Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi

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Letters

Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi

Ergosterol has recently been used as a biomass indicator to compare the growth of different arbuscular mycorrhizal (AM) fungi (Hart & Reader, 2002a,b). Here, we show that ergosterol is not a suitable biochemical marker for estimating the biomass of AM fungi and that the comparison of biomass between different fungal taxa is very difficult using any kind of currently available biochemical marker.

Because they are usually degraded rapidly after cell death and because membrane area is assumed to be well correlated with the biovolume of microbial cells (Tunlid & White, 1992), membrane compounds, such as sterols, are attractive biomass indicators of microorganisms in environmental samples. Furthermore, sterols seem to represent a rather constant part of the fungal biomass, constituting somewhere between 5 and 15 mg g^{-1} in most fungal groups (Weete & Gandhi, 1996). In particular, ergosterol is specific to the fungal kingdom (Weete & Gandhi, 1996) and occurs mainly as a membrane constituent. Ergosterol has been used to indicate the fungal biomass in soil (Grant & West, 1986; Frostegård & Bååth, 1996), pathogenic fungi in roots (Bindler *et al.*, 1988), fungi in cereal grains (Seitz *et al.*, 1972), saprophytic fungi in decaying plant material (Newell *et al.*, 1988) and ectomycorrhizal fungi in roots (Salmanowicz & Nylund, 1988; Wallander *et al.*, 1997) and soil (Ek *et al.*, 1994; Ekblad *et al.*, 1995).

The occurrence of ergosterol is generally restricted to the more advanced fungal taxa, while the more primitive taxa contain other sterols (Weete & Gandhi, 1996). Thus, it is the dominating sterol in ascomycetes and basidiomycetes. By contrast, the picture is rather more complex within the phylum Zygomycota where members of Mucorales contain ergosterol, while *Mortierella* contain desmosterol, but no ergosterol (Weete & Gandhi, 1999). In a similar way, most members of the newly identified phylum Glomeromycota (Schüssler *et al.*, 2001), fungal obligate symbionts forming arbuscular mycorrhizas (AM), seem to contain sterols other than ergosterol. No ergosterol was detected in several studies in which gas chromatography-mass spectrometry (GC-MS) analysis was carried out on spores or extraradical mycelium of either *Glomus* or *Acaulospora* species (Beilby & Kidby, 1980; Beilby, 1980; Nordby *et al.*, 1981; Grandmougin-Ferjani *et al.*, 1999; Fontaine *et al.*, 2001) or mature spores of *Gigaspora margarita* (Grandmougin-Ferjani *et al.*, 1999). However, Frey *et al.* (1992, 1994) identified ergosterol in roots colonised by *Glomus intraradices* using GC-MS, but not in noncolonised roots, and they proposed the ergosterol content in extraradical hyphae of this fungus to be 0.063 mg per g mycelium. More recently, Fujiyoshi *et al.* (2000) found that the mycelium collected around roots colonised by *Gigaspora margarita* had 0.63 mg ergosterol per g of mycelium. Nevertheless, neither of the former two studies was carried out under *in vitro* conditions, and thus ergosterol from contaminating fungi could hardly be avoided. The slightest contamination may have a significant effect on the results because of the high ergosterol content in many saprophytic fungi.

Despite the fact that ergosterol has been shown to be absent in AM fungi on several occasions, high performance

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Table 1 Ergosterol content of fungal mycelia or monoxenic carrot root colonised with AM fungi or nonmycorrhizal

Biological materials	Ergosterol (mg g^{-1}) HPLC	GC-MS-MS
Glomalean fungi (extraradical mycelium)		
Glomus intraradices	< 0.025	< 0.001
Gigaspora margarita	< 0.16	< 0.001
Saprophytic zygomycetes		
Rhizopus arrhizus	2.9	4.8
Zygorrhyncus heterogamus	3.1	3.9
Ascomycetes		
Penicillium roqueforti (mainly conidia)	0.07	0.41
Cenococcum geoforme	4.2	3.5
Ectomycorrhizal basidiomycete		
Paxillus involutus	8.5	5.9
Carrot roots		
Nonmycorrhizal	< 0.006	< 0.0001
Glomus intraradices	< 0.002	< 0.0001
Gigaspora margarita	< 0.010	< 0.0001

Seven different mycelia of *G. intraradices* was analysed and one of *Gi. margarita*. Ergosterol was measured either with high performance liuquid chromatography (HPLC) or with tandem mass spectrometry (GC-MS-MS).

