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CHAPTER 3.3.45

Ecophysiology of the Genus *Shewanella*

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Introduction

The genus *Shewanella* is comprised of more than 20 species inhabiting a wide range of environments including spoiled food, oil field wastes, redox interfaces in marine and freshwater, cold waters and sediments of the deep sea, and mesophilic ones all around the planet. Much of the recent interest in the shewanellae stems from their almost notorious abilities in the area of anaerobic respiration; these bacteria appear to be able to use nearly any electron acceptor more electronegative than sulfate, including oxygen. However, the genus is much more than just a group of respiratory specialists, as will be discussed here.

As with many contemporary genera, the *Shewanella* have experienced a rocky road to their present status, as evidenced by the species *S. putrefaciens*. Originally isolated as an active agent in food spoilage (Derby and Hammer, 1932), this organism was first called "*Achromobacter putrefaciens*," then *Pseudomonas putrefaciens* (Shewan et al., 1960), *Alteromonas putrefaciens* (Lee et al., 1977), and finally *Shewanella putrefaciens* (MacDonell and Colwell, 1985). One notable strain of *S. putrefaciens* called "MR-1" was isolated from Oneida Lake, New York, as a metal reducer, and after genome sequencing was begun, the epithet was changed to *S. oneidensis* on the basis of molecular data, including 16S rRNA sequence analyses, DNA gyrase sequence analyses, lipid analyses, and DNA/DNA hybridization (Venkateswaran et al., 1999).

Several *Shewanella* species have been found in Antarctic Continental shelf sediments (Bozal et al., 2002) as well as in sea-ice microbial communities (adapted to grow at temperatures below 4°C) isolated from McMurdo, Antarctica (Brown and Bowman, 2001). Some of these (like *S. livingstonensis*; Bozal et al., 2002) are cultivated strains, while others (such as McMurdo.10) are as yet uncultivated and exemplify psychrophilic Antarctic strains identified only via sequence analysis of 16S rDNA (Fig. 1). Some of the psychrophilic strains are also tolerant of high pressures, i.e., piezotolerant (Kato and Nogi, 2001). To date, only a few genera are known to have piezophilic members, and the regulation and growth under high pressure conditions of two notable species *S. benthica* (Kato et al., 1998) and *S. violacea* (Nogi et al., 1998; Nakasone et al., 1999) are under intensive study (Tamegai et al., 1998; Yamada et al., 2000).

Finally, the carbon source of many *Shewanella* strains is quite restricted, mainly fermentation end products such as lactate, some amino acids, formate, and hydrogen. Members of this group thus live a syntrophic lifestyle, in rich environments, and in association with fermentative communities that supply them the needed nutrients.

The placement of the various species within the genus *Shewanella* has largely been via the similarity of 16S rRNA sequences (Fig. 1), and while the organisms are indeed taxonomically linked by this criterion, they represent a wide range of physiological types, inhabiting many different niches (Table 1). This issue will arise several times, as it appears that the linkage on the basis of 16S rRNA may have the effect of removing virtually every physiological trait and ecological characteristic used as identifiers of the genus.

Physiology and Habitats of the Shewanellae

The type strain of *Shewanella putrefaciens* is a Gram-negative γ-Proteobacteria that is motile by a single polar flagellum. It produces hydrogen sulfide when grown anaerobically on thiosulfate or polysulfide, is incapable of glycolysis and fermentation, grows on lactate, pyruvate, formate, and a few amino acids, and is capable of respiratory growth on oxygen as well as a variety of different electron acceptors, including nitrate, thiosulfate, elemental sulfur, iron oxide, and manganese oxide. This combination of traits is nearly diagnostic for many (but not all) of the shewanellae. However as more *Shewanella* species are characterized and grouped according to their 16S rDNA sequence similarities, it has become increasingly clear that these traits do not

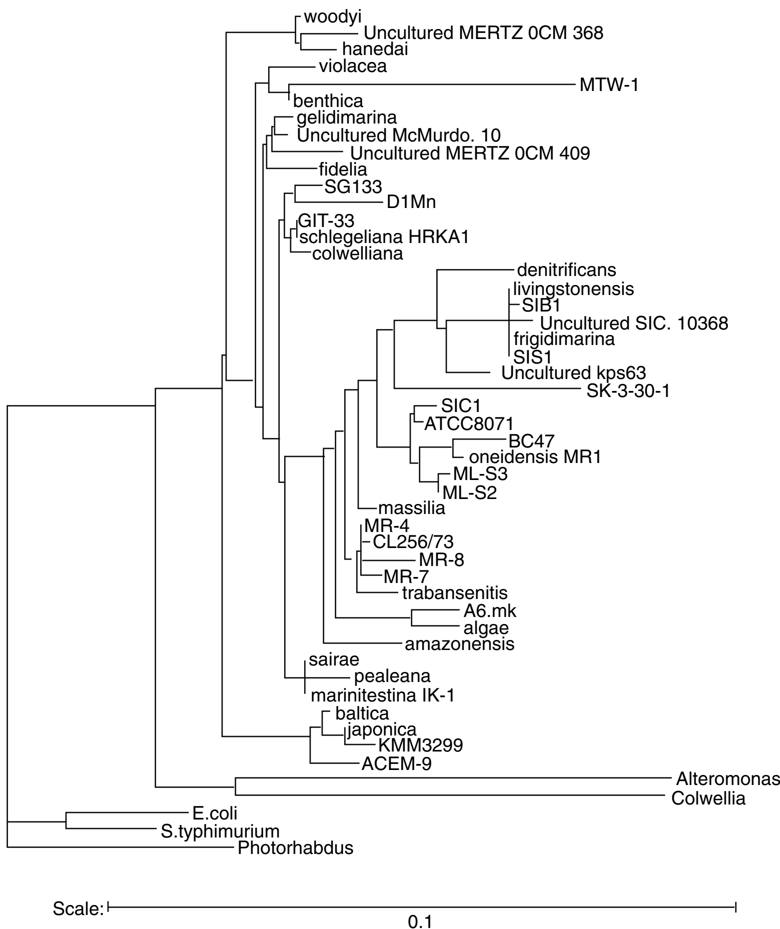

Fig. 1. Phylogenetic tree of about 40 *Shewanella* was constructed on the basis of 16S rRNA DNA sequences from both cultured clones and polymerase chain reaction (PCR) amplicons from a wide range of environments. The sequences were obtained from {the National Center for Biotechnology and Information website}. The tree was constructed using the online alignment, matrix calculation and tree building tools of the (Ribosomal Database Project (RDP-II); http://rdp.cme.msu.edu) at Michigan State University in East Lansing, Michigan (Maidak et al., 2001). All sequences are in the γ-Proteobacteria, and are linked to *Altermonas* and *Colwellia* on one side, and *Escherichia*, *Salmonella* and *Photorhabdus* on the other. The tree was constructed from 400 nucleotide positions using the Phylip program. The maximum likelihood distance matrix was utilized with *Photorhabdus* being used as the outgroup for construction of the tree.

define all the members of the genus. Thus, to elucidate the ecophysiological features (if any) that may be common to all, the physiological traits that distinguish various *Shewanella* species are discussed before environments where they are common and abundant.

Carbon and Energy Utilization

The carbon utilization of many isolates of *Shewanella* has been screened, and in general, ability to use complex carbon sources is absent. There is a strong preference for lactate as a carbon source, and an apparent inability to utilize many complex carbon sources under anaerobic conditions (Myers and Nealson, 1988; Venkateswaran et al., 1999). Overall, strains prefer lactate, pyruvate, or simple amino acids (Ringo et al., 1984), rarely utilize glucose, and are nonfermentative. By contrast, some of the recent psychrophilic isolates in the *frigidimarina* and other groups not only utilize glucose and other sugars, but are reported to ferment them as well (Bowman et al., 1997; Venkateswaran et al., 1999; Reid and Gordon, 1999; Kato and Nogi, 2001). Given that their metabolism is based on the respiration of simple carbon sources (lactate, pyruvate, formate, and some amino acids) and/or hydrogen, it is reasonable that, like many sulfate reducers, the shewanellae should be syntrophic partners of fermentative microbes, driving anaerobic metabolism forward via the removal of fermentation end products (Fig. 2). In addition, under anaerobic conditions, when grown on lactate, the shewanellae release CO_2 and acetate as end products. Again, the excretion of acetate is similar to that seen for many sulfate reducers, and suggestive of a lifestyle involving syntrophic partners capable of acetate utilization. Unlike the sulfate reducers, however, the shewanellae are capable of growth on a wide range of electron acceptors, including oxygen, are almost uniformly capable of the reduction of elemental sulfur, and are incapable of growth on sulfate as an electron acceptor.

