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Alkali-extractable soil organic matter: An important factor affecting the mycelial growth of ectomycorrhizal fungi

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ABSTRACT

Significant effects of two fractions of alkali-extractable soil organic matter (AEOM) extracted from three different soils (agricultural soil, soil from deciduous forest, soil from spruce monoculture) on mycelial growth of 17 isolates of ectomycorrhizal fungi were observed. Growth of *Lactarius deterrimus*, *Meliniomyces bicolor* and one of the isolates of *Leccinum aurantiacum* was significantly stimulated by acid-insoluble fraction extracted from all three soils. The stimulatory effects were frequent but inhibition of mycelial growth of some isolates was also observed. The fungal response to the presence of the organic extract in the nutrient medium was isolate-specific rather than species-specific. Organic matter extracted from different source soils affected differently the mycelial growth, the largest number of stimulatory effects being observed in an experiment where the extract was richest in trace elements Zn and Cu. At the same time, the observed stimulatory effects were not attributable to increased concentrations of trace elements in the nutrient medium. The results indicate that soil may be used as a source of extractable organic fractions which, when used as a cultivation medium additive, may significantly improve the growth of responsive fungal isolates. Under natural conditions, AEOM (traditionally designated humic substances) represent a potential factor affecting the composition of cenosis of ectomycorrhizal fungi in soil.

1. Introduction

Soil contains variable concentration of organic matter that is an important factor affecting growth and physiological activity of many organisms, providing carbon compounds and energy for their nutrition. Not only saprotrophic organisms but also ectomycorrhizal fungi (e.g. Baar and Elferink, 1996; Rumberger et al., 2004) and obligate biotrophic arbuscular mycorrhizal fungi (St John et al., 1983; Rydlová and Vosátka, 2000) are substantially affected by this important soil component.

Organic matter in the soil undergoes a humification process which leads to the production of relatively stable complex compounds, traditionally called humic substances, which can be efficiently extracted from the soil with alkali. This fraction of organic matter, rich in aromatic groups, possesses some biological activity (O'Donnell, 1973; Nardi et al., 1994) and is frequently in contact with the mycelium of ectomycorrhizal fungi.

The effects of alkali-extractable soil organic matter (AEOM) on ectomycorrhizal fungi and vice versa have not been studied extensively. It can affect soil environment because it can act as ion exchanger and thus stabilize inorganic ion concentrations in the soil solution (Nordén and Dabek-Zlotorzynska, 1996). Further, it can affect plants in different ways (Nardi et al., 2002a) and its effects may depend on its solubility. Under natural conditions, the components of this organic fraction are solubilized in the presence of root exudates (''breaking out'', Nardi et al., 2002b). Laboratory extraction might partially simulate this natural process.

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AEOM is not constituted exclusively by the residues of decomposition of plant materials, but it is also enriched by products of various microorganisms, probably including ectomycorrhizal fungi. This was documented on the formation of a ''humic-like material'' in laboratory culture of *Pisolithus tinctorius* (Tan et al., 1978).

Our previous work (Gryndler et al., 2005) evaluated the effects of AEOM on the arbuscular mycorrhizal fungus *Glomus claroideum* in a soil-free hydroponic culture by focusing on two fractions: acid-insoluble fraction (''humic acid'', HA) and acid soluble fraction (''fulvic acid'', FA). We found stimulation of mycorrhizal colonization and growth of mycelium by FA at 600 mg/L, and substantial stimulation of mycelial growth by an alkali-free preparation of HA. These results differ from that of Vallini et al. (1993), probably because the mentioned authors used soluble sodium salt of HA instead of insoluble sodium-free preparation. Possible physiological alkalinity of the sodium salt may be the reason of the inhibition effects reported in their work.

Unlike arbuscular mycorrhizal fungi, ectomycorrhizal fungi are often not obligate mutualists and, inhabiting soils often very rich in humus (e.g. litter, O_F or O_H horizons in forest soils, Dickie et al., 2002), they possess considerable metabolic activity directed to exploitation of this source of carbon nutrition (Burke and Cairney, 2002). They might thus be more sensitive to the presence of organic compounds (including AEOM fractions) in the soil solution.

Tan and Nopamornbodi (1979) were the first to observe a response (stimulation) of growth of the ectomycorrhizal fungus *P. tinctorius* to FA at concentrations of 640–1600 mg/L. Their results indicate that soluble humic substances, having a polysaccharidic component, may be an important source of nutrition for fungal growth.

AEOM is chemically heterogeneous and, when extracted from different soils, may show different effects on ectomycorrhizal fungi. The response of different fungal species to the presence of a specific substance under study may also be variable. This can, in principle, constitute one of the selection factors leading to the development of specific community of ectomycorrhizal fungi in an ecotope.

