Rapid and reliable species identification of wild mushrooms by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS)

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ABSTRACT

Mushrooms are a favourite natural food in many countries. However, some wild species cause food poisoning, sometimes lethal, due to misidentification caused by confusing fruiting bodies similar to those of edible species. The morphological inspection of mycelia, spores and fruiting bodies have been traditionally used for the identification of mushrooms. More recently, DNA sequencing analysis has been successfully applied to mushrooms and to many other species. This study focuses on a simpler and more rapid methodology for the identification of wild mushrooms via protein profiling based on matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS). A preliminary study using 6 commercially available cultivated mushrooms suggested that a more reproducible spectrum was obtained from a portion of the cap than from the stem of a fruiting body by the extraction of proteins with a formic acid-acetonitrile mixture (1+1). We used 157 wild mushroom-fruiting bodies collected in the centre of Hokkaido from June to November 2014. Sequencing analysis of a portion of the ribosomal RNA gene provided 134 identifications of mushrooms by genus or species, however 23 samples containing 10 unknown species that had lower concordance rate of the nucleotide sequences in a BLAST search (less than 97%) and 13 samples that had unidentifiable poor or mixed sequencing signals remained unknown. MALDI-TOF MS analysis yielded a reproducible spectrum (frequency of matching score ≥ 2.0 was ≥ 6 spectra from 12 spectra measurements) for 114 of 157 samples. Profiling scores that matched each other within the database gave correct species identification (with scores of ≥ 2.0) for 110 samples (96%). An in-house prepared database was constructed from 106 independent species, except for overlapping identifications. We used 48 wild mushrooms that were collected in autumn 2015 to validate the in-house database. As a result, 21

mushrooms were identified at the species level with scores ≥ 2.0 and 5 mushrooms at the genus level with scores ≥ 1.7, although the signals of 2 mushrooms were insufficient for analysis. The remaining 20 samples were recognized as “unreliable identification” with scores < 1.7. Subsequent DNA analysis confirmed that the correct species or genus identifications were achieved by MALDI-TOF MS for the 26 former samples, whereas the 18 mushrooms with poorly matched scores were species that were not included in the database. Thus, the proposed MALDI-TOF MS coupled with our database could be a powerful tool for the rapid and reliable identification of mushrooms; however, continuous updating of the database is necessary to enrich it with more abundant species.

Keywords:

Mass spectrometry

Species identification

Mushroom

Natural toxin

Food poisoning

1. Introduction

Wild mushrooms have considerable worldwide culinary popularity. Recently, interest in them has increased not only because of their unique textures and tastes but also because they can be used as foods for specified health uses (FOSHU) [1]. Indeed, some recently isolated components have antioxidant, antitumoural and immunomodulatory properties [1]. However, from a food safety viewpoint, it is also true that toxic mushrooms are a serious risk to human health and are sometimes lethal [2, 3]. Actually, nearly one hundred people are poisoned by toxic mushrooms every year in Japan, but only a few poisonings result in death [4]. These official figures in government reports represent the lowest verifiable incidence; therefore, the number of actual poisonings might be much higher. The incidence of poisoning is primarily due to the confusion of toxic mushrooms with an edible species that has a morphologically similar fruiting body [2]. To avoid health risks, the exact identification of collected wild mushrooms is essential.

The most prevalent and practical method for the identification of a mushroom species is macroscopic or morphological observation of each part of the fruiting body, such as the pileus, stipe, lamella and volva, on an empirical basis [5]. Microbiological analysis consisting of the microscopic inspection of tissues and spores is also an important approach because mushrooms are taxonomically classified as fungi. Chemical composition has also been evaluated for the species identification of mushrooms [5]. These methods each have their advantages of course; however, the procedures are somewhat complicated and their application to various groups of mushrooms is rather limited.

At present, DNA sequencing analysis of portions of the ribosomal RNA gene (rDNA) is the most reliable identification method for mushrooms as well as for many other organisms

[6-8]. DNA analysis is the method of choice for the retrospective analysis of left-over food or the vomitus of patients for example, but it is not practical to ensure the edibility of a mushroom suspected to be toxic. A remarkable development in DNA sequencing has been mainly achieved instrumentally [9, 10], but sample pre-treatment, such as DNA extraction and PCR amplification, is a barrier for easy and rapid identification of wild mushrooms on the same day they are collected.

Recently, an approach that uses matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) is increasing in many fields including food analysis, for example, in the species identification of shrimp [11] or scallops [12]. MALDI-TOF MS has also been successfully adapted for the rapid identification of microbial species from a single colony on a culture plate, based on the mass spectrum, which mainly reflects the diversity of ribosomal RNA proteins [13]. However, the application of MALDI-TOF MS to mushrooms has been limited to the mycelial form. In fact, several databases are available for yeast or fungi identification, but they never focus on mushrooms or their fruiting bodies [14-18]. The most attractive advantage in introducing MALDI-TOF MS for species identification is its rapidity; it can be implemented in a time as short as 10 min only for measurement or 30 min if pre-treatment steps are included. The use of MALDI-TOF MS thus makes it possible to identify a mushroom species on the day it is collected and brought into an average laboratory that has the instrument.

In this study, we tried to determine the fundamental conditions necessary to obtain reproducible spectra output from fruiting bodies of wild mushrooms and to then construct a database. The proposed MALDI-TOF MS system with our in-house generated database would be useful for the rapid and reliable identification of mushrooms and contribute to the

prevention of food poisoning resulting from the misidentification of edible and toxic mushrooms.

2. Materials and methods

2.1. Mushroom samples

A total of 157 wild mushroom samples collected in the centre of Hokkaido from June to November 2014 were used to construct an in-house database. The surface of fruiting body was gently washed with tap water to remove attached material such as soil and leaves. The mushrooms were left on paper towels to remove extra water. An additional 6 species of cultivated mushrooms were purchased at a retail store as models for the optimization of the basic analytical conditions. We also used 48 wild mushrooms that were collected in the same region from September to October 2015 to validate the in-house database.

2.2. Species identification by DNA sequencing

Approximately 5 mm^3 of the fruiting body was dissected by a No.10 surgical blade (Futaba, Tokyo, Japan), and DNA was extracted using an ISOIL Beads Beating kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. Internally transcribed spacer (ITS) regions of rDNA, approximately 300 bp each in length, were amplified by PCR using the primer pairs ITS 1/2 (5' TCC GTA GGT GAA CCT GCG G / 5' GCT GCG TTC ATC GAT GC) and/or ITS 3/4 (5' GCA TCG ATG AAG AAC GCA GC / 5' TCC GCT TAT TGA TAT GC) [19] under conventional reaction conditions with an annealing temperature of 55 ℃ and a cycling number of 30. Each PCR product was purified with a NucleoSpin Gel

and PCR clean-up kit (Macherey-Nagel, Düren, Germany) and submitted to a DNA sequencing service (Fasmac, Kanagawa, Japan). Mushroom species were identified by comparing the nucleotide sequences of the samples to sequences deposited in Genebank and the basic local alignment search tool (BLAST). A sample was considered as the same species with a 97% or greater concordance rate of the nucleotide sequences.

