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# Diverse manganese(II)-oxidizing bacteria are prevalent in drinking water systems

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## Summary

**Manganese (Mn) oxides are highly reactive minerals that influence the speciation, mobility, bioavailability and toxicity of a wide variety of organic and inorganic compounds. Although Mn(II)-oxidizing bacteria are known to catalyze the formation of Mn oxides, little is known about the organisms responsible for Mn oxidation *in situ*, especially in engineered environments. Mn(II)-oxidizing bacteria are important in drinking water systems, including in biofiltration and water distribution systems. Here, we used cultivation dependent and independent approaches to investigate Mn(II)-oxidizing bacteria in drinking water sources, a treatment plant and associated distribution system. We isolated 29 strains of Mn(II)-oxidizing bacteria and found that highly similar 16S rRNA gene sequences were present in all culture-independent datasets and dominant in the studied drinking water treatment plant. These results highlight a potentially important role for Mn(II)-oxidizing bacteria in drinking water systems, where biogenic Mn oxides may affect water quality in terms of aesthetic appearance, speciation of metals and oxidation of organic and inorganic compounds. Deciphering the ecology of these organisms and the factors that regulate their Mn(II)-oxidizing activity could yield important insights into how microbial communities influence the quality of drinking water.**

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## Introduction

The presence of manganese (Mn) in source waters used for drinking water (DW) production is a common concern, primarily due to the impact of Mn on the aesthetic quality of finished water (Kohl and Medlar, 2006). Mn is regulated by the U.S. Environmental Protection Agency at a nonenforceable secondary maximum contaminant level (MCL) of 0.05 mg/l (EPA, 2013). Regardless of the possible direct effects of Mn on human health (Bouchard *et al*., 2011; Khan *et al*., 2012), the presence of Mn oxides in DW can strongly alter other aspects of water chemistry (Tebo *et al*., 2004), thus they may indirectly influence the quality of DW.

Mn oxides can rapidly oxidize other metals and metalloids (metal(loid)s hereafter), affecting their speciation, mobility and toxicity. For example, Mn oxides can convert highly toxic trivalent arsenic into the less acutely toxic pentavalent form (Tournassat *et al*., 2002). In addition to their strong redox activity, Mn oxides have a high capacity for cation exchange, sorption and coprecipitation of metal(loid)s (Tebo *et al*., 2004). Thus, they can sequester and immobilize a wide array of heavy metal(loid)s and have been used for this purpose in DW (Driehaus *et al*., 1995; Bajpai and Chaudhuri, 1999). Mn oxides contribute to the decomposition of complex organic matter (Sunda and Kieber, 1994) and effectively degrade some endocrine disruptors (de Rudder *et al*., 2004; Furgal *et al*., 2015). Mn oxides often have detoxifying effects on pollutants, however they can also catalyze reactions that increase the toxicity of some metal(loid)s. Particularly relevant to DW is chromium (Cr), which is rapidly oxidized by biogenic Mn oxides from a relatively harmless form, Cr(III), to a highly mobile, bioavailable and carcinogenic form, Cr(VI) (Murray *et al*., 2005; Costa and Klein, 2006).

Under oxic and circumneutral pH conditions, the abiotic oxidation of aqueous Mn(II) to solid Mn oxides is thermodynamically favorable but kinetically slow relative to bacterially mediated Mn(II) oxidation (Tebo *et al*., 2004). Thus, the oxidation of Mn(II) to Mn(III) and Mn(IV) in the environment is believed to be largely catalyzed by

bacteria (Tebo *et al*., 2007). In contrast, Mn removal during DW treatment is commonly achieved through abiotic oxidation of dissolved Mn(II) with chlorine or ozone followed by filtration of particulate and colloidal Mn oxides (Kohl and Medlar, 2006). Biological Mn removal as an alternative to traditional chemical processes is increasing in popularity (Tekerlekopoulou *et al*., 2013) and removal efficiencies of over 98% have been demonstrated (Tekerlekopoulou *et al*., 2013; Hoyland *et al*., 2014). The documented presence of Mn(II)-oxidizing microorganisms in DW systems that are not specifically operated for biological Mn oxidation (Sly *et al*., 1988; Cerrato *et al*., 2010) raises the question of their broader role and utility in DW systems.

Characterizing the role of microorganisms in mediating the formation of Mn oxides in complex systems, including DW systems, is challenging due to the extensive diversity of Mn(II)-oxidizing bacteria and mechanisms by which Mn(II) oxidation can be catalyzed. Distantly related bacteria from phyla such as *Firmicutes*, *Actinobacteria* and *Proteobacteria* can oxidize Mn(II) (Tebo *et al*., 2005), and the ability to oxidize Mn(II) is not always conserved between phylogenetically closely related bacteria (Dick *et al*., 2008; Anderson *et al*., 2009).