The objective of this work was to evaluate the effect of AEOM fractions (HA and FA) extracted from three different soils on the growth of cultures of 17 isolates of ectomycorrhizal fungi. We used these materials as components of complete MMN cultivation medium (Marx, 1969) ordinarily used for laboratory cultivation of many ectomycorrhizal fungi. AEOM thus did not served as an important source of organic carbon and energy because culture growth in the MMN medium is not limited by carbon or mineral nutrition. Under these conditions, non-nutritional biological effects may be expected to come to the fore.

2. Materials and methods

2.1. Soils

Three different soils were used for extraction of AEOM fractions in our work:

Soil R: tilled clay-loam orthic luvisol, pH 6.5, developed on dilluvial sediments mixed with loess (Prague – Ruzyně, Research Institute of Plant Production);
Soil X: intact cambisol (layers $O_H + O_F$, pH 4.0) from oak–beech mixed forest (Prague – Xaverov);
Soil B: intact podzol (layers $O_H + O_F$, pH 4.0) from spruce monoculture (Prague – Strnady, Forestry and Management Research Institute Prague).

2.2. Extraction of FA and HA fractions of AEOM

Both AEOM fractions were extracted from 4 kg soil R previously shaken in 8 L 0.1 M HCl for 30 min, and washed twice in 8 L deionised water. The washed soil was extracted with 8 L 0.1 M NaOH for 2 h and centrifuged for 20 min at 3000 rpm. The extract was acidified to pH 2.6 and centrifuged again. Sediment (HA), washed with deionised water, and supernatant (HCl-neutralized FA in mineral solution) were used in the experiments. Only a half amount of soils and chemical reagents were used to extract humic substances from soils B and X. Concentrations of trace elements Fe, Mn, Zn, Co and Cu were measured in FA and HA after dilution to working concentrations using atomic absorption spectrometry (instrument Aurora AI1200, Aurora Instruments Ltd., Canada) and are shown in Table 1. Wavelengths used for measurements of Fe, Mn, Zn, Co and Cu concentrations were 248.3, 279.5, 213.9, 240.7 and 324.7 nm, respectively.

2.3. Fungi

Isolates of ectomycorrhizal fungi, described in Table 2, were used for experiments. The identity of all these isolates was determined on the basis of morphology of the fruitbodies from which they were obtained, only the identity of the isolate *Meliniomyces bicolor* (MBI2), was based on colony micro- and macromorphology. Partial sequences of ribosomal RNA gene cassette of the most responsive isolates *Lactarius deterrimus* (LD1), *Leccinum aurantiacum* (KR5) and *M. bicolor* (MBI2), as well as of *Boletus edulis* (BE1) and *Cantharellus cibarius* (CC1) have been submitted to GenBank (accession numbers EF517301, EF517299, EF517302, EF517300 and EF546767, respectively) and compared with existing records. On the basis of sequence similarity (99, 95, 98, 99 and 94%, respectively) with the closest GenBank records referring to identified fungi, the identity of all five isolates was confirmed. This excluded a possible contamination of isolates during isolation. The aligned sequences were 401, 1020, 503, 643 and 1398 bp long, respectively. The

Table 1 – Concentration of trace elements in AEOM fractions at final dilution used in experiments

Soil	Fraction	Final element concentration (mg/L)				
		Zn	Cu	Mn	Fe	Co
R	HA	0.208	0.294	0.157	43.64	0.027
R	FA	0.094	0.282	0.961	1.44	0.049
B	HA	0.012	0.026	0.061	4.91	0.000
B	FA	0.037	0.013	0.463	1.44	0.027
X	HA	0.016	0.087	0.099	9.00	0.000
X	FA	0.046	0.013	0.329	1.40	0.000