2.3. Sample preparation for MALDI-TOF MS

Approximately 5 mm^3 of a cap- or stem-portion of the fruiting body was dissected with No.10 surgical blades (Futaba), and proteins were extracted in 400 μL of a formic acid-acetonitrile mixture (1+1) including glass beads, 300 mg of BZ-01, 0.1 mm diameter together with 2 pieces of BZ-5, 5 mm diameter (AS ONE, Osaka, Japan) in a 2 mL screw-cap tube with an O-ring seal T-204 (BM Equipment, Tokyo, Japan). The sample was homogenized at 2,000 r min^{-1} for 3 min with a BC-20 shaker (Central Scientific commerce, Tokyo, Japan) and then centrifuged (20,000 g, 2 min). An approximately 50 μL portion of the supernatant was transferred to a new tube as the extracted solution and used on the same day it was prepared.

2.4. Measurement of mass spectrum

One microliter of the extracted solution was spotted on a polished steel target plate (Bruker Daltonics, Billerica, MA) in quadruplet for database spectrum or duplicate for inspection spectrum and air dried at room temperature. For each spot, 1 μL of the matrix solution of α-cyano-4-hydroxycinnimic acid (HCCA) (255344, Bruker Daltonics) was

spotted over the extracted solution and air dried again. Protein spectra were obtained using a MALDI-TOF MS Autoflex with Flex Control software (Bruker Daltonics).

2.5. Generation of the in-house database

The four spots prepared for a reference sample were measured 3 times each with the MALDI-TOF MS. The 12 spectra obtained, at most, were averaged for each reference sample using Flex Analysis software (Bruker Daltonics) and deposited in the in-house database as the species identified by DNA sequencing analysis. If 6 or more spectra were finally obtained, we used an average spectrum as a reference, although some spots or repeated measurements without sufficient signals to process were removed.

2.6. Species identification by MALDI-TOF MS

For the inspection of a sample, duplicate spots were measured by MALDI-TOF MS with the Flex Control software, and the obtained spectra were matched in parallel by MALDI Biotyper Real Time Classification (RTC) software (Bruker Daltonics) to those in the in-house database. We used the higher score value in duplicate measurements. According to the manufacturer's instructions, matching score values of 2.30 to 3.00 were classified as "highly probable species identification", 2.0 to 2.29 were "secure genus, probable species identification", 1.70 to 1.99 were "probable genus identification" and 0.00 to 1.69 were "unreliable identification". In practice, score values of 2.0 or greater were considered to be a candidate species for inspection.

2.7. Improvement of efficiency in protein extraction

We experienced a lack of a spectrum signal rather frequently in 2015. Therefore, extra studies were conducted by both focusing on the sampling portion from the cap to the lamella and introducing sonication (10 min) between the homogenization and centrifugation steps in section 2.3. The protein concentration in the formic acid-acetonitrile extract was measured by a BCA protein assay kit (Thermoscientific, Rockford, IL, USA). An analysis of variance (ANOVA), calculated by Prism 6 (GraphPad, San Diego, CA, USA), was used for testing the differences between groups.

2.8. Verification of in-house database

We used 48 wild mushrooms collected in 2015 for validation of the in-house database constructed using 106 species of mushrooms collected in 2014. Sample preparation was adopted based on the improved protocol described in section 2.7. Species identification by MALDI-TOF MS followed the same procedure described in section 2.6.

3. Results

3.1. Sampling portion

Unique and consistent mass spectra were obtained by MALDI-TOF MS for fruiting bodies of mushrooms covering a molecular mass range of 2,000 - 12,000. The sampling portions were compared in 4 samples with 3 species of cultivated mushrooms. The cap-portion appeared to give a higher and more diverse signal than the stem-portion of the same mushroom (Fig. 1). The matching scores between cap and stem of the same fruiting bodies were more than 2.0 for *Hypsizygus marmoreu;* however, values of 1.7 were recorded

for *Pleurotus eryngii* from one spot. We therefore decided to use the cap-portion for sampling at this time.

3.2. Reproducibility for different fruiting bodies

The reproducibility of the spectrum was evaluated for multiple samples of cultivated *Lentinula edodes*. Three samples purchased on different days, which were different lots from the same manufacturer, gave matching scores greater than 2.4 (Mean ±SD = 2.50 ±0.10, n=3). Four samples obtained from different manufacturers also gave matching scores greater than 2.5 (2.63±0.08, n=6). Thus, the spectra did not differ from one individual to the next within the same species of cultivated mushrooms.

3.3. Storage conditions

The effects of storage conditions on the spectrum were evaluated for cultivated *Lentinula edodes* and *Pleurotus eryngii*. Storage at 4°C in a plastic bag to avoid drying kept the matching scores greater than 2.0 even after 7 days for both mushrooms. However, storage at room temperature or -30°C decreased the matching scores to less than 2.0 after 4 days. In addition, 7 day storage at room temperature caused fungal growth on the surface of the fruiting body and rendering it unusable for the following measurement step.

3.4. Matching scores among cultivated mushrooms

The spectra obtained from 6 species of cultivated mushrooms were registered in a temporary database. The matching scores against the database were greater than 2.5 for the same species of all mushrooms and did not exceed 1.3 among different species (Table 1).

3.5. In-house database of wild mushrooms

The collected samples of 157 wild mushrooms were at first tentatively identified based on the morphological features of the fruiting bodies (Table S1). The following DNA sequencing analysis provided 134 identifications of mushrooms to the genus or species level, although 23 samples consisting of 10 unknown species with a lower concordance in a BLAST search (less than 97%) and 13 samples with unidentifiably poor or mixed sequencing signals remained unidentified. By the MALDI-TOF MS analysis, reproducible spectra were obtained for 114 of 157 samples. The matching scores within the database showed correct species identifications for 110 samples (96%). Only for the two pairs of *Inocybe maculata* (14-061) versus *I. lanatodisca* (14-062) and *Hypholoma sublateritium* (14-059) versus *H. capnoides* (14-056, 057) were the species not distinguishable with a matching score greater than 2.0. The in-house database was finally constructed from 106 independent species except for 4 overlapped samples (or strains) with the same species.

The MALDI-TOF MS with the in-house database was successfully applied for the species differentiation among morphologically or genetically similar mushrooms such as the toxic *Hypholoma fasciculare* (14-058) versus the edible *H. sublateritium* (14-059) or *Pholiota microspora* (14-099) (Fig. 2).