The presence and role of Mn(II)-oxidizing bacteria in DW systems has been investigated only to a limited extent. In some cases, well characterized Mn(II)-oxidizing groups such as *Pseudomonas*, *Bacillu*s and *Leptothrix* have been identified in DW systems (Katsoyiannis and Zouboulis, 2004; Burger *et al*., 2008; Cerrato *et al*., 2010; Farkas *et al*., 2013), but it remains unclear if they are abundant and to what extent they contribute to Mn cycling. Despite recent advances in DNA sequencing technologies and in understanding the molecular mechanisms of bacterial Mn(II) oxidation, identifying Mn(II)-oxidizing bacteria with culture-independent methods remains challenging since there are no known universal molecular markers for Mn(II) oxidation. Therefore, cultivation remains an important means of identifying Mn(II)-oxidizing bacteria. This poses a significant hurdle in identifying environmentally relevant Mn(II)-oxidizing bacteria as only a small portion of community members are typically able to be cultivated (Staley and Konopka, 1985). However, for those Mn(II)-oxidizing bacteria that are amenable to laboratory growth, high throughput DNA sequencing can be used to elucidate their abundance (or the abundance of closely related taxa) within complex microbial consortia.

In this study, we cultured and characterized Mn(II)-oxidizing bacteria from one DW treatment plant and two surface water sources used for DW production. We describe several novel Mn(II)-oxidizing bacteria, and found that some were abundant members of the analyzed DW treatment system's microbial communities, suggesting a high capacity for biological Mn(II) oxidation in DW systems.

## Results

### *Isolation and phylogenetic characterization of Mn(II)-oxidizing bacteria*

We isolated Mn(II)-oxidizing bacteria from surface sediments of Lake Erie and the Huron River (Ann Arbor, MI), and from the DW treatment and distribution system (DWDS) in Ann Arbor, MI. A total of 29 Mn(II)-oxidizing bacteria were isolated, and 21 of the 16S rRNA gene sequences obtained were unique (shared a maximum of 99% sequence identity; Table 1). The isolates are taxonomically diverse and fall within the high and low GC gram-positive bacteria (*Actinobacteria* and *Bacillaceae*, respectively) and *Alpha*-, *Beta*- and *Gammaproteobacteria*. Many of these groups were previously known to harbor Mn(II)-oxidizing bacteria (Tebo *et al*., 2005). However, as discussed below in more detail, we also identified bacteria in three genera not previously associated with Mn(II) oxidation (Table 1).

Phylogenetic analysis of 16S rRNA gene sequences was used to investigate relationships between the Mn(II)-oxidizing bacteria found in this study and those previously reported in the literature. The majority of *Firmicutes* and *Pseudomonas* (Phylum: *Proteobacteria*, Class: *Gammaproteobacteria*) isolates fell into lineages that contain previously identified Mn(II)-oxidizing bacteria. Four of the unique *Firmicutes* isolates fell within the genus *Bacillus*; one clustered with organisms isolated from marine sediments that oxidize Mn(II) as metabolically dormant spores (Francis and Tebo, 2002; Dick *et al*., 2006), two were closely related to *Bacillus muralis*, and one to *Bacillus niacini*. The remaining two phylogenetically distinct *Firmicutes* isolates clustered tightly with *Lysinibacillus* (Supporting Information Fig. S1), which are known to oxidize Mn(II) (Johnson *et al*., 2011).

Two of the Mn(II)-oxidizing *Pseudomonas* isolates clustered with two separate clades of Mn(II)-oxidizing bacteria isolated from cave deposits (Carmichael *et al*., 2013). Two isolates were in a clade with *Pseudomonas chlororaphis*, and *Pseudomonas extremorientalis*; the latter was isolated from a DW reservoir (Ivanova *et al*., 2002) (Supporting Information Fig. 2). All of the Mn(II)-oxidizing *Pseudomonas* isolates have 97–98% 16S rRNA gene sequence identity to *Pseduomonas putida* strains Mn-B1 and GB-1. Strain Mn-B1 was isolated from a Mn-encrusted DW pipe in Germany (Schweisfurth, 1973) and both organisms have long been used as model systems for studying bacterial Mn(II) oxidation (Caspi *et al*., 1998; Geszvain and Tebo, 2010).

**Table 1.** Sequencing effort and number of Mn(II)-oxidizing bacteria isolated.

| | | | | Location | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | LE | HR | AA-W | AA-In | AA-Flt | AA-Ef | AA-Rs | AA-DS | | |
| # 454 seqs: | | | | 0 | 35,986 | 24,419 | 23,220 | 45,901 | 43,916 | 29,666 | 389,053 | | |
| # Sanger seqs: | | | | 0 | 0 | 0 | 0 | 51 pairs | 0 | 0 | 49 | # Total isolates | # Unique isolates |
| **Number of isolates for each taxonomic group** | Actinobacteria | Microbacteriaceae; Microbacterium | | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 |
| | | Microbacteriaceae; **Agromyces** | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | | Mycobacteriaceae; Mycobacterium | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | | Micrococcaceae; Arthrobacter | | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| | Firmicutes | Bacillaceae; Bacillus | | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 7 | 4 |
| | | Bacillaceae; Lysinibacillus | | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 2 |
| | Proteobacteria | Gammaproteobacteria; Pseudomonas | | 1 | 4 | 1 | 0 | 0 | 0 | 1 | 0 | 7 | 4 |
| | | Betaproteobaceria; Burkholderiales; Comamonadaceae; **Hydrogenophaga** | | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 4 | 3 |
| | | Alphaproteobact. | **Rhodospirillales**; **Reyranella** | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| | | | Rhizobiales; Bradyrhizobiaceae, Afipia | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | | | Rhizobiales; Bradyrhizobiaceae, Bosea | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |

Bolded taxa are taxonomically novel Mn(II) oxidizers. Phylogenetically equivalent isolates were 100% identical to one another. AA, *Ann Arbor*; LE, *Lake Erie*; HR, *Huron River*; W, *Well*; In, *Filter Influent*; Flt, *Filter*; Ef, *Filter Effluent*; Rs, *Reservoir*; DS, *Distribution System*; # tot. isol., Number of Total Isolates; # uni. isol., Number of Unique Isolates.

