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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.

[1]Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, Czech Republic.

2.3. Experiments

2.3.1. Experiment 1: Ability of selected microfungal species to modify FA and HA as only nutrient sources, complemented by glucose and FA complemented by minerals

The experiment had a randomized two-factorial design with the fungal species as one factor (*A. alternata, C. rosea, P. lilacinus, Phoma* sp.) and substrate added to the FA or HA medium as the second factor (no additive-control or glucose for HA; no additive-control, glucose or minerals for FA). FA and HA datasets were processed separately. Three replicates from each treatment were arranged. A sample without fungal biomass was used as blank for measurements. Measurements of absorbance at 280 nm and OC in treatments supplied with glucose are not comparable with that in treatments without glucose because glucose itself affects the value of both parameters.

A mycelial suspension of each microfungal species was prepared by mixing 50 ml distilled water with aerial mycelium scraped from the surface of 15 fungal colonies growing at 25 °C in 5 Petri dishes for 1 week on malt-extract agar (Pitt, 1979). This ensured that the fungal cultures of the same age, cultivated under identical environmental conditions, were used for inoculation of experimental treatments. One-half milliliter of homogenized mycelial suspension was transferred to three 100 ml Erlenmeyer flasks with 10 ml FA or HA solution. Half milliliter of mineral stock solution was added in a third of treatments and glucose monohydrate (200 mg/l) to another third to study the effect of minerals and easily available carbon source on the utilization of HA and FA by fungal strains. Mineral stock solution contained 2920 mg $Ca(NO_3)_2 \cdot 4H_2O$, 1957 mg $MgSO_4.7H_2O$, 972 mg $CaCl_2.2H_2O$, 704 mg Na_2SO_4, 193 mg KNO_3, 45 mg K_2HPO_4, 25 mg NH_4NO_3 and 6 mg KH_2PO_4 in 1000 ml water.

After 1 month of incubation, the solution was filtered through filter paper to separate FA and HA from fungal biomass. The filtrate was further characterized by absorbance at 280 nm characteristic for aromatic compounds and by the A_4/A_6 ratio (calculated from the absorbances at 465 and 665 nm) referring to molecular weight of HA (Chen et al., 1977). Also absorbance at 665 nm that could inform about HA and FA decolorization (Koukol et al., 2004) was measured. The OC amount in HA and FA solutions was determined according to (Sims and Haby 1971) with some modifications. A volume of 0.5 ml of either HA or FA, 2 ml 1% $K_2Cr_2O_7$ in concentrated sulfuric acid and 0.5 ml of water were mixed and incubated at 25 °C for 1 h. Samples were then heated to 110 °C for 3 h, left to cool and their absorbance was measured at 580 nm. The content of OC was always calibrated against anhydrous glucose (0, 10, 30, 100, 1000 mg glucose/l). Spectrophotometric measurements were done using a Ultrospec III UV–VIS spectrophotometer (Pharmacia LKB, Sweden). Fungal biomass, retained on filter paper, was oven-dried at 105 °C to constant weight. In addition, one replicate per treatment of FA solution complemented by minerals and incubated with microfungi was analyzed by MECC.

MECC was performed at 30 °C in 50 mM borate buffer (pH 9) as a working electrolyte containing 50 mM SDS in the Beckman P/ACE 5000 capillary electrophoresis instrument, with fused silica capillary (internal diameter 75 μm, effective length 30 cm), 110 μA current at electric field intensity 270 V/cm for 30 min. Organic compounds were detected as absorbance peaks measured at 214 nm.

2.3.2. Experiment 2: Comparison of abilities of selected soil microfungi and white-rot fungi to modify FA

The experiment had one-factorial design with uninoculated control and treatments inoculated with 10 fungal isolates (microfungi: *A. alternata, E.* cf. *salmonis, F. coeruleum, F. redolens, C. rosea, P. lilacinus, P. canescens, Phoma* sp., white-rot fungi: *P. chrysosporium* and *T. versicolor*). Three replicates per each treatment were arranged. All the species were incubated in FA complemented by minerals where the fungal biomass grew better than in FA itself or FA complemented by glucose. The experimental conditions and FA characterization were the same as in Experiment 1. MECC was not used.