Some detailed studies of carbon metabolism provide insights into the metabolism of the shewanellae, and the following statements paint a general picture of the group. Under aerobic conditions, *S. oneidensis* MR-1 utilizes a standard tricarboxylic acid (TCA) cycle for carbon metabolism, while under anaerobic conditions, other

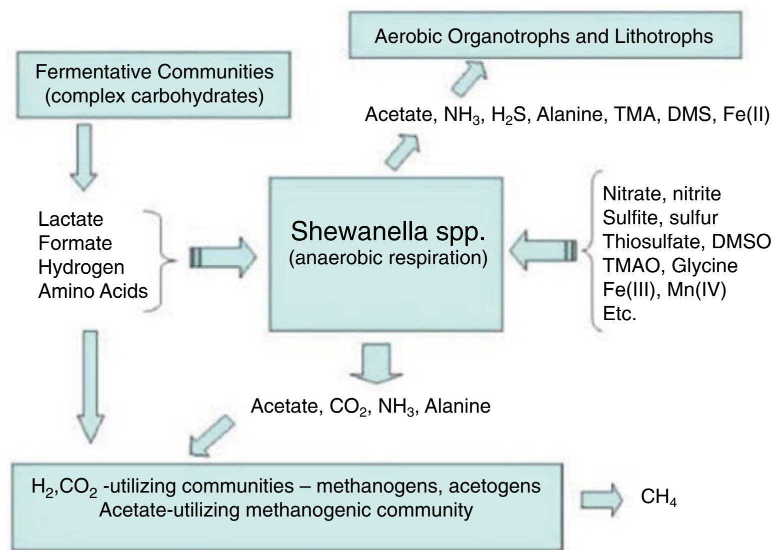

Fig. 2. Syntrophic opportunities for the shewanellae. Under anaerobic conditions, the shewanellae utilize many of the products produced by fermentative communities, enhancing the breakdown processes. If growing on lactate, they excrete copious amounts of acetate on one hand, and reduced electron acceptors on the other. The acetate can be utilized by many anaerobic organisms, especially methanogens, while both the acetate and the reduced inorganics can be utilized by aerobic organisms.

Table 1. Molar growth yields and products excreted by *S. putrefaciens* growing anaerobically with TMAO as electron acceptor.

Substrate	Growth yield[a]	Generation time (h)	CO_2[b]	Acetate[b]	Alanine[b]	NH_3[b]	%C recovered
Serine	17.5	12	2.8	0.0	0.11	0.9	104
Cysteine	17.5	12	2.7	0.0	0.10	1.1	98
Lactate	11.5	7	2.0	0.42	0.06	0.3	100
Formate	5.0	13	nd	0.0	0.0	0.2	nd

Abbreviations: TMAO, trimethylamine oxide; and n.d., no data.
[a]Molar growth yield as ∝g dry weight/∝mole of substrate oxidized.
[b]Product excreted is expressed ∝mol/∝mole of substrate oxidized.
Data from Ringo et al. (1984).

pathways are used (Scott and Nealson, 1994). Under anoxic conditions, during growth on lactate, acetate accumulates in the growth medium, probably via the acetyl-phosphate pathway, and is not further utilized. These results are in accord with the work of Ringo et al. (1984), who showed that with lactate, the incomplete oxidation pattern of carbon to CO_2 observed under anoxic conditions was inconsistent with the operation of a complete TCA cycle. It is also consistent with isotopic measurements that show a difference in carbon fractionation between aerobically and anaerobically grown cells (Teece et al., 1999).

Since growth is fastest with lactate as the carbon source, lactate is often the preferred substrate for anaerobic studies of the shewanellae. Reports of the growth on lactate vary, with some reports of anaerobic growth showing a 1 : 1 stoichiometry of lactate conversion to acetate and CO_2 (Lovley et al., 1989; Caccavo et al., 1992), and an earlier report that about 40% of the lactate (on a molar basis) was excreted as acetate (Ringo et al., 1984) by *S. putrefaciens* NCMB1735. The latter authors also noted that growth on amino acids such as serine or cysteine resulted in higher molar growth yields, with no excretion of organic acids (see Table 1). Growth on formate resulted in low molar growth yields and no excretion of organic acids.

Shewanella putrefaciens (Lovley et al., 1989) and *S. algae* (Caccavo et al., 1992) can use H_2 as an energy source, and the ability of these species to remove hydrogen is such that they can outcompete sulfate reducers for hydrogen. On the basis of studies on the binding properties of uptake hydrogenases in *Shewanella putrefaciens*, at least in soil systems, Klüber and Conrad (1993) concluded that *S. putrefaciens* does not play an important role in hydrogen oxidation in that environment.

In another study of a *Shewanella* isolate (almost certainly not *S. putrefaciens*, on the basis of carbon source utilization), glucose was used as the carbon source, and nitrate as the electron acceptor (Samuelsson, 1985). While the organism showed no growth on glucose alone, it was capable of nitrate reduction, with conversion to either nitrogen or ammonia, depending on the

redox status of the medium. Such studies demonstrate the complexities of this group of organisms, and the dangers of generalization.

Electron Acceptor Utilization

Many bacteria are somewhat versatile with regard to respiration, but few exhibit the range of activity seen in the shewanellae. The list is probably not complete, but so far, shewanellae have been shown to reduce more than 20 different electron acceptors (Table 2). This versatility is reflected in the number of cytochromes, especially *c*-type cytochromes, and in the number of cytochrome genes seen in the sequenced genome of *S. oneidensis* MR-1; {The Institute of Genomic Research [TIGR] Web site}. So far, over 40 *c*-type cytochromes are known, a great many in comparison to other bacteria of similar genome size. While the number is large, few duplicates seem to exist, and this anomaly should be addressable via mutagenesis experiments, now underway in many different laboratories.

Iron and Manganese Oxide Reduction

With regard to electron acceptor utilization, the shewanellae are known have a series of abilities not common to other aerobic bacteria: namely, the dissimilatory reduction of solid iron and manganese oxides, and polysulfide (see below). We say this with some caution, inasmuch as the dissimilatory reduction of metals was shown many years after the deposition of the type strain of this organism in the American Type Culture Collection, and the same could easily be true of other groups of bacteria already in "captivity." The ability to reduce iron was reported many years ago, even for some of the strains of *S. putre-*

Table 2. Electron acceptors utilized by shewanellae isolates.

