	2	2	6	5	3	6	-	4	3	4	1	5

Table 3. Comparison of gene presence involved in N metabolism. Comparative presence or absence of genes involved in N fixation, urea transport and catabolism, ammonium uptake, cellular N signaling, and nitrate metabolism for all *Desulfovibrio* genomes examined in this study. (*) Indicates the presence of one or more plasmids for that organism, numbers indicate the gene copy number for genes listed to the left, and (h) indicates that a homolog was found by hand. Genes were only tabulated if at least one representative was predicted to have that gene, in order to conserve space. In addition to what is shown here all representatives were assessed for the presence of alternate nitrogenases (*anf* and *vnf* gene families), urea amidolyases, assimilatory nitrate reductases (*nas* gene cluster), membrane-bound respiratory nitrate reductases (*nar* gene cluster), and periplasmic dissimilatory nitrate reductases (*nap* gene cluster), respiratory NO forming nitrite reductase (*nirS/nirK*), dissimilatory cytoplasmic NH₄⁺ forming nitrite reductase (*nirBD*) and nitric oxide reductases (*nor* gene cluster).

	<i>D. longus</i>	<i>D. magnificus</i> str. RS1*	<i>D. oxycliniae</i>	<i>D. piezophilus</i>	<i>D. piger</i>	<i>D. putredinis</i>	<i>D. soldevicens</i>	<i>D. sp. A2</i>	<i>D. sp. FW1012B*</i>	<i>D. sp. U5L</i>	<i>D. vulgaris</i> str. D94*	<i>D. vulgaris</i> str. Hildenborough*	<i>D. vulgaris</i> str. Miyazaki F	<i>D. vulgaris</i> str. RC111*	<i>D. zosterae</i>
✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
1	-	1	1	-	1	3	-	2	1	2	2	2	1	1	
1	1	1	2	-	1	1	1	1	1	1	1	1	2	1	
1	1	1	1	-	2	1	1	1	2	1	1	1	1	1	
1	1	1	1	-	2	1	1	1	2	1	1	1	2	1	
1	1	1	1	-	2	1	1	1	2	1	1	1	1	1	
1	1	1	1	-	2	1	1	1	1	1	1	1	1	1	
1	1	1	1	-	2	1	1	1	1	1	1	1	1	1	
-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	-	✓	✓	-	✓	-	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
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-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	2	2	2	1	2	2	2	3	3	1	1	2	1	2	
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	4	5	4	1	6	4	4	5	8	3	3	4	3	4	

Predicted nitrate/nitrite transport		✓	✓	✓	✓	-	-	✓	✓	-	✓	✓	-	✓	-	✓	-	-	-	-	-
	MFS transporter, NNP family, nitrate/nitrate	1	1	1	1	-	-	1	1	-	1	1	-	1	-	1	-	-	-	1	-
Predicted dissimilatory nitrate reduction		-	-	-	-	-	-	-	-	-	-	-	-	✓	-	-	✓	✓	-	-	-
	<i>napA</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-	-	-
	<i>napC</i>	1	1	1	1	-	-	-	-	-	1	-	-	1	-	-	1	-	-	-	-
	<i>napD</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-
	<i>napG</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	<i>napH</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	<i>napM</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Predicted respiratory nitrite reduction		-	-	-	✓	✓	✓	-	-	-	✓	-	✓	✓	-	-	✓	✓	-	-	✓
	<i>nrfA</i>	-	-	-	1	1	1	-	-	-	1	-	1	1	-	-	1	1	-	-	1
	<i>nrfH</i>	-	-	-	1	1	1	-	-	-	1	-	1	1	-	-	1	1	-	-	1
Predicted respiratory nitric oxide reduction		-	✓	-	-	-	-	-	-	✓	-	-	✓	-	-	-	-	-	-	-	-
	<i>norB</i>	-	1	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-	-	-	-

-	-	✓	-	-	-	-	✓	-	-	✓	✓	✓	-	-
1	-	1	-	-	-	-	1	-	-	1	1	1	-	-
-	-	-	-	-	-	-	✓	-	-	-	-	-	-	-
-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
-	-	-	-	-	-	-	3	-	-	1	1	-	1	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	✓	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
-	1	-	-	1	1	1	2	1	1	1	1	2	1	-
-	1	-	-	1	1	1	2	1	1	1	1	2	1	-
-	-	-	-	-	✓	-	-	✓	✓	-	-	-	-	-
-	-	-	-	-	1	-	-	1	1	-	-	-	-	-

Supplemental Protocol 1

Complete DNA Extraction Protocol

Reagents Needed

1. Qiagen DNeasy Minikit for Plant Tissue
2. Phenol/chloroform/isoamyl alcohol (PCI) solution (25:24:1), molecular biology grade, pH 7.8-8.2
3. Chloroform/isoamyl alcohol (24:1), molecular biology grade
4. Elution Buffer (10mM Tris-HCl, pH 8.5)
5. Ammonium acetate, concentrated solution
6. 100% Ethanol
7. 70% Ethanol

Part 1-Modified Qiagen DNeasy Minikit for Plant Tissue

Using filter membrane or ~.30 g glass beads as starting materia

1. Add 400uL buffer AP1 to starting material in 2mL microcentrifuge tube.
2. If extracting from a filter, add .5um and .1um silica beads to each tube. If extracting from beads, add only .1um silica beads.
3. Add 4 uLs RNase A to each tube
4. Vortex to mix
5. Incubate at 65°C for 10min, mixing 2-3 times during incubation by inverting tubes.
6. Bead beat each tube for 1 minute
7. Add 130uL of Buffer AP2 to lysate, vortex to mix
8. Incubate at 4°C for 5 minutes
9. Centrifuge lysate for minutes at 14,000 rpm
10. If filter is present, remove. If not, continue.

Part 2-Phenol/Chloroform/Isoamyl Alcohol Extraction

Expect ~400uL of supernatant from step 10, split into two 2mL microcentrifuge tubes of 200uL each. If supernatant is less than 400uL, aliquot 200uL to one tube and bring up the volume of the 2nd tube to 200uL using elution buffer (10mM Tris-HCl, pH 8.5).

11. Add 200uL of phenol/choloroform/isoamyl alcohol solution (25:24:1) to tubes containing the supernatant.
12. Bead beat tubes 1min
13. Centrifuge tubes at 15,000 rpm for 5 minutes
14. Remove ~180uL of the top aqueous layer and place into a new 2mL microcentrifuge tube. Avoid picking up any of the phenol/chloroform/isoamyl alcohol phase. Set aside.

15. Add 200uL of EB to the first tube, still containing the phenol/chloroform isoamyl alcohol.
16. Repeat steps 12 and 13
17. Remove as much of the top aqueous layer as possible and add it to what is already in the second tube. Discard first tube.

Part 3-Chloroform Back Extraction

18. Add equal volumes (~800uL) chloroform/isoamyl alcohol solution (24:1) to second tube containing the aqueous layer
19. Bead beat 1min
20. Centrifuge at 15,000 for 5 minutes
21. Remove as much of the top aqueous solution as possible and place into a new 2mL microcentrifuge tube. Avoid picking up any of the chloroform/isoamyl alcohol phase.

Part 4-Ethanol Precipitation

At this stage samples originating from the same source, or samples that needed to be split in earlier stages because of large volumes, can be pooled together.

22. Combine any samples needing to be pooled into a new centrifuge tube
23. Add 0.1xvol 5M ammonium acetate
24. Add 2.5xvol 100% EtOH
25. Precipitate overnight at -20 °C
26. Centrifuge at 4 °C at maximum speed for 1hr
27. Decant supernatant
28. Add 750mL 70% EtOH
29. Centrifuge 5 min at maximum speed and pour off EtOH
30. Add 750mL 100% EtOH
31. Centrifuge 5 min at maximum speed and pipette out EtOH. Be careful not to disturb pellet (may or may not be visible)
32. Dry pellet 4-5 min
33. Elute DNA in 20-100uL of elution buffer or PCR-grade water, depending on desired DNA concentration

If contaminant EPS are still present, Part 4 may be repeated

Supplemental Protocol 2

Preparation of Anaerobic Glycerol Stocks

Reagents needed

Glycerol Solution

30% Glycerol

69.8% ddH₂O

.2% of 0.2% Resazurin solution

Preparation of Glycerol Vials

1. Prepare glycerol solution in a gas tight serum bottle. Place glycerol solution in 50°C water bath for 5 minutes and let cool while gassing with N₂. Flush and evacuate bottle.
2. Flush and evacuate all 10 ml serum vials to be used with N₂.
3. Transfer 1 ml of glycerol solution to each vial via syringe.
4. Flush and evacuate all vials repeatedly.
5. Autoclave 30 min.

Freezing Cells

1. Grow up a fresh culture of bacteria to high density.
2. Add a drop or two of an appropriate reducing agent (such as sodium sulfide) to vials until the resazurin turns colorless. Wrap label tape completely around vial.
3. Using aseptic technique, add 1 ml of culture by syringe to each vial. Quickly place vial into a dry ice/ethanol bath or liquid nitrogen.
4. Store frozen culture stocks at -80°C.

Figure S1. Calculations Used for Biomass Estimates

$$C_T = G_T = \text{DNA}_{CT} / G_M$$

Where:

1. DNA_{TE} = Total Extracted DNA = DNA concentration of sample x volume of sample
2. DNA_{GB} = DNA per gram of glass beads = DNA_{TE} x mass of glass beads extracted
3. DNA_{CT} = DNA per culture tube = DNA_{GB} x grams of glass beads in culture tube
4. Mass_{NP} = Average mass of nucleotide pair = 650 Daltons (g/mol)₁ = 1.1×10^{-12} ng
5. G_S = Size of *Desulfovibrio* sp. NAR1 Genome = ~3,500,000 base pairs
6. G_M = Mass of genome = Mass_{NP} x G_S = 0.00000385 ng
7. G_T = Genomes per tube = DNA_{CT} / G_M
8. C_T = Total cells = G_T

1. New England Biolabs (<https://www.neb.com/tools-and-resources/usage-guidelines/nucleic-acid-data>)

Contig	length	average coverage	total reads	reads in pairs	single reads
DESnar1_contig1	627453.0	649.6	2186959.0	871350.0	1315609.0
DESnar1_contig2	456096.0	630.8	1527705.0	611490.0	916215.0
DESnar1_contig3	169857.0	649.2	588479.0	234526.0	353953.0
DESnar1_contig4	9397.0	632.8	32374.0	12612.0	19762.0
DESnar1_contig5	353712.0	755.5	1426648.0	574344.0	852304.0
DESnar1_contig6	327788.0	734.1	1308218.0	517404.0	790814.0
DESnar1_contig7	133415.0	815.6	592379.0	234126.0	358253.0
DESnar1_contig8	20195.0	777.3	86064.0	33994.0	52070.0
DESnar1_contig9	127540.0	909.1	625948.0	248282.0	377666.0
DESnar1_contig10	340071.0	764.1	1408497.0	559778.0	848719.0
DESnar1_contig11	103006.0	766.5	429425.0	170686.0	258739.0
DESnar1_contig12	8343.0	710.0	32539.0	13058.0	19481.0
DESnar1_contig13	362963.0	725.4	1418561.0	563138.0	855423.0
DESnar1_contig14	248880.0	665.6	890749.0	354300.0	536449.0
DESnar1_contig15	204429.0	652.1	719542.0	282760.0	436782.0
DESnar1_contig16	1207.0	339.2	2258.0	1106.0	1152.0
DESnar1_contig17	1581.0	579.7	4998.0	2090.0	2908.0

Table S1. Contig Statistics NAR1. Table displaying statistics for individual contigs of the final draft genome assembly of *Desulfovibrio* sp. NAR1. The last three columns refer to reads that mapped back to the respective contigs.

Contig	length	average coverage	total reads	reads in pairs	single reads
DESnar2_contig1	23563.0	858.2	104701.0	102634.0	2067.0
DESnar2_contig2	1122304.0	788.2	4619612.0	4555582.0	64030.0
DESnar2_contig3	1879303.0	705.1	6896898.0	6803450.0	93448.0
DESnar2_contig4	669951.0	789.4	2752059.0	2714686.0	37373.0
DESnar2_contig5	179092.0	825.2	770800.0	759902.0	10898.0
DESnar2_contig6	11013.0	752.8	43358.0	41914.0	1444.0
DESnar2_contig7	22162.0	1875.7	220441.0	212364.0	8077.0

Table S2. Contig statistics NAR2. Table displaying statistics for individual contigs of the final draft genome assembly of *Desulfovibrio* sp. NAR2. The last three columns refer to reads that mapped back to the respective contigs.