2.3.3. Experiment 3: Activities of lignolytic enzymes produced by microfungi growing in FA medium

The experiment had one-factorial design with uninoculated control and treatments inoculated with four microfungal isolates (*A. alternata, C. rosea, P. lilacinus, Phoma* sp.). Four replicates per each treatment were arranged. The lignolytic enzymes laccase, MnP and LiP were assessed during the experimental period of 1-month incubation of the four microfungal species in 50 ml of FA containing 0.5 ml of microbial suspension (see above). The samples were shaken at 22 °C. Activities of enzymes were measured at 3–4 d intervals.

Activity of MnP was assessed spectrophotometrically according to Ngo and Lenhoff (1980), activity of LiP according to Tien and Kirk (1988) and laccase activity was detected according to Niku-Paavola et al. (1990).

2.3.4. Experiment 4: Measuring of FA degradation caused by selected microfungi by carbon dioxide release

The experiment was designed as one-factorial with uninoculated control and treatments inoculated with four fungal isolates (*A. alternata, C. rosea, P. lilacinus, Phoma* sp.). Five replicates from each treatment were arranged.

Half milliliter of mycelial water suspension (see above) was added to a medium containing 1 g perlite, 0.3 ml mineral stock solution and 2.5 ml FA as the only carbon source. In parallel, the same volume of inoculum was added to 10 g of sterile (γ-irradiated, 50 kGy; pH 6.5, 30% humidity) soil sieved through a 2-mm sieve. The samples were incubated at room temperature (ca 23 °C). Perlite containing FA solution was used rather than FA solution alone in order to simulate gas fluxes in soil. All the samples

were incubated in 100 ml glass bottles sealed with tight rubber stoppers.

At 3–4 d intervals, a sample of 500 µl of headspace air was taken from each container using a 2 ml syringe. The carbon dioxide concentration in the air sample was measured using a Perkin-Elmer 8500 gas chromatograph (2 m column Chromosorb 102/80-100, temperature 150 °C, molecular nitrogen as carrier gas at a flow rate of 19 ml/min, thermal conductivity detector at 350 °C). After 4 weeks, FA (pH 5.5) was isolated from incubated samples and characterized as described above.

2.4. Data analysis

Analysis of variance (ANOVA) with $P \leqslant 0.05$ was used in order to evaluate the significance of effects of fungal species, available minerals and glucose on HA- and FA-characterizing parameters.

3. Results

3.1. Ability of selected microfungal species to modify FA and HA as only nutrient sources, complemented by glucose and FA complemented by minerals (Experiment 1)

All four fungal species, *A. alternata, C. rosea, P. lilacinus* and *Phoma sp*., tended to increase the A_4/A_6 ratio of HA after 1 month of incubation. Compared to uninoculated control, significant increase in this parameter was caused only by *C. rosea* (+12.2%) and *P. lilacinus* (+7.3%, Table 1). Glucose addition significantly decreased the values of this parameter. Absorbance of HA at 665 and 280 nm was significantly decreased by all fungi. In both cases, the most effective strains were again *C. rosea* and *P. lilacinus*, which decreased the absorbance of HA at 665 nm, compared to uninoculated control, by 27.5% and 22.2% Table 1 and the absorbance of HA at 280 nm by nearly 19.2% and 16%, respectively Table 2. OC in HA was not significantly affected by microfungi.

In FA solution, none of the four fungal species caused significant changes in the absorbance at 665 nm Table 1, but when minerals were added, the absorbance strongly increased (+89%). However, the presence of glucose caused no changes in this parameter. Absorbance at 280 nm was significantly affected only by *A. alternata* (−9.1%). The added minerals significantly increased (+8.3%) the absorbance at 280 nm Table 1. Dry mycelial biomass determined after 1 month was too small to be evaluated, except in FA complemented by minerals. One species, *Phoma* sp., changed significantly the pH of the FA solution Table 1. As with HA, OC in FA was not significantly affected by microfungi.

All the tested species of microfungi, notably *C. rosea, A. alternata* and mainly *Phoma* sp., modified the electrokinetic profile of FA as revealed by MECC (Fig. 1), causing, e.g., size reduction of some absorbance peaks.