Electron acceptor	Reduction products[a]	References[b]
Oxygen	H_2O	Derby and Hammer, 1931
Nitrate	NO_2^-, NO, N_2O, N_2, and NH_4^+	Samuelsson, 1985 Krause and Nealson, 1997
Nitrite	NO, N_2O, N_2, and NH_4^+	Samuelsson, 1985
Mn(IV) solid	Mn(II) soluble	DiChristina et al., 1988 Myers and Nealson, 1988
Mn(III) chelate	Mn(II) soluble	Kostka et al., 1995
Mn(III) solid	Mn(II) soluble	Larsen et al., 1998
Fe(III) chelate	Fe(II) soluble	Arnold et al., 1988 Myers and Nealson, 1988
$Fe(OH)_3$ ferrihydrite	Fe(II) soluble	Roden and Zachara, 1996 Urrutia et al., 1998, 1999
FeO(OH) goethite	Fe(II) soluble	Roden and Zachara, 1996 Urrutia et al., 1998, 1999
Fe_2O_3 hematite	Fe(II) soluble	Roden and Zachara, 1996 Urrutia et al., 1998, 1999
Fe_3O_4 magnetite	Fe(II) soluble	Kostka and Nealson, 1995 Dong et al., 2000
Fe(III) clay smectite	Fe(II) soluble	Kostka et al., 1996, 1999a, b
SO_3^{-2}	H_2S	Perry et al., 1993
$S_2O_3^{-2}$	H_2S	Perry et al., 1993
S^0	H_2S	Perry et al., 1993 Moser and Nealson, 1996
U(VI) soluble	U(IV) solid	Wade and DiChristina, 2000 Fredrickson et al., 2000
Cr(VI) soluble	Cr(III) solid	K. H. Nealson, unpublished observation
Selenite	Se^0 solid	Taratus et al., 2000
Arsenate	Arsenite, As^0	D. K. Newman, personal communication
Tc(VII) soluble	Tc(IV) solid	Wildung et al., 2000
Iodate	Iodide	Ferrenkopf et al., 1997
Trimethylamine-*N*-oxide	Trimethyl amine	Ringo et al., 1984
Dimethylsulfoxide	Dimethylsulfide	Myers and Nealson, 1988
Fumarate	Succinate	Myers and Nealson, 1988
Glycine	Alanine	Myers and Nealson, 1988

[a]The products of reduction are the primary products. For the metals, a variety of products are formed, depending on environmental conditions.

[b]As near as possible, these represent the first reports of specific electron acceptor utilization, and other useful references. The list is not complete.

faciens (Obuekwe et al., 1981; Obuekwe and Westlake, 1982; Semple and Westlake, 1987), but the unequivocal evidence that metal reduction was coupled to cellular metabolism and growth in the shewanellae was not reported until the late 1980s (DiChristina et al., 1988; Myers and Nealson, 1988). Since then, many metal reducers in a wide range of bacteria and archaea have been reported to be dissimilatory metal-reducing bacteria (DMRB). Now, reduction of a wide range of iron oxides as well as iron rich clays has been shown (Table 2).

The ability to reduce metal oxides is one that was doubted for many years because of the fact that most of the oxidized metals available in abundance in nature are solids (iron and manganese oxides form a range of insoluble forms, and virtually no soluble forms with the exception of organic ligands of Mn[III] and Fe[III]). The existence of the solid oxides within a metal's cycle provides an interesting twist for two reasons. First, in stratified water bodies like meromictic lakes or fjords, because the oxidized solid forms of iron and manganese sink, the cycling of these metals is in effect an oxidant pump, delivering oxidizing equivalents to the deeper waters. It is essentially a gravity-driven redox cycle (Fig. 3). In sedimentary environments, on the other hand, the layers of oxidized metals are fixed into the soils or sediments, and if not completely reduced by the next cycle of nutrients (usually on a yearly cycle in temperate zones), they can leave a layer of oxidized iron and/or manganese in the sediments—a record of previous microbial metal cycling in the soils.

One of the predictable outcomes of interacting with solid surfaces as oxidants is that the reaction rates can be a function of surface area rather than cell number. Burdige et al. (1992) studied reduction of a series of manganese oxides by various shewanellae, and have shown that surface area of the oxides is of great importance with regard to predicting rates of reaction. In fact, some "aged" oxides like pyrolusite are virtually unavailable to the shewanellae as electron acceptors, owing likely to a combination of low surface area and high crystallinity (Burdige et al., 1992). Similar conclusions have been reached with studies of iron oxides by a number of different workers (Roden and Zachara, 1996b; Urrutia et al., 1998), concluding that surface area and crystallinity are the major factors that determine the rates of reduction. Such information is of more than idle interest to those wishing to study these processes in that positive results are difficult to obtain if highly crystalline oxides of low surface area are used for experiments, especially for initial enrichments.

Another important part of the metal cycle is the ability of metal oxides to act as natural ligands for other metals. This is particularly true for manganese oxides (notorious for their ability

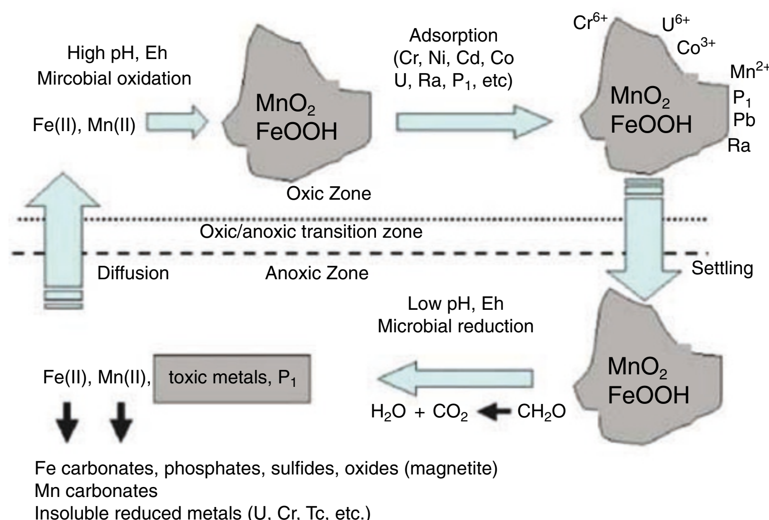

Fig. 3. The major processes occurring during the biogeochemical cycling of iron and manganese oxides across the oxic/anoxic boundary. Under conditions of high Eh and pH, iron and manganese are oxidized, and form hydrous metal oxides and oxyhydroxides. These fresh oxides are strong ligands for a variety of transition metals and actinides, as well as some heavy metals, and phosphorous, so that the insoluble oxides act as sinks for these other metals, transporting them to sedimentary environments via precipitation. In the anoxic zone, reduction occurs, either spontaneously via Eh/pH effects, or via microbial catalysis. This results in the release of the bound metals as well as Fe(II) and Mn(II), and the recycling of iron and manganese via diffusion to the oxic zone. In addition, secondary metals can be formed, depending on the chemistry of the anoxic environment in which the reduction is occurring.

to bind cations of all kinds) and for iron oxides, which strongly bind phosphate. Reduction of these oxides can then result in the release of a number of other metals into solution. Phosphate can be beneficial, and in oxic soils or marine environments, the availability of iron-reducing bacteria as symbionts may well be an important strategy in avoiding phosphorous limitation (Fig. 3). A recent proposal suggests that there have been major times in the Earth's history when productivity of the oceans was limited by phosphate, and that the relief of this limitation was achieved via iron reduction (Bjerrum and Canfield, 2002). In other cases of metal reduction, potentially toxic metals may be released, causing potential problems, or at least allowing them to diffuse to other sites. An example of such a case is shown in Fig. 4, where the cycle of uranium (U) in the Mississippi Delta is shown to be strongly regulated by the reduction of Mn oxides (Nealson et al., 2002).

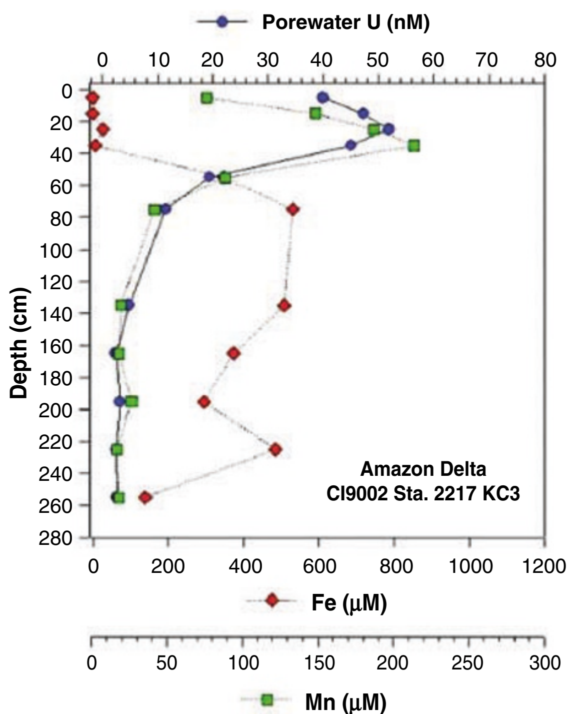

Fig. 4. Release of uranium during metal reduction. This profile plotted from data obtained during a cruise to the Amazon Delta region (Nealson et al., 2002), shows the porewater profiles of soluble iron, manganese and uranium. As seen, the uranium, which is in its soluble, oxidized (U[VI]) state, tracks almost perfectly the profile seen for Mn. This is because as the sedimentary Mn oxides are reduced by dissimilatory metal reducing bacteria (DMRB), the uranium, which is loosely bound to the Mn oxides is released into the sediments. In contrast, there appears to be no relationship between the profile of soluble iron and uranium.