Sequence	Database Retrieved	RefSeq	DSM	ATCC	
<i>Desulfovibrio acrylicus</i>	IMG	NZ_AULY00000000.1	10141	----	
<i>Desulfovibrio aespoeensis</i> str. Aspo2	GenBank	NC_014844.1	10631	700646	
<i>Desulfovibrio africanus</i> str. PCS	IMG	NZ_AOSV00000000.1	----	----	Source DNA available from Romy Chakraborty at Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
<i>Desulfovibrio africanus</i> str. Walvis Bay	GenBank	NC_016629.1	----	19997	
<i>Desulfovibrio alaskensis</i> str. DSM 16109	IMG	NZ_AXWQ00000000.1	16109	----	
<i>Desulfovibrio alaskensis</i> str. G20	GenBank	NC_007519.1	----	----	Source DNA and bacteria available from Judy Wall (wallj@missouri.edu)
<i>Desulfovibrio alcoholivorans</i>	IMG	NZ_JNJA00000000.1	5433	----	
<i>Desulfovibrio alkalitolerans</i>	IMG	NZ_ATHI00000000.1	16529	----	
<i>Desulfovibrio aminophilus</i>	IMG	NZ_AUMA00000000.1	12254	----	
<i>Desulfovibrio cf. magneticus</i>	IMG	NZ_JAGC00000000.1	----	----	Source DNA and organism available from Christopher W. Schadt (schadtcw@ornl.gov)
<i>Desulfovibrio cuneatus</i>	IMG	NZ_AUCY00000000.1	11391	----	
<i>Desulfovibrio desulfuricans</i> str. ND132	GenBank	NC_016803.1	----	----	Source DNA and organism available from Steven D. Brown (Oak Ridge National Laboratory, ORNL)
<i>Desulfovibrio desulfuricans</i> subsp. aestuarii	IMG	NZ_ARQF00000000.1	17919	29578	
<i>Desulfovibrio desulfuricans</i> subsp. desulfuricans	IMG	NZ_ATUZ00000000.1	642	29577	
<i>Desulfovibrio desulfuricans</i> subsp. desulfuricans str. ATCC 27774	GenBank	NC_011883.1	----	27774	
<i>Desulfovibrio frigidus</i>	IMG	NZ_JONL00000000.1	17176	----	
<i>Desulfovibrio fructosivorans</i> str. JJ	IMG	NZ_AECZ00000000.1	----	----	Source DNA and organism available from Terry C. Hazen (TCHazen@lbl.gov)
<i>Desulfovibrio gigas</i>	GenBank	NC_022444.1	1382	19364	
<i>Desulfovibrio hydrothermalis</i> str. AM13	GenBank	NC_020055.1	14728	----	
<i>Desulfovibrio longus</i>	IMG	NZ_ATVA00000000.1	6739	51456	
<i>Desulfovibrio magneticus</i> str. RS1	GenBank	NC_012796.1	----	----	
<i>Desulfovibrio magneticus</i> str. RS1 plasmid pDMC1	GenBank	NC_012797.1	----	----	
<i>Desulfovibrio magneticus</i> str. RS1 plasmid pDMC2	GenBank	NC_012795.1	----	----	Microbial strain and DNA available through the NBRC (http://www.nbrc.nite.go.jp/e/index.html)
<i>Desulfovibrio oxycilinae</i>	IMG	NZ_AQXE00000000.1	11498	----	
<i>Desulfovibrio piezophilus</i>	GenBank	NC_020409.1	21447	----	
<i>Desulfovibrio piger</i>	IMG	NZ_ABXU00000000.1	----	29098	
<i>Desulfovibrio putalis</i>	IMG	NZ_AUBQ00000000.1	16056	----	
<i>Desulfovibrio salexigens</i>	GenBank	NC_012881.1	2638	----	
<i>Desulfovibrio</i> sp. A2	IMG	NZ_AGFG00000000.1	----	----	Source DNA and organism available from Marc Solioz, Dept. Clinical Research, University of Berne, Murtenstrasse 35, 3010 Berne, Switzerland.
<i>Desulfovibrio</i> sp. FW1012B	IMG	NZ_CM001368.1	----	----	
<i>Desulfovibrio</i> sp. FW1012B plasmid pFW10101	IMG	NZ_CM001369.1	----	----	
<i>Desulfovibrio</i> sp. FW1012B plasmid pFW10102	IMG	NZ_CM001370.1	----	----	Source DNA and organism available from Terry C. Hazen (TCHazen@lbl.gov)
<i>Desulfovibrio</i> sp. U5L	IMG	NZ_AHMC00000000.1	----	----	Source DNA and organism available from Terry C. Hazen (TCHazen@lbl.gov)
<i>Desulfovibrio vulgaris</i> str. DP4	IMG	NC_008751.1	----	----	
<i>Desulfovibrio vulgaris</i> str. DP4 pDVUL01	IMG	NC_008741.1	----	----	Source DNA and organism available from Christopher Walker (cbwalkdr@u.washington.edu)
<i>Desulfovibrio vulgaris</i> str. Hildenborough	GenBank	NC_002937.3	29579	----	
<i>Desulfovibrio vulgaris</i> str. Hildenborough plasmid pDV	GenBank	NC_005863.1	29579	----	
<i>Desulfovibrio vulgaris</i> str. Miyazaki F	IMG	NC_011769.1	----	----	Source DNA and organism available from Terry C. Hazen (TCHazen@lbl.gov)
<i>Desulfovibrio vulgaris</i> str. RCH1	IMG	NC_017310.1	----	----	
<i>Desulfovibrio vulgaris</i> str. RCH1 plasmid pDEVAL01	IMG	NC_017311.1	----	----	Source DNA and organism available from Terry C. Hazen (TCHazen@lbl.gov)
<i>Desulfovibrio zosterae</i>	IMG	NZ_AUDC00000000.1	11974	----	

Table S3. *Desulfovibrio* genomes used in comparison. Table displaying organism names, source databases, RefSeq accession numbers, and culture collection information for all additional *Desulfovibrio* genomes examined in this study.

Gene name	Gene product
infB	translation initiation factor-2
rplB	50S ribosomal protein L2
rplD	50S ribosomal protein L4
rplE	50S ribosomal protein L5
rplF	50S ribosomal protein L6
rplK	50S ribosomal protein L11
rplN	50S ribosomal protein L14
lepA	GTP binding protein LepA
pheS	phenylalanyl-tRNA synthetase, alpha subunit
rpsB	30S ribosomal protein S2
rpsC	30S ribosomal protein S3
rpsE	30S ribosomal protein S5
rpsG	30S ribosomal protein S7
rpsH	30S ribosomal protein S8
rpsI	30S ribosomal protein S9
rpsK	30S ribosomal protein S11
rpsL	30S ribosomal protein S12
rpsM	30S ribosomal protein S13
rpsQ	30S ribosomal protein S17
rpsS	30S ribosomal protein S19

Table S4. Genes used to construct multigene phylogeny. Information regarding genes used to construct our multigene phylogeny, including gene name and product.

CONCLUSION

N-fixation by heterotrophic diazotrophs residing in estuarine sediments has recently been shown to be a significant source of N to the overall N budget of these ecosystems under certain environmental conditions (Fulweiler et al., 2007). Sulfate reducing bacteria, such as members of the *Desulfovibrionaceae*, have been found to be one of the dominant groups of diazotrophs in a variety of estuarine systems including the sediments of the Narragansett Bay (Brown and Jenkins, 2014; Fulweiler et al., 2013), Eckernförde Bay (Baltic Sea) (Bertics et al., 2012), and Chesapeake Bay (Burns et al., 2002). The habitats in which these sulfate reducers dominate sediment N-fixation are typically replete for sources of combined N, the presence of which is believed to suppress nitrogen fixation in the environment. Although it has been known for some time that *Desulfovibrio* are capable of fixing N (Sisler & ZoBell, 1951), questions regarding controls on their N fixation behavior, such as why they fix N in seemingly N rich environments, and other questions about their autecology, have remained largely unanswered, in part due to a lack of cultivated representatives.

This thesis sought to couple bacterial genome analysis and comparative genomics with N fixation rate measurements under various combined N conditions in order to examine the response of nitrogenase activity to the presence of different N sources, and link that to the genomic potential (i.e. gene content) of members of the *Desulfovibrionaceae*. Nitrogen fixation rates for pure cultures of *D. sp. NAR1* grown under a variety of combined N conditions were examined using the acetylene reduction assay (Capone, 1993). Cultures without any reactive N source were found to reduce acetylene to ethylene, with peak ethylene production occurring between 5-10

days after inoculation. Cultures with added 12 mM nitrate were also found to reduce acetylene to ethylene, with peak ethylene production occurring 10 days after inoculation and with rates that were comparable to the reactive-N free control. Cultures with added 12 mM urea were found to reduce acetylene to ethylene, again with peak ethylene production occurring 10 days after inoculation but with rates that were slightly higher than the reactive-N free control and nitrate sets. Increased NA for NAR1 in the presence of urea is something that was observed in earlier pilot experiments, and is a result for which there is currently no explanation or known cause. Cultures with added 12 mM ammonia were not found to reduce acetylene to ethylene, exhibiting no measureable NA, which agrees with what is widely accepted regarding the behavior of the nitrogenase enzyme (Mackerras, 1986). The genomes of both NAR1 and NAR2 isolates were further found to lack genes necessary for catabolism of either nitrate or urea, but were found to possess genes necessary for assimilating and catabolizing ammonia and deaminating amino acids, which agrees with our ARA based observations. Additionally, these gene-level observations held true for the majority of the *Desulfovibrio* genomes that were assessed in comparison to our environmental isolates, suggesting that an inability to utilize forms of reactive N other than ammonia may be characteristic of the *Desulfovibrio*. This inability could in turn be part of why we observe N fixation by this group even in environments that are not limited for sources of combined N, such as Narragansett Bay.

In addition to providing insight regarding the N fixation behavior of members of the *Desulfovibrio*, this study also sought to add to the body of knowledge and collection of representative genomes for this genus by sequencing and annotating the

genomes of two novel *Desulfovibrio* isolates, *Desulfovibrio* sp. NAR1 and *Desulfovibrio* sp. NAR2, and characterizing additional aspects of their physiology and genome content outside of that which directly pertained to N fixation. The NAR1 isolate was found to produce a biofilm under nearly all growth conditions, where NAR2 exhibited no biofilm formation. The biofilm formation in NAR1 was also reflected at the genome level, with an evolutionary gene network revealing genes found only in NAR1 that were involved in sensing and synthesizing c-di-GMP, a cell-to-cell signaling molecule that has been shown to be involved in biofilm formation and persistence (Römmling et al., 2013; Hunter et al., 2014; Bellows et al., 2012). This could have important implications for future work involving *D. sp. NAR1*, or other biofilm forming *Desulfovibrio*, as the mechanism and genes involved in biofilm formation for this genus are not well characterized. The NAR1 isolate was also found to possess genes for mercury methylation, an environmentally harmful process that is primarily restricted to anaerobic sulfate reducing bacteria (Compeau & Bartha, 1985; Gilmour et al., 1992). Both isolates were found to have a significant number of genes involved in bacterial defense and heavy metal resistance, which is common for members of the *Desulfovibrio*, however, the presence of these genes may still provide a specific advantage for survival in Narragansett Bay sediments.

This study is meaningful to increase our understanding of the N metabolism and nitrogenase activity of members of the *Desulfovibrionaceae*, who are one of the primary groups responsible for heterotrophic N fixation in Narragansett Bay, RI sediments (Fulweiler et al., 2013). It is also advantageous to add to the body of molecular knowledge regarding this genus, as they are important to several

environmental processes and have additional industry applications (Fulweiler et al., 2013; Parks et al., 2013; Muyzer & Stams, 2008; Hulshoff et al., 1998; Martins et al., 2009; Gutierrez-Sanchez et al., 2011). By increasing our overall understanding of this important diazotrophic group through detailed examination of cultivated representatives and their N fixation profiles, and comparing those results to environmental field observations, we can gain a more comprehensive view of factors controlling prevalence and N fixation behavior of this group. This will then allow for better predictions of how N fixation by this group will respond to changing environmental conditions and could ultimately serve to better inform policies regarding environmental waste and water management.

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Appendix A. List of proteins contained in *Desulfovibrio sp.* NAR1 filtered network.

