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The effect of oxygen on fatty acid composition of soil micromycetes

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ABSTRACT

The aim of this work was to describe changes in fatty acid profiles of fungi growing under artificial conditions of oxygen depletion. In total, 133 fungal strains belonging to eight orders were isolated from cattle impacted soils and tested. The analysis of the ten most frequent fatty acids revealed significant shift in fatty acids composition as a result of decreasing oxygen level. Taxonomic- as well as aeration-dependent changes in the amounts of fungal biomarker fatty acids (18:1ω9 and 18:2ω6,9) were found. Therefore, the ratio of these two fatty acids could be considered as an indicator of anaerobic, microaerobic or aerobic conditions in soil. Moreover, fatty acid-based estimation of fungal biomass in soils should be performed as a sum of both biomarker fatty acids and with respect to the soil characteristics as well as to the composition of fungal community.

1. Introduction

Fatty acids (FA) in microorganisms occur as a homologous series ranging in carbon chain length from C10 to C25, with even-numbered carbon chains predominating in fungi (Weete and Gandhi, 1992). From all cellular FA, only phospholipid fatty acids (PLFA) provide quantitative insight into the viable microbial biomass because PLFA are important components of the membrane constitution (Pupin et al., 2000) and are rapidly degraded after cell death (Kaur et al., 2005). However, neutral lipid fatty acids (NLFA) also can be used as biomarkers, e.g. of energy storage compounds in plants, animals, and fungi (Ruess and Chamberlain, 2010). Various sets of FA are used as biomarkers for microorganisms (Ruess and Chamberlain, 2010). The most frequently used FA are cyclopropyl and monounsaturated FA for Gram-negative bacteria; iso and anteiso isomers of 15:0 and 17:0 for Gram-positive bacteria; polyunsaturated FA for protozoa; and/or 10-methyl branched FA for actinomycetes. FA 18:1ω9 and 18:2ω6,9 are commonly used as fungal biomarkers in soil microbial community studies (e.g. Joergensen and Wichern, 2008). In addition, changes in composition of the membrane provide an opportunity to microorganisms to respond to altering environment conditions (Kaur et al., 2005). Therefore, the sets of FA, which are characteristic of environment stress, are used as indicators for environmental monitoring and assessment (Guckert et al., 1986; Heipieper et al., 1996). Stress dependency, however, might also affect the FA analyses of microbial communities in disturbed soils.

Some FA can be found in more than one group of organisms, e.g. fungal marker FA 18:2ω6 represents one of the major fatty acids in plants (Kaur et al., 2005). Nevertheless, FA-based analyses were reported to be efficient, sensitive, and reproducible way to monitor effects of treatments on the soil community and to increase the understanding of the soil ecosystem (Frostegård et al., 2011).

The detailed analyses of the fatty acid composition of fungi cultured under defined conditions determine the potential of fatty acids to be used as specific indicators (Devi et al., 2006). The aim of the work was to describe changes in fatty acid profiles of fungi growing under conditions of oxygen depletion. In natural ecosystems, such conditions can prevail in soils, where compaction of soil or surface flooding appears, e.g. at the overwintering pasture with gradient of cattle impact (trampling, soil compaction, excrement deposition).

2. Methods

2.1. Soil sampling

Soil fungi were isolated from soils of upland pasture used for overwintering cattle (Šimek et al., 2006; Jirout et al., 2013). Soil samples (homogenized soil from the top 15 cm soil layer) were collected in triplicates at six sites, differing in the management history and intensity of animal impact in May 2013, several days after the cattle left the winter pasture.

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2.2. Isolation and identification of soil micromycetes

Soil micromycetes were isolated from 2 g of fresh soil as described by Jirout et al. (2013) using the plate dilution method (Sabouraud dextrose agar (BD Difco™) amended with rose bengal). Morphologically distinct isolates were transferred onto fresh plate to obtain pure fungal isolates, subsequently stored using silica gel storage method (Perkins, 1962).

The identification of isolates was provided by sequencing of the ITS rDNA. DNA from fungal mycelium was extracted using NucleoSpin® Soil kit (Macherey-Nagel, Germany). PCR was performed with primers ITS1F-ITS4 (White et al., 1990; Gardes and Bruns, 1993). After purification, PCR products were commercially sequenced with ITS1F as sequencing primer. Sequence similarity search was performed using nucleotide BLAST (NCBI web interface). For a purpose of this study, only higher taxa (genera and orders) of fungi were assigned based on the maximum query coverage and identities of nucleotides.

2.3. Incubation experiment

Fungal propagules on silica gel were directly inoculated into 100 mL flasks filled with 20 mL of sterile liquid medium containing ($g\,L^{-1}$ of distilled water): glucose, 10.00; KH_2PO_4, 1.00; Na_2HPO_4, 0.43; KCl, 0.52; $MgSO_4$, 0.52; $NaNO_2$, 0.69; solution of trace elements, 1 mL (Kurakov et al., 2000). The effect of decreasing oxygen level on fungal isolates was assessed in three treatments for each isolate: *Aerobic*—cellulosic stopper; *Microaerobic*—butyl rubber stopper, screw cap, headspace atmosphere unchanged; *Anaerobic*—butyl rubber stopper, screw cap, headspace atmosphere replaced by argon. For each isolate, one replicate per treatments was used. Flasks were incubated at 28 °C in a horizontal shaker. After 168 h, whole cell fatty acids were extracted using Instant fatty acids methyl esters (FAME) protocol (MIDI Inc., USA). There are five steps in the Instant FAME™ protocol, originally described for preparation of extracts from bacterial cells. Procedures were conducted in 2 mL GC vial as follows: (i) fungal pellets harvesting (approximately 15 mg of fresh fungal biomass) and mechanical disruption; (ii) extraction of FA from cells with 250 μL of Reagent 1 and 10 s vortexing; (iii) transfer of FA from aqueous to organic phase with 250 μL of Reagent 2 and 3 s vortexing; (iv) separation of phases with 250 μL of Reagent 3; and (v) removal of 100 μL of the top layer into a new vial with insert for GC analysis.

