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Proteome Analysis of *Desulfovibrio desulfuricans* G20 Mutants Using the Accurate Mass and Time (AMT) Tag Approach

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Abundance values obtained from direct LC−MS analyses were used to compare the proteomes of six transposon-insertion mutants of *Desulfovibrio desulfuricans* G20, the lab strain ($G20_{lab}$) and a sediment-adapted strain ($G20_{sediment}$). Three mutations were in signal transduction histidine kinases, and three mutations were in other regulatory proteins. The high-throughput accurate mass and time (AMT) tag proteomic approach was utilized to analyze the proteomes. A total of 1318 proteins was identified with high confidence, approximately 35% of all predicted proteins in the *D. desulfuricans* G20 genome. Proteins from all functional categories were identified. Significant differences in the abundance of 30 proteins were detected between the $G20_{lab}$ strain and the $G20_{sediment}$ strain. Abundances of proteins for energy metabolism, ribosomal synthesis, membrane biosynthesis, transport, and flagellar synthesis were affected in the mutants. Specific examples of proteins down-regulated in mutants include a putative tungstate transport system substrate-binding protein and several proteins related to energy production, for example, 2-oxoacid:acceptor oxidoreductase, cytochrome c-553, and formate acetyltransferase. In addition, several signal transduction mechanism proteins were regulated in one mutant, and the abundances of ferritin and hybrid cluster protein were reduced in another mutant. However, the similar abundance of universal stress proteins, heat shock proteins, and chemotaxis proteins in the mutants revealed that regulation of chemotactic behavior and stress regulation might not be observed under our growth conditions. This study provides the first proteomic overview of several sediment fitness mutants of G20, and evidence for the difference between lab strains and sediment-adapted strains at the protein level.

Keywords: *Desulfovibrio desulfuricans* G20 • accurate mass and time (AMT) tag • proteome of mutants • regulatory genes

Introduction

Sulfate-reducing bacteria (SRB), which use sulfate as a terminal electron acceptor to oxidize both organic and inorganic compounds, are ubiquitous in soils and sediments.[1] Since *Desulfovibrio* species were found to be capable of immobilizing uranium[2] and other heavy metals[3,4] in natural environments, their physiology and molecular genetics have been extensively studied in the laboratory.[5,6] SRB have also been broadly studied *in situ* and often show different physiological characteristics in the environment. For example, actively sulfate reducing *Desulfovibrio* species were present in the photooxic zone,[7] while laboratory pure cultures of *Desulfovibrio* species can only be grown under anaerobic conditions. Therefore, it is clear that SRB exhibit a differentiated set of reactions to adapt to different conditions. It has also been observed that sediment-adapted strains have different physiological features from their corresponding well-adapted lab strains;[8] however, there is no study that compares the protein production between these two types of strains.

Desulfovibrio desulfuricans G20, originally isolated from a petroleum producing well, serves as a model organism for the study of sediment survival and molecular genetics in SRB. The genome of *D. desulfuricans* G20 has been sequenced (NCBI accession No. NC_007519), and the bacterium is easy to cultivate for biochemical studies. Genomic information provides an opportunity to obtain further insights into the metabolism of this organism in lab medium and in sediment. In a previous study, more than 5000 transposon-insertion mutants were generated,[9] and about 100 genes were identified as important for sediment fitness.[10] Among them, several mutations occurred within general regulatory genes, and these

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Table 1. List of G20 Mutants Used for Proteomic Analysis

mutant	sediment recovery (%)[10]	interrupted gene locus	protein description	contained domain	potential function
A1(pD3)	0	Dde_0289 (633 aa)	Transcriptional regulator, Fis family	RocR (~500 aa)	Activates rRNA transcription, regulation of virulence factors, inhibition of the initiation of DNA replication[71−73]
B8(pB6)	0	Dde_3047 (397 aa)	Serine phosphatase RsbU, regulator of sigma subunit		Regulates important virulence factors, influences the survival ability [56]
D12(pE9)	0.3	Dde_1614 (224 aa)	Regulatory protein GntR, HTH	FadR (~215 aa)	Regulates various biological processes, repressor for *uxuRBA* operon[59]
H9(pF8)	0	Dde_0602 (711 aa)	Multi-sensor signal transduction histidine kinase	BaeS (~330 aa)	Involved in two-component regulatory system, domain found in heat shock protein Hsp90[47]
D12(pB8)	0	Dde_3715 (653 aa)	Multi-sensor signal transduction histidine kinase	BaeS (~250 aa)	Involved in two-component regulatory system, domain found in heat shock protein Hsp90[47]
G5(pA9)	0.3	Dde_1945 (676 aa)	Putative PAS/PAC sensor protein	AtoS (~125 aa)	Associated with a histidine kinase or a sensor protein, involved in lesion formation, swarming and production of extracellular protease[51]

mutants were unable to survive in sediment. Several histidine kinases were identified. They belong to two-component regulatory systems (TCSs), which are known to mediate responses to a variety of environmental signals. To adapt to and survive complex environmental changes in nature, it may be especially important that different TCSs form regulatory networks. However, little is known about whether functional interactions between different TCSs form signaling networks in *D. desulfuricans* G20. In addition, little information is available on the expression of these regulatory genes, or on which pathways they regulate.

Studies using microarray technology have increased our knowledge of global transcriptomic regulation within *Desulfovibrio* species under different stresses, including salt,[11] heat,[12] and nitrite.[13] These studies have identified genes of energy metabolism pathways and genes related to stress adaptation and have shown us the versatility of this microorganism in its ability to adapt to various stresses. Previous work has mainly focused on comparing growth phases,[14] electron donors,[15] and stressors at the mRNA level. However, mRNA abundance is not always correlated with protein abundance;[16] furthermore, information of post-transcriptional and post-translational modification processes is not available from the transcriptomic analyses. With the development of capillary liquid chromatography and tandem mass spectrometry technology, high-throughput proteomic analysis has been applied to many organisms[17−19] to identify and compare protein abundance under different growth conditions.

The goals of this investigation were to elucidate proteome abundance information for *D. desulfuricans* G20 strains, to examine the proteins involved in sediment fitness, and to identify possible regulatory pathways involved in these TCSs. To accomplish this, the accurate mass and time (AMT) tag approach[19,20] was used which allows peptides to be identified quantitatively with high confidence and high-throughput. We analyzed the proteomes of *D. desulfuricans* $G20_{lab}$ and $G20_{sediment}$ strains, as well as several transposon mutants that did not survive in active sulfate reducing sediment. The AMT abundance values were used as a quantitative measurement of relative protein abundances within individual samples. Normalized *z*-scores were used to quantify the relative protein abundances. *Z*-score differences between samples of at least 1.5 or greater were considered significant.

