Invited commentary: lubricating the rusty wheel, new insights into iron oxidizing bacteria through comparative genomics

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A commentary on

Comparative genomics of freshwater Fe-oxidizing bacteria: implications for physiology, ecology, and systematics
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Iron is the fourth most abundant mineral in the Earth's lithosphere (Weber et al., 2006; Emerson et al., 2013), where it is present at a mean concentration of 5% (Hedrich et al., 2011). Iron can exist in two oxidation stages as ferrous (Fe(II)) and ferric (Fe(III)) iron and some bacteria and archaea have evolved to use iron as an obligate or facultative energy source, giving them the name "Iron Oxidizing Bacteria" (FeOB) and "Iron Oxidizing Archaea" (FeOA), respectively (**Figure 1**). The oxidation from Fe(II) to Fe(III) can occur under both oxic and anoxic conditions within a pH range between 0.5 to 8.4 (Edwards et al., 2000; Weber et al., 2006; Hedrich et al., 2011). Here, the microbially mediated oxidation of iron under (micro-)aerobic circum-neutral conditions will be discussed.

The biogeochemistry of iron poses several challenges to iron oxidizing microbes (for a detailed review see Weber et al., 2006). In circum-neutral environments and in the presence of oxygen, rapid abiotic oxidation of Fe(II) occurs, with half-life times of less than 60 s (Emerson et al., 2010). This abiotic oxidation to poorly soluble iron-oxyhydroxides reduces the availability of Fe(II) for biological process significantly. Microbial oxidation of Fe(II) therefore generally occurs in microaerophilic environments, which extend the half-life time of ferrous iron while at the same time increasing the amount of soluble Fe(II) (Bonnefoy and Holmes, 2012). Further, the oxidation from ferrous to ferric iron under low oxygen partial pressure increases the Gibbs free energy yield from 29 to -90 kJ mol^{-1} Fe(II) (Emerson et al., 2010).

Iron oxidizing bacteria and archaea have contributed on a global scale to shape the lithosphere (Emerson and Moyer, 2002; Weber et al., 2006). Evidence of their impact on the global iron cycle has been dated back to Pre-Cambrian age where they are thought to have potentially aided in the deposition of banded iron formations (Hedrich et al., 2011). Even today they have a major impact on terrestrial and aquatic systems. FeOB and FeOA can be found in habitats ranging from deep sea vents to freshwater systems and the rhizosphere and can form thick mats and deposits from a few millimeters to tens of centimeters thick. More recently, these microbes have made their appearance in man-made environments and industrial processes (Valdes et al., 2008). Most commonly known may be their contribution to biofouling and corrosion where the build-up of ferric iron can lead to a reduction in the water flow rates and water quality (Hedrich et al., 2011; Mcbeth et al., 2011).

Iron oxidizing microbes and their geological significance were first described in the 19th century (Ehrenberg, 1837; Harder, 1919). They are of key importance to global biogeochemical processes and more recently have gained interest for their impact on man-made environments (Hedrich et al., 2011). Yet, progress in understanding the ecology, physiology and genetic analysis of iron oxidizing microbes has been slow (Weber et al., 2006). Although iron oxidizing microbes can be readily observed in nature due to their striking morphology (such as the iconic stalk-forming *Gallionella*) and ability to form large microbial mats, they have proven elusive to cultivation under laboratory conditions. This elusiveness has caused confusion in the past, where taxonomy was inferred exclusively by morphological and ecological characteristics (Emerson et al., 2010). Recent advances in culturing techniques (Fabisch et al., 2013; Tischler et al., 2013) and culture-independent high-throughput DNA sequencing methods are providing a clearer phylogenetic picture of these bacteria (Emerson et al., 2010).