2.4. FAME profiles analyses

The whole cell FAME profiles were assessed by gas chromatography (Agilent 7980A, Agilent Technologies, USA) with a flame ionization detector on a capillary column (Ultra 2, 25 m × 0.20 mm × 0.33 μm, Agilent Technologies, USA). Samples were injected in split mode (1:30) at an injection temperature of 250 °C using hydrogen as the carrier gas. The GC-temperature regime was 170 °C–5 °C min^{-1}–260 °C–40 °C min^{-1}–310 °C–1.5 min. The RTSBA6 library (MIS Sherlock 6.2) was used for identification of the FAME peaks.

2.5. Statistical analyses

Differences in fatty acids composition of fungal orders under three levels of oxygen were analyzed using Principal component analysis (PCA) (CANOCO for Windows, ver. 4.5). Changes in relative, log-transformed abundances of FA under three levels of oxygen were tested by one-way ANOVA with post-hoc Tukey HSD test (STATISTICA 6.0, StatSoft, Inc., USA). The level of significance for all analyses was $p \leq 0.05$.

Table 1
Differences in the abundance of the ten most frequent fatty acids among all fungal isolates (mean ± SD, $n = 133$) tested under the decreased oxygen level conditions regardless the taxonomy. O—aerobic; M—microaerobic; A—anaerobic. Letters (a–c) indicate significant differences between treatments significance level $\alpha \leq 0.05$.* Un 10.95 stands for FA of unknown structure with Equivalent Chain Lengths (ECL) between 10 and 11 carbons.

Fatty acid	Treatment		
	O	M	A
	% Of total fatty acid content (mean ± SD)		
14:0	0.43 ± 0.79a	0.67 ± 0.84b	1.11 ± 0.93b
16:0	17.03 ± 3.39b	18.44 ± 3.65a	17.27 ± 3.91b
17:0	0.28 ± 0.42a	0.27 ± 0.40a	0.24 ± 0.23a
18:0	3.42 ± 3.53a	4.07 ± 3.61a	5.41 ± 3.35b
16:1ω7c	0.90 ± 0.90a	1.17 ± 1.43ab	1.40 ± 1.28b
17:1ω8c	0.45 ± 0.89a	0.38 ± 0.49a	0.34 ± 0.32a
18:1ω7c	2.20 ± 4.49ab	3.14 ± 4.71a	1.14 ± 2.90b
18:1ω9c	16.17 ± 15.16a	19.56 ± 13.38a	37.49 ± 14.71b
18:2ω6,9c	54.21 ± 21.34a	48.04 ± 17.24b	30.64 ± 14.43c
*Un 10.95	0.20 ± 0.54a	0.25 ± 0.64a	0.52 ± 1.35b
18:2ω6,9c/18:1ω9c	2.88 ± 2.40a	2.05 ± 1.87b	1.02 ± 0.67c

3. Results

In total, 133 fungal isolates, belonging to 27 genera of eight orders of fungi (Supplementary Table S1), were tested for FA composition under decreasing oxygen levels. The principal component analysis (PCA) of the ten most frequent fatty acids in whole-cell FA profiles revealed significant shift in FA composition of the fungal orders as a result of decreasing oxygen level (Supplementary Fig. S2). The FA profiles under anaerobic conditions (A) were significantly different from FA profiles under aerobic (O) ($p = 0.015$) as well as microaerobic conditions (M) ($p = 0.021$).

Among all tested fungal isolates, differences in the abundance of the ten most frequent FA were found along with the decreasing oxygen level (Table 1). The amount of straight saturated FA (s-STFA; 14:0, 18:0) was higher under anaerobic (A) ($p < 0.05$) and/or microaerobic (M) ($p < 0.05$) conditions comparing to aerobic ones (O). Same trend was observed also in some straight monounsaturated FA (s-MUFA; 18:1ω9c, 16:1ω7). Contrary, the amount of polyunsaturated FA (s-PUFA; 18:2ω6,9) was significantly ($p < 0.05$) the lowest under anaerobic conditions (A) (Table 1).

Therefore, ratio of the two most abundant FA in fungal FA profiles (i.e. 18:1ω9 and 18:2 ω6,9) could be suggested as a possible indicator of oxygen level during incubation of fungal isolates. The ratio of 18:2/18:1 significantly decreased ($F = 39.94$; $n = 133$; $p < 0.001$) along with the decreasing oxygen level under aerobic conditions (O), microaerobic (M) and anaerobic (A), respectively (Table 1).

Moreover, the taxonomic-dependent differences in the amounts of fungal biomarker FA (18:1ω9c, 18:2ω6,9) as well as some other FA were observed (Table 2). Comparing to other Ascomycetes, the orders Capnodiales (Ascomycetes), Mucorales and Mortierellales (Zygomycetes) showed substantially lower amounts of FA 18:2ω6,9 as well as quite high amounts of FA 18:1ω9c, even under aerobic conditions. This discrepancy became more apparent with decreasing oxygen availability (Table 2).

4. Discussion

This study presented aeration—as well as taxonomic-dependent changes in the abundance of the most frequent fatty acids in fungi. Observed trends matched with trends reported by Ruenwai et al. (2010), i.e. accumulation of saturated FA and limited amounts of polyunsaturated ones in fungal cells under anaerobic conditions as a response to lack of aerobic desaturases. The limited amount of FA