Table 2 The content of the PLFA 18 : 2ω6,9 and ergosterol (± SD) in mycelia of *Suillus* spp. (n = 8, including two isolates of each species) and *Paxillus involutus* (n = 12, including three isolates) growing in pure culture on agar, and the ratio of PLFA 18 : 2ω6,9 to ergosterol

Fungal species	Ergosterol (mg g^{-1})	PLFA 18 : 2ω6,9 (μmol g^{-1})	Ratio PLFA 18 : 2ω6,9/ ergosterol (μmol mg^{-1})
Paxillus involutus	4.5 ± 1.6	2.1 ± 0.5	0.45
Suillus bovinus	5.4 ± 3.5	24.3 ± 4.4	4.5
Suillus variegatus	1.8 ± 0.7	21.5 ± 2.2	12

The PLFA content was measured with GC (Olsson *et al*., 1995) and ergosterol with HPLC.

liquid chromatography (HPLC) estimation of ergosterol was recently used as a means of estimating and comparing the fungal biomass of various AM fungi in soil and roots (Hart & Reader, 2002a,b). In the same studies, the ergosterol content of the AM fungal inocula was used as a means of equalising the amount of inoculum added. In order to ascertain whether ergosterol can be used to estimate AM fungal biomass at all, we investigated ergosterol content in monoxenically (*in vitro*) grown AM fungi (Petri dish systems with carrot root cultures) where no contaminating fungi could affect the results.

We collected the extraradical mycelium of *G. intraradices* developing in liquid medium of monoxenic cultures (Olsson *et al*., 2002) and *Gi. margarita* in solid medium (Bago *et al*., 2002) as well as colonised and noncolonised roots. The ergosterol contents of both extraradical mycelium and colonised roots were estimated by HPLC separation and the specific detection of ergosterol using a UV detector (Nylund & Wallander, 1992), which is a commonly used method for ergosterol determination as a fungal biomass indicator. Other sterols are retained in the purified sample but only ergosterol is detectable at 280 nm because of a conjugated pair of double bonds (Nylund & Wallander, 1992). A newly developed highly specific method in which ergosterol is analysed using tandem mass spectrometry (GC-MS-MS; Larsson & Saraf, 1997) was also used. Irrespective of the method, we found no detectable ergosterol in *G. intraradices* or *Gi. margarita* (Table 1), which is in accordance with earlier studies.

The fatty acid 16 : 1ω5 has been used as a biomarker for AM fungi in many studies (Olsson *et al*., 1995; Olsson, 1999), but even with this method it is difficult to make comparisons between species because the variation between species and between genera may be considerable (Graham *et al*., 1995). In order to use any biochemical marker for estimating AM fungal biomass a conversion factor for each species must first be obtained, ideally from monoxenic cultures. The large variation that can be found in any biochemical marker compound is furthermore exemplified by the variation we

found in the content of the phospholipid fatty acid (PLFA) 18 : 2ω6,9 between *Suillus* spp. and *Paxillus involutus*, while both fungi contained similar amounts of ergosterol (Table 2).

We conclude that: ergosterol cannot be used as biomass indicator for glomalean fungi; and regardless of which biochemical marker is used, it is very difficult to make comparisons of biomass between different species because there are always taxonomic-based differences in content of any signature compound. Ideally, a specific conversion factor would have to be developed for each taxa when the aim is to compare the growth of different species.

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