 

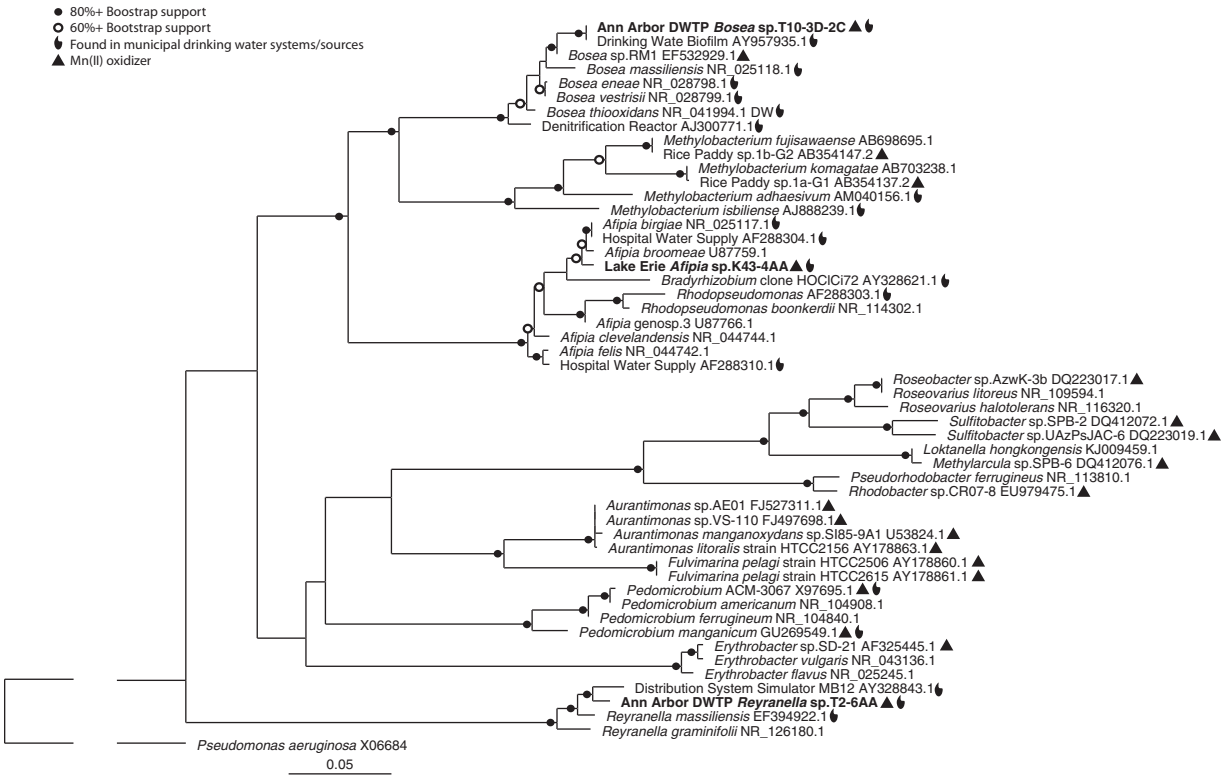


**Fig. 1.** Maxmimum likelihood phylogenetic tree of 16S rRNA gene sequences for the class *Alphaproteobacteria*. Sequences in bold are from this study. Trees were bootstrapped 1000 times using RAxML (Stamatakis, 2014) with the GTRGAMMA model of nucleotide substitution. The dashed branch to the outgroup was condensed.

Mn(II)-oxidizing bacteria were identified in three genera of *Alphaproteobacteria*, including *Afipia*, *Bosea* and *Reyranella* (Fig. 1). To our knowledge, *Reyranella* has not been implicated in Mn(II) oxidation previously. Mn(II)-oxidizing *Afipia* strains have previously been found in rock varnishes from the Atacama desert in Chile (Kuhlman *et al*., 2008) and New Mexico (Northup *et al*., 2010). A single Mn(II)-oxidizing *Bosea* culture ('RM1') was obtained from a stream containing abundant Mn oxides in the United Kingdom and was subsequently used to evaluate Mn(II) removal in experimental reactors (Mariner *et al*., 2008). *Alphaproteobacteria* are commonly capable of Mn(II) oxidation, and many of these isolates or their close relatives have been observed in DW systems (Fig. 1).

