Review TRENDS in Microbiology Vol.13 No.9 September 2005



# Geomicrobiology of manganese(II) oxidation

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**Mn(II)-oxidizing microbes have an integral role in the biogeochemical cycling of manganese, iron, nitrogen, carbon, sulfur, and several nutrients and trace metals. There is great interest in mechanistically understanding these cycles and defining the importance of Mn(II)-oxidizing bacteria in modern and ancient geochemical environments. Linking Mn(II) oxidation to cellular function, although still enigmatic, continues to drive efforts to characterize manganese biomineralization. Recently, complexed-Mn(III) has been shown to be a transient intermediate in Mn(II) oxidation to Mn(IV), suggesting that the reaction might involve a unique multicopper oxidase system capable of a two-electron oxidation of the substrate. In biogenic and abiotic synthesis experiments, the application of synchrotron-based X-ray scattering and spectroscopic techniques has significantly increased our understanding of the oxidation state and relatively amorphous structure (i.e. δ–$MnO_2$-like) of biogenic oxides, providing a new blueprint for the structural signature of biogenic Mn oxides.**

## Dynamics of Mn biogeochemistry

Manganese (Mn) (II)-oxidizing microorganisms, primarily bacteria and fungi, accelerate the rate of Mn biomineralization several orders of magnitude faster than either abiotic catalysis on mineral surfaces or homogeneous oxygenation in aqueous solution [1]. This biogeochemical process has gained much attention in recent years because Mn(III,IV) oxide minerals are abundant in terrestrial and marine environments. Serving as major sources or sinks for bioavailable Mn, these Mn oxide minerals affect a variety of biological processes, including photosynthesis, carbon fixation and scavenging of reactive oxygen species (ROS). Recent structural studies have shown that biogenic Mn oxides are nanoparticulate, cryptocrystalline materials and are representative of the most highly reactive and important Mn oxide phases in the environment [2,3]. Next to oxygen, Mn oxides are some of the strongest naturally occurring oxidizing agents in the environment. They participate in numerous redox and sorption reactions, thereby controlling the distributions of many other trace and contaminant elements, in addition to serving as terminal electron acceptors for bacterial respiration.

Mn(II)-oxidizing bacteria are ubiquitous in nature (Table 1). Isolated from virtually any environmental sample, they are most conspicuous when there is an adequate supply of reduced Mn(II), such as in oxic–anoxic transition zones or at hydrothermal vents. They might have a greater impact on local biogeochemistry, however, in environments in which Mn cycling is rapid – that is, where Mn(II)↔Mn(III,IV) reactions proceed quickly without the build-up of a particular product or reactant. Such environments include soils and sediments, where newly formed Mn oxides can react quickly with other reduced substances, in turn becoming reduced in the process. This rapid redox cycling of Mn might also influence other biogeochemical cycles (Fe, S, C) without leaving evidence in the geochemical profiles. For example, it has been estimated that Mn might recycle 100–300 times during organic matter remineralization in coastal sediments before it is ultimately buried [4].

The focus of this review is on bacterial Mn(II) oxidation, highlighting some of the major unanswered microbiological questions and the relevance of bacterial Mn biomineralization to Mn cycling in the geosphere. For a more thorough review of the mechanism and products of bacterial Mn(II) oxidation, the reader is referred to another recent review [1].

## Why do Mn(II)-oxidizing bacteria oxidize Mn(II)?

Although bacterial Mn(II) oxidation is widespread, we know little about why bacteria oxidize Mn(II). Indeed, Mn(II) oxidation might be an 'accidental' occurrence, the result of nonspecific interactions with cellular or extracellular products. It has also been suggested that rather than serving some explicit biological role, metal oxidation in some species might be an evolutionary holdover that no longer has physiological relevance [5]. Yet, because so

**Table 1. Environments likely to harbor Mn(II)-oxidizing bacteria**

| |
|---|
| Basalt glasses (weathered surfaces) |
| Hydrothermal vent plumes |
| Hydrothermal vent deposits |
| Metalliferous sediments associated with midocean ridge-spreading centers |
| Ferromanganese nodules and concretions in oceans, lakes, soils |
| Oxic–anoxic interfaces (Black Sea, sediments, fjords) |
| Desert varnish |
| Hyporheic zones (river and stream sediments) |
| Water pipes |

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Available online 28 July 2005

 0966-842X/$ - see front matter  doi:10.1016/j.tim.2005.07.009

many different bacteria oxidize Mn(II), and many bacteria with specific enzymatic activities become encrusted in Mn oxides, we presume that there could be good evolutionary reasons to possess this trait. The potential benefits of Mn(II) oxidation are numerous, ranging from protective mechanisms to storage of an electron acceptor [6]. It has long been proposed that bacteria might be able to derive energy, possibly for chemolithoautotrophic growth, by oxidizing Mn(II). However, although the oxidation of Mn(II) to either Mn(III) or Mn(IV) oxides is thermodynamically favorable, there is no unequivocal evidence directly linking Mn(II) oxidation to energy conservation, nor has current research attempted to settle this question.