3.2. Comparison of abilities of selected soil microfungi and white-rot fungi to modify FA (Experiment 2)

The presence of any of the eight microfungi or two species belonging to basidiomycetes under study in FA complemented by minerals (Experiment 2) did not affect the A_4/A_6 ratio or absorbance at 665 nm (Table 3). Absorbance at 280 nm was lower in treatments inoculated by microfungus *P. lilacinus* (−10.4%) and white-rot fungus *T. versicolor* (−11.8%) than in uninoculated control. No significant differences were observed in the biomass produced by individual microfungi, although the highest biomass was recorded for *F. coeruleum, E.* cf. *salmonis* and

Table 1
Average values of parameters characterizing humic substances, their final pH and fungal biomass accumulated after 1-month incubation of four microfungal species in fulvic and humic acid solutions available as only source of nutrients, complemented by glucose or minerals

	FA: A_{280}	FA:A_4/A_6	FA: A_{665}	FA: pH	HA: biomass(g)	HA: A_4/A_6	HA: A_{665}	HA: pH
Inoculation								
A. alternata	11.9b[1] ± 0.5	27.1 ± 35.2	0.044 ± 0.022	6.47ab ± 0.11	0.034a ± 0.018	6.12c ± 0.00	0.690b ± 0.033	6.59 ± 0.15
C. rosea	12.8a ± 0.6	13.8 ± 5.4	0.056 ± 0.019	6.54a ± 0.10	0.033a ± 0.024	6.80a ± 0.32	0.585d ± 0.023	6.80 ± 0.16
P. lilacinus	12.6a ± 0.6	19.1 ± 17.0	0.053 ± 0.024	6.53a ± 0.14	0.027a ± 0.009	6.50ab ± 0.52	0.628cd ± 0.092	6.65 ± 0.31
Phoma sp.	12.5a ± 0.8	32.2 ± 37.2	0.046 ± 0.026	6.40b ± 0.03	0.026a ± 0.016	6.34bc ± 0.19	0.660bc ± 0.021	6.52 ± 0.17
Control	13.1a ± 0.8	13.1 ± 6.3	0.040 ± 0.017	6.50a ± 0.00	0	6.06c ± 0.14	0.807a ± 0.028	6.50 ± 0.00
Medium								
0	12.1b ± 0.6	28.2 ± 28.6	0.036b ± 0.036	6.50 ± 0.09	0.023 ± 0.017	6.50a ± 0.45	0.665 ± 0.100	6.63 ± 0.17
Glucose	[2]	24.0 ± 29.9	0.039b ± 0.090	6.50 ± 0.10	0.025 ± 0.021	6.22b ± 0.26	0.683 ± 0.076	6.59 ± 0.24
Minerals	13.1a ± 0.6	11.1 ± 6.3	0.068a ± 0.067	6.47 ± 0.12	nd	nd	nd	nd

0—FA solution, glucose—FA complemented by glucose, minerals—FA complemented by minerals, A_{280}—absorbance at 280 nm, A_4/A_6—A_4/A_6 ratio, A_{665}—absorbance at 665 nm, biomass—final dry biomass stated in g, FA—fulvic acid, HA—humic acid.

[1]a,b,c,d—significant differences exist where only different letters occur, within a factor (columns) and between fungal species or between media. Duncan's multiple range test has been used.

[2]Measurements of absorbance at 280 nm in treatments supplied with glucose are not comparable with that in treatments without glucose because glucose itself affects the value of both parameters. The values are presented by one-factorial design (Table 2). Also values of OC in FA are presented in Table 2 because there was an interaction between the factors when the data were processed by two-way ANOVA. nd: not determined.

Table 2
Average values of parameters characterizing humic acid after 1 month incubation with four microfungal species