Table 2 – Isolates of ectomycorrhizal fungi used in the study

Acronym	Species	Collection	Locality characteristics
AMU1	*Amanita muscaria* (L.) Lam.	DMS	Modrava, under *Picea abies*, podzol on granite
AM17	*Amanita muscaria* (L.) Lam.	LFB	Libava, under mixed *Picea abies–Fagus sylvatica*, cambisol on sandstone
AC11	*Amanita citrina* (Schaeff.) Pers.	LFB	Voznice, under mixed *Picea abies–Fagus sylvatica*, cambisol
BE1	*Boletus edulis* Rostk	LFB	Pařez, under *Pinus sylvestris*, cambisol on quartz sand.
BE13	*Boletus edulis* Rostk.	LFB	Libava, under mixed *Picea abies–Fagus sylvatica*, cambisol on sandstone
CC1	*Cantharellus cibarius* Fr.	LFB	Souš, under mixed *Picea abies–Quercus robur*, heterogeneous substrate
LD1	*Lactarius deterrimus* Gröger	LFB	Voznice, under mixed *Picea abies–Populus* sp., cambisol on slate
KR5	*Leccinum aurantiacum* (Bull.) Gray	LFB	Libava, under mixed *Betula pendula.–Salix* sp., poorly developed soil on sandstone bedrock
KR20	*Leccinum aurantiacum* (Bull.) Gray	LFB	Libava, under mixed *Betula pendula.–Salix* sp., poorly developed soil on sandstone bedrock
LV1	*Leccinum vulpinum* Watling	LFB	Soběslav, dike of the Rožberk pond, under mixed *Betula–Populus–Quercus–Pinus–Picea*, heterogeneous substrate
MBI2	*Meliniomyces bicolor* Hambleton & Sigler	DMS	Unidentified ectomycorrhiza, probably of *Picea abies*
PIN2	*Paxillus involutus* (Batsch) Fr.	DMS	Hungary, host and substrate unknown
PTI1	*Pisolithus tinctorius* (Pers.) Coker & Couch	DMS	Portugal, under mixed *Eucalyptus globulus–Pinus pinaster*, sandy soil low in organic matter (=isolate PT-R01 by R. S. Oliveira)
SG1	*Suillus grevillei* (Klotzsch) Winter	LFB	Jablonec nad Nisou, under *Larix decidua*, podzol on granite
BB3	*Xerocomus badius* (Fr.) Kühner	LFB	Voznice, under *Fagus sylvatica*, cambisol on slate
BB9	*Xerocomus badius* (Fr.) Kühner	LFB	Voznice, under *Fagus sylvatica*, cambisol on slate
HZ	*Xerocomus chrysenteron* (Bull.) Quél.	LFB	Prague-Krč, under mixed *Betula–Quercus–Larix*, dystric cambisol on slate

Fungi were isolated from fruitbodies, with exception of the isolate MBI2 obtained from unidentified ectomycorrhiza. Desiccates of fruitbodies, from which the isolates deposited in LFB collection were obtained, are deposited in Laboratory of Fungal Biology, Institute of Microbiology ASCR, Prague, Czech Republic. If not otherwise indicated, the localities are located on the territory of the Czech Republic. Acronym: identifier of the isolate used in the text, identical with collection culture specimen identifier. Collection: abbreviation of the name of culture collection in which the isolate is currently maintained: DMS—Collection of isolates, Department of mycorrhizal symbioses, Institute of Botany CAS, Pruhonice, Czech Republic, LFB—Collection of basidiomycetes, Laboratory of Fungal Biology, Institute of Microbiology ASCR, Prague, Czech Republic. Locality characteristics: the locality in the nature from where the isolate was obtained.

sequence from isolate LD1 comprised ITS1, 5.8S rDNA gene and ITS2, sequences from isolates KR5 and CC1 comprised part of 18S rDNA gene, ITS1, 5.8S rDNA gene, ITS2 and part of 28S rDNA gene, the sequence from isolate MBI2 comprised ITS1, 5.8S rDNA gene, ITS2 and part of 28S rDNA gene and the sequence from the isolate BE1 comprised part of 18S rDNA gene, ITS1, 5.8S rDNA gene and ITS2.

2.4. Culture media, inoculation and conditions of cultivation

The extracts of the three soils contained different amounts of HA and FA. To ensure a defined concentration of phenolic components of humic substances in the nutrient media, the resulting solutions had to be diluted to reach the same final absorbance equal to 0.287 and 0.233 (at a wavelength of 460 nm) for HA and FA, respectively (see Section 4).

The concentration of HA extracted from soils R, X and B and diluted to the working concentration was measured gravimetrically (after drying an aliquot at 105 °C) and was equal to 1340, 480 and 334 mg HA per L, respectively.

The concentration of oxidizable carbon was measured in FA diluted to the working concentration using bichromate wet combustion (Hršelová et al., 1999). This concentration was 240, 202 and 177 mg of anhydrous glucose equivalent per L of FA extracted from soils R, X and B, respectively.

For laboratory experiments, the above solutions of HA or FA were supplied with components of MMN medium at concentrations 250 mg $(NH_4)_2HPO_4$, 500 mg KH_2PO_4, 150 mg $MgSO_4{\cdot}7H_2O$, 50 mg $CaCl_2{\cdot}2H_2O$, 25 mg NaCl, 20 mg FeNa-EDTA, 10 g glucose, 3 g malt extract and 0.1 mg thiamine-HCl per L (Marx, 1969). Medium MMN without HA as described above and medium MMN without FA containing additional 100 mM NaCl to balance the concentration of Na^+ and Cl^- ions present in the solution of FA were used as controls.

Effects of AEOM fractions extracted from the three source soils on ectomycorrhizal fungi were evaluated in three separate experiments established during a period of 3 months. The effects of FA on isolates LV1 and CC1 were not tested. In all treatments, the medium MMN contained 6 g per L Phytagel (Sigma) as a gelling agent. The initial pH was set to 6.5 (controls and FA treatments) or 7.0 (HA treatments). The pH of autoclaved media containing HA extracted from the soils R, X and B always decreased to the value 6.5, the pH of autoclaved media containing FA extracted from the soils R, X and B decreased to the value 6.2, 6.1 and 6.3, respectively. The pH of autoclaved control media in the three experiments was 6.4 (controls without additional NaCl) or 6.3 (controls with additional NaCl).