As a trace nutrient, Mn is required for a large number of cellular functions. Therefore, it is worth considering whether Mn(II) oxidation is related to the role of Mn in cellular functioning. For example, intracellular Mn(II) is known to act as an antioxidant, protecting some cells from ROS, such as superoxide, even in cells that do not possess superoxide dismutase [7,8]. In fact, the scavenging of superoxide radicals by Mn(II) might also provide a mechanism for *Deinococcus* to resist high levels of ionizing radiation [9,10]. The reaction of Mn(II) with superoxide can produce Mn(III) or Mn(IV) species [10]. These reactions of Mn(II) are likely to occur on the inner membrane, where ROS are produced as by-products of respiration. Thus, the question that arises for the Mn(II)-oxidizing bacteria which precipitate Mn oxides extracellularly, is whether Mn(II) oxidation is related to this intracellular ROS scavenging, or whether the bacteria are simply taking advantage of similar chemistry to protect themselves from oxidants in the environment.

By coating themselves with Mn oxides, Mn(II)-oxidizing bacteria can protect themselves from other environmental insults, such as UV radiation, predation, viral attack or heavy metal toxicity. For example, recent Fourier transform infrared spectroscopy experiments have found that biomass accumulates at a faster rate in a monoculture biofilm actively oxidizing Mn(II) than in the absence of Mn oxides [11]. Bacteriogenic Mn(IV) oxides might also provide the bacteria and the microbial community at large with a means to derive energy from refractory organic matter. Mn(IV) oxides are known to degrade humic substances oxidatively to low molecular weight compounds that can be used as substrates for microbial growth [12]. If these compounds were to be used by the Mn(II)-oxidizing bacteria, or the microbial community, the energetic benefit, and evolutionary advantage, of Mn(II) oxidation would be apparent. Should this process occur, it would have important implications for the biogeochemical cycling of carbon. Alternatively, in lean times, Mn(IV)-oxides might act as the storage of an electron acceptor, until carbon and energy again become available [6].

## Which bacteria oxidize Mn(II)?

Mn(II)-oxidizing bacteria have been identified in a growing number of divergent phylogenetic lineages in the bacterial domain, such as Firmicutes, Proteobacteria and Actinobacteria (Figure 1). This broad phylogenetic diversity mirrors the physiological diversity of Mn(II)-oxidizing bacteria, as demonstrated by the well-studied model organisms: the Gram positive spore-forming *Bacillus* sp. strain SG1 [13], the γ-proteobacteria *Pseudomonas putida* MnB1 and GB-1 [14], and the β-proteobacterium sheath-forming *Leptothrix discophora* strain SS-1 [15]. As efforts to cultivate Mn(II)-oxidizing microbes increase, bacteria with distinct and varied traits continue to be reported. Takeda *et al*. have described a poly (3-hydroxybutyrate)-degrading thermophilic β-proteobacterium that oxidizes Mn(II) [16]. Within the α-proteobacteria, strain SD-21, a relative of the aerobic anoxygenic phototrophic genus *Erythrobacter*, grows to a higher cell density when Mn(II) is present under both light and dark conditions, in spite of light inhibiting Mn(II) oxidation [17]. In contrast to most strains, which oxidize during stationary phase, the budding α-proteobacterium *Pedomicrobium* sp. ACM 3067 oxidizes Mn(II) during early- to mid-log phase [18]. Recently, several heterotrophic Mn(II) oxidizers associated with the rinds of deep sea basaltic glass recovered from the Loihi Seamount, an active submarine volcano off of the Island of Hawaii, have been described [19]. Based upon 16S rRNA analysis, *Pseudoaltermonas* species were the most common isolates of the γ-proteobacteria. Numerous α-proteobacteria in the *Rhodobacter* group were also isolated [19]. The role of these bacteria in the dissolution of volcanic glasses and the oxidation of rock-derived Mn(II) remains to be shown. Nevertheless, the study indicates the presence and abundance of phylogenetically diverse Mn(II)-oxidizing bacteria in oligotrophic deep sea environments. In addition to the bacterial domain, fungi have also been implicated in catalyzing the formation of Mn oxides [20,21] (Box 1).

Clone libraries from environmental sites rich in Mn oxides and from Mn(II)-oxidizing enrichment cultures have provided indirect evidence of Mn(II)-oxidizing bacteria. This approach has been used [22,23] to examine the microbial community associated with Lechuguilla and Spider Caves, environments rich in ferromanganese deposits formed during the weathering of Mn(II)-bearing carbonate host rock.

Analysis of the 16S rRNA clone libraries derived from the caves is intriguing because 56 of the 78 clones analyzed were found to be Archaea [22]. Although the role of these Archaea in Mn(II) or Fe(II) oxidation has not been directly demonstrated, and no Mn(II)-oxidizing Archaea have been described, these caves might prove worthy of further enrichment for this class of 'new' Mn(II) oxidizers. In addition to the Archaeal sequences, other sequences with homology to known Mn(II)-oxidizing bacteria were identified, such as *Pedomicrobium manganicum* and a *Hyphomicrobium* species [22]. The *Pedomicrobium* species identified was consistent with the budding cell morphology that had been described in the ferromanganese deposits.