Materials and Methods

Strains, Media, and Culture Conditions. *D. desulfuricans* G20, a spontaneous nalidixic acid resistant strain, was obtained from Dr. Judy Wall at the University of Missouri-Columbia. Cultures were grown in lactate-sulfate (LS) medium, prepared as described by Rapp and Wall,[21] with N_2 in the headspace. The medium contained 50 mM sulfate and 66 mM lactate, and vitamin and metal solutions as described elsewhere.[22] Prior to autoclaving, the pH was adjusted to 7.2. After autoclaving, 8 mM bicarbonate and 0.025% cysteine were added from anaerobic stock solutions. The lab strain has been cultured in this medium for several years. To generate a sediment-adapted strain, approximately 10^5 cells of the lab strain were inoculated into a microcosm with 2 g of sulfidogenic subsurface sediment and incubated in the dark at room temperature for 1 week. Inoculum-free microcosms were set up as controls. Cells were extracted from the sediment and plated onto LS solid medium with 200 μg/mL nalidixic acid and 1.5% agar. Colonies were obtained from the plates and inoculated into LS liquid medium. Glycerol stocks were prepared after a 24-h incubation period. This strain was designated as $G20_{sediment}$, as mentioned previously.[9] No colonies were obtained from uninoculated microcosms. Strain $G20_{sediment}$ was used as a parent strain for conjugation experiments to obtain transposon-insertion mutants as described previously.[9] Six mutants obtained during our previous work were used for this study. Details on the mutants are available in Table 1.

All of these strains were cultivated from glycerol stocks and grown in LS medium to mid-log phase ($OD_{600} = 0.5$). Cells were harvested anaerobically by centrifugation at 8000 rpm for 10 min at 4 °C, supernatants were decanted, and pellets were resuspended in 1 mL of washing buffer.[9] Cell pellets were placed into liquid N_2 immediately, and stored at −80 °C until shipment. The cells were shipped to Pacific Northwest National Laboratory (PNNL) on dry ice.

Peptide Preparation. The cell pellets were resuspended in 2 vol of 50 mM ammonium bicarbonate, pH 7.8. The cell suspensions were lysed by bead-beating the mixture with 0.1 mm zirconia/silica beads in a mini-bead beater (Biospec, Bartlesville OK) for 3 min at 4500 rpm. Lysates were collected and placed immediately on ice to inhibit proteolysis. The protein solutions were digested with trypsin, and the digested peptides were desalted using Supelco (St. Louis, MO) Supel-

clean C-18 tubes as described elsewhere.[23] Peptide concentrations were determined with the BCA assay (Pierce, Rockford, IL) using a bovine serum albumin standard.

Capillary LC Separations. All peptide mixtures from the whole cell lysate, as well as off-line strong cation exchange (SCX) fractions of the same lysate, were then separated by an automated in-house designed HPLC system as described elsewhere.[23,24] The separated samples were fractionated into as many as 100 and as few as 25 fractions.[25] Fractions were concentrated to about 1 μg/μL for direct analysis by LC−MS/MS.

MS/MS Acquisition. Eluate from the HPLC was directly transferred into an ion trap MS (LCQ, ThermoFinnigan, San Jose, CA) using electrospray ionization (ESI). For the MS/MS detection, a total of 10 μg (1 μg/μL) of total peptide were loaded onto the reversed-phase column for each analysis. The mass spectrometer operated in a data-dependent MS/MS mode over a series of seven smaller segmented m/z ranges (400−700, 700−900, 900−1100, 1100−1300, 1300−1500, 1500−1700, 1700−2000), and in a 400−2000 m/z range for several SCX fractions obtained from many cultures of *D. desulfuricans* G20. The details for the generation of initial mass and time tag database are described elsewhere.[26] This database serves as a "look up" table for subsequent high-resolution experiments. The MS/MS spectra were analyzed using the peptide identification software SEQUEST[27] in conjunction with the annotated protein translations from the genome sequence of *D. desulfuricans* G20. Filtered identifications were based on peptide identification that had minimum Xcorr values of 1.9, 2.2, and 3.5 for charge states of 1+, 2+, and 3+, respectively, if detected at least twice in all the analyses. For fully, partially, and non-tryptic terminal peptides only detected once, filtered identifications were based on peptide values that had a minimum Xcorr value of 1.9, 2.2, and 3.75 for charge states of 1+, 2+, and 3+, respectively. Additionally, all peptides had to have a minimum DeltaCn value of 0.1. The DeltaCn value is the difference between the XCorr values for the best match and the second best match. A larger DeltaCn value gives us more confidence in identification of the peptide.

AMT Tag Validation. With the use of 5 μg (0.5 μg/μL) of total peptide from each of the same samples, intact peptide mass data were obtained using the same HPLC system, but using an FTICR mass spectrometer for MS detection. A mass calibration mixture was infused at the end of each analysis, and the masses of the compounds in the mixture were used to calibrate all of the spectra within the analyses. The mass tags identified from the tandem mass spectrometry analysis were stored in the mass tag database and were matched against unique peptides detected with the FTICR, as described previously.[20] Search tolerances for peptide peak matching were ±6 ppm for the mass and ±2% of the total analysis time for the elution time. Those PMT tags that matched the closest with the FTICR and elution time data were validated as AMT tags.

Data Analysis. The acquired AMT peptide data were further filtered, by setting the spatially localized confidence (SLiC) score >0.5 as a cutoff, to increase the confidence levels of peptide identification. If a peptide was detected in only one of three replicate LC−FTICR runs, that peptide was excluded from the analyses.

The reproducibility of LC−FTICR MS analyses was focused on evaluating protein abundance values by comparing triplicate analyses of the same biological sample and analyses of triplicate biological samples, prepared independently from mid-log phase cells. In the latter case, the average AMT abundance from three measurements per sample was used for comparison. Statistical analyses showed good reproducibility between replicate runs and biological replicates. The results were exemplified by $G20_{sediment}$ samples shown in supplementary Figure 1 in Supporting Information.

The relative protein abundances were estimated by averaging the abundances of multiple peptides for a given protein, and only those peptides whose intensities were ≥33% of the most abundant peptide for the given protein chosen.[28] Z-scores were calculated for each protein from the $\log_2$ of the abundance were used to compare these estimated abundances across all the strains, and then were clustered by using the hierarchical clustering algorithms available in Spotfire (Spotfire Inc., Cambridge, MA).

For the calculation of the "Codon Adaptation Index" (CAI), a measure of the expressivity of a given gene based on its codon usage, 3775 gene sequences of *D. desulfuricans* G20 were downloaded from the NCBI database. CAI calculation required a determination of relative adaptiveness value ω for *D. desulfuricans* G20, which was obtained from the University of Maryland, Baltimore County (UMBC, http://www.evolvingcode.net/codon/cai/cai.php). CAI values for each ORF of total proteins of G20 and 1318 identified proteins from this study were computed using a calculator available from the same Web site.

Results and Discussion

1. Overview of Proteomic Analyses. We chose a single growth condition for this study and analyzed the protein profiles of $G20_{sediment}$, $G20_{lab}$, and six insertion mutants that were deficient in sediment fitness. One of our goals was to provide global protein diversity information for *D. desulfuricans* G20 using the high-throughput AMT tag approach. To address these issues, protein abundance value profiles were generated using the mass analyses obtained from the LC−FTICR MS runs.

A total of 1318 proteins was identified, representing 35% of the predicted gene products encoded in the *D. desulfuricans* G20 genome. For the majority (98%) of the proteins, two or more unique peptides were detected per protein. With high confidence for the identification of peptides, proteins identified by only one unique peptide were considered valid, because some proteins only have one detectable tryptic peptide when using a mass spectrometer. The presence of a single peptide for identification of the corresponding protein was confirmed in all data sets. Proteins have been identified that are involved in all functional categories (Figure 1). A large number of identified proteins also fell into the category of proteins with unknown functions, including the hypothetical, conserved hypothetical, and unassigned functional proteins. This finding is consistent with previous work[18,28] on the importance of "hypothetical proteins" in cell function.

