



Ecosystems and Networks Integrated with Genes and Molecular Assemblies

Comparative genomics analysis and phenotypic characterization of *Shewanella putrefaciens* W3-18-1: Anaerobic respiration, bacterial microcompartments, and lateral flagella



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K-1770

INTRODUCTION

Respiratory versatility and psychrophily are the hallmarks of *Shewanella*. The ability to utilize a wide range of electron acceptors for respiration is due to the large number of *c*-type cytochrome genes present in the genome of *Shewanella* strains. More recently the dissimilatory metal reduction of *Shewanella* species has been extensively and intensively studied for potential applications in the bioremediation of radioactive wastes of groundwater and subsurface environments. Multiple *Shewanella* genome sequences are now available in the public databases (Fredrickson *et al.*, 2008). Most of the sequenced *Shewanella* strains were isolated from marine environments and this genus was believed to be of marine origin (Hau and Gralnick, 2007). However, the well-characterized model strain, *S. oneidensis* MR-1, was isolated from the freshwater lake sediment of Lake Oneida, New York (Myers and Nealson, 1988) and similar bacteria have also been isolated from other freshwater environments (Venkateswaran *et al.*, 1999). Here we comparatively analyzed the genome sequence and physiological characteristics of *S. putrefaciens* W3-18-1 and *S. oneidensis* MR-1, isolated from the marine and freshwater lake sediments, respectively. The anaerobic respirations, carbon source utilization, and cell motility have been experimentally investigated. Large scale horizontal gene transfers have been revealed and the genetic divergence between these two strains was considered to be critical to the bacterial adaptation to specific habitats, freshwater or marine sediments.

RESULTS

The divergent *c*-type cytochrome genes and respiration chains:

(1) W3-18-1 harbors only 32 *c*-type cytochrome genes which are all shared by other *Shewanella* strains, while 42 are present in the genome of MR-1. W3-18-1 lacks gene clusters coding for sulfite hydrogenase SorAB, octaheme tetrathionate reductase Otr, trimethylamine N-oxide reductase (TMAO), dimethylamine N-oxide reductase (DMSO), and the secondary metal reductase MtrDEF (Table 1), which may account for its inability or decreased ability to utilize those electron acceptors (TMAO, DMSO, and some metals and metalloids) or donor (sulfite) as demonstrated in our experiments. Ironically, the prototype *S. putrefaciens* is named for the iconic offensive putrid odor caused by trimethylamine, a reduction product of the abundant osmolyte TMAO in seafood. However, W3-18-1 harbors tetrathionate reductase TrABC found in *Salmonella* and another periplasmic nitrate reductase, NapEDABC, which is functionally redundant with NapDAGHB in dissimilatory nitrate reduction because deletion of each operon did not affect nitrate reduction. While W3-18-1 lacks the Fe-only hydrogenase and its Ni-Fe hydrogenase genes were separated into two clusters, it still could use hydrogen as electron donor.

(2) NADH and formate are major electron donors for respiratory electron transport chains. *Shewanella* strains differ in the NADH and formate dehydrogenases and the *fdh-O* and *nqr* gene clusters seem to have duplicated in most *Shewanella* strains (Table 1), which may represent an adaptation strategy in respiration of *Shewanella*. The NDH-I (NuoA-N) is unique to MR-1 among the *Shewanella* strains, indicating that acquisition of this NADH dehydrogenase gene cluster may be related to bacterial adaptation to freshwater environments.

Table 1 Comparative genomics analyses of components of diverse respiration and electron transport chains between W3-18-1 and MR-1

