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THE GENETICS OF GEOCHEMISTRY

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■ **Abstract** Bacteria are remarkable in their metabolic diversity due to their ability to harvest energy from myriad oxidation and reduction reactions. In some cases, their metabolisms involve redox transformations of metal(loid)s, which lead to the precipitation, transformation, or dissolution of minerals. Microorganism/mineral interactions not only affect the geochemistry of modern environments, but may also have contributed to shaping the near-surface environment of the early Earth. For example, bacterial anaerobic respiration of ferric iron or the toxic metalloid arsenic is well known to affect water quality in many parts of the world today, whereas the utilization of ferrous iron as an electron donor in anoxygenic photosynthesis may help explain the origin of Banded Iron Formations, a class of ancient sedimentary deposits. Bacterial genetics holds the key to understanding how these metabolisms work. Once the genes and gene products that catalyze geochemically relevant reactions are understood, as well as the conditions that trigger their expression, we may begin to predict when and to what extent these metabolisms influence modern geochemical cycles, as well as develop a basis for deciphering their origins and how organisms that utilized them may have altered the chemical and physical features of our planet.

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INTRODUCTION

Picking up an article in a typical geology or geochemistry journal, one might stumble across sentences such as the following: "Elementary sedimentary cycles are. . . 'genetic sequences' or 'genetic units'" (135), or, "This paper. . .assesses the genetic relationships between intrusion and geothermal circulation" (140). Although these usages of the word "genetic" at first blush may seem a bit bizarre to a geneticist, contextualized, they convey a more familiar meaning: Rocks or gases have a certain genesis, just as enzymatic activities spring from genes. "Genetic" stemming from the Greek word, *gignesthai*, pertains to the origin of something.

Making a connection between genes and rocks is more than just an issue of etymology, however; the relationship between the activity of particular microorganisms (both present and past) and the chemistry of their environment lies at the heart of the field of geomicrobiology (126). For centuries now, scientists working at this interface have changed our perception of what is possible for life and the significance of life for geology (6, 48, 84, 191). Virtually every month new discoveries are made about the occurrence of life in extreme environments, ranging from the freezing to the boiling point (80, 139). Microbial metabolisms are now known to span those that derive power from the sun to those that are driven by geochemical reactions occurring deep within the surface of the Earth (24, 47). While the interactions between life and the Earth are highly complex, it is by now axiomatic that the emergence of critical forms of life profoundly and irreversibly changed the chemistry of the planet (e.g., cyanobacterial producion of O_2). Despite this recognition, we rarely understand microbial geochemical activities today well enough to be able to quantify, predict, or control them. Moreover, little is understood about microbially mediated chemical changes that occurred on the early Earth.

The subject of this review is the genetics of geochemistry. By this we mean the science of applying classical principles of bacterial genetics to understanding geochemically relevant microbial metabolisms. Be it in a modern or ancient setting, unless we know what to look for, it is impossible to determine whether a specific microbial activity is having or had an impact on a particular environment. The reductionist power of genetics provides a means to determine how geochemically significant metabolisms work (e.g., identify specific molecules that catalyze a reaction of interest) and how they are regulated (e.g., the environmental conditions that trigger their expression), which ultimately may allow us to detect and quantify their activity today and/or identify traces of their activity in the ancient rock record. In addition, to the extent that DNA itself is a "fossil" and that life and the Earth have coevolved, identification of genes that are linked to specific biogeochemical processes may provide us with a window into the evolution of metabolism.

The number of microbial metabolisms that affect environmental geochemistry is enormous, and includes those that change the composition of the hydro-, atmo-, and lithospheres. A few examples of where genetics has been used to explore geochemically relevant microbial metabolisms include studies of methanogenesis (88, 98, 108, 180), sulfate-reduction (16), manganese oxidation (20, 174), nitrogen-cycling (105), radionuclide processing (38, 93), and phosphorus cycling (106). In the interests of space, however, we limit this review to metabolisms that alter the redox state of iron (Fe) or arsenic (As) (e.g., photosynthetic and respiratory processes). We chose these metabolisms for several reasons: (*a*) Fe and As biogeochemical cycles are relatively well understood and related to one another (Figure 1); (*b*) the reactions catalyzed by key microbial players in these cycles are known (Table 1); (*c*) representative organisms that are involved in these cycles are already in culture (Figure 2); and (*d*) the genetic tractability of these organisms varies, allowing us to illustrate the challenges of doing genetics in recently discovered environmental isolates.

Our discussion begins with ferrous iron [Fe(II)] oxidation, about which the least is known at the genetic level. Next we consider ferric iron [Fe(III)] reduction, for which several genetically tractable model systems exist and a wealth of genetic information is accumulating. We end by reviewing what is known about As oxidation and reduction, and provide an example of how genetic information can be taken back to the environment to determine whether a particular metabolic process is shaping its geochemistry.

FE(II) OXIDATION

Environmental and Geological Significance

Microbial Fe(II) oxidation is an important component of the Fe geochemical cycle. In modern environments, microorganisms that are able to oxidize Fe(II) are ubiquitous, inhabiting and affecting a wide variety of environments where Fe(II) is present. These environments include marine coastal sediments and brackish water lagoons (171, 173); sediments from freshwater creeks, ponds, lakes, and ditches (65, 68, 69, 188); low-pH environments associated with acid mine waters (30, 45); groundwater springs (52); sediments, and the rhizosphere of plants from freshwater wetlands (54, 167); the seafloor near active hydrothermal fields (46, 53); and swine-waste lagoons (25).

Microorganisms that oxidize Fe(II) are diverse in their phylogeny and overall physiology (Table 1). Representative examples of bacteria and archaea capable of coupling Fe(II) oxidation to growth include psychro-, hyperthermo-, and mesophiles that couple Fe(II) oxidation to the reduction of nitrate at neutral pH (13, 172), or to the reduction of oxygen at either low (45, 175), or neutral pH (52), and the anaerobic Fe(II)-oxidizing phototrophs (47, 69, 188) (Table 1). For a complete list of specific organisms able to oxidize Fe(II) enzymatically and more

TABLE 1 Reactions catalyzed by Fe and As-metabolizing microorganisms

Metabolism	Reaction	Genes
Acidophilic iron oxidation	$4Fe^{2+} + 4H^+ + O_2 \rightarrow 4Fe^{3+} + 2H_2O$	*iro*, *cyc1*, *cyc2*, *coxA*,*B*,*C*,*D*, *rus*
Phototrophic iron oxidation	$4Fe^{2+} + HCO_3^- + 10H_2O \rightarrow 4Fe(OH)_3 + (CH_2O) + 7H^+$	None known
Neutrophilic iron oxidation	$4Fe^{2+} + 10H_2O + O_2 \rightarrow 4Fe(OH)_3 + 8H^+$	None known
Nitrate-dependent iron oxidation	$10Fe^{2+} + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18H^+$	None known
Lactate-mediated iron reduction	$CH_3CHOHCOO^- + 4Fe(OH)_{3(am)} + 7H^+ \rightarrow CH_3COO^- + 4Fe^{2+}\ 10H_2O + HCO_3^-$	*men*, *cymA*, *cctA*, *mtrA*, *mtrB*, *omcB*, *omcA*, *gsp*
Acetate-mediated iron reduction	$CH_3COO^- + 8Fe(OH)_{3(am)} + 15H^+ \rightarrow 2HCO_3^- + 8Fe^{2+}\ 20H_2O$	*omcB*, PPCA
Lactate-mediated arsenate reduction	$CH_3CHOHCOO^- + 2HAsO_4^{2-} + 3H^+ \rightarrow CH_3COO^- + 2HAsO_2 + 2H_2O + HCO_3^-$	*arrA*, *arrB*
Acetate-mediated arsenate reduction	$CH_3COO^- + 4HAsO_4^{2-} + 7H^+ \rightarrow 2HCO_3^- + 4HAsO_2 + 4H_2O$	*arrA*, *arrB*
Nitrate-dependent arsenite oxidation	$HAsO_2 + NO_3^- + H_2O \rightarrow HAsO_4^{2-} + NO_2^- + 2H^+$	None known
Oxygen-dependent arsenite oxidation	$2HAsO_2 + 2H_2O + O_2 \rightarrow 2HAsO_4^{2-} + 4H^+$	*aroA*, *aroB*

details on these organisms, we refer the reader to a number of extensive reviews (49, 58, 121, 170).

In addition to contributing to Fe cycling in modern environments, Fe(II)-oxidizing bacteria may have affected the Fe cycle over geological time. For example, both direct photoautotrophic Fe(II) oxidation and indirect Fe(II) oxidation mediated by cyanobacteria (27) have been proposed as possibly being responsible for the deposition of Banded Iron Formations, a class of ancient sedimentary iron ore deposits (63, 85, 188). However, in distinguishing these two biological processes from each other in the rock record, as well as from other proposed abiotic mechanisms of Fe(II) oxidation, biological signatures that uniquely represent the activity of Fe(II)-oxidizing organisms must be identified. A first step toward this is understanding the molecular mechanisms of Fe(II) oxidation by extant relatives of ancient Fe(II)-oxidizing bacteria. The use of Fe(II) as an electron donor in anoxygenic photosynthesis likely arose early in Earth history (17, 36, 197); therefore, understanding the mechanisms of Fe(II) oxidation by anoxygenic photoautotrophic bacteria is of particular interest.

Mechanisms of Fe(II) Oxidation

OVERVIEW The genetics of Fe(II)-oxidizing bacteria is primitive compared with other bacterial species. This is largely due to the challenges inherent in growing these organisms. For example, aerobic neutrophilic Fe(II)-oxidizers must outcompete the rate of abiotic oxidation of Fe(II) by molecular oxygen (O_2) to harvest energy for growth. The requirement of specific O_2 and Fe(II) concentrations for these bacteria is met by growing them in tubes of solid medium with opposing gradients of Fe(II) and O_2 (52). Such culturing requirements are not easily amenable to large-scale genetic screens. Additionally, many Fe(II)-oxidizing organisms have been discovered only in the past ~10 or less years. Most of what we know about Fe(II) oxidation at a genetic level comes from studies of the acidophilic Fe(II)-oxidizing organism *Acidithiobacillus ferrooxidans*. Recently, our laboratory has begun to make progress in using genetic approaches to elucidate the molecular mechanism of phototrophic Fe(II) oxidation. Our success derives from the fact that some isolated Fe(II)-oxidizing phototrophs are members of the purple nonsulfur group of bacteria, for which genetic systems exist in non-Fe(II)-oxidizing species such as *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, and *Rhodopseudomonas palustris* (42, 134). The remainder of this section focuses on what has been learned about Fe(II) oxidation from genetic analysis of *A. ferrooxidans* and on the genetic approaches to study Fe(II) oxidation by *Rhodobacter* and *Rhodopseudomonas* species.

ACIDITHIOBACILLUS FERROOXIDANS *A. ferrooxidans* is a gram-negative, mesophillic, obligately autotrophic and acidophilic bacterium capable of aerobic respiration on Fe(II) and reduced forms of sulfur (H_2S, S^o, $S_2O_3^{2-}$) (49, 143). Because it can grow chemolithoautotrophically on sulfide ores, this bacterium solubilizes

a variety of valuable metals such as copper, uranium, cobalt, and gold that are embedded within the ores (142). Given this property, understanding its metabolism is particularly interesting to industries wishing to use this strain (or genetically modified derivatives) for leaching purposes (4, 141, 192).

