Environmental, Biogeographic, and Biochemical Patterns of Archaea of the Family Ferroplasmaceae

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About 10 years ago, a new family of cell wall-deficient, iron-oxidizing archaea, Ferroplasmaceae, within the large archaeal phylum Euryarchaeota, was described. In this minireview, I summarize the research progress achieved since then and report on the current status of taxonomy, biogeography, physiological diversity, biochemistry, and other research areas involving this exciting group of acidophilic archaea.

Microorganisms thrive remarkably under various conditions, including high temperatures, extremely high osmosis, and very acidic pH, that would generally be considered hostile or limiting to higher organisms. Studying and understanding the uniqueness of extremophilic organisms and the biochemical and cellular processes underlying their functioning and role in biogeochemical processes comprise an emerging research area in modern bioscience. Of special interest for various biotechnological applications are enzymes produced by extremophiles, the so-called extremozymes, which exhibit a high activity and stability under extreme physical-chemical conditions. The representatives of the “third domain of life,” the archaea, are unique contributors to this area of special interest. Several factors make the archaea an attractive subject of study, including their relatively recent history of discovery, ability (often much higher than that of bacteria) to adapt to harsh environments, enigmatic nature, and low cultivability.

This minireview focuses on acidophiles representing the euryarchaeal family Ferroplasmaceae, first described about a decade ago (25), and on some aspects relevant to their physiology, geographic distribution, and taxonomic diversity. Representatives of this family are cell wall-lacking extreme and obligate acidophiles that are able to grow at pH values around 0. Together with their closest phylogenetic neighbors, from the family Picophy- llaceae, they comprise a group of the most extreme acidophilic organisms known. Furthermore, the Ferroplasmaceae thrive in systems with high concentrations of iron, copper, zinc, and other metals. Archaea of the family Ferroplasmaceae coexist in their natural habitats with other acidophilic or acid-tolerant prokaryotes, namely, members of the bacterial phyla Firmicutes, Proteobacteria, Actinobacteria, and Nitrospirae, representatives of the Crenarchaeota, and other Euryarchaeota that are important drivers in environmental acid generation and in global cycling of iron and sulfur. Accordingly, being important iron oxidizers, the organisms of the family Ferroplasmaceae significantly contribute to these processes.

Biogeography. The members of the family Ferroplasmaceae are distributed worldwide (Fig. 1) and can be found in a variety of acidic environments with very stable chemical conditions, such as ore deposits, mines, and acid mine drainage systems (natural or man-made), and in areas with geothermal activity. Detection or quantification of the Ferroplasmaceae in these environments was done mostly using small subunit (SSU) rRNA-targeting analyses, such as 16S rRNA gene clone libraries’ sequencing, amplified rRNA gene restriction analysis (ARDRA) profiling, real-time quantitative PCR, restriction fragment length polymorphism (RFLP) or fluorescence in situ hybridization (FISH), and oligonucleotide microarray analysis, all of which revealed the presence of these archaea in a number of pyritic/arsenopyritic, gold-arsenopyritic/chalcopirctic, and lead-, zinc-, and copper-containing mines across all continents (11, 24, 26, 45, 46, 51, 55, 56, 57, 58). Clones related to the family Ferroplasmaceae have also been documented in further natural sulfide-rich ecosystems, e.g., in the snottiates, i.e., the stalactite-like formations of microbial origin taken from the walls of Frasassi Cave and in the Rio Garrafo cave systems, both located in Italy, where acidic microenvironments were formed as a result of sulfide oxidation (38). Acid mine drainage systems of Richmond Mine on Iron Mountain (California) and Rio Tinto (Spain) have been extensively studied as environments hosting the Ferroplasmaceae (1, 3, 18, 23, 29). For years the family Ferroplasmaceae was represented by a single monospecific genus, Ferroplasma, containing only a single species with a validly published name, Ferroplasma acidiphilum V\textsuperscript{T} DSM 12658\textsuperscript{T} (25). Further isolates belonging to the genus Ferroplasma have been obtained, such as “Ferroplasma acidarmanus” fer1 (18) and a few others, with SSU rRNA identical to that of F. acidiphilum or just with few mismatches (12, 16, 40, 41; D. B. Johnson, personal communication) (Fig. 2). A new genus, Acidiplasma, of the family Ferroplasmaceae has very recently been described as containing two species, named Acidiplasma aeolicum V\textsuperscript{T} (DSM 18409\textsuperscript{T} = JCM 14615\textsuperscript{T}) and A. cupricumulans (DSM 16551\textsuperscript{T} = JCM 13668\textsuperscript{T}), isolated from the hydrothermal pool located on Vulcano Island (Italy) and from chalcolite/copper-containing heaps (Myanmar), respectively (28, 30, 31). A few other Acidiplasma-like strains have been isolated from pyrite- and chalcolyte-leaching bioreactors (59). It should also be noted that A. aeolicum is not the only representative of the family Ferroplasmaceae isolated from sites with high geothermal activity, rich in ferrous sulfides, hydrogen sulfides, and sulfur dioxide. There are quite a number of similar moderately thermophilic strains isolated by our laboratory from South Europe’s stratovolcanos, with high hydrothermal activity observed on the sites in forms of hot springs along the costs, solfataras, and fumaroles (Fig. 3).
Sequences of archaea of the family Ferroplasmaceae were also recovered from Red Sea samples taken from Atlantis II, the world’s largest marine polymetallic ore body (R. Z. Abdallah, personal communication). Many questions arise in connection with these local environmental conditions, such as atmosphere, salinity, pH range, and general features of seawater promoting buffering capacity. However, the findings summarized above undoubtedly represent only a very small proportion of still-undetected and uncharacterized family members.