	FA: A_{280}	FA: carbon	FA: biomass	HA: A_{280}	HA: carbon
A. alternata					
0	[1]	135.3±2.7	nd	34.0bc[2]±0.55	328.7±23.7
Glucose	12.3±0.09	256.4±203.3	nd	35.0b±0.77	283.1±4.7
Minerals	—	129.9±14.5	0.034±0.024	nd	nd
C. rosea					
0	—	127.4±63.5	nd	34.2bc±0.54	286.2±19.1
Glucose	11.8±0.19	143.1±10.9	nd	32.1c±1.98	253.2±23.3
Minerals	—	124.4±14.7	0.036±0.007	nd	nd
P. lilacinus					
0	—	136.8±26.3	nd	32.2c±1.39	308.3±35.4
Glucose	12.0±0.37	151.0±12.5	nd	34.6bc±2.85	346.0±118.5
Minerals	—	121.6±5.9	0.035±0.027	nd	nd
Phoma sp.					
0	—	143.1±11.9	nd	34.9b±0.92	297.2±32.7
Glucose	11.7±0.27	140.0±5.4	nd	33.5bc±0.2	270.5±11.9
Minerals	—	134.1±11.1	0.055±0.030	nd	nd
Control					
0	—	127.4±1.2	nd	39.2bc±0.01	293.5±1.0
Glucose	11.9±0.02	136.7±1.4	nd	40.3a±0.01	307.1±1.3
Minerals	—	121.6±0.9	0	nd	nd

0—FA solution, glucose—FA complemented by glucose, minerals—FA complemented by minerals, carbon—total oxidizable carbon stated in mg/l, A_{280}—absorbance at 280 nm, biomass—final dry biomass stated in g, FA—fulvic acid, HA—humic acid.
[1]Values are presented in two-factorial design (see Table 1). nd—not determined.
[2]a,b,c—see Table 1.

Phoma sp. Treatments inoculated with the species belonging to basidiomycetes, mainly *T. versicolor*, exhibited much lower biomass.

3.3. Activities of lignolytic enzymes produced by microfungi growing in FA medium (Experiment 3)

No activity of LiP was detected in any of all the microfungal species studied.

Low activities of other (i.e. Mn-independent) peroxidases were recorded. Similarly, negligible laccase activities were detected in all the studied fungal species (Table 4).

MnP activity was the highest of all measured enzymes. It was also determined in all four species of microfungi studied, being highest in *A. alternata* and *Phoma sp.*

Differences in enzyme activities among fungal species and over time were non-significant. Mean values of enzyme activities over the time (from day 3 to the last day of measurement) and standard deviations are given in the Table 4.

3.4. Measuring of FA degradation caused by selected microfungi by carbon dioxide release (Experiment 4)

A significant ($P \leqslant 0.05$) negative correlation was noted between total CO_2 produced from sterile soil inoculated by microfungi within 1 month and three characteristics of FA, OC ($R = -0.9907$), absorbance at 665 nm ($R = -0.9236$) and 280 nm ($R = -0.9602$).

Carbon dioxide concentration measured in sealed flasks containing FA medium in perlite or sterile soil inoculated by any fungal species under study increased during the 1 month of incubation. From day 20, the CO_2 concentrations increased only marginally, except for *A. alternata*, which showed the highest CO_2 concentrations (Table 5). This species produced approximately a double of total produced CO_2, compared to that produced by the other fungi growing on FA. *P. lilacinus* was the least effective producer and, in sterile soil, produced approximately three to five times less CO_2 than the other fungal species.

Because it was not possible to reisolate FA from perlite and characterize it after the experiment finished, only CO_2 release from this substrate was recorded.

4. Discussion

The utilization of HA and FA has so far been studied in wood- and litter-decomposing basidiomycetes (e.g. Blondeau, 1989; Dehorter and Blondeau, 1992; Steffen et al., 2002; Yanagi et al., 2002) while the involvement of soil microfungi in this process was studied only rarely. In agreement with our results, Gramss et al. (1999) and Koukol et al. (2004) found that common microfungal species are able to modify some physico-chemical properties of HA. We showed it by changes in absorbances at three wavelengths and by MECC. Tested soil microfungi decolorized HA, decreased molecular weight of HA and some of them also aromaticity of HA and FA. On the other

hand, the OC in HA and also in FA was not affected by any microfungal species, although all the tested microfungal species released the CO_2 from FA retained in perlite.