Gene or cluster	Functions	W3-18-1	MR-1	Presence in other <i>Shewanella</i> strains
<i>fdh-N</i>	Nitrate reductase-linked formate dehydrogenase, proton pump	Absent	SO_0101 to SO_0113	ANA-3, MR-7, MR-4, OS185, OS223, OS195, Spea, HAW-EB4
<i>fdh-O</i>	Formate dehydrogenase-O, proton pump, encoded by two contiguous <i>fdhGH</i> cassettes	Sputw3181_3870 to 3873 Sputw3181_3874 to 3877	SO_4508 to SO_4511 SO_4512 to SO_4515	All except OS217
<i>fdh-H</i>	Hydrogenase-linked formate dehydrogenase	Sputw3181_0894	SO_0988	All except OS217
<i>hydAB</i>	Fe-only hydrogenase, hydrogen production	Absent	SO_3920 and SO_3921	MR-4, ANA-3, HAW-EB4
<i>hyaAB</i>	Ni-Fe hydrogenase, hydrogen utilization	Sputw3181_1919 to 1924 Sputw3181_2173 to 2178	SO_2089 to SO_2099	All except OS217
<i>ndh-I</i> (nuoA-nuoN)	NADH dehydrogenase I, proton pump	Absent	SO_1009 to SO_1021	Absent
<i>ndh-II</i>	NADH dehydrogenase II	Sputw3181_3093	SO_3517	All
<i>sorAB</i>	Sulfite hydrogenase SorAB and monoheme c	Absent	Deleted in MR-1	WP3, Spea, HAW-EB3, HAW-EB4, NCIMB 400, PV-4
<i>otr</i>	Octaheme tetrathionate reductase	Absent	SO_0714 to SO_0717	OS155, OS195, OS223, WP3, Spea, Swoo, HAW-EB4
<i>ttr</i>	Tetrathionate reductase TrABC	Sputw3181_3510 to 3512	Absent	CN-32, ANA-3, OS155, OS185, OS195, OS223, MR-4
<i>nap-alpha</i>	Periplasmic nitrate reductase (NapEDABC)	Sputw3181_2103 to 2107	Absent	All including OS217
<i>nap-beta</i>	Periplasmic nitrate reductase (NapDAGHB)	Sputw3181_0792 to 0796	SO_0845 to SO_0849	All except OS217
<i>nqrABCDFE-1</i>	Sodium ion translocating NADH dehydrogenase I	Sputw3181_3324 to 3319	SO_0902 to SO_0907	All except OS217
<i>nqrABCDFE-2</i>	Sodium ion translocating NADH dehydrogenase II	Sputw3181_3236 to 3231	SO_1103 to SO_1108	All
<i>rnfABCDGE</i>	Electron transfer complex RnfABCDGE	Sputw3181_2159 to 2164	SO_2508 to SO_2513	All
<i>cyoABCDE</i>	Cytochrome <i>bo</i> terminal oxidase, proton pump	Sputw3181_0091 to 0096	Absent	CN-32, 200, OS185, OS155, OS223, OS195,
<i>tatECADSTR</i>	Trimethylamine N-oxide reductase	Absent	SO_1228 to SO_1234	All except OS217, CN-32 and 200
<i>dms</i>	Dimethyl sulfoxide reductase I, Dms-1 Dimethyl sulfoxide reductase II, Dms-2	Absent	SO_1427 to 1432 SO_4357 to 4362	One, two or multiple <i>dms</i> operons present in other <i>Shewanella</i> strains except OS217
<i>mtrABC</i>	Dissimilatory metal reductase MtrABC	Sputw3181_2623 to 2625	SO_1776 to SO_1778	All except OS217
<i>mtrDEF</i>	Secondary metal reductase MtrDEF	Absent	SO_1780 to SO_1782	OS155, OS185, OS195, OS223, ANA-3, MR-4, MR-7, PV-4, Spea, HAW-EB3, HAW-EB4

ACKNOWLEDGEMENT

ENIGMA is a Scientific Focus Area Program supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL Foundational Science through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

MATERIALS AND METHODS

Bacterial strains: *Shewanella putrefaciens* W3-18-1 was isolated from the deep marine sediments underlying 670 m of water off the Washington state coast by our laboratory (Murray *et al.*, 2001; Stapleton *et al.*, 2005). The control strain *S. oneidensis* MR-1 was isolated from the sediment of Lake Oneida, New York by Ken Nealson.

Genome sequencing and annotation: The DNA sequencing, assembly, and annotation of the W3-18-1 genome were conducted by Joint Genome Institute (Copeland *et al.*, 2006).

Carbon source utilization assays: BioLog microplate assays and MRI minimum media (supplemented with different carbon sources) were used to identify the utilization of carbon sources in *Shewanella*.

Transposon mutagenesis and isolation of motility defective mutants: The *mariner* transposon mutagenesis (pminiHmar RB1, courtesy by Dr. Daad Saffarini) was conducted to isolate mutants (Km^r) of *Shewanella putrefaciens* W3-18-1 that are defective in motility on the LM soft agar plates (0.4% agar, w/v). The transposon insertion sites were mapped as previously described (Bouhenni *et al.*, 2005).

In-frame deletion: The two step protocol of selection (single cross-over, antibiotics resistance) and counter-selection (double crossover, sucrose sensitivity) was applied for in-frame deletion of specific genes using suicide vector pDS3.0 (R6K replicon, *sacB*, Gm^r)-based constructs carrying a fusion of upstream and downstream sequences of target genes.

