Manganese and Iron Oxidation by Fungi Isolated from Building Stone

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Abstract. Acid and nonacid generating fungal strains isolated from weathered sandstone, limestone, and granite of Spanish cathedrals were assayed for their ability to oxidize iron and manganese. In general, the concentration of the different cations present in the mineral salt media directly affected Mn(IV) oxide formation, although in some cases, the addition of glucose and nitrate to the culture media was necessary. Mn(II) oxidation in acidogenic strains was greater in a medium containing the highest concentrations of glucose, nitrate, and manganese. High concentrations of Fe(II), glucose, and mineral salts were optimal for iron oxidation. Mn(IV) precipitated as oxides or hydroxides adhered to the mycelium. Most of the Fe(III) remained in solution by chelation with organic acids excreted by acidogenic strains. Other metabolites acted as Fe(III) chelators in nonacidogenic strains, although Fe(III) deposits around the mycelium were also detected. Both iron and manganese oxidation were shown to involve extracellular, hydrosoluble enzymes, with maximum specific activities during exponential growth. Strains able to oxidize manganese were also able to oxidize iron. It is concluded that iron and manganese oxidation reported in this work were biologically induced by filamentous fungi mainly by direct (enzymatic) mechanisms.

Introduction

Iron and manganese are important components in the majority of rocks present on the earth’s crust, and essential for oxidative and reductive microbial metabolism, which plays an important role in the natural cycling of these elements. Although the ability of fungi to oxidize both manganese and iron has been widely reported [2, 11, 21, 22, 24], the selective advantage conferred by such oxidation is not clear [13, 14].

Fungi are known to participate in the biodeterioration of rocks by metal biotransfer [10, 19]. Iron and manganese oxidation may contribute to many biodeterioration
and crust exfoliation processes in stone monuments [15], and under certain environmental conditions may lead to the formation of protective coatings, as reported by Krumbein and Jens [9] and Petersen et al. [16].

The biological oxidation of both iron and manganese can be either direct (enzymatic) or indirect [4, 13, 14]. Although enzymatic oxidation of manganese has been studied by Glenn and Gold [5] and Glenn et al. [6] with the white rot fungus *Phanerochaete chrysosporium*, there is still little information available about such direct fungal oxidation of mineral elements.

In the present work we have studied the capacity of filamentous fungal strains isolated from weathered Spanish monuments to oxidize both Mn and Fe elements.

**Materials and Methods**

**Mn(II) Oxidation**

*Organisms.* The fungal strains studied (Table 1) were those most frequently isolated and widespread along the façades of Salamanca (SA) and Toledo (TO) cathedrals (Spain). Two groups were distinguished according to their ability to produce organic acids in culture [23].

*Cultivation Media and Methods.* Four different culture media were made (Table 2). In medium 3, Mn concentration was adjusted according to Timonin et al. [22]. Twenty milliliters of Mn(II)-containing media in 100-ml conical flasks was inoculated with 1 ml of spore suspension (10⁵–10⁶ cells/ml) of each fungal strain, or 1 ml of distilled and sterilized water in controls, and incubated at 27°C, in the dark, for periods up to 28 days.

*Spectroscopic Procedures.* Spectra and absorbance values were recorded on a Hitachi U-1100 spectrophotometer at room temperature using a spectral bandwidth of 1.0 nm and quartz cuvettes with a 0.5 cm light path for oxidation enzyme assays and 1 cm for oxidation assays.

*Assay of Mn(IV).* The contents of the conical flasks were harvested periodically, centrifuged at 4°C for 20 min at 15,000 rpm, and the pH of the supernatant determined. Mn(IV) was assayed according to Krumbein and Altman [8].

**Fe(II) Oxidation**

*Organisms.* The fungal strains were *Cladosporium cladosporoides* (TO), *Alternaria alternata* (SA), *Phoma glomerata* (SA), *Penicillium frequentans* (SA), and *Penicillium steckii* (TO) (Table 1).

*Cultivation Media and Methods.* The strains were cultivated in Czapek-Dox saline medium (g l⁻¹): NaNO₃ (2), K₂HPO₄ (0.1), KCl (0.05), MgSO₄ · 7H₂O (0.05), FeSO₄ (0.05), and glucose (30); pH was adjusted to 4.42 to avoid Fe(II) self-oxidation. Glucose was sterilized by steaming for 20 min on each of three successive days, separately from mineral medium. Mineral medium was sterilized by filtration to avoid Fe(II) self-oxidation at high temperature. Cultures were grown for up to 21 days in darkness at 27°C in 100-ml conical flasks containing 20 ml of medium and inoculated with 1 ml of spore suspension (10⁵–10⁶ cells/ml) of each strain. Controls were prepared by substituting the fungal inoculum with an equivalent volume of distilled and sterilized water.

*Assay of Fe(III).* The contents of the conical flasks were harvested periodically and the mycelium separated by filtration through Whatman no. 1 paper. The filtrate was passed through Millipore filters (0.22 µm pore size) and its pH, Fe(III), and total Fe concentrations were quantified, the last according