In seeking an explanation for why the Ferroplasmaceae are so globally ubiquitous (which is reflected in numerous literature references and sequence databases’ entries), one might consider the global abundance of iron, which is the fourth most abundant chemical element in the Earth’s crust and is found in concentrated deposits widely distributed across all continents. The iron-containing mineral pyrite is the most ubiquitous sulfide mineral on Earth, accumulated in sites of hydrothermal origin and found in igneous, sedimentary, and metamorphic
rocks. It is quite obvious that different acidic ecosystems with extremely low or moderate-value pHs and high concentrations of iron favorable to iron-oxidizing acidophiles exist in many locations on our Planet. It may also be suggested that there is a sampling bias that favors acid mine drainage (AMD) systems or ore deposits because of biotechnological/commercial importance and their contribution to environmental pollution. Indeed, AMD streams are the cause of a significant negative impact on the surrounding ecosystems due to their contribution of highly acidic waters containing high concentrations of

FIG. 3. Examples of sites of recent isolation or enrichments of Ferroplasmaceae. The sample sites are marked with white arrows. (A) Rock outcrops/sample at the old copper mine Parys Mountain (Anglesey, United Kingdom). The enrichment in the medium 9K contains an organism closely related to \textit{F. acidiphilum} (O. V. Golyshina, unpublished). (B) The hydrothermal pool on Vulcano Island (Italy), the origin of the isolation of \textit{Acidiplasma aeolicum} \textsuperscript{V1}. The culture was initially enriched from the sand/gravel-containing material (right panel) (28). (C) The outlet (right panel) of the hot spring (left panel) on São Miguel Island (Azores, Portugal). The 16S rRNA clone library from the enrichment established with the surface sulfur-containing sample contained sequences closely related to \textit{Acidiplasma aeolicum} (Golyshina, unpublished).
toxic soluble metals to the rivers, seas, and oceans. Further, volcanic acidic environments associated with tectonic activities are another sampling “hot spot” and attract the increasing attention of microbiologists who seek unusual microorganisms and an understanding of their biogeochemical activities. Vulcano Island is an example of an extensively sampled yet unexhausted geothermally active site, where a few dozen new species of thermophilic archaea and bacteria have already been isolated and described. On the other hand, a feasible explanation for the occurrence of the Ferroplasmaceae in such environments, with conditions that are relatively consistent in terms of pH and high concentrations of soluble iron, are their relatively small genomes, predetermining metabolism and narrowing their niche, as discussed earlier (26). The Ferroplasmaceae exhibit a combination of physiological traits compared to other acidophilic prokaryotes that will further be discussed in detail below (see “Physiological variability within Ferroplasmaceae”).