The enzymatic mechanism(s) leading to metal reduction is not yet fully elucidated (Newman, 2001), and will not be reviewed here. Suffice it to say that many advances have been made, including investigations of many enzymes, genes coding for these enzymes, regulation of metal reduction, and the possibility that extracellular electron shuttles are utilized for metal reduction. From the work of several laboratories, some of the enzymes involved in metal reduction are clearly located on the outer cell wall of the shewanellae (Myers and Myers, 1992b; Myers and Myers, 1993a; Myers and Myers, 1998; Myers and Myers, 2001; Beliaev and Saffarini, 1998; Field et al., 2000; Gordon et al., 2000; Beliaev et al., 2001; DiChristina et al., 2002). Given the early reports that the reduction of solid metal oxides required cell contact with the oxides, and the more recent reports that reduction of iron requires cell adhesion (Caccavo, 1999; Das and Caccavo, 2000, Das and Caccavo, 2001), the location of such enzymes is reasonable, although the mechanism(s) whereby energy is conserved during reduction will of necessity be different from the more standard redox systems of other bacteria.

The role of quinones is not entirely elucidated, but through the work of several laboratories, it is clear that under anaerobic conditions, menaquinones are synthesized and play a major role in metal reduction. Mutants deficient in quinone production are defective in metal reduction as well as the reduction of several other substrates (Myers and Myers, 1993b; Saffarini et al., 2002). The shewanellae, along with a few other species of primarily aerobic bacteria produce methylmenaquinones (MMKs; Venkateswaran et al., 1999), and the presence of specific MMKs has been proposed as a taxonomic trait of these groups (Akagawa-Matsushita et al., 1992). Interest in quinone physiology has recently increased with the reports of Newman and Kolter (2000) and Hernandez and Newman (2001) that surface contact is not always necessary for metal reduction. Instead, they postulate that external "electron shuttles" in the form of quinones are utilized for metal reduction. These workers have shown that it is possible to isolate *S. oneidensis* mutants that cannot reduce solid iron unless supplied with quinones of the proper type. The resolution of this issue has important implications for the ecology of the metal-reducing shewanellae, as their interaction with surfaces may be a significant part of their strategy for survival and success. If surface interaction is unnecessary, or necessary only at certain times or in certain niches, these details of metal reduction will need to be determined to understand the ecophysiology of this group.

Environmental Effects of Fe and Mn Oxide Reduction

In carbon-rich sediments, metal reduction can be a major process and account for much of the carbon cycling. As shown in Table 3, a number of environments are now characterized where from 20% to virtually all of the carbon oxidized can be accounted for by metal reduction (see Thamdrup, 2000a for a comprehensive review). In general, metal cycling can play a major role in carbon-rich aquatic sediments, and even in marine systems, where it is usually assumed that sulfate reduction dominates. This view of the role of metals in the overall carbon cycling of sedimentary environments constitutes a major change in thinking, which has taken place over the last decade. Prior to this, the solid metals were considered to be rather inactive, and their role in carbon mineralization at the most was considered to be stoichiometric. It is now clear from work like that cited in Table 3 that metals may turn over many times before being buried, and can be a dynamic component of many sedimentary environments.

In addition to the cycling of organic carbon, metal reduction has physical effects—solid metal oxides are solubilized and allowed to diffuse away so that the very texture of an environment is changed. A laboratory example (see Fig. 5) is a culture of *S. oneidensis* solubilizing the solid MnO_2. The complete solubilization of metal oxide occurs in a few days, and in systems where oxygen is available, Mn reoxidation can occur rapidly, resulting in a cyclic oxidation similar to that shown in Fig. 2. Another physical effect is that seen with smectite clays, which upon iron reduction can change their swelling capacity, charge, and other physical properties resulting in major changes to the soil environment (Kostka et al., 1996; Kostka et al., 1999b; Kostka et al., 1999c).

Finally, as noted in Fig. 3, metal reducing bacteria interact via the formation of new minerals upon reduction of iron and/or Mn oxides. When iron oxides are reduced, the fate of the Fe(II) is specified by the environment as much or more than by the bacterium. Thus, one can alter the iron to form siderite (iron carbonate), magnetite (a mixed phase iron oxide), pyrite or other iron sulfides, or vivianite (iron phosphate), and perhaps many other products, simply by growing the cells in media with different buffer systems (Nealson and Saffarini, 1995a; Roden and Zachara, 1996b; Urrutia et al., 1998; Urrutia et al., 1999; Bjerrum and Canfield, 2002). The introduction of Fe(II) or Mn(II) and reformation into new mineral forms is of course potentially very important with regard to the ecosystem dynamics.

Reduction of Sulfur Compounds

The shewanellae are unusual, perhaps unique, as aerobic bacteria, in their ability to grow at the expense of elemental sulfur (in the form of polysulfide; Moser and Nealson, 1996). They produce hydrogen sulfide (H_2S) from thiosulfate, sulfite, or polysulfide, and are remarkably resistant to H_2S (Perry et al., 1993). As such, they are as "comfortable" at interfaces with sulfate-reducing bacteria as they are with aerobic heterotrophs, and span a very wide range of ecological niches in layered communities like those of the Black Sea (Nealson et al., 1991). Very little work has been done with the sulfur-reducing systems in the shewanellae, and sulfur reduction (especially in marine systems) may be one of the potentially important roles of such organisms.

Reduction of Nitrogen Compounds

The shewanellae are capable of reduction of nitrate to nitrite, nitrous oxide, ammonium, or dinitrogen, depending on conditions (Samuelsson, 1985). Brettar and Höfle (1993) identified *S. putrefaciens* (now named "*S. baltica*"; Ziemke et al., 1998) as the major organism present and

Table 3. Oxidation of organic carbon by the reduction of Mn and/or Fe.

Location[a]	% of total C oxidation due to metal reduction	Metal used as electron acceptor	Reference
Panama Basin	~100	Mn	Aller, 1990
Skagerrak	~90	Mn	Canfield et al., 1993a,b
Black Sea Shelf	23–73	Mn	Thamdrup et al., 2000
Oneida Lake	25–75	Mn	Aguilar and Nealson, 1990
Talladega wetland	38–55	Fe	Roden and Wetzel, 1996
Amazon shelf	>40	Fe	Aller et al., 1991
Skagerrak	32–51	Fe	Canfield et al., 1993a,b
Gulf of Trieste	14–73	Fe	Hines et al., 1997
Norwegian fjords	10–26	Fe	Kostka et al., 1999
Chile margin	12–29	Fe	Thamdrup and Canfield, 1996

[a]For details of study sites, temperatures, and more examples, see Thamdrup (2000).