A codon usage-based approach, the Codon Adaptation Index (CAI) was used to determine whether the detection of proteins was biased toward those that were more highly expressed. The CAI uses a set of highly expressed genes from a species as references to assess the relative merits of each codon, and a score for a gene sequence is calculated from the frequency of use of all codons in that gene sequence. The index is useful for predicting the level of expression of a gene, and gives an approximate indication of the likely success for heterologous gene expression.[29] CAI values range from about 0.1 to close to

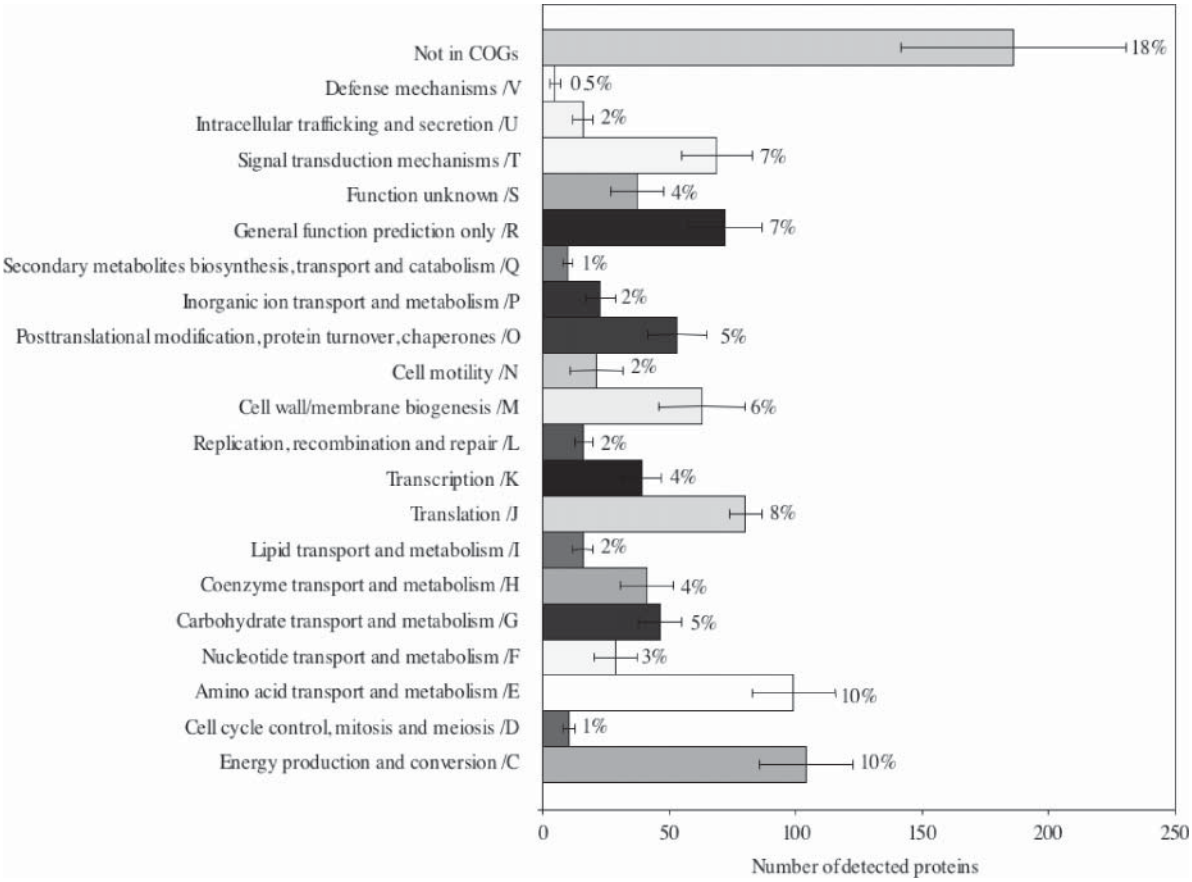

Figure 1. Functional classes of proteins detected by the AMT approach. Each bar represents the average number of proteins detected in all samples, and the average percentage of each functional category (http://www.ncbi.nlm.nih.gov/COG/grace/fiew.cgi) is shown next to each bar. Error bar represents the range of detected proteins in different samples. The proteins are grouped in their functional categories according to the NCBI annotation (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome&cmd=Retrieve&dopt=Protein+Table&list_uids=18914).

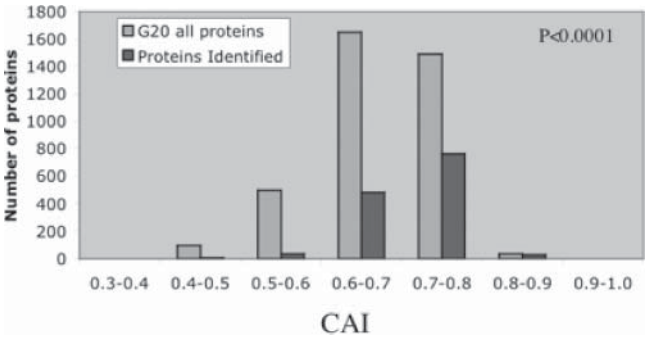

Figure 2. Distribution of CAI values for all encoding genes in the *D. desulfuricans* G20 genome and for genes encoding proteins identified by this study.

1.0 with higher values representing more highly expressed proteins. The CAI for all predicted proteins was determined and compared to CAI values for those that were measured in cell extracts (Figure 2). Kolmogorov-Smirnov tests ($P < 0.0001$) indicated that these two distributions were different. The results showed that 60% of the identified proteins had CAI values larger than 0.7 and only 3% had CAI values smaller than 0.6. This suggests that, although the LC−FTICR MS demonstrated the ability to detect relatively low abundance proteins, the majority of proteins identified in this study still represent relatively abundant proteins in *D. desulfuricans* G20.

For the systematic analyses, the proteomic profiles of six insertion mutants and two controls of G20 were generated using the AMT tag approach with a *z*-score normalization to compare relative protein abundance. There are many sources of systematic variation in the LC−MS-based proteomic experiments that affect protein abundance measurement. These sources include variations in sample preparation, LC separation, electrospray ionization efficiency, and mass spectrometer performance. Normalization is the term used to describe the process of removing or correcting for such variations.[19] Protein abundance values with $\log_2$ ratios of $|z| > 1.5$ were considered significant.[14,18] All of the enzymes required for the metabolism of sulfate (ATP sulfurylase, inorganic pyrophosphatase, adenylyl sulfate (APS) reductase, and bisulfite reductase) were detected in all of the samples with relatively high abundance (Supplementary Table 1 in Supporting Information). No significant differences were observed between samples. Similar abundance values for all of the samples may explain the reason the strains grew similarly in lactate−sulfate medium. It also indicates that these mutated genes do not regulate sulfate reduction in G20.