It is interesting to mention that the strains V\textsuperscript{T} (A. aeolicum) and BH2\textsuperscript{T} (A. cupricumulans), isolated from geographically distinct regions and from geologically and geochemically distinct ecosystems (Vulcano Island [Italy] and a mine in Myanmar; acidic volcanic pool and chalcocite- and copper-containing ore, correspondingly) exhibit no mismatches in their 16S rRNA gene sequence; however, the results of DNA-DNA hybridization revealed that the strains indeed belong to two different species (28).

The same is also true for many members of the Ferroplasmaceae isolated from various locations (Fig. 3): most members of Ferroplasma spp. (16) and Acidiplasma spp. exhibited identical 16S rRNA gene sequences within corresponding genera. This does not necessarily mean that all strains belong to the same species and are not distinct physiologically, since the natural variability of mineral substrates, pH, different types of ores, etc., in isolation sites represents a very powerful natural force for evolution and speciation.

In the past few years, numerous examples of uncultured Thermoplasmatales, the so-called “alphabet plasmas,” detected in clone libraries or found in metagenomic sequencing data sets derived from a great variety of environments, have emerged (for a few, see references 3, 38, and 45). SSU rRNA gene sequences of “alphabet plasmas” have a very broad phylogenetic diversity across the order Thermoplasmatales. It is not clear if the epithet “plasma” is appropriate in the context of cellular morphology, i.e., whether these organisms lack the cell wall, since even within Thermoplasmatales, the archaea from genus Picrophilus spp. have a rigid cellular envelope (49, 50) and thus cannot be considered “plasmas.” I therefore believe, to avoid further confusion, these organisms must be defined as “members of Thermoplasmata/Thermoplasmatales” or another taxon, depending on their affiliation within a commonly recognized taxonomic boundary.

**Physiological variability within Ferroplasmaceae.** All members of the family Ferroplasmaceae, with minor exceptions, share quite similar physiological traits, which to some extent makes it difficult to distinguish isolates for provision of taxonomic descriptions. According to the opinion of Valentine (53), the physiology and phylogeny are generally more cohesive in archaea than in bacteria. For example, Valentine places the phylogenetically coherent halophilic archaea that form a phylogenetically tightly clustering group of 22 genera within just one class, known as Halobacteria, which is in sharp contrast to phylogenetically diverse halophilic bacteria that span more than 10 different classes of Bacteria. All members of the family Ferroplasmaceae that have been physiologically characterized share extreme acidophily (optimal pH range of 0.8 to 1.8), a ubiquitous capacity for ferrous iron oxidation (within this family, there are no isolates known that are unable to oxidize ferrous iron), and a strict dependence on a low concentration of yeast extract, common for a vast majority of archaea. Some variations among strains of the two genera comprising the Ferroplasmaceae are known in relation to the ability for chemooorganotrophy, aerobic or facultative anaerobic growth, or temperature growth optima (mesophilic or moderately thermophilic). Mesophilic or moderate thermophilic Ferroplasmaceae exhibit growth at optimal temperatures from 35°C for the most mesophilic strains to 55°C for the majority of thermophilic isolates. Being able to outperform the competitors at moderate temperatures or across a relatively wide temperature range is an important option for these iron oxidizers since iron/pyrite oxidation is an exothermic process. CO\textsubscript{2} fixation rates in F. acidiphilum strains Y\textsuperscript{T} and Y-2 were reported to be relatively low, approximately 12 times lower than those in the optimally grown, iron-oxidizing bacterium Acidithiobacillus ferroxidans (41), a margin similar to that observed for growth rates which are generally low in all members of the Ferroplasmaceae (16, 25). However, Acidiplasma aeolicum Y\textsuperscript{T} grown chemooorganotrophically on glucose and yeast extract, while exhibiting lower growth rates, produces higher biomass yields per unit of consumed substrate (28). Karavaiko and coauthors (34) suggested that the occurrence of more-diverse variants of metabolism, e.g., mixotrophy and general reduction of autotrophy in environments with higher temperatures, is a function of the lower solubility of both oxygen and CO\textsubscript{2} required for iron oxidation and autotrophy. Heterotrophic metabolism in some members of the Ferroplasmaceae may function as an ecological advantage, enabling this group to operate as do Thermoplasma spp., by scavenging organic matter produced in microbial biofilms that are typically formed in iron-based natural environments.