Three phylogenetically unique Mn(II)-oxidizing *Betaproteobacteria* were isolated belonging to the genus *Hydrogenophaga*, which had not previously been known to oxidize Mn(II) (Fig. 2). From the order *Burkholderiales*, three genera of Mn(II)-oxidizing bacteria have previously been reported: *Burkholderia*, *Caldimonas* and *Leptothrix*, all of which have previously been found in DW sources or systems (Zanetti *et al*., 2000; Laseke, 2006; de Vet *et al*., 2009; Laseke *et al*., 2010). Mn(II)-oxidizing *Actinobacteria* clustered within the genera *Mycobacterium*, *Arthrobacter*, *Agromyces* and *Microbacterium* (Supporting Information Fig. S3). To our knowledge, Mn(II) oxidation within the genus *Agromyces* is novel.

### *Abundance of Mn(II)-oxidizing taxa in bacterial communities of DW systems*

To investigate the abundance of Mn(II)-oxidizing bacteria in DW associated microbial communities, 16S rRNA gene sequences retrieved from isolates were compared to culture-independent 16S rRNA gene datasets from the DW system in Ann Arbor, MI. The majority of the Ann Arbor data was obtained by 454 GS-FLX sequencing of the V4-V5 region (Pinto *et al*., 2014). However, two Sanger sequenced clone libraries were created and analyzed to corroborate the findings from 454 amplicon sequencing over additional 16S rRNA gene positions. Given the disparity of sequence lengths between 454 amplicons and assembled near full-length Sanger sequences, an *in silico* comparison of sequence similarites for the V4-V5 region and near full-length 16S rRNA gene sequences was conducted using Silva references. A description of the method can be found in Supporting Information along with obtained results (Supporting

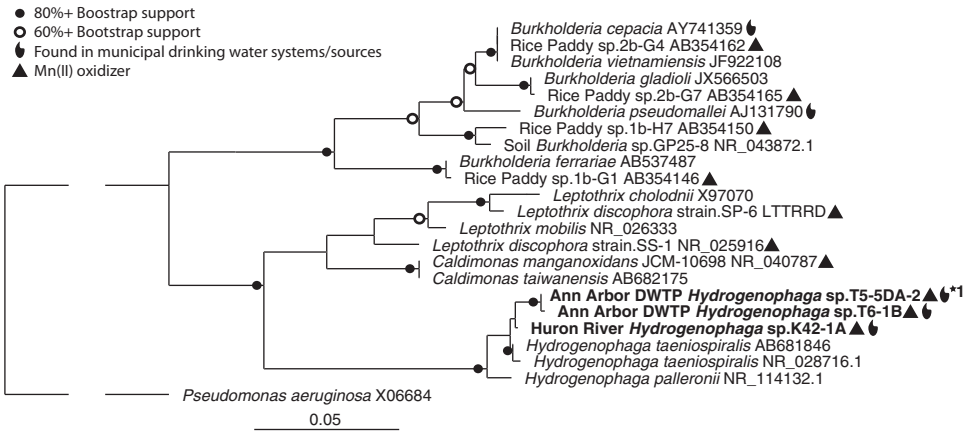


**Fig. 2.** Maxmimum likelihood phylogenetic tree of 16S rRNA gene sequences for the class *Betaproteobacteria*. Sequences in bold are from this study. Trees were bootstrapped 1000 times using RAxML (Stamatakis, 2014) with the GTRGAMMA model of nucleotide substitution. The dashed branch to the outgroup was condensed. ⋆1: one additional isolate with 100% sequence identity was obtained from the AA DW treatment plant.

Information Table S1). Briefly, we found that phylogenetic conservation between the V4-V5 region and the near full-length 16S rRNA gene sequence varied by phylogenetic group.

At a BLASTN threshold of 99% identity over 280 bp, 12 of the 21 phylogenetically unique Mn(II)-oxidizing isolates matched at least 0.5% of recovered 454 bacterial sequences in at least one of the seven Ann Arbor DW system locations (Supporting Information Tables S2 and S3). The two most abundant bacterial genera were *Hydrogenophaga* and *Bosea*. *Hydrogenophaga* sp. K42-1A, which was isolated from Huron River surface sediments, was especially abundant in the filter and filter effluent, where in some samples it represented approximately half of the community sequences recovered (Fig. 3A). The abundance of *Hydrogenophaga* sp. K42-1A was much lower in source water (river and well) and was intermediate in the filter influent, reservoir and distribution system samples. Two additional *Hydrogenophaga* isolates satisfied the 0.5% community abundance threshold specified above, but were consistently much less abundant than *Hydrogenophaga* sp. K42-1A (Supporting Information Table S3).

*Bosea* sp. T10-3D-2C, which was isolated from a filter influent sample, was the second most abundant isolate throughout the DW system recovered in the culture independent dataset (Fig. 3B). In contrast with *Hydrogenophaga* sp. K42-1A, *Bosea* sp. T10-3D-2C was most abundant in the distribution system. The abundance of other isolates recovered in this study that made up at least 0.5% of the total community in one sample can be found in Supporting Information Table S3.