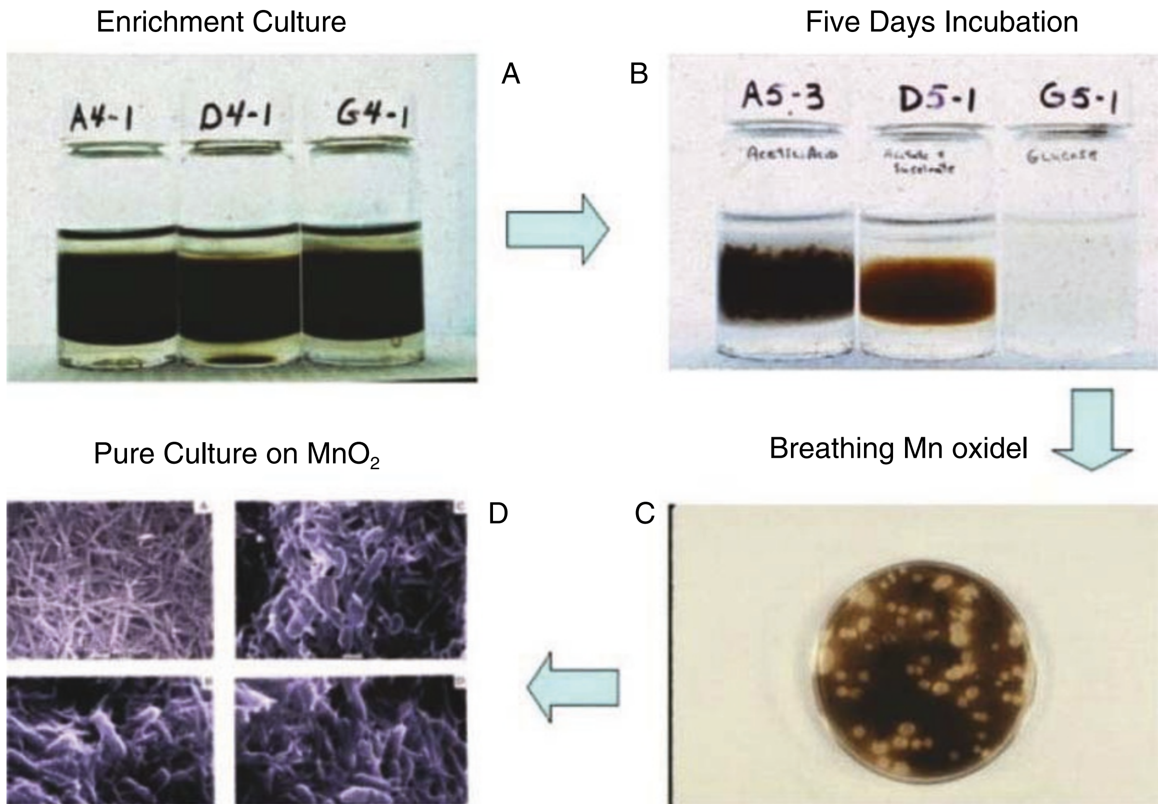

Fig. 5. *Shewanella oneidensis* and manganese reduction. A. Enrichment cultures set up for isolation of dissimilatory metal-reducing bacteria (DMRB)—soft agar vials or tubes containing MnO_2 as the only electron acceptor. Enrichments are covered with a layer of mineral oil to keep them anaerobic, and contain one or more carbon sources. B. Enrichments after several days of growth. Poisoned or uninoculated samples show no change, while those with inocula show variable results depending on the carbon sources added. Here acetate, acetate plus succinate, and glucose utilized as carbon sources are seen. The glucose has resulted in metal reduction owing to acid production. C. Enrichments on lactate-media plates are incubated with an overlay of MnO_2-containing top agar. Clearings in the top agar layer are due to reduction of the MnO_2 by dissimilatory metal-reducing bacteria (DMRB). D. Scanning electron microscopic views of pure DMRB (*S. oneidensis*) cultures growing on MnOOH surfaces. Upper left = uninoculated surface, Others = bacteria attached to surface.

responsible for organic carbon cycling and nitrous oxide production at the oxic/anoxic interface in the Central Baltic Sea, and proposed that it was a major factor in the carbon/nitrogen cycle in this marine environment. As with the sulfur compounds, much work is yet to be done on the physiology of nitrogen oxide reduction by the shewanellae, but the abundance of these organisms at nitrate-rich interfaces in the Black Sea (Nealson et al., 1991) and other marine environments, as well as the above-cited work leads to the conclusion that shewanellae will have major environmental impacts on the nitrogen cycle and its coupling to the oxidation and cycling of organic carbon.

Reduction of Other Inorganic Electron Acceptors

As shown in Table 2, the shewanellae are capable of reducing a seemingly endless array of electron acceptors down to the level of sulfate, but not including either sulfate or CO_2. This includes oxidized forms of chromium, selenium, arsenic, uranium and technetium (Fredrickson and Gorby, 1996; Fredrickson et al., 2000; Wade and DiChristina, 2000; Wildung et al., 2000). The ecological importance of these reactions is unknown, but they all have potential bioremedial uses, as reduction of these toxic metals almost always alters their solubility.

Types of Habitats

Considering the above traits, the kinds of niches where shewanellae might be expected to be found include environments: 1) that are energy rich in which fermentation is occurring and energy is continuously being deposited via sedimentation; 2) where the redox conditions might change rapidly and shift the dominance of electron acceptors, including between oxic and

anoxic states; and 3) in which other partners are present to remove the acetate produced via anaerobic respiration. These general notions fit well with the recorded isolations of shewanellae from a wide variety of mostly energy-rich niches appropriate for multispecies anaerobic metabolism, and many of which are subject to periodic redox changes.

To some extent, the ability to use the products of other anaerobes and aerobes, and a wide variety of different electron acceptors, and the ability to sense and move towards electron acceptors, are all traits that make the shewanellae ideal gradient organisms (microbes not only tolerant of, but well suited to the "gradient lifestyle") and would appear to be best adapted to syntrophic coexistence with other bacteria. Figure 6 is a schematic showing where the shewanellae have been found; some of these niches will be discussed in detail below. Of particular note is the expected (and observed) ability of the shewanellae to utilize oxygen, and thus not only tolerate rapid changes in redox status of the environment, but also thrive in them.

REDOX-RELATED HABITATS Considering the above, it is reasonable to expect that the redox interfaces, which exist nearly everywhere that biomass is produced and cycled, in the habitats occupied by the shewanellae are truly cosmopolitan, and include a wide variety of freshwater and marine niches, especially energy-rich and dynamic environments. Particularly in marine stratified communities, these versatile bacteria comprise major parts of the populations at redox interfaces (Brettar and Höfle, 1993; Ziemke et al., 1997; Ziemke et al., 1998). For example, in the Black Sea, in which redox strata occur at intervals of many meters, viable counts of bacteria revealed shewanellae accumulated at anaerobic interfaces, and were below limits of detection in the intervening depths (Nealson et al., 1991). Similarly, Höfle and Brettar (1996) have reported the abundance of shewanellae at redox interfaces in the stratified water column of the North Sea, consequently labeling them with the very appropriate epithet "interface organisms."

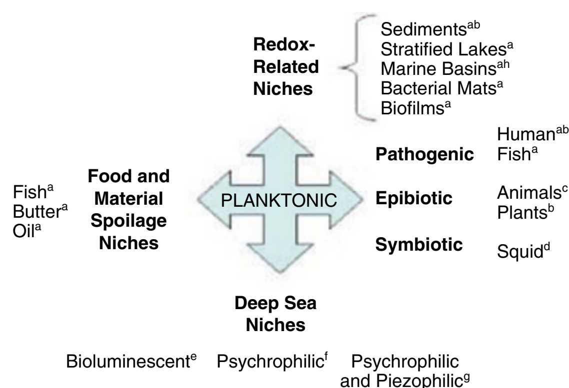

Fig. 6. The major niches of the shewanellae. As with many aquatic microbes, the niche often sampled is planktonic, but the niches are diverse. Superscripts have been added to indicate the major groups of the shewanellae found in each niche subdivision. These are a = *S. putrefaciens*, *S. oneidensis*; *b* = *S. algae*; c = *S. sairae*, *S. schlegeliana*, *S. marinintestina* ??; d = *S. pealeana*; e = *S. woodyi*, *S. hanedai*; f = *S. frigidimarina*, *S. gelidimarina*; g = *S. benthica*, *S. violaceae*; h = *S. baltica*; i = ??; j = *S. schlegi*, etc.