3.6. Improvement of signal acquisition

The frequency of the insufficient signal in MALDI-TOF MS analysis was reduced from 27 % (42/157 samples in Table S1) in the original procedure to 4 % (2/48 in Table 2) in the improved procedure sampling from the lamella portion with sonication treatment. The protein concentrations of eight different species (15-08, 12, 15, 17, 21, 32, 33 and 36 in Table 2) without sonication were much higher in the lamella (2,400 μg mL^{-1}) than in the cap and

stem (approximately 500 μg mL^{-1}) (Fig. 3). The concentration was specifically increased by sonication to 3,200 μg mL^{-1} in the lamella, in contrast to no increase in the cap and stem. The two-way ANOVA demonstrated a significant difference ($p < 0.001$) among sampling portions; however, no significant difference ($p = 0.537$) was observed between the cases with and without sonication.

3.7. Verification of in-house database

The in-house database was validated by 48 samples of wild mushrooms collected in 2015. Twenty-one samples were identified at the species level with scores ≥ 2.0, whereas 5 mushrooms were limited to the genus level with scores ≥ 1.7 (Table 2). These 26 mushrooms represented 54 % of the total samples. Two cases of limited identification at the genus level—*Hypholoma* sp. (15-14) and *Pleurella* sp. (15-38)—were not identified at the species level by DNA sequencing. Two mushrooms (15-10 and 15-44) did not provide sufficient signals for analysis. The remaining 20 samples were classified as "unreliable identification" with scores < 1.7. Subsequent DNA analysis confirmed that correct species or genus identification was achieved by MALDI-TOF MS for 26/26 samples, whereas 18/20 of the mushrooms with poorly matched scores were species that were not included in the database (Table 2). *Lepista sordida* (15-22) and *Pholiota adiposa* (15-28) were not identified correctly with scores < 1.7, although their corresponding spectra data were registered in the in-house database.

4. Discussion

DNA analysis is the most prevalent approach for mushroom-species identification [6-8]; however, it includes some time-consuming steps. The purpose of this study is the rapid identification just after collection followed by cooking of wild mushrooms as well as morphological and empirical inspection. We achieved reliable and practical identification of mushroom species through the use of MALDI-TOF MS with an in-house database within 30 min including a sample pre-treatment step. This study is the first report, as far as we know, that fruiting body rather than the mycelial form of mushroom is applicable for MALDI-TOF MS measurement [16, 17].

The cap-portion was better than the stem-portion for obtaining an informative spectrum accompanied with various and/or higher signal peaks (Fig. 1). This seems to be due to differences in protein composition or amount between the portions. Minor amounts of total protein in the extraction solution of the stem-portion compared to the cap-portion were observed by SDS-PAGE; additionally, the weaker signal was not improved by desalting purification nor inhibition of ionization [11] (data not shown). The tendency for a lower spectrum signal in the stem-portion may be due to this factor. The measurements of protein concentration in the extracts by the original and improved procedure support this speculation. The lamella-portion could provide many more abundant proteins in the extracts than the cap and stem portions with or without sonication (Fig. 3). In the lamella, the presence of basidia, active multiplication cells, may contribute to the richness of the protein concentration [20, 21]. The in-house database was constructed based on protein spectra extracted from the cap portion. At the beginning of this study, we paid no attention to the discrimination of the cap from the lamella. Therefore, some portions of the lamella might have been contaminated in cap sampling and have mainly contributed to the spectrum measurement. The higher matching score with the improved signal acquisition rate in Table 2 using spectra from the

lamella, not the cap, will support this hypothesis. We decided to incorporate sonication into the extraction step because it increases the extraction efficiency by approximately 30 % in the lamella (Fig. 3).

Matching scores of greater than 2.5 were achieved with all the 6 cultivated mushroom species (Table 1). In addition to these high scores, no differences were observed between either lots or manufacturers, so reliable and reproducible identification is possible by MALDI-TOF MS. It is also practically important to test mushrooms stored in a home refrigerator for a week at most before starting a MALDI-TOF MS analysis. Many wild mushrooms are collected over a holiday and brought into the laboratory after several days of storage.

For the 157 samples of wild mushrooms, the results of the morphological inspection did not necessarily coincide with those of the DNA sequencing analysis (Table S1). These discrepancies are mainly ascribed to somewhat ambiguous identification in both approaches. The in-house database of 106 wild mushrooms for MALDI-TOF MS was constructed based on species or genera identified with surety by DNA sequencing analysis. The matching scores of mushrooms in the database showed that as much as 96 % of the wild mushroom species were correctly identified, including toxic species such as *Omphalotus japonicus* (14-090) and *Hypholoma fasciculare* (14-058). The most important advantage of MALDI-TOF MS identification is enabling us to immediately bring attention to these poisonous mushrooms, which are often misidentified as edible species (Fig. 2).

The reproducible identification within the same species for cultivated mushrooms was confirmed (Table 1) in turn, a similar consistency for wild mushrooms should be validated because they grow under much more divergent circumstances. We conducted this study for 48 mushroom samples collected during autumn 2015 (Table 2). Of these, 26 mushrooms

(54 %) were correctly identified at the species or genus level against the in-house database. Mushrooms with “unreliable identification” totaled 20 samples, of which 18 (90 %) were novel species not registered in the in-house database (Table S1). The sum of these figures (44 / 48 samples) means that 92 % of the mushrooms collected beyond different seasons would be identifiable by MALDI-TOF MS if the database were continually updated with more abundant species and strains. We continue to enrich our in-house database to make it publicly available for local governmental laboratories responsible for mushroom identification requested from citizens.

Introduction of the proposed MALDI-TOF MS identification of wild mushroom species will contribute to the avoidance of a serious risk of food poisoning; as a result, it is expected to make it easier and safer to enjoying the taste of wild mushrooms.

5. Conclusion

We showed that MALDI-TOF MS with an in-house database was applicable for the identification of wild mushroom species using fruiting bodies. The advantages of this approach are a rapidity close to that achieved by a morphological inspection and a reliability similar to DNA sequencing analysis.

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Table 1

Matching scores of cultivated mushrooms against a temporary database.

Mushroom	*Pholiota microspora*	*Flammulina velutipes*	*Pleurotus citrinopileatus*	*Hypsizygus marmoreus*	*Lentinula edodes*	*Pleurotus eryngii*
Pholiota microspora	2.7 [a]	0.6	0.8	0.4	0.3	0.2
Flammulina velutipes	0.6	2.8 [a]	1	0.3	0.4	1
Pleurotus citrinopileatus	0.9	0.8	2.6 [a]	0.7	0.7	0.9
Hypsizygus marmoreus	1.1	0.6	0.8	2.7 [a]	0.1	0.1
Lentinula edodes	1.3	0	0.2	0.2	2.7 [a]	0.3
Pleurotus eryngii	0.5	0.8	0.8	0	0.2	2.7 [a]

Figure meanings,

2.30 to 3.00: highly probable species identification, 2.0 to 2.29: secure genus, probable species identification, 1.70 to 1.99: probable genus identification, 0.00 to 1.69: unreliable identification

[a] Between the same species.

