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Soil Biology & Biochemistry 40 (2008) 1364–1369

Soil Biology & Biochemistry

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Microbial DNA extraction and analyses of soil iron–manganese nodules

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Received 7 November 2006; received in revised form 18 December 2006; accepted 2 January 2007
Available online 8 February 2007

Abstract

Iron–manganese (Fe–Mn) nodules and concretions are soil new growth, reflecting soil environmental conditions during their formation. Bacteria play a dominant role in the oxidation of dissolved Mn(II) in aqueous systems and the formation of marine and freshwater Fe–Mn nodules. However, the role and significance of bacteria in soil Fe–Mn nodule formation have not been well recognized. In this paper, microbial DNA was directly extracted from two Fe–Mn nodule samples collected from Wuhan and Guiyang in central China. The extracted DNA was amplified by polymerase chain reaction (PCR) and cloned. The clones were then screened by amplified ribosomal DNA restriction analysis (ARDRA). Twenty patterns were obtained for Wuhan sample and Guiyang sample, respectively. DNA sequencing and phylogenetic analyses revealed that the bacterial compositions of the Fe–Mn nodules were mainly belonged to Firmicutes, β-proteobacteria, γ-proteobacteria branches of the domain bacteria. These divisions had close relativeness with Mn(II)-oxidizing bacteria identified from marine Fe–Mn nodules, implying the possible contributions of these bacteria to soil Fe–Mn nodule formation.

Keywords: Soil Fe–Mn nodules; DNA extraction; 16S rDNA; ARDRA; Mn(II)-oxidizing bacteria

1. Introduction

Fe and Mn oxides (including hydroxides and oxyhydroxides), widespread in soils and sediments, play a critical role in the fate and transport of heavy metals and organic pollutants through sorptive, catalytic and oxidative processes. The formation of Fe–Mn nodules and concretions in soil is regarded as the result of drying–wetting alternations of soil moisture and the corresponding oxidation and reduction cycles (Burns and Burns, 1975). Fe and Mn oxides release Fe(II) andMn (II) ions into the soil solution under the reducing conditions, and the Fe(II) and Mn(II) are oxidized into Fe(III) and Mn(III, IV) and precipitated when the soil is dried out (McKenzie, 1989). Manganese is more mobile than Fe and requires a higher oxidation potential than Fe(II), resulting in the formation of alternate layers of Fe-rich and Mn-rich materials observed in many nodules (White and Dixon, 1996). The elemental composition and distribution characteristics within the nodules may reflect their forming redox history of the pedoenvironment (McKenzie, 1989; Liu et al., 2002).

Microorganisms, especially bacteria, are known to catalyze the oxidation of Mn(II) and the formation of Mn(III, IV) oxide minerals. Biological Mn(II) oxidation is generally much faster (by up to 10^5 times) than abiotic Mn(II) oxidation processes, suggesting that biological Mn(II) oxidation dominates in the environment (Kim et al., 2003; Tebo et al., 2004). A number of investigations at specific field sites have shown that the biological processes are responsible for Mn(II) oxidation at those locations (e.g., Tebo and Emerson, 1985, 1986; Cowen et al., 1986, 1990; Tebo, 1991; Wehrli et al., 1995; Harvey and Fuller, 1998; van Cappellen et al., 1998; Fuller and Harvey, 2000; Kay et al., 2001). For these reasons, the majority of naturally occurring environmental Mn oxides are believed to be derived either directly from biogenic Mn(II) oxidation processes or from the subsequent

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0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2007.01.004

alteration of the biogenic oxides (Tebo et al., 2004). Significant advances have been made in the last 10 years in the molecular biology of Mn(II) oxidation by three phylogenetically distinct bacteria representatives of different aqueous environmental settings: a marine *Bacillus* sp. strain SG-1; *Leptothrix discophora* strains SS-1 and SP-6, common in wetlands and in iron seeps and springs; and *Pseudomonas putida* strain MnB1, representative of freshwaters (Tebo et al., 1997). However, very little information is available about biological Mn oxidation in the soil environment. Douka (1977) isolated two Mn(II) oxidizing bacteria from manganese concretions of an alfisol soil of West Peloponnese in Greece. The bacteria were identified as *Pseudomonas* sp. nov. and *Citrobacter freundii* and their cell and the cell-free extracts could catalyze the formation of Mn precipitates. Sullivan and Koppi (1992) observed cell-like substances on the surface of manganese oxide coatings of a black earth (Typic Pellustert) in Australia using light microscopy and electron microscopy, suggesting microbial oxidation of Mn(II) contributed to the formation of the manganese oxide coatings in this soil.