Bioinformatics tools: The orthologs are identified by using bidirectional BLASTP (best hits) between W3-18-1 and other *Shewanella* strains and also based on synteny. The paralog(s) of polypeptides were found by BLAST against the same genome (>70% sequence similarity).

RESULTS

Comparative genomics:

W3-18-1 belongs to the *Shewanella putrefaciens* clade (Konstantinidis *et al.*, 2009) and its genome (4,708 kb) is substantially smaller than that of MR-1 (5,130 kb). These two strains share a large core genome including 3,162 genes. However, there are few genes homologous to those encoded by the plasmid pMR-1 of MR-1. About 700 genes, encoding bacterial microcompartments, lateral flagella, bacteriophages, and other functions, from W3-18-1 are absent in MR-1. The strain-specific (unique to W3-18-1) and species-specific (also present in *S. putrefaciens* CN-32 and 200 other strains) gene cassettes indicate recent lateral gene transfer events (Table 2). The SXT/R391 ICE and SGI1-like prophages may have been recently acquired because, among the sequenced *Shewanella* genomes, they are only present in W3-18-1 and are also found in pathogenic bacteria such as *Vibrio* and *Salmonella*. Several gene clusters that are absent in MR-1 are related to the osmotic stress response and sodium ion-dependent energy transduction (sodium ion translocating oxaloacetate decarboxylase) and transport, which may be crucial for surviving in marine habitats.

Table 2 Some of W3-18-1-harbored genetic loci that are absent in the genome of MR-1

No.	Locus	Length	Genes	Predicted functions	Presence in other strains
1	Sputw3181_0088-0096	10.4 kb	Cytochrome <i>bo</i> oxidase genes <i>cyoABCDEF</i>	Electron transfer and energy transduction	CN-32, 200, OS155, OS185, OS195, OS223
2	Sputw3181_0197-0204	9.1 kb	Anion transporter, fumarate, and fumarate reductase genes <i>rfaABCD</i>	Nutrient uptake and fumarate reduction	Putrescine
3	Sputw3181_0095-0100	27 kb	Degenerate type III secretion system	Degenerate type III secretion system	CN-32, 200
4	Sputw3181_0041-0067	27.8 kb	Drug efflux genes, metal resistance genes	Toxin and heavy metal resistance	Absent
5	Sputw3181_0408-0445	82.4 kb	Bacterial microcompartment operon and PTS system genes	1,2-propanediol utilization and sugar uptake	CN-32
6	Sputw3181_0454-0493	35.8 kb	Lateral flagella operon	Motility and colonization	CN-32, OS155, OS217, WP3, HAW-EB3, HAW-EB4, Spea
7	Sputw3181_0554-0559	8.6 kb	Tannase/ferrolase, outer member protein, and fumarate reductase	Motility and colonization	CN-32, 200, OS185, NCIMB 400
8	Sputw3181_0862-0868	8.5 kb	Potassium ion transporting ATPase operon and two-component system	Osmotic stress response	CN-32, 200
9	Sputw3181_1072-1183	108.6 kb	SXT/R391 ICE (integrating conjugative elements)-like prophage	Mobile element, prophage	Absent
10	Sputw3181_1380-1395	4.3 kb	Acyltransferase-cyclidyltransferase and sugar nucleotidyltransferase	Utilization of amino sugars	CN-32
11	Sputw3181_1948-1966	32.8 kb	L-arabinose and polymer utilization operon	Uptake and degradation of L-arabinose and arabinan	All except MR-1
12	Sputw3181_2102-2107	4.6 kb	Periplasmic nitrate reductase operon <i>napEDABC</i> (<i>nap-alpha</i>)	Nitrate reduction and anaerobic respiration	All except MR-1
13	Sputw3181_2184-2212	37.7 kb	CRISPR elements	Plasmid and phage restriction	CN-32, 200, OS185, OS195
14	Sputw3181_2399	2.5 kb	Retron and PNA-directed DNA polymerase gene	Absent	Absent
15	Sputw3181_2877-2921	37.5 kb	Pil phase element	Motility	MR-7, OS155, HAW-EB4
16	Sputw3181_2930-2954	24.1 kb	Mu phage element and arsenate reductase genes	Arsenate resistance	Absent
17	Sputw3181_3133-3136	3.5 kb	Sodium ion translocating oxaloacetate decarboxylase genes	Energy transduction	CN-32, 200, OS155, OS185, OS195, OS223, MR-4, MR-7
18	Sputw3181_3204-3212	7.2 kb	Mercury resistance operon	Mercury resistance	CN-32, 200
19	Sputw3181_3508-3513	9.7 kb	Tetrathionate reductase and two-component system	Tetrathionate reduction	CN-32, ANA-3, OS155, OS185, OS195, OS223, MR-4
20	Sputw3181_3902-3909	11.8 kb	Pseudomonas Cup (chaperone and usher pathway) type pilus operon	Biofilm formation	All except MR-1
21	Sputw3181_3982-3996	20.9 kb	Proline biosynthesis and sodium proline symporter genes	Proline uptake and osmotic stress response	All except MR-1
22	Sputw3181_4067-4090	20.7 kb	Prophage genes, similar to SGI1 element from <i>Salmonella</i>	DNA modification and restriction	Absent

