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The volatile organic compounds from the mycelium of *Tuber borchii* Vitt.

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Abstract

The mycelium of *T. borchii* (characterized by DNA analysis) grown in sterile liquid medium produced some VOCs. The VOCs were retained on carbographs by passing a flow of helium, isolated and characterized in a GC–MS equipment after a thermal desorption. The compounds present in the VOCs from the mycelium cultures, but not in the VOCs from the control cultures, contained 29 compounds. The main compounds were 1,3-ditertbutylbenzene (16.1 ng/l), 3-methylheptane (9.2 ng/l), butan-2-one (8.8 ng/l), ethynylbenzene (5.6 ng/l), and octan-3-one (4.9 ng/l). © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Tuber borchii Vitt. (*Tuberales*) is an edible species of the genus *Tuber*. It is morphologically similar to *Tuber magnatum* Pico, the finest truffle species, but lacks the latter's typical sulphureous fragrance (Pelusio et al., 1995; Bellesia et al., 1996, 1998a,b,c).

Many volatile organic compounds (VOCs) from *T. borchii* ascocarps have been isolated: some of them derive from various degradation patterns while others are of bacterial origin (Talou et al., 1987; Fiecchi, 1988).

Our study reports, for the first time, the isolation and characterization of VOCs produced by the mycelium of *T. borchii* grown in sterile liquid medium. The great majority of the compounds recorded are not present in *T. borchii* ascocarps, and 27 of them have not been reported previously in the genus *Tuber*.

2. Results and discussion

Sterile mycelia of *Tuber borchii* were inoculated in fresh liquid medium and 11 days later a helium flux was applied; 172.2 ng/l of volatile organic compounds (VOCs) were extracted from the mycelium liquid cultures. The desorption was carried by backflushing the traps, all components were eluted at temperatures lower than ca. 130–150°C. Extensive studies on the thermal desorption with carbograph 2, carbograph 1, and carbograph 5 set in series did not show any substantial artifacts of VOCs (Ciccioli et al., 1992; Larsen et al., 1997; Brancaleoni et al., 1999). The compounds present in the VOCs from the mycelium cultures, but not in the VOCs from the control cultures, contained 29 compounds. The major compounds were 1,3-ditertbutyl benzene (16.1 ng/l), 3-methylheptane (9.2 ng/l), butan-2-one (8.8 ng/l), ethynylbenzene (5.6 ng/l), and octan-3-one (4.9 ng/l). Of the recovered compounds, 28.6 ng/l were aromatics, 24.1 ng/l hydrocarbons (cyclic: 2.1 ng/l), and 16,0 ng/l ketones. One alcohol (dodecan-1-ol) and one sulphur compound (dimethyltrisulfide) were also present. 2,2,5,5-tetramethyltetrahydrofuran was the

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only heterocyclic compound to be detected. α Terpinolene was the only terpenoid compound to be detected. Table 1 reports the volatile organic compounds emission (ng×g dry wt.$^{-1}$×h^{-1}) from the sterile mycelium of *T. borchii*.

The presence of dimethyltrisulfide seems to derive from the conversion of 2,4-dithiapentane to dimethyl disulfide through a hydrolytic-oxidative pathway already observed in *T. magnatum* (Bellesia et al., 1998c). None of these compounds has been found to be produced by *T. borchii* ascocarps (Bellesia et al., 1996); butan-2-one has been found to be produced by *T. melanosporum* Vitt. (Bellesia et al., 1998b) and *T. magnatum* Pico ascocarps (Bellesia et al., 1998c); dimethyltrisulfide has been found to be produced by *T. magnatum* Pico ascocarps (Bellesia et al., 1998c). All the other compounds were reported for the first time in the genus *Tuber*.

3. Experimental

3.1. Mycelium growth

The mycelium of *T. borchii* was cultured in Melin-Norkrans liquid medium modified as follows: $CaCl_2$ 0.45 mM; NaCl 0.43 mM; KH_2PO_4 3.4 mM; $(NH_4)_2HPO_4$ 1.89 M; $MgSO_4{\cdot}7H_2O$ 0.6 mM; malt extract 10 g/l; pH = 6.6. The medium was autoclaved (121°C; 1.2 bar; 15 min) and after cooling to room temperature, Sequestrene F 330 0.2 g/l and thiamine HCl 1 mg/l were added from filter sterilized stock solutions. Erlenmeyer flasks, each containing 50 ml of liquid medium, were inoculated with 0.2 g of fresh mycelium, incubated (23°C, 30 μE s^{-1} m^{-2}, 12-h photoperiod) without agitation and sub-cultured at monthly intervals.