Network protein number	Contig assignment	Functional annotation
3_DESnar1	13	1,4-alpha-glucan branching enzyme
5_DESnar1	12	1-acyl-sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.51)
11_DESnar1	6	2'-5' RNA ligase
14_DESnar1	15	2,3-bisphosphoglycerate-independent phosphoglycerate mutase, archaeal type (EC 5.4.2.1)
20_DESnar1	13	2-heptaprenyl-1,4-naphthoquinone methyltransferase (EC 2.1.1.163)
21_DESnar1	10	2-heptaprenyl-1,4-naphthoquinone methyltransferase (EC 2.1.1.163)
22_DESnar1	7	2-hydroxychromene-2-carboxylate isomerase/DsbA-like thioredoxin domain
27_DESnar1	2	2-oxoglutarate oxoreductase, alpha subunit (EC 1.2.7.3)
34_DESnar1	3	2-oxoglutarate oxoreductase, delta subunit, putative (EC 1.2.7.3)
44_DESnar1	1	3-demethylubiquinone-9 3-O-methyltransferase
56_DESnar1	13	3-polypropenyl-4-hydroxybenzoate carboxy-lyase UbiX (EC 4.1.1.-)
58_DESnar1	1	4-amino-6-deoxy-N-Acetyl-D-hexosaminyl-(Lip carrier) acetyltrasferase
60_DESnar1	1	4-hydroxy-2-oxovalerate aldolase (EC 4.1.3.39)
64_DESnar1	2	4-hydroxybenzoyl-CoA thioesterase family active site
67_DESnar1	6	4Fe-4S binding domain/4Fe-4S dicluster domain
69_DESnar1	10	4Fe-4S dicluster domain
70_DESnar1	2	4Fe-4S ferredoxin, iron-sulfur binding domain protein
76_DESnar1	10	5-formyltetrahydrofolate cyclo-ligase (EC 6.3.3.2)
81_DESnar1	10	6-phosphogluconolactonase (EC 3.1.1.31), eukaryotic type
83_DESnar1	6	7,8 dihydropteroate synthase (methanopterin)
84_DESnar1	9	7,8 dihydropteroate synthase (methanopterin)
85_DESnar1	1	8-amino-7-oxononanoate synthase (EC 2.3.1.47)
87_DESnar1	6	16S rRNA processing protein RimM
88_DESnar1	6	16S rRNA processing protein RimM
89_DESnar1	6	115 kDa surface antigen
90_DESnar1	7	115 kDa surface antigen precursor
92_DESnar1	15	18K peptoglycan-associated outer membrane lipoprotein; Peptoglycan-associated lipoprotein precursor
104_DESnar1	1	A/G-specific adenine glycosylase (EC 3.2.2.-)
105_DESnar1	10	AAA domain containing protein
106_DESnar1	1	ABC transport system, permease component YbhR
107_DESnar1	1	ABC transport system, permease component YbhS
109_DESnar1	5	ABC transporter ATP-binding protein
110_DESnar1	14	ABC transporter
114_DESnar1	1	ABC transporter multrug efflux pump, fused ATP-binding domains
117_DESnar1	6	ABC transporter related
125_DESnar1	14	ABC Transporter ATP-binding protein
129_DESnar1	6	ABC Transporter permease protein
131_DESnar1	13	ABC-type amino ac transport, signal transduction systems, periplasmic component/domain
132_DESnar1	13	ABC-type amino ac transport, signal transduction systems, periplasmic component/domain
133_DESnar1	1	ABC-type amino ac transport, signal transduction systems, periplasmic component/domain
134_DESnar1	15	ABC-type amino ac transport, signal transduction systems, periplasmic component/domain
135_DESnar1	13	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
136_DESnar1	2	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
137_DESnar1	2	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
138_DESnar1	5	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
139_DESnar1	14	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
140_DESnar1	10	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
141_DESnar1	15	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
142_DESnar1	15	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
144_DESnar1	2	ABC-type amino ac transport/signal transduction systems, periplasmic component/domain
146_DESnar1	1	ABC-type branched-chain amino ac transport systems, periplasmic component
147_DESnar1	10	ABC-type branched-chain amino ac transport systems, periplasmic component
150_DESnar1	1	ABC-type metal ion transport system, periplasmic component/surface adhesin
151_DESnar1	1	ABC-type metal ion transport system, periplasmic component/surface adhesin
152_DESnar1	1	ABC-type multrug transport system, permease component
153_DESnar1	1	ABC-type nitrate/sulfonate/bicarbonate transport systems, periplasmic components
156_DESnar1	1	ABC-type polysacchare/polyol phosphate export systems, permease component
157_DESnar1	13	ABC-type sugar transport system, ATPase component
158_DESnar1	1	ABC-type sugar transport system, periplasmic binding protein YcjN

160_DESnar1	13	ABC-type transport system involved in resistance to organic solvents
162_DESnar1	1	ABC-type tungstate transport system, ATP-binding protein
166_DESnar1	1	Abortive infection C-terminus
167_DESnar1	1	Acetate kinase (EC 2.7.2.1)
180_DESnar1	5	Acetyl-CoA synthetase (ADP-forming) alpha chain (EC 6.2.1.13)
187_DESnar1	10	Acetylornithine aminotransferase (EC 2.6.1.11)
190_DESnar1	6	Acetyltransferase YpeA
191_DESnar1	6	Acetyltransferases
192_DESnar1	1	Acetyltransferases
193_DESnar1	2	Acetyltransferases
196_DESnar1	2	ACP:hemolysin acyltransferase (hemolysin-activating protein)
197_DESnar1	6	Acriflavin resistance protein
199_DESnar1	14	Acriflavin resistance protein
200_DESnar1	7	Acriflavin resistance protein
201_DESnar1	9	Acriflavin resistance protein
203_DESnar1	14	Activator of (R)-2-hydroxyglutaryl-CoA dehydratase
206_DESnar1	13	Acyl-ACP thioesterase
209_DESnar1	6	Acylphosphate phosphohydrolase (EC 3.6.1.7), putative
210_DESnar1	5	Acylphosphate phosphohydrolase (EC 3.6.1.7), putative
211_DESnar1	1	Acyltransferase family protein
216_DESnar1	2	Adenosylcobinamide-phosphate synthase
218_DESnar1	13	Adenylate cyclase (EC 4.6.1.1)
219_DESnar1	3	Adenylate cyclase (EC 4.6.1.1)
220_DESnar1	1	Adenylate cyclase (EC 4.6.1.1)
221_DESnar1	2	Adenylate cyclase (EC 4.6.1.1)
222_DESnar1	2	Adenylate cyclase
223_DESnar1	14	Adenylate cyclase
224_DESnar1	1	Adenylate cyclase, family 3 (some proteins contain HAMP domain)
228_DESnar1	1	Adenylylsulfate kinase (EC 2.7.1.25)
233_DESnar1	9	ADP-heptose synthase (EC 2.7.-.-) / D-glycero-beta-D-manno-heptose 7-phosphate kinase
235_DESnar1	15	ADP-heptose--lipooligosacchare heptosyltransferase II (EC 2.4.1.-)
240_DESnar1	6	Alanine racemase (EC 5.1.1.1)
243_DESnar1	14	Alcohol dehydrogenase (EC 1.1.1.1)
245_DESnar1	2	Aldehyde dehydrogenase, iron-sulfur subunit
247_DESnar1	5	Aldose 1-epimerase (EC 5.1.3.3)
250_DESnar1	5	Alkaline phosphatase (EC 3.1.3.1)
255_DESnar1	10	Alkyl hydroperoxide reductase subunit C-like protein
256_DESnar1	5	Alkylphosphonate utilization operon protein PhnA
258_DESnar1	10	Alpha-acetolactate decarboxylase (EC 4.1.1.5)
259_DESnar1	2	Alpha-glucosases, family 31 of glycosyl hydrolases
260_DESnar1	9	Alpha/beta hydrolase family
262_DESnar1	2	Amohydrolase family protein
266_DESnar1	13	Amino ac ABC transporter substrate-binding protein, PAAT family (TC 3.A.1.3.-)
267_DESnar1	1	Amino ac ABC transporter substrate-binding protein, PAAT family (TC 3.A.1.3.-)
268_DESnar1	15	Amino ac ABC transporter substrate-binding protein, PAAT family (TC 3.A.1.3.-)
271_DESnar1	10	Amino ac ABC Transporter ATP-binding protein
272_DESnar1	10	Amino ac ABC Transporter periplasmic amino ac-binding protein
273_DESnar1	13	Amino ac ABC Transporter periplasmic precursor
274_DESnar1	10	Amino ac ABC Transporter permease protein
279_DESnar1	13	amino ac/ame ABC transporter substrate-binding protein, HAAT family (TC 3.A.1.4.-)
281_DESnar1	9	Amioxychorismate lyase (EC 4.1.3.38)
293_DESnar1	2	Anaerobic dehydrogenases, typically selenocysteine-containing
300_DESnar1	10	Ankyrin repeats
305_DESnar1	2	Apolipoprotein N-acyltransferase (EC 2.3.1.-) / Copper homeostasis protein CutE
307_DESnar1	5	Arabinose efflux permease
309_DESnar1	6	AraC-type DNA-binding domain-containing proteins
311_DESnar1	14	AraC-type DNA-binding domain-containing proteins
312_DESnar1	10	AraC-type DNA-binding domain-containing proteins
313_DESnar1	11	AraC-type DNA-binding domain-containing proteins
320_DESnar1	13	Arsenical pump-driving ATPase (EC 3.6.3.16)
321_DESnar1	13	Arsenical resistance operon repressor
322_DESnar1	14	Arsenical resistance operon repressor

325_DESnar1	1	Arylesterase precursor (EC 3.1.1.2)
326_DESnar1	5	Arylsulfotransferase (ASST)
327_DESnar1	5	AsmA protein
328_DESnar1	5	asparagine synthase (glutamine-hydrolyzing)
329_DESnar1	5	Asparagine synthetase [glutamine-hydrolyzing] (EC 6.3.5.4)
348_DESnar1	9	At2g5740/F3N11.19
366_DESnar1	5	ATP-dependent DNA helicase pcrA (EC 3.6.1.-)
370_DESnar1	13	ATP-dependent exoDNase (exonuclease V) alpha subunit - helicase superfamily I member
371_DESnar1	2	ATP-dependent helicase
372_DESnar1	10	ATP-dependent helicase
381_DESnar1	1	ATP-dependent transcriptional regulator-like protein
382_DESnar1	9	ATP-utilizing enzyme of the PP-loop superfamily
383_DESnar1	1	ATPase
385_DESnar1	3	ATPase component STY3233 of energizing module of queuosine-regulated ECF transporter
387_DESnar1	2	ATPase YjeE, predicted to have essential role in cell wall biosynthesis
391_DESnar1	13	Autolysis histine kinase LytS
397_DESnar1	11	Bacterial RNase P class A
399_DESnar1	15	Bacteriophage CI repressor helix-turn-helix domain
400_DESnar1	2	Bacteriophage tail assembly protein
401_DESnar1	2	Baseplate assembly protein J
403_DESnar1	6	Beta-hexosaminase (EC 3.2.1.52)
406_DESnar1	9	Beta-propeller domains of methanol dehydrogenase type
407_DESnar1	13	Bifunctional protein: zinc-containing alcohol dehydrogenase; quinone oxoreductase
408_DESnar1	1	binding-protein-dependent transport systems inner membrane component
412_DESnar1	5	Biopolymer transport protein ExbD/TolR
420_DESnar1	2	Biotin-(acetyl-CoA carboxylase) ligase
421_DESnar1	2	Biotin-protein ligase (EC 6.3.4.15)
423_DESnar1	1	Branched-chain amino ac ABC Transporter amino ac-binding protein (TC 3.A.1.4.1)
440_DESnar1	9	Butyrate kinase (EC 2.7.2.7)
442_DESnar1	15	CGCAxxGCC family protein
444_DESnar1	6	CAAX prenyl protease 1, putative
446_DESnar1	3	Candidate gene for the hypothesized phosphomevalonate decarboxylase; COG1355
447_DESnar1	1	Capsular polysacchare ABC Transporter ATP-binding protein KpsT
448_DESnar1	1	Capsular polysacchare export system inner membrane protein KpsE
449_DESnar1	1	Capsular polysacchare export system periplasmic protein KpsD
450_DESnar1	1	Capsular polysacchare export system protein KpsC
451_DESnar1	1	Capsular polysacchare export system protein KpsS
455_DESnar1	15	Carbon monoxide dehydrogenase CooS subunit (EC 1.2.99.2)
456_DESnar1	15	Carbon monoxide-responsive transcriptional activator CooA
457_DESnar1	13	Carbon starvation protein A
458_DESnar1	13	Carbon starvation protein, predicted membrane protein
463_DESnar1	3	Cardiolipin synthase
464_DESnar1	3	Cardiolipin synthetase (EC 2.7.8.-)
465_DESnar1	2	Catalase (EC 1.11.1.6)/Peroxase (EC 1.11.1.7)
466_DESnar1	2	Cation/multitrug efflux pump
472_DESnar1	1	Cell division inhibitor
482_DESnar1	13	Cell division protein FtsQ
491_DESnar1	5	Cell wall-associated hydrolases (invasion-associated proteins)
492_DESnar1	1	Cephalosporin hydroxylase
494_DESnar1	14	CgeB family protein
495_DESnar1	15	CgeB family protein
497_DESnar1	10	Channel-forming transporter/cytolysins activator of TpsB family
502_DESnar1	10	Chemotaxis protein CheD
505_DESnar1	5	Chemotaxis protein cheV (EC 2.7.3.-)
509_DESnar1	5	Chemotaxis protein methyltransferase CheR (EC 2.1.1.80)
512_DESnar1	3	Chemotaxis regulator-transmits chemoreceptor signals to flagellar motor components CheY
514_DESnar1	2	Chemotaxis regulator-transmits chemoreceptor signals to flagellar motor components CheY
521_DESnar1	1	Chemotaxis signal transduction protein
522_DESnar1	5	Chitin catabolic cascade sensor histine kinase ChiS
526_DESnar1	1	Choline-glycine betaine transporter
533_DESnar1	9	Chromosome (plasm) partitioning protein ParB
534_DESnar1	1	Chromosome initiation inhibitor