Two clone libraries were created to validate the trends observed from 454 community data. Five of 49 near full-length 16S rRNA gene sequences obtained from multiple pooled Ann Arbor DWDS samples matched Mn(II)-oxidizing isolates with BLASTN cutoffs of 97% ID over 1200 bp. All five hits matched sequences of isolates from either the Ann Arbor DW system or source water. This includes four BLASTN matches to *Hyrogenophaga* spp. isolated from Huron River sediments and the Ann Arbor DW treatment plant intake well and one BLASTN match to *Reyranella* sp. T2-6AA isolated from the Ann Arbor filters. The results confirmed the presence of environmental sequences that matched *Hydrogenophaga* sp. K42-1A with high sequence specificity over more than just the V4-V5 region of the 16S rRNA gene.

A single Ann Arbor filter sample was selected for an additional clone library on the basis of the elevated abundance (53%) of sequences closely related to *Hydrogenophaga* sp. K42-1A in the 454 amplicon data. This library yielded 13 partial 16S rRNA gene sequence clones (out of a total of 51) with at least 97% sequence identity over 800 bp to isolates obtained from the Ann Arbor DW system. Eleven of these clones matched *Hydrogenophaga* spp. (K42-1A, T6-1B and T5-5DA-2), one matched only *Hydrogenophaga* spp. K42-1A and T5-5DA-2 and one matched *Bosea* sp. T10-3D-2C. Thus, both Ann Arbor clone libraries showed sizeable proportions of sequences closely related to *Hydrogenophaga* isolates, corroborating the 454 GS-FLX derived abundance estimates.

*Hydrogenophaga* sp. K42-1A obtained from an Ann Arbor DW source is closely related to one of the most dominant operational taxonomic units (OTUs) in the DW system (Pinto *et al*., 2012; Pinto *et al*., 2014), raising the question of whether such organisms deposit Mn oxides in DW systems. *Hydrogenophaga* species are commonly identified as abundant members of DW microbial

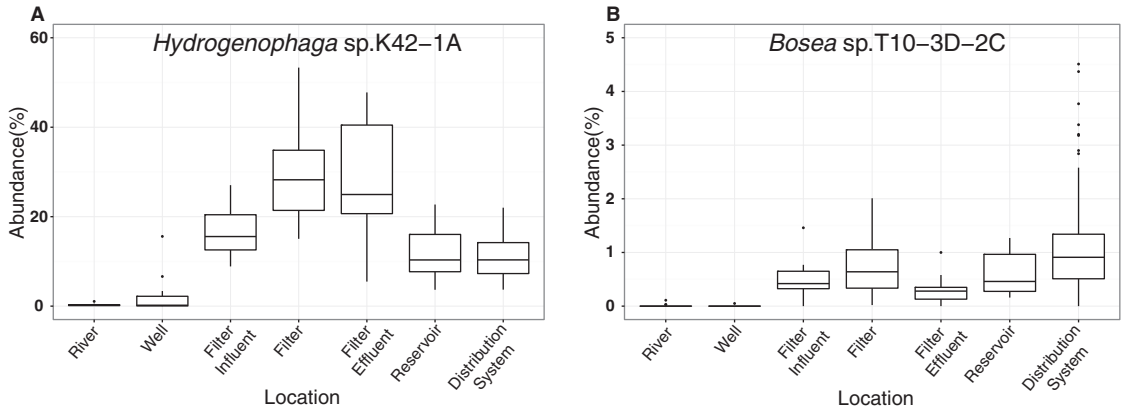


**Fig. 3.** Box and whisker relative abundance plot of pyro tag sequences from Ann Arbor DW system satisfying a 99% identity threshold over at least 280 bp when BLASTN aligned against (A) *Hydrogenophaga* sp. K42-1A and (B) *Bosea* sp. T10-3D-2C.

communities, thus their impact on Mn cycling and associated chemical processes could be widespread and substantial. In bulk water collected from a model, non-chlorinated DWDS in Denmark, nearly 20% of all isolates obtained were *Hydrogenophaga*, and it was the single most abundant taxon identified by almost a factor of two (Martiny *et al*., 2005). In Greece, *Hydrogenophaga* amounted to approximately 8% of all clones recovered from a DWDS (Kormas *et al*., 2010). In a comparison of microbial communities from GAC filters in multiple DW treatment plants, approximately 14% of all isolates obtained were *Hydrogenophaga* (Magic-Knezev *et al*., 2009). Consistent with our findings, Hydrogenophaga has been documented as a dominant community member in numerous DW communities around the world. From source waters through the various stages of DW treatment, there is an increased abundance of taxa associated with Mn(II) oxidation, such as *Hydrogenophaga* (Fig. 3), consistent with major changes in community structure due to treatment processes (Pinto *et al*., 2012). The Ann Arbor DW treatment plant employs ozonation for disinfection prior to filtration (Pinto *et al*., 2012). This process oxidizes natural organic matter into simpler compounds (Becker and O'Melia, 2001) that are more bioavailable. Hence, organisms that survive the highly selective ozonation process could rapidly proliferate in an environment of reduced competition and with readily biodegradable compounds available.

In this study, we isolated Mn(II)-oxidizing bacteria from a conventional DW system and showed that some of the taxonomic groups are novel and some are abundant in communities of bacteria within a DW system. Although previous studies have isolated Mn(II)-oxidizing bacteria from DW systems (Schweisfurth, 1973; Sly *et al*., 1988; Sly *et al*., 1990; Cerrato *et al*., 2010), they have not reported that such bacteria can be abundant members of DW bacterial communities. These results contrast those of other environments where bacterial Mn(II) oxidation is prevalent but cultured Mn(II)-oxidizing bacteria were at such low abundance that they remained undetected with culture-independent methods (Dick and Tebo, 2010). Indeed, few studies have cultured Mn(II)-oxidizing bacteria that were abundant members of their source communities (Bräuer *et al*., 2011).