The recent availability of metagenomic data in particular has greatly accelerated our understanding of iron oxidizing microbes (Wang et al., 2011; Yelton et al., 2013). Only 54 bacterial and 13 archaeal genomes of FeOB are publicly accessible to date (Weber et al., 2006; Cardenas et al., 2010; Emerson et al., 2010; Hedrich et al., 2011), on which the emerging sequence-based taxonomic framework is built upon
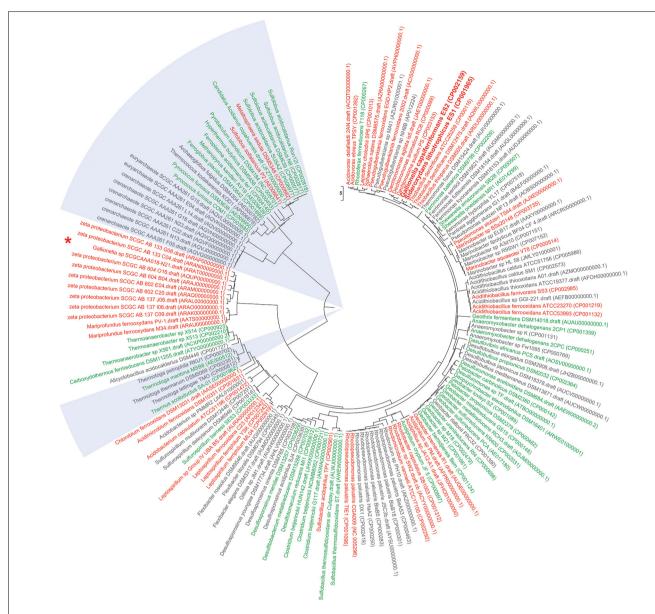
FIGURE 1 | Functional Genome Distribution Tree of iron oxidizing and iron reducing microbes. 158 genomes of publicly available known (and predicted) iron oxidizing and iron reducing bacteria and archaea were included into a Functional Genome Distribution (FGD) analysis (Altermann, 2012). Briefly, draft genomes were downloaded in FASTA format, concatenated and their respective gene models predicted through an updated version of the GAMOLA annotation pipeline (Altermann and Klaenhammer, 2003). Complete genomes were downloaded in Genbank format. All 158 genomes were then subjected to an FGD analysis using the compACTor software. Briefly, within an FGD analysis complete ORFeomes are compared to each other, providing a snapshot of genome-to-genome similarities, rather than an ancestral outlook. In summary, the 158 genomes comprised 501,624 open reading frames (ORFs) and a total of 79,881,099 individual BLAST analyses were carried out to calculate the level of similarities between all genomes. The resulting dissimilarity matrix was then visualized in Mega6 (Tamura et al., 2013) using the UPGMA algorithm to infer a dissimilarity tree. The early draft sequence of *Leptothrix ochracea* L12 was found to be too incomplete and was removed from the final tree. Iron oxidizing bacteria and archaea are shown in red; iron reducing bacteria (FeRB) and archaea (FeRA) are depicted

in green; likely iron oxidizing/reducing candidate microbes are indicated in gray. Archaeal iron oxidizers and reducers are highlighted in light blue sections. Accession numbers for respective Genbank or assembly entries are listed in brackets behind strain designations. *Sideroxydans lithotrophicus* ES1 and *Gallionella capsiferriformans* ES2 are shown in bold. Interestingly, *Sideroxydans lithotrophicus* ES1 and *Gallionella capsiferriformans* ES2 form their own sub-cluster and are most similar to *Thiobacillus* and *Dechloromonas* genomes. The recently published draft genome of *Gallionella* sp SCGCAAA018-N21 (marked by "*") does not fall into the ES1/ES2 cluster, but groups within the larger zeta proteobacterium cluster. This may indicate the need to further investigate whether N21 represents a true *Gallionella* strain. In most instances, iron oxidizing and iron reducing microbes form their own separate cluster. There are notable exceptions (e.g., *Rhodoferax ferrireducens* T118 or *Geobacter bremensis* R1) and it may be possible that those microbes are able to carry out both iron oxidation and iron reduction, depending on their respective environmental circumstances. Most candidate iron oxidizing or iron reducing bacteria and archaea fall within distinct clusters that harbor examples of confirmed phenotypes. This may assist future research in systematically test for iron oxidizing or iron reducing phenotypes.

(**Figure 1**). New information from culti-vated strains still refines this framework and the recently introduced class of marine Zetaproteobacteria (Emerson et al., 2007) is an excellent example of this rapid taxo-nomic evolution. An overview of publicly available genomes of iron oxidizing (and reducing) microbes and their relation to each other is presented in **Figure 1**.

While metagenomic datasets are valu-able in improving our understanding of the presence and composition of iron oxi-dizing microbial communities (Ionescu et al., 2012; Kato et al., 2013; Singer et al., 2013), they rely on a robust gene-based taxonomic framework. Such a framework and detailed metabolic recon-struction of individual microbial strains require access to (draft) genome sequences and, consequently, to pure strains of iron oxidizing microbes (Kato et al., 2013). Despite the requirement for new strains of iron oxidizing microbes, iso-lation and characterization is challeng-ing and time consuming. For example, *Gallionella* were first described in 1837 (Ehrenberg, 1837) and are amenable to cultivation. However, it was not until the mid-1990s where cultivation of two further strains, *Gallionella ferruginea and Gallionella capsiferriformans* ES-2, was successful (Hallbeck et al., 1993; Emerson and Moyer, 1997). Similarly, the isolation and characterization of other freshwater Fe(II) oxidizing Betaproteobacteria such as *Sideroxydans* sp. ES-1, that is metabol-ically flexible and can utilize reduced sulfur compounds and fix nitrogen, were also achieved only in the 1990s (Emerson and Moyer, 1997).