Interestingly, the clone libraries from the ferromanganese cave deposits and the enrichment cultures showed some similarity to the clone libraries and purified cultures from ferromanganese micronodules of Green Bay sediments [24]. Marine Crenarchaeota represented the Archaeal clones in libraries from both sites. In addition, a *Comamonas* isolate from the ferromanganese

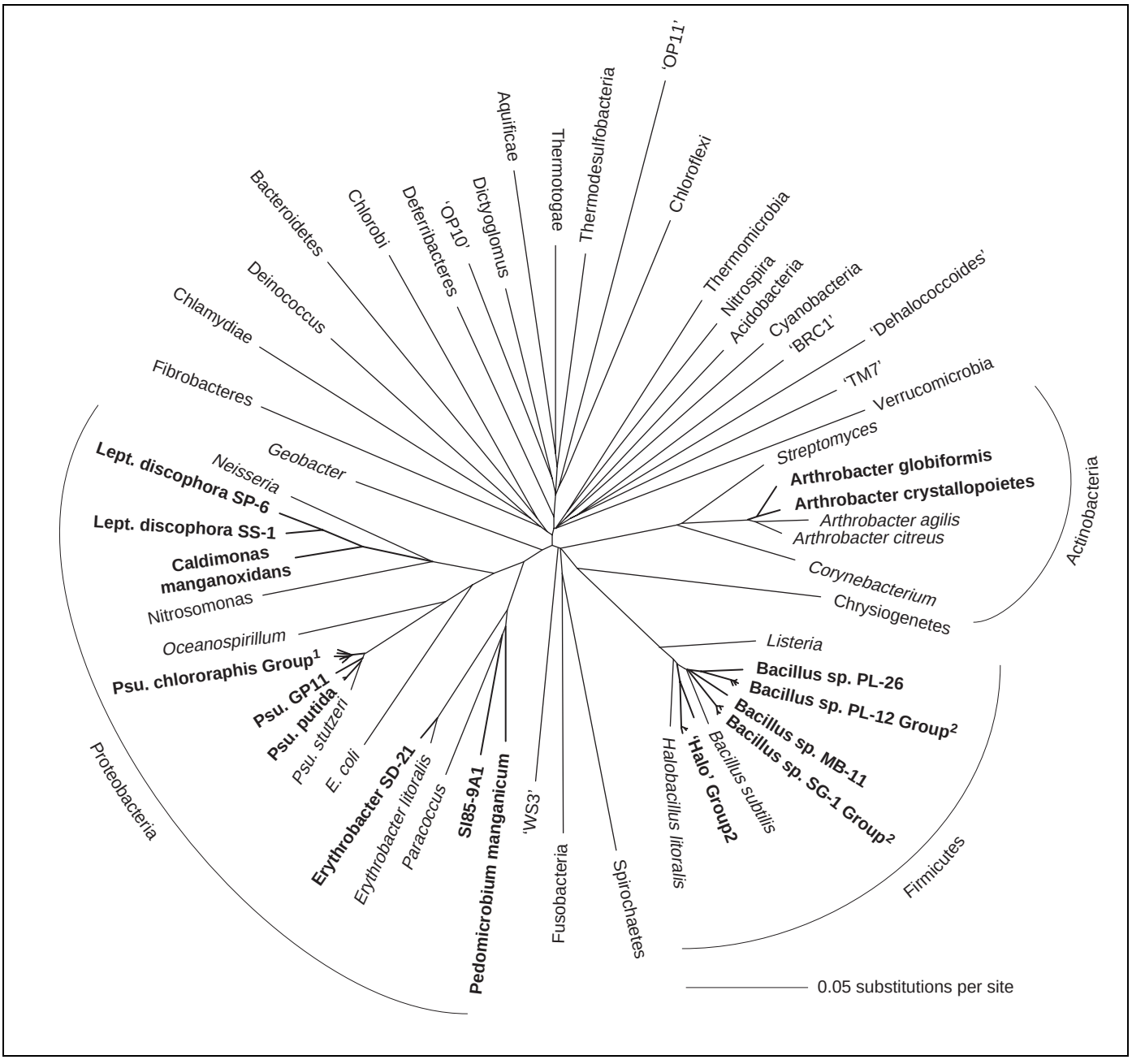


**Figure 1.** Neighbor-joining, unrooted phylogram of the domain bacteria showing representative Mn(II)-oxidizing bacteria. 16S rRNA sequences aligned over 1536 positions were obtained from the Ribosomal Database Project (RDP; http://rdp.cme.msu.edu) and analyzed using PAUP (phylogenetic analysis using parsimony; ver. 4.0b10). Mn(II)-oxidizing bacterial strains for which sequences appear in the RDP are shown in bold. Sequence similarity is only an estimate of evolutionary relationships and is expressed here as the distance between any two branch points; sequences that appear next to each other are not necessarily close relatives. Phyla in quotes are considered to be taxa of uncertain affiliation and have few (if any) cultivated members. Abbreviations: *Lept.*, *Leptothrix*; *Psu.*, *Pseudomonas*. [1]The *Psu. chlororaphis* group includes the Mn(II)-oxidizing strains ISO1, ISO6, GB-13, MG1 and PCP but not all similarly close relatives of *Psu. chlororaphis* are known to oxidize Mn(II). [2]*Bacillus* species groups are labeled according to Francis and Tebo [56]. Reproduced, with permission, from Ref. [1].

micronodules of Green Bay would align closely in a phylogenetic tree to the *Variovorax* clone in a clone library from cave ferromanganese deposits.