Most of what is known about the mechanism of Fe(II) oxidation by *A. ferrooxidans* stems from biochemical studies, yet how the different components of the electron transport pathway (Figure 3*A*) work together is uncertain and controversial (4, 15, 76, 199). Comparatively little is known about the genetics of Fe(II) oxidation in *A. ferrooxidans* because genetic analysis has been constrained by the culturing requirements for this organism. For example, a number of antibiotics are inactive at low-pH and high-Fe(II) concentrations (192), resulting in a dearth of suitable selective markers. To circumvent this problem, toxic metal resistance genes have been used as selective markers, but only with limited success (86). Additionally, while some of the standard tools of genetics (e.g., appropriate shuttle vectors and transformation methods) have been developed and/or optimized for various strains of *A. ferrooxidans* (86, 136, 142), until recently (92), these methods have not been used for the construction of mutants. Consequently, no defined mutants defective in Fe(II) oxidation exist, although spontaneous mutants that have lost the ability to oxidize Fe(II) have been identified (158). The recent report of the construction of a *recA* mutant of *A. ferrooxidans* strain ATCC 33020 via marker exchange mutagenesis represents a step toward improved genetic analysis of this strain (92).

Nevertheless, several genes thought to be involved in Fe(II) oxidation are known. The majority have been identified using degenerate primers derived from N-terminal sequences of purified proteins. The first gene to be identified using reverse genetics was the *iro* gene of *A. ferrooxidans* strain Fe1. This gene encodes a high-potential Fe-S protein that is homologous to the soluble ferredoxins commonly found in purple photosynthetic bacteria. Northern blot and RNA primer extension analyses suggest that this gene is transcribed on its own, but expression studies under different growth conditions have not yet been conducted (87). Because of its high redox potential, Fe(II)-cytochrome *c*-552 oxidoreductase activity and acid stability in vitro, it has been proposed that the product of this gene catalyzes the first step in the transfer of electrons from Fe(II) to O_2 (57, 87, 200). However, arguments have been made against this claim (4), and genetic evidence to support this function does not exist.

The next gene thought to encode a protein involved in Fe(II) oxidation by *A. ferrooxidans* is the *rus* gene. Again, using reverse genetics, the *rus* gene was cloned from *A. ferrooxidans* ATCC 33020 (12, 61). This gene encodes the small, type 1 blue copper protein, rusticyanin, a protein that has received much attention in biochemical studies given that it represents up to 5% of the total soluble protein of *A. ferrooxidans* cells when grown on Fe(II) and also displays a high degree of acid tolerance and a high redox potential (34, 75). In the region upstream of the *rus* gene, a sequence similar to a rho-independent terminator and two potential *Escherichia coli*-like σ^{70}-specific promoter sequences are present. Downstream

of the gene are two putative stem-loop structures, one of which is followed by a T-rich region. This suggests that the *rus* gene can be transcribed from its own promoter (12). Further investigations of *rus* gene transcription by Northern, RT-PCR, and primer extension analyses have shown that this gene is part of an operon comprising eight genes, of which *rus* is the last (4, 4a, 12). Putative promoters in this operon have been identified both by sequence and primer extension analyses. Primer analysis with RNA extracted from cells grown on sulfur or Fe(II) indicates that two promoters upstream of *cyc2* and one promoter upstream of *rus* are active in cells grown on sulfur, whereas only one of the promoters upstream of *cyc2* is active in Fe(II)-grown cells (4). Additionally, it has been observed that while the *rus* transcript is present in both Fe(II)- and sulfur-grown cells, it is more abundant in Fe(II)-grown cells and present throughout all growth phases (in contrast to sulfur-grown cells, where it appears only in exponential phase) (202).

Since the discovery that *rus* is cotranscribed with several other genes in an operon (Figure 4*A*), the other genes in this operon have been analyzed (3, 4, 201). Strikingly, seven of the eight genes seem to encode redox proteins. The *cyc2* gene encodes a high-molecular-weight outer-membrane *c*-type cytochrome (3, 201), whereas *cyc1* encodes a *c*4-type cytochrome with a signal peptide sequence indicative of translocation to the periplasm (3). *coxB*, *coxA*, and *coxC* encode proteins with homology to subunits II, I, and III, respectively, of an

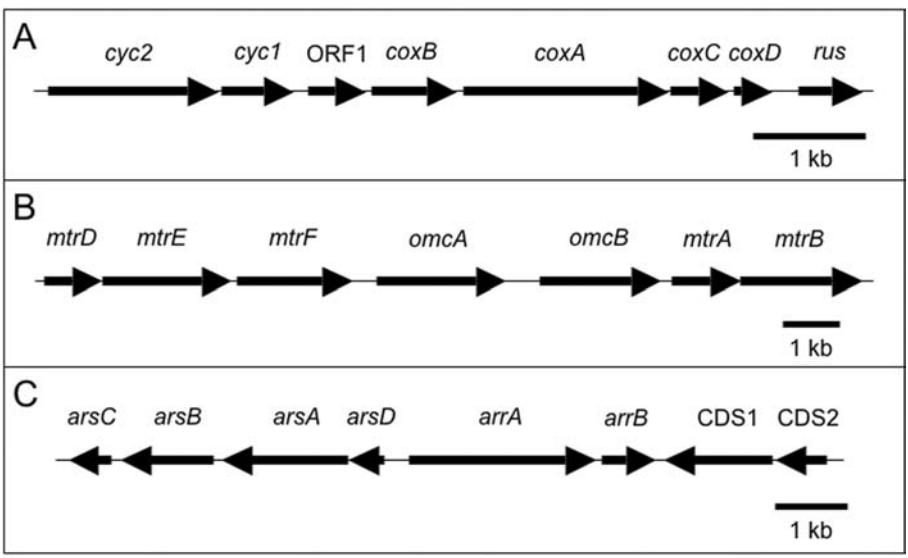

Figure 4 Representative gene clusters involved in metal(loid) transformations. (*A*) Putative Fe(II)-oxidation genes from *Acidithiobacillus ferrooxidans* strain ATCC 33020. (*B*) Fe(III) reduction genes from *Shewanella oneidensis* strain MR-1. (*C*) As(V) reduction genes from *Shewanella* species strain ANA-3.

*aa*3-type cytochrome *c* oxidase. The protein encoded by *coxD* shares similarity with nothing in the database; however, given the position of this gene relative to the other *cox* gene homologues and what is known about the organization of these genes in other organisms, it was assumed that *coxD* represents subunit IV of an *aa*3-type cytochrome *c* oxidase (4). Lastly, ORF1 encodes a putative protein of unknown function with a terminal signal sequence, suggesting that it is translocated to the periplasm. Given that biochemical studies have implicated proteins of these types in Fe(II) oxidation (14, 29, 34, 57, 75, 182, 186), it is assumed that they are involved in the electron transport pathway (Figure 3*A*) (4). This requires confirmation through the construction and analysis of mutants using the newly described method (92). Moreover, additional components of the Fe(II) oxidization pathway remain unknown, including all the components involved in the regulation of these genes. The ability to perform a large-scale screen is thus a goal for future genetic analysis of *A. ferrooxidans*.

Genetics in Fe(II)-Oxidizing Phototrophs

Recently, we have attempted to identify the genes required for phototrophic Fe(II) oxidation. Our approach has varied depending on the growth characteristics of the Fe(II)-oxidizing strain. One of the best-studied Fe(II)-oxidizing phototrophs is *Rhodobacter* strain SW2 (47). Unfortunately, it does not readily form colonies on plates, which means that generating and screening for mutants that lose the function of Fe(II) oxidation is impractical. Accordingly, we have taken a gain-of-function approach that exploits the close relatedness of strain SW2 to *Rhodobacter capsulatus* (36, 47). In particular, we use strain SB1003, which does not oxidize Fe(II) but is genetically tractable. To provide strain SB1003 with genes from SW2 so that it gains the ability to oxidize Fe(II), we constructed a genomic cosmid library of strain SW2. By complementing a tryptophan auxotroph of *Rhodobacter sphaeroides* [strain CM06, (99)], we first demonstrated that genes from the SW2 library could be expressed successfully in other *Rhodobacter* species, and have recently identified four putative clones with genes required for Fe(II) oxidation (L.R. Croal, Y. Jiao & D.K. Newman, unpublished).

Although heterologous expression appears to be successful for strains that do not form colonies on plates, ultimately, the construction and analysis of gene deletion or disruption mutants will be required to demonstrate that a particular gene is involved in this metabolism. To that end, we have recently enriched and purified a new Fe(II)-oxidizing photoautotroph from School Street Marsh in Woods Hole, Massachusetts. This strain, *Rhodopseudomonas* strain TIE1, forms colonies aerobically on plates, and can be randomly mutagenized by a hyperactive mariner transposon (26). A screen of 12,000 random transposon mutants has just been completed, and candidate mutants are currently being retested and sequenced (Y. Jiao, L.R. Croal, A. Kappler & D.K. Newman, unpublished).

Once these genes are identified, we will be able to address whether the Fe(II)-oxidation system is similar to that in other Fe(II)-oxidizing bacteria such as *A. ferrooxidans* and begin to uncover the degree to which electron transfer from

Fe(II) is conserved among phylogenetically divergent species. In addition, knowing which genes are involved in the pathway will help us address where in the cell Fe(II) is oxidized, and whether Fe(III) chelators are produced to prevent encrustation by poorly crystalline Fe(III)-(hydr)oxides [$Fe(OH)_3$], the products of this metabolism. This information will aid interpretation of Fe-isotopic fractionation by these organisms, which is relevant to identifying traces of Fe(II) oxidation in the rock record (36, 79).

DISSIMILATORY FE(III) REDUCTION

Environmental and Geological Significance

Although many bacteria reduce Fe(III) for the purpose of assimilation, here we consider only those that engage in dissimilatory Fe(III) reduction: the process of using Fe(III) as a terminal electron acceptor in anaerobic respiration. The environmental context and physiology of dissimilatory Fe(III) reduction have been discussed in detail elsewhere (see 96, 122, 123, 170). Fe(III)-reducing bacteria inhabit both freshwater and marine environments and significantly affect the biogeochemical cycling of carbon, hydrogen, and a variety of trace metal(loid)s. Moreover, Fe(III)-reducing bacteria are useful for bioremediation (e.g., oxidizing toxic organic compounds to CO_2 and/or reductively immobilizing radionuclides in groundwaters) as well as generating power from marine sediments (2, 18, 72, 95). In addition to their importance today, it seems likely that Fe-reducers, like Fe-oxidizers, influenced the geochemical cycling of Fe on the ancient Earth [e.g., helping to define the mineralogy of Banded Iron Formations (79)].

The dissimilatory Fe(III)-reducing organisms most studied are mesophiles that grow at circumneutral pH and belong to the *Shewanella* and *Geobacter* genera, although hyperthermophiles and even extreme hyperthermophiles are also capable of Fe(III) reduction (80, 183). *Geobacter* species couple the oxidation of acetate to CO_2 with the reduction of Fe(III) to Fe(II) (97), whereas *Shewanella* species cannot utilize acetate as a sole source of carbon and energy, and are more often grown on lactate (Table 1). *Geobacter* species are more versatile with respect to carbon source utilization, whereas *Shewanella* species are more versatile with respect to terminal electron acceptors. Both *Geobacter* and *Shewanella* species have been identified in diverse locales around the world (28, 184), with *Geobacter* species often dominating in sedimentary environments (145, 166). Recently, sequences derived from the Sargasso Sea indicated that *Shewanella* species are present in high abundance in this region (185).