Table 2.

Validation of the the in-house database using wild mushrooms collected in 2015

No.	Morphological estimation	Identified by			
		DNA sequencing [a]	MALDI-TOF MS	Score [b]	No. in Table S1 [c]
15-01	*Amanita imazekii*	*Amanita imazekii*	*Amanita imazekii*	2.48, 2.49	14-010
15-02	*Amanita muscaria*	*Amanita muscaria*	*Amanita muscaria*	2.29, 2.37	14-011
15-03	*Boletopsis leucomelas*	*Boletopsis grisea*	(unreliable)	< 1.70	-
15-04	*Boletus reticulatus*	*Boletus edulis*	*Boletus edulis*	2.21, 1.93	14-020
15-05	*Boletus calopus*	*Boletus smithii*	(unreliable)	< 1.70	-
15-06	*Clitocybe connata*	*Clitocybe connata*	*Clitocybe connata*	2.37, 2.41	14-027
15-07	*Clitocybe nebularis*	*Clitocybe nebularis*	*Clitocybe nebularis*	2.08, 2.40	14-081
		C. robusta			
		Leucopaxillus tricolor			
15-08	*Cortinarius trivialis*	*Cortinarius trivialis*	(unreliable)	< 1.70	-
		C. favrei			
15-09	*Tricholoma saponaceum*	*Cortinarius perplexus*	(unreliable)	< 1.70	-
		C. intricatus			
15-10	*Auricularia auricula-judae*	*Exidia recisa*	(not identified)	no peak	-
15-11	*Flammulina velutipes*	*Flammulina rossica*	(unreliable)	< 1.70	-
15-12	*Hygrophorus olivaceoalbus*	*Hygrophorus olivaceoalbus*	*Hygrophorus olivaceoalbus*	2.16, 2.47	14-050
15-13	*Hygrophorus lucorum*	*Hygrophorus* sp.	(unreliable)	< 1.70	-
15-14	*Hypholoma capnoides*	*Hypholoma capnoides*	*Hypholoma capnoides*	2.12, 2.14	14-057
		H. sublateritium	*H. sublateritium*	2.14, 2.14	14-059
15-15	*Inocybe geophylla*	*Inocybe geophylla*	(unreliable)	< 1.70	-
15-16	*Lactarius laeticolorus*	*Lactarius deliciosus*	*Lactarius deliciosus*	2.11, 1.99	14-065
15-17	*Lactarius necator*	*Lactarius necator*	(unreliable)	< 1.70	-
15-18	*Lactarius flavidulus*	*Lactarius alnicola*	(unreliable)	< 1.70	-
		L. leonis			
		L. scrobiculatus			
15-19	*Leccinum scabrum*	*Leccinum callitrichum*	*Leccinum versipelle* [d]	1.78, 1.90	14-077
		L. versipelle			
15-20	*Lentinellus cochleatus*	*Lentinellus sinensis*	(unreliable)	< 1.70	-
		L. suelineolatus			
		L. vulpinus			
15-21	*Lepista nuda*	*Lepista nuda*	*Lepista nuda*	2.20, 2.44	14-79
15-22	*Lepista sordida*	*Lepista sordida*	(unreliable)	< 1.70	14-80
15-23	*Leucoagaricus sp.*	*Leucoagaricus leucothites*	(unreliable)	< 1.70	-
15-24	*Lyophyllum semitale*	*Lyophyllum semitale*	(unreliable)	< 1.70	-
15-25	*Hypomyces sp.*	*Mortierella hyalina*	(unreliable)	< 1.70	-
15-26	*Paxillus involutus*	*Paxillus involutus*	(unreliable)	< 1.70	-
15-27	*Phaeolepiota aurea*	*Phaeolepiota aurea*	(unreliable)	< 1.70	-
15-28	*Pholiota aurivella*	*Pholiota adiposa*	(unreliable)	< 1.70	14-095
15-29	*Pholiota squarrosa*	*Pholiota adiposa*	*Pholiota adiposa*	2.12, 2.28	14-095
		P. aurivella			
		P. limonella			
		P. squarrosoides			
15-30	*Pholiota lenta*	*Pholiota lenta*	*Pholiota lenta*	2.09, 2.16	14-096
		P. lubrica			
15-31	*Pholiota lubrica*	*Pholiota lubrica*	*Pholiota lubrica*	2.12, 2.17	14-097
15-32	*Pholiota microspora*	*Pholiota microspora*	*Pholiota microspora*	2.06, 2.42	14-099
15-33	*Pholiota spumosa*	*Pholiota spumosa*	*Pholiota spumosa* [d]	< 1.70, 1.75	14-101
15-34	*Pholiota terrestris*	*Pholiota squarrosa*	*Pholiota squarrosa*	2.19, 2.44	14-102
15-35	*Pholiota terrestris*	*Pholiota squarrosa*	*Pholiota squarrosa*	2.09, 2.27	14-102
		P. lundbergii			
15-36	*Phygrophorus pudorinus*	*Phygrophorus pudorinus*	(unreliable)	< 1.70	-
15-37	*Phygrophorus pudorinus*	*Phygrophorus pudorinus*	(unreliable)	< 1.70	-
15-38	*Pleurella ardesiaca*	*Pleurella ardesiaca* (87)	*Pleurella ardesiaca* [d]	1.73, 1.86	14-104
15-39	*Polyporellus badius*	*Polyporellus badius*	*Polyporellus badius*	2.10, 2.16	14-107
15-40	*Russula emetica*	*Russula cavipes*	*Russula cavipes*	2.26, 1.99	14-115
15-41	*Sarcomyxa serotina*	*Sarcomyxa serotina*	*Sarcomyxa serotina*	2.14, 1.75	14-120
15-42	*Suillus granulatus*	*Suillus luteus*	(unreliable)	< 1.70	14-127
		S. brevipes			
15-43	*Tricholoma flavovirens*	*Tricholoma flavovirens*	*Tricholoma flavovirens*	2.07, 2.28	14-134
		T. equestre			
15-44	*Tricholoma japonicum*	*Tricholoma japonicum*	(not identified)	no peak	-
15-45	*Tricholoma portentosum*	*Tricholoma portentosum*	(unreliable)	< 1.70	-
		T. saponaceum			
		T. sejunctum			
15-46	*Tricholoma sejunctum*	*Tricholoma sejunctum* (91)	*Tricholoma sejunctum*	2.02, 2.08	14-138
		T. porentosum (91)			
15-47	*Tricholoma virgatum*	*Tricholoma terreum*	*Tricholoma terreum*	2.05, 2.28	14-141
15-48	*Tyromyces chioneus*	*Tyromyces chioneus*	*Tyromyces chioneus* [d]	no peak, 1.76	14-143

[a] Concordance rate of BLAST is shown in parentheses when less than 97%.