Although the function of biogenic Mn(II) oxidation remains enigmatic, the widespread presence of manganese in myriad ecological niches suggests that the diversity of microbial Mn(II) oxidizers will continue to increase. In addition, as our understanding of the mechanism of Mn(II) oxidation improves (as discussed later), our greater ability to detect bacteria that oxidize Mn(II) will probably lead to the discovery of new phylotypes.

## How do bacteria oxidize Mn(II)?

The biochemical mechanism of Mn(II) oxidation has not yet been described because neither native purification nor heterologous overexpression of putative Mn(II) oxidases has been successful to date. However, numerous details of a regulated, functional pathway have emerged. Multi-copper oxidase (MCO)-type enzymes have an integral role in Mn(II) oxidation in diverse species, and genetic studies indicate that the site of Mn(II) oxidation in vegetative cells, as was shown with *Bacillus* sp. strain SG1 spores, is likely to be at the cell surface. In addition, the mechanism

**Box 1. Mn(II) oxidation by fungi**

Although bacteria have an important role in the formation of biogenic Mn oxides, the same is also true for fungi. Both fresh water and marine systems have yielded fungal Mn oxide producers. Based upon 18S rRNA, these fungi have been identified as ascomycetes. The mechanism of the Mn oxide deposition is unknown but it is likely to be similar to that studied in bacteria. Biogenic Mn oxide formation in the anamorphic ascomycete strain KR21–2 was investigated by Tani *et al.* [57,58]. Similarly to findings in *L. discophora* SS-1, an extracellular protein was involved in Mn oxide formation. These studies show that the Mn-oxidizing protein can also oxidize known laccase substrates such as *p*-phenylenediamine and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid). Azide and *o*-phenanthroline (a copper chelator), known bacterial Mn oxidation inhibitors, inhibit Mn oxidation in KR21–2 as well, suggesting a role for an MCO-type enzyme in fungal Mn oxide formation. Fungi also oxidize Mn(II) by heme-containing Mn peroxidases in the degradation of lignin. In the presence of a suitable Mn(III) chelator, Mn(II) is oxidized by a peroxide-oxidized enzyme to Mn(III). If insoluble Mn oxides are formed from this reaction, it is suspected to be via disproportionation under chelator-limiting conditions. Another enzyme used in lignin degradation, laccase, also an MCO, can oxidize Mn. Purified laccases from *Trametes versicolor* [59] and *Stropharia rugosoannulata* [30] can oxidize Mn(II) to Mn(III). Whether all fungal laccases are capable of this reaction is unknown but, based upon the lower substrate specificity of these enzymes, we would expect most laccases to be capable of Mn(II) oxidation.

of Mn(II) oxidation proceeds via a Mn(III) intermediate – with potentially intriguing implications [25].

In model organisms for biogenic Mn(II) oxidation, many genes, identified by mutagenesis as loss-of-function phenotypes, have been shown to affect bacterial Mn(II) oxidation (Table 2). Genes encoding an MCO, a family of enzymes characterized by conserved Cu-binding sites and a catalytic mechanism that involves a sequential one-electron oxidation of the substrate with the concomitant reduction of $O_2$ to $H_2O$, were found in *Bacillus* sp. SG-1, *P. putida* MnB1 and GB-1, and *L. discophora* SS1: *mnxG*, *cumA* and *mofA*, respectively, are presumed to encode the catalytic Mn(II) oxidases. The involvement of an MCO in Mn(II) oxidation is consistent with what is known of other Mn- and Fe-oxidizing MCOs (see later). The underlying mechanism, as Webb *et al.* [25] point out in experiments tracking the possibility of the existence of soluble Mn(III) intermediates in the Mn(II)→Mn(IV) oxidation reaction, is significantly different. Using the exosporium isolated from *Bacillus* sp. SG-1, which contains the Mn(II)-oxidizing factor, and the trapping chemistry of pyrophosphate, the study demonstrates that a Mn(III) intermediate is produced during the oxidation of Mn(II) (Figure 2). The presence of the soluble Mn(III) intermediate implies that bacterial Mn(II) oxidation occurs as a sequence of two enzymatically mediated, one-electron transfer reactions, and indicates that the *Bacillus* sp. SG-1 exosporium enzyme MnxG is required for both oxidation steps. Although the nature of the Mn(III) enzyme intermediate and the mechanism of the two-step catalysis by the MCO remain unknown, the findings suggest that the oxidation of Mn(II) involves a unique MCO system that is capable of catalyzing the overall two-electron oxidation of the substrate [25].