Solidified media in 90 mm Petri dishes (four replicates per treatment) were inoculated with approximately 5 mm × 10 mm agar blocks cut from fungal cultures grown for 5 weeks on MMN medium (isolates AC11, BB3, BB9, BE1, BE13, HZ, KR5, KR20, LV1, SG1) or 50% potato dextrose agar (isolates AMU1, AM17, CC1, LD1, MBI2, PIN2, PTI1). Cultures were kept for 6 weeks at 25 °C in the dark.

To check separately the effects of trace elements, an additional experiment with five fungal isolates (AM17, BE13, KR5, LD1, MBI2) was performed using the same methodology (without AEOM), including two treatments: control and a

treatment supplied with trace elements at the doses recommended for ectomycorrhizal fungi (Brundrett et al., 1996). Micronutrients supplied treatment received (in addition to above described MMN medium components) 3.1 mg H_3BO_3, 10.1 mg $MnSO_4 \cdot H_2O$, 2.0 mg $ZnSO_4 \cdot 7H_2O$, 3.0 mg $CuSO_4 \cdot 5H_2O$, 2 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.025 mg $CoCl_2 \cdot 6H_2O$ and 0.75 mg KI per L of the medium.

2.5. *Evaluation of fungal growth and final pH of the medium*

The growth of fungi under study was evaluated in terms of mycelial biomass production. The part of the medium containing fungal mycelium was melted according to the modified protocol by Doner and Bécard (1991) in 0.1 M citric acid (pH 6.0, set by NaOH) for 24–48 h. The fungal mycelia were dried for 3 h at 105 °C, weighed and 95% confidence limits were calculated for all mean values of dry biomass produced per Petri dish.

An aliquot of colony-surrounding solid medium was homogenized by spatula and used for determination of pH using a combination pH electrode (type G-P Combo-w/RJ, Corning, USA). The mean value of final pH calculated for control without an AEOM fraction was subtracted from the value calculated for the treatment involving FA or HA and the same fungal isolate as in the control. The result is referred to as experimental pH shift. Similarly, the mean value of fungal biomass calculated for control without an AEOM fraction was subtracted from the value calculated for the treatment involving a FA or HA and the same fungal isolate. The result is referred to as the growth response to AEOM.

2.6. *Statistical methods*

Coefficients of correlation between experimental pH shift and the growth response to AEOM were calculated separately for HA and FA, always involving the pairs of means from all three experiments. Coefficient of correlation between final pH of the media and fungal growth was also calculated from means of the treatments, separately for treatments with or without 100 mM Na ions supplement. 95% confidence limits are given for all the mean values.

3. Results

3.1. *Effects of HA*

Fig. 1 displays the effect of HA on mycelial growth of the ectomycorrhizal fungi in terms of dry biomass per Petri dish. When the overlap of 95% confidence limits was taken as a criterion of statistical significance, the isolates KR5 (*L. aurantiacum*), LD1 (*L. deterrimus*) and MBI2 (*M. bicolor*) were significantly stimulated by HA extracted from all the three soils. Both isolates of *B. edulis*, BE1 and BE13, were stimulated by HA obtained from soils R and B only but no significant stimulation of their growth by HA obtained from soil X was noted. Significant growth stimulation by HA extracted from soil R was also observed for fungal isolates BB3 (*Xerocomus badius*) and AM17 (*Amanita muscaria*).