2. $G20_{sediment}$ versus $G20_{lab}$ Strains. The second goal of this investigation was to compare the protein profiles of *D. desulfuricans* $G20_{sediment}$ to the $G20_{lab}$ strain. It is clear that all bacterial cultures undergo genetic change in the laboratory, although perhaps it is not immediately obvious from the phenotype.[8] However, several researchers have proposed the idea of returning frequently to the natural environment to

Table 2. Proteins with Significant Changes in Strain $G20_{sediment}$

locus	protein description	functional category	changes in $G20_{sediment}$[a]
Dde_0046	Pyruvate ferredoxin/flavodoxin oxidoreductase family protein	C	−1.62
Dde_1359	Elongator protein 3/MiaB/NifB	C	*1.94*
Dde_3055	Formate acetyltransferase 2	C	*2.54*
Dde_2744	Aminotransferase, class V	E	2.52
Dde_2852	Sodium:alanine symporter	E	**−2.96**
Dde_2285	1,4-alpha-glucan branching enzyme	G	*2.09*
Dde_3685	HPCH/HPAI aldolase family protein	G	4.89
Dde_2839	Valyl-tRNA synthetase, class Ia	J	−1.66
Dde_2844	Queuine/ other tRNA-ribosyltransferase:Queuine tRNA-ribosyltransferase	J	**−3.45**
Dde_0553	Hypothetical protein	K	*2.52*
Dde_0047	Elongator protein 3/MiaB/NifB	L	*3.55*
Dde_0542	Formamidopyrimidine-DNA glycolase	L	*1.76*
Dde_3571	ATP-dependent DNA helicase, UvrD/REP family	L	**−3.26**
Dde_0367	3-deoxy-D-manno-octulosonic-acid transferase, putative	M	*1.50*
Dde_0426	Glycosyl transferase, group 1/2 family protein	M	*2.50*
Dde_0831	Chain length determinant family protein	M	*3.62*
Dde_1711	Flagellar hook protein FlgE	N	*1.81*
Dde_2099	ATPase	O	*1.80*
Dde_3546	Conserved hypothetical protein	O	*1.94*
Dde_1189	Conserved hypothetical protein	P	3.39
Dde_1774	Conserved hypothetical protein	R	*1.94*
Dde_2055	GatB/Yqey family protein	S	−1.90
Dde_3139	Conserved hypothetical protein	S	*1.73*
Dde_0160	ATP-binding region, ATPase-like	T	*2.78*
Dde_2716	HD domain protein	T	*2.02*
Dde_2213	Hypothetical protein		**−2.62**
Dde_2284	Asparaginase family protein		*2.26*
Dde_2886	Methyltransferase FkbM		*1.83*
Dde_3026	Hypothetical protein		**−2.53**
Dde_3515	Formate dehydrogenase formation protein FdhE, putative		*1.77*

[a] Represents the difference in *Z*-score between the $G20_{sediment}$ and $G20_{lab}$. Number in italic means the protein was detected in $G20_{sediment}$ sample only, number in bold means the protein was detected in $G20_{lab}$ sample only.

obtain samples of bacteria that have not been separated from their evolutionarily important selective pressures.[30] It seems that there is a tradeoff between having long-term, well-characterized cultures and recent cultures that probably reflect the natural environment more accurately. A recent study with *Bacillus subtilis* has identified genetic differences between newly isolated and a lab-adapted strain.[31] In this study, we compared the proteomes of the well-adapted lab strain of G20 and the same strain conditioned to grow in sediments, in order to look at differences in global protein abundances.

Among the 1097 and 995 proteins detected in the $G20_{sediment}$ and $G20_{lab}$ samples, respectively, 955 proteins were detected in both samples. Proteins with significant changes are listed in Table 2. One of the eight proteins with lower abundance in $G20_{sediment}$ is a pyruvate ferredoxin/flavodoxin oxidoreductase (Dde_0046), although its abundance value (0.27) was relatively high when compared to other proteins found in both samples. Pyruvate ferredoxin oxidoreductase (POR) catalyzes the oxidation of pyruvate to acetyl-CoA in the cytoplasm and has also been purified from *Desulfovibrio africanus*.[32] Its low abundance in our $G20_{sediment}$ strain may suggest that pyruvate is not the primary carbon source in sediments. It is also possible that this protein may not be involved in pyruvate metabolism due to its low homology to known POR genes. Three ribosomal proteins queuine/other tRNA-ribosyltransferase (Dde_2844), valyl-tRNA synthetase (Dde_2839), and GatB/Yqey family protein (Dde_2055) were also observed in low abundance in the $G20_{sediment}$. The valyl-tRNA synthetase in *Escherichia coli* plays an important role in correcting misactivation and misacylation errors by regulating a tRNA (Val)-dependent editing reaction.[33] GatB is the β subunit of a glutamyl-tRNA amidotransferase protein, which in *B. subtilis* controls the formation of correctly charged Gln-$tRNA^{Gln}$ through the transamidation of misacylated Glu-$tRNA^{Gln}$.[34]

Several proteins (22) were more abundant in $G20_{sediment}$, for example, two different elongator proteins 3/MiaB/NifB (Dde_1359 and Dde_0047), methyltransferase (Dde_2886), and aminotransferase (Dde_2744). Most aminotransferases have multiple functions in different pathways and exemplify the class of enzymes with ambiguous substrates;[35] while some aminotransferases are unique catalysts for a particular reaction, for example, in *E. coli*, mutations in ilvE, hisC, or bioA lead to auxotrophic requirements for isoleucine, histidine, or biotin, respectively.[36−38] Since no elevated levels of gene products of ilvE, hisC, or bioA were observed in the strain $G20_{sediment}$, the elevated abundance level of this aminotransferase (Dde_2744) suggests that this protein plays general functions in transamination reactions in G20. Methyltransferases may acts as methylating agents modifying DNA and may produce lethal and mutagenic lesions. The use of methyltransferases is one of the mechanisms to correct modified DNA strands and repair potentially lethal lesions.[39] MiaB protein is a bifunctional radical-*S*-adenosylmethionine enzyme involved in thiolation and methylation of tRNA in bacteria.[40] Elevated flagellar hook protein (Dde_1711) abundances in the $G20_{sediment}$ were expected since there are potential benefits to cell motility including increased nutrient acquisition, toxic substance avoidance, and the ability to migrate to optimal colonization sites.[41] Formate dehydrogenase formation protein (FdhE) also showed elevated abundance levels in the $G20_{sediment}$ sample. and FdhE has been found to be involved in the formation of respiratory formate dehydrogenase in *E. coli*.[42]

3. Possible Gene Regulation in G20 Mutants. The third goal of this investigation was to identify possible regulatory roles

of the gene products from identified genes. That is, to understand the reason these mutants were unable to survive in sediment, while there was no obvious change of growth characteristics in LS medium, indicated by the similar doubling time relative to $G20_{lab}$ (data not shown).

Three mutants had insertion mutations in open reading frames annotated as signal transduction histidine kinases (HKs). HKs usually belong to two-component systems (TCSs), which are required for innumerable adaptive responses in bacteria. TCSs are widespread and exist not only in prokaryotes, but also in eukaryotes.[43] A typical TCS includes a HK and a partner response regulator (RR). The HK acts as a sensor and is autophosphorylated in response to an input signal, and the histidine-to-aspartate phosphotransfer to the RR results in a cellular output response.

The *D. desulfuricans* G20 genome encodes 44 HK proteins, 42 RR proteins, and 8 hybrid HK proteins. In at least 10 cases, pairs of HK and RR proteins are encoded by adjacent or nearby genes that are often arranged in operons. However, some are encoded by genes that appear to be separated on the chromosome. The interrupted HK gene in the mutant D12(pB8) is located in a nine-gene operon that includes a HK, a RR, a nitrogen regulation protein, a universal stress protein, three transmembrane proteins, and two conserved hypothetical proteins. In mutant H9(pF8), the HK gene (locus Dde_0602) is located in an operon that includes the periplasmic component of an ABC-type phosphate/phosphonate transport system and a nitrogen regulation protein. While in mutant G5(pA9), the HK gene (locus Dde_1945) is a single gene, with the corresponding RR perhaps located in a separate part of the genome.