The genomic analysis of *P. putida* sp. KT2440 describes an unexpectedly large variety of genes predicted to be involved in metal(loid) transport, tolerance and resistance [26]. Mn(II)-oxidizing *P. putida* strains GB-1 and MnB1 are considered very close relatives of *P. putida* sp. KT2440. Therefore, given the homology of the MCO gene *cumA* (the gene encoding the putative Mn oxidase), and the arrangement of the neighboring open reading frames [27] in GB-1, MnB1 and KT2440, much can be inferred from the KT2440 genome regarding the transport of Mn in the pseudomonads that oxidize Mn(II).

As it has been difficult directly to link the genes shown to be necessary for Mn(II) oxidation [1,28] to the catalytic enzymes that are presumed to drive the oxidation reaction, it is useful to consider how Mn and its periodic neighbor Fe are oxidized in other systems. In some fungi, oxidized Mn is used to degrade lignin (Box 1). Two enzymes catalyze the reaction: Mn peroxidase oxidizes Mn(II) to Mn(III) with $H_2O_2$ [29], and a fungal MCO, laccase, oxidizes Mn(II) to Mn(III) directly [30]. Other

**Table 2. Genes involved in Mn(II) oxidation**

| Gene | Description | Strain | Refs |
|---|---|---|---|
| *ccmE* | c-type cytochrome biogenesis | *P. putida* MnB1 | [51] |
| *ccmF* | c-type cytochrome biogenesis | *P. putida* MnB1 and GB-1 | [51,52] |
| *ccmA* | c-type cytochrome biogenesis | *P. putida* MnB1 | [51] |
| *sdhABCD* | Succinate dehydrogenase complex | *P. putida* MnB1 | [51] |
| *aceA* | Lipoate acetyltransferase (subunit of pyruvate dh complex) | *P. putida* MnB1 | [51] |
| *icd* | Isocitrate dh | *P. putida* MnB1 | [51] |
| *trpE* | Subunit of anthranilate synthetase | *P. putida* MnB1 | [51] |
| *xcpA* | General secretory pathway | *P. putida* GB-1 | [27] |
| *xcpT* | General secretory pathway | *P. putida* GB-1 | [27] |
| *cumA* | MCO | *P. putida* GB-1 | [53] |
| *mnxA* | No homology | *Bacillus* sp. SG-1 | [54] |
| *mnxB* | No homology | *Bacillus* sp. SG-1 | [54] |
| *mnxC* | Homology to scoI, required for cytochrome c oxidase activity | *Bacillus* sp. SG-1 | [54] |
| *mnxD* | No homology | *Bacillus* sp. SG-1 | [54] |
| *mnxE* | No homology | *Bacillus* sp. SG-1 | [54] |
| *mnxF* | No homology | *Bacillus* sp. SG-1 | [54] |
| *mnxG* | MCO | *Bacillus* sp. SG-1 | [54] |
| *mofA* | MCO | *L. discophora* SS-1 | [55] |
| *mofB* | Peptidyl-prolyl-cis-trans isomerase (involved in protein folding) and macrophage infectivity potentiator | *L. discophora* SS-1 | [28] |
| mofC | Cytochrome c family protein | *L. discophora* SS-1 | [28] |