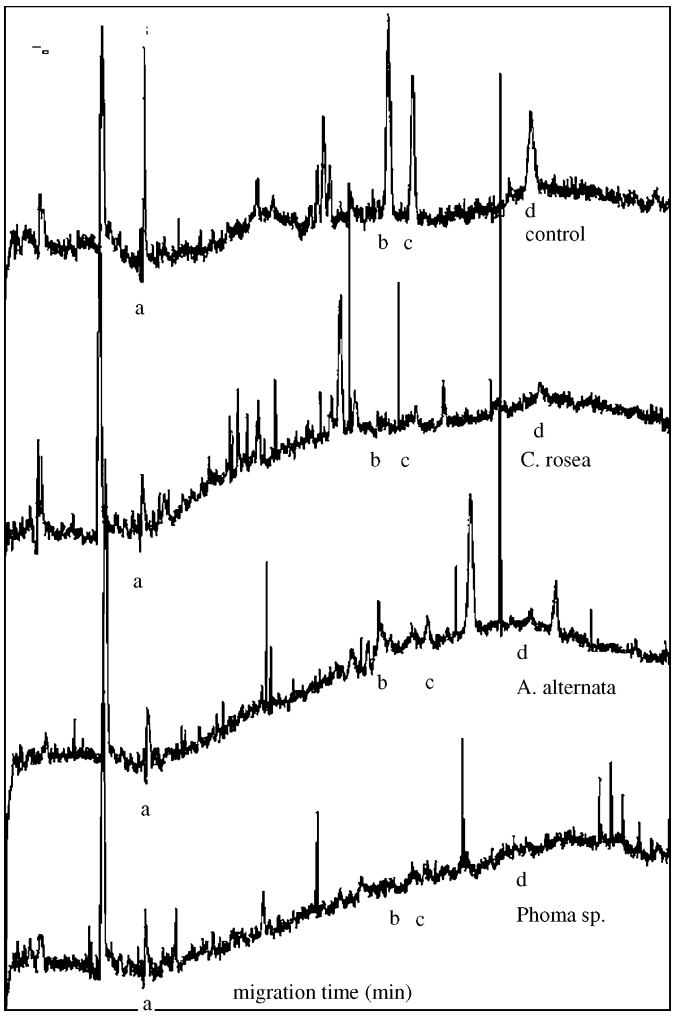

Fig. 1. Changes in FA caused by microfungi assessed by MECC. Peaks correspond to an electrokinetic motility. Peaks that are evidently different from peaks of control FA are identified by a letter below. The same letter represent the same peak.

This discrepancy is probably caused by different sensitivities of the methods used to measure released CO_2 and OC. Highly sensitive gas chromatography was used to detect large differences in CO_2 concentration whereas the less sensitive colorimetric method of estimation of OC failed to detect relatively very small differences of OC corresponding to CO_2 amounts evolved.

Although one would expect a ready utilization of the smaller molecules of easily available FA, much more parameters characterizing HA were affected by the microfungi compared to those characterizing FA. This could possibly be caused by the FA concentration used in the experiments being lower or by insufficient sensitivity of the methods used to characterize HA and FA. However, this result is in agreement with the gel permeation chromatographic data of Gramss et al. (1999) showing that HA are more readily degraded than the smaller FA molecules.

Although HA degradation is considered to be a cometabolic event (Zavarzina et al., 2004), we did not find any significant effect of glucose on the utilization of either HA or FA by microfungi, except for differences in A_4/A_6 ratio of HA referring to molecular size (Chen et al., 1977). Our expectation that mineral nutrients will support FA modifications by the way of supporting microfungal biomass generation was not proven. Instead of this, mineral nutrients decreased the degradation rate measured as decrease rate of FA aromaticity. The effect of other available nutrient sources on either HA or FA utilization was studied rarely. Gramss et al. (1999) detected a reduction of the decolorization rates in media with humic material as a sole carbon source; the rate dropped by 25–50% relative to the rate found in media with malt-extract supplements. Koukol et al. (2004) found higher fungal biomass if organic nitrogen was added to a medium containing HA as a source of carbon and energy.

Unlike the results obtained by other authors with two known HA degraders among basidiomycetes, *T. versicolor* and *P. chrysosporium* (Blondeau, 1989; Dehorter and Blondeau, 1992), our data showed that the modifications

Table 3
Average values of parameters characterizing FA after 1-month incubation of microfungal and white-rot species in FA solution complemented by minerals