Interestingly, we found that the abundances of several proteins were affected by the deletion of more than one TCS. For example, high-abundance values for the glutamyl-$tRNA^{Gln}$ amidotransferase A subunit (Dde_1020) were observed in mutant D12(pB8), H9(pF8), and G5(pA9). Glutamyl-tRNA amidotransferase converts Glu-$tRNA^{gln}$ to Gln-$tRNA^{gln}$ in bacteria not able to form Gln-tRNA directly from glutamine.[34] High abundance of methyltransferase GidB was observed only in mutant H9(pF8) and G5(pA9), but not in mutant D12(pB8). GidB is a family of bacterial, glucose-inhibited division proteins involved in the regulation of cell division.[44] Fifteen other common proteins with lower abundance were observed in mutants G5(pA9), H9(pF8), and D12(pB8) (data not shown), indicating that many TCSs may have common physiological roles in *Desulfovibrio*.[45]

The histidine kinase in two mutants, H9(pF8) and D12(pB8), have BaeS domains. BaeS sensor kinase and BaeR RR control the expression of the *spy* gene in response to envelope stress in *E. coli*.[46] The BaeS/BaeR system is also thought to control genes for an efflux pump (*mdtABC*) in *E. coli*.[47,48] The *E. coli* baeSR mutants showed increased sensitivity to myricetin, gallic acid, nickel chloride, and especially sodium tungstate.[49] A putative tungstate transport system substrate-binding protein was in low abundance in mutant H9(pF8). This protein may be involved in tungstate efflux; thus, its low abundance could cause the mutant to be more sensitive to tungstate, or an analog in sediment. These two interrupted histidine kinases have a 27% identity in amino acid sequence, and they down-regulated 18 proteins in common. However, the up-regulated proteins were very different in these mutants (see Table 3). In the same mutant H9(pF8), two cell envelope biogenesis proteins, methyltransferase GidB (Dde_2293) and glycosyltransferase (Dde_2890), constituted a large percentage of the proteins identified. This is consistent with the previous finding that the BaeS/BaeR system controls an envelope stress pathway.[46] While in mutant D12(pB8), several proteins involved in energy production were highly abundant, for example, alcohol dehydrogenase (Dde_3523), flavodoxin (Dde_3667), and [NiFe] hydrogenase, small subunit (Dde_2137). It is uncertain whether this sensor protein also senses electron status in the cell. The differentially regulated proteins imply that several different sets of genes are controlled by different networks of TCSs.[50]

The mutated signal transduction histidine kinase in mutant G5(pA9) has an AtoS domain. AtoS-AtoC also belongs to a TCS family, and AtoS functions both as a transcriptional and post-translational regulator.[51] In a typical AtoS-AtoC system, the *atoS* gene coding for a sensor kinase is located just upstream of *atoC*. AtoS/AtoC deletion mutants of *E. coli* showed increased use of glucuronamide as a carbon source.[49] The *ato*SC mutants also showed increased sensitivity to sodium chloride and were more susceptible to changes in osmolarity, some membrane active agents, aminoglycosides, and a respiratory inhibitor.[49] A recent transcriptome analysis of the *E. coli* two-component systems revealed that possible roles of the AtoS-AtoC system included flagellar synthesis and chemotactic behavior.[49] This histidine kinase gene in G20 is a single gene, which does not form a typical *ato*SC operon. Proteins with significant changes in mutant G5(pA9) are shown in Table 3. Several proteins with decreased abundance in this mutant were related to energy production, for example, 2-oxoacid:acceptor oxidoreductase (Dde_1793), cytochrome c-553 (Dde_1821), formate acetyltransferase (Dde_3055), and ethanolamine utilization protein eutM precursor (Dde_3270). Cytochrome *c*-553 from sulfate-reducing bacteria is a low-oxidoreduction-potential cytochrome that lacks primary sequence homology to other cytochrome *c* proteins.[52] The redox potential of this protein is abnormally low (+20 mV) when compared with a normal range of approximately 200−450 mV for the cytochrome *c* family.[52] Low abundances of cytochrome *c*-553 in mutants may explain why these mutants were not competitive in sediment.

The insertion in mutant A1(pD3) was within Dde_0289, encoding a protein that contains a RocR domain. RocR is a member of the NtrC/NifA family of regulators and a member of a family of prokaryotic enhancer-binding proteins that act together with sigma 54 factors. In *B. subtilis*, the positive regulatory protein RocR is required for the expression of both *rocABC* and *rocDEF*, two operons involved in arginine and ornithine catabolism.[53] However, our data set did not further elucidate the regulation in this pathway. Instead, we found three signal transduction mechanism proteins (with loci: Dde_2148, Dde_0160, and Dde_2716) with low abundance and three signal transduction mechanism proteins (with loci: Dde_1399, Dde_2457, and Dde_1697) with high abundance in this mutant. This may suggest that this transcriptional regulator is involved in the regulation of a signal transduction network. The complete list of proteins with significant abundance changes are shown in Table 3. One probable rubredoxin (Dde_2749) was also found to be in low abundance in this mutant. Rubredoxin in *Desulfovibrio* species plays an important role, not only in electron transport, but also in the protection from oxidative stress.[54] It has been shown that, during periods of oxidative stress, rubredoxin could divert electron flow from the electron transport chain of *D. vulgaris* to rubrerythrin and superoxide reductase, thereby simultaneously protecting autooxidizable redox enzymes and lowering intracellular hydro-