Cell motility:

A common feature for *Shewanella* strains is the large number of methyl accepting chemotaxis sensory transducer genes (38 in W3-18-1), indicating the importance of active chemotaxis for bacterial survival. W3-18-1 exhibited a higher motility and its colonies could swarm on the 0.4% (w/v) soft agar plates while MR-1 could not. We have isolated a series of transposon mutants which exhibited defective motility on soft agar (0.4%) and then quantitatively compared the cell motility of different mutants on 0.3% soft agar plates. The lateral flagella, polar flagellum, and MSHA pili (twitching motility) are all involved in cell motility in W3-18-1 (Table 3 and Figure 1), which is crucial for survival in the viscous marine habitats. On the other hand, the gene cluster (Sputw3181_3902 to 3909, 11.8 kb) coding for the Cup (Chaperone usher pathway) type IV pili may be only involved in biofilm formation as previously described in *Pseudomonas aeruginosa*.

Table 3 Motility of wild type strain and mutants of W3-18-1 on soft agar (0.3%) plates

Strain	Genotype	Colony diameter at 12 hrs (Slowing-down rate %)	Colony diameter at 18 hrs (Slowing-down rate %)	Colony diameter at 24 hrs (Slowing-down rate %)
W3-18-1	Wild type	15.5 mm (0%)	21.8 mm (0%)	28.3 mm (0%)
MD05	<i>mshM::Km^r</i> (MSHA pili)	7.5 mm (51.6%)	11.0 mm (49.5%)	14.0 mm (50.5%)
MD01	<i>flhF::Km^r (laf)^{1/2}</i>	12.0 mm (22.6%)	15.0 mm (31.2%)	18.0 mm (36.4%)
MD11	<i>flhF::Km^r (laf)^{2/3}</i>	12.0 mm (22.6%)	15.0 mm (31.2%)	18.0 mm (36.4%)
MD13	<i>ecfE::Km^r (pgf)^{1/2}</i>	7.0 mm (54.8%)	9.0 mm (58.7%)	11.0 mm (61.1%)
MD10	<i>flhD::Km^r (ppl)</i>	7.0 mm (54.8%)	9.1 mm (58.3%)	10.9 mm (61.5%)

The data represent the average of replicates. ^{1/2}Pfd and ^{2/3}laf represent the polar flagellum and lateral flagella, respectively.

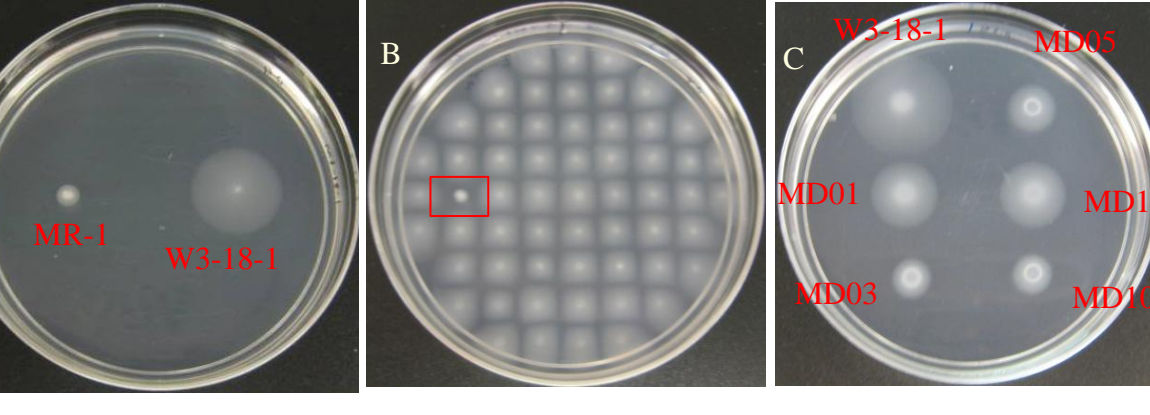


Figure 1 Cell motility assays on soft agar plates. A. Comparison of motility between MR-1 and W3-18-1 (0.4% agar, w/v); B. Isolate of motility-defective mutants (0.4% agar); C. Quantification of motility of different mutants (See Table for details).