As environments become more oligotrophic, the occurrence and abundance of shewanellae tends to decline. For example, shewanellae are typically absent in low energy groundwater or vadose zone environments with constant conditions and low nutrient flow. Studies of North American lakes have supported this view (DiChristina and DeLong, 1993; MacGregor et al., 1997; Stein et al., 2001). In Green Bay or Oneida Lake sediments where sediment deposition rates are high, shewanellae are abundant. However, as one moves to environments with successively lower fluxes of organic carbon, e.g., to deeper parts of Green Bay and then to Lake Michigan, the shewanellae disappear and are replaced by other metal-reducing microbes (predominantly bacilli; C. Aguilar and K.H. Nealson, unpublished observation).

PATHOGENIC HABITATS Several different shewanellae have been implicated as opportunist pathogens of humans (Khashe and Jauda, 1988; Kim et al., 1989; Nozue et al., 1992; Dominguez et al., 1996; Holt et al., 1997; Iwata et al., 1999), and extensive work by Gram and colleagues (Gram et al., 1999; Vogel et al., 2000) has shown that the clinical isolates are virtually identical to those seen in environmental samples. The predominant organism capable of human infection is *S. algae*, which is also a remarkably versatile redox-active organism isolated from a variety of marine environments. This species, implicated in a case of human septicemia in Japan (Iwata et al., 1999), may well possess characteristics making it particularly suited as a human secondary or opportunistic pathogen. To this end, the shewanellae have been shown to produce only hydroxamate-type siderophores (Gram, 1994), one of which has been structurally characterized and designated as "putrebactin" (Ledyard and Butler, 1997). The siderophores of *S. algae* have not been characterized, but a slight difference in structure or regulation from that of *S. putrefaciens* could easily account for the apparently more virulent nature of this species. Only one isolate has been implicated in symbiosis with eukaryotic organisms: *S. pealeana* (Leonardo et

al., 1997), which was isolated from the nidamental gland of a marine squid. Several others, however, have been isolated as epibionts from both plant and animal materials (see below).

Food Spoilage Habitats *Shewanella* species as a food spoilage organism was first reported by Derby and Hammer (1931), and the number of such reports has continued to grow since then. The shewanellae become dominant organisms on stored marine fish materials, and are probably one of the major organisms responsible for odor production via both trimethylamine oxide (TMAO) reduction to trimethylamine, and production of hydrogen sulfide (Levin, 1968; Gram et al., 1987, Gram et al., 1990; Jorgensen and Huss, 1989; Stenstrom and Molin, 1990; Subasinghe and Shariff, 1992; Gram, 1993). It seems likely that the rich nature of the food environment provides the type of habitat needed for the shewanellae, and the abundant TMAO (and sulfur compounds) in marine fish may make these habitats ideal for the shewanellae. Of particular interest are the species that can grow well near 0°C, such as *S. frigidimarina* and *S. gelidimarina* (Fig. 1), and their potential role in food quality degradation. Many of these species are capable of a much wider range of carbon metabolism than that normally seen in the mesophilic shewanellae (Bowman et al., 1997; Bowman et al., 2000).

Deep Sea, Psychrophilic, Epibiotic and Bioluminescent Shewanellae Very little is known about the ecophysiology of the bacteria that inhabit these niches, and virtually nothing is known about the nature of the microniches or the numbers of organisms present. Recent work by Nichols et al. (2000), and Bowman et al. (Bowman et al., 1997; Bowman et al., 2000) suggests a critical feature of the deep sea cold- and pressure-tolerant microbes is their ability to produce eicosapentaenoic acid (EPA). However the strains adapted to pressure and cold-temperature appear to be biochemically and phylogenetically related. Also, these traits appear to be associated with the ability of shewanellae to survive as epibiotic strains in the tissues of various marine animals.

Several epibiotic strains of *Shewanella* have been identified and characterized including *Shewanella marinintestina*, *S. schlegeliana* and *S. sairae* from intestines of various marine animals (Satomi et al., 2003). While *S. sarie* and *S. marinintestina* cluster closely with *S. pealeana*, *S. schlegeliana* clusters closely with *S. cowelliana*. Biochemical analysis of the epibiotic strains indicates that EPA can make up a significant portion of their unsaturated lipids (Satomi et al., 2003). Though EPA may play an important role in the colonization of the host, there is no strong support for such a conjecture at this point. In fact there is strong evidence that the presence of EPA may be related to temperature adaptation. Recently it was demonstrated that when grown at 28°C, *Shewanella pealeana* appeared not to produce detectable levels of EPA (Leonardo et al., 1997). However, when grown at a lower temperature (20°C), the same strain was shown to produce significant levels of EPA (Satomi et al., 2003). Therefore the presence of EPA may have little direct influence on the host-epibiont relationship.

The trait of bioluminescence is found in only two species of shewanellae, *S. hanedai* and *S. woodyi*, both isolated from deep cold water, and both capable of growth at 4°C. The ability to emit visible light is consistent with location in the dark, deep sea, and given the association of *S. pealeana* with squid, a symbiotic luminous habitat for these species might be imagined. Both the mechanism of the light emission (bacterial luciferase), and wavelengths emitted are similar to that seen in the luminous vibrios and photobacteria. However, these two species were isolated as planktonic bacteria and nothing else is known of their luminous niches or ecophysiology. One item of interest is that these bacteria lack the capacity for widespread redox chemistry so characteristic of most of the other shewanellae.

Kato and Nogi (2001) have proposed that the shewanellae be divided into two groups on the basis of ability to adapt to and grow in the deep sea. This would put several closely related organisms like *S. benthica*, *S. violacea*, *S. pealeana*, *S. gelidimarina*, marine epibionts, and the luminous shewanellae into a separate subgroup, with *S. violacea* and *S. benthica* being placed in a branch with the piezophilic strains. As the authors note, this division has some difficulties, e.g., those tying the 16S rDNA sequence data to the physiological traits of the shewanellae in general. Nakasone et al. (K. Nakasone et. al., personal communication) are in the process of completing the genomic sequence for one of these bacteria. As discussed later, this sequence may provide major insights into the differences among the shewanellae, as both the 16S rRNA sequence (Fig. 1) and deep-sea niche of *S. benthica* and *S. oneidensis* MR-1 are widely separated.

Isolation and Characterization of the Shewanellae

Several notable features of most shewanellae can be used for enrichment: 1) the metabolism of lactate via anaerobic respiration of several

electron acceptors including thiosulfate, nitrate and hydrous metal oxides; 2) aerobic metabolism; and 3) the production of H_2S from thiosulfate during anaerobic growth. Along with a few other groups of metal-reducing bacteria, the shewanellae can also use molecular oxygen as electron acceptors. Thus the aerobic growth cycle can be used to speed up initial enrichments, although the shewanellae are so abundant in many metal-reducing environments, and grow so well in enrichment cultures, that such procedures are not necessary. However, for teaching and demonstration purposes, enrichment cultures can be achieved with any of several general media as follows: growing organisms anaerobically using lactate (20 mM) as the carbon source, and nitrate or fumarate (5–50 mM) as electron acceptor, then transferring them first to aerobic medium with lactate as carbon source, second to anaerobic medium with thiosulfate (50 mM) as electron acceptor, and lactate as electron donor, and lastly to anaerobic medium with iron or manganese oxide (solids at approximately 100 mM) as sole electron acceptor, and lactate as electron donor. At each stage of the enrichment, the cells are plated out on metal overlay plates (see below) and the colonies are picked and checked for H_2S production. Thiosulfate-containing medium with Fe(II) is used so that any H_2S formed through thiosulfate reduction results in the formation of a black (iron sulfide) precipitate. This trait is nearly diagnostic for the shewanellae.