## Conclusions

Overall, our results have several important implications for understanding bacterial Mn(II) oxidation and its impact on the chemistry of DW. First, the observed abundance Mn(II)-oxidizing bacteria in DW systems suggests that biogenic Mn oxides produced by these organisms could influence DW quality. Highly reactive biogenic Mn oxides (i) alter the speciation, toxicity and mobility of metal(loid)s such as chromium and arsenic and (ii) degrade refractory organic compounds into labile forms, which may influence bacterial regrowth in DW distribution systems (Escobar and Randall, 2001). Second, our results suggest that DW treatment strongly selects for taxa capable of Mn(II) oxidation, potentially amplifying their role in chemical transformations such as those indicated above. Third, the mechanisms of Mn(II) oxidation has not been studied in many of the Mn(II)-oxidizing bacteria isolated here, including the dominant community member *Hydrogenophaga*. This presents an opportunity to investigate bacterial Mn(II) oxidation both in pure culture and *in situ* within environmental communities. An increased understanding of the physiological function and environmental controls of bacterial Mn(II) oxidation may help guide strategies for optimizing microbial community function to improve DW quality.

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## References


Anderson, C.R., Dick, G.J., Chu, M.L., Cho, J.C., Davis, R.E., Bräuer, S.L., and Tebo, B.M. (2009) *Aurantimonas manganoxydans*, sp nov and *Aurantimonas litoralis*, sp nov.: Mn(II) oxidizing representatives of a globally distributed clade of alpha-proteobacteria from the Order Rhizobiales. *Geomicrobiol J* **26**: 189–198.

Bajpai, S., and Chaudhuri, M. (1999) Removal of arsenic from ground water by manganese dioxide-coated sand. *J Environ Eng* **125**: 782–784.

Becker, W.C., and O'Melia, C.R. (2001) Ozone: its effect on coagulation and filtration. *Water Sci Technol* **1**: 81–88.

Bouchard, M.F., Sauve, S., Barbeau, B., Legrand, M., Brodeur, M.E., Bouffard, T., *et al*. (2011) Intellectual impairment in school-age children exposed to manganese from drinking water. *Environ Health Perspect* **119**: 138–143.

Bräuer, S.L., Adams, C., Kranzler, K., Murphy, D., Xu, M., Zuber, P., *et al*. (2011) Culturable rhodobacter and Shewanella species are abundant in estuarine turbidity maxima of the Columbia River. *Environ Microbiol* **13**: 589–603.

Burger, M.S., Krentz, C.A., Mercer, S.S., and Gagnon, G.A. (2008) Manganese removal and occurrence of manganese oxidizing bacteria in full-scale biofilters. *J Water Supply Res Technol –AQUA* **57**: 351–359.

Carmichael, M.J., Carmichael, S.K., Santelli, C.M., Strom, A., and Bräuer, S.L. (2013) Mn(II)-oxidizing bacteria are abundant and environmentally relevant members of ferromanganese deposits in caves of the Upper Tennessee River Basin. *Geomicrobiol J* **30**: 779–800.

Caspi, R., Tebo, B.M., and Haygood, M.G. (1998) c-Type cytochromes and manganese oxidation in Pseudomonas putida MnB1. *Appl Environ Microbiol* **64**: 3549–3555.

Cerrato, J.M., Falkinham, J.O., III, Dietrich, A.M., Knocke, W.R., McKinney, C.W., and Pruden, A. (2010) Manganese-oxidizing and -reducing microorganisms isolated from biofilms in chlorinated drinking water systems. *Water Res* **44**: 3935–3945.

Costa, M., and Klein, C.B. (2006) Toxicity and carcinogenicity of chromium compounds in humans. *Crit Rev Toxicol* **36**: 155–163.

Dick, G.J., and Tebo, B.M. (2010) Microbial diversity and biogeochemistry of the Guaymas Basin deep-sea hydrothermal plume. *Environ Microbiol* **12**: 1334–1347.

Dick, G.J., Lee, Y.E., and Tebo, B.M. (2006) Manganese(II)-oxidizing bacillus spores in Guaymas Basin hydrothermal sediments and plumes. *Appl Environ Microbiol* **72**: 3184–3190.

Dick, G.J., Podell, S., Johnson, H.A., Rivera-Espinoza, Y., Bernier-Latmani, R., McCarthy, J.K., *et al*. (2008) Genomic insights into Mn(II) oxidation by the marine alphaproteobacterium *Aurantimonas* sp strain SI85-9A1. *Appl Environ Microbiol* **74**: 2646–2658.

Driehaus, W., Seith, R., and Jekel, M. (1995) Oxidation of arsenate(iii) with manganese oxides in water-treatment. *Water Res* **29**: 297–305.

