

Impact of zinc on the biomineralization of fungal manganese oxides by a novel Mn-oxidizing fungus (*Curvularia lunata* TC-1): implications for metal bioremediation

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Manganese (Mn) oxides are widespread minerals in terrestrial and aquatic environments, exerting control over the uptake and release of various metals through diverse mechanisms. In natural settings, microbiological activities predominantly facilitate the formation of Mn oxides, achieved through direct enzymatic or metabolite-mediated oxidation of aqueous Mn(II), such as by superoxide. These biogenic Mn oxides manifest as highly reactive phyllo-manganates, exhibiting a substantial capacity to sequester other metals from diverse aqueous environments. This underscores their potential for the bioremediation of environments contaminated with multiple metal co-contaminants. Mining and industrial wastewaters, characterized by complex water chemistry, often contain a mixture of metals. Zinc (Zn), an essential element for animals and humans but toxic in excess, commonly coexists with Mn(II) in these contaminated environments. Zn can interact with Mn oxides through surface adsorption and structural incorporation processes. While previous studies have demonstrated that Zn(II) can inhibit the formation of bacterial Mn oxides by altering the structure of oxidation-related enzymes, such as multi-copper oxidases, similar inhibition in fungal systems has not been reported previously. Remarkably, our ongoing research has observed that, under environmentally relevant conditions, the presence of Zn(II) inhibits the formation of Mn oxides by a recently isolated Mn-oxidizing fungus, *Curvularia lunata* TC-1 from the legacy lead-zinc mining district in Oklahoma, at a relatively low concentration. Importantly, this inhibition does not affect the growth of the fungus. To elucidate this phenomenon, we are conducting a series of experiments to uncover the Mn(II) oxidation pathways of the fungus TC-1 and how Zn(II) impedes the biomineralization of Mn oxides. Standard native PAGE will be employed to separate extracellular metabolites and intracellular Mn oxide-related enzymes from the fungus grown with or without Zn(II), and identification will be carried out using LC/MS/MS. Results from this study have the great potential to unveil a novel pathway in the biogenic Mn(II) oxidation process, providing valuable insights for advancing future methodologies utilizing native fungi in metal bioremediation strategies.