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Modifications of degradation-resistant soil organic matter by soil saprobic microfungi

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Abstract

Modifications of humic (HA) and fulvic (FA) acids in their solutions and in sterile soil by microfungal species and two well-known HA degraders were studied by measurement of total oxidizable carbon (OC), absorbances, enzyme activities and CO_2 release. The effect of glucose on FA and HA, and also minerals on FA utilization was also observed. Microfungi affected HA more than FA. Common microfungal species decolorized HA and decreased their molecular size (evaluated in terms of A_4/A_6 ratio). Some of them decreased aromaticity of HA and FA as the only carbon sources. They did not affect OC, although released CO_2 from FA. Under higher availability of mineral nutrients, the FA aromaticity increased and FA decolorization decreased. The molecular size of HA decreased in the presence of glucose. In the FA medium complemented by minerals, the known basidiomycete HA degrader, *Trametes versicolor,* decreased the amount of aromatic compounds in contrast to microfungal species *Alternaria alternata*, *Clonostachys rosea*, *Exophiala* cf. *salmonis*, *Fusarium coeruleum*, *F. redolens*, *Penicillium canescens, Phoma* sp. and another basidiomycete *Phanerochaete chrysosporium*. No microfungal species exhibited lignin peroxidase activity. On the other hand, activities of manganese peroxidase (MnP) were recorded for all species incubated in FA. Carbon dioxide produced from soil inoculated by microfungi negatively correlated with the decolorization, aromaticity and OC of/in FA reisolated from the soil. The results support the hypothesis that soil microfungi can attack both HA and FA and can represent an important factor in their transformations in arable soils. The enzyme involved in FA modifications is probably fungal MnP. We enriched a group of known HA and FA degraders and showed some abilities of a few frequent soil microfungal species. This can be one of the first but important step towards learning the functioning of carbon release from the big reservoir represented by humic substances in arable soils.

Keywords: Soil microfungi; Fulvic acid; Humic acid; Enzyme activities; CO_2 production; Effect of glucose; Mineral nutrients; Spectrophotometry

1. Introduction

Humic substances (HS) present in soil, water and sediments are the product of biotransformation of plant and animal residues. They constitute not only a major pool of organic carbon in the global ecosystem but are also able to bind nutrients for plants and remobilize sedimented heavy metals. In addition, HS influences soil water-holding capacity and the degree of soil particle aggregation and are thus considered to be essential for soil stability (Kästner, 2000).

Investigation into the organisms responsible in HS biotransformation is important because in some regions, current global warming can lead to increasing degradation and mineralization of humus by soil organisms (Zavarzina et al., 2004).

White-rot fungi, brown-rot basidiomycetes, terricolous basidiomycetes, ectomycorrhizal fungi, soil-borne microfungi and bacteria were found to be able to decolorize humic acids (HA) (Gramss et al., 1999). Studies of HS utilization have so far mostly focused on a few model

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species of the ligninolytic wood-decay fungi of the white-rot type (e.g. *Phanerochaete chrysosporium* or *Trametes versicolor*, Blondeau, 1989; Dehorter and Blondeau, 1992), which are not considered to play a key role in utilization of HS, e.g. in arable soils, because they grow mostly on compact wood or woody debris and cannot survive in soil for longer periods (Martens and Zadrazil, 1992). Bacteria have also been found to be able to decolorize HA medium but the decolorization seemed to end after 48 h and eubacteria grown in a rich nutrient broth were ineffective degraders of soil HA compounds (Gramss et al., 1999). Though scant attention has so far been paid to microfungi, their abilities to utilize resistant soil organic matter (SOM) were noted (Gramss et al., 1999; Strnadová et al., 2004) and some microfungal species, e.g. *Chalara longipes*, were found to decolorize spruce litter HA more effectively than the basidiomycetes *Coriolus consors*, *Coriolus hirsutus* and *Lenzites betulina* (Koukol et al., 2004).

The ability of fungi to modify HS is associated with their extracellular system of non-specific lignolytic enzymes; lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Dehorter and Blondeau, 1992; Steffen et al., 2002). Moreover, degradation of HA in vivo is considered to be a cometabolic event (Zavarzina et al., 2004).