EPA (2013) *Secondary drinking water regulations: guidance for nuisance chemicals* [WWW document]. URL http://water.epa.gov/drink/contaminants/secondarystandards.cfm

Escobar, I.C., and Randall, A.A. (2001) Case study: Ozonation and distribution system biostability. *J Amer Water Works Assoc* **93**: 77–89.

Farkas, A., Dragan-Bularda, M., Muntean, V., Ciataras, D., and Tigan, S. (2013) Microbial activity in drinking water-associated biofilms. *Cent Eur J Biol* **8**: 201–214.

Francis, C.A., and Tebo, B.M. (2002) Enzymatic Manganese(II) oxidation by metabolically dormant spores of diverse Bacillus species. *Appl Environ Microbiol* **68**: 874–880.

Furgal, K.M., Meyer, R.L., and Bester, K. (2015) Removing selected steroid hormones, biocides and pharmaceuticals from water by means of biogenic manganese oxide nanoparticles in situ at ppb levels. *Chemosphere* **136**: 321–326.

Geszvain, K., and Tebo, B.M. (2010) Identification of a two-component regulatory pathway essential for Mn(II) oxidation in pseudomonas putida gb-1. *Appl Environ Microbiol* **76**: 1224–1231.

Hoyland, V.W., Knocke, W.R., Falkinham, J.O., III, Pruden, A., and Singh, G. (2014) Effect of drinking water treatment process parameters on biological removal of manganese from surface water. *Water Res* **66**: 31–39.

Ivanova, E.P., Gorshkova, N.M., Sawabe, T., Hayashi, K., Kalinovskaya, N.I., Lysenko, A.M., *et al*. (2002) Pseudomonas extremorientalis sp nov., isolated from a drinking water reservoir. *Int J Syst Evol Microbiol* **52**: 2113–2120.

Johnson, K.W., Carmichael, M.J., McDonald, W., Rose, N., Pitchford, J., Karatan, E., and Bräuer, S.L. (2011) Increased abundance of Gallionella spp., Leptothrix spp., and total bacteria in response to enhanced Mn and Fe concentrations in a distrubed southern appalachian high elevation wetland. *Geomicrobiol J* **29**: 124–138.

Katsoyiannis, I.A., and Zouboulis, A.I. (2004) Biological treatment of Mn(II) and Fe(II) containing groundwater: kinetic considerations and product characterization. *Water Res* **38**: 1922–1932.

Khan, K., Wasserman, G.A., Liu, X.H., Ahmed, E., Parvez, F., Slavkovich, V., *et al*. (2012) Manganese exposure from drinking water and children's academic achievement. *Neurotoxicology* **33**: 91–97.

Kohl, P., and Medlar, S. (2006) *Occurrence of Manganese in Drinking Water and Manganese Control*. Colorado (CO), USA: American Water Works Association.

Kormas, K.A., Neofitou, C., Pachiadaki, M., and Koufostathi, E. (2010) Changes of the bacterial assemblages throughout an urban drinking water distribution system. *Environ Monit Assess* **165**: 27–38.

Kuhlman, K.R., Venkat, P., La Duc, M.T., Kuhlman, G.M., and McKay, C.P. (2008) Evidence of a microbial community associated with rock varnish at Yungay, Atacama Desert, Chile. *J Geophys Res Biogeosci* **113**: G04022.

Laseke, I. (2006) Identification and characterization of bacterial communities in warm groundwater aquifers. Masters Thesis. University of Cincinnati: Cincinnati, Ohio.

Laseke, I., Korte, J., Lamendella, R., Kaneshiro, E.S., Marciano-Cabral, F., and Oerther, D.B. (2010)