537_DESnar1	14	Citrate synthase (si) (EC 2.3.3.1)
538_DESnar1	1	Class III cytochrome C family
540_DESnar1	15	CO dehydrogenase accessory protein CooC (nickel insertion)
552_DESnar1	6	Cobalt-precorrin-6 synthase, anaerobic
562_DESnar1	6	Cobalt-precorrin-6 synthase, anaerobic
563_DESnar1	5	CofD-related protein, GAK system
566_DESnar1	2	COG0398: uncharacterized membrane protein/PF00070 family
572_DESnar1	10	COG1242: Predicted Fe-S oxoreductase
575_DESnar1	5	COG1720: Uncharacterized conserved protein
576_DESnar1	3	COG2740: Predicted nucleic-ac-binding protein implicated in transcription termination
577_DESnar1	1	COG2827: putative endonuclease containing a URI domain
579_DESnar1	1	COG4123: Predicted O-methyltransferase
582_DESnar1	14	Cold-shock DNA-binding protein family
583_DESnar1	13	Competence protein F homolog, phosphoribosyltransferase domain; protein YhgH
588_DESnar1	10	Conserved hypothetical protein
589_DESnar1	2	Conserved protein, permease-related
603_DESnar1	1	CTP:molybdopterin cytylyltransferase
604_DESnar1	2	CTP:molybdopterin cytylyltransferase
605_DESnar1	10	Cupin 2, conserved barrel domain protein
606_DESnar1	10	Cyclic nucleotide-binding protein
608_DESnar1	5	Cyclolysin secretion ATP-binding protein
610_DESnar1	14	Cysteine desulfurase (EC 2.8.1.7)
617_DESnar1	1	Cytochrome B561
619_DESnar1	6	cytochrome c family protein
620_DESnar1	1	Cytochrome c family protein
626_DESnar1	1	Cytochrome c-type biogenesis protein DsbD, protein-disulfide reductase (EC 1.8.1.8)
628_DESnar1	3	Cytochrome c3
629_DESnar1	10	Cytochrome C553 (soluble cytochrome f)
636_DESnar1	6	D-alanyl-D-alanine carboxypeptase
637_DESnar1	15	D-glycero-D-manno-heptose 1,7-bisphosphate phosphatase
644_DESnar1	5	DegT/DnrJ/EryC1/StrS aminotransferase family protein
647_DESnar1	6	Deoxyguanosinetriphosphate triphosphohydrolase (EC 3.1.5.1)
656_DESnar1	14	diguanylate cyclase (GGDEF) domain
658_DESnar1	6	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
659_DESnar1	6	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
660_DESnar1	6	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
661_DESnar1	6	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
662_DESnar1	1	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
665_DESnar1	1	Dihydrofolate synthase (EC 6.3.2.12) / Folylpolyglutamate synthase (EC 6.3.2.17)
677_DESnar1	1	Dinucleotide-utilizing enzymes involved in molybdopterin and thiamine biosynthesis family 2
678_DESnar1	1	Dinucleotide-utilizing enzymes involved in molybdopterin and thiamine biosynthesis family 2
686_DESnar1	1	DNA internalization-related competence protein ComEC/Rec2
703_DESnar1	10	DNA topoisomerase IV subunit A
704_DESnar1	1	DNA-3-methyladenine glycosylase (EC 3.2.2.20)
709_DESnar1	1	DNA-binding protein, CopG family
710_DESnar1	2	DNA-binding protein, CopG family
714_DESnar1	1	DNA-binding response regulator LuxR family
715_DESnar1	5	DNA-cytosine methyltransferase (EC 2.1.1.37)
716_DESnar1	5	DNA-cytosine methyltransferase (EC 2.1.1.37)
717_DESnar1	5	DNA-cytosine methyltransferase (EC 2.1.1.37)
723_DESnar1	6	Dolichol-phosphate mannosyltransferase (EC 2.4.1.83)
726_DESnar1	10	Dolichol-phosphate-mannose-protein mannosyltransferase family protein
727_DESnar1	1	Domain of unknown function (DUF303)
728_DESnar1	5	Domain of unknown function (DUF1837)
729_DESnar1	3	Domain of unknown function (DUF1987)
733_DESnar1	1	dTDP-4-dehydrorhamnose 3,5-epimerase (EC 5.1.3.13)
735_DESnar1	1	dTDP-4-dehydrorhamnose 3,5-epimerase (EC 5.1.3.13)
736_DESnar1	1	dTDP-4-dehydrorhamnose reductase (EC 1.1.1.133)
739_DESnar1	1	dTDP-glucose 4,6-dehydratase (EC 4.2.1.46)
742_DESnar1	14	EBNA-1 nuclear protein
744_DESnar1	14	electron transfer flavoprotein beta subunit-like protein
750_DESnar1	1	Electron transport complex protein RnfC

751_DESnarl	5	Electron transport complex protein RnfC
762_DESnarl	6	Endonuclease/exonuclease/phosphatase
770_DESnarl	5	Energy-conserving hydrogenase (ferredoxin), subunit F
773_DESnarl	1	Epoxide hydrolase (EC 3.3.2.9)
774_DESnarl	2	ERF superfamily
775_DESnarl	5	ErfK/YbiS/YcfS/YnhG family protein
787_DESnarl	14	Exopolyphosphatase (EC 3.6.1.11)
788_DESnarl	14	Exopolyphosphatase
791_DESnarl	13	Extracellular ligand-binding receptor
792_DESnarl	5	Extracellular ligand-binding receptor
793_DESnarl	6	ExtraCellular Mutant; Ecm15p
794_DESnarl	14	Extracellular solute binding protein ScrB
795_DESnarl	15	Extracellular solute-binding protein, family 3/GGDEF domain protein
796_DESnarl	13	extracellular solute-binding protein, putative
800_DESnarl	1	Fatty ac desaturase
801_DESnarl	1	Fatty-ac desaturase (EC 1.14.99.-)
813_DESnarl	1	Fe ²⁺ /Zn ²⁺ uptake regulation proteins
816_DESnarl	6	Ferredoxin
817_DESnarl	3	Ferredoxin
820_DESnarl	14	Ferredoxin
823_DESnarl	10	Ferredoxin
826_DESnarl	1	Ferric iron ABC Transporter ATP-binding protein
828_DESnarl	2	Ferric serophore transport system, periplasmic binding protein TonB
831_DESnarl	1	Ferric uptake regulation protein FUR
832_DESnarl	14	Ferric uptake regulation protein FUR
840_DESnarl	1	FIG000233: metal-dependent hydrolase
843_DESnarl	9	FIG000605: protein co-occurring with transport systems (COG1739)
848_DESnarl	14	FIG004453: protein YceG like
850_DESnarl	9	FIG004694: Hypothetical protein
851_DESnarl	13	FIG022606: AAA ATPase
854_DESnarl	1	FIG049111: Hypothetical protein in pyoverdine gene cluster
855_DESnarl	3	FIG057251: Fe-S oxoreductase
856_DESnarl	13	FIG059250: hypothetical protein
862_DESnarl	3	FIG146085: 3'-to-5' oligoribonuclease A, Bacillus type
865_DESnarl	1	FIG00440303: hypothetical protein
870_DESnarl	2	FIG00602277: hypothetical protein
871_DESnarl	3	FIG00602295: hypothetical protein
874_DESnarl	3	FIG00602413: hypothetical protein
875_DESnarl	15	FIG00602413: hypothetical protein
876_DESnarl	10	FIG00602432: hypothetical protein
877_DESnarl	2	FIG00602438: hypothetical protein
880_DESnarl	2	FIG00602496: hypothetical protein
881_DESnarl	2	FIG00602510: hypothetical protein
883_DESnarl	15	FIG00602569: hypothetical protein
885_DESnarl	15	FIG00602578: hypothetical protein
887_DESnarl	2	FIG00602628: hypothetical protein
889_DESnarl	2	FIG00602688: hypothetical protein
892_DESnarl	6	FIG00602793: hypothetical protein
896_DESnarl	2	FIG00602866: hypothetical protein
897_DESnarl	1	FIG00602945: hypothetical protein
899_DESnarl	15	FIG00602963: hypothetical protein
906_DESnarl	1	FIG00603294: hypothetical protein
911_DESnarl	3	FIG00603589: hypothetical protein
916_DESnarl	15	FIG00603666: hypothetical protein
917_DESnarl	2	FIG00603746: hypothetical protein
922_DESnarl	5	FIG00603852: hypothetical protein
929_DESnarl	2	FIG00604212: hypothetical protein
930_DESnarl	5	FIG00604258: hypothetical protein
937_DESnarl	3	FIG00604763: hypothetical protein
942_DESnarl	1	FIG00605110: hypothetical protein
945_DESnarl	5	FIG00605436: hypothetical protein
947_DESnarl	5	FIG00605486: hypothetical protein