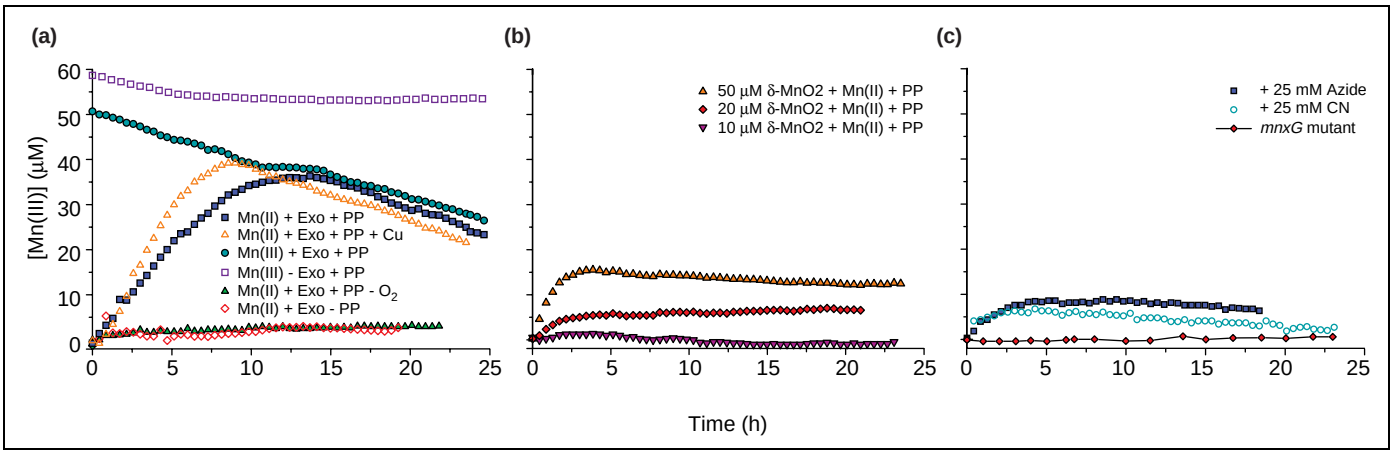


**Figure 2.** Evidence for the Mn(III) intermediate in the Mn oxidation reaction in marine Bacillus sp. SG-1. Measurements of Mn(III)–pyrophosphate (PP) complexes under various experimental conditions. **(a)** Control experiments are given by the open diamonds (no PP present) and solid triangles (no oxygen present). In these conditions, little Mn(III)–PP signal is observed and is attributed to drift of the spectrometer. The Mn(III)–PP complex is stable, as shown by the data indicated by open squares. Production and decay of Mn(III)–PP complexes in systems with exosporium plus Mn(II) is shown by the filled squares. The decay of Mn(III) by the exosporium can also be achieved by direct addition of Mn(III) to the exosporium suspension, as indicated by the solid circles. The effect of addition of 50 nM $CuCl_2$ is shown by the open triangles. **(b)** Measurements of Mn(III)–PP produced by the abiotic reactions of Mn(II) and Mn(IV) as a function of Mn(IV) concentration. [δ-$MnO_2$] = 50 μM (triangles), [δ-$MnO_2$] = 20 μM (diamonds), [δ-$MnO_2$] = 10 μM (upside-down triangles). **(c)** Measurements of the inhibition of Mn(III)–PP formation in the presence of metalloenzyme inhibitors such as sodium azide (squares) and sodium cyanide (open circles). Measurements performed with exosporium derived from spores with transposon insertions into the *mnxG* gene show no production of Mn(III) (diamonds). All measurements performed with 0.5 mM PP, 20 mM HEPES, pH ~ 7.6. Reproduced, with permission, from Ref. [25].

MCOs such as ceruloplasmin (humans), Fet3 (yeast) and a bacterial ferroxidase [31] are known to oxidize Fe(II) to Fe(III). Although the oxidation of Mn(II) and Fe(II) show many similarities, it is worth noting that *L. discophora* SS-1, which can oxidize both Mn(II) and Fe(II), is suspected of having two independent oxidizing factors [32].

Small endogenous molecules and polypeptides might also have a role in the oxidation reaction, serving as Mn(III) chelators, similarly to pyrophosphate in the recent trapping studies [25]. Recently, Parker *et al.* [33] demonstrated that the *Pseudomonas* siderophore pyoverdine binds Mn(III) with a slightly greater affinity than Fe(III). The Mn(III)–Fe(III) competition for siderophores suggests that the production of Mn(III) could benefit the bacterium in Fe-limiting conditions because it might help it acquire Fe from another inaccessible Fe–siderophore complex. The finding also suggests that a complicated relationship might exist between Mn(II) oxidation and Fe homeostasis.

The accumulation of Mn oxides on the cell surface is the final step in the oxidation reaction with Mn(III,IV) species interacting with catalytic proteins and/or retained within the extracellular polymeric substances that make up sheaths (*L. discophora*), exosporium (*Bacillus* SG-1) and biofilms (*P. putida* GB-1 and MnB1) common to Mn(II)-oxidizing bacteria. In spore-forming *Bacillus* sp. SG-1, both oxidation steps take place on the exosporium, a loose-fitting, outer spore layer found only in certain *Bacillus* and *Clostridium* species [34]. In *P. putida* GB-1 and MnB1, the evidence for localization of the entire reaction to the outer membrane is more ambiguous. Protein transport experiments have indicated that CumA, the putative Mn oxidase, is transported to the cell surface by way of a novel general secretory pathway-related pathway [27]. Earlier research, however, found that part of the Mn(II)-oxidizing activity was soluble and occurred at an intracellular or inaccessible (to outside exposure) cellular location [14]. Recent near-edge X-ray absorption fine structure spectroscopy has shown that a Mn(III) intermediate, presumably membrane bound rather than a solution-phase Mn(III) complex, accumulates on or between *P. putida* MnB1 cells at the beginning of oxidation [35]. These results, as a whole, further suggest that a more complex, perhaps multiple enzyme, reaction pathway might exist in vegetative cells, and point to new areas of research that will help to illuminate the intriguing details of this process.

## What is the biogeochemical importance of Mn oxidation?

Recent field studies focused on Mn oxide recycling in the Orca Basin [36], the Black Sea [37] and acid-mine drainage systems [38] have highlighted the fact that net geochemical fluxes and rates of element cycling are mechanistically linked to Mn(II) oxidation. However, despite new information on the diversity of Mn(II)-oxidizing bacteria and the molecular mechanisms likely to be involved in Mn(II) oxidation, factors controlling the distribution, activity and biochemical function of Mn(II)-oxidizing bacteria remain unknown. Several major questions that are currently under investigation include: what are the direct links between Mn oxide biomineralization and the transformation and sequestration of Fe, C, N, S, nutrients and trace metals; are there chemolithoautotrophic Mn-oxidizing bacteria that can use oxygen or nitrate as an electron acceptor during Mn(II) oxidation and conserve energy for growth – this is particularly interesting when there is a supply of Mn(II) derived from geological processes; and are there biogenic Mn oxide minerals that can be used to identify microbial Mn(II) oxidation in diverse geochemical environments? More importantly, are these oxides preserved in the geological record as recognizable microfossils, particularly when the minerals coat unique morphological forms such as sheaths and capsules?