Since the initial description of both *Gallionella capsiferriformans* ES-2 and *Sideroxydans lithotrophicus* ES-1 a num-ber of publications have investigated their morphological, phenotypical and industrial characteristics (Hallbeck and Pedersen, 1995; De Vet et al., 2012; Krepski et al., 2012), but no information was avail-able on their genetic blueprint. The recent publication by Emerson et al. (2013), presenting the comparative analysis of genomic sequences of both *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsi-ferriformans* ES-2, has filled this gap. By combining morphological characteristics with *in silico* comparative genomic analy-ses the authors were able to demonstrate

that, although isolated from the same habitat, an iron-mat from ferruginous groundwater in Michigan (Emerson and Moyer, 1997), both strains belong to dif-ferent genera in the recently proposed order Gallionellales. Importantly, the genomic comparison and metabolic path-way reconstruction highlighted a number of similarities and differences between both strains: 60% of the predicted genes were homologous at the 30% similarity level and both strains share a number of core metabolic capabilities for envi-ronmental sensing (chemotaxis), motility (pili), the uptake of nutrients through ABC transporters, energy metabolism, and detoxification of heavy metals, although strain ES-2 harbors a greater array of detoxification systems than ES-1 implying a superior ability to persist in habitats rich in environmental toxins. Notably, ~10% of both genomes is dedicated to environ-mental sensing and signal transduction which is a significantly higher number of genes than found in most other microbial genomes. Both strains harbor complete sets of chemotaxis genes and in combi-nation with the elaborate environmental sensing array, controlled movement along the opposing gradients of Fe(II) and oxy-gen may facilitate optimal positioning of individual cells.

One of the problems aerobic Fe(II) oxidizing microbes must overcome is the presence of highly reactive oxygen species (ROS) that are produced via Fenton chem-istry in the presence of oxygen and iron. Catalase and superoxide dismutase are the most common defense mecha-nisms against ROS and surprisingly both strains feature only a minimal set of cata-lase and peroxidase genes. This may be compensated by a number of truncated hemoglobins that are highly conserved and found in other FeOB such as *M. ferrooxy-dans* PV-1. These hemoglobins may rep-resent an intracellular oxygen buffer that reduces the occurrence of ROS by binding oxygen under microaerophilic conditions and high concentrations of Fe(II).

Interestingly, strains ES-1 and ES-2 differ in their strategies to generate ATP. While ES-2 relies on the alterna-tive complex III and a bacterial ATPase, ES-1 employs Cytochrome bc1, and a bacterial/archaeal-type ATPase in addition to the bacterial ATPase. The advantages of

a second ATPase are presently unknown and the distribution of such redundant systems throughout the genomes of iron oxidizing microbes may provide clues to their respective niche adaptation in the future.

Both strains harbor prophage that may have been acquired independently and do not share homologous genes. However, the ES-1 prophage shares significant nucleic acid similarity (24%) to a prophage gene cluster identified in *Mariprofundus fer-rooxydans PV-1* (Singer et al., 2011), pos-sibly indicating shared hosts in the past. Neither of the two strains features CRISPR sequences that have long been associated with phage defense, posing an interesting question on the evolutionary pressure of bacteriophage in iron oxidizing communi-ties. It is generally accepted that phage and their respective hosts are locked into an ongoing arms race that shapes their evo-lution (Stern and Sorek, 2011). Emerging genomes of iron oxidizing microbes will provide deeper insights into the defense mechanisms these microbes deploy to evade phage infection and killing.

Neither of the two strains features any of the unique extracellular structures such as sheaths or stalks often associated with FeOB. Genome analysis revealed that strain ES-1 feature a single large gene cluster dedicated to exopolymer synthe-sis, while ES-2 harbors two separate large EPS clusters in addition to two gene clus-ters possibly involved in cellulose syn-thesis. The presence of exopolysaccharide (EPS) gene clusters may provide clues how those strains prevent self-encrustation by iron-oxyhydroxides but further *in vitro* research is required to unravel these survival strategies.

Recently, the draft genome sequences of *Gallionella* sp. SCGC AAA018-N21 have been released (BioSample: SAMN02256456) and a future compar-ative genomics analyses will undoubtedly reveal further insights in lifestyle adap-tation and contribute to a more robust taxonomic framework.

The research presented by Emerson et al. contributes significantly to our understanding of neutrophilic freshwater iron-oxidizing bacteria and their taxo-nomic relations to each other. However, the intriguing features found in both genomes—either commonly shared or

specific to the individual strains—have created a large, new research space waiting to be investigated.

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