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A molecular method to evaluate basidiomycete laccase gene expression in forest soils

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Abstract

Fungal laccases, exo-enzymes lacking high substrate specificity, play a central role in cycling of soil organic matter. In a precedent work based on a PCR technique on soil DNA extracts, we showed that the highest diversity of laccase genes occurred in the most organic horizon (i.e., O_h) of a brown forest soil. In the present article, we develop a method of RNA extraction, RT-PCR, and semi-quantitative PCR to analyze the expression of Basidiomycete laccase genes. We performed a very first assessment of the methodological approach on five cores of the O_h horizon of the same brown forest soil. The level of laccase transcripts was heterogeneous amongst the soil cores. Two samples gave strong expression levels, two showed very faint ones, and the last replicate presented no detectable laccase transcripts. A control with a transcript analysis of the actin gene, which is constitutively expressed in fungi, allowed to rule out that the differences in the transcript level of laccases were due to experimental failures or inhibiting substances in the RNA extracts. A cladistic analysis showed that most laccase transcript sequences detected were grouped in two clades closely related to the ectomycorrhizal fungus *Xerocomus chrysenteron*. Comparison between DNA laccase sequences found in our previous study and RNA laccase sequence profiles found here showed that less than 30% of the laccases genes detected in a soil core were expressed. This preliminary study demonstrates the potential of RT-PCR for gene expression profiling in forest soils. The number of analyzed samples cannot allow us to draw definitive ecological conclusions, but there are some indications that differences between rhizospheric and bulk soil samples (polyphenol abundances, microbial densities, etc.) might be a potential explanation for the variable laccase expression observed.
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Keywords: Laccases; Soil RNA extraction; RT-PCR; Semi-quantitative PCR; Basidiomycetes; Brown forest soil

1. Introduction

A great part of the terrestrial carbon (C) is bound as organic residues in soils (Killham, 1994). This C-pool is maintained quite constant by several processes, such as humification, recycling, and mineralization. Soil microorganisms have been shown to

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be largely involved in these processes, and their high biodiversity contributes to the soil function in ecosystems (Emmerling et al., 2002). As contribution to the present *Geoderma* special issue about the international conference "Mechanisms and Regulation of Organic Matter Stabilization in Soils," this article deals with a methodological development to characterize the expression in soils of fungal laccases, key enzymes involved in both mineralization and humification.

Fungi are one of the most important soil microorganism groups involved in the carbon cycle in many regards. With 40–200 g of mycelial dry matter per square meter of soil, they represent a dominant part of the soil biomass (Thorn, 1997; Dighton and Kooistra, 1993). Due to their mycelial structure, they can explore soil compartments and reach organic matter fractions not accessible to many other organisms (Read and Perez-Moreno, 2003). As partners of mycorrhizal symbioses, they contribute to the transfer of 10–20% of the global photo-assimilates into soils (Smith and Read, 1997). Based on their nutritional mode, fungi can be divided in three functional groups (saprophytes, symbionts, and parasites), in each of which many species are able to produce oxidative exo-enzymes playing an important role in the formation and decomposition of soil organic matter (SOM) (Chefetz et al., 1998; Read and Perez-Moreno, 2003).

Laccases, lignin, and manganese peroxidases are the main oxidative exo-enzymes produced by fungi (Gramss et al., 1998; Chen et al., 2003). Apart from a lack of substrate specificity common to other exo-enzymes, laccases have the widest distribution among fungi (Gramss et al., 1998). Both features allow them to be involved in many biosynthetic processes such as formation of humic acids (Chefetz et al., 1998) and biodegradation of complex organic and aromatic substances (Eggert et al., 1997). It was also shown that laccases can oxidize nonphenolic components in the presence of appropriate radical mediators (Bourbonnais et al., 1995, 1998) and so completely degrade lignin (Eggert et al., 1996).

The development of molecular methods opened new perspectives for investigating and understanding the function of microorganisms in soils. They in particular allow determining the respective role of target groups of microorganisms by characterizing more relevant species and by assessing whether a loss of diversity might disturb soil functions (Emmerling et al., 2002). Direct DNA extraction from soils and its use for PCR amplifications with specific primers has been developed from years for bacteria (Holben et al., 1988; Wintzingerode et al., 1997). Application of this method to fungi mainly focus on polymorphic regions of the ribosomal DNA to assess the diversity and spatial distribution of fungal communities in soils (Dickie et al., 2002; Tedersoo et al., 2003), but studies aiming to analyze protein-coding genes are scarce (Lyons et al., 2003).

In a precedent work (Luis et al., 2004), we optimized a PCR-based method on DNA extracted from different soil horizons of a brown forest soil to analyze the diversity of Basidiomycete laccase genes. The gene diversity profiling provided a glimpse of the oxidative potential of fungi with laccase genes. But, as all genes might not be continually expressed, their distribution gave no information on the real fungal laccase activity in the soil. The aim of the present study was to develop a procedure to analyze the expression of Basidiomycete laccase genes. This procedure was assessed on five soil samples on which the gene diversity had been characterized previously (Luis et al., 2004). This allowed comparing the already known diversity with the expression pattern revealed here. To our knowledge this kind of comparison was never reported for soil fungi previously. We also assessed the respective part of the ectomycorrhizal and saprophytic Basidiomycetes in the revealed expression pattern using a cladistic analysis of detected sequences.

2. Materials and methods

2.1. Samples of soils

Five cores (S1–S5) with 12 cm diameter and 35 cm height of a brown forest soil under a mixed oak beech forest of the "Steigerwald" (Northern Bavaria, 49°52′26″ N, 10°27′54″ E) that belongs to an experimental station of the Institute of Ecosystem Research (BITÖK) from the University of Bayreuth were randomly collected in September 2002 within a plot of 6×6 m. The position of the cores and their contact to the tree vegetation were noted. Two of the cores (S2

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