Table 1
Volatile organic compounds emission (ng×g dry wt.$^{-1}$×h^{-1}) from the sterile mycelium of *Tuber borchii*

Compound	RRt	Rl	Emission
2-Butyne	3.61	482	0.002
1-Pentene	3.64	484	0.008
2-Methyl-1-butene	3.77	494	0.004
trans-2-Pentene	4.03	506	0.005
cis-2-Pentene	4.19	511	0.003
2-Methyl-2-butene	4.31	515	0.005
3-Penten-1-yne	4.65	525	0.012
Butan-2-one	6.34	578	0.094
2-Ethoxy-2-methylpropane	8.11	620	0.040
Methylcyclopentane	8.15	621	0.012
4-Methyl-2-pentanone	14.05	726	0.024
Propylcyclopentane	14.17	728	0.007
2,2,5,5-Tetramethyltetrahydrofuran	16.11	759	0.014
3-Methylheptane	17.00	773	0.098
2,3,5-Trimethylhexane	19.61	817	0.026
3-Methyloctane	20.80	839	0.010
Ethynylbenzene	22.09	863	0.060
Trimethylheptane	22.80	876	0.006
Dimethyltrisulfide	26.16	949	0.003
iso-decane	26.82	964	0.018
Octan-3-one	27.14	972	0.052
iso-decane	27.18	973	0.013
2,2,4,6,6-Pentamethylheptane	28.07	994	0.023
1H-Indene	29.47	1031	0.032
α-Terpinolene	31.45	1084	0.003
1,3-Ditertbutylbenzene	36.83	1249	0.172
Dodecan-1-ol	41.15	1398	0.036
Acenaphthylene	42.40	1445	0.010
2,4-Bis(1,1-dimethylethyl)-phenol	43.92	1502	0.032

3.2. Molecular characterization of the mycelium

DNA was isolated from the mycelium and PCR amplified as described by Paolocci et al. (1999) using the universal ribosomal primers ITS1/ITS4 (White et al., 1990). The amplification product was purified through a G50 Sephadex column (Pharmacia Biotech) according to the suppliers' instructions and directly sequenced by using the Big Dye Terminator Kit (PE Biosystem). For sequencing, the following primers were used: ITS1, ITS4, 5.8SB and 5.8SF (Paolocci et al., 1997). The reaction products were run on an ABIPRISM 310 Genetic Analyser PE Biosystem automated sequencer. The Blasta similarity search program confirmed that the ITS sequence of the in vitro cultivated mycelium did belong to *T. borchii*.

3.3. Sampling of gases evolved by mycelia

Mycelia of *T. borchii* were sealed into 0.5 l glass cuvettes with inlet and outlet lines fitted with stopcocks to prevent the diffusion of gases and vapours into the air. After 11 days the organic components were sampled by connecting the inlet line with a helium cylinder equipped with a gas regulation system and a filter for removing particles and bacteria larger than 0.22 μm. By passing a flow of helium of 100 ml min^{-1} through the glass cuvette, all organic compounds with a carbon number larger than 4 were fully retained on glass traps (16 cm long, 0.3 cm i.d.) filled with carbograph 2 (118 mg), carbograph 1 (60 mg) and carbograph 5 (115 mg) set in series. These materials were all supplied by LARA S.p.a. (Rome, Italy) in the particle range of 20–40 mesh. They were selected for their ability to retain the widest possible range of volatile organic compounds. A total volume of 1 l was passed through the cuvettes. After sample collection, the stopcocks were turned off, the traps were removed and transferred to the GC–MS system for analysis. After recovery of the VOCs, the mycelia were dried, giving a solid residue of 0.355 g/l culture broth.

3.4. Gas chromatography

Traps were connected to a thermal desorption system (PTI- Chrompack, Middleburg, The Netherlands) for

injecting the sample into a capillary column. Gases and vapours were desorbed under programmed conditions by rising the temperature from 25° to 250°C in 3.5 min under a flow of helium. They were cryofocused into a fused-silica liner kept at −150°C by using a helium flow rate of 20 ml min^{-1}. After the whole sample was condensed into the liner, it was transferred into the GC column by rising the temperature of the liner from −150 to 200°C in 5 min. The separation of the mixture was accomplished in a 50 m×0.32 mm i.d. capillary column coated with a thin film (0.40 μm) of CP-Sil-5 CB-MS supplied by Chrompack. The column was connected to a Hewlett-Packard (Palo Alto, CA, USA) gas chromatograph (model 5890) coupled to a 5970B mass selective detector (MSD) supplied by the same company. To optimize the separation of volatile compounds, the column was maintained at 0°C for 3 min after the injection and then the temperature was first raised to 50°C at a rate of 3°C min^{-1} then from 50 to 220°C at a rate of 5°C min^{-1}. The final temperature was maintained for 30 min.

The MSD unit was operated in the scan mode by collecting all ions ranging from m/z 20 to 200. Whenever possible, identification was performed by analyzing the entire spectrum. For coeluted compounds or species present at trace levels, positive identification was achieved by combining the retention index and reconstructed mass chromatogram information. Selective ions and time sequences of volatile compounds can be found in Ciccioli et al. (1993).

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