RESULTS

Carbon source utilization:

Like MR-1, W3-18-1 did not utilize most of sugars tested (Table 4) and it lacks the 6-phosphofructokinase gene required for glucose utilization. W3-18-1 could grow well on defined minimal media supplemented with carbon sources, suggesting that it can synthesize all the amino acids, nucleotides, and cofactors for cellular metabolisms. W3-18-1 could utilize more organic acids (Table 4), which may be due to the presence of relevant transporter genes encoded in its genome (Table 2). For example, W3-18-1 could utilize L-malic acid and succinic acid, which are intermediates of citric acid cycle, but the transporters remain to be defined in W3-18-1. MR-1 did not utilize the external formate for respiration, though it does harbor multiple formate dehydrogenases (Table 1). The formate transporter genes are different between W3-18-1 and MR-1. Though their utilization of carbon sources is slightly different, the cellular metabolism of W3-18-1 and MR-1 are predicted to be very similar because very few of the strain-specific genes encode metabolic enzymes.

Metabolic pathways:

Based on the genome sequence comparison and the evidence that W3-18-1 could use all of the carbon sources that MR-1 utilized (Table 4), we predicted that W3-18-1 may share features of cellular metabolism computationally and experimentally disclosed in MR-1, including a complete TCA cycle under both aerobic and anaerobic conditions. W3-18-1 also contains the galactokinase and ribokinase genes, but it does not utilize galactose or ribose, which may be due to the absence of relevant sugar uptake systems. There is only one PTS system shared by the *Shewanella* strains.

Bacterial microcompartment:

W3-18-1 and CN-32 contain a large laterally transferred element (82.4 kb, sputw3181_0408 to 0445) coding for bacterial microcompartments and another PTS system similar to the PTS system for mannose/fructose/ sorbose. However, W3-18-1 did not exhibit significant utilization of either 1,2-propanediol or ethanolamine in BioLog or minimal media-based assays in the presence of vitamin B₁₂. W3-18-1 does not utilize more tested sugars than MR-1 and the functions of this PTS system needs to be further investigated.

Arabinan and arabinose utilization:

Some strains of *Shewanella*, including W3-18-1, could utilize L-arabinose as sole carbon and energy source, which is due to a large gene cluster (32.8 kb, sputw3181_1944 to 1966) that is absent in MR-1. However, this operon does not contain the gene coding for the regulatory protein AraC, and instead has more genes involved in uptake and degradation of polymers.

SUMMARY

- (1) The marine sediment isolate W3-18-1 and freshwater sediment strain MR-1 share a large core genome (3,162 genes), including multiple *c*-type cytochrome genes involved in diverse anaerobic respirations. However, W3-18-1 possesses only 32 putative cytochrome *c* genes (10 fewer than MR-1) and could not utilize either dimethylsulfoxide (DMSO) or trimethylamine-N-oxide (TMAO) as electron acceptors. Though the Ni-Fe hydrogenase operon is split into two clusters in W3-18-1, it could still use hydrogen as electron donor.
- (2) W3-18-1 harbors multiple laterally transferred elements (LGT), including five prophages, CRISPR, and other cassettes encoding the four-member D-type fumarate reductase complex and a second PTS system, bacterial microcompartments and B₁₂-independent PFL2/DhaB enzymes, lateral flagella, and type IV chaperone-usher pathway pili. The STX-like ICE1 and SGI1-like prophages have been well-characterized in pathogenic strains of *Vibrio* and *Salmonella*.
- (3) Lateral flagella and MSHA pili (twitching motility) enhance the bacterial motility of W3-18-1.
- (4) W3-18-1 harbors multiple gene clusters with functional redundancy, including nitrate reductase (*napDAGHB* and *napEDABC*), nitrite reductase (*nrfa*), and arsenate reductase gene clusters.
- (5) W3-18-1 utilizes more four-carbon compounds than MR-1 and its arabinan-degradation pathway allows for aerobic utilization of L-arabinose.
- (6) On the other hand, MR-1 harbors a series of species-specific gene clusters, encoding NADH dehydrogenase I, phosphate ABC transporter, and the proton driven flagellar motors, which may be related to its adaptation to freshwater environments. The presented genome divergence will provide insight into bacterial adaptation to specific niches, genome evolution, and bioremediation.