Because the process of HA and fulvicacid (FA) degradation is induced by enzymes produced by microorganisms, it is useful to combine the viewpoint of soil microbiology, enzymology and chemistry in one approach. Obviously, because of extremely high complexity of the system studied, it is not possible to observe and identify all the organisms and their enzyme activities as well as all the changes in HS induced by them. We thus had to choose important parameters describing the process of HS modification as a prerequisite of degradation.

In the present study, the abilities of common soil microfungal species to modify HA and FA were assessed by measurements of the physico-chemical properties of HA and FA by spectrophotometric methods, measurement of concentration of total oxidizable carbon (OC), carbon dioxide release and by micellar electrokinetic capillary chromatography (MECC). We investigated if the tested microfungi are able to modify HA and FA and how they do it. It was studied at different conditions: during the growth of microfungi in HA and FA as the only nutrient sources, in FA and HA complemented by glucose and in FA complemented by minerals. The hypothesis that glucose will support HA and FA utilization by supplying of easily available energy in a first phase of mycelium formation, and that minerals increase the degradation by supporting creation of biomass was tested. The ability of microfungi to utilize FA was compared with two well-known HA degraders, white-rot fungi *T. versicolor* and *P. chrysosporium*. The hypothesis that white-rot fungi will more effectively degrade FA than soil microfungi because of their well-developed enzyme system centered on lignine-like substances was tested by measurements of FA absorbances and OC concentration in FA.

2. Material and methods

2.1. Organisms

The following microfungal species were tested: *Alternaria alternata* (Fr.) Keissl. (CCF [1]3529), *Clonostachys rosea* f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams (CCF 3532), *Exophiala* cf. *salmonis* J. W. Carmich, *Fusarium coeruleum* Lib. ex Sacc., *Fusarium redolens* Wollenw., *Paecilomyces lilacinus* (Thom) Samson (CCF 3531), *Penicillium canescens* Sopp and *Phoma* sp. (CCF 3530).

Fungal strains used in the present work were obtained from the arable soil (clay-loam orthic luvisol, pH (water) 6.97, 1.77% OC, 0.13% total N, 15.3 mg/kg available P) collected in experimental field of the Research Institute of Crop Production (Prague, Czech Republic). All of them were considered to utilize FA because they were obtained from soil by using an isolation medium containing silicate-gel, minerals and FA as the only source of carbon. Moreover, all strains were capable of producing lignolytic enzymes (non-specific peroxidases) which participate in HA degradation. The production was detected by a spot test for the presence of oxidative enzymes according to Gramss (Gramss et al., 1998).

Phanerochaete chrysosporium Burds. (CCBAS 854) and *Trametes versicolor* Lloyd (1920) (CCBAS 614), known HS degraders (Dehorter and Blondeau, 1992), were tested for comparison. They were obtained from the Culture Collection of Basidiomycetes CCBAS of the Institute of Microbiology, CAS.

2.2. Preparation of FA and HA

HS were prepared following the procedure of Gryndler et al. (2003). One kilogram soil from the above-mentioned field in Prague, sieved through a 2-mm sieve, was shaken with 2 l of 0.1 M HCl for 20 min, washed twice with 2 l of distilled water and extracted with 2 l of 0.5 M NaOH for 20 h at room temperature. Further, the extract was filtered through filter paper, acidified to pH 2.6 with HCl and precipitated HA was retained on filter paper. The precipitate was dissolved in 0.1 M NaOH. Precipitate-free FA-containing filtrate was acidified to pH 1.5 and its FA was adsorbed in a polyvinyl polypyrrolidone column (Sigma P6755, 10 cm height, 4.5 cm diameter). After washing with 300 ml deionized water, FA was eluted from the column by a minimum volume of 0.01 M NaOH. Final pH of the eluate was adjusted to soil pH (pH 6.5).

For analytical purposes (Experiment 4), only 1 g soil, 2 ml of 0.1 M HCl, 2 ml of distilled water and 2 ml of 0.5 M NaOH were used for preparation of FA from each soil sample.

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