Most of the strains of the Ferroplasmaceae (a majority of F. acidiphilum strains, A. cupricumulans BH2, A. aeolicum V, and further strains of A. aeolicum [O. V. Golyshina, unpublished]) have been found and isolated from solid ores and minerals, volcanic soils, ash particles, or sand/gravel samples or from biofilms and microbial mats (e.g., “F. acidarmanus” fer1 [18]), indicating that the solid-phase-attached, rather than planktonic, cell forms are prevalent in nature. In this relation, the high level of adhesion of F. acidiphilum Y\textsuperscript{T} cells to the surface of pyrite under acidic conditions was studied and explained through the prism of the Deryagin-Landau-Verwey-Overbeek (DLVO) theory, i.e., considering the interaction of two charged surfaces via a thin liquid layer (19). Two different types of biofilm morphology have been found to be produced by the strain “F. acidarmanus” fer1, and further prevalence of the anaerobic type of metabolism has been observed in mature biofilms (7). However, no common quorum-sensing signaling molecules have been detected by the authors in the aqueous phase of bioreactors. Given that the Ferroplasmaceae are non-motile, they may not necessarily require an extra chemical
signal to adhere. Additional studies will be of a great importance for further understanding the mechanisms and kinetics of cellular attachment of the Ferroplasmaceae to solid surfaces.

**Lipid biomarkers.** Archaeal tetraether-based lipids are considered to play a pivotal role in maintaining the pH gradient across the cellular membrane (36, 54). The chemical composition of membrane lipids is therefore relevant, if not the only useful, marker for chemotaxonomy in those archaea. Major membrane lipids of the family members are dibiphytanyl-based tetraether lipids. The polar lipids were reported to be mostly single phosphoglycolipid derivatives based on a galactosyl dibiphytanyl phosphoglycerol tetraether, with minor amounts of mono- and diglycosyl dibiphytanyl ether lipids; and the main respiratory quinones were found to be naphthoquinone derivatives (8, 28). *F. acidiphilum* Y\(^T\) and Y-2 have been reported to have a β-d-glucose moiety in their major glycosyl dibiphytanyl phosphoglycerol lipid (8), whereas the sugar residue in *A. aeolicum* Y\(^T\) was reported to be β-galactopyranosyl (28). Undoubtedly, it is hard to overstate the role of chemotaxonomy in provision of an unambiguous identity of new isolates for their affiliation at the genus level within Ferroplasmaceae and within hierarchically higher taxonomic divisions, especially as a result of poor resolution of SSU rRNA within a single genus.