Table 3. Proteins with Significant Changes in Mutants

locus[a]	protein description	functional category[b]	changes in mutant [c]					
			D12(pB8)	H9(pF8)	G5(pA9)	A1(pD3)	B8(pB6)	D12(pE9)
Dde_1793	2-oxoacid:acceptor oxidoreductase, beta subunit, pyruvate/2-ketoisovalerate	C	−0.81	**−1.68**	**−1.51**	−0.81	−0.60	**−1.61**
Dde_3523	Alcohol dehydrogenase, iron-containing	C	**2.95**	0.15	0.12	−0.01	**3.99**	0.24
Dde_1821	Cytochrome *c*-553	C	−0.32	**−1.96**	**−1.59**	−0.83	−0.33	−1.34
Dde_1359	Elongator protein 3/MiaB/NifB	C	**−1.95**	0.51	0.10	**−2.03**	0.02	**−1.78**
Dde_3775	Ferredoxin I	C	−0.80	−0.83	0.88	0.66	−0.83	**2.56**
Dde_3667	Flavodoxin, short chain	C	**1.59**	0.15	0.12	−0.01	1.42	0.24
Dde_3055	Formate acetyltransferase 2	C	**−2.55**	**−2.47**	**−2.50**	0.05	−0.08	0.45
Dde_2642	Iron-sulfur cluster-binding protein	C	−0.08	−1.42	**−1.50**	−0.88	0.09	**−1.56**
Dde_2137	Ni−Fe hydrogenase, small subunit:Twin-arginine translocation pathway signal	C	**2.42**	0.15	0.12	−0.01	**2.40**	0.24
Dde_0086	Oxygen-insensitive NAD(P)H nitroreductase	C	0.07	0.15	**2.09**	−0.01	0.15	**2.29**
Dde_2749	Probable rubredoxin	C	−0.07	−0.84	−0.29	**−3.00**	0.07	−1.08
Dde_1792	Pyruvate ferredoxin oxidoreductase, alpha subunit	C	−1.21	−1.45	−1.49	−0.83	−1.35	**−1.53**
Dde_0046	Pyruvate ferredoxin/flavodoxin oxidoreductase family protein	C	0.09	0.31	0.17	0.22	0.22	**1.66**
Dde_2744	Aminotransferase, class V	E	**−2.63**	**−2.47**	**−2.40**	0.00	**−2.64**	**−2.46**
Dde_3552	Aspartate ammonia-lyase, putative	E	−0.03	0.05	**1.72**	0.42	0.04	0.14
Dde_2664	Diaminopimelate decarboxylase	E	**1.67**	0.45	0.42	0.75	**1.59**	0.24
Dde_1305	Peptidase, M24 family	E	**−2.62**	0.41	0.44	0.02	0.00	0.12
Dde_2282	Hydantoinase/oxoprolinase family protein	EQ	−0.98	−1.06	−1.26	−0.20	**−1.76**	**−1.67**
Dde_2285	1,4-alpha-glucan branching enzyme	G	0.31	0.35	0.64	**−2.18**	0.58	**−1.93**
Dde_2353	Alpha amylase, catalytic subdomain	G	**4.29**	0.15	0.12	−0.01	0.15	0.24
Dde_3736	Glyceraldehyde-3-phosphate dehydrogenase, type I	G	−0.53	−1.13	−1.05	−0.89	−0.46	**−1.69**
Dde_3685	HPCH/HPAI aldolase family protein	G	−1.36	−0.15	−0.44	0.52	**−5.45**	**−5.20**
Dde_0234	ABC transporter, putative tungstate transport system substrate-binding protein	H	−1.02	**−1.72**	−1.02	−0.78	−1.12	−0.33
Dde_0801	Precorrin-4 C11-methyltransferase region	H	**−1.73**	**−1.65**	**−1.68**	−0.03	−0.22	**−1.56**
Dde_2722	Protein of unknown function UPF0004	J	0.00	**−1.56**	**−1.59**	−0.16	0.00	−0.43
Dde_2378	Protein of unknown function UPF0004:tRNA-i(6)A37 modification enzyme MiaB	J	0.83	−1.36	−1.39	**−1.52**	−1.37	−1.27
Dde_2844	Queuine/other tRNA-ribosyltransferase:Queuine tRNA-ribosyltransferase	J	0.07	0.15	0.12	0.01	0.15	**4.43**
Dde_2982	Ribosomal protein L31	J	**−1.87**	0.83	0.41	0.34	0.45	0.43
Dde_2289	Sua5/YciO/YrdC/YwlC	J	**−2.42**	**−2.34**	**−2.37**	−0.01	**−2.34**	**−2.25**
Dde_0553	Hypothetical protein	K	**−2.53**	**−2.45**	**−2.48**	**−2.61**	**−2.45**	0.07
Dde_3426	Hypothetical protein	K	−0.47	**−2.10**	**−2.13**	0.42	0.08	0.80
Dde_0349	Transcriptional regulator, LysR family	K	**−2.65**	**−2.57**	0.14	0.20	**−2.58**	**−2.49**
Dde_1399	ATPase	KT	0.07	0.15	0.12	**1.78**	0.15	0.24
Dde_2457	HDIG	KT	**1.55**	0.15	0.12	**1.54**	0.15	**1.58**
Dde_3571	ATP-dependent DNA helicase, UvrD/REP family	L	−0.04	0.15	0.22	0.18	0.15	**3.10**
Dde_0047	Elongator protein 3/MiaB/NifB	L	0.01	**−3.48**	**−3.51**	0.02	**−3.49**	0.30
Dde_1491	Endonuclease III/Nth	L	−0.35	**−2.27**	**−2.31**	**−2.43**	**−2.28**	−0.20
Dde_0542	Formamidopyrimidine-DNA glycolase	L	0.49	**−1.69**	**−1.72**	**−1.85**	1.01	**−1.60**
Dde_0367	3-deoxy-D-manno-octulosonic-acid transferase, putative	M	**−1.52**	−1.44	−1.47	**−1.59**	−1.44	−1.35
Dde_0831	Chain length determinant family protein	M	0.20	−0.21	−0.11	**−2.91**	0.28	**−2.26**
Dde_2565	Conserved hypothetical membrane protein	M	−0.83	−0.84	−1.04	**−2.14**	**−1.62**	−0.03
Dde_0426	Glycosyl transferase, group 1/2 family protein	M	**−2.51**	**−2.43**	**−2.46**	0.22	**−2.43**	**−2.34**
Dde_1433	Heptosyltransferase family protein	M	0.07	0.15	**2.76**	−0.01	0.15	0.24
Dde_2293	Methyltransferase GidB	M	0.07	**2.45**	**2.35**	−0.01	0.15	0.24
Dde_2203	Peptidase, M23/M37 family	M	0.07	0.15	**1.75**	0.03	0.15	0.24
Dde_2890	Similar to Glycosyltransferase	M	0.76	**1.52**	1.12	−0.01	0.15	0.24
Dde_0431	Similar to Putative glycosyl/glycerophosphate transferases involved in teichoic acid biosynthesis TagF/TagB/EpsJ/RodC	M	**−2.62**	**−2.54**	**−2.57**	0.23	**−2.55**	0.09
Dde_2892	Hypothetical protein	M/S	0.07	**1.96**	**1.96**	−0.03	**2.29**	0.24
Dde_2734	Bacterial chemotaxis sensory transducer	NT	0.24	−0.26	−0.26	−0.30	−0.73	**−1.79**
Dde_2148	Chemotaxis protein CheZ, putative	NT	0.09	**−1.68**	0.45	**−1.50**	−0.11	0.41
Dde_0305	AhpC/TSA family protein	O	−0.46	**−1.56**	**−1.60**	−0.32	**−1.57**	−1.48
Dde_2560	Alkyl hydroperoxide reductase	O	0.11	−1.28	−1.17	−0.77	0.02	**−2.09**
Dde_2099	ATPase	O	**−1.81**	**−1.63**	**−1.76**	**−1.89**	**−1.53**	**−1.64**
Dde_3546	Conserved hypothetical protein	O	−0.32	0.24	0.14	−0.05	−0.22	**−1.78**

