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Detection and Identification of Microbial Volatile Organic Compounds of the Green Mold Disease:
MVOC Profile on Different Media

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ABSTRACT

Button mushrooms are one of the most commonly cultivated mushroom species facing different risks e.g.: viral, bacterial and fungal diseases. One of the most common problems is caused by Trichoderma aggressivum, or 'green mould' disease. The presence or absence of mushroom disease-related moulds can sufficiently be detected from the air by headspace solid-phase microextraction coupled gas chromatography-mass spectrometry (HS SPME GC-MS) via their emitted microbial volatile organic compounds (MVOCs). In the present study, HS SPME GC-MS was used to explore the volatile secondary metabolites released by T. aggressivum f. europaeum on different nutrient-rich and -poor media. The MVOC pattern of green mould was determined, then media-dependent and independent biomarkers were also identified during metabolomic experiments. The presented results provide the basics of a green mould identification system which helps producers reducing yield loss, new directions for researchers in mapping the metabolomic pathways of T. aggressivum and new tools for policy makers in mushroom quality control.

KEYWORDS

Biomarkers, Different Media, Environmental Chemistry, MVOC (Microbial Volatile Organic Compound) Pattern, SPME (Solid-Phase Microextraction) Sampling, Trichoderma Aggressivum

LITERATURE REVIEW

Mushroom production showed an increasing tendency in the past few years. Button mushroom (*Agaricus bisporus*) gives one-third of the total amount of mushroom production (Chang & Miles, 2004; Largeteau & Savoie, 2010). Unfortunately, it is excessively sensitive to different diseases, such

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as viral, fungal (*Lecanicillium fungicola* – dry bubble disease, *Mycogene perniciosa* – wet bubble disease, *Trichoderma aggressivum* – green mould disease), and bacterial diseases (*Pseudomonas* spp.) (Fletcher, 1990).

Trichoderma aggressivum is known as the most harmful mould in mushroom production. Green mould is able to proliferate on mushroom compost, while the mushroom mycelium growth is obstructed, therefore it causes retarded, low-quality mushroom fruiting body instead of healthy, high-quality products (O'Brien, Grogan, & Kavanagh, 2014). Moreover, *T. aggressivum* produces extracellular enzymes, toxic secondary metabolites, and volatile organic compounds. As a consequence, not only the quality and the amount of yield decreases but the use of crop models is also obstructed (Papajorgji, Clark, & Jallas, 2009). Green mould disease is hard to recognize in the initial days during its long vegetative growth phase, the mould lawn is white (button mushroom mycelium is white as well); the colour of mould changes only when the green spores occur (after 2-4 days) (Largeteau & Savoie, 2010). *Trichoderma* species educed metabolite control system, which enables them to survive extreme environmental conditions, like low oxygen (Silva, Steindorff, & Monteiro, 2014). Green mould is also known as the most aggressive mould species in mushroom cultivation. At the initial phase, mould mycelium grows simultaneously with mushroom mycelium; mould can use compost substrates as carbon source via extracellular enzymes (Krupke, Castle, & Rinker, 2003). As soon as *Trichoderma* produce green spores, the mushroom growth is decreased, while the growth of green mould rapidly increases. After green mould sporulation, the button mushroom mycelium is obstructed by the mould (Górski, Sobieralski, Siwulski, Frąszczak, & Sas-Golak, 2014; Mamoun, Lapicco, Savoie, & Olivier, 2000; Mamoun, Savoie, & Olivier, 2000; Williams, Clarkson, Mills, & Cooper, 2003). One mushroom growth inhibitor compound produced by *T. aggressivum* is 3,4-dihydro-8-hydroxy-3-methylisocoumarin. This compound has not been noticed in not aggressive *Trichoderma* isolates (Krupke et al., 2003). In order to preserve the high quality of mushroom compost, this harmful microorganism must be detected as soon as possible and the quality of the compost should be continuously monitored and controlled.

A novel approach to fight against infections is volatile organic compound (VOC) examination. VOCs can be used as specific biomarkers or ecological indicators to describe or identify different species or groups of fungi (Muller et al., 2013). Microbial volatile organic compounds (MVOCs) are emitted during microorganism's metabolite pathways. Several microorganisms emit volatile compounds to evolve interactions (Tirranen & Gitelson, 2006). In 2013, Lemfack and his co-workers built an MVOC database (Lemfack, Nickel, Dunkel, Preissner, & Piechulla, 2014), which contains more than 10 000 species and their VOCs, and it is online available.

Several paper deals with examination of *Trichoderm*a fungi's volatile compounds, however, only *T. atroviride* (2-heptanone; 1-octen-3-ol; 3-octanone; 2-pentyl furan 3-octanol; 6-α-phellandrene; α-terpinene; β-phellandrene; 2-nonanone; phenylethyl alcohol; β-farnesene; α-curcumene (Stoppacher, Kluger, Zeilinger, Krska, & Schuhmacher, 2010)) and *T. harzianum* (butyric acid, ethyl ester; 2-methyl butyric acid, ethyl ester; phenylethanol; 2,6,-dimethyl-2,4,6-octatriene (Fiedler, Schütz, & Geh, 2001)) have been examined in most of the cases. Volatile metabolites of *T. aggressivum* were only researched by Krupke and his co-workers in 2003 (Krupke et al., 2003).

Trichoderma species emitted mostly different terpenes (Berg, Kemami Wangun, Nkengfack, & Schlegel, 2004; Cardoza et al., 2011; Reino, Guerrero, Hernández-Galán, & Collado, 2008). The diversity of *Trichoderma* species can be demonstrated according to its metabolite profile (Gupta et al., 2014). Alternative identification of different mould species can be done using their emitted volatile metabolite compounds (Naznin et al., 2014; Zhang, Askim, Zhong, Orlean, & Suslick, 2014).

Several fungi species can be distinguished using different coupled analytical techniques. MVOCs emitted by fungi can be captured, analysed and monitored with headspace solid-phase microextraction combination with gas chromatography-mass spectrometry (HS-SPME-GC-MS) from the air directly above the sample (Claeson, Levin, Blomquist, & Sunesson, 2002; Dong et al., 2015; Kluger, Zeilinger, Wiesenberger, Schöfbeck, & Schuhmacher, 2013; Matysik, Herbarth, & Mueller,

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