Highly mobile Mn(II) is initially released into the environment from hydrothermal fluids or weathering of

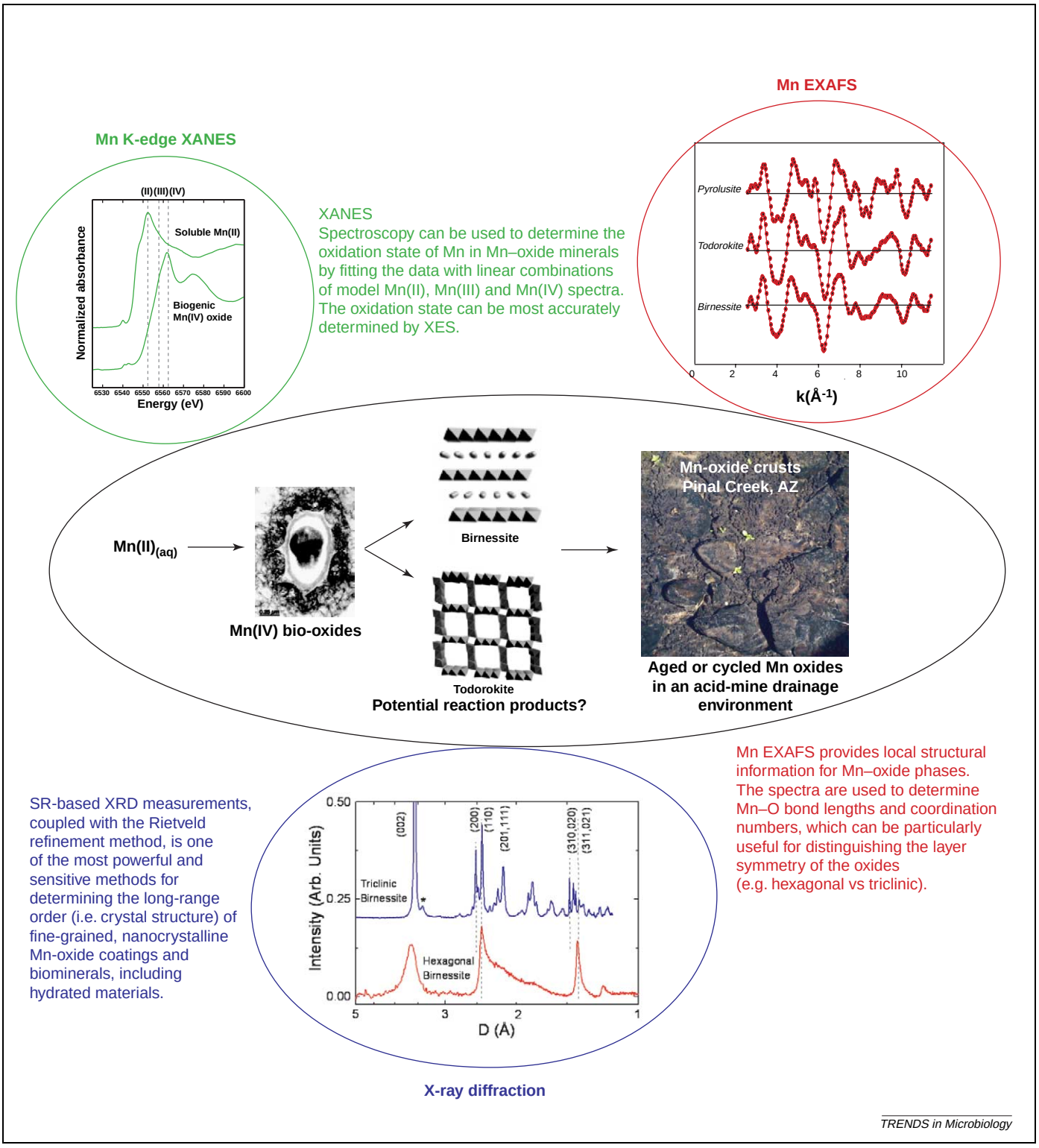


**Figure 3.** Synchrotron-based X-ray diffraction and spectroscopy. Increased application of synchrotron (SR)-based X-ray diffraction (XRD) and spectroscopic techniques has enabled the characterization (structure and oxidation state) of Mn oxide minerals previously considered to be relatively 'X-ray amorphous', such as nanocrystalline natural phases and biogenic oxides. These techniques can be used in time-course studies to determine the initial phases formed and the subsequent reaction products during continued Mn(II) oxidation. Transmission electron microscopy, selected area electron diffraction and neutron diffraction techniques can complement these synchrotron-based approaches. Abbreviations: EXAFS, extended X-ray absorption fine-structure spectroscopy; XANES, X-ray near-edge structure; XES, X-ray emission spectroscopy.