Table 3. (Continued)

locus[a]	protein description	functional category[b]	changes in mutant [c]					
			D12(pB8)	H9(pF8)	G5(pA9)	A1(pD3)	B8(pB6)	D12(pE9)
Dde_2946	HflK	O	0.54	0.37	0.06	0.81	0.53	**1.51**
Dde_2068	Peptidase M22, glycoprotease	O	0.39	1.01	1.02	1.13	0.90	**1.58**
Dde_1189	Conserved hypothetical protein	P	**−3.49**	−0.40	−0.24	**−3.34**	**−3.38**	**−3.28**
Dde_1791	Ferritin	P	−0.35	−0.92	−1.05	−0.45	−0.44	**−1.53**
Dde_3248	Rhodanese-like	P	0.07	0.15	0.12	−0.01	**4.50**	0.24
Dde_3270	Ethanolamine utilization protein eutM precursor	QC	−1.06	−1.46	**−1.71**	−0.55	−1.12	−1.31
Dde_1774	Conserved hypothetical protein	R	−0.95	**−1.88**	**−1.91**	**−2.03**	−1.15	**−1.79**
Dde_0799	Conserved hypothetical protein	R	**−2.67**	**−2.59**	**−2.62**	0.22	**−2.59**	−0.25
Dde_0793	RNA-binding region RNP-1 (RNA recognition motif)	R	0.86	**2.23**	0.88	**2.00**	0.15	**2.32**
Dde_1822	Similar to Uncharacterized protein SCO1/SenC/PrrC involved in biogenesis of respiratory and photosynthetic systems	R	0.07	0.15	0.12	−0.01	0.15	**1.79**
Dde_3468	Tyrosine protein kinase:Serine/threonine protein kinase	RTKL	**−2.71**	−1.22	−0.63	−0.03	−0.06	**−2.54**
Dde_3139	Conserved hypothetical protein	S	**−1.74**	**−1.66**	**−1.70**	−0.35	**−1.67**	−0.40
Dde_3214	Conserved hypothetical protein	S	0.07	**1.65**	1.26	−0.01	0.15	0.24
Dde_0773	Conserved hypothetical protein	S	1.36	**1.56**	**1.83**	−0.01	0.14	0.24
Dde_1497	Conserved hypothetical protein	S	**2.61**	0.15	0.12	−0.01	0.15	0.24
Dde_2303	Hypothetical protein	S	−0.61	**−2.80**	**−1.87**	−1.14	−0.51	**−2.71**
Dde_1116	MJ0042 finger-like region	S	0.07	0.15	0.12	1.30	0.15	**2.40**
Dde_0160	ATP-binding region, ATPase-like	T	**−2.80**	**−2.72**	−0.30	**−2.87**	−0.63	**−2.63**
Dde_3535	ATP-binding region, ATPase-like:Histidine kinase A, N-terminal	T	**−3.09**	**−1.90**	**−3.04**	−0.91	−0.87	**−1.52**
Dde_2716	HD domain protein	T	**−2.04**	0.38	0.30	**−2.11**	**−1.96**	**−1.87**
Dde_1016	Helix-turn-helix, Fis-type	T	**−2.20**	**−2.12**	**−2.16**	−0.79	−1.02	**−2.03**
Dde_0695	Response regulator receiver	T	**2.50**	0.15	0.12	−0.01	0.15	0.24
Dde_1697	Response regulator receiver:ATP-binding region, ATPase-like:Histidine kinase A, N-terminal	T	0.85	0.15	0.19	**1.67**	0.64	0.82
Dde_2519	Zn-finger, prokaryotic DksA/TraR C4 type	T	0.07	1.19	1.28	−0.01	0.15	**1.92**
Dde_0560	Cell division transporter substrate-binding protein FtsY	U	1.36	1.34	**1.51**	−0.01	1.36	1.19
Dde_2284	Asparaginase family protein	-	**−2.28**	**−2.20**	**−2.23**	**−2.35**	**−2.20**	**−2.11**
Dde_2038	Conserved hypothetical protein	-	**−2.58**	**−2.50**	−0.07	0.03	−2.51	**−2.41**
Dde_2169	Conserved hypothetical protein	-	−0.05	−1.38	−1.18	−0.70	0.12	**−2.05**
Dde_0807	Conserved hypothetical protein	-	**−1.63**	−1.45	−1.31	−1.39	**−1.66**	−1.30
Dde_2627	Conserved hypothetical protein	-	0.31	0.46	0.71	0.55	**2.03**	**2.36**
Dde_1811	Conserved hypothetical protein	-	0.10	0.81	0.63	0.35	−0.38	**−1.60**
Dde_0194	Conserved hypothetical protein	-	**−3.56**	**−3.57**	**−3.55**	−1.04	**−3.52**	−0.52
Dde_1621	Conserved hypothetical protein	-	0.62	0.76	**−3.27**	0.54	0.75	1.06
Dde_1384	Conserved hypothetical protein	-	0.07	**1.74**	0.12	−0.01	0.15	1.22
Dde_2610	Conserved hypothetical protein	-	**1.64**	0.15	**2.26**	1.16	**1.87**	**2.30**
Dde_3430	Excisionase/Xis, DNA-binding	-	**2.17**	**2.54**	**1.99**	−0.01	0.15	0.24
Dde_3515	Formate dehydrogenase formation protein FdhE, putative	-	**−1.79**	**−1.71**	**−1.74**	−0.75	**−1.71**	**−1.62**
Dde_1020	Glutamyl-tRNA(Gln) amidotransferase A subunit	-	**1.77**	**1.85**	**1.75**	0.01	**1.75**	0.21
Dde_0409	Glycerol uptake facilitator protein	-	**−1.60**	0.51	0.13	**−1.68**	0.31	−0.87
Dde_2752	Histone-like bacterial DNA-binding protein	-	−0.90	−0.94	−1.14	−0.29	**−1.72**	−0.20
Dde_2641	Hybrid cluster protein	-	−0.53	**−1.71**	**−1.53**	−1.12	−0.59	**−2.64**
Dde_3184	Hypothetical protein	-	**2.36**	0.15	0.12	−0.01	0.15	0.24
Dde_0877	Hypothetical protein	-	**2.32**	**1.91**	**1.96**	−0.01	**1.70**	0.24
Dde_2315	Hypothetical protein	-	−0.73	−1.44	−1.40	**−1.67**	−0.74	**−2.12**
Dde_2270	Hypothetical protein	-	**3.40**	−0.01	0.06	0.09	−0.31	−0.16
Dde_0446	Hypothetical protein	-	0.07	0.15	**2.02**	−0.01	0.15	0.24
Dde_3014	Hypothetical protein	-	0.06	**−2.73**	−0.64	0.07	−0.67	0.33
Dde_2224	Hypothetical protein	-	**2.28**	**2.47**	**2.47**	−0.01	**2.61**	**2.48**
Dde_3026	Hypothetical protein	-	0.07	0.15	**3.16**	**2.50**	**2.82**	**3.34**
Dde_2886	Methyltransferase FkbM	-	0.97	1.34	1.38	0.38	0.91	**1.64**
Dde_0447	TonB dependent receptor domain protein	-	0.07	**4.36**	0.12	0.46	0.15	0.24

[a] Gene locus in bold represents that the protein abundance level changed significantly in all the mutants. [b] Minus, (-) represents function uncategorized. [c] Represents the difference in *Z*-score between the mutant and $G20_{sediment}$. Number in bold means the protein abundance level changed significantly in the corresponding mutant.

gen peroxide and superoxide levels.[54] It is possible that this protein plays similar roles in strain G20.

Mutant B8(pB6) has a mutation in Dde_3047 serine phosphatase RsbU, a regulator of the sigma subunit. RsbU is thought

to play a role in activating SigB but may also play some unknown additional roles.[55] Both RsbU and SigB were found to regulate important virulence factors in *Staphylococcus aureus*, thereby contributing significantly to the outcome of staphylococcal infection.[55,56] Previous experiments suggested that SigB or RsbU influences the ability of *S. aureus* to survive in the bloodstream and reach the joints, rather than the ability to multiply in the joint and cause inflammation.[55] In addition, RsbU and SigB are also involved in the regulation of virulence determinant production. Many virulence proteins are part of cell envelopes.[57,58] We therefore expected to find a lowered abundance of cell envelope biogenesis related proteins in this mutant. Three proteins with significant changes include a conserved hypothetical membrane protein (Dde_2565), glycosyl transferase (Dde_0426), and glycosyl/glycerophosphate transferase (Dde_0431) that may be involved in teichoic acid biosynthesis. Significant changes in proteins of this mutant are shown in Table 3.