Genetics in *Shewanella* and *Geobacter*

S. oneidensis [formerly *S. putrefaciens* (184)] strain MR-1 is the strain most commonly used for genetic studies, though other strains have also been used [e.g., *Shewanella algae* BrY (23), *Shewanella frigidimarina* (138), and *Shewanella putrefaciens* 200 (21, 40, 41)]. A major advantage to performing genetics in *S. oneidensis*

is its rapid generation time when grown aerobically in rich medium. Targeted mutagenic techniques have only recently been developed for *G. sulfurreducens* (33, 89, 94), so less is known about which of its genes are involved in Fe(III) respiration. The complete genome sequences are available for both *G. sulfurreducens* (107) and *S. oneidensis* (67), facilitating rapid identification of transposon mutants, construction of targeted gene knockouts, and the ability to use PCR to clone genes for complementation. The genome sequences also permit comparisons between the genes involved in Fe(III) reduction in *Shewanella* and *Geobacter*, and the use of microarray technology to study whole-genome expression (10, 11, 176). Currently, neither *S. oneidensis* nor *G. sulfurreducens* has a generalized transducing phage that can be used for suppressor analysis and strain construction. Both lambda and Mu-like phages have been identified in the genome sequence of *S. oneidensis* (67), which could facilitate the further development of genetics in this organism.

Mechanisms of Mineral Reduction

OVERVIEW At near-neutral pH, Fe(III) exists in the form of very poorly soluble minerals [e.g., $Fe(OH)_3$], thus Fe(III)-reducing bacteria face the challenge of delivering electrons to an external and solid electron acceptor. Different Fe(III)-reducing bacteria appear to take one of three strategies to accomplish this task: (*a*) They can attach to and transfer electrons to the mineral presumably via proteins present in the outer membrane (122); (*b*) they can use/release Fe(III) chelators to bring Fe into the cell where it can then be reduced (124); or (*c*) they can use/release extracellular electron shuttles to transfer electrons back and forth between the cell and the mineral (71). The last two strategies enable bacteria to reduce Fe(III) at a distance, whereas the first requires direct contact between the cell and the mineral; it has been suggested that the latter are most relevant for biofilm communities (M.E. Hernandez, D.P. Lies, L.A. Kappler, R.E. Mielke & D.K. Newman, manuscript in preparation; 71, 124). Currently, very little is understood about Fe reduction at a distance: We do not yet know the structure of the molecule(s) involved, how and where in the cell they are reduced, which genes control their biosynthesis, nor how these genes are regulated. Accordingly, we focus this review on what is known about the "direct-contact" pathway. Because historically more genetic work has been done with *Shewanella* than with *Geobacter*, we focus our discussion on what we know about the electron transfer pathway in *Shewanella*, making a brief comparison to *Geobacter* at the end of this section. We first present what is known about the regulation of the electron transfer components, proceed to elaborate on their specific roles, and briefly discuss what is known about their maturation.

SPECIFIC GENES

Regulation Two mutant strains isolated as being deficient in anaerobic respiration had mutations in the *crp* gene, encoding the cyclic AMP receptor protein (CRP) (148). These mutants could still use trimethylamine N-oxide as an electron acceptor, but had significant defects on all other anaerobic electron acceptors tested,

including Fe(III)-citrate and manganese oxide minerals (e.g., MnO_2). The addition of cyclic AMP to aerobic cultures of *S. oneidensis* resulted in a modest increase in soluble Fe(III) reductase activity, tripling the activity of aerobic cultures alone, but only to about half of the activity of anaerobic cultures (148). Regulation of genes known to be involved in mineral reduction was not addressed. In addition to CRP, other unidentified components may play a role in regulating the metal-reduction pathway(s) in *S. oneidensis*. Anoxic conditions appear to trigger the induction of genes required for Fe(III) reduction (111), and CRP may help modulate expression (148), but the traditional system used by *E. coli* and *Salmonella enterica* to regulate anaerobic respiration [FNR, reviewed in (82)] does not appear to be required by *Shewanella* for mineral respiration (101).

Menaquinone requirement As for most respiratory chains, the pathway of electron transfer begins with standard dehydrogenases and quinones, and branches to more specific electron carriers that convey electrons to Fe(III) (Figure 3*B*). The pool of quinones consists of two main types, ubiquinones and menaquinones (MQ). Defects in MQ biosynthesis nearly eliminate the ability of *S. oneidensis* to respire Fe(III) and Mn(IV) minerals (112, 127, 147). Myers & Myers showed that defects in reduction of dissolved Fe(III) associated with the MQ-deficient strain CMA-1 were reversed by supplementation with MQ or an intermediate in the MQ biosynthetic pathway, 1,4-dihydroxy-2-napthoate (112). Mutations in *menC* (encoding O-succinylbenzoate-CoA synthase) were isolated in a screen for mutants unable to reduce anthraquinone-2,6-disulfonate (AQDS), a redox-active analog for quinone moieties in humic substances (127). As expected, this phenotype was reversed when mutants were supplemented with MQ or 1,4-dihydroxy-2-napthoate. Additionally, a defect in $Fe(OH)_3$ reduction in a *menC* mutant strain was described, although the data were not shown (127). Mutations in *menB* and *menD* have also been characterized with respect to their metal reduction defects (147). Membrane fractions derived from these mutant strains are deficient in Fe(III)-citrate reduction, which could be reversed by the addition of MQ (147).

CymA The likely role of CymA in metal reduction (and in anaerobic respiration in general) is to pass electrons from the MQ pool to periplasmic electron carriers (160). The *cymA* gene encodes an inner-membrane tetraheme cytochrome and is postulated to have quinol dehydrogenase activity (55, 113, 159, 160). Initial reports of a defect associated with lesions in *cymA* (113) were complicated by the realization that MQ pools had been severely decreased, apparently resulting from a rifampicin resistance mutation present in the genetic background of the parent strain (118). Mutations in *cymA* result in defects in anaerobic respiration of Fe(III)-citrate, MnO_2, nitrate, fumarate (118), dimethyl sulfoxide (DMSO), and nitrite (160).

CctA A small tetraheme *c*-type cytochrome was purified from the periplasm of *S. frigidimarina* NCIMB400, characterized biochemically, and a mutant defective

in the gene encoding the protein (*cctA*) was generated (59). This mutant strain had decreased Fe(III)-citrate reductase activity and was unable to use dissolved Fe(III) as an electron acceptor for growth (59). A homolog of CctA has been identified, purified, and the structure solved from *S. oneidensis* (90, 178, 179). However, a role for *cctA* (SO2727) in Fe(III) reduction in *S. oneidensis* has not yet been established through mutagenesis. It has been suggested that CctA passes electrons from CymA to the periplasmic decaheme cytochrome MtrA, based on the dissolved Fe(III) reduction phenotypes of strains lacking these proteins (9) (Figure 3*B*).

MtrA A targeted gene disruption was generated in the *mtrA* gene based on its proximity to *mtrB* (Figure 4*B*), and resulted in a defect in Fe(III)-citrate reduction (8). The gene product was predicted (8) and shown biochemically (138) to be a soluble decaheme *c*-type cytochrome. Expression of this protein alone in *E. coli* (with additional cytochrome maturation factors) enabled the strain to reduce dissolved forms of Fe(III) (138), indicating that MtrA can receive electrons from a variety of electron donors. Although MtrA reduces dissolved Fe(III) biochemically, it may also have a role in passing electrons to outer-membrane proteins that reduce $Fe(OH)_3$ (138).

MtrB The first gene isolated as being involved in metal reduction was *mtrB* (8). Mutations in this gene significantly decrease the capacity of *S. oneidensis* to reduce Fe(III)-citrate, $Fe(OH)_3$, and MnO_2 (8, 44, 115). Shyu et al. (163) demonstrated that *mtrB* mutants are also defective in reducing AQDS. Although several interesting defects have been attributed to *mtrB* mutants, the function of MtrB is unknown. Motif analysis of the protein sequence suggests that it is localized to the outer membrane and that it contains a transmembrane domain near the C terminus (8, 115, 116). A CxxC motif has been postulated as a putative metal-binding domain (8), although this hypothesis has yet to be tested. Protein alignments of MtrB sequences from *S. oneidensis*, *S. frigidimarina*, and environmental sequences from the Sargasso Sea (185) indicate that the CxxC motif is 100% conserved, suggesting that these residues may be important for the function of the protein. Outer-membrane *c*-type cytochromes appear to be mislocalized in the *mtrB* mutant background, including OmcA and OmcB. This implies that MtrB may have a role in scaffolding or maintenance of a terminal reductase complex (115) (Figure 3*B*).

OmcB (MtrC) In the first reports detailing the disruption of this gene, one group referred to the locus as *mtrC* [for metal reduction, (9)] and another group referred to it as *omcB* [for outer-membrane cytochrome, (119)]. Beliaev et al. named the gene based on its proximity to, and its potential inclusion in, an operon with *mtrA* and *mtrB* (9) (Figure 4*B*). Myers & Myers argued that the predicted cellular location and heme content of the protein encoded by this locus merited the name *omcB* (119). For purposes of continuity, this locus should subsequently be referred to as *omcB*, as to this is the annotated name in the genome of *S. oneidensis* (http://www.tigr.org). OmcB was predicted (9, 119) and shown to be present on

the outer membrane of *S. oneidensis* (116). Computational analysis of the primary amino acid sequence suggests that OmcB is a decaheme *c*-type cytochrome, and therefore has the potential to be directly involved in electron transfer reactions to $Fe(OH)_3$. Mutations in *omcB* have been isolated by both random mutagenesis (9) and by directed gene knockout (119). These mutants are defective in both $Fe(OH)_3$ (9, 120) and MnO_2 (9, 119) reduction. Additionally, *omcB* mutants have a slower rate in reducing Fe(III)-citrate, compared with wild type (9, 120). It was also shown that *omcB* null strains contain ~15% of the OM cytochrome content compared with MR-1 (119). One possible explanation for this phenotype is that OmcB forms an anchoring unit for additional OM cytochromes, possibly a mineral reductase complex, as has been suggested for MtrB (115).

OmcA An 83-kDa outer-membrane *c*-type cytochrome was purified and N-terminal sequence was used to identify *omcA* (114, 117). Sequence analysis suggests that the protein encoded by *omcA* contains 10 heme-binding domains. Although OmcA and OmcB are both decaheme cytochromes, the proteins themselves do not appear to be related (based on BLAST). Analysis of the *omcA* transcript through Northern blotting suggests that this gene is monocistronic and that insertional inactivation will not have polar effects on downstream genes (117) (Figure 4*B*). A mutation in *omcA* was isolated by directed mutagenesis and found to cause a defect in MnO_2 reduction (119). Metal reduction phenotypes associated with *omcB* mutants appear to be stronger than *omcA* mutants, suggesting that both outer-membrane cytochromes contribute to mineral reduction (119).

Type II secretion A lesion preventing Fe(III) and Mn(IV) reduction was mapped by complementation to a gene involved in type II secretion in *S. putrefaciens* strain 200 (41). DiChristina et al. named this locus *ferE* (for Fe reduction); its homologue in *S. oneidensis* is annotated as *gspE* (for general secretory pathway). The *fer/gsp* genes encode components of the type II protein secretory pathway that is used to move mature proteins from the periplasm to the outside of the cell (reviewed in 154). A *c*-type cytochrome with Fe(III)-citrate reductase activity is mislocalized from the outer membrane to the cytoplasmic membrane in *ferE* (*gspE*) mutants (41).

Comparisons to Geobacter Although little is currently known about the genes required for dissimilatory iron reduction in *Geobacter* species, a recently developed single-step gene disruption technique is now being used for targeted mutagenesis (33). Two genes, *ppcA* and *omcB*, have been shown to be required for Fe(III) reduction. *ppcA* encodes a periplasmic 9.6-kDa *c*-type cytochrome (94) and *omcB* encodes a putative outer-membrane multiheme *c*-type cytochrome (89). OmcB from *S. oneidensis* does not appear to be related to OmcB from *G. sulfurreducens* (89). This suggests that these organisms use different enzymes, and perhaps altogether different mechanisms, to pass electrons to $Fe(OH)_3$ and MnO_2 (89, 124). The recent discovery that *G. sulfurreducens* is not a strict anaerobe (91) may facilitate large-scale random mutagenic analysis of this organism in the future.