949_DESnar1	10	FIG00605567: hypothetical protein
955_DESnar1	1	FIG00606315: hypothetical protein
957_DESnar1	14	FIG00606843: hypothetical protein
961_DESnar1	1	FIG00607845: hypothetical protein
963_DESnar1	13	FIG00609972: hypothetical protein
964_DESnar1	5	FIG00640255: hypothetical protein
966_DESnar1	3	FIG00765372: hypothetical protein
967_DESnar1	14	FIG00765372: hypothetical protein
968_DESnar1	3	FIG00824701: hypothetical protein
970_DESnar1	10	FIG01160836: hypothetical protein
972_DESnar1	5	FIG01161872: hypothetical protein
973_DESnar1	1	FIG01163484: hypothetical protein
977_DESnar1	14	Flagellar assembly protein FliH
978_DESnar1	3	Flagellar basal-body P-ring formation protein FlgA
980_DESnar1	14	Flagellar basal-body rod protein FlgB
984_DESnar1	3	flagellar basal-body rod protein, putative
989_DESnar1	1	Flagellar biosynthesis protein FliL
996_DESnar1	6	Flagellar hook protein FlgE
1001_DESnar1	14	Flagellar hook-basal body complex protein FliE
1002_DESnar1	11	Flagellar hook-length control protein FliK
1012_DESnar1	2	Flagellar motor rotation protein MotB
1020_DESnar1	3	Flagellar protein FlgJ [peptoglycan hydrolase] (EC 3.2.1.-)
1024_DESnar1	13	Flagellin protein FlaA
1025_DESnar1	10	Flagellin protein FlaA
1029_DESnar1	6	Flavodoxin domain/4Fe-4S binding domain
1033_DESnar1	5	Flp pilus assembly protein TadD, contains TPR repeat
1036_DESnar1	13	FOG: GGDEF domain
1037_DESnar1	9	FOG: GGDEF domain
1039_DESnar1	7	FOG: HEAT repeat
1040_DESnar1	10	FOG: HPt domain
1042_DESnar1	1	FOG: PAS/PAC domain
1044_DESnar1	5	FOG: TPR repeat, SEL1 subfamily
1045_DESnar1	7	Foldase protein PrsA precursor (EC 5.2.1.8)
1048_DESnar1	13	Formate dehydrogenase chain D (EC 1.2.1.2)
1050_DESnar1	13	Formate dehydrogenase formation protein FdhE
1051_DESnar1	10	Formate dehydrogenase formation protein FdhE
1060_DESnar1	5	Formate dehydrogenase-O, major subunit (EC 1.2.1.2)
1067_DESnar1	10	Fosfomycin resistance protein FosX
1071_DESnar1	9	Fructose-2,6-bisphosphatase
1075_DESnar1	1	Fumarate reductase cytochrome b subunit
1076_DESnar1	6	GAF domain/HD domain protein
1077_DESnar1	5	Galactokinase (EC 2.7.1.6)
1079_DESnar1	1	Galactose O-acetyltransferase (EC 2.3.1.18)
1081_DESnar1	13	GCN5-related N-acetyltransferase
1082_DESnar1	6	GCN5-related N-acetyltransferase
1083_DESnar1	3	GDP-L-fucose synthetase (EC 1.1.1.271)
1088_DESnar1	5	Geranylgeranyl reductase (EC 1.3.1.83)
1089_DESnar1	6	GGDEF domain protein
1093_DESnar1	14	GGDEF domain protein
1094_DESnar1	10	GGDEF/PAS/PAC-domain containing protein
1095_DESnar1	13	Gll2461 protein
1101_DESnar1	1	Glucose-1-phosphate cytylyltransferase (EC 2.7.7.33)
1102_DESnar1	1	Glucose-1-phosphate cytylyltransferase
1118_DESnar1	2	Glutamine ABC Transporter periplasmic glutamine-binding
1121_DESnar1	10	Glutamine cyclotransferase
1136_DESnar1	13	Glycose hydrolase, family 13 domain protein
1137_DESnar1	9	Glycosyl transferase 4-like domain
1138_DESnar1	1	Glycosyl transferase
1139_DESnar1	1	Glycosyl transferase family 2
1140_DESnar1	1	Glycosyl transferase group 1
1141_DESnar1	1	Glycosyl transferase, family 2
1142_DESnar1	1	Glycosyl transferase, family 2

1144_DESnar1	5	Glycosyl transferase, family 2
1145_DESnar1	1	Glycosyl transferase, group 1
1146_DESnar1	14	Glycosyl transferase, group 1
1147_DESnar1	11	Glycosyl transferase, group 1
1150_DESnar1	1	Glycosyl transferase, group 2-Jan family protein
1151_DESnar1	6	Glycosyl transferase, group 2 family protein
1152_DESnar1	6	Glycosyl transferase, group 2 family protein
1153_DESnar1	3	Glycosyl transferase, group 2 family protein
1154_DESnar1	1	Glycosyl transferase, group 2 family protein
1156_DESnar1	11	Glycosyl transferase, group 2 family protein
1157_DESnar1	6	Glycosyltransferase (EC 2.4.1.-)
1158_DESnar1	3	Glycosyltransferase (EC 2.4.1.-)
1159_DESnar1	1	Glycosyltransferase (EC 2.4.1.-)
1160_DESnar1	5	Glycosyltransferase (EC 2.4.1.-)
1161_DESnar1	7	Glycosyltransferase (EC 2.4.1.-)
1162_DESnar1	3	Glycosyltransferase
1163_DESnar1	3	Glycosyltransferase
1164_DESnar1	3	Glycosyltransferase
1165_DESnar1	1	Glycosyltransferase
1166_DESnar1	1	Glycosyltransferase
1167_DESnar1	1	Glycosyltransferase
1168_DESnar1	5	Glycosyltransferase
1169_DESnar1	15	Glycosyltransferase
1170_DESnar1	6	Glycosyltransferases involved in cell wall biogenesis
1171_DESnar1	1	Glycosyltransferases involved in cell wall biogenesis
1172_DESnar1	1	Glycosyltransferases involved in cell wall biogenesis
1177_DESnar1	13	Glyoxalase/bleomycin resistance protein/dioxygenase
1180_DESnar1	11	GMP synthase [glutamine-hydrolyzing] (EC 6.3.5.2)
1192_DESnar1	2	HAD-superfamily hydrolase, subfamily IA, variant 1
1194_DESnar1	10	HAMP domain/GAF domain/HD domain protein
1197_DESnar1	11	HD domain protein
1209_DESnar1	1	heat shock protein, Hsp20 family
1210_DESnar1	2	heat shock protein, Hsp20 family
1212_DESnar1	5	Helicase conserved C-terminal domain protein
1216_DESnar1	6	Hemerythrin-like iron-binding protein
1217_DESnar1	13	Hemolysin-type calcium-binding region
1221_DESnar1	2	heptosyltransferase family protein
1223_DESnar1	10	HesA/MoeB/ThiF family protein
1224_DESnar1	6	HesB-like domain
1234_DESnar1	13	High-affinity choline uptake protein BetT
1236_DESnar1	15	High-affinity leucine-specific transport system
1237_DESnar1	13	High-molecular-weight cytochrome c precursor
1238_DESnar1	13	HipA protein
1240_DESnar1	10	His Kinase A (phospho-acceptor)
1241_DESnar1	13	His Kinase A (phospho-acceptor)
1242_DESnar1	9	histine kinase
1244_DESnar1	13	Histine kinase-, DNA gyrase B-, and F
1250_DESnar1	5	HlyD family secretion protein
1253_DESnar1	5	HNH endonuclease
1264_DESnar1	2	Hydrolase (HAD superfamily)
1265_DESnar1	15	Hydrolase, alpha/beta fold family
1268_DESnar1	9	Hydroxyethylthiazole kinase (EC 2.7.1.50)
1269_DESnar1	9	Hydroxyethylthiazole kinase, sugar kinase family
1273_DESnar1	2	Hydroxymethylpyrimine phosphate kinase Th (EC 2.7.4.7)
1277_DESnar1	5	hypothetical cytosolic protein
1278_DESnar1	6	Hypothetical iron-sulfur cluster binding protein YccM
1279_DESnar1	6	Hypothetical iron-sulfur cluster binding protein YccM
1281_DESnar1	6	hypothetical membrane protein
1282_DESnar1	13	hypothetical protein
1283_DESnar1	13	hypothetical protein
1284_DESnar1	13	hypothetical protein
1285_DESnar1	13	hypothetical protein

1989_DESnarl	14	hypothetical protein
1990_DESnarl	14	hypothetical protein
1991_DESnarl	14	hypothetical protein
1992_DESnarl	14	hypothetical protein
1993_DESnarl	14	hypothetical protein
1994_DESnarl	14	hypothetical protein
1995_DESnarl	14	hypothetical protein
1997_DESnarl	14	hypothetical protein
1999_DESnarl	14	hypothetical protein
2000_DESnarl	14	hypothetical protein
2001_DESnarl	14	hypothetical protein
2002_DESnarl	14	hypothetical protein
2003_DESnarl	14	hypothetical protein
2006_DESnarl	14	hypothetical protein
2008_DESnarl	14	hypothetical protein
2009_DESnarl	14	hypothetical protein
2010_DESnarl	14	hypothetical protein
2011_DESnarl	14	hypothetical protein
2013_DESnarl	14	hypothetical protein
2015_DESnarl	14	hypothetical protein
2018_DESnarl	14	hypothetical protein
2019_DESnarl	14	hypothetical protein
2021_DESnarl	14	hypothetical protein
2023_DESnarl	14	hypothetical protein
2024_DESnarl	14	hypothetical protein
2026_DESnarl	14	hypothetical protein
2027_DESnarl	14	hypothetical protein
2029_DESnarl	14	hypothetical protein
2030_DESnarl	14	hypothetical protein
2031_DESnarl	14	hypothetical protein
2050_DESnarl	7	hypothetical protein
2051_DESnarl	7	hypothetical protein
2052_DESnarl	7	hypothetical protein
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2058_DESnarl	7	hypothetical protein
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2061_DESnarl	7	hypothetical protein
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2063_DESnarl	7	hypothetical protein
2064_DESnarl	7	hypothetical protein
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2069_DESnarl	7	hypothetical protein
2070_DESnarl	7	hypothetical protein
2071_DESnarl	7	hypothetical protein
2072_DESnarl	7	hypothetical protein
2073_DESnarl	7	hypothetical protein
2074_DESnarl	7	hypothetical protein
2075_DESnarl	7	hypothetical protein
2076_DESnarl	7	hypothetical protein
2079_DESnarl	7	hypothetical protein
2080_DESnarl	7	hypothetical protein
2081_DESnarl	7	hypothetical protein
2082_DESnarl	7	hypothetical protein
2083_DESnarl	7	hypothetical protein
2084_DESnarl	7	hypothetical protein
2085_DESnarl	7	hypothetical protein

2091_DESnar1	7	hypothetical protein
2109_DESnar1	8	hypothetical protein
2110_DESnar1	8	hypothetical protein
2112_DESnar1	8	hypothetical protein
2116_DESnar1	9	hypothetical protein
2117_DESnar1	9	hypothetical protein
2118_DESnar1	9	hypothetical protein
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2144_DESnar1	9	hypothetical protein
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2160_DESnar1	10	hypothetical protein
2161_DESnar1	10	hypothetical protein
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2165_DESnar1	10	hypothetical protein
2166_DESnar1	10	hypothetical protein
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2178_DESnar1	10	hypothetical protein
2179_DESnar1	10	hypothetical protein
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2200_DESnar1	10	hypothetical protein

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2304_DESnar1	11	hypothetical protein
2305_DESnar1	11	hypothetical protein
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2313_DESnar1	11	hypothetical protein
2315_DESnar1	11	hypothetical protein
2316_DESnar1	11	hypothetical protein
2317_DESnar1	11	hypothetical protein
2320_DESnar1	11	hypothetical protein
2340_DESnar1	2	Hypothetical protein Cj1505c
2341_DESnar1	10	hypothetical protein YaeJ
5614_DESnar1	1	Imazole glycerol phosphate synthase amotransferase
5616_DESnar1	1	Imazole glycerol phosphate synthase cyclase
5618_DESnar1	7	Imazolonepropionase and related amohydrolases
5621_DESnar1	1	Import inner membrane translocase, subunit Tim44
5622_DESnar1	15	Inactive homolog of metal-dependent proteases
5631_DESnar1	2	Inositol-1-monophosphatase (EC 3.1.3.25)