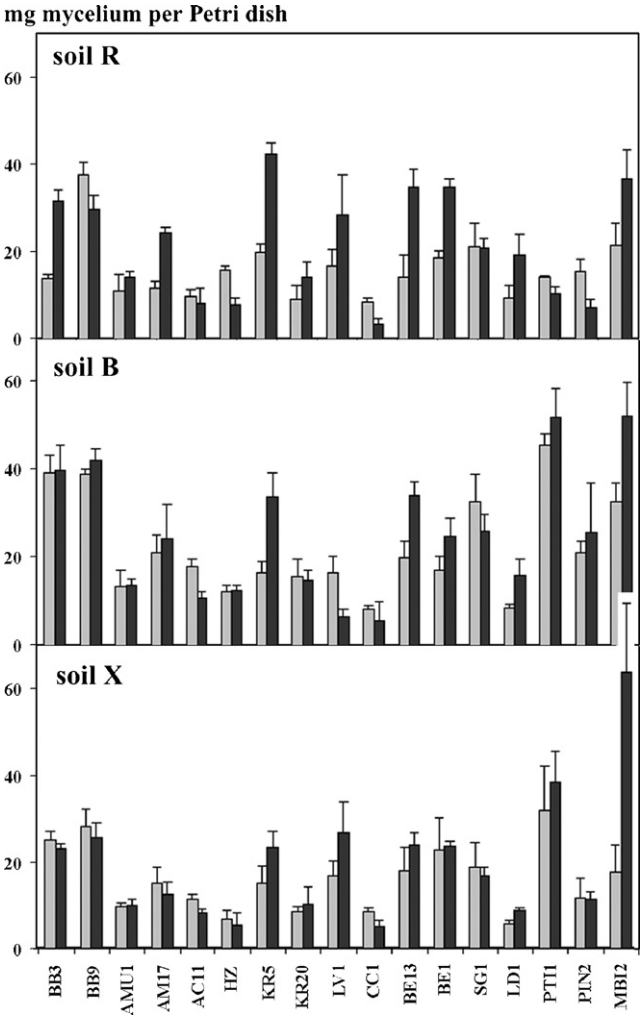

Fig. 1 – Effect of HA extracted from soils R, B and X on the growth of 17 ectomycorrhizal fungi in vitro, expressed as mg mycelial dry weight per Petri dish (20 mL MMN medium) produced in control medium (gray columns) and in medium containing HA (black columns). For explanation of isolate identifiers see Table 2. Error bars indicate 95% confidence limit, $n = 4$.

The only inhibitory effects of HA on biomass produced by the studied ectomycorrhizal fungi significant consistently over at least two HA source soils were growth depressions of the isolate AC11 (*Amanita citrina*) caused by HA extracted from soils B and X and significant inhibition of mycelial growth of the isolate CC1 (*C. cibarius*) by HA extracted from soils R and X. Four other fungal isolates were inhibited by HA extracted from the soil R: BB9 (*X. badius*), HZ (*Xerocomus chrysenteron*), PTI1 (*P. tinctorius*) and PIN2 (*Paxillus involutus*). The growth of isolate LV1 (*Leccinum vulpinum*) was decreased by HA extracted from soil B.

3.2. *Effects of FA*

None of the fungal isolates under study showed significant and consistent growth stimulation by FA extracted from all the three soils (Fig. 2). Almost all the isolates but BB3 (*X. badius*), AC11 (*A. citrina*), KR5 (*L. aurantiacum*) and HZ (*X. chrysenteron*)

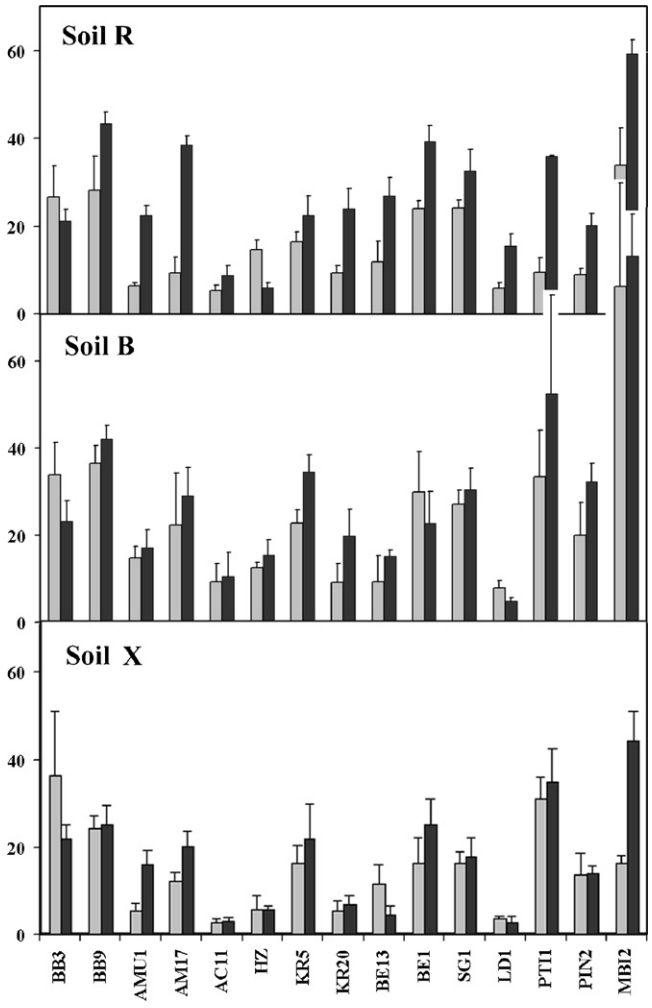

Fig. 2 – Effect of FA extracted from soils R, B and X on the growth of 15 ectomycorrhizal fungi in vitro, expressed as mg mycelial dry weight per Petri dish (20 mL MMN medium) produced in control medium (gray columns) and in medium containing FA (black columns). Isolate identifiers as in Fig. 1. Error bars indicate 95% confidence limit, $n = 4$.

showed increased growth rate in the presence of FA extracted from soil R (compared to control medium with NaCl). FA extracted from soil B stimulated the growth of three fungi: KR5 (*L. aurantiacum*), KR20 (*L. aurantiacum*), and PIN2 (*P. involutus*). FA extracted from soil X supported the growth of isolates AMU1 (*A. muscaria*), AM17 (*A. muscaria*) and MBI2 (*M. bicolor*).