**Viral control in acidophilic microbial communities.** Microbial life in acidic ecosystems is controlled by factors similar to those in any other microbial community: competitive interactions, predation, and syntrophic and mutualistic interactions, to name a few (32). Viruses seem to play an important role in environments populated by the Ferroplasmaceae. In some acidic systems, e.g., geothermally active sites, environments with temperatures above 80°C have been known to have lower numbers of viruses than other ecosystems due to challenging low pH and high temperatures. Nonetheless, even under such extreme conditions, viruses can effectively control microbial densities, significantly influencing biogeochemical cycles and acting as drivers of microbial evolution (43, 47). At least two viral morphotypes and virus-host associations have been observed in a cryo-electron microscopy study of ultrasmall archaean “ARMAN” cells from biofilm samples from the Richmond Mine at Iron Mountain (California) (13). A further confirmation of the presence of viruses in the environments populated *inter alia* by members of the Ferroplasmaceae is referred to in publications about the presence of sequences of CRISPR, CRISPR-associated *cas* genes, and prophage-associated genes identified in the process of metagenomic data mining from samples from Richmond Mine (Iron Mountain) by the group led by J. F. Banfield. CRISPR-containing loci were initially identified in large metagenome assemblies to harbor approximately 2,400 unique spacer regions, which were further compared to all contigs and unassembled reads. After that, the spacer-based reconstruction of genome fragments of viruses was performed, and finally, the pairing matches between hosts and viruses were established. The observed prevalent matching of only very recently acquired spacers to corresponding viruses suggested a high rate of resistance emergency in acid mine drainage systems (2, 15). About 24 putative prophage-associated genes have been identified in the genome of the strain “*F. acidarmanus*” fer1; prophage-associated genes were also present in the 8-Mbp metagenomic sequencing data set obtained by shotgun sequencing of small-insert libraries derived from the DNA sample from Richmond Mine; importantly, from the isolation site of the strain “*F. acidarmanus*” fer1 (1). Analysis showed that the gene insertion and loss of genes, possibly of phage origin, and the presence of numerous transposases are important factors for increasing heterogeneity within the local population (1).

**Iron-containing proteins in Ferroplasma.** Since organisms from the family Ferroplasmaceae exhibit a number of unusual physiological traits, one can assume that the biochemistry underlying their ability to exist at the known limits of life is also rather unusual. Indeed, a number of studies have been undertaken to gain a deeper insight into the structural-functional peculiarities of proteins from cultured representatives of the Ferroplasmaceae. Investigations on helicase Rad3 from “*F. acidarmanus*” showed the importance of the FeS cluster for “the folding and stability of the auxiliary domain uniquely characteristic to the Rad3 helicase” (44). A number of remarkable characteristics have also been discovered in proteins from *F. acidiphilum* Y\(^T\). Interestingly, a few cloned and heterologously expressed (in *E. coli*) intracellular or membrane-associated enzymes, namely, three alpha-glycosidases, esterase, and DNA-ligase, exhibited very low pH optima *in vitro*, or “pH optimum anomaly” (26, 27). Cloned enzymes were most active in the pH range 1.7 to 4.0, which is unusually low. A possible explanation was that the low pH *in vitro* is mimicking positive charges of amino acids and cations accumulating in the cytoplasm to electrostatically compensate the high proton gradient across the membrane via establishing a high positive membrane potential (∆Ψ) (inside positive), which is the known mechanism for adaptation to the acidity described for eukaryotic cellular structures such as positively charged secretory vacuoles, lysosomes, and mitochondria, where an acidic pH is necessary for certain activities. An alternative but rather speculative explanation was a possible cytoplasmic heterogeneity or cellular compartmentalization (27). Among proteins from *Ferroplasma* spp. characterized thus far, perhaps the most striking was the DNA ligase from *F. acidiphilum* Y\(^T\) (LigFa), the evolutionarily very conserved enzyme important for maintenance of the integrity of DNA in the cell, especially in the context of damaging effects of acid and oxidative stress caused by reactive forms of iron. Comparative characterization of DNA ligases from *F. acidiphilum* and from other hyperacidophilic archaea and phylogenetic neighbors, namely, from *Thermoplasma acidophilum* and *Picrophilus torridus*, from *Sulfolobus acidocaldarius* (Crenarchaeota, inhabiting acidic, metal- and sulfur-rich high-temperature ecosystems), and from an acidophilic iron-oxidizing bacterium, *Acidithiobacillus ferrooxidans*, revealed the uniqueness of the purple-colored LigFa enzyme with respect to its extremely low pH optimum *in vitro* and unusual ferric iron dependency, which contrasts with the magnesium or potassium requirement common in DNA ligases (21). The LigFa is phylogenetically related to DNA ligases from other members of the Thermoplasmatales (T. *acidophilum* and *P. torridus*), and as it turned out, all of them depend on both ATP and NAD\(^{+}\), whereas ligases from *S. acidocaldarius* and *A. ferrooxidans* were exclusively ATP dependent. The dual cofactor dependence has also been described for members of other euryarchaeotic orders, *Thermococcales*, *Thermococcus kodakaraensis*, and *Thermococcus junicolans*, archaea phylogenetically very close to Thermoplasmatales and functioning at high
or very high temperatures (39, 48). The LigFa together with other ligases from archaea of the order Thermoplasmatales is, rather, phylogenetically placed within a cluster of ligases from Crenarchaeota, which is indeed an indication of a special and somewhat unclear placement of Thermoplasmatales on the overall archaeal map. Coincidentally, some proteins present in almost all members of the Euryarchaeota were reported to be missing in the Thermoplasmatales, and conversely, there is a certain core of proteins encoded in the genomes of the Thermoplasmatales which allows one to speculate about a deeper branching of this order within the phylum Euryarchaeota (22). Sequence analysis revealed about 77 archaeon-specific proteins uniquely present only in Thermoplasmata, of which 17 unique proteins were shared by P. torridus and “F. acidarmanus”; almost all of those were annotated as hypothetical proteins. Gao and Gupta (22) have indicated that the members of the order Thermoplasmatales are the closest euryarchaeota to the branching point where the Euryarchaeota and Crenarchaeota emerged, a concept supported by data on distribution of specific archaeal proteins encoded by representative genomes.