5634_DESnar1	10	Integral membrane protein, DUF6
5636_DESnar1	1	Integrase
5637_DESnar1	2	Integrase
5638_DESnar1	2	Integrase
5639_DESnar1	5	Integrase
5641_DESnar1	1	integrase family protein
5645_DESnar1	15	iron (III) ABC transporter ATP-binding
5648_DESnar1	13	Iron(III) dicitrate-binding protein
5651_DESnar1	3	Iron-sulfur cluster assembly scaffold protein
5653_DESnar1	10	iron-sulfur cluster binding protein
5654_DESnar1	1	Iron-sulfur cluster-binding protein
5656_DESnar1	14	iron-sulfur cluster-binding protein
5658_DESnar1	2	iron-sulfur flavoprotein
5660_DESnar1	13	iron-sulfur flavoprotein, putative
5662_DESnar1	6	Isochorismatase (EC 3.3.2.1)
5669_DESnar1	6	Kinesin light chain
5679_DESnar1	1	L-serine dehydratase (EC 4.3.1.17)
5687_DESnar1	10	Lhr-like helicases
5688_DESnar1	1	Lip A export ATP-binding/permease protein MsbA
5689_DESnar1	9	Lip carrier : UDP-N-acetylgalactosaminyltransferase
5695_DESnar1	13	Lipopolysacchare biosynthesis chain length determinant
5702_DESnar1	6	lipoprotein, putative
5703_DESnar1	2	lipoprotein, putative
5707_DESnar1	9	lipoprotein, putative
5710_DESnar1	14	Long-chain acyl-CoA synthetases (AMP-forming)
5711_DESnar1	1	Long-chain fatty ac transport protein
5713_DESnar1	1	Long-chain-fatty-ac--CoA ligase (EC 6.2.1.3)
5741_DESnar1	10	LSU ribosomal protein L25p
5747_DESnar1	13	LSU ribosomal protein L32P
5753_DESnar1	2	LysE type translocator
5756_DESnar1	16	Lysine exporter protein (LYSE/YGGA)
5758_DESnar1	6	Lysophospholipase L1 and related esterases
5761_DESnar1	5	M. jannaschii predicted coding region MJ0455
5762_DESnar1	3	Mac 1
5764_DESnar1	2	Magnesium and cobalt efflux protein CorC
5766_DESnar1	13	major facilitator superfamily MFS 1
5768_DESnar1	10	major facilitator superfamily MFS 1
5771_DESnar1	1	Malonyl CoA-acyl carrier protein transacylase
5773_DESnar1	1	Manganese transport system ATP-binding protein
5775_DESnar1	3	Mannose-1-phosphate guanylyltransferase (GDP)
5777_DESnar1	9	MATE efflux family protein
5779_DESnar1	6	Membrane fusion protein of RND family multrug efflux pump
5780_DESnar1	2	Membrane fusion protein of RND family multrug efflux pump
5781_DESnar1	13	membrane protein
5782_DESnar1	6	membrane protein
5783_DESnar1	14	Membrane protein
5789_DESnar1	13	membrane protein, putative
5790_DESnar1	3	membrane protein, putative
5793_DESnar1	5	membrane protein, putative
5796_DESnar1	10	Membrane proteins related to metalloendopeptases
5798_DESnar1	1	Membrane-associated lipoprotein
5800_DESnar1	6	Membrane-bound lysozyme inhibitor of c-type lysozyme
5802_DESnar1	1	Membrane-bound lytic murein transglycosylase A precursor
5803_DESnar1	1	Membrane-bound lytic murein transglycosylase B precursor
5807_DESnar1	1	Membrane-bound lytic murein transglycosylase E (EC 3.2.1.-)
5809_DESnar1	7	Membrane-fusion protein
5811_DESnar1	6	Menaquinone via futasoline step 2
5814_DESnar1	2	Mercuric ion reductase (EC 1.16.1.1)
5815_DESnar1	6	metal dependent phosphohydrolase
5818_DESnar1	11	Metal-dependent hydrolase (EC 3.-.-.-)
5819_DESnar1	1	Metal-dependent hydrolase
5821_DESnar1	5	Metal-dependent hydrolases of the beta-lactamase superfamily II

5822_DESnar1	1	Metal-dependent phosphohydrolase
5823_DESnar1	9	Metal-dependent phosphohydrolase
5828_DESnar1	10	metallophosphoesterase
5835_DESnar1	6	Methionine transporter MetT
5839_DESnar1	3	Methyl-accepting chemotaxis protein
5840_DESnar1	1	Methyl-accepting chemotaxis protein
5841_DESnar1	1	Methyl-accepting chemotaxis protein
5842_DESnar1	2	Methyl-accepting chemotaxis protein
5843_DESnar1	2	Methyl-accepting chemotaxis protein
5844_DESnar1	2	Methyl-accepting chemotaxis protein
5845_DESnar1	2	methyl-accepting chemotaxis protein
5846_DESnar1	5	Methyl-accepting chemotaxis protein
5847_DESnar1	14	methyl-accepting chemotaxis protein
5849_DESnar1	9	Methyl-accepting chemotaxis protein
5850_DESnar1	13	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5851_DESnar1	13	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5852_DESnar1	1	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5853_DESnar1	2	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5854_DESnar1	2	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5855_DESnar1	14	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5856_DESnar1	14	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5857_DESnar1	10	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5858_DESnar1	10	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
5859_DESnar1	13	methyl-accepting chemotaxis sensory transducer with Cache sensor
5860_DESnar1	5	methyl-accepting chemotaxis sensory transducer with Cache sensor
5861_DESnar1	14	methyl-accepting chemotaxis sensory transducer with Cache sensor
5862_DESnar1	2	methyl-accepting chemotaxis sensory transducer with Pas/Pac sensor
5864_DESnar1	13	Methylase involved in ubiquinone/menaquinone biosynthesis
5865_DESnar1	10	Methylated-DNA--protein-cysteine methyltransferase (EC 2.1.1.63)
5866_DESnar1	2	methylated-DNA--protein-cysteine methyltransferase-related protein
5871_DESnar1	1	Methyltransferase
5872_DESnar1	5	Methyltransferase RD1 738
5873_DESnar1	1	methyltransferase, FkbM family domain protein
5874_DESnar1	1	methyltransferase, putative
5880_DESnar1	10	Mll3258 protein
5881_DESnar1	13	Mlr1851 protein
5882_DESnar1	13	Mlr1851 protein
5883_DESnar1	10	MobD
5884_DESnar1	5	Mobile element protein
5885_DESnar1	5	Mobile element protein
5889_DESnar1	2	Molybdenum ABC Transporter periplasmic molybdenum-binding protein
5890_DESnar1	5	Molybdenum ABC Transporter periplasmic molybdenum-binding protein
5893_DESnar1	1	Molybdenum cofactor biosynthesis protein MoaA
5896_DESnar1	1	Molybdenum cofactor biosynthesis protein MoaB
5898_DESnar1	2	Molybdenum transport ATP-binding protein ModC (TC 3.A.1.8.1)
5899_DESnar1	5	Molybdenum transport ATP-binding protein ModC (TC 3.A.1.8.1)
5900_DESnar1	10	Molybdenum transport ATP-binding protein ModC (TC 3.A.1.8.1)
5902_DESnar1	5	Molybdenum transport system permease protein ModB (TC 3.A.1.8.1)
5904_DESnar1	2	molybdenum-pterin binding domain
5905_DESnar1	2	molybdenum-pterin binding domain protein/site-specific recombinase
5913_DESnar1	7	Molybdopterin biosynthesis protein MoeA
5915_DESnar1	2	Molybdopterin oxoreductase, membrane subunit
5916_DESnar1	9	Molybdopterin-guanine dinucleotide biosynthesis protein A
5919_DESnar1	7	Molybdopterin-guanine dinucleotide biosynthesis protein MobB
5921_DESnar1	5	MotA/TolQ/ExbB proton channel family protein
5923_DESnar1	1	multi-sensor hybr histine kinase
5924_DESnar1	14	multi-sensor hybr histine kinase
5935_DESnar1	1	N-acetyl sugar amotransferase
5936_DESnar1	6	N-Acetyl-D-glucosamine ABC transport system, sugar-binding protein
5938_DESnar1	1	N-acetylglucosamine-1-phosphate uryltransferase
5941_DESnar1	3	N-acetylmannosaminyltransferase (EC 2.4.1.187)
5944_DESnar1	1	N-Acetylneuraminate cytylyltransferase (EC 2.7.7.43)

5945_DESnar1	1	N-Acetylneuraminate cytylyltransferase (EC 2.7.7.43)
5946_DESnar1	5	N-Acetylneuraminate cytylyltransferase (EC 2.7.7.43)
5947_DESnar1	1	N-acetylneuraminate synthase (EC 2.5.1.56)
5949_DESnar1	1	N-carbamoylputrescine amase (3.5.1.53)
5959_DESnar1	5	NAD binding oxoreductase
5960_DESnar1	3	NAD kinase (EC 2.7.1.23)
5964_DESnar1	2	NAD(P)H oxoreductase YRKL (EC 1.6.99.-)
5965_DESnar1	2	NAD(P)H-nitrite reductase
5966_DESnar1	2	NAD(P)HX epimerase / NAD(P)HX dehydratase
5986_DESnar1	13	NADPH:quinone reductase
5989_DESnar1	11	NHL domain/cytochrome c family protein
5994_DESnar1	15	Nicotinamide-nucleotide adenyltransferase, NadM family
5995_DESnar1	15	Nicotinate-nucleotide adenyltransferase (EC 2.7.7.18)
6013_DESnar1	13	Nitrogenase FeMo-cofactor synthesis FeS core scaffold
6014_DESnar1	13	Nitroreductase
6017_DESnar1	10	Nitroreductase family protein
6023_DESnar1	3	Nucleose triphosphate pyrophosphohydrolase MazG
6024_DESnar1	3	Nucleose-diphosphate-sugar epimerases
6025_DESnar1	1	Nucleose-diphosphate-sugar epimerases
6026_DESnar1	5	Nucleose-diphosphate-sugar epimerases
6032_DESnar1	14	O-methyltransferase
6033_DESnar1	13	O-Methyltransferase involved in polyketide biosynthesis
6034_DESnar1	1	O-methyltransferase, family 2
6035_DESnar1	10	O-methyltransferase, family 2
6036_DESnar1	1	Octaheme tetrathionate reductase
6037_DESnar1	1	Octaheme tetrathionate reductase
6050_DESnar1	2	Ornithine cyclodeaminase (EC 4.3.1.12)
6053_DESnar1	8	Osmosensitive K ⁺ channel histine kinase KdpD
6054_DESnar1	10	Osmotically inducible protein Y precursor
6055_DESnar1	1	Outer membrane autotransporter barrel
6056_DESnar1	1	outer membrane efflux protein
6058_DESnar1	10	Outer membrane lipoprotein
6059_DESnar1	6	Outer membrane lipoprotein omp16 precursor
6060_DESnar1	15	Outer membrane protein
6062_DESnar1	10	Outer membrane protein
6063_DESnar1	1	Outer membrane protein H precursor
6064_DESnar1	1	Outer membrane protein H precursor
6066_DESnar1	1	Outer membrane receptor for ferrienterochelin and colicins
6067_DESnar1	14	Outer membrane receptor proteins, mostly Fe transport
6068_DESnar1	14	Outer membrane receptor proteins, mostly Fe transport
6072_DESnar1	3	Oxoreductase (EC 1.1.1.-)
6074_DESnar1	1	oxoreductase, Gfo/h/MocA family
6076_DESnar1	1	Oxoreductase, short-chain dehydrogenase/reductase family
6079_DESnar1	6	Pantoate--beta-alanine ligase (EC 6.3.2.1)
6081_DESnar1	15	PAP2 family protein
6082_DESnar1	15	PAP2 superfamily
6083_DESnar1	9	Para-aminobenzoate synthase, amotransferase component
6084_DESnar1	9	Para-aminobenzoate synthase, aminase component
6085_DESnar1	15	Paraquat-inducible protein B
6086_DESnar1	2	Parvulin-like peptyl-prolyl isomerase
6088_DESnar1	3	PAS domain S-box
6089_DESnar1	1	PAS domain S-box
6090_DESnar1	5	PAS domain S-box
6091_DESnar1	5	PAS domain S-box
6092_DESnar1	14	PAS domain S-box
6093_DESnar1	14	PAS domain S-box
6094_DESnar1	9	PAS domain S-box
6095_DESnar1	10	PAS domain S-box
6096_DESnar1	10	PAS domain S-box
6098_DESnar1	1	PAS domain S-box/diguanylate cyclase (GGDEF) domain
6099_DESnar1	1	PAS modulated sigma54 specific transcriptional regulator
6100_DESnar1	1	PDZ domain protein