ARSENIC TRANSFORMATIONS

Environmental and Geological Significance

Arsenic (As) is best known as a poison, but has also been used in metallurgy, wood preservation, painting, medicine, pest control, and as an additive to chicken feed, where it increases growth (130, 165). Although anthropogenic sources contribute to some incidents of As contamination (169), volcanic action, low-temperature volatilization, and natural weathering (5, 189) of As-containing minerals are the greatest sources of As in the environment. As can exist in the form of arsine gas, arsenosugars, or other organoarsenicals, such as arsenobetaine, but the major species present in natural waters are arsenate [$HAsO_4^{2-}$; As(V)] and arsenite [H_3AsO_3; As(III)] (56). These two oxyanions readily interconvert, and their different chemical properties determine whether they are sequestered in solid form or mobilized into the aqueous phase (127).

Though As is only the twentieth most abundant element in the Earth's crust, being present at about 1.8 ppm (83), its toxicity is problematic in many parts of the world. The World Health Organization recommends As guideline values of 10 μg/L (193), but As concentrations in water supplies in Argentina, Chile, Canada, Bangladesh, Cambodia, Vietnam, West Bengal, and the United States often exceed this limit. In Bangladesh alone, where As concentrations reach as high as 1000 μg/L (129), as many as 50–60 million people are exposed to As-contaminated drinking water, and thousands of cases of arsenicosis are diagnosed each year. Microbial metabolisms that convert As between As(V) and As(III) contribute in large part to As speciation in the environment (37, 125, 133). Because both As(V) and As(III) adsorb onto $Fe(OH)_3$, the fate of As is often linked to that of Fe (Figure 1). In sedimentary environments that are not dominated by Fe, however, As(III) is generally more mobile than As(V); thus, understanding what stimulates As(III) oxidation and/or limits As(V) reduction is relevant for bioremediation of contaminated sites (64).

Microorganisms that transform As between As(V) and As(III) are diverse in their phylogeny and overall physiology (Table 1). By far the most genetic work on As(V) reduction comes from studies of the As detoxification systems in *Staphylococcus aureus* and *E. coli* (19, 39, 66, 109, 164, 196, 198). These systems do not generate energy. Microorganisms that utilize As for energy fall into two classes: the chemolithoautotrophic As(III) oxidizers and the heterotrophic As(V) reducers. Chemolithoautotrophs gain energy from coupling the oxidation of As(III) to the reduction of oxygen (74, 155) or nitrate (132, 161). They include members of the *Alcaligenes, Pseudomonas*, and *Thermus* genera. Not all As(III)-oxidizing bacteria conserve energy from As(III) oxidation, however; several As(III)-oxidizing heterotrophs do not appear to utilize As(III) as an electron donor for respiration, suggesting that As(III) oxidation may be incidental or a form of detoxification in these strains (1, 51, 149). A diverse group of heterotrophic bacteria can utilize As(V) as a terminal electron acceptor for respiration. These organisms comprise

members of the gamma-, delta-, epsilon-*Proteobacteria*, gram-positive bacteria, thermophilic Eubacteria, and *Crenarchaeota* (133). Most As(V)-respiring strains couple the oxidation of lactate to acetate to support As(V) reduction to As(III) (Table 1), although some isolates can mineralize acetate to CO_2 (100) and/or use H_2 as an electron donor (73). Although most known As(V) respirers are obligate anaerobes, a few are facultative aerobes, and one, *Shewanella* species strain ANA-3, has been particularly useful in genetic studies. For more details about As transforming organisms, we refer the reader to a number of extensive reviews (50, 125, 133, 165).

Mechanisms of As(V) Reduction and As(III) Oxidation

DETOXIFICATION: THE *ARS* GENES As(V) enters Gram-negative cells through nonselective porins in the outer membrane. Because arsenate is an analog of phosphate, it is thought to enter into the cytoplasm mainly through the Pit phosphate transport system, a low-affinity/high-velocity system (164). *E. coli* that rely on the Pit system cannot grow in the presence of 10-mM arsenate, whereas *pit* mutants that rely on the Pst phosphate transport system—which has a higher affinity for phosphate than the Pit system—can grow (190). Once As(V) enters the cells, it uncouples oxidative phosphorylation and inhibits ATP synthesis. In contrast, As(III) appears to enter the cell through aquaglyceroporins (146), one of which, GlpF, was identified in a screen for genes responsible for antimonite [Sb(III)] sensitivity (153). As(III) is much more toxic than As(V) owing to its strong affinity for sulfhydryl groups in proteins (133). Paradoxically, although As(III) is more toxic than As(V), As(V) reduction to As(III) may provide cells with an advantage in exporting As (165).

The best-studied As detoxification system involves the *ars* operon from *E. coli* plasmid R773 (66). This operon encodes five genes, *arsRDABC*, that are cotranscribed from one promoter region. *arsR* and *arsD* encode two regulatory elements. *arsR* encodes a helix-turn-helix repressor that binds the operator region of the *ars* operon as a dimer until As(III) or Sb(III) binds to it and induces its release (195, 198). *arsD* is believed to be a *trans*-acting inducer-independent secondary regulator (196). It is expressed in the presence of As(III), and has no effect on the expression of the operon when ArsR is repressing the system (196). *arsA* encodes a membrane-associated ATPase subunit that interacts with the *arsB* gene product (81, 177). ArsB is the efflux pump responsible for the extrusion of As(III) and Sb(III). In association with ArsA, it uses the energy from ATP hydrolysis for this function; however, when ArsA is not present, ArsB is still functional (39). *arsC* encodes a small cytoplasmic arsenate reductase.

Not every *ars* operon contains these five genes, however. Most of the operons identified in bacteria contain only three genes, *arsRBC*. In addition, although almost all of the operons contain the small As(V) reductase encoded by *arsC*, two distinct families of these reductases exist. The *E. coli* ArsC receives reducing equivalents from glutathione and glutaredoxin (162) whereas the *S. aureus* ArsC

receives them from thioredoxin (77). Additional genes, such as *arsH* from *Yersinia* (128) and ORF2 from *Bacillus subtilis* (157), have been identified in *ars* operons, and sometimes *arsB* and *arsH* are transcribed in the opposite direction, suggesting complex regulation (22). A wide variety of bacteria contain *ars* operons on their chromosomes (22, 157, 168).

OXIDATION: THE AOX AND ARO GENES Considerably less is known about As(III) oxidation. Transposon mutagenesis in strain ULPAs1 (187), a chemoorganotrophic member of the beta *Proteobacteria*, identified two genes (*aoxA* and *aoxB*) required for As(III) oxidation (110). Biochemical studies and sequence analyses indicate that these genes encode a heterodimer that is a member of the DMSO reductase family (1, 32, 104, 156). Additionally, protein purification and N-terminal sequencing allowed the identification of another pair of As(III) oxidoreductase genes (*aroA* and *aroB*) from strain NT-26, a chemolithoautotrophic As(III)-oxidizing bacterium; deletion of *aroA* resulted in the loss of As(III) oxidation (156). *aroA* is 48% identical at the amino acid level to *aoxB*, and *aroB* is 52% identical to *aoxA*. Translated sequences of these genes share high identity with proteins from *A. faecalis*, another As(III) oxidizer whose genes have been sequenced (L.T. Phung, unpublished), and putative proteins from the archaea *Aeropyrum pernix* and *Sulfolobus tokodaii* and the phototroph *Chloroflexus aurantiacus* (156).

RESPIRATORY REDUCTION: THE ARR GENES Recently, our laboratory established a genetic system in *Shewanella* species strain ANA-3 (152) that led to the discovery that this organism has two independent systems for reducing As(V) (Figure 4*C*). The first system belongs to the *ars* detoxification family and is not required for anaerobic respiration (although it provides an advantage at high arsenic concentrations) (150). The second system comprises two genes, *arrA* and *arrB*, required for respiratory arsenate reduction. The operon encoding these genes is proximal to the *ars* operon on the chromosome, but divergently transcribed (152). These genes appear to be under the control of a promotor that senses anaerobiosis, and preliminary data suggest that an additional As-specific activator helps to up-regulate *arr* gene expression (C.W. Saltikov & D.K. Newman, unpublished). Additionally, the sequence region at the start of the *arrA* gene contains a putative twin arginine signal sequence (TAT) known to be involved in Sec-independent transport of folded proteins with metal cofactors, suggesting that the *arr* genes are translocated to the periplasm. In support of this prediction, a translational *gfp* fusion to the *arrA* gene expresses GFP in the periplasm (D. Malasarn, C.W. Saltikov & D.K. Newman, unpublished). The *arrA* gene is predicted to encode a protein that shares characteristics with molybdopterin oxidoreductases: It has a cysteine-rich motif that typically binds iron sulfur clusters and a canonical molybdopterin binding domain. *arrB* is downstream of *arrA* and its translated product is predicted to be similar to proteins that contain iron sulfur clusters. *c*-type cytochromes have recently been shown to be involved in the electron transport pathway to As(V) in strain ANA-3 by transposon mutagenesis (C.W. Saltikov, Y. Jiao & D.K. Newman, unpublished). Our current model of the respiratory complex is that it interacts with

a CymA homologue in ANA-3 or an as-yet unidentified membrane anchor protein that receives electrons from a membrane-bound *c*-type cytochrome (Figure 3*C*).

Surprisingly, the *arr* genes appear to be highly conserved among phylogenetically diverse As(V)-respiring bacteria. For example, genomic database searches reveal ORFs in the gram-positive bacterium *Desulfitobacterium hafniense* whose translated products exhibit 51% identity, 68% similarity to ArrA (ANA-3) and 53% identity, 61% similarity to ArrB (ANA-3). When the predicted *arrA* and *arrB* genes from *D. hafniense* are introduced into an ANA-3 *arrA* mutant, partial complementation is observed. The *D. hafniense arrAB* genes appear to be part of a larger operon, comprising several additional regulatory units and a putative anchor protein. Homologues have also been identified in *Bacillus selenitireducens* and *Chrysiogenes arsenatis* (152). Whether these genes were spread horizontally has yet to be determined.

From Genes to the Environment

Previous studies have indicated that biological As(V) reduction is an important component of the As geochemical cycle in a variety of environments (43, 62, 131, 204). Because the *arr* genes appear to be ubiquitous and highly conserved, they afford a means to use genetic information to determine whether respiratory As(V) reduction [as opposed to As(V) reduction catalyzed by detoxification enzymes] plays a role in shaping the geochemistry of a particular environment. To this end, we have recently developed a set of degenerate primers based on the *arrA* gene to detect the presence and/or expression of this gene in environmental samples. These primers were used to amplify an internal region of the *arrA* gene from a diverse group of isolated As(V) reducers, as well as uncultured As(V)-respiring organisms from Haiwee Reservoir, an arsenic-contaminated water body in eastern California. In addition, *arrA*-specific mRNA transcripts appear to be expressed in Haiwee Reservoir sediments in zones where As(V) reduction is thought to occur. These results represent the first time the expression of specific functional genes has been shown to correlate with redox transformations of As in the environment (D. Malasarn, C.W. Saltikov, K.M. Campbell, J.M. Santini, J. Herring & D.K. Newman, unpublished).

FINAL COMMENTS

In this review, we have provided three examples of how genetics affords geomicrobiologists a means to understand how geochemically significant microbial metabolisms work. Even in the three cases we have discussed, where genetic systems have been established, our understanding of the genes that control these processes and their regulation is still primitive. With increasing involvement of geneticists in geomicrobiology, we expect rapid advances in the genetic tractability of environmentally relevant model systems, leading to an improvement in our understanding of how these organisms operate. Geneticists are particularly needed now as more and more genomes are sequenced and an increasing number of

genes of unknown function appear in the database (181, 185). Although functional genomic approaches have led to exciting discoveries about microbial metabolic activities in the environment (7, 144), isolation and genetic study of strains from the environment are still imperative if we seek to understand their physiology in detail. Increasingly, creative approaches are being used to isolate environmentally significant organisms (31, 60, 203), challenging the conventional wisdom that only 1% of organisms in the environment can be cultured. This, and the fact that sequencing microbial genomes is now routine, provides geneticists with an unprecedented opportunity to uncover and study novel pathways that catalyze a wide variety of geochemically significant reactions.