Apart from individually cloned and characterized enzymes, iron-containing proteins have further been identified in the total proteome of F. acidiphilum YT (20). About 85% of all proteins in the analyzed proteome retained iron in stoichiometric amounts, and as revealed by the experimental analysis of a subset of proteins picked at random, this element was essential for protein structure and for function. Among the analyzed items were many housekeeping proteins never reported to contain iron or other metals. Importantly, the observed ubiquity of iron-containing proteins was not observed in phylogenetically or physiologically related microorganisms, either in other members of the Thermoplasmatales or in iron-grown A. ferrooxidans: it was an exclusive attribute of F. acidiphilum YT. Based on this observation, a hypothesis was proposed that iron became a major structure-determining component in Ferroplasma proteins, since these organisms permanently thrived in (and never escaped from) the iron-rich environments that were dominant under primordial conditions on Earth but have become extinct since then. In other microorganisms that further evolved outside iron-rich niches, the structural function of iron had to be substituted for by other elements or mechanisms due to the low availability of the former. According to this hypothesis, Ferroplasma represents a relic form of life with a very special evolutionary trajectory.

**Copper and arsenic resistance.** The Ferroplasmaceae often function at high concentrations of iron, and depending on the mineral composition of ores or rocks, copper and arsenic may also be present in quantities toxic for most prokaryotes. Obviously, adaptation mechanisms are essential for archaea operating under such conditions, especially in bioleaching systems. Studies of resistance mechanisms have been carried out with cultures of “F. acidarmanus” fer1 (5, 6). Using proteome analysis, multiple pathways of resistance have been recorded to be activated at copper concentrations of up to 20 g Cu²⁺ liter⁻¹; the induction of DNA repair proteins, such as RadA, type II 60-kDa chaperonins, and chaperone DnaK homologs. Transcription analysis revealed an increase in two mRNA species, transcribed from the genes copZ, encoding the metal binding protein, and copB, encoding the copper-transporting P-type ATPase, in response to high levels of copper (5). Arsenic resistance of the strain “F. acidarmanus” fer1 against up to a 133 mM concentration of arsenite was suggested to be determined by a known As(III) reduction mechanism, whereas no genes for arsenate reductase have been found in the draft genome of “F. acidarmanus” fer1 and the pathway to cope with As(V) was proposed to be entirely novel (6).