6104_DESnar1	7	peptase S24, S26A and S26B
6105_DESnar1	1	peptase, M16 family
6108_DESnar1	2	peptase, M23/M315 family
6110_DESnar1	10	Peptase, M23/M315 family
6122_DESnar1	14	Peptyl-prolyl cis-trans isomerase (EC 5.2.1.8)
6124_DESnar1	10	Peptyl-prolyl cis-trans isomerase PpiA precursor (EC 5.2.1.8)
6126_DESnar1	6	Peptyl-prolyl cis-trans isomerase ppiC (EC 5.2.1.8)
6130_DESnar1	14	Periplasmic amino ac-binding protein-related protein
6131_DESnar1	1	Periplasmic aromatic aldehyde oxoreductase
6132_DESnar1	15	periplasmic binding protein, putative
6133_DESnar1	15	periplasmic binding protein, putative
6134_DESnar1	1	periplasmic chaperone for outer membrane proteins Skp
6135_DESnar1	11	Periplasmic component of the Tol biopolymer transport system
6138_DESnar1	1	Periplasmic protein involved in polysacchare export
6143_DESnar1	10	Permease of the drug/metabolite transporter (DMT) superfamily
6145_DESnar1	11	Permease of the drug/metabolite transporter (DMT) superfamily
6151_DESnar1	2	Phage baseplate assembly protein
6153_DESnar1	2	Phage baseplate assembly protein W
6154_DESnar1	2	Phage DNA packaging protein, Nu1 subunit of terminase
6155_DESnar1	10	Phage integrase
6156_DESnar1	2	Phage major tail tube protein
6157_DESnar1	2	Phage portal protein
6158_DESnar1	2	Phage protein
6159_DESnar1	2	Phage protein D
6160_DESnar1	2	Phage protein U
6161_DESnar1	1	phage regulatory protein, rha family
6162_DESnar1	1	Phage Rha protein
6164_DESnar1	2	Phage tail protein
6165_DESnar1	2	phage tail protein, P2 protein I family
6166_DESnar1	2	Phage tail sheath monomer
6167_DESnar1	2	Phage terminase, large subunit
6168_DESnar1	2	Phage-related protein
6169_DESnar1	15	Phage-related tail protein
6175_DESnar1	2	Phenylacetate-coenzyme A ligase (EC 6.2.1.30)
6177_DESnar1	14	Phenylacetic ac degradation protein PaaD, thioesterase
6198_DESnar1	5	Phosphoenolpyruvate synthase (EC 2.7.9.2)
6205_DESnar1	1	Phosphoesterase, PA-phosphatase related
6209_DESnar1	9	Phosphoglycerate mutase family
6210_DESnar1	2	phosphoglycerate mutase family protein
6212_DESnar1	13	Phospholip methyltransferase
6214_DESnar1	2	phosphomethylpyrimine kinase
6215_DESnar1	13	Phosphonate ABC transporter phosphate-binding
6233_DESnar1	1	Phosphosulfolactate phosphohydrolase and related enzymes
6236_DESnar1	6	Phosphotransferase system
6239_DESnar1	2	Polyferredoxin NapH (periplasmic nitrate reductase)
6240_DESnar1	15	Polymyxin resistance protein
6244_DESnar1	1	Positive regulator of CheA protein activity
6247_DESnar1	1	possible ATLS1-like light-inducible protein
6250_DESnar1	1	Possible sterol desaturase
6252_DESnar1	10	Potassium efflux system KefA protein
6258_DESnar1	1	Predicted acyltransferases
6266_DESnar1	1	Predicted dehydrogenases and related proteins
6270_DESnar1	5	Predicted Fe-S-cluster oxoreductase
6273_DESnar1	3	Predicted glycosyltransferases
6276_DESnar1	1	Predicted hydrolases or acyltransferases
6279_DESnar1	1	Predicted membrane fusion protein (MFP)
6280_DESnar1	13	Predicted membrane protein
6281_DESnar1	6	Predicted membrane protein
6283_DESnar1	15	Predicted metal-dependent hydrolase
6286_DESnar1	10	Predicted O-methyltransferase
6292_DESnar1	3	Predicted regulator of STY3230 transporter operon
6297_DESnar1	9	Predicted signal transduction protein

6302_DESnarl	1	Predicted Zn-dependent peptases
6309_DESnarl	1	Probable 2-phosphosulfolactate phosphatase (EC 3.1.3.71)
6310_DESnarl	10	Probable ATP /GTP binding protein
6311_DESnarl	3	probable beta-D-galactosase
6314_DESnarl	9	Probable Co/Zn/Cd efflux system membrane fusion protein
6315_DESnarl	7	probable exported protein STY2149
6316_DESnarl	3	probable glycosyl transferase
6318_DESnarl	10	probable membrane protein YPO22915
6319_DESnarl	5	probable methyltransferase
6320_DESnarl	1	Probable poly(beta-D-mannuronate) O-acetylase (EC 2.3.1.-)
6321_DESnarl	1	probable selenium-dependent hydroxylase accessory protein YqeC
6322_DESnarl	2	probable tail fiber assembly protein
6324_DESnarl	9	PROBABLE TRANSMEMBRANE PROTEIN
6326_DESnarl	1	Probable two-component sensor
6333_DESnarl	1	Protein containing aminopeptase domain
6337_DESnarl	5	Protein of unknown function (DUF1847)
6339_DESnarl	13	Protein of unknown function (DUF3108)
6340_DESnarl	3	Protein of unknown function CGGC region
6341_DESnarl	10	protein of unknown function DUF45
6346_DESnarl	10	protein of unknown function DUF606
6347_DESnarl	5	protein of unknown function DUF885
6356_DESnarl	1	Protein-N(5)-glutamine methyltransferase PmC
6358_DESnarl	1	Pseudaminic ac biosynthesis protein PseA
6359_DESnarl	1	pseudaminic ac biosynthesis-associated protein PseG
6360_DESnarl	2	Pseudourylate synthases, 23S RNA-specific
6366_DESnarl	6	PTS system, mannose-specific I component (EC 2.7.1.69)
6369_DESnarl	1	Putative activity regulator of membrane protease YbbK
6370_DESnarl	5	Putative amino ac ABC Transporter
6372_DESnarl	6	Putative cytochrome C
6373_DESnarl	14	Putative cytoplasmic protein
6376_DESnarl	9	putative efflux protein, MATE family
6377_DESnarl	5	putative exonuclease
6379_DESnarl	1	putative glycosyltransferase
6380_DESnarl	13	Putative hemagglutinin/hemolysin-related protein
6384_DESnarl	10	Putative intracellular protease/amase
6385_DESnarl	11	Putative L-asparaginase (EC 3.5.1.1) (L-asparagine amohydrolase)
6386_DESnarl	13	Putative L-xylulose-5-phosphate 3-epimerase
6389_DESnarl	5	putative lipoprotein
6390_DESnarl	1	putative LPS biosynthesis protein WbpG
6391_DESnarl	6	putative membrane protein
6392_DESnarl	1	putative membrane protein
6393_DESnarl	9	putative membrane protein
6394_DESnarl	5	Putative metal chaperone, involved in Zn homeostasis, GTPase
6395_DESnarl	6	putative methionine Transporter NhaC family (TC 2.A.35.1.-)
6396_DESnarl	2	Putative Nudix hydrolase YfcD (EC 3.6.-.-)
6398_DESnarl	13	PUTATIVE PERIPLASMIC PROTEIN
6400_DESnarl	2	Putative periplasmic protein YibQ
6401_DESnarl	14	putative PQQ enzyme repeat
6403_DESnarl	10	Putative PQQ enzyme repeat
6405_DESnarl	1	putative protein
6407_DESnarl	5	putative secreted lipase
6409_DESnarl	1	putative sulfonate/nitrate transport system substrate-binding protein
6414_DESnarl	6	Pyroxamine 5'-phosphate oxase-related, FMN-binding
6420_DESnarl	13	Pyrroline-5-carboxylate reductase (EC 1.5.1.2)
6431_DESnarl	9	radical activating enzyme
6440_DESnarl	5	Radical SAM domain protein
6442_DESnarl	5	Radical SAM domain protein
6446_DESnarl	10	Radical SAM domain protein
6449_DESnarl	1	radical SAM/Cys-rich domain protein
6457_DESnarl	11	Related to MCBG protein
6462_DESnarl	10	Resolvase
6463_DESnarl	2	Respiratory arsenate reductase cytoplasmic chaperone

6464_DESnar1	2	Respiratory arsenate reductase FeS subunit
6468_DESnar1	5	Response regulator
6470_DESnar1	14	Response regulator
6476_DESnar1	10	Response regulator
6477_DESnar1	10	Response regulator
6479_DESnar1	14	Response regulator containing a CheY-like receiver domain
6484_DESnar1	13	Response regulator of zinc sigma-54-dependent
6488_DESnar1	14	Response regulator of zinc sigma-54-dependent
6500_DESnar1	14	response regulator receiver protein
6505_DESnar1	10	response regulator receiver protein
6507_DESnar1	10	response regulator receiver protein
6511_DESnar1	5	Restriction endonuclease
6514_DESnar1	7	Rhodanese-related sulfurtransferase
6516_DESnar1	6	Rhombo family protein
6540_DESnar1	2	Ribosomal large subunit pseudourine synthase B
6541_DESnar1	5	Ribosomal large subunit pseudourine synthase C
6542_DESnar1	2	Ribosomal large subunit pseudourine synthase D
6571_DESnar1	6	RNA-binding protein Jag
6583_DESnar1	3	RsbR, positive regulator of sigma-B
6598_DESnar1	1	SAM-dependent methyltransferases
6601_DESnar1	5	Sell repeat
6605_DESnar1	6	Selenoprotein O and cysteine-containing homologs
6608_DESnar1	3	sensor histine kinase
6610_DESnar1	5	sensor histine kinase
6611_DESnar1	10	sensor histine kinase
6613_DESnar1	10	sensor histine kinase
6618_DESnar1	14	sensory box histine kinase/Response regulator
6619_DESnar1	10	sensory box histine kinase/Response regulator
6621_DESnar1	14	Sensory box/GGDEF family protein
6622_DESnar1	1	sensory transduction regulatory protein
6624_DESnar1	10	Ser/Thr protein phosphatase family
6627_DESnar1	3	Serine phosphatase RsbU, regulator of sigma subunit
6629_DESnar1	7	Serine phosphatase RsbU, regulator of sigma subunit
6631_DESnar1	2	Serine protease precursor MucD/AlgY
6638_DESnar1	1	Shikimate kinase I (EC 2.7.1.71)
6639_DESnar1	3	Short C-terminal domain
6640_DESnar1	10	Shufflon-specific DNA recombinase
6641_DESnar1	10	Shufflon-specific DNA recombinase
6644_DESnar1	1	Sigma-54 dependent DNA-binding Response regulator
6645_DESnar1	1	Sigma-54 dependent DNA-binding Response regulator
6646_DESnar1	14	sigma-54 dependent DNA-binding Response regulator
6649_DESnar1	3	sigma-54 dependent transcriptional regulator/Response regulator
6652_DESnar1	5	sigma-54 dependent transcriptional regulator/Response regulator
6653_DESnar1	2	Sigma-70, region 4
6656_DESnar1	1	Signal peptase-like protein
6665_DESnar1	5	Signal transduction histine kinase
6667_DESnar1	14	Signal transduction histine kinase
6668_DESnar1	14	Signal transduction histine kinase
6670_DESnar1	10	Signal transduction histine kinase
6671_DESnar1	10	signal transduction histine kinase
6672_DESnar1	1	Signal transduction histine kinase CheA (EC 2.7.3.-)
6676_DESnar1	1	similar to ATPase (AAA+ superfamily)
6677_DESnar1	14	Similar to carbon monoxide dehydrogenase
6678_DESnar1	1	Similar to CDP-glucose 4,6-dehydratase (EC 4.2.1.45)
6680_DESnar1	5	Similar to TadZ/CpaE, associated with FliP pilus assembly
6686_DESnar1	15	Sirohydrochlorin cobaltochelatase CbiK (EC 4.99.1.3)
6687_DESnar1	1	Site-specific recombinase XerD
6688_DESnar1	10	Site-specific recombinase XerD
6690_DESnar1	1	Site-specific recombinases, DNA invertase Pin homologs
6693_DESnar1	1	Sodium-dependent phosphate transporter
6698_DESnar1	7	SOS-response transcriptional repressors
6721_DESnar1	9	Staphylococcus nuclease (SNase) domain

