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Differentiation of basidiospores by MALDI-TOF lipid profiling

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ABSTRACT

Lipid profiles of microorganisms, such as bacteria and fungi, have been studied intensively and have shown great potential for species differentiation. However, lipid profiles of basidiospores are seldom reported in the literature. In this study, the MALDI-TOF mass spectra of lipids extracted from basidiospores of *Auricularia auricula*, *Agaricus bisporus*, *Hypsizygus marmoreus*, *Lentinus edodes*, *Pleurotus ostreatus*, and *Volvariella volvacea* were obtained using a laboratory-built MALDI-TOF mass spectrometer, equipped with an aerodynamic lens assembly as a direct deposition interface. The sample preparation strategies included matrices, solvents, and analyte/matrix ratios, which were optimized to acquire reproducible and informative mass spectra of lipid mixtures. Phospholipids, diacylglycerols, and triacylglycerols were observed by mixing the lipid extracts with equal volumes of 2,4-DHB/methanol solution (10 mg/mL). Species-specific lipid profiles of basidiospores were obtained. The experimental results demonstrate that the lipid profiles can be used to differentiate basidiospores.

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1. Introduction

Basidiospores are the sexual reproductive spores released by the basidiomycete fungi. The estimated average emission rate of basidiospores worldwide is ~17 Tg/yr, which accounts for one third of the total fungal spore emission (~50 Tg/yr) [1]. Airborne spores can cause significant adverse effects on health [2–4], and can affect climate by acting as cloud condensation nuclei and ice nuclei [5]. Therefore, the development of rapid methods to differentiate aerosolized fungal spores is of great importance.

Traditional approaches for fungal spores differentiation, such as cultivation methods and microscopic analyses, have been based on the morphological and phenotypic characteristics of microorganisms. These methods usually suffer from pleiotropism [6] and require highly specialized skills because of the wide array of airborne spores. Various modern analytical approaches, including enzyme-linked immunosorbent analysis [7], PCR amplification [8,9], fluorescent and Raman spectroscopies [10,11], HPLC tandem mass spectrometry and GC–MS [12], can differentiate aerosolized fungal spores according to their optical characteristics or biomarkers, such as the presence of saccharine and nucleic acids. However, these modern approaches with multifaceted procedures are time-consuming [13].

Lipids comprise a group of organic molecules that include fatty acyls, glycerolipids, glycerophospholopids, and others [14]. These molecules can act as major structural components of cell membranes as well as exert important biological functions for energy storage and signaling [15]. Lipid profiling involves the determination of lipid distributions in living organisms. The qualitative [16] and quantitative [17] differences contained in the lipid profiles of different organisms have been used to discriminate microorganisms. Black et al. reported on the chemotaxonomic differentiation between the *Bacillus cereus* group and *Bacillus subtilis* by analyzing phospholipid (PL) extracts with electrospray ionization tandem mass spectrometry [16]. We recently reported on the rapid differentiation of the *Bacillus* genus [18] and pollen grains [19] via MALDI-TOF lipid profiling.

Fungal spores can be classified as roughly globose, oval, or polygonal with diameters ranging from 2 μm to 50 μm, indicating that these molecules are larger than most individual bacterial cells but smaller than pollen grains [20]. These spores are coated with specific sugar, sterol, and hydrophobin compounds [21]. The water-repellent surface could aid in dispersal, prevent desiccation, and may provide a barrier to the entry of toxicants [22]. The fungal spore wall comprises multiple layers enclosing two nuclei, ribosomes, mitochondria, and other organelles [23–25]. Lipid bodies were observed in some mature basidiospores, such as *A. bisporus* and *Rhizopogon roseolus* spores [23,25], but not in *Agrocybe acericola* spores [24]. Chromatography, GC–MS, HPLC, and Raman spectroscopy have been used to analyze the lipids of fungal spores as well as identify fatty acids, glycerolipids, glycerophospholipids, and sterol lipids [26–29].

Glycerolipids, such as mono-, di-, and triglycerides, are the dominant neutral lipids in fungal spores and can act as energy reserves for future spore germination [30]. Fatty acids provide the carbon source and energy for spore germination [26]. Fatty acid

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Table 1
Morphological properties of the investigated basidiospores.

Scientific name	English name	Size (μm)	Shape	Color
A. auricula	Tree-ear	9–16 × 5–7.5	Sausage-shaped	Colorless
A. bisporus	Button mushroom	6–8.5 × 5–6	Ellipsoid	Brown
H. marmoreus	Beech mushroom	4–5 × 3.5–4	Sphere-like	White
L. edodes	Shiitake mushroom	4.5(5) × 2(2.5)	Ellipsoid	Colorless
P. ostreatus	Oyster mushroom	7–10 × 2.5–3.6	Cylinder	Colorless
V. volvacea	Straw mushroom	6–8 × 4–5	Ellipsoid	Colorless

Quoted from Huang [40].