Precautions and Recommendations

1) Mn oxides are easy to reduce and may give false positives. Because Mn oxides are more quickly reduced, demonstration of Mn oxides reduction is a good teaching tool
2) Addition of reductants to the medium (e.g., cysteine) to lower the redox, or production of organic acids, H_2S, or Fe(II) can result in Mn(IV) reduction
3) Fe oxides are more stable, and difficult to reduce abiotically
4) Highly crystalline metal oxides (Fe or Mn) purchased from most supply houses have low surface area and low reactivity. Thus, make your own
5) Metal oxides (especially Mn oxides) stored in liquid suspension tend to change (coagulate and "age") with time, leading to differences between experiments and ultimately to very unreactive metal oxides
6) Fe(III) can be prepared in a number of soluble forms, using chelators such as NTA (nitriloacetic acid), citric acid, ammonium citrate, or others. Such approaches reduce the complexities seen when solid surfaces are added to experiments

Preparation of Manganese and Iron Oxides

MANGANESE OXIDES Mn oxides are in general very easy to prepare (see Kostka and Nealson, 1997). There are a number of methods, all of which produce acceptably active, amorphous or poorly crystalline MnO_2. One method is as follows:

1) Dissolve 8 g of $KMnO_4$ in 200 ml of distilled water
2) Heat to 90°C while stirring continuously
3) Add 10 ml of 5 N NaOH
4) In a separate flask, dissolve 15 g of $MnCl_2 \cdot 4H_2O$ in 75 ml of distilled water. Add this solution slowly to the basic permanganate solution. This results in a precipitate being rapidly formed
5) Continue heating and stirring for one hour
6) After cooling, the MnO_2 is washed several times by centrifugation and resuspension in distilled water
7) After the final resuspension, the solid is freeze dried and stored as a fine dried powder

This material is referred to as "δMnO_2" or "vernadite" (Balistrieri and Murray, 1982), but unless the mineralogy is determined, it is more appropriate to refer to it as "amorphous MnO_2" or "manganate," a generic term for Mn oxides of the formula MnO_2.

If weighed subsamples are stored, they can be rehydrated to similar concentrations, and the reactivity of the manganate is reproducible. Samples can also be autoclaved before lyophilization, and stored as sterile dry subsamples for later use. A slight variation of this method is presented in Kostka and Nealson (1997).

IRON OXIDES Iron oxides range from amorphous $Fe(OH)_3$ (ferrihydrite or rust), to highly crystalline forms such as goethite, and magnetite. Like the Mn oxides, the iron oxides can change with time, forming a wide range of intermediate complex forms.

A complete treatise on the preparation and properties of iron oxides is available (and recommended) if detailed knowledge of iron oxides is required (Schwertmann and Cornell, 1991). Because ferrihydrite is commonly used in enrichments, one of the many ways of preparing it (Schwertmann and Cornell, 1991) is given below.

1) Dissolve 40 g of $Fe(NO_3)_3 \cdot 9H_2O$ in 500 ml of distilled water
2) Add KOH (approximately 330 ml of a 1.0 M solution) to bring the pH to 7.8. As pH approaches 7.8, add last few ml dropwise with constant monitoring and stirring

3) Ferrihydrite suspension is centrifuged and washed six times in distilled water
4) Use on day of preparation, as goethite or hematite will form with age
5) Ferrihydrite can be lyophilized and stored dry with only minor alterations

This method gives a good general, high surface area, reactive iron oxide for enrichment cultures.

Basal Medium (M1)

M1 basal medium is used, but simply using complex medium (peptone-yeast extract media of almost any type) with lactate as the carbon source will suffice for general isolation of shewanellae. It is critical that lactate be used as the carbon source for enrichment. As far as is known, all *Shewanella* species can use lactate, which reduces competing contaminants, and also diminishes any nonspecific metal reduction due to organic acid production, etc.

M1 Medium

Component	Amount
$(NH_4)_2SO_4$	1.19 g
PB	15 ml
BSS	100 ml
Distilled water	875 ml
MSS	0.1 ml
$NaHCO_3$	10 ml
AA	10 ml
Carbon substrates	variable
Electron acceptors	variable

For one liter of medium, $(NH_4)_2SO_4$ is dissolved in a solution containing phosphate buffer (PB), basal salts solution (BSS), and distilled water in a 2-liter Erlenmeyer flask. Then, 0.1 ml of trace metals supplement (MSS) is added before adjusting to pH 7.0 with 10 N NaOH or 10 N HCl as needed. For solid media, 15 g of agar are added. After autoclaving for 20 min, some turbidity may appear, but it will clear up with cooling. The 0.2 mM $NaHCO_3$ solution is added after cooling and the medium stored at room temperature. Before use, amino acids (AA), carbon substrate, and electron acceptor(s) are added as desired. Ten ml of AA is usually used, while carbon sources (10–20 mM) and electron acceptors (4–100 mM) are added as needed.

To stimulate growth, the medium may be supplemented with yeast extract (0.01–0.05%), and peptone or casamino acids to similar or higher levels. The medium is normally buffered with 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) at a final concentration of 10–50 mM before adjusting the pH of the basal medium.

Phosphate Buffer

Component	Amount
KH_2PO_4	30.0 g
K_2HPO_4	66.1 g
Distilled water	800 ml

Adjust to pH 7.0, and bring final volume to 1 liter. Check and adjust pH again, and store at 4°C in a plastic container (or freeze for long-term storage).

Trace Metals Supplement

Component	Amount
$CoSO_4 \cdot 7H_2O$	1.41 g
$Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$	1.98 g
NaCl	0.58 g
Distilled water	100 ml

Autoclave for 20 min and store at 4°C.

Basic Salts Solution

Component	Amount
Sterile MSS (see above)	10 ml
Distilled water	800 ml
$MgSO_4 \cdot 7H_2O$	2.0 g
$CaCl_2 \cdot 2H_2O$	0.57 g
Ethylene diaminetetraacetate (EDTA), disodium salt	0.20 g
$FeSO_4 \cdot 7H_2O$	0.012 g

Filter sterilize and store in glass bottle at 4°C.

Amino Acid Mixture Add 0.2 g each of L-arginine, L-serine and L-glutamic acid to 100 ml of distilled water. Autoclave for 20 min, and store in a sterile glass bottle at 4°C.

Carbon Substrates Carbon substrates are prepared as filter-sterilized solutions of 1 M, with pH adjusted to 7.0. They are stored in glass bottles.

Electron Acceptors Soluble electron acceptors are prepared as stock solutions, sterilized, and stored in glass jars as sterile solutions. Insoluble electron acceptors are prepared as described above, and stored as sterile dried powders.

Applications

Bioremediation of Metal Pollutants

The shewanellae as a group have been considered excellent model systems for the study of bioremediation of toxic metals. Their ability to reduce U(VI) to insoluble U(IV), and Cr(VI) to insoluble Cr(III) have made them prime candidates for use in contaminated systems, where addition of nutrients and/or addition of microorganisms might be utilized for the *in situ* immobilization of toxic elements. Such approaches might be particularly valuable in storage tanks or other locations where high volumes of dilute waste was present. With the advent of the discovery of many other metal-reducing bacteria, it seems almost certain that this approach will be adopted for *in situ* and *ex situ* bioremediation of toxic metal contaminants. The shewanellae are well suited to some applications, being tolerant to oxygen and thus reasonably robust for intro-

duction to polluted environments of different oxygen concentrations. Some strains, but not all, have very limited versatility with regard to electron donor utilization, so success might depend on the choice of strains.

Reduction of other metals, such as the oxidized forms of selenium, arsenic, and technetium (Table 2) have also been demonstrated for some shewanellae, and may offer some yet-to-be-gained insights into the remediation of these compounds. Little has been done, other than to demonstrate these abilities. Sulfide formation has received little attention as a method for remediation of metal contamination, particularly insoluble sulfide formation as a method of removing transition and heavy metals. The shewanellae may offer some interesting variations on this theme via the production of sulfide from thiosulfate, a process that can be regulated by the addition of other electron acceptors.