Isolates HZ (*X. chrysenteron*) and LD1 (*L. deterrimus*) were the only organisms whose mycelial growth was significantly inhibited by FA extracted from soil R or B, respectively. Isolate BE13 (*B. edulis*) showed negative growth response to the presence of FA extracted from soil X.

In media containing FA, the largest biomass was always produced by the isolate MBI2 (*M. bicolor*).

3.3. *Effect of micronutrients*

Introduction of trace elements into the nutrient medium did not significantly affect the production of mycelial biomass. An exception was biomass reduction (−84.9%) in the case of the isolate AM 17 in the treatment receiving trace elements, compared to the control treatment.

3.4. *Sodium ion supplementation, experimental pH shift and final pH of the medium*

MMN medium containing HA tended to have larger positive experimental pH shift than the medium containing FA. A weak negative correlation (coefficient of correlation is equal to −0.297 and is statistically significant at $P \leq 0.05$) between experimental pH shift and growth response to HA indicates that the medium tends very weakly to be acidified in the presence of actively growing mycelium in proportion to growth rate.

The final pH values of the media containing 100 mM sodium concentration (treatments containing FA plus their controls) varied between 3.60 and 5.47. The final pH values of the media without 100 mM sodium concentration (treatments containing HA plus their controls) varied between 3.26 and 5.95.

Correlation between mycelial growth and final pH of the medium was insignificant (coefficients of correlation being equal to −0.154 and −0.103 for media with or without 100 mM Na ion supplement, respectively). The mean value of mycelial growth in all treatments on media with or without 100 mM Na ion supplement was 23.55 or 22.28 mg per Petri dish, respectively, indicating that increased salt concentration does not show a general inhibitory effect on mycelial growth.

In some cases (isolates BB3, MBI2), the growth of the mycelium in control treatments differed between experiments (see Figs. 1 and 2 for mycelial growth in control treatments established for evaluation of effects of HA and FA extracted from the three source soils). Because the same experimental conditions as well as chemicals for preparation of the media were always used, we attribute these differences to the seasonal changes of the mycelial growth rate in some isolates.

4. Discussion

In our experiments we used AEOM extracted from three different soils (tilled soil R without typically developed humus form vs. soils B and X with ''moder'' form of humus), with the idea to detect some general effects on fungi. We thus had to use the concentrations of AEOM which had to be ''standardized'' in terms of concentration of a characteristic component. Characteristic components of AEOM (humic substances) are aromatic compounds in a various degree of oxidative polymerization (production of chinoid structures during the humification process) which show absorbance within a broad range of UV and visible light (e.g. Kumke et al., 2001; Tan, 2003).

In common, absorbance of AEOM at wavelengths around 650 and 460 nm is measured as their physicochemical characteristic (Tan, 2003; Řezáčová et al., 2006), the high absorbance at approximately 650 nm being an indicator of the highest degree of polymerization. Absorbance at approximately 460 nm is much less dependent on the molecular weight of AEOM so that the ratio of absorbances measured at

460–465 and 650–665 nm can be used to describe the degree of humification (Flaig et al., 1975; Chen et al., 1977). We standardized the concentration of AEOM in our media by absorbance at 460 nm, which is weakly dependent on the degree of humification and is also used to detect humic substances in aqueous solutions (Corin et al., 1996).

Our observations indicate that AEOM (HA and FA) affect significantly the growth of mycelium of ectomycorrhizal fungi. We could observe three isolates, belonging to the species *L. aurantiacum*, *L. deterrimus* and *M. bicolor,* showing consistently positive growth response to HA extracted from three different soils. Because no discoloration of the medium in Petri dishes was visually observed (in contrast to the results of Tan and Nopamornbodi, 1979), these isolates probably do not consume HA in considerable amounts and growth effects of HA are thus mainly of non-nutritional character.

Negative effects of HA on mycelial growth were less frequent than the positive ones and none of the isolates showed consistently a negative response to all the three different HA's. This indicates that mycelial growth depression caused by the HA is not a general feature of the studied isolates of ectomycorrhizal fungi.

The effects of FA differed strongly according to the source soil; for example, *A. muscaria* grew faster when FA extracted from soil R or X was added into the medium while no such an effect was noted with FA extracted from soil B. Similarly, the growth of some isolates (BE13, LD1) was stimulated, inhibited or unaffected by FA, depending on its origin.

Our results thus suggest that biological effects of AEOM fractions strongly depend on the soil from which they were extracted, probably reflecting the diversity of the source of AEOM, humus, which is formed from different organic sources. In the nature this may represent a factor affecting the composition of fungal community because a fungus which does not tolerate a specific humus component may be replaced by another one or may disappear, as it is demonstrated by lower diversity of ectomycorrhizal fungi in intact soil in comparison with soil where litter and the humic soil horizons are removed (Baar and ter Braak, 1996; Smit et al., 2003). For example, humic substances formed under some ericaceous plants may contain allelopathic phenolic compounds (e.g. *p*-hydroxyacetophenone, *p*-hydroxybenzoic acid, catechol, protocatechuic acid) which show negative effects on ectomycorrhizal fungi even at low concentrations (Pellisier, 1993; Souto et al., 2000).