Mutant D12(pE9) has a mutation in a transcriptional regulator (Dde_1614) with an FadR domain. The FadR regulon is globally connected to the universal stress response of *E. coli*, an important function in bacteria. Global regulator FadR is required for specific alterations in the membrane lipid-fatty acid composition for survival of the cell during fluctuating environmental conditions. FadR, in conjuction with a long-chain fatty acyl-CoA, long-chain acyl-ACP, ppGpp, and cAMP, are key players in regulating the activities of enzymes and expression of genes involved in fatty acid and phospholipid metabolism in dividing and aging *E. coli* cells.[59] Mutations in FadR result in an aseptate morphology during rapid growth, accumulation of cardiolipin, cell lysis, and poor stasis survival. However, no universal stress proteins or phospholipid metabolism-related proteins were observed to undergo significant changes in this mutant. Interestingly, the abundances of ferritin (Dde_1791) and hybrid cluster protein (Dde_2641) were reduced in this mutant. Ferritins are widespread in all domains of life, including aerobic and anaerobic organisms. Ferritins function by detoxifying iron or protecting against O_2 and its radical products.[60] Hybrid cluster proteins (HCPs) contain two types of Fe−S clusters and a novel type of hybrid cluster. The first HCPs were isolated from sulfate-reducing bacteria. Subsequent studies found that they were present in a wide range of organisms, including aerobic, anaerobic, and facultative cells from all domains of life.[61] Studies conducted with *E. coli* show that transcription of HCP was induced by hydrogen peroxide, and this induction was regulated by the redox-sensitive transcriptional activator, OxyR. The *E. coli* oxyR mutant exhibited higher sensitivity to hydrogen peroxide.[61] These results may indicate that HCP is involved in oxidative stress protection. Significant changes including 17 proteins with high-abundance values and 35 proteins with low-abundance values in this mutant are shown in Table 3. For most proteins, only one of the subunits was detected in this study. However, in this mutant, both subunits (α and β) of pyruvate:ferredoxin/flavodoxin oxidoreductase encoded by Dde_1792 and Dde_1793 were detected with significant low abundance.

4. Other Proteins. 4.1. Proteins Involved in Energy Metabolism. Seventy-four proteins detected in the samples were involved in energy production and conversion. Key proteins involved in energy metabolism were detected and are listed in Supplementary Table 2 in Supporting Information. The majority of observed proteins showed no significant changes, and the consistent protein levels in these mutants indicate that they are not regulated by these regulatory genes.

4.2. Stress Proteins. We suspected that some of the regulated proteins would be universal stress proteins and heat shock proteins, due to their functions related to the stress response. Universal stress proteins were found to be important for the protection of cells under salt, oxygen, heat, or osmotic stresses.[62,63] The production of these protein were found to be stimulated by a large variety of conditions, such as stationary phase, and carbon starvation.[62,64] Heat shock proteins (HSPs) also play important roles in the responses to heat and other environmental stressors. In some cases, HSPs are also linked to resistance to DNA-damaging agents and to respiratory uncouplers.[12,63,65] Both the specific protection against an acute emerging stress and the nonspecific, prospective protection against future stress are adaptive functions crucial for surviving stress and low-nutrient conditions in nature.

Recent transcriptomic analyses done with *D. vulgaris* showed that many genes have augmented expression levels under salt stress, nitrite stress, and heat stress.[11−13] Several universal stress proteins and heat shock proteins were detected in the proteome of strain G20 (Supplementary Table 3 in Supporting Information). No significant changes in stress proteins were observed in our proteomic data. This may indicate that, under our growth condition, the cells are not experiencing sudden changes, and thus, changes in stress proteins cannot be revealed in this study. This result also indicates that during exponential phase, cells did not encounter similar significant stresses as cells that enter stationary phase.[66,67]

4.3. Ribosomal Proteins. Ribosomal proteins were predicted to be the most abundant proteins in the bacterial cells during the exponential phase and were expected to be present at high levels. In agreement with this prediction, we identified 50 ribosomal proteins (see Supplementary Table 4 in Supporting Information), representing 91% of the total number of predicted ribosomal proteins. Ribosomal protein synthesis consumes more than 50% of the cells' total energy.[68] However, we are not aware of any study that proposes a role for ribosomal proteins in sediment growth or adaptation. Indeed, ribosomal proteins exist in certain ratios when forming ribosomes,[69] with most ribosomal proteins being present in a single copy. Therefore, you would expect that all of the ribosomal proteins should be present in similar amounts in the cell. However, our proteomic data do not support this idea, as some ribosomal proteins are more abundant than others. The $G20_{sediment}$ strain had similar abundance values for ribosomal proteins when compared to the $G20_{lab}$ strain. On the other hand, the mutants had a few ribosomal proteins at either higher or lower abundance. It is unknown whether synthesizing ribosomal proteins at the different ratio is a disadvantage when cells are growing in the sediment. In addition, ribosomal-associated proteins, for example, amino-acyl-tRNA synthetases and translational elongation factors, were also detected in this study. A total of 32 genes encoding putative amino-acyl-tRNA synthetase has been found in the *D. desulfuricans* G20 genome, and 21 of their proteins were detected in this study (see Supplementary Table 4 in Supporting Information). Amino-acyl-tRNA synthetases are essential proteins for ensuring the fidelity of transfer of genetic information from the DNA into the protein.[70] Their similar abundances in exponential phase samples from all mutants may explain the similar growth rate in laboratory media.

Conclusions

Our proteomic profiles of $G20_{sediment}$ and $G20_{lab}$ provide us with the direct evidence that, at the protein level, well-adapted lab strains have different features from less adapted sediment strains. Proteomic analyses of sediment fitness mutants provide the opportunity to look at the global regulation of genes involved in many biological processes, although the observed phenomena may not always be a direct consequence of interruption of each TCS. Indeed, single protein changes may reflect complex shifts in cellular physiology. However, further studies to identify the nature of the suggested functional interactions between TCSs should shed light on the TCS networks in G20. The limitation of this study is that we only used one growth phase and one growth condition for culturing and comparing the different mutants. Many TCSs, operating under specific growth conditions, may not have been strongly induced under the growth condition we used. In general, no attempt was made to ascertain whether these differences were attributable to the loss of the two-component system *per se,* or to the loss of the function of a nearby gene(s). Further studies may elucidate specific functions.

The AMT tag approach is a powerful tool that may help identify the regulatory networks of signal transduction systems since growth condition changes and their corresponding effects can be monitored for a high number of proteins over a large dynamic range.

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Supporting Information Available: Supplementary Figure 1, scatter plot of protein abundance of $G20_{sediment}$ samples, and Supplementary Tables 1−4, listing the abundance of sulfate-related proteins in different mutants, proteins involved in energy metabolism, the abundance charges of detected stress proteins, and the ribosomal and ribosomal-associated proteins detected in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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