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LITERATURE CITED

1. Anderson GL, Williams J, Hille R. 1992. The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. *J. Biol. Chem.* 267:23674–82
2. Anderson RT, Vrionis HA, Ortiz-Bernad I, Resch CT, Long PE, et al. 2003. Stimulating the in situ activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer. *Appl. Environ. Microbiol.* 69:5884–91
3. Appia-Ayme C, Bengrine A, Cavazza C, Giudici-Orticoni MT, Bruschi M, et al. 1998. Characterization and expression of the co-transcribed *cyc1* and *cyc2* genes encoding the cytochrome *c4* (*c*552) and a high-molecular-mass cytochrome c from *Thiobacillus ferrooxidans* ATCC 33020. *FEMS Microbiol. Lett.* 167:171–77
4. Appia-Ayme C, Guiliani N, Ratouchniak J, Bonnefoy V. 1999. Characterization of an operon encoding two *c*-type cytochromes, an *aa*(3)-type cytochrome oxidase, and rusticyanin in *Thiobacillus ferrooxidans* ATCC 33020. *Appl. Environ. Microbiol.* 65:4781–87
5. Armienta MA, Villasenor G, Rodriguez R, Ongley LK, Mango H. 2001. The role of arsenic-bearing rocks in groundwater pollution at Zimapan Valley, Mexico. *Environ. Geol.* 40:571–81
6. Beijerinck MW. 1901. Ueber oligonitrophile Mikroben. *Zentralbl. Bakteriol.* 7:561–82
7. Beja O, Aravind L, Koonin EV, Suzuki MT, Hadd A, et al. 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science* 289:1902–6
8. Beliaev AS, Saffarini DA. 1998. *Shewanella putrefaciens mtrB* encodes an outer membrane protein required for Fe(III) and Mn(IV) reduction. *J. Bacteriol.* 180:6292–97
9. Beliaev AS, Saffarini DA, McLaughlin JL, Hunnicutt D. 2001. MtrC, an outer

membrane decahaem c cytochrome required for metal reduction in *Shewanella putrefaciens* MR-1. *Mol. Microbiol.* 39:722–30

10. Beliaev AS, Thompson DK, Fields MW, Wu L, Lies DP, et al. 2002. Microarray transcription profiling of a *Shewanella oneidensis etrA* mutant. *J. Bacteriol.* 184:4612–16
11. Beliaev AS, Thompson DK, Khare T, Lim H, Brandt CC, et al. 2002. Gene and protein expression profiles of *Shewanella oneidensis* during anaerobic growth with different electron acceptors. *Omics* 6:39–60
12. Bengrine A, Guiliani N, Appia-Ayme C, Jedlicki E, Holmes DS, et al. 1998. Sequence and expression of the rusticyanin structural gene from *Thiobacillus ferrooxidans* ATCC33020 strain. *Biochim. Biophys. Acta* 1443:99–112
13. Benz M, Brune A, Schink B. 1998. Anaerobic and aerobic oxidation of ferrous iron at neutral pH by chemoheterotrophic nitrate-reducing bacteria. *Arch. Microbiol.* 169:159–65
14. Blake RC 2nd, Shute EA. 1994. Respiratory enzymes of *Thiobacillus ferrooxidans*. Kinetic properties of an acid stable iron:rusticyanin oxidoreductase. *Biochemistry* 33:9220–28
15. Blake RC 2nd, Shute EA, Waskovsky J, Harrison APJ. 1992. Respiratory components in acidophilic bacteria that respire iron. *Geomicrobiol. J.* 10:173–92
16. Blanchard L, Marion D, Pollock B, Voordouw G, Wall J, et al. 1993. Overexpression of *Desulfovibrio vulgaris* Hildenborough cytochrome c553 in *Desulfovibrio desulfuricans* G200. Evidence of conformational heterogeneity in the oxidized protein by NMR. *Eur. J. Biochem.* 218:293–301
17. Blankenship RE, Hartman H. 1998. The origin and evolution of oxygenic photosynthesis. *Trends Biochem. Sci.* 23:94–97
18. Bond DR, Lovley DR. 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.* 69:1548–55
19. Broer S, Ji G, Broer A, Silver S. 1993. Arsenic efflux governed by the arsenic resistance determinant of *Staphylococcus aureus* plasmid pI258. *J. Bacteriol.* 175:3480–85
20. Brouwers GJ, Vijgenboom E, Corstjens P, De Vrind JPM, de Vrind-de Jong EW. 2000. Bacterial Mn^{2+} oxidizing systems and multicopper oxidases: an overview of mechanisms and functions. *Geomicrobiol. J.* 17:1–24
21. Burnes BS, Mulberry MJ, DiChristina TJ. 1998. Design and application of two rapid screening techniques for isolation of Mn(IV) reduction-deficient mutants of *Shewanella putrefaciens*. *Appl. Environ. Microbiol.* 64:2716–20
22. Butcher BG, Deane SM, Rawlings DE. 2000. The chromosomal arsenic resistance genes of *Thiobacillus ferrooxidans* have an unusual arrangement and confer increased arsenic and antimony resistance to *Escherichia coli*. *Appl. Environ. Microbiol.* 66:1826–33
23. Caccavo F, Das A. 2002. Adhesion of dissimilatory Fe(III)-reducing bacteria to Fe(III) minerals. *Geomicrobiol. J.* 19: 161–77
24. Chapelle FH, O'Neill K, Bradley PM, Methe BA, Ciufo SA, et al. 2002. A hydrogen-based subsurface microbial community dominated by methanogens. *Nature* 415:312–15
25. Chaudhuri SK, Lack JG, Coates JD. 2001. Biogenic magnetite formation through anaerobic biooxidation of Fe(II). *Appl. Environ. Microbiol.* 67:2844–48
26. Chiang SL, Rubin EJ. 2002. Construction of a mariner-based transposon for epitope-tagging and genomic targeting. *Gene* 296:179–85
27. Cloud P. 1973. Paleoecological significance of banded iron-formation. *Econ. Geol.* 68:1135–43
28. Coates JD, Phillips EJ, Lonergan DJ,

Jenter H, Lovley DR. 1996. Isolation of *Geobacter* species from diverse sedimentary environments. *Appl. Environ. Microbiol.* 62:1531–36

29. Cobley JG, Haddock BA. 1975. The respiratory chain of *Thiobacillus ferrooxidans*: the reduction of cytochromes by Fe^{2+} and the preliminary characterization of rusticyanin a novel 'blue' copper protein. *FEBS Lett.* 60:29–33
30. Colmer AR, Temple KL, Hinkle ME. 1949. An iron-oxidizing bacterium from the drainage of some bituminous coal mines. *J. Bacteriol.* 59:317–28
31. Connon SA, Giovannoni SJ. 2002. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Appl. Environ. Microbiol.* 68:3878–85
32. Conrads T, Hemann C, George GN, Pickering IJ, Prince RC, Hille R. 2002. The active site of arsenite oxidase from *Alcaligenes faecalis*. *J. Am. Chem. Soc.* 124:11276–77
33. Coppi MV, Leang C, Sandler SJ, Lovley DR. 2001. Development of a genetic system for *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* 67:3180–87
34. Cox JC, Boxer DH. 1978. The purification and some properties of rusticyanin, a blue copper protein invloved in Fe(II) oxidation from *Thiobacillus ferro-oxidans*. *Biochem. J.* 174:497–502
35. Deleted in proof
36. Croal LR, Johnson CJ, Bread BL, Newman DK. 2004. Iron isotope fractionation by Fe(II)-oxidizing photoautotrophic bacteria. *Geochim. Cosmochim. Acta* 68: 1227–42
37. Cullen WR, Reimer KJ. 1989. Arsenic speciation in the environment. *Chem. Rev.* 89:713–64
38. De Luca G, de Philip P, Dermoun Z, Rousset M, Vermeglio A. 2001. Reduction of technetium(VII) by *Desulfovibrio fructosovorans* is mediated by the nickel-iron hydrogenase. *Appl. Environ. Microbiol.* 67:4583–87
39. Dey S, Rosen BP. 1995. Dual mode of energy coupling by the oxyanion-translocating ArsB protein. *J. Bacteriol.* 177:385–89
40. DiChristina TJ, DeLong EF. 1994. Isolation of anaerobic respiratory mutants of *Shewanella putrefaciens* and genetic analysis of mutants deficient in anaerobic growth on Fe^{3+}. *J. Bacteriol.* 176:1468–74
41. DiChristina TJ, Moore CM, Haller CA. 2002. Dissimilatory Fe(III) and Mn(IV) reduction by *Shewanella putrefaciens* requires *ferE*, a homolog of the *pulE* (*gspE*) type II protein secretion gene. *J. Bacteriol.* 184:142–51
42. Donohue TJ, Kaplan S. 1991. Genetic techniques in *Rhodospirillaceae*. *Methods Enzymol.* 204:459–85
43. Dowdle PR, Laverman AM, Oremland RS. 1996. Bacterial dissimilatory reduction of arsenic(V) to arsenic(III) in anoxic sediments. *Appl. Environ. Microbiol.* 62:1664–69
44. Dubiel M, Hsu CH, Chien CC, Mansfeld F, Newman DK. 2002. Microbial iron respiration can protect steel from corrosion. *Appl. Environ. Microbiol.* 68:1440–45
45. Edwards KJ, Bond PL, Gihring TM, Banfield JF. 2000. An archaeal iron-oxidizing extreme acidophile important in acid mine drainage. *Science* 287:1796–99
46. Edwards KJ, Rogers DR, Wirsen CO, McCollom TM. 2003. Isolation and characterization of novel psychrophilic, neutrophilic, Fe-oxidizing, chemolithoautotrophic alpha- and gamma-proteobacteria from the deep sea. *Appl. Environ. Microbiol.* 69:2906–13
47. Ehrenreich A, Widdel F. 1994. Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Appl. Environ. Microbiol.* 60:4517–26
48. Ehrlich HL. 1998. Geomicrobiology: its significance for geology. *Earth Sci. Rev.* 45:45–60
49. Ehrlich HL. 2002. Geomicrobiology of

iron. In *Geomicrobiology,* HL Ehrlich, pp. 345–429. New York: Dekker.