On the other hand, a stimulatory activity of an AEOM fraction was observed in our experiment with relatively large number of fungal entities (species, isolates). In the nature it may result in increased abundance of ectomycorrhizal fungi in soil. This would agree with some field reports indicating a significant positive effect of site/humus type on the diversity and number of ectomycorrhizae, particularly if the humus is rich in mineral nutrients (Rumberger et al., 2004).

The stimulation of mycelial growth of *M. bicolor* by HA is important because it appears in an ectomycorrhizal ascomycete (Hambleton and Sigler, 2005), which is taxonomically different from the other tested fungi. Our observation suggests that the positive influence of AEOM is not restricted to ectomycorrhizal basidiomycetes but may apply to at least some ectomycorrhizal ascomycetes.

It is interesting that significant stimulation of fungal isolates by FA was most frequently observed when this material was extracted from the soil R. It is richest in manganese, copper and cobalt ions as well as in oxidizable organic matter. However, the observed stimulatory effect of AEOM fractions cannot be attributed simply to enrichment of the MMN medium by these trace elements because significant stimulation of mycelial growth of responsive isolates was not observed when the microelements were supplied as mineral salts in the medium without added AEOM. Nevertheless, AEOM components, as chelating agents, could improve the bioavailability of nutrients (Mackowiak et al., 2001) which might result in the stimulatory effects observed.

According to our observations, the fungal response to an AEOM fraction is strain-specific rather than species-specific. This is documented for example on two isolates of *L. aurantiacum*. Whereas the isolate KR5 responded positively to any HA applied, the isolate KR20 showed no significant reaction to any HA. Similarly, the reaction of two studied isolates of *B. edulis* to FA of different origin was also different. This infraspecific variability may reflect the genotype variability of the species under natural conditions, resulting in selection for specific strains of a given fungal species.

We cannot attribute the positive response of a fungal isolate to an AEOM fraction as a result of adaptation of the organism to the presence of increased concentrations of organic matter because one of the best responding isolates, *L. aurantiacum* KR5 was isolated from a raw soil (bedrock with removed organic horizons) almost lacking organic matter.

Cultivation of the fungi in MMN-based media always resulted in a decrease in pH, but there was no clear relationship between this parameter and produced mycelial biomass, indicating that the pH decrease during the cultivation period is not an invariant characteristic related to active biomass of all fungal isolates/species. A perceptible correlation between experimental pH shift and growth response to HA was too weak to allow general explanations of growth reaction of different isolates to this AEOM fraction as a result of growth limitation by pH change.

The results of our work show that AEOM may affect positively the mycelial growth of some ectomycorrhizal fungi and may be applied as a supplement to media used for cultivation of responsive isolates. At the same time, the different sensitivity of fungal isolates to AEOM may act as a selection factor influencing the formation of the cenosis of ectomycorrhizal fungi in the soil containing humus components.

Further information is necessary to describe how the extraction procedure (chemical modification of humus components in strongly alkaline extractant) affects the biological activity of AEOM. Future research should also be directed to the effects of humus formed from the litter under different plant species under conditions simulating soil environment. This may partially elucidate the specificity of ectomycorrhizal fungi in relation to soil conditions.

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REFERENCES

Baar, J., Elferink, M.O., 1996. Ectomycorrhizal development on Scotch pine (*Pinus sylvestris* L.) seedlings in different soils. Plant Soil 179, 287–292.

Baar, J., ter Braak, C.J.F., 1996. Ectomycorrhizal sporocarp occurrence as affected by manipulation of litter and humus layers in Scots pine stands of different age. Appl. Soil Ecol. 4, 61–73.

Brundrett, M., Bougher, N., Dell, B., Grove, T., Malajczuk, N., 1996. Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research Monograph No 32, Canberra, 374 pp.

Burke, R.M., Cairney, J.W.G., 2002. Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza 12, 105–116.

Chen, Y., Senesi, N., Schnitzer, M., 1977. Information provided on humic substances by E_4/E_6 ratios. Soil Sci. Soc. Am. J. 41, 352–358.

Corin, N., Backlund, P., Kulovaara, M., 1996. Degradation products formed during UV radiation of humic waters. Chemosphere 33, 245–255.

Dickie, I.A., Xu, B., Koide, R.T., 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytol. 156, 527–535.

Doner, L.W., Bécard, G., 1991. Solubilization of gellan gels by chelation of cations. Biotechnol. Tech. 5, 25–28.