[b] Matching scores in duplicate measurements of lamella-portion extracts againt the in-house database constructed in 2014.

[c] No. in the in-house database corresponding to the identified species by DNA sequencing.

[d] Identification in genus level with scores between 1.7 to 1.99.

Table S1.

In-house database obtained by MALDI-TOF-MS for wild mushrooms collected in 2014 (1/3)

No.	Morphological estimation	DNA sequencing identification			Frequency of
		Scientific name [a]	Common name	Family	Score ≥ 2.0 [b]
14-001	*Agaricus arvensis*	*Agaricus albolutescens*	-	*Agaricaceae*	0
14-002	*Tricholoma album*	*Agaricus campestris*	Meadow mushroom	*Agaricaceae*	≥ 6
		A. californicus	California agaricus		
14-003	*Agrocybe paludosa*	*Agrocybe praecox*	Spring fieldcap	*Bolbitiaceae*	≥ 6
		A. erebia	Dark fieldcap		
14-004	*Aleuria aurantia*	*Aleuria aurantia*	Orange peel fungus	*Pyronemataceae*	≥ 6
14-005	*Aleuria rhenana*	*Aleuria rhenana*	Stalked orange peel fungus	*Pyronemataceae*	≥ 6
14-006	*Amanita citrina*	*Amanita citrina*	False deathcap	*Amanitaceae*	≥ 6
14-007	*Amanita pantherina*	*Amanita ibotengutake*	-	*Amanitaceae*	≥ 6
14-008	*Amanita pantherina*	*Amanita ibotengutake*	-	*Amanitaceae*	≥ 6
		A. pantherina	Panther cap		
14-009	*Amanita imazekii*	*Amanita imazekii*	-	*Amanitaceae*	1 to 5
14-010	*Amanita imazekii*	*Amanita imazekii*	-	*Amanitaceae*	≥ 6
14-011	*Amanita muscaria*	*Amanita muscaria*	*Fly amanita*	*Amanitaceae*	≥ 6
14-012	*Amanita virosa*	*Amanita oberwinklerana*	Oberwinkler's destroying angel	*Amanitaceae*	0
14-013	*Leucocortinarius bulbiger*	*Amanita silvifuga* (88%)	-	-	≥ 6
14-014	*Ampulloclitocybe clavipes*	*Ampulloclitocybe clavipes*	Club-footed clitocybe	*Tricholomataceae*	≥ 6
14-015	*Clitocybe maxima*	*Ampulloclitocybe clavipes*	Club-footed clitocybe	*Tricholomataceae*	≥ 6
14-016	*Armillaria mellea*	*Armillaria gallica*	Bulbous honey fungus	*Tricholomataceae*	≥ 6
		with other 7 candidate species			
14-017	*Armillaria ostoyae*	*Armillaria sinapina*	-	*Tricholomataceae*	≥ 6
		with other 5 candidate species			
14-018	*Armillaria mellea*	*Armillaria sinapina*	-	*Tricholomataceae*	0
		with other 6 candidate species			
14-019	*Tylopilus eximius*	*Boletaceae sp.*	-	*Boletaceae*	0
14-020	*Boletus edulis*	*Boletus edulis*	King bolete	*Boletaceae*	≥ 6
14-021	*Cortinarius balteatocumatilis*	*Boletus edulis*	King bolete	*Boletaceae*	≥ 6
14-022	*Leccinum holopus*	*Boletus edulis*	King bolete	*Boletaceae*	≥ 6
14-023	*Leccinum scabrum*	*Boletus edulis*	King bolete	*Boletaceae*	1 to 5
14-024	*Catathelasma ventricosum*	*Catathelasma imperiale*	Imperial mushroom	*Biannulariaceae*	≥ 6
		C. ventricosum	-		
14-025	*Chalciporus piperatus*	*Chalciporus piperatus*	Peppery bolete	*Boletaceae*	≥ 6
14-026	*Chroogomphus rutilus*	*Chroogomphus rutilus*	Brown slimecap	*Gomphidiaceae*	≥ 6
14-027	*Clitocybe connata*	*Clitocybe connata*	-	*Lyophyllaceae*	≥ 6
14-028	*Mycena galericulata*	*Clitocybula familia*	-	*Marasmiaceae*	1 to 5
14-029	*Rhodophyllus abortivus*	*Clitopilus abortivus*	Aborted entoloma	*Entolomataceae*	≥ 6
14-030	*Collybia dryophila*	*Collybia dryophila*	Russet toughshank	*Tricholomataceae*	≥ 6
14-031	*Collybia dryophila*	*Collybia dryophila*	Russet toughshank	*Tricholomataceae*	≥ 6
14-032	*Coprinus micaceus*	*Coprinellus disseminatus*	Fairy inkcap	*Psathyrellaceae*	≥ 6
14-033	*Coprinopsis atramentaria*	*Coprinopsis atramentaria*	Common inkcap	*Coprinaceae*	0
14-034	*Cortinarius balteatocumatilis*	*Cortinarius balteatus*	-	*Cortinariaceae*	≥ 6
		C. balteatocumatilis	Oak webcap		
14-035	*Cortinarius crocolitus*	*Cortinarius ophiopus*	-	*Cortinariaceae*	≥ 6
		C. crocolitus	-		
14-036	*Cortinarius crocolitus*	*Cortinarius ophiopus*	-	*Cortinariaceae*	≥ 6
		C. triumphans	Birch webcap		
14-037	*Cortinarius crocolitus*	*Cortinarius ophiopus*	-	*Cortinariaceae*	≥ 6
		C. triumphans	Birch webcap		
14-038	*Polyporus brumalis*	*Daedaleopsis confragosa*	Blushing bracket	*Polyporaceae*	0
		Polyporus brumalis	Winter polypore		
14-039	*Entoloma abortivum*	*Entoloma abortivum*	Aborted entoloma	*Entolomataceae*	≥ 6
14-040	*Rhodophyllus rhodopolius*	*Entoloma sinuatum* (92%)	-	-	≥ 6
14-041	*Gymnopilus penetrans*	*Flammula alnicola*	-	*Ranunculaceae*	≥ 6
		Pholiota pinicola	-	*Strophariaceae*	
14-042	*Flammulina velutipes*	*Flammulina fennae*	-	*Tricholomataceae*	≥ 6
14-043	*Astraeus hygrometricus*	*Geastrum saccatum* (95%)	-	-	1 to 5
14-044	*Grifola frondosa*	*Grifola frondosa*	Hen of the woods	*Meripilaceae*	1 to 5
14-045	*Grifola frondosa*	*Grifola frondosa*	Hen of the woods	*Meripilaceae*	1 to 5
14-046	*Gymnopilus liquiritiae*	*Gymnopilus liquiritiae*	-	*Cortinariaceae*	≥ 6
		G. picreus	-		
14-047	*Gymnopilus penetrans*	*Gymnopilus penetrans*	Common rustgil	*Cortinariaceae*	≥ 6
14-048	*Gymnopilus liquiritiae*	*Gymnopilus penetrans*	Common rustgil	*Cortinariaceae*	≥ 6
		G. hybridus	-		
14-049	*Gymnoplius spectabilis*	*Gymnoplius spectabilis*	Laughing gym	*Cortinariaceae*	≥ 6
14-050	*Hygrophorus olivaceoalbus*	*Hygrophorus olivaceoalbus*	Olive wax cap	*Hygrophoraceae*	≥ 6
14-051	*Hygrophorus pudorinus*	*Hygrophorus pudorinus*	Rosy woodwax	*Hygrophoraceae*	1 to 5
14-052	*Hygrophorus purpurascens*	*Hygrophorus pudorinus* (90%)	-	-	≥ 6
14-053	*Camarophyllus virgineus*	*Hygrophorus queletii*	-	*Hygrophoraceae*	1 to 5
14-054	*Hygrophorus russula*	*Hygrophorus russula*	Pinkmottle woodwax	*Hygrophoraceae*	0
14-055	*Hygrophorus lucorum*	*Hygrophorus sp.*	-	*Hygrophoraceae*	≥ 6