50. Ehrlich HL. 2002. Geomicrobiology of iron. See Ref. 49, pp. 303–43
51. Ellis PJ, Conrads T, Hille R, Kuhn P. 2001. Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two crystal forms at 1.64 Å and 2.03 Å. *Structure* 9:125–32
52. Emerson D, Moyer C. 1997. Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. *Appl. Environ. Microbiol.* 63:4784–92
53. Emerson D, Moyer CL. 2002. Neutrophilic Fe-oxidizing bacteria are abundant at the Loihi Seamount hydrothermal vents and play a major role in Fe oxide deposition. *Appl. Environ. Microbiol.* 68:3085–93
54. Emerson D, Weiss JV, Megonigal JP. 1999. Iron-oxidizing bacteria are associated with ferric hydroxide precipitates (Fe-plaque) on the roots of wetland plants. *Appl. Environ. Microbiol.* 65:2758–61
55. Field SJ, Dobbin PS, Cheesman MR, Watmough NJ, Thomson AJ, Richardson DJ. 2000. Purification and magneto-optical spectroscopic characterization of cytoplasmic membrane and outer membrane multiheme c-type cytochromes from *Shewanella frigidimarina* NCIMB400. *J. Biol. Chem.* 275:8515–22
56. Francesconi KA, Kuehnelt D. 2002. Arsenic compounds in the environment. See Ref. 56a, pp. 51–94

56a. Frankenberger WT Jr, ed. 2002. *Environmental Chemistry of Arsenic*. New York/Basel: Marcel Dekker

57. Fukumori Y, Yano T, Sato A, Yamanaka T. 1988. Fe(II)-oxidizing enzyme purified from *Thiobacillus ferrooxidans*. *FEMS Microbiol. Lett.* 50:169–72
58. Ghiorse WC. 1984. Biology of iron- and manganese-depositing bacteria. *Annu. Rev. Microbiol.* 38:515–50
59. Gordon EH, Pike AD, Hill AE, Cuthbertson PM, Chapman SK, Reid GA. 2000. Identification and characterization of a novel cytochrome *c*(3) from *Shewanella frigidimarina* that is involved in Fe(III) respiration. *Biochem. J.* 349:153–58
60. Graber JR, Leadbetter JR, Breznak JA. 2004. Description of *Treponema azotonutricium* sp. nov. and *Treponema primitia* sp. nov., the first spirochetes isolated from termite guts. *Appl. Environ. Microbiol.* 70:1315–20
61. Hall JF, Hasnain SS, Ingledew WJ. 1996. The structural gene for rusticyanin from *Thiobacillus ferrooxidans*: cloning and sequencing of the rusticyanin gene. *FEMS Microbiol. Lett.* 137:85–89
62. Harrington JM, Fendorf SE, Rosenzweig RF. 1998. Biotic generation of arsenic(III) in metal(loid)-contaminated freshwater lake sediments. *Environ. Sci. Technol.* 32: 2425–30
63. Hartman H. 1984. The evolution of photosynthesis and microbial mats: a speculation on banded iron formations. In *Microbial Mats: Stromatolites*, ed. Y Cohen, RW Castenholz, HO Halvorson, pp. 451–53. New York: Liss
64. Harvey CF, Swartz CH, Badruzzaman AB, Keon-Blute N, Yu W, et al. 2002. Arsenic mobility and groundwater extraction in Bangladesh. *Science* 298:1602–6
65. Hauck S, Benz M, Brune A, Schink B. 2001. Ferrous iron oxidation by denitrifying bacteria in profundal sediments of a deep lake (Lake Constance). *FEMS Microbiol. Ecol.* 37:127–34
66. Hedges RW, Baumberg S. 1973. Resistance to arsenic compounds conferred by a plasmid transmissible between strains of *Escherichia coli*. *J. Bacteriol.* 115:459–60
67. Heidelberg JF, Paulsen IT, Nelson KE, Gaidos EJ, Nelson WC, et al. 2002. Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. *Nat. Biotechnol.* 20:1118–23
68. Heising S, Richter L, Ludwig W, Schink B. 1999. *Chlorobium ferrooxidans* sp. nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in

coculture with a "*Geospirillum*" sp. strain. *Arch. Microbiol.* 172:116–24
69. Heising S, Schink B. 1998. Phototrophic oxidation of ferrous iron by a *Rhodomicrobium vannielii* strain. *Microbiology* 144:2263–69
70. Deleted in proof
71. Hernandez ME, Newman DK. 2001. Extracellular electron transfer. *Cell Mol. Life Sci.* 58:1562–71
72. Holmes DE, Bond DR, Lovley DR. 2004. Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes. *Appl. Environ. Microbiol.* 70:1234–37
73. Huber R, Sacher M, Vollmann A, Huber H, Rose D. 2000. Respiration of arsenate and selenate by hyperthermophilic archaea. *Syst. Appl. Microbiol.* 23:305–14
74. Ilyaletdinov AN, Abdrashitova SA. 1981. Autotrophic oxidation of arsenic by a culture of *Pseudomonas arsenitoxidans*. *Microbiology* 50:135–40
75. Ingledew WJ, Cobley JG. 1980. A potentiometric and kinetic study on the respiratory chain of ferrous-iron-grown *Thiobacillus ferrooxidans*. *Biochim. Biophys. Acta* 590:141–58
76. Ingledew WJ, Houston A. 1986. The organization of the respiratory chain of *Thiobacillus ferrooxidans*. *Biotechnol. Appl. Biochem.* 8:242–48
77. Ji G, Silver S. 1992. Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of *Staphylococcus aureus* plasmid pI258. *Proc. Natl. Acad. Sci. USA* 89:9474–78
78. Deleted in proof
79. Johnson CM, Beard BL, Beukes NJ, Klein C, O'Leary JM. 2003. Ancient geochemical cycling in the Earth as inferred from Fe isotope studies of banded iron formations from the Transvaal Craton. *Contrib. Mineral. Petrol.* 144:523–47
80. Kashefi K, Lovley DR. 2003. Extending the upper temperature limit for life. *Science* 301:934
81. Kaur P, Rosen BP. 1992. Plasmid-encoded resistance to arsenic and antimony. *Plasmid* 27:29–40
82. Kiley PJ, Beinert H. 1998. Oxygen sensing by the global regulator, FNR: the role of the iron-sulfur cluster. *FEMS Microbiol. Rev.* 22:341–52
83. Klein C. 2002. *Mineral Science*. New York: Wiley
84. Kluyver AJ. 1924. Eenheid en verscheidenheid in de stofwisseling der microben. *Chemisch. Weekbl.* 21:266
85. Konhauser KO, Hamade T, Raiswell R, Morris RC, Ferris FG, et al. 2002. Could bacteria have formed the Precambrian banded iron formations? *Geology* 30: 1079–82
86. Kusano T, Sugawara K, Inoue C, Takeshima T, Numata M, Shiratori T. 1992. Electrotransformation of *Thiobacillus ferrooxidans* with plasmids containing a mer determinant. *J. Bacteriol.* 174:6617–23
87. Kusano T, Takeshima T, Sugawara K, Inoue C, Shiratori T, et al. 1992. Molecular cloning of the gene encoding *Thiobacillus ferrooxidans* Fe(II) oxidase. High homology of the gene product with HiPIP. *J. Biol. Chem.* 267:11242–47
88. Lange M, Ahring BK. 2001. A comprehensive study into the molecular methodology and molecular biology of methanogenic *Archaea*. *FEMS Microbiol. Rev.* 25:553–71
89. Leang C, Coppi MV, Lovley DR. 2003. OmcB, a c-type polyheme cytochrome, involved in Fe(III) reduction in *Geobacter sulfurreducens*. *J. Bacteriol.* 185:2096–103
90. Leys D, Meyer TE, Tsapin AS, Nealson KH, Cusanovich MA, Van Beeumen JJ. 2002. Crystal structures at atomic resolution reveal the novel concept of "electron-harvesting" as a role for the small tetraheme cytochrome c. *J. Biol. Chem.* 277:35703–11
91. Lin WC, Coppi MV, Lovley DR. 2004. *Geobacter sulfurreducens* can grown with oxygen as a terminal electron acceptor. *Appl. Environ. Microbiol.* 70:2525–28

92. Liu Z, Guiliani N, Appia-Ayme C, Borne F, Ratouchniak J, Bonnefoy V. 2000. Construction and characterization of a recA mutant of *Thiobacillus ferrooxidans* by marker exchange mutagenesis. *J. Bacteriol.* 182:2269–76
93. Lloyd JR. 2003. Microbial reduction of metals and radionuclides. *FEMS Microbiol. Rev.* 27:411–25
94. Lloyd JR, Leang C, Hodges Myerson AL, Coppi MV, Cuifo S, et al. 2003. Biochemical and genetic characterization of PpcA, a periplasmic c-type cytochrome in *Geobacter sulfurreducens*. *Biochem. J.* 369:153–61
95. Lloyd JR, Lovley DR, Macaskie LE. 2003. Biotechnological application of metal-reducing microorganisms. *Adv. Appl. Microbiol.* 53:85–128
96. Lovley DR. 1993. Dissimilatory metal reduction. *Annu. Rev. Microbiol.* 47:263–90
97. Lovley DR, Giovannoni SJ, White DC, Champine JE, Phillips EJ, et al. 1993. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. *Arch. Microbiol.* 159:336–44
98. Luo Y, Wasserfallen A. 2001. Gene transfer systems and their applications in *Archaea*. *Syst. Appl. Microbiol.* 24:15–25
99. Mackenzie C, Simmons AE, Kaplan S. 1999. Multiple chromosomes in bacteria. The yin and yang of *trp* gene localization in *Rhodobactersphaeroides* 2.4.1. *Genetics* 153:525–38
100. Macy JM, Nunan K, Hagen KD, Dixon DR, Harbour PJ, et al. 1996. *Chrysiogenes arsenatis* gen nov, sp nov; a new arsenate-respiring bacterium isolated from gold mine wastewater. *Int. J. Sys. Bacteriol.* 46:1153–57
101. Maier TM, Myers CR. 2001. Isolation and characterization of a *Shewanella putrefaciens* MR-1 electron transport regulator *etrA* mutant: reassessment of the role of EtrA. *J. Bacteriol.* 183:4918–26
102. Deleted in proof
103. Deleted in proof
104. McNellis L, Anderson GL. 1998. Redox-state dependent chemical inactivation of arsenite oxidase. *J. Inorg. Biochem.* 69: 253–57
105. Merrick MJ, Edwards RA. 1995. Nitrogen control in bacteria. *Microbiol. Rev.* 59: 604–22
106. Metcalf WW, Wolfe RS. 1998. Molecular genetic analysis of phosphite and hypophosphite oxidation by *Pseudomonas stutzeri* WM88. *J. Bacteriol.* 180:5547–58
107. Methe BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W, et al. 2003. Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments. *Science* 302:1967–69
108. Meuer J, Kuettner HC, Zhang JK, Hedderich R, Metcalf WW. 2002. Genetic analysis of the archaeon *Methanosarcina barkeri* Fusaro reveals a central role for Ech hydrogenase and ferredoxin in methanogenesis and carbon fixation. *Proc. Natl. Acad. Sci. USA* 99:5632–37
109. Mobley HL, Chen CM, Silver S, Rosen BP. 1983. Cloning and expression of R-factor mediated arsenate resistance in *Escherichia coli*. *Mol. Gen. Genet.* 191:421–26
110. Muller D, Lievremont D, Simeonova DD, Hubert JC, Lett MC. 2003. Arsenite oxidase aox genes from a metal-resistant beta-proteobacterium. *J. Bacteriol.* 185: 135–41
111. Myers CR, Myers JM. 1993. Ferric reductase is associated with the membranes of anaerobically grown *Shewanella putrefaciens* MR-1. *FEMS Microbiol. Lett.* 108:15–22
112. Myers CR, Myers JM. 1993. Role of menaquinone in the reduction of fumarate, nitrate, iron(III) and manganese(IV) by *Shewanella putrefaciens* MR-1. *FEMS Microbiol. Lett* 114:215–22
113. Myers CR, Myers JM. 1997. Cloning and sequence of *cymA*, a gene encoding a

tetraheme cytochrome *c* required for reduction of iron(III), fumarate, and nitrate by *Shewanella putrefaciens* MR-1. *J. Bacteriol.* 179:1143–52