Flaig, W., Beutelspacher, H., Rietz, E., 1975. Chemical composition and physical properties of humic substances. In: Gieseking, J.E. (Ed.), Soil Components. I. Organic Components. Springer Verlag, Berlin/Heidelberg/New York, pp. 1–211.

Gryndler, M., Hršelová, H., Sudová, R., Gryndlerová, H., Řezáčová, V., Merhautová, V., 2005. Hyphal growth and mycorrhiza formation by the arbuscular mycorrhizal fungus *Glomus claroideum* BEG 23 is stimulated by humic substances. Mycorrhiza 15, 483–488.

Hambleton, S., Sigler, L., 2005. *Meliniomyces*, a new anamorph genus for root-associated fungi with phylogenetic affinities to *Rhizoscyphus ericae* (=*Hymenoscyphus ericae*), Leotiomycetes. Stud. Mycol. 53, 1–27.

Hršelová, H., Chvátalová, I., Vosátka, M., Klír, J., Gryndler, M., 1999. Correlation of abundance of arbuscular mycorrhizal fungi, bacteria and saprophytic microfungi with soil carbon, nitrogen and phosphorus. Folia Microbiol. 44, 683–687.

Kumke, M.U., Specht, C.H., Brinkmann, S.T., Frimmel, F.H., 2001. Alkaline hydrolysis of humic substances—spectroscopic and chromatographic investigations. Chemosphere 45, 1023–1031.

Mackowiak, C.L., Grossl, P.R., Bugbee, B.G., 2001. Beneficial effects of humic acid on micronutrient availability to wheat. Soil Sci. Soc. Am. J. 65, 1744–1750.

Marx, D.H., 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59, 153–163.

Nardi, S., Panuccio, M.R., Abenavoli, M.R., Muscolo, A., 1994. Auxin-like effects of humic substances extracted from faeces of *Allolobophora caliginosa* and *A. rosea*. Soil Biol. Biochem. 26, 1341–1346.

Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A., 2002a. Physiological effects of humic substances on higher plants. Soil Biol. Biochem. 34, 1527–1536.

Nardi, S., Sessi, E., Pizzeghello, D., Sturaro, A., Rella, R., Parvoli, G., 2002b. Biological activity of soil organic matter mobilized by root exudates. Chemosphere 46, 1075–1081.

Nordén, M., Dabek-Zlotorzynska, E., 1996. Study of metal–fulvic acid interactions by capillary electrophoresis. J. Chromatogr. A 739, 421–429.

O'Donnell, R.W., 1973. The auxin-like effects of humic preparations from leonardite. Soil Sci. 116, 106–112.

Pellisier, F., 1993. Allelopathic effect of phenolic acids from humic solutions on two spruce mycorrhizal fungi: *Cenococcum graniforme* and *Laccaria laccata*. J. Chem. Ecol. 19, 2105–2114.

Řezáčová, V., Hršelová, H., Gryndlerová, H., Mikšík, I., Gryndler, M., 2006. Modifications of degradation–resistant soil organic matter by soil saprobic fungi. Soil Biol. Biochem. 38, 2292–2299.

Rumberger, M.D., Münzenberger, B., Bens, O., 2004. Changes in diversity and storage function of ectomycorrhiza and soil organoprofile dynamics after introduction of beech into Scots pine forests. Plant Soil 264, 111–126.

Rydlová, J., Vosátka, M., 2000. Sporulation of symbiotic arbuscular mycorrhizal fungi inside dead seeds of a non-host plant. Symbiosis 29, 231–248.

Souto, C., Pellisier, F., Chiapusio, G., 2000. Allelopathic effects of humus phenolics on growth and respiration of mycorrhizal fungi. J. Chem. Ecol. 26, 2015–2023.

Smit, E., Veenman, C., Baar, J., 2003. Molecular analysis of ectomycorrhizal basidiomycete communities in a *Pinus sylvestris* L. stand reveals long-term increased diversity after removal of litter and humus layers. FEMS Microbiol. Ecol. 45, 49–57.

St John, T.V., Coleman, D.C., Reid, C.P.P., 1983. Association of vesicular-arbuscular mycorrhizal hyphae with soil organic particles. Ecology 64, 957–959.

Tan, K.H., 2003. Humic Matter in Soil and the Environment. Marcel Dekker, New York/Basel, 386 pp.

Tan, H.K., Nopamornbodi, V., 1979. Fulvic acid and the growth of the ectomycorrhizal fungus *Pisolithus tinctorius*. Soil Biol. Biochem. 11, 651–653.

Tan, H.K., Sihanoth, P., Todd, R.L., 1978. Formation of humic acid like compounds by the ectomycorrhizal fungus, *Pisolithus tinctorius*. Soil Sci. Soc. Am. J. 42, 906–908.

Vallini, G., Pera, A., Avio, L., Valdrighi, M., Giovannetti, M., 1993. Influence of humic acids on laurel growth, associated rhizospheric microorganisms, and mycorrhizal fungi. Biol. Fertil. Soils 16, 1–4.