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Obtaining a spore free fungal community composition

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ABSTRACT

Understanding basic ecological processes within fungal communities is complicated by the cryptic and often below-ground habitat of most fungi. Up to now, molecular methods, enabling analyses of community processes and interaction strategies, have been mainly based on internal transcribed spacer (ITS) DNA analyses. The fact that these DNA profiles are contaminated by dormant propagules and dead material is a well known draw-back, obscuring sound conclusions. Recently, precursor ITS rRNA was suggested as a solution to this problem, as it was hypothesised that dormant and dead material contains little or no precursor rRNA. Our results show that basidiospores do not contain precursor ITS rRNA and thus confirm this hypothesis. This implies that the precursor ITS rRNA should be used for analysis and characterization of active species composition, when contamination of ungerminated basidiospores should be avoided.

Introduction

Molecular approaches have resulted in a progress-leap in fungal ecological research (Anderson and Cairney, 2004; Buée et al., 2009; Horton and Bruns, 2001). However, studies on below-ground microbial communities, including fungi, have been up to now mainly based on ribosomal DNA (rDNA) (Hirsch et al., 2010; Wallander et al., 2013). For community studies on Basidiomycota and Ascomycota the internal transcribed spacer (ITS) region of the tandem repeated ribosomal RNA has been the most popular marker of choice (Anderson and Cairney, 2004; Abarenkov et al., 2010). One of the major concerns for the research on soil-borne fungi is that no distinction is made between DNA derived from active mycelium, dead material and spores (Dickie et al., 2002; Guidot et al., 2003; Koide et al., 2005; Landeweert et al., 2005). Developments in molecular approaches have dramatically increased the sensitivity of detection techniques (Buée et al., 2009; Hirsch et al., 2010), however, no improvements have been made to avoid the inclusion of spore DNA. At best avoiding spores has been attempted by sampling at least several centimetres below the soil surface (Guidot et al., 2003; Koide et al., 2005; Van der Linde et al., 2012) or by sampling before or after the sporocarp fruiting season (Dickie et al., 2002; Landeweert et al., 2005).

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In terms of community assembly and niche theory it is important to exclude propagules since they have a very low chance of becoming adult individuals and thus are not expected to have an influence on actual community processes (Tilman, 2004). In other words, spores and other propagules contaminating community compositions derived from environmental DNA extracts and should be avoided if the aim is to assess the actual active community.

Anderson and Parkin (2007) were the first to show that it is possible to amplify fungal ITS precursor rRNA and consecutively several studies have been performed showing distinct differences between environmental DNA and RNA profiles (Anderson and Parkin, 2007; Anderson et al., 2008; Baldrian et al., 2012; Bastias et al., 2007; Korkama-Rajala et al., 2008; Rajala et al. 2011; Van der Linde et al., 2010, 2012). In these studies, it was assumed that inactive mycelium and spores produce little or no RNA and that thus the active community composition was analysed. However, basidiospores of *Pisolithus microcarpus* have been shown to store RNA delivered by the basidia shortly before spore maturation (Campos and Costa, 2010). We hypothesized that precursor ITS rRNA is not synthesized in dormant (i.e. ungerminated) basidiospores. However, we expected that as soon as spores become active and hyphae start to grow, precursor ITS rRNA is formed and detectable. We tested if precursor ITS rRNA is extractable and amplifiable from spores collected from sporocarps of different basidiomycete species. Subsequently, it was tested if precursor ITS rRNA was detectable in germinated spores from the same fungal sporocarps.

Materials and methods

Spore collection and germination

Sporocarps of 13 different basidiomycete species were collected in 2012 at different locations in Switzerland and Germany (Table 1), in addition we obtained sporocarps of cultivated *Pleurotus ostreatus* and *Agaricus bisporus* (Wauwiler Champignons, Wauwil, Switzerland). We aimed to obtain a wide phylogenetic variety of the Basidiomycota with 13 species covering the Boletales and three of the six major clades of the Agaricales as defined by Matheny et al. (2006) (Table 1).

Stipes were removed from the cap, and the caps were placed overnight above a paper surface with the hymenium facing downwards. The resulting spore print was scraped off with a microscope slide and eluted in 200 μl desalted water, these elutions were stored at 4 °C until further use.

To initiate germination, 50 μl of spore elution was mixed with 50 μl Penicillin-Streptomycin (Sigma–Aldrich, St. Louis MO, USA) and then transferred to a Petri dish with potato dextrose agar (PDA) (Sigma–Aldrich, St. Louis MO, USA). The spore PDA plates were inoculated at room temperature (24 °C) in the dark and periodically checked for germination.

Nucleic acid extractions

The RNeasy Plant mini kit (Qiagen, Hilden, Germany), in combination with cell lysis by bead beating with ceramic beads, was used to extract total RNA from the spore and germinating spore samples. The germinating spores were scraped off the PDA with a razor blade, taking as little agar medium as possible. Amongst the germinating spores there was always a minority of non-germinated spores, we did not try to separate these spores from the germinating spores. Only plates with germinating spore samples with single unbranched hyphae were used, plates with branching hyphae were discarded (these were considered past the germination stage). The ungerminated spore samples never contained germinating spores. Fifty microlitres of the spore elution or all spores scraped off the PDA plates (not germinated and germinating respectively) were transferred to a lysing matrix E-tube (MP Biomedicals, Illkirch, France) together with 100 μl RLT lysis buffer and then lysed twice for 30 s at 30 Hz with a MM400 mixer mill (Retsch, Haan, Germany). After lysing a supplemental 300 μl RLT lysis buffer was added and subsequently the manufacturers' protocol was used including the optional DNase step. As a positive control for the lysing method the DNeasy Plant mini kit (Qiagen, Hilden, Germany) in combination with cell lysis by bead beating with ceramic

Table 1 – Sporocarps collected in Switzerland (CH) and Germany (GER) including information on the phylogenetic variety based on the phylogenetic clade.

Species name	Location	Collection date	Phylogenetic clade	Spore germination
Hymenopellis radicata	Bruderholz, Basel, CH	Sep 12	Agaricales, marasmioid clade	Y
Tapinella atrotomentosa	Hanke, Basel, CH	Sep 12	Boletales	Y
Hypholoma fasciculare	Spitalwald, Basel, CH	Oct 12	Agaricales, agaricoid clade	Y
Marasmius oreades	Hanke, Basel, CH	Nov 12	Agaricales, marasmioid clade	Y
Pleurotus ostreatus	Wauwiler Champignons, Wauwil, CH	Nov 12	Agaricales, pluteoid clade	Y
Amanita crocea	Hottingen, Rickenbach, GER	Sep 1	Agaricales, pluteoid clade	N
Scleroderma citrinum	Hottingen, Rickenbach, GER	Sep 1	Boletales	N
Coprinus sp.	Hanke, Basel, CH	Sep 12	Agaricales, agaricoid clade	N
Amanita phalloides	Dent les Morcles, Martigny, CH	Oct 12	Agaricales, pluteoid clade	N
Macrolepiota procera	Brontallo, Vallemaggia, CH	Oct 13	Agaricales, agaricoid clade	N
Armillaria mellea	Hanke, Basel, CH	Oct 21	Agaricales, marasmioid clade	N
Megacollybia platyphylla	Hanke, Basel, CH	Oct 21	Agaricales, marasmioid clade	N
Agaricus bisporus	Wauwiler Champignons, Wauwil, CH	Nov 12	Agaricales, agaricoid clade	N