114. Myers CR, Myers JM. 1997. Outer membrane cytochromes of *Shewanella putrefaciens* MR-1: spectral analysis, and purification of the 83-kDa c-type cytochrome. *Biochim. Biophys. Acta* 1326: 307–18
115. Myers CR, Myers JM. 2002. MtrB is required for proper incorporation of the cytochromes OmcA and OmcB into the outer membrane of *Shewanella putrefaciens* MR-1. *Appl. Environ. Microbiol.* 68:5585–94
116. Myers CR, Myers JM. 2003. Cell surface exposure of the outer membrane cytochromes of *Shewanella oneidensis* MR-1. *Lett. Appl. Microbiol.* 37:254–58
117. Myers JM, Myers CR. 1998. Isolation and sequence of *omcA*, a gene encoding a decaheme outer membrane cytochrome *c* of *Shewanella putrefaciens* MR-1, and detection of *omcA* homologs in other strains of *S. putrefaciens*. *Biochim. Biophys. Acta* 1373:237–51
118. Myers JM, Myers CR. 2000. Role of the tetraheme cytochrome CymA in anaerobic electron transport in cells of *Shewanella putrefaciens* MR-1 with normal levels of menaquinone. *J. Bacteriol.* 182:67–75
119. Myers JM, Myers CR. 2001. Role for outer membrane cytochromes OmcA and OmcB of *Shewanella putrefaciens* MR-1 in reduction of manganese dioxide. *Appl. Environ. Microbiol.* 67:260–69
120. Myers JM, Myers CR. 2003. Overlapping role of the outer membrane cytochromes of *Shewanella oneidensis* MR-1 in the reduction of manganese(IV) oxide. *Lett. Appl. Microbiol.* 37:21–25
121. Nealson KH. 1983. The microbial iron cycle. In *Microbial Geochemistry*, ed. WE Krumbein, pp. 159–89. Boston: Blackwell Sci.
122. Nealson KH, Belz A, McKee B. 2002. Breathing metals as a way of life: geobiology in action. *Antonie Van Leeuwenhoek J. Microbiol. Serol.* 81:215–22
123. Nealson KH, Saffarini D. 1994. Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. *Annu. Rev. Microbiol.* 48:311–43
124. Nevin KP, Lovley DR. 2002. Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentans*. *Appl. Environ. Microbiol.* 68:2294–99
125. Newman DK, Ahmann D, Morel FMM. 1998. A brief review of microbial arsenate respiration. *Geomicrobiology* 15:255–68
126. Newman DK, Banfield JF. 2002. Geomicrobiology: How molecular-scale interactions underpin biogeochemical systems. *Science* 296:1071–77
127. Newman DK, Kolter R. 2000. A role for excreted quinones in extracellular electron transfer. *Nature* 405:94–97
128. Neyt C, Iriarte M, Thi VH, Cornelis GR. 1997. Virulence and arsenic resistance in *Yersiniae*. *J. Bacteriol.* 179:612–19
129. Nickson R, McArthur J, Burgess W, Ahmed KM, Ravenscroft P, Rahman M. 1998. Arsenic poisoning of Bangladesh groundwater. *Nature* 395:338
130. Nriagu J. 2002. Arsenic poisoning through the ages. See Ref. 56a, pp. 1–26
131. Oremland RS, Dowdle PR, Hoeft SE, Sharp JO, Schaefer JK, et al. 2000. Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California. *Geochim. Cosmochim. Acta* 64:3073–84
132. Oremland RS, Hoeft SE, Santini JA, Bano N, Hollibaugh RA, Hollibaugh JT. 2002. Anaerobic oxidation of arsenite in Mono Lake water and by facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. *Appl. Environ. Microbiol.* 68:4795–802
133. Oremland RS, Stolz JF. 2003. The ecology of arsenic. *Science* 300:939–44
134. Page MD, Sockett RE. 1999. Molecular genetic methods in *Paracoccus* and

Rhodobacter with particular reference to the analysis of respiration and photosynthesis. In *Methods in Microbiology*, ed. MCM Smith, RE Sockett, pp. 427–66. San Diego: Academic

135. Pawellek T, Aigner T. 2003. Stratigraphic architecture and gamma ray logs of deeper ramp carbonates (Upper Jurassic, SW Germany). *Sediment. Geol.* 159:203–40
136. Peng JB, Yan WM, Bao XZ. 1994. Plasmid and transposon transfer to *Thiobacillus ferrooxidans*. *J. Bacteriol.* 176:2892–97
137. Deleted in proof
138. Pitts KE, Dobbin PS, Reyes-Ramirez F, Thomson AJ, Richardson DJ, Seward HE. 2003. Characterization of the *Shewanella oneidensis* MR-1 decaheme cytochrome MtrA: Expression in *Escherichia coli* confers the ability to reduce soluble Fe(III) chelates. *J. Biol. Chem.* 278:27758–65
139. Priscu JC, Fritsen CH, Adams EE, Giovannoni SJ, Paerl HW, et al. 1998. Perennial Antarctic lake ice: an oasis for life in a polar desert. *Science* 280:2095–98
140. Rae AJ, Cooke D, Phillips M, Zaide-Delfin M. 2004. The nature of magmatism at Palinpinon geothermal field, Negros Island, Philippines: implications for geothermal activity and regional tectonics. *J. Volcanol. Geotherm. Res.* 129:321–42
141. Rawlings DE. 1994. Molecular genetics of *Thiobacillus ferrooxidans*. *Microbiol. Rev.* 58:39–55
142. Rawlings DE. 2001. The molecular genetics of *Thiobacillus ferrooxidans* and other mesophilic, acidophilic, chemolithotrophic, iron- or sulfur-oxidizing bacteria. *Hydrometallurgy* 59:187–201
143. Robertson LA, Gijs Kuenen J. 2002. The genus *Thiobacillus*. In *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. Release 3.9*, ed. M Dworkin. New York: Springer-Verlag. http://141.150.157.117:8080/prokPUB/index.htm
144. Rondon MR, Goodman RM, Handelsman J. 1999. The Earth's bounty: assessing and accessing soil microbial diversity. *Trends Biotechnol.* 17:403–9
145. Rooney-Varga JN, Anderson RT, Fraga JL, Ringelberg D, Lovley DR. 1999. Microbial communities associated with anaerobic benzene degradation in a petroleum-contaminated aquifer. *Appl. Environ. Microbiol.* 65:3056–63
146. Rosen BP. 2002. Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 133:689–93
147. Saffarini DA, Blumerman SL, Mansoorabadi KJ. 2002. Role of menaquinones in Fe(III) reduction by membrane fractions of *Shewanella putrefaciens*. *J. Bacteriol.* 184:846–48
148. Saffarini DA, Schultz R, Beliaev A. 2003. Involvement of cyclic AMP (cAMP) and cAMP receptor protein in anaerobic respiration of *Shewanella oneidensis*. *J. Bacteriol.* 185:3668–71
149. Salmassi TM, Venkateswaran K, Satomi M, Nealson KH, Newman DK, Hering JG. 2002. Oxidation of arsenite by *Agrobacterium albertimagni*, AOL15, sp nov., isolated from Hot Creek, California. *Geomicrobiol. J.* 19:53–66
150. Saltikov CW, Cifuentes A, Venkateswaran K, Newman DK. 2003. The ars detoxification system is advantageous but not required for As(V) respiration by the genetically tractable *Shewanella species* strain ANA-3. *Appl. Environ. Microbiol.* 69:2800–9
151. Deleted in proof
152. Saltikov CW, Newman DK. 2003. Genetic identification of a respiratory arsenate reductase. *Proc. Natl. Acad. Sci. USA* 100:10983–88
153. Sanders OI, Rensing C, Kuroda M, Mitra B, Rosen BP. 1997. Antimonite is accumulated by the glycerol facilitator GlpF in *Escherichia coli*. *J. Bacteriol.* 179:3365–67

154. Sandkvist M. 2001. Biology of type II secretion. *Mol. Microbiol.* 40:271–83
155. Santini JM, Sly LI, Schnagl RD, Macy JM. 2000. A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: phylogenetic, physiological, and preliminary biochemical studies. *Appl. Environ. Microbiol.* 66:92–97
156. Santini JM, vanden Hoven RN. 2004. Molybdenum-containing arsenite oxidase of the chemolithoautotrophic arsenite oxidizer NT-26. *J. Bacteriol.* 186:1614–19
157. Sato T, Kobayashi Y. 1998. The *ars* operon in the skin element of *Bacillus subtilis* confers resistance to arsenate and arsenite. *J. Bacteriol.* 180:1655–61
158. Schrader JA, Holmes DS. 1988. Phenotypic switching of *Thiobacillus ferrooxidans*. *J. Bacteriol.* 170:3915–23
159. Schwalb C, Chapman SK, Reid GA. 2002. The membrane-bound tetrahaem c-type cytochrome CymA interacts directly with the soluble fumarate reductase in *Shewanella*. *Biochem. Soc. Trans.* 30:658–62
160. Schwalb C, Chapman SK, Reid GA. 2003. The tetraheme cytochrome CymA is required for anaerobic respiration with dimethyl sulfoxide and nitrite in *Shewanella oneidensis*. *Biochemistry* 42:9491–97
161. Senn DB, Hemond HF. 2002. Nitrate controls on iron and arsenic in an urban lake. *Science* 296:2373–76
162. Shi J, Vlamis-Gardikas A, Aslund F, Holmgren A, Rosen BP. 1999. Reactivity of glutaredoxins 1, 2, and 3 from *Escherichia coli* shows that glutaredoxin 2 is the primary hydrogen donor to ArsC-catalyzed arsenate reduction. *J. Biol. Chem.* 274:36039–42
163. Shyu JB, Lies DP, Newman DK. 2002. Protective role of *tolC* in efflux of the electron shuttle anthraquinone-2,6-disulfonate. *J. Bacteriol.* 184:1806–10
164. Silver S, Budd K, Leahy KM, Shaw WV, Hammond D, et al. 1981. Inducible plasmid-determined resistance to arsenate, arsenite, and antimony (III) in *Escherichia coli* and *Staphylococcus aureus*. *J. Bacteriol.* 146:983–96
165. Silver S, Phung LT, Rosen BP. 2002. Arsenic metabolism: resistance, reduction, and oxidation. See Ref. 56a, pp. 247–72
166. Snoeyenbos-West OL, Nevin KP, Anderson RT, Lovley DR. 2000. Enrichment of Geobacter species in response to stimulation of Fe(III) reduction in sandy aquifer sediments. *Microb. Ecol.* 39:153–67
167. Sobolev D, Roden EE. 2001. Suboxic deposition of ferric iron by bacteria in opposing gradients of Fe(II) and oxygen at circumneutral pH. *Appl. Environ. Microbiol.* 67:1328–34
168. Sofia HJ, Burland V, Daniels DL, Plunkett G, Blattner FR. 1994. Analysis of the *Escherichia coli* genome. V. DNA sequence of the region from 76.0 minutes to 81.5 minutes. *Nucleic Acids Res.* 22:2576–86
169. Spliethoff HM, Hemond HF. 1996. History of toxic metal discharge to surface waters of the Aberjona Watershed. *Environ. Sci. Technol.* 30:121–28
170. Straub KL, Benz M, Schink B. 2001. Iron metabolism in anoxic environments at near neutral pH. *FEMS Microbiol. Ecol.* 34:181–86
171. Straub KL, Benz M, Schink B, Widdel F. 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Appl. Environ. Microbiol.* 62:1458–60
172. Straub KL, Buchholz-Cleven BE. 1998. Enumeration and detection of anaerobic ferrous iron-oxidizing, nitrate- reducing bacteria from diverse European sediments. *Appl. Environ. Microbiol.* 64: 4846–56
173. Straub KL, Rainey FA, Widdel F. 1999. *Rhodovulum iodosum* sp. nov. and *Rhodovulum robiginosum* sp. nov., two new marine phototrophic ferrous-iron-oxidizing purple bacteria. *Int. J. Syst. Bacteriol.* 49:729–35
174. Tebo BM, Ghiorse WC, vanWaasbergen

LG, Siering PL, Caspi R. 1997. Bacterially mediated mineral formation: Insights into manganese(II) oxidation from molecular genetic and biochemical studies. *Rev. Mineral.* 35:225–66

