METAGENOMIC ANALYSIS OF NITRATE-DEPENDENT IRON OXIDIZING ENRICHMENT CULTURES. S. He[1], A. Kappler[2], S. Behrens[3], and E.E. Roden[1], [1]Department of Geoscience, University of Wisconsin-Madison, 1215 W Dayton St, Madison, WI 53706, USA, [2]Center for Applied Geosciences (ZAG), Eberhard-Karls-University Tuebingen, Sigwartstrasse 10, D-72076 Tuebingen, Germany, [3]Department of Civil, Environmental, and Geo-Engineering, University of Minnesota, 117 Pleasant Street SE, Minneapolis, MN 55455, USA

Introduction: Aqueous and solid-phase ferrous iron (Fe(II)) compounds can serve as an electron donor for chemolithotrophic iron-oxidizing microorganisms under both oxic and anoxic conditions. Fe-based chemolithotrophy may have played a role in past (and possibly, present) life on Mars, whose crust has a relatively rich content of Fe(II)-bearing silicate minerals, as well as Fe(II)-bearing phyllosilicate minerals formed during weathering of primary silicates. Nitrate-dependent ferrous iron oxidation (NDFO) is well-recognized chemolithotrophic pathways in terrestrial and marine anaerobic sediments. A chemolithoautotrophic enrichment culture, originally obtained from freshwater sediment [1], has been used as a model system for physiological and geochemical studies of NDFO [2]. However the primary iron oxidizer in this enrichment culture, a bacterium closely related to *Sideroxydans lithotrophicus* ES-1 in the family of *Gallionellaceae* has not been isolated despite extensive efforts attempting to culture it. In this study, we conducted a metagenomic analysis of this enrichment culture in order to understand the electron transfer pathways involved in nitrate-dependent iron oxidation and the roles of the flanking community members, and to gain insights on why it is difficult to isolate this ES-1 related iron oxidizer.

Method: The metagenomic analyses was conducted on two versions of NDFO enrichment culture. One version has been maintained at the University of Tuebingen (Germany) since the initial enrichment, and the other has been independently maintained at the University of Wisconsin, Madison (WI, USA) since 1998. Next-generation high throughput sequencing was performed on DNA extracted from both cultures, sequences were assembled, binned to different organisms, and draft genomes from the abundant community members were recovered.

Results: From the metagenomes, we found that community composition differed between the two enrichments. However, the primary iron oxidizer, i.e. the bacterium in the *Gallionellaceae* family, remained as the predominant community member, and presumably is the core (essential) member of the enrichment cultures. Due to its high abundance, we obtained its near-complete genome. Draft genomes were also obtained from flanking community members belonging to the family *Comamonadaceae*, and the genera *Bradyrhizobium*, *Rhizobium*, and *Rhodanobacter*, respectively.

We search for putative extracellular electron transfer pathways in these draft genomes. In *Gallionellaceae* sp., we found homologs of *MtoAB*, which were suggested to be involved in aerobic Fe(II) oxidation by ES-1 [3]. Specifically, *MtoA* in *Gallionellaceae* sp. encodes a c-type cytochrome with 10 heme-biding sites, and *MtoB* is predicted to be an outer membrane porin-like protein with 28 transmembrane regions, consistent with their hypothesized role in extracellular Fe(II) oxidation. Similar porin-cytochrome c model was also found in some flanking community members, as well as a novel porin-multicopper oxidase model, suggesting that some of these flanking members may be also involved in Fe(II) oxidation.

Draft genomes also enable us to learn more about their metabolisms and potential interactions. For example, RuBisCo and related genes required for carbon fixation are present in *Gallionellaceae* sp. draft genome. Together with the scarcity of transporters for organic substrates, this confirms its autotrophic life style. The RuBisCo system was also found in each of the flanking community members except for the heterotrophic *Comamonadaceae* sp., which may rely on other autotrophic community members for a fixed carbon source. As a nitrate reducer, dissimilatory nitrate reductase and nitrite reductase genes were found in *Gallionellaceae* sp. as expected. However, nitric oxide and nitrous oxide reductase genes are missing. Instead, the complete denitrification pathway is present in the flanking populations. Therefore, we hypothesize that the flanking populations play an important role in consuming nitric oxide to prevent its toxic build-up. This might explain the difficulty in isolating *Gallionellaceae* sp. since its genome does not seem to lack any amino acid or vitamin biosynthesis pathways.

Implications: This study revealed putative extracellular electron transfer pathways in NDFO enrichment bacteria, their metabolic potential and interspecies interactions. Our results significantly expand our knowledge of the metabolic diversity of FeOB, and provide a range of new targets for genomics-based analysis of the evolutionary relationships, molecular mechanisms, and environmental regulation of Fe(II)-oxidizing chemolithotrophs.

References: [1] Straub K.L. et al. (1996) *Appl Environ Microbiol, 62,* 1458-1460. [2] Blothe M. and Roden E.E. (2009) *Appl Environ Microbiol, 75*, 6937–6940. [3] Liu J. et al. (2012) *FMICB*, *3*, Article 37.