6722_DESnar1	1	Structural elements; Cell Exterior; surface polysacchares/antigens
6723_DESnar1	15	Sua5 YciO YrdC YwlC family protein
6724_DESnar1	6	Substrate-specific component BioY of biotin ECF transporter
6726_DESnar1	3	Substrate-specific component STY3230
6729_DESnar1	1	succinate dehydrogenase subunit C (EC 1.3.5.1)
6737_DESnar1	5	Sulfate adenylyltransferase subunit 2 (EC 2.7.7.4)
6741_DESnar1	5	Sulfate permease
6745_DESnar1	2	Sulfite reductase assimilatory-type (EC 1.8.-.-)
6752_DESnar1	1	Sulfotransferase
6753_DESnar1	3	Sulfotransferase domain
6754_DESnar1	1	Sulfotransferase domain
6755_DESnar1	1	Sulfotransferase domain
6756_DESnar1	1	Sulfur carrier protein adenylyltransferase ThiF
6757_DESnar1	10	Sulfur carrier protein adenylyltransferase ThiF
6758_DESnar1	10	Superfamily I DNA/RNA helicase protein
6759_DESnar1	14	Superfamily II DNA and RNA helicases
6761_DESnar1	13	Surface lipoprotein
6762_DESnar1	2	Survival protein SurA precursor
6763_DESnar1	2	Tail fiber protein
6764_DESnar1	2	Tail protein I
6766_DESnar1	13	Tetraacyldisacchare 4'-kinase (EC 2.7.1.130)
6767_DESnar1	13	Tetracenomycin polykete synthesis O-methyltransferase tcmP
6768_DESnar1	14	Tetracenomycin polykete synthesis O-methyltransferase tcmP
6771_DESnar1	14	Tetratricopeptide repeat
6780_DESnar1	10	ThiJ/PfpI family protein
6788_DESnar1	10	Thioredoxin family protein
6802_DESnar1	5	TonB family C-terminal domain
6804_DESnar1	6	TonB-dependent receptor
6805_DESnar1	1	TonB-dependent receptor
6806_DESnar1	5	TonB-dependent receptor
6807_DESnar1	14	TonB-dependent receptor
6808_DESnar1	14	TonB-dependent receptor
6809_DESnar1	14	TonB-dependent receptor
6810_DESnar1	14	TonB-dependent receptor
6811_DESnar1	7	TonB-dependent receptor
6812_DESnar1	10	TonB-dependent receptor
6813_DESnar1	10	TonB-dependent receptor
6814_DESnar1	6	TPR domain protein
6818_DESnar1	10	TPR domain protein
6820_DESnar1	14	TPR domain protein, putative component of TonB system
6821_DESnar1	14	TPR domain protein, putative component of TonB system
6823_DESnar1	2	TPR repeat repeat-containing protein
6825_DESnar1	11	TPR repeat repeat-containing protein
6826_DESnar1	2	TPR repeat containing exported protein; Putative periplasmic protein
6827_DESnar1	2	TPR repeat containing exported protein; Putative periplasmic protein
6828_DESnar1	13	TPR repeat precursor
6830_DESnar1	6	TPR repeat-containing protein
6831_DESnar1	6	TPR repeat-containing protein
6835_DESnar1	11	TPR repeat-containing protein
6846_DESnar1	1	Transcriptional regulator
6847_DESnar1	9	Transcriptional regulator
6848_DESnar1	15	Transcriptional regulator
6850_DESnar1	2	Transcriptional regulator YbiH, TetR family
6851_DESnar1	6	Transcriptional regulator AraC family
6852_DESnar1	1	Transcriptional regulator AraC family
6853_DESnar1	2	Transcriptional regulator AraC family
6854_DESnar1	5	Transcriptional regulator AraC family
6855_DESnar1	14	Transcriptional regulator AraC family
6856_DESnar1	14	Transcriptional regulator AraC family
6858_DESnar1	7	Transcriptional regulator AraC family
6859_DESnar1	10	Transcriptional regulator AraC family
6860_DESnar1	10	Transcriptional regulator AraC family

6861_DESnarl	10	Transcriptional regulator AraC family
6862_DESnarl	15	Transcriptional regulator AraC family
6864_DESnarl	11	Transcriptional regulator AraC family
6868_DESnarl	14	Transcriptional regulator AsnC family
6871_DESnarl	6	Transcriptional regulator GntR family
6872_DESnarl	5	Transcriptional regulator GntR family
6877_DESnarl	13	Transcriptional regulator IclR family
6879_DESnarl	6	Transcriptional regulator LysR family
6880_DESnarl	1	Transcriptional regulator LysR family
6881_DESnarl	9	Transcriptional regulator LysR family
6883_DESnarl	9	Transcriptional regulator LysR family
6884_DESnarl	15	Transcriptional regulator LysR family
6885_DESnarl	10	Transcriptional regulator MarR family
6886_DESnarl	13	Transcriptional regulator MarR family
6887_DESnarl	13	Transcriptional regulator MarR family
6888_DESnarl	6	Transcriptional regulator MarR family
6889_DESnarl	2	Transcriptional regulator MarR family
6890_DESnarl	14	Transcriptional regulator MarR family
6891_DESnarl	14	Transcriptional regulator MarR family
6892_DESnarl	14	Transcriptional regulator MarR family
6893_DESnarl	9	Transcriptional regulator MarR family
6895_DESnarl	17	Transcriptional regulator MarR family
6897_DESnarl	11	Transcriptional regulator MerR family
6901_DESnarl	13	Transcriptional regulator TetR family
6904_DESnarl	6	Transcriptional regulator TetR family
6907_DESnarl	9	Transcriptional regulator TetR family
6908_DESnarl	10	Transcriptional regulator TetR family
6909_DESnarl	15	Transcriptional regulator TetR family
6911_DESnarl	6	Transcriptional regulators
6913_DESnarl	3	Transcriptional regulators
6914_DESnarl	5	Transcriptional regulators
6915_DESnarl	14	Transcriptional regulators
6918_DESnarl	10	Transcriptional regulators of sugar metabolism
6919_DESnarl	13	Transcriptional regulatory protein
6921_DESnarl	2	transfer-messenger RNA Contig
6923_DESnarl	1	Transketolase C-terminal section (EC 2.2.1.1)
6924_DESnarl	1	Transketolase N-terminal section (EC 2.2.1.1)
6936_DESnarl	1	Transmembrane component BioN
6937_DESnarl	2	Transmembrane component NikQ
6938_DESnarl	13	Transporter
6943_DESnarl	9	Transporter LysE family
6944_DESnarl	15	Transporter LysE family
6945_DESnarl	5	Transporter putative
6946_DESnarl	3	Transposase and inactivated derivatives
6947_DESnarl	3	Transposase and inactivated derivatives
6949_DESnarl	5	Transposase and inactivated derivatives
6951_DESnarl	10	Transposase and inactivated derivatives
6952_DESnarl	17	Transposase and inactivated derivatives
6954_DESnarl	5	Transposon Tn15 transposition protein tnsA
6955_DESnarl	5	Transposon Tn15 transposition protein tnsA
6956_DESnarl	5	Transposon Tn15 transposition protein tnsB
6957_DESnarl	5	Transposon Tn15 transposition protein tnsB
6958_DESnarl	5	Transposon Tn15 transposition protein tnsC
6960_DESnarl	5	Transposon Tn15 transposition protein tnsD
6961_DESnarl	5	Transposon Tn15 transposition protein tnsD
6962_DESnarl	5	Transposon Tn15 transposition protein tnsE
6963_DESnarl	5	Transposon Tn15 transposition protein tnsE
6967_DESnarl	6	TRAP transporter solute receptor TAXI family precursor
6971_DESnarl	13	TRAP transporter solute receptor unknown substrate
6973_DESnarl	1	TRAP-type C4-dicarboxylate transport system, periplasmic
6975_DESnarl	1	TRAP-type C4-dicarboxylate transport system, large permease
6977_DESnarl	1	TRAP-type C4-dicarboxylate transport system, periplasmic

6978_DESnar1	1	TRAP-type transport system, small permease component
6986_DESnar1	13	tRNA (5-methylaminomethyl-2-thiourylate)-methyltransferase
6996_DESnar1	2	tRNA nucleotyltransferase (EC 2.7.7.21) (EC 2.7.7.25)
7001_DESnar1	2	tRNA threonylcarbamoyl adenosine modification protein YjeE
7003_DESnar1	2	tRNA(Ile)-lysine synthetase
7004_DESnar1	2	tRNA(Ile)-lysine synthetase, N-terminal domain
7012_DESnar1	2	Trypsin-like serine proteases, typically periplasmic
7016_DESnar1	10	Tryptophan synthase beta chain like (EC 4.2.1.20)
7023_DESnar1	1	Tungsten-containing aldehyde:ferredoxin oxoreductase (EC 1.2.7.5)
7024_DESnar1	1	Tungsten-containing aldehyde:ferredoxin oxoreductase (EC 1.2.7.5)
7026_DESnar1	14	Tungsten-containing aldehyde:ferredoxin oxoreductase (EC 1.2.7.5)
7028_DESnar1	15	Twin-arginine translocation protein TatB
7029_DESnar1	15	Twin-arginine translocation protein TatC
7034_DESnar1	5	two-component hybr sensor and regulator
7035_DESnar1	9	two-component hybr sensor and regulator
7036_DESnar1	2	two-component Response regulator
7038_DESnar1	10	Two-component Response regulator
7044_DESnar1	5	type I secretion system ABC Transporter HlyB family
7046_DESnar1	5	Type II R/M system (EC 2.1.1.72)
7048_DESnar1	5	Type II/IV secretion system ATPase TadZ/CpaE
7051_DESnar1	5	type III restriction enzyme, res subunit
7052_DESnar1	5	Type III restriction-modification system methylation subunit
7053_DESnar1	5	Type IV prepilin peptase TadV/CpaA
7056_DESnar1	1	UBA/THIF-type NAD/FAD binding protein
7057_DESnar1	7	UbiE/COQ5 methyltransferase
7060_DESnar1	5	UDP-2-acetamo-3-amino-2,3-deoxy-D- glucuronic ac acetyltransferase
7064_DESnar1	1	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase
7065_DESnar1	1	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase
7066_DESnar1	1	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase
7067_DESnar1	1	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase
7068_DESnar1	1	UDP-glucose 4-epimerase (EC 5.1.3.2)
7069_DESnar1	1	UDP-glucose 4-epimerase (EC 5.1.3.2)
7070_DESnar1	5	UDP-glucose 4-epimerase (EC 5.1.3.2)
7071_DESnar1	5	UDP-glucose 4-epimerase (EC 5.1.3.2)
7072_DESnar1	7	UDP-glucose 4-epimerase (EC 5.1.3.2)
7079_DESnar1	5	UDP-N-acetylglucosamine 4,6-dehydratase (EC 4.2.1.-)
7080_DESnar1	13	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide)
7084_DESnar1	13	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D-alanine
7085_DESnar1	3	Uncharacterised ArCR, COG2043
7086_DESnar1	1	Uncharacterised ArCR, COG2043
7087_DESnar1	2	Uncharacterized component of anaerobic dehydrogenases
7088_DESnar1	10	Uncharacterized component of anaerobic dehydrogenases
7090_DESnar1	14	Uncharacterized conserved protein
7091_DESnar1	14	Uncharacterized conserved protein
7092_DESnar1	14	Uncharacterized conserved protein
7093_DESnar1	14	Uncharacterized conserved protein
7098_DESnar1	9	Uncharacterized Fe-S protein PflX, homolog of pyruvate formate lyase
7100_DESnar1	13	Uncharacterized protein conserved in bacteria (DUF2320)
7102_DESnar1	6	Uncharacterized protein conserved in bacteria
7104_DESnar1	3	Uncharacterized protein conserved in bacteria
7106_DESnar1	1	Uncharacterized protein conserved in bacteria
7108_DESnar1	10	Uncharacterized protein conserved in bacteria
7110_DESnar1	13	Uncharacterized protein involved in exopolysacchare biosynthesis
7111_DESnar1	1	Uncharacterized protein involved in outer membrane biogenesis
7112_DESnar1	13	Uncharacterized protein MA0381
7113_DESnar1	1	Uncharacterized protein MJ0202
7118_DESnar1	5	Uncharacterized stress protein (general stress protein 26)
7122_DESnar1	13	Undecaprenyl-phosphate galactosephosphotransferase (EC 2.7.8.6)
7128_DESnar1	5	unknown
7138_DESnar1	10	UspA domain protein
7139_DESnar1	13	UTP--glucose-1-phosphate urylyltransferase (EC 2.7.7.9)
7141_DESnar1	13	VacJ family lipoprotein

7143_DESnarl	13	VCBS repeat
7144_DESnarl	13	VCBS repeat
7145_DESnarl	15	Vitamin B12 ABC Transporter permease component BtuC
7147_DESnarl	1	Xanthine and CO dehydrogenases maturation factor, XdhC/CoxF family
7148_DESnarl	1	Xanthine and CO dehydrogenases maturation factor, XdhC/CoxF family
7149_DESnarl	2	Xanthine and CO dehydrogenases maturation factor, XdhC/CoxF family
7151_DESnarl	1	Xanthine dehydrogenase iron-sulfur subunit (EC 1.17.1.4)
7152_DESnarl	1	Xanthine dehydrogenase, molybdenum binding subunit (EC 1.17.1.4)
7154_DESnarl	11	YbbL ABC transporter ATP-binding protein
7157_DESnarl	2	YefM protein (antitoxin to YoeB)
7160_DESnarl	2	YoeB toxin protein
7162_DESnarl	9	Zinc ABC Transporter periplasmic-binding protein ZnuA
7164_DESnarl	13	Zinc resistance-associated protein
7167_DESnarl	13	Zn-dependent protease with chaperone function PA4632