In the present study, we improved soil DNA extraction methods and successfully extracted microbial DNA from soil Fe–Mn nodules. The extracted DNA was polymerase chain reaction (PCR) amplified and cloned. The clones were then screened and sequenced. Phylogenetic analyses revealed that the bacterial compositions of the Fe–Mn nodules were mostly belonged to Firmicutes, β-proteobacteria, γ-proteobacteria branches of the domain bacteria. Some of them had close relativeness with Mn(II)-oxidizing bacteria identified from the marine Fe–Mn nodules, implying the possible contributions of these bacteria to the formation of soil Fe–Mn nodules.

2. Materials and methods

2.1. Sample collection

Fe–Mn nodules were collected from two different locations of Wuhan, Hubei Province and Guiyang, Hunan Province in central China. Wuhan sample (WH) was collected at a 40-cm depth from the subsoil horizon of a subacid orthic agrudalf developed from Quaternary siliceous and alluvial sediments, and Guiyang sample (GY) was collected at 20–100 cm depth of an alt-udic ferrisol developed from rammell. Nodules were separated from the soil by wet sieving and stored at room temperature. The morphological properties, mineralogy, and chemical composition of the nodules have reported previously (Tan et al., 2000; Liu et al., 2002).

2.2. DNA extraction from the Fe–Mn nodules

Before DNA extraction, 50 g of each sample was surface sterilized by rinsing in sterile distilled water for five times, immersing in 0.1% NaClO for 1 min, and then followed by five rinses in sterile distilled water. The nodules were ground to powder using a pestle and a mortar under asepsis condition. The control powder was autoclaved at 121 °C for 30 min twice and dried at 100 °C for 6 h. DNA extraction was carried out with a combination of physical bead beating, chemical and biological lyses as described by Zhang et al. (2005) and He et al. (2005). Fe–Mn nodule powder was suspended in 143 ml extraction buffer (200 mM NaCl, 200 mM Tris, 2 mM sodium citrate, 10 mM $CaCl_2$, 50 mM EDTA, adjusted to pH 8.0), and then 1 ml poly(A) (10 mg ml^{-1}) and 4.5 ml 10% pyrophosphate were added. The suspensions were treated with bead beater (Biospec Products, Bartlesville, OK) for 3 min in an ice bath after mixing with 40 g of 1-mm-diameter silica beads and 5 g of 0.1-mm-diameter glass beads. They were incubated at 37 °C for 2 h after 2 ml lysozyme (50 mg ml^{-1}) was added, and then 1 ml protease K (20 mg ml^{-1}) and 10 ml 20% SDS were added into. Subsequently, the suspensions were shaken at 250 rpm for 1 h at 37 °C and then incubated in a 65 °C water bath for 1 h with end-over-end inversions every 10 min and centrifuged at 6000*g* for 15 min. The supernatants were extracted with an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1, vol:vol:vol) and precipitated by adding 0.1 volume of 3 mol l^{-1} sodium acetate (pH 5.2) and equal volume of cold isopropanol, washed with 75% ethanol, and resuspended in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). The resuspended solution was re-extracted with phenol–chloroform–isoamyl alcohol (25:24:1) and precipitated with ethanol again (He et al., 2005). The control sample was subjected to the same DNA extraction procedures.

2.3. PCR amplification, cloning, and amplified ribosomal DNA restriction analysis (ARDRA)

For amplification of 16S rRNA gene of bacteria, the primers 27F (5′-AGA GTT TGA TCM TGG CTC AG) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T) (Lane, 1991) were used in a standard 35-cycles PCR with an annealing temperature of 50 °C. The DNA extracts were 10-fold diluted and used as a template. The 50-μl reaction mixtures contained 1 × PCR buffer, 400 nM each primer, 2 mM $MgCl_2$, 250 μM each dNTP, 2.5 U *Taq* DNA polymerase, 1 μl of 20 mg ml^{-1} bovine serum albumin (BSA), and 2 μl of DNA template. The amplified 16S rRNA gene fragments were ligated into the pGEM-T Easy Vector (Promega, Madison, WI), and the resulting ligation products were used for transformating into *E. coli* JM109 competent cells following the instructions of the manufacturer. 16S rRNA gene libraries were constructed and 40 randomly chosen colonies per sample were PCR re-amplified using the primers of T7 and SP6. The amplicons were analyzed by restriction digestion with *Hae* III, *Rsa* I, and *Hha* I (New England Biolabs). The digested DNA fragments were run in 2% agarose gel electrophoresis. The ARDRA patterns were grouped by similarity and 1 or 2 representative clones from each group were sequenced.