	A_{280}	A_4/A_6	A_{665}	Biomass (mg)
Control	13.65a[1] ± 0.01	11.35 ± 0.00	0.047 ± 0.001	0
A. alternata	13.12a ± 0.69	10.37 ± 1.06	0.078 ± 0.008	35.6abc ± 23.8
C. rosea	13.07a ± 0.32	11.00 ± 1.12	0.073 ± 0.008	34.7abc ± 6.5
P. lilacinus	12.23b ± 0.50	11.16 ± 0.20	0.067 ± 0.004	33.8abc ± 27.4
Phoma sp.	13.22a ± 0.38	11.09 ± 1.25	0.073 ± 0.010	55.4a ± 29.8
P. canescens	13.61a ± 0.53	12.96 ± 0.17	0.063 ± 0.004	45.0ab ± 5.0
E. cf. *salmonis*	13.16a ± 0.77	11.88 ± 2.54	0.070 ± 0.018	55.6a ± 43.1
F. coeruleum	13.69a ± 0.36	11.01 ± 0.88	0.077 ± 0.008	75.3a ± 21.3
Fusarium redolens	13.78a ± 0.30	11.16 ± 0.95	0.075 ± 0.009	45.0ab ± 1.9
P. chrysosporium	13.51a ± 0.53	10.09 ± 0.79	0.083 ± 0.010	8.8bc ± 0.7
T. versicolor	12.04b ± 0.66	11.50 ± 2.71	0.065 ± 0.017	2.4c ± 0.3

A_{280}—absorbance at 280 nm, A_4/A_6—A_4/A_6 ratio, A_{665}—absorbance at 665 nm, biomass—final dry biomass expressed in mg
[1]a,b,c—see Table 1.

Table 4
Average values and standard errors of laccase and peroxidases activities (mU/ml) calculated from the activities measured from the third to 28th day of incubation of microfungal species in FA solution

	MnP	Other peroxidases	laccase
A. alternata	0.66±0.29	0.03±0.03	0.04±0.04
C. rosea	0.45±0.28	0.02±0.03	0.11±0.07
P. lilacinus	0.43±0.41	0.02±0.03	0.07±0.07
Phoma sp.	0.91±0.52	0.15±0.15	0.04±0.03

Table 5
Average values of carbon dioxide concentration in the atmosphere (%) after 1-month incubation of microfungi in FA and sterile soil

	FA + perlite	Sterile soil
A. alternata	1.48a[1]±0.26	3.24a±0.66
C. rosea	0.79b±0.43	2.42a±0.77
P. lilacinus	0.64b±0.23	0.81b±1.13
Phoma sp.	0.83b±0.26	2.74a±0.78

Carbon dioxide concentrations in control treatments (average values 0.13% and 0.15% for sterile FA in perlite and sterile soil, respectively) have been subtracted.
[1]a,b—see Table 1.

of FA by microfungi involved only the degree of aromaticity. Here, *T. versicolor* caused a much higher decrease in absorbance at 280 nm than the other fungi under study, except *P. lilacinus*. A significantly lower biomass was, however, found for *T. versicolor* than the other tested microfungi. On the other hand, our results showed that the extent of FA utilization by soil microfungi and white-rot fungus *P. chrysosporium* is comparable. Similarly, Gramss et al. (1999) found strong decrease of absorbance at 340 nm by white-rot basidiomycetes in comparison with the microfungi, while Koukol et al. (2004) noted higher decolorization of HA by the litter-inhabiting anamorphic ascomycete *Chalara longipes* than by some basidiomycetes.

Growing on FA, all microfungal species produced the lignolytic enzyme MnP that is considered to be involved in HA degradation by fungi (Dehorter and Blondeau, 1992). The values were mostly even higher than that found by Tomšovský and Homolka (2004) for various species of white-rot fungi of the genus *Trametes*. The result corresponds with the data of Steffen et al. (2002) who found MnP as a key enzyme in HA degradation by the litter-decomposing basidiomycete *Collybia dryophila*. Similarly, Gramss et al. (1999) noted a positive correlation between decolorization of HA and MnP activity.

Negative correlation between CO_2 release from soil inoculated by microfungi and OC or absorbances of FA reisolated from the same soil could point to an important role of these fungi in FA degradation in natural environment. We do not dare to affirm that there is a direct correlation between e.g., activity of particular enzymes produced by soil microfungi and HS modifications in the field. Even though our results support this hypothesis, it should be tested in further experiments.

Our work enriched a group of known possible HA and FA degraders in arable soils and showed some abilities of several frequent soil microfungal species. Here, the important degraders of lignin-like substances which belong to white-rot basidiomycetes cannot survive for a longer time and soil microfungi may take their role.

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