175. Temple KL, Colmer AR. 1951. The autotrophic oxidation of iron by a new bacterium, *Thiobacillus ferrooxidans*. *J. Bacteriol.* 62:605–11
176. Thompson DK, Beliaev AS, Giometti CS, Tollaksen SL, Khare T, et al. 2002. Transcriptional and proteomic analysis of a ferric uptake regulator (*fur*) mutant of *Shewanella oneidensis*: possible involvement of fur in energy metabolism, transcriptional regulation, and oxidative stress. *Appl. Environ. Microbiol.* 68:881–92
177. Tisa LS, Rosen BP. 1990. Molecular characterization of an anion pump. The ArsB protein is the membrane anchor for the ArsA protein. *J. Biol. Chem.* 265:190–94
178. Tsapin AI, Nealson KH, Meyers T, Cusanovich MA, Van Beuumen J, et al. 1996. Purification and properties of a low-redox-potential tetraheme cytochrome c3 from *Shewanella putrefaciens*. *J. Bacteriol.* 178:6386–88
179. Tsapin AI, Vandenberghe I, Nealson KH, Scott JH, Meyer TE, et al. 2001. Identification of a small tetraheme cytochrome *c* and a flavocytochrome *c* as two of the principal soluble cytochromes *c* in *Shewanella oneidensis* strain MR1. *Appl. Environ. Microbiol.* 67:3236–44
180. Tumbula DL, Whitman WB. 1999. Genetics of *Methanococcus*: possibilities for functional genomics in *Archaea*. *Mol. Microbiol.* 33:1–7
181. Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, et al. 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* 428:37–43
182. Valkova-Valchanova MB, Chan SH. 1994. Purification and characterization of two new *c*-type cytochromes involved in Fe^{2+} oxidation from *Thiobacillus ferrooxidans*. *FEMS Microbiol. Lett.* 121:61–69
183. Vargas M, Kashefi K, Blunt-Harris EL, Lovley DR. 1998. Microbiological evidence for Fe(III) reduction on early Earth. *Nature* 395:65–67
184. Venkateswaran K, Moser DP, Dollhopf ME, Lies DP, Saffarini DA, et al. 1999. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int. J. Syst. Bacteriol.* 49(Part 2):705–24
185. Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, et al. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304:66–74
186. Vernon LP, Mangum JH, Beck JV, Shafia FM. 1960. Studies on a ferrous-ion-oxidizing bacterium. II. Cytochrome composition. *Arch. Biochem. Biophys.* 88: 227–31
187. Weeger W, Lievremont D, Perret M, Lagarde F, Hubert JC, et al. 1999. Oxidation of arsenite to arsenate by a bacterium isolated from an aquatic environment. *Biometals* 12:141–49
188. Widdel F, Schnell S, Heising S, Ehrenreich A, Assmus B, Schink B. 1993. Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature* 362:834–36
189. Wilkie JA, Hering JG. 1998. Rapid oxidation of geothermal arsenic(III) in streamwaters of the eastern Sierra Nevada. *Environ. Sci. Technol.* 32:657–62
190. Willsky GR, Malamy MH. 1980. Characterization of two genetically separable inorganic phosphate transport systems in *Escherichia coli*. *J. Bacteriol.* 144:356–65
191. Winogradsky S. 1889. Recherches physiologiques sur les sulfobacteries. *Ann. Inst. Pasteur* 3:49–60
192. Woods DR, Rawlings DE, Barros ME, Pretorius I, Ramesar R. 1986. Molecular genetic studies of *Thiobacillus ferrooxidans*: the development of genetic

systems and the expression of cloned genes. *Biotechnol. Appl. Biochem.* 8:231–41

193. World Health Organization. 2001. *Fact sheet, No. 210: Arsenic in drinking water*
194. Deleted in proof
195. Wu J, Rosen BP. 1991. The ArsR protein is a trans-acting regulatory protein. *Mol. Microbiol.* 5:1331–36
196. Wu J, Rosen BP. 1993. The *arsD* gene encodes a second trans-acting regulatory protein of the plasmid-encoded arsenical resistance operon. *Mol. Microbiol.* 8:615–23
197. Xiong J, Fischer WM, Inoue K, Nakahara M, Bauer CE. 2000. Molecular evidence for the early evolution of photosynthesis. *Science* 289:1724–30
198. Xu C, Shi W, Rosen BP. 1996. The chromosomal *arsR* gene of *Escherichia coli* encodes a *trans*-acting metalloregulatory protein. *J. Biol. Chem.* 271:2427–32
199. Yamanaka T, Fukumori Y. 1995. Molecular aspects of the electron transfer system which participates in the oxidation of ferrous ion by *Thiobacillus ferrooxidans*. *FEMS Microbiol. Rev.* 17:401–13
200. Yamanaka T, Yano T, Kai M, Tamegai H, Sato A, Fukumori Y. 1991. The electron transfer system in an acidophilic iron-oxidizing bacterium. In *New Era of Bioenergetics*, ed. Y Mukohata, pp. 223–46. Tokyo: Academic
201. Yarzabal A, Brasseur G, Ratouchniak J, Lund K, Lemesle-Meunier D, et al. 2002. The high-molecular-weight cytochrome *c* Cyc2 of *Acidithiobacillus ferrooxidans* is an outer membrane protein. *J. Bacteriol.* 184:313–17
202. Yarzabal A, Duquesne K, Bonnefoy V. 2003. Rusticyanin gene expression of *Acidithiobacillus ferrooxidans* ATCC 33020 in sulfur- and in ferrous iron media. *Hydrometallurgy* 71:107–14
203. Zengler K, Toledo G, Rappe M, Elkins J, Mathur EJ, et al. 2002. Cultivating the uncultured. *Proc. Natl. Acad. Sci. USA* 99:15681–86
204. Zobrist J, Dowdle PR, Davis JA, Oremland RS. 2000. Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. *Environ. Sci. Technol.* 34:4747–53

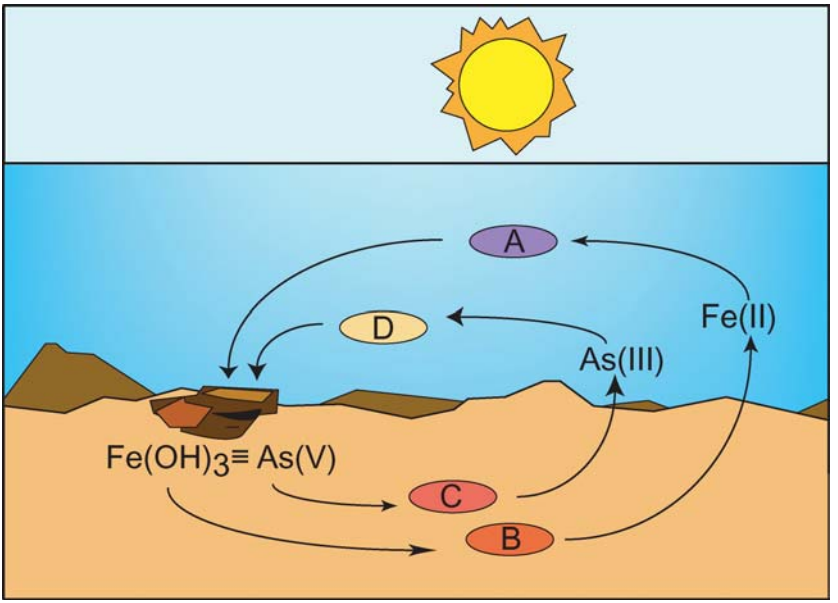

Figure 1 Simplified cartoon of the Fe-As biogeochemical cycle, showing the effect of representative microbial processes that either oxidize or reduce metal(loids). The speciation of Fe and As are neglected (with the exception of $Fe(OH)_3$), and only the dominant oxidation states are represented. The organism labeled *A* catalyzes Fe(II) oxidation through a light-dependent process (i.e., Fe(II)-based photoautotrophy). This results in the conversion of soluble Fe(II) to $Fe(OH)_3$, which is re-reduced by the activity of Fe(III)-respiring microorganisms (*B*). As(V) as well as As(III) strongly adsorb onto $Fe(OH)_3$. Because of this, Fe(III)-reducing bacteria can affect As biogeochemistry by catalyzing the release of As(V) from $Fe(OH)_3$, making it more available for reduction by As(V)-reducing bacteria (*C*). As(V)-reducers, in turn, can promote the release of As(III) into the water column if $Fe(OH)_3$ is not abundant. Finally, the As biogeochemical cycle is completed by As(III)-oxidizing bacteria (*D*) that convert As(III) to As(V) coupled to the reduction of oxygen or nitrate.

Figure 2 Representative cultures of Fe(II)-oxidizing, Fe(III)-reducing, and As(V)-reducing bacteria. The bottle on the left contains a culture of *Rhodobacter* species strain SW2 grown photoautotrophically on Fe(II). The rusty color indicates the precipitation of $Fe(OH)_3$. The center bottle contains a culture of *Shewanella oneidensis* strain MR-1 grown on lactate with $Fe(OH)_3$ as the electron acceptor. With time, Fe(II) accumulates in the culture and adsorbs onto the parent mineral, creating a mixed-valent Fe mineral that is dark in color (e.g., magnetite). The bottle on the right contains a culture of *Shewanella* species strain ANA-3 grown on lactate with As(V) as the electron acceptor. When sulfide is added to the medium and the pH is less than 7, yellow arsenic trisulfide (As_2S_3) precipitates from solution.

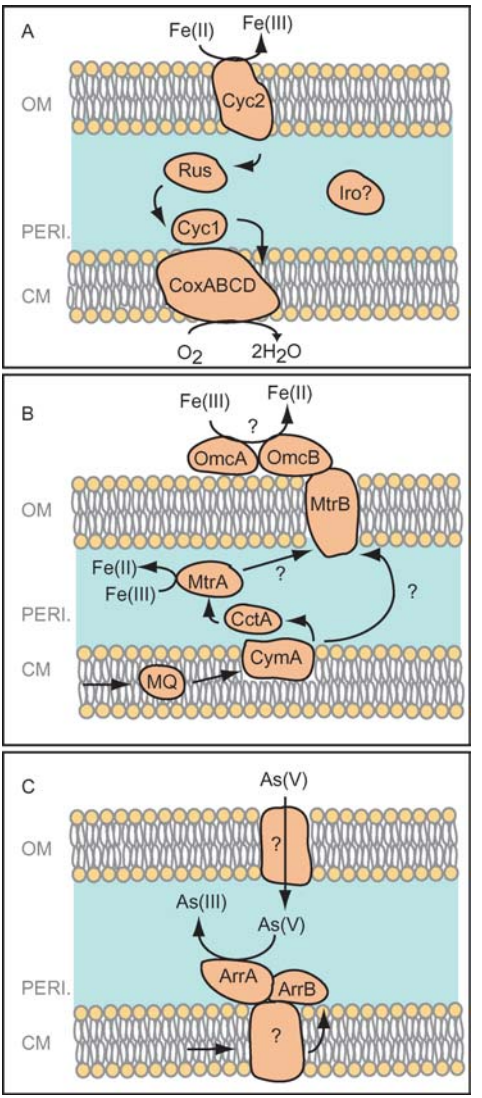

Figure 3 Cartoon drawings of model pathways showing the major components involved in electron transfer for: (*A*) Fe(II) oxidation by *Acidithiobacillus ferrooxidans* strain ATCC 33020; (*B*) Fe(III) reduction by *Shewanella oneidensis* strain MR-1; and (*C*) As(V) reduction by *Shewanella* species strain ANA-3. The arrows between the components point in the direction of electron flow (e.g., from the reductant to the oxidant). Question marks indicate unidentified components and/or proteins for which the role is unclear. The text elaborates on the degree to which these models are (or are not) supported by genetic analyses.

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