Bioremediation of Organic Pollutants

With the exception of remediation of some of the methyl halides (Picardel et al., 1993; Petrovskis et al., 1994), organic pollutant remediation by the shewanellae has been little investigated. The potential of shewanellae (as a group) in the anaerobic bioremediation of organic pollutants (at the expense of iron reduction) has been largely ignored because their ability to take up and utilize complex organics is limited. Given the plethora of metal-reducing microbes (including new isolates of shewanellae) now being isolated, and their wide range of organic carbon utilization capability, it is almost certain that this process will be useful. The role(s) of the shewanellae in such processes are simply not known at this time.

Food Spoilage

The shewanellae were first isolated as food spoilage organisms, and remain important today, especially in marine systems where TMAO predominates, and where anaerobic fish spoilage is equated with TMAO reduction. The regulatory system of the shewanellae, and the controls on TMAO-dependent anaerobic respiration, are currently being investigated (Gon et al., 2002) and will have potential strong implications with regard to food spoilage.

Shewanella Strains as Expression Vectors

Ozawa and coworkers have shown that *S. oneidensis* MR-1 is an excellent vehicle for the expression of genes for heme proteins from *Desulfovibrio vulgaris* Miyazaki F (Ozawa et al., 2000), and have followed this report with more details of the expression system (Ozawa et al., 2001). The advantage of the system shown to date is not so much that higher yields are obtained (although the yields are substantially better than in other expression systems), but that the cells can be grown rapidly to high densities under aerobic conditions, then switched to anaerobic conditions, and large amounts of protein produced. For potentially valuable cytochromes, or for proteins needed in high amounts for analysis, such a system offers obvious advantages.

Controversy and Perspectives

Piezophilic Strains

Shewanellae in the groups *S. benthica* and *S. violacea* have been isolated from a number of deep sites and been shown to be not only tolerant of high pressures and low temperatures, but in many cases, to be highly adapted to and dependent upon high pressure. The characteristics of these organisms, referred to as "piezophiles," are reviewed in a recent paper by Kato and Nogi (2001), in which the authors propose that the shewanellae should be broken up into two subgroups, one containing the piezophilic and the other piezotolerant strains. Whether or not this suggestion stands the test of time (and more isolates with new phenotypes), the impact of these strains on our understanding of temperature and pressure as regulatory signals is unquestionable.

Requirement for Surface Attachment for Metal Reduction

One of the most interesting recent developments in understanding this system has been the realization that while the iron and manganese oxides are solids, and surface contact was necessary for reduction (according to initial reports; Myers and Nealson, 1988; Lovley et al., 1989; Caccavo et al., 1990), there are conditions under which this is clearly not the case. Lovley et al. (1996) reported a substantially increased rate of metal reduction after adding "artificial humic substances," e.g., anthraquinone disulfonate, which acts as an electron shuttle, allowing metal reduction without bacterial/mineral contact. Newmann and Kolter (2000) followed this work with observations that shewanellae incapable of metal reduction could be crossfed by wildtype cells, indicating that the shewanellae were producing reductants of their own that allowed extracellular reduction. This led to a model in which extracellular electron shuttles act as reductants (Hernandez and Newmann, 2000), and contrasts with reports indicating that specific proteins are

made by the shewanellae for attachment to metal surfaces. Given that Caccavo (1999) and Das and Caccavo (Das and Caccavo, 2000; Das and Caccavo, 2001) have shown the ability of *S. algae* to attach specifically to various surfaces, and that Lower et al. (2001) have shown physical changes upon contact with solid surfaces, it seems very likely that there will be conditions under which each approach might be favored, and studies with genomic arrays and targeted gene systems should lead to a resolution of this interesting conflict. With regard to some of the proposed applications discussed above, the use of such electron shuttles may offer particularly nice tools with which to manipulate or direct the activities of the shewanellae or other dissimilatory metal-reducing bacteria.

Distribution and Abundance of the Shewanellae

Various *Shewanella* species have been identified from a wide variety of different environments using direct isolation, enrichment cultures, and molecular identification methods. It is thus of some curiosity that a small but directed controversy appears to have arisen around the existence of this bacterial group in environments where metal reduction occurs. This issue relates to the comparative levels of members of the group called "Geobacteriaceae" (containing several genera), which are routinely found either by isolation or molecular methods in many vadose zone and ground water environments. In these environments, the shewanellae are either absent or not abundant, leading to the assertion that the shewanellae are inconsequential organisms in "areas of metal reduction" (Lovley, 2000). Surely this issue will be settled with time, but notably, the shewanellae are clearly abundant in many widespread environments where metal reduction is a major process (with metal reduction rates that may be orders of magnitude higher than those in the vadose zones). Such places include the redox interfaces of the Baltic Sea, the redox interfaces of the Black Sea, marine sediments of the Amazon, Panama and Mississippi deltas, lake sediments, and probably many others. As more is learned about these bacteria, we believe that their ecological role(s) will become clear, as will the reasons for their abundance in environments of very high metal reduction activity.

Genomics

The analysis of the MR-1 genome has already begun to have major effects on our knowledge of this organism, and with the completion of a second genome (*S. violacea* DSS12; K. Nakasone, personal communication), this should continue. The areas that have been impacted by the genomic information fall into two classes: first the recognition of genes and gene types within the genome that can now be specifically mutated and studied, and second the use of expression arrays to study large numbers of genes responding to changes in growth conditions. With regard to the first area, many advances can now be made that previously would have been extremely difficult and time-consuming. This will be an area of great activity, as individual systems are mutated and studied singly and in combination. As for the second area, a number of preliminary studies have already been initiated, and early results suggest that gene arrays can be routinely utilized for MR-1 (Murray et al., 2001; Beliaev et al., 2002a; Beliaev et al., 2002b; Thompson et al., 2002). The work of Murray et al. compares expression of a number of different shewanellae, using an MR-1 array. The results suggest that though some genes are highly conserved, a lot of modification has occurred, to the point that a single shewanella array for environmental work is not a feasible approach. The other reports are the beginning of a large effort to elucidate this organism's global regulation mechanisms, regarded by many individual reports as recent developments. This global approach under various physiological conditions, should lead to some resolution of how this organism interacts with its environment, including other species of bacteria.

Evolution of Metabolism

When MR-1 was isolated, and the range of electron acceptors it can utilize were recognized (Myers and Nealson, 1988), there was some excitement and anticipation that detailed study of the genes and enzymes involved in these processes might lend insights into the sequence of evolution of anaerobic respiration. As the genome of MR-1 is completed, it should be possible to recognize which genes have evolved by duplication and which acquired by horizontal transfer. The ability to extract evolutionary insights from the sequences will almost certainly be enhanced by the comparisons between the MR-1 genome and other bacteria, as well as partial or full genomes of other shewanellae (note again that the complete genome sequence of a second shewanella species, *S. violaceae* strain DSS12 is nearly complete; K. Nakasone, personal communication). This deep-sea strain can grow at low and medium temperatures, and at low and high pressures. Its 16S rRNA sequence widely separates it from strain MR-1 (Fig. 1), and this strain should thus be a good candidate for comparative sequence analyses.

With regard to gene location and transfer, it was noted several years ago that a large megaplasmid (~200 kbp) is present in MR-1, and that genes involved in anaerobic respiration are present on this plasmid (Saffarini et al., 1994). These initial observations have been confirmed with the identification on the plasmid of structural and regulatory genes for respiration (Heidelberg et al., 2002). Thus, the issue of large-scale horizontal transfer of genes involved both in general anaerobic respiration and in metal reduction via such a megaplasmid must be considered. Such a possibility might account for some of the major differences seen in the different ecological groups of the shewanellae.

16S rDNA Taxonomy and Phylogeny

We would be remiss in this article not to mention the difficulties that have arisen with the genus *Shewanella* as more species have been added to the group on the basis of 16S rDNA similarities. As a result, the genus has virtually no unifying phenotypic or ecological features that can accurately define it. Classical microbial taxonomy would not have placed these organisms together, and given the differences in ecophysiology, either they will be separated into two or more genera, or other aspects of their ecophysiology will be elucidated to justify the grouping of these species into a single genus.

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