**Methanethiol production.** The microbial production of methanethiol is important from an ecological point of view as a contribution to sulfur cycling and considering its potential role as a greenhouse gas; the degradation of methanethiol in some environments, such as freshwater sediments, has also been proposed to be linked to methanogenic activity (37). An observation about methanethiol production by “F. acidarmanus” has been presented (10). Radiolabeled methionine, cysteine, and sulfate were used for methanethiol production by heterotrophically or chemolithotrophically grown “F. acidarmanus”. This study, together with another survey of a high typical sulfate concentration for growth of this archaeon, contributes to the understanding of the role of these archaea in the sulfur cycle in the biosphere (9). The ecological role of methanethiol production by “F. acidarmanus” was proposed to be as a substrate or chemotactic signal for the other community members (10). That compound seems to be an additional (physiological) link between phylogenetically/evolutionarily related methanogenes and members of the order Thermoplasmatales, where the family Ferroplasmaceae is accommodated as well.

**Genomics of Ferroplasmaceae.** Members of the Ferroplasmaceae are widely represented in acidic environments that were recently subjected to extensive environmental genomics studies (4, 52). Later, the genome sequencing of the isolate “F. acidarmanus” fer1 was conducted (1). The genome sequence assembly of “F. acidarmanus” fer1 was obtained from 41,779 small-insert library reads and additional 1,537 end sequences delivered from a fosmid library. The resulting draft genome contained a number of gaps, which have been filled using the metagenome data. The size of the genome was reported to be 1.94 Mb, with 1,963 predicted open reading frames (ORFs), of which 69.4% were genes with functional predictions and 15.3% and 15.2% were represented by conserved hypothetical and hypothetical proteins. The study was accompanied by comparative genome analysis of the fer1 isolate with its environmental population, fer1(env), from the same sampling site. Given the high abundance of the Ferroplasmaceae in the sample, the total of 103 Mb of combined environmental sequencing data from different samples covered almost 92% of the fer1 genome. The difference between fer1 and fer1(env) genomes was in just 45 genes present in the environmental population but missing in the fer1 isolate and 152 genes occurring in the isolate and not present in the composite fer1(env) data. Corresponding gene products were mostly conserved hypothetical and hypothetical proteins, integrases, transposases, restriction/modification, DNA repair, and transport proteins, and glycosyltransferases. Furthermore, there were clear indications of genomic heterogeneity within the natural population, with a predominance of a small number of sequence types and a strong stabilizing selection for almost all genes (1). Apparent genome rearrangements in the isolate fer1 detected after comparison of Sanger sequencing data sets from a small-insert library and fosmids established a
few years after the latter were the likely cause of gaps in the genome assembly. Such genome instability was reported earlier for *Ferroplasma acidiphilum* isolates (35). The availability of genomic data for *Ferroplasma* spp. has stimulated a number of studies for experimental validation of physiological implications of genome-based *in silico* metabolic reconstructions conducted in earlier investigations pioneered by Tyson and colleagues (52). In particular, blue copper-heme sulfocyanin and cytochrome *ccbb* have been suggested to play an important role in ferrous oxidation and to act as a terminal electron acceptor, respectively (17). A very recent proteome study has pinpointed a number of proteins involved in iron homeostasis in the *Ferroplasmaceae* (42).

Concluding remarks. The wide geographical dissemination across all continents, their ecological importance in iron and sulfur cycling, and the uniqueness of their biochemical machinery make the microorganisms comprising the family *Ferroplasmaceae* an important subject for studies in general microbiology, biochemistry, microbial evolution, origins of life, astrobiology, and biotechnology. Although a great deal of progress has been made, I anticipate that future research will greatly expand our knowledge of this archaeal group. Comprehensive studies of chemical composition variations in membrane lipids and detailed protein structural-functional studies will help in gaining a deeper insight into the mechanisms of adaptation to low pH. The cultivation and description of new members, especially representatives of the *Ferroplasmaceae* from a marine milieu, will undoubtedly contribute to our understanding of how these organisms were, and are, disseminated across remote geographical regions isolated by oceans yet express highly similar genomic composition and possess identical molecular taxonomy markers. Whether the ocean floor expresses highly similar genomic composition and possesses identical genomics and proteomics studies on AMD communities characterized by indigenous acidophilic bacteria. *Hydrometallurgy* 85:184–194.


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