Identification of Naegleria fowleri in warm ground water aquifers. *J Environ Qual* **39**: 147–153.
Magic-Knezev, A., Wullings, B., and Van der Kooij, D. (2009) Polaromonas and Hydrogenophaga species are the predominant bacteria cultured from granular activated carbon filters in water treatment. *J Appl Microbiol* **107**: 1457–1467.
Mariner, R., Johnson, D.B., and Hallberg, K.B. (2008) Characterisation of an attenuation system for the remediation of Mn(II) contaminated waters. *Hydrometallurgy* **94**: 100–104.
Martiny, A.C., Albrechtsen, H.J., Arvin, E., and Molin, S. (2005) Identification of bacteria in biofilm and bulk water samples from a nonchlorinated model drinking water distribution system: detection of a large nitrite-oxidizing population associated with Nitrospira spp. *Appl Environ Microbiol* **71**: 8611–8617.
Murray, K.J., Mozafarzadeh, M.L., and Tebo, B.M. (2005) Cr(III) oxidation and Cr toxicity in cultures of the manganese(II)-oxidizing Pseudomonas putida strain GB-1. *Geomicrobiol J* **22**: 151–159.
Northup, D.E., Snider, J.R., Spilde, M.N., Porter, M.L., van de Kamp, J.L., Boston, P.J., *et al*. (2010) Diversity of rock varnish bacterial communities from Black Canyon, New Mexico. *J Geophys Res Biogeosci* **115**: G02007.
Pinto, A.J., Xi, C.W., and Raskin, L. (2012) Bacterial community structure in the drinking water microbiome is governed by filtration processes. *Environ Sci Technol* **46**: 8851–8859.
Pinto, A.J., Schroeder, J., Lunn, M., Sloan, W., and Raskin, L. (2014) Spatial-temporal survey and occupancy-abundance modeling to predict bacterial community dynamics in the drinking water microbiome. *Mbio* **5**: e01135–14.
de Rudder, J., Van de Wiele, T., Dhooge, W., Comhaire, F., and Verstraete, W. (2004) Advanced water treatment with manganese oxide for the removal of 17 alpha-ethynylestradiol (EE2). *Water Res* **38**: 184–192.
Schweisfurth, R. (1973) Manganoxydierende bakterien. i. isolierung und bestimmung einiger stamme von mangan-bakterien. *Z Allg Mikrobiol* **13**: 341–347.
Sly, L.I., Arunpairojana, V., and Hodgkinson, M.C. (1988) Pedomicrobium-manganicum from drinking-water distribution-systems with manganese-related dirty water problems. *Syst. Appl Microbiol* **11**: 75–84.
Sly, L.I., Hodgkinson, M.C., and Arunpairojana, V. (1990) Deposition of manganese in a drinking-water distribution-system. *Appl Environ Microbiol* **56**: 628–639.
Staley, J.T., and Konopka, A. (1985) Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu Rev Microbiol* **39**: 321–346.
Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
Sunda, W.G., and Kieber, D.J. (1994) Oxidation of humic substances by manganese oxides yields low-molecular-weight organic substrates. *Nature* **367**: 62–64.
Tebo, B.M., Bargar, J.R., Clement, B.G., Dick, G.J., Murray, K.J., Parker, D., *et al*. (2004) Biogenic manganese oxides: properties and mechanisms of formation. *Annu Rev Earth Planet Sci* **32**: 287–328.
Tebo, B.M., Johnson, H.A., McCarthy, J.K., and Templeton, A.S. (2005) Geomicrobiology of manganese(II) oxidation. *Trends Microbiol* **13**: 421–428.
Tebo, B.M., Clement, B.G., and Dick, G.J. (2007) Biotransformations of manganese. In *Manual of Environmental Microbiology*. Hurst, C.J., Crawford, R.L., Garland, J.L., Lipson, D.A., Mills, A.L., and Stetzenbach, L.D. (eds). Washington, DC, USA: ASM Press, pp. 1223–1238.
Tekerlekopoulou, A.G., Pavlou, S., and Vayenas, D.V. (2013) Removal of ammonium, iron and manganese from potable water in biofiltration units: a review. *J. Chem. Technol. Biotechnol* **88**: 751–773.
Tournassat, C., Charlet, L., Bosbach, D., and Manceau, A. (2002) Arsenic(III) oxidation by birnessite and precipitation of manganese(II) arsenate. *Environ Sci Technol* **36**: 493–500.
de Vet, W., Dinkla, I.J.T., Muyzer, G., Rietveld, L.C., and van Loosdrecht, M.C.M. (2009) Molecular characterization of microbial populations in groundwater sources and sand filters for drinking water production. *Water Res* **43**: 182–194.
Zanetti, F., De Luca, G., and Stampi, S. (2000) Recovery of *Burkholderia pseudomallei* and B-cepacia from drinking water. *Int J Food Microbiol* **59**: 67–72.

## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** Maxmimum likelihood phylogenetic tree of 16S rRNA gene sequences for the phylum *Firmicutes*. Sequences in bold are from this study. Trees were bootstrapped 1000 times using RAxML (Stamatakis, 2014) with the GTRGAMMA model of nucleotide substitution. The dashed branch to the outgroup was condensed. ⋆1: one additional isolate with 100% sequence identity was obtained from Lake Erie. ⋆2: two additional isolates with 100% sequence identity were obtained from Lake Erie.

**Fig. S2.** Maxmimum likelihood phylogenetic tree of 16S rRNA gene sequences for the class *Gammaproteobacteria*. Sequences in bold are from this study. Trees were bootstrapped 1000 times using RAxML (Stamatakis, 2014) with the GTRGAMMA model of nucleotide substitution. The dashed branch to the outgroup was condensed. ⋆1: *Pseudomonas* sp. K43-2B isolated from Lake Erie is 100% identical over all high quality bases. ⋆2: two additional isolates with 100% sequence identity were obtained from the Huron River.

**Fig. S3.** Maxmimum likelihood phylogenetic tree of 16S rRNA gene sequences for the phylum *Actinobacteria*. Trees were bootstrapped 1000 times using RAxML (Stamatakis, 2014) with the GTRGAMMA model of nucleotide substitution. The dashed branch to the outgroup was condensed. ⋆1: one additional isolate with 100% sequence identity was obtained from the Huron River.

**Table S1.** The V4-V5 region from representative isolates was aligned to the Silva SSU NR database. Near full-length Silva representatives that satisfied alignment criteria over

the V4-V5 region were then compared to the near full-length sequences of the same isolates.

**Table S2**. Locations in the Ann Arbor drinking water system where isolates matched a minimum of 0.5% of community reads at 99% ID or greater over a minimum of 280bp in at least one sample. Abbreviations: HR, Huron river, W, well; In, filter influent; Flt, filter; Ef, filter effluent; Rs, reservoir; DS, distribution system.

**Table S3.** See external document.