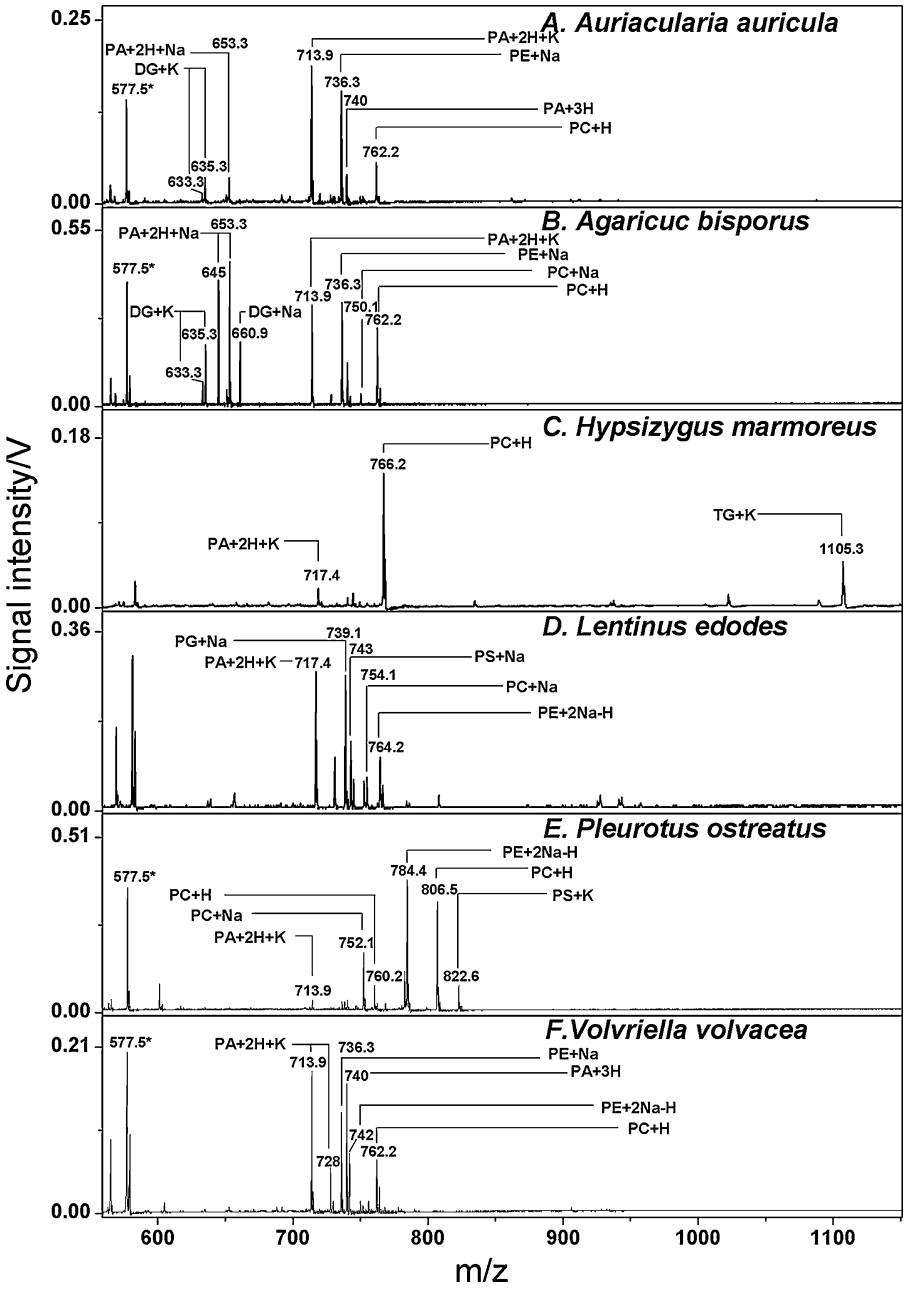

Fig. 1. MALDI-TOF mass spectra of the lipids extracted from spores of *A. auricula*, *A. bisporus*, *H. marmoreus*, *L. edodes*, *P. ostreatus*, and *V. volvacea* (A–F). The samples were prepared by mixing the lipid extract with an equal volume of 2,4-DHB/methanol solution (10 mg/mL).

chains usually contain 9–24 carbon atoms and are either saturated or unsaturated with one or two unsaturated bonds [26,31]. Oleic (C18:1), palmitoleic (C16:1), and linoleic (C18:2) acids are the major constituents of lipid reserves in *Pisolithus* and *Lactarius* basidiospores [26,27], ganoderma spores [32], and *Hypholoma capnoides* spores [31], respectively. PLs are major components of bilayer membranes and highly relevant to the second messenger molecules in a living organism [33]. However, PLs are found in very low quantities in fungal spores. O'Sullivan and Lösel indicated that *A. bisporus* spores have a low PL content of 0.12% [29]. Sterols are

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