[a] Concordance rate of BLAST is shown in parentheses when less than 97%.

[b] Frequency of matching score 2.0 or greater from 12 spectra measurements was classified as 6 or greater (≥ 6), less than 6 and more than 1 (1 to 5) and none (0).

Table S1.

In-house database obtained by MALDI-TOF-MS for wild mushrooms collected in 2014 (2

No.	Morphological estimation	DNA sequencing identification
		Scientific name [a]
14-056	*Hypholoma capnoides*	*Hypholoma capnoides*
14-057	*Hypholoma capnoides*	*Hypholoma capnoides*
14-058	*Hypholoma fasciculare*	*Hypholoma fasciculare*
14-059	*Hypholoma sublateritium*	*Hypholoma sublateritium*
14-060	*Hypsizygus marmoreus*	*Hypsizygus ulmarius*
14-061	*Inocybe fastigiata*	*Inocybe lanatodisca*
14-062	*Psathyrella candolleana*	*Inocybe maculata*
14-063	*Laccaria laccata*	*Laccaria laccata*
		L. trichodermophora
14-064	*Lacrymaria lacrymabunda*	*Lacrymaria glareosa*
		L. lacrymabunda
14-065	*Lactarius deliciosus*	*Lactarius deliciosus*
14-066	*Lactarius porninsis*	*Lactarius deliciosus*
14-067	*Lactarius akahatsu*	*Lactarius fennoscandicus*
		L. deterrimus
		L. aurantiosordidus
14-068	*Lactarius chrysorrheus*	*Lactarius lacunarum*
14-069	*Lactarius chrysorrheus*	*Lactarius lilacinus*
14-070	*Lactarius uvidus*	*Lactarius uvidus*
14-071	*Lactarius volemus*	*Lactarius volemus*
		Lactarius crocatus
14-072	*Laetiporus cremeiporus*	*Laetiporus cremeiporus*
14-073	*Lactarius flavidulus*	*Lapista flaccida*
14-074	*Leccinum scabrum*	*Leccinum melaneum*
		L. rotundifoliae
		L. scabrum
14-075	*Leccinum scabrum*	*Leccinum scabrum*
14-076	*Leccinum scabrum*	*Leccinum schistophilum*
		L. palustre
14-077	*Leccinum holopus*	*Leccinum versipelle*
		L. roseotinctum
14-078	*Lepista flaccida*	*Lepista flaccida*
14-079	*Lepista nuda*	*Lepista nuda*
14-080	*Lepista irina*	*Lepista sordida*
14-081	*Clitocybe nebularis*	*Leucopaxillus tricolor*
		Clitocybe robusta
		Clitocybe nebularis
14-082	*Lycoperdon perlatum*	*Lycoperdon perlatum*
14-083	*Lyophyllum decastes*	*Lyophyllum decastes*
14-084	*Lyophyllum decastes*	*Lyophyllum decastes*
14-085	*Lyophyllum sykosporum*	*Lyophyllum deliberatum*

14-086	*Marasmius maximus*	*Marasmius purpureostriatus* (95%)
14-087	*Pluteus atricapillus*	*Megacollybia marginata*
14-088	*Mucidula brunneomarginata*	*Mucidula brunneomarginata*
14-089	*Mycena pura*	*Mycena pura*
		with other 4 candidate species
14-090	*Omphalotus japonicus*	*Omphalotus japonicus*
14-091	*Peziza vesiculosa*	*Otidea onotica*
14-092	*Paxillus involutus*	*Paxillus sp*.
14-093	*Dictyophora duplicata*	*Phallus indusiatus* (80%)
14-094	*Pholiota adiposa*	*Pholiota abietis*
14-095	*Pholiota aurivella*	*Pholiota adiposa*
		P. aurivella
		P. limonella
14-096	*Pholiota lenta*	*Pholiota lenta*
		P. lubrica
		P. spumosa
14-097	*Pholiota lubrica*	*Pholiota lubrica*
14-098	*Pholiota lubrica*	*Pholiota lubrica*
14-099	*Pholiota microspora*	*Pholiota microspora*
14-100	*Pholiota destruens*	*Pholiota mixta* (96%)
14-101	*Pholiota spumosa*	*Pholiota spumosa*
14-102	*Pholiota terrestris*	*Pholiota squarrosa*
14-103	*Pholiota terrestris*	*Pholiota squarrosa*
14-104	*Clitocybula esculenta*	*Pleurella ardesiaca* (90%)
14-105	*Hohenbuehelia geogenia*	*Pleurotus pulmonarius*
14-106	*Pluteus atricapillus*	*Pluteus pouzarianus*
14-107	*Polyporus badius*	*Polyporus badius*
14-108	*Leucopaxillus septentrionalis*	*Porpoloma macrocephalum* (96%)

[a] Concordance rate of BLAST is shown in parentheses when less than 97%.

[b] Frequency of matching score 2.0 or greater from 12 spectra measurements was (and none (0).