Mn(II)-bearing rocks, such as mafic silicates and Mn carbonates. Dense plumes of Mn oxide particulates coat microbial cells wherever reduced fluids rich in Mn(II) react with oxygenated seawater (e.g. Guaymas Basin hydrothermal fluids). Rate measurements of Mn(II) oxidation, often using $^{54}$Mn(II) as a radiotracer, also suggest bacterial processes [39]. When these rates are too high to be explained by abiotic processes, are inhibited by poisons or show a temperature optimum for particulate Mn formation, microbial activity is inferred. Linking targeted culturing efforts of Mn(II)-oxidizing bacteria with phylogenetic data obtained from the natural Mn

oxides (e.g. small subunit rRNA clone libraries from DNA associated with Mn oxides) is necessary to confirm this relationship. In addition, structural determination of the 'fresh' environmental oxides relative to those preserved after dissolution or reprecipitation processes is needed to establish further the importance of Mn(II)-oxidizing bacteria in the geosphere.

The average residence times of Mn oxides in soil and aquatic systems might be relatively short, as a result of their catalytic role in the oxidation of reduced Fe, S and C. Current efforts to understand the structural properties of these freshly formed Mn oxides will help to illuminate the controls on reactivity towards an enormous suite of trace elements (e.g. Cu, Co, Cd, Zn, Ni, Sn, Pb, Ca, Fe, Ra, Hg, U, Pu, Po, As, Se and Th), and determine whether metal ions are sorbed or structurally incorporated into crystalline and amorphous oxides phases. Unfortunately, defining the mineralogy of environmental Mn(III or IV) oxide minerals has been constrained by the complex mixtures of minerals present and the small (often nanometer) crystal size, poor crystallinity and high disorder of these minerals [40]. However, recent applications of high-resolution electron microscopy, time-of-flight neutron diffraction and synchrotron-based X-ray spectroscopy have led to breakthroughs in the characterization of the Mn mineralogy in lake sediments [41], marine nodules [42] and dendritic rock varnish [43], as just a few examples (Figure 3).

These techniques have also been applied to recent laboratory-based biogenic and abiotic synthesis experiments [3,25,44–48]. For example, biogenic Mn oxides produced by *Bacillus* sp. SG-1 [2] and *P. putida* MnB1 [3] have been carefully characterized. Combined scattering and spectroscopy data have demonstrated that the Mn bio-oxides are typically high average oxidation state [i.e. structural Mn(IV)] layer-type oxides with very high specific surface areas and poor crystallinity, most closely resembling δ-$MnO_2$ or $H^+$-birnessite. These studies are helping to establish the criteria necessary to infer the biogenic origin of Mn oxides, and to link the structure of the bio-oxides to their reactivity. As new insights are gained regarding the modes and mechanisms of metal association with Mn oxides [49,50], the next step will be to understand trace metal speciation in natural, diagenetically altered Mn oxide products that have been dynamically recycled during dissolution or reprecipitation processes. This would help in the interpretation of trace metal accumulation within (banded) Mn oxide accumulations and could possibly be used to develop temporal records of metal inputs into given biogeochemical environments [42].

The improved structural determination of environmental and biogenic oxides is also important for the (re-) interpretation of how a wide variety of Mn oxide deposits are formed, such as ferromanganese varnishes, crusts and nodules in ocean, freshwater, rock and soil environments. The presence of Mn(III) or Mn(IV) oxides often serves as a likely indicator of biological activity under conditions relevant to 'low-temperature' geochemistry. In the geological context, a key question, then, is whether Mn oxide phases preserved in the rock record might serve as a 'biomarker'. Can microfossils be definitively recognized, and can they be used not only to infer microbial origin but also to define paleoenvironmental conditions, particularly in terms of oxygen concentration, pH and temperature? Todorokite, buserite and birnessite are some of the most commonly preserved Mn oxides and are also routinely invoked as products of microbial Mn(II) oxidation. However, the links between the relatively amorphous microbial oxide δ-$MnO_2$-like 'precursor' and the Mn minerals preserved in geological systems after 'aging' and diagenetic alteration, particularly the Mn oxides interlayered with Fe-oxide minerals in ferromanganese crusts, need to be established. There is the potential that understanding the biochemical mechanism as well as the chemical and structural signatures of Mn oxide biomineralization might help to recognize and decipher major environmental and biological events in early earth history, such as the mechanism of formation of the Kalahari Mn oxide deposits in South Africa formed 2.2 billion years ago.

**Acknowledgements**

We gratefully acknowledge support from the National Science Foundation: CRAEMS (Collaborative Research Activities in Environmental Molecular Science) grant CHE-0089208 (see http://mnbiooxides.ucsd.edu/) and our collaborators John Bargar, Garrison Sposito and Tom Spiro, who have greatly influenced our thinking on bacterial Mn(II) oxidation. We appreciate the critical comments of five anonymous reviewers. We also acknowledge other funding from NSF (CHE-9910572, MCB-0422232; MCB-0348668; OCE-0221500; OCE-0352081; OCE-0433692), the Superfund Basic Research Program (NIEHS grant ES10337 to UCSD) and the University of California Toxic Substances Research and Teaching Program.

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