2/3)

Common name	Family	Frequency of Score ≥ 2.0 [b]
Conifer tuft	*Strophariaceae*	≥ 6
Conifer tuft	*Strophariaceae*	≥ 6
Sulphur tuft	*Strophariaceae*	≥ 6
Brick cap	*Strophariaceae*	≥ 6
Elm oyster	*Tricholomataceae*	≥ 6
-	*Inocybaceae*	≥ 6
Frosty fibrecap	*Inocybaceae*	≥ 6
Waxy laccaria -	*Tricholomataceae*	≥ 6
-	*Coprinaceae*	≥ 6
Weeping window		
Saffron milkcap	*Russulaceae*	1 to 5
Saffron milkcap	*Russulaceae*	0
-	*Russulaceae*	≥ 6
False saffron milkcap		
-		
-	*Russulaceae*	1 to 5
Lilac milkcap	*Russulaceae*	≥ 6
Shiner	*Russulaceae*	≥ 6
Weeping milkcap	*Russulaceae*	0
-		
-	*Polyporaceae*	≥ 6
Tawny funnel	*Tricholomataceae*	≥ 6
-	*Boletaceae*	0
-		
Brown birch bolete		
Brown birch bolete	*Boletaceae*	1 to 5
-	*Boletaceae*	≥ 6
-		
Orange birch bolete	*Boletaceae*	≥ 6
-		
Tawny funnel	*Tricholomataceae*	≥ 6
Wood blewit	*Tricholomataceae*	≥ 6
Sordid blewit	*Tricholomataceae*	≥ 6
-	*Tricholomataceae*	≥ 6
-		
Cloud funnel		
Common puffball	*Agaricaceae*	1 to 5
Fried chicken mushroom	*Lyophyllaceae*	≥ 6
Fried chicken mushroom	*Lyophyllaceae*	≥ 6
-	*Lyophyllaceae*	≥ 6

-	-	≥ 6
-	*Porotheleaceae*	≥ 6
-	*Tricholomataceae*	≥ 6
-	*Tricholomataceae*	≥ 6
Moonlight mushroom	*Omphalotaceae*	≥ 6
Hare's ear	*Pyronemataceae*	≥ 6
-	*Paxillaceae*	1 to 5
-	-	0
-	*Strophariaceae*	≥ 6
-	*Strophariaceae*	≥ 6
Golden scalycap		
-		
-	*Strophariaceae*	≥ 6
-		
-		
-	*Strophariaceae*	≥ 6
-	*Strophariaceae*	≥ 6
Nameko	*Strophariaceae*	≥ 6
-	-	≥ 6
-	*Strophariaceae*	≥ 6
Shaggy scalycap	*Strophariaceae*	≥ 6
Shaggy scalycap	*Strophariaceae*	≥ 6
-	-	≥ 6
Indian oyster	*Pleurotaceae*	≥ 6
-	*Pluteaceae*	≥ 6
Black-footed polypore	*Polyporaceae*	≥ 6
-	-	1 to 5

classified as 6 or greater (≥ 6), less than 6 and more than 1 (1 to 5)

Table S1.

In-house database obtained by MALDI-TOF-MS for wild mushrooms collected in 2014 (3/3)

No.	Morphological estimation	DNA sequencing identification			Frequency of Score ≥ 2.0 [b]
		Scientific name [a]	Common name	Family	
14-109	*Psathyrella candolleana*	*Psathyrella candolleana*	Pale brittlestem	*Coprinaceae*	≥ 6
14-110	*Psathyrella candolleana*	*Psathyrella candolleana*	Pale brittlestem	*Coprinaceae*	≥ 6
14-111	*Psathyrella multissima*	*Psathyrella microrhiza*	Rootlet brittlestem	*Coprinaceae*	1 to 5
14-112	*Pseudoclitocybe cyathiformis*	*Pseudoclitocybe cyathiformis*	Goblet funnel cap	*Typhulaceae*	≥ 6
14-113	*Rhodocollybia butyracea*	*Rhodocollybia butyracea*	Buttery collybia	*Tricholomataceae*	≥ 6
14-114	*Stropharia aeruginosa*	*Russula aeruginosa*	-	*Russulaceae*	≥ 6
14-115	*Russula emetica*	*Russula cavipes*	-	*Russulaceae*	≥ 6
14-116	*Russula foetens*	*Russula foetens*	Stinking brittlegill	*Russulaceae*	0
14-117	*Russula cyanoxantha*	*Russula heterophylla*	Greasy green brittlegill	*Russulaceae*	≥ 6
14-118	*Russula emetica*	*Russula vinacea*	-	*Russulaceae*	0
		R. atropurpurea	Purple brittlegill		
14-119	*Sarcodon leucopus*	*Sarcodon leucopus*	-	*Thelephoraceae*	≥ 6
14-120	*Sarcomyxa serotina*	*Sarcomyxa serotina*	Olive oystering	*Tricholomataceae*	≥ 6
14-121	*Sarcomyxa serotina*	*Sarcomyxa serotina*	Olive oystering	*Tricholomataceae*	≥ 6
14-122	*Stropharia aeruginosa*	*Stropharia aeruginosa*	Verdigris roundhead	*Strophariaceae*	≥ 6
14-123	*Stropharia aeruginosa*	*Stropharia aeruginosa*	Verdigris roundhead	*Strophariaceae*	≥ 6
14-124	*Suillus laricinus*	*Suillus aeruginascens*	-	*Boletaceae*	1 to 5
14-125	*Boletus auripes*	*Suillus grevillei*	Larch bolete	*Boletaceae*	≥ 6
14-126	*Suillus grevillei*	*Suillus grevillei*	Larch bolete	*Boletaceae*	≥ 6
14-127	*Suillus granulatus*	*Suillus luteus*	Slippery jack	*Boletaceae*	≥ 6
14-128	*Suillus luteus*	*Suillus luteus*	Slippery jack	*Boletaceae*	1 to 5
14-129	*Suillus luteus*	*Suillus luteus*	Slippery jack	*Boletaceae*	0
14-130	*Suillus placidus*	*Suillus sibiricus*	Siberian slippery jack	*Boletaceae*	≥ 6
14-131	*Tremella foriacea*	*Tremella foriacea*	Leafy brain	*Tremellaceae*	≥ 6
14-132	*Cantharellus cibarius*	*Tremiscus helvelloides*	Apricot jelly	*Auriculariaceae*	≥ 6
14-133	*Tremiscus helvelloides*	*Tremiscus helvelloides*	Apricot jelly	*Auriculariaceae*	0
14-134	*Tricholoma flavovirens*	*Tricholoma flavovirens*	Yellow knight	*Tricholomataceae*	≥ 6
14-135	*Cortinarius balteatocumatilis*	*Tricholoma pessundatum*	Tacked knight	*Tricholomataceae*	1 to 5
		T. fulvum	Birch knight		
		T. muricatum	-		
14-136	*Tricholoma ustale*	*Tricholoma populinum*	Poplar knight	*Tricholomataceae*	≥ 6
14-137	*Tricholoma ustale*	*Tricholoma populinum*	Poplar knight	*Tricholomataceae*	≥ 6
		T. ustale	Burnt knight		
14-138	*Tricholima sejunctum*	*Tricholoma portentosum*	Charbonnier	*Tricholomataceae*	≥ 6
		T. griseoviolaceum	-		
		T. sejunctum	Yellow blusher		
14-139	*Tricholoma orirubens*	*Tricholoma saponaceum*	Soap-scented toadstool	*Tricholomataceae*	≥ 6
14-140	*Tricholoma saponaceum*	*Tricholoma saponaceum*	Soap-scented toadstool	*Tricholomataceae*	≥ 6
14-141	*Tricholoma virgatum*	*Tricholoma terreum*	Grey knight	*Tricholomataceae*	≥ 6
14-142	*Tricholoma psammopus*	*Tricholoma vaccinum*	Scaly knight	*Tricholomataceae*	≥ 6
		T. imbricatum	Matt knight		
14-143	*Tyromyces chioneus*	*Tyromyces chioneus*	White cheese polypore	*Polyporaceae*	0
14-144	*Tyromyces chioneus*	*Tyromyces chioneus* (96%)	-	-	1 to 5
14-145	*Psilocybe argentipes*	not identified [c]	-	-	1 to 5
14-146	*Gymnopilus liquiritiae*	not identified [c]	-	-	≥ 6
14-147	*Tricholoma album*	not identified [c]	-	-	1 to 5
14-148	*Polyporus badius*	not identified [c]	-	-	≥ 6
14-149	*Tricholomopsis rutilans*	not identified [c]	-	-	0
14-150	*Helvella crispa*	not identified [c]	-	-	1 to 5
14-151	*Sparassis crispa*	not identified [c]	-	-	0
14-152	*Lentinellus cochleatus*	not identified [c]	-	-	0
14-153	*Bondarzewia montana*	not identified [c]	-	-	0
14-154	*Russula chloroides*	not identified [c]	-	-	≥ 6
14-155	*Tricholoma imbricatum*	not identified [c]	-	-	≥ 6
14-156	*Inonotus obliquus*	not identified [c]	-	-	0
14-157	*Leucocortinarius bulbiger*	not identified [c]	-	-	≥ 6

[a] Concordance rate of BLAST is shown in parentheses when less than 97%.

[b] Frequency of matching score 2.0 or greater from 12 spectra measurements was classified as 6 or greater (≥ 6), less than 6 and more than 1 (1 to 5) and none (0).

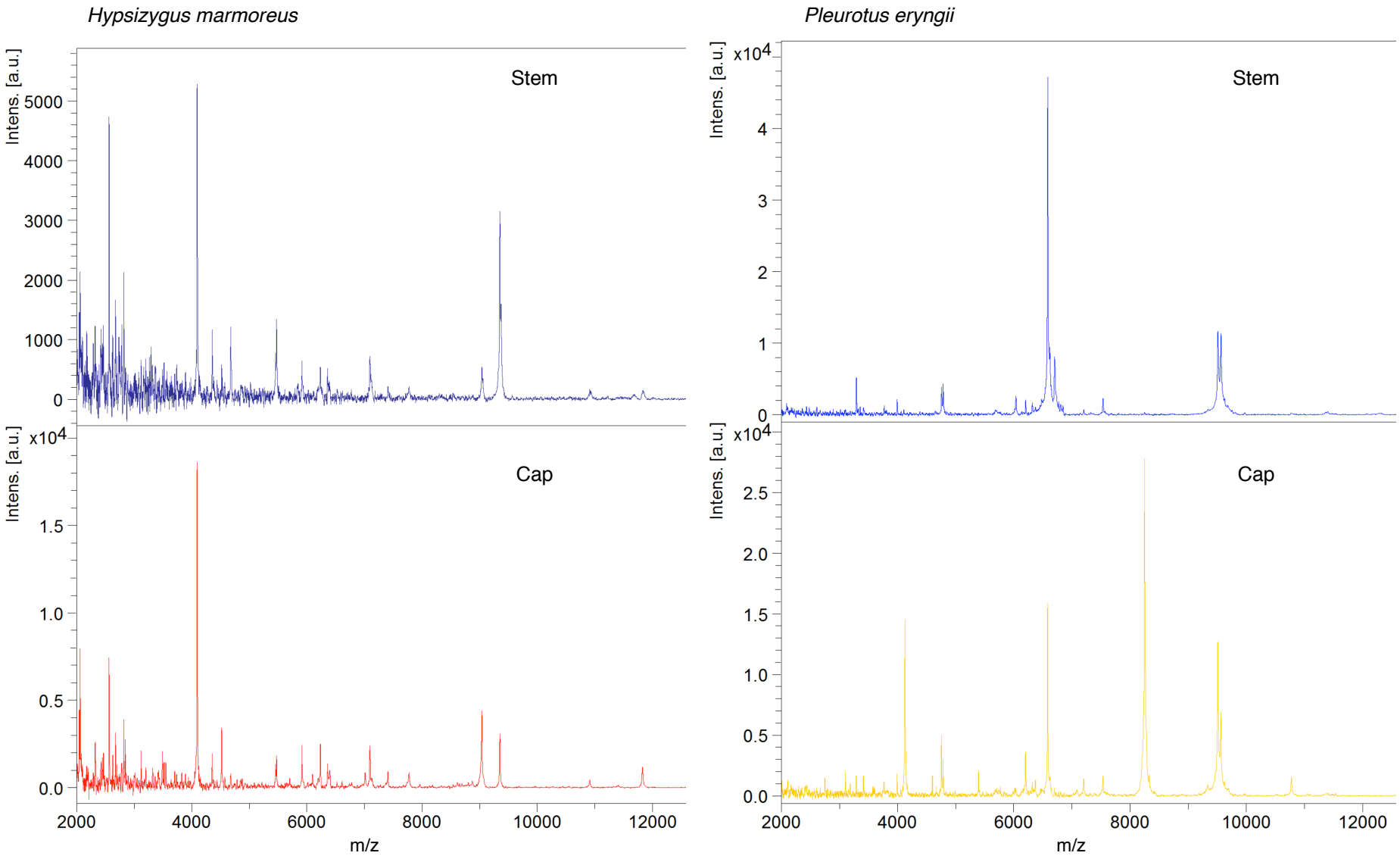
[c] Species were unidentified because of PCR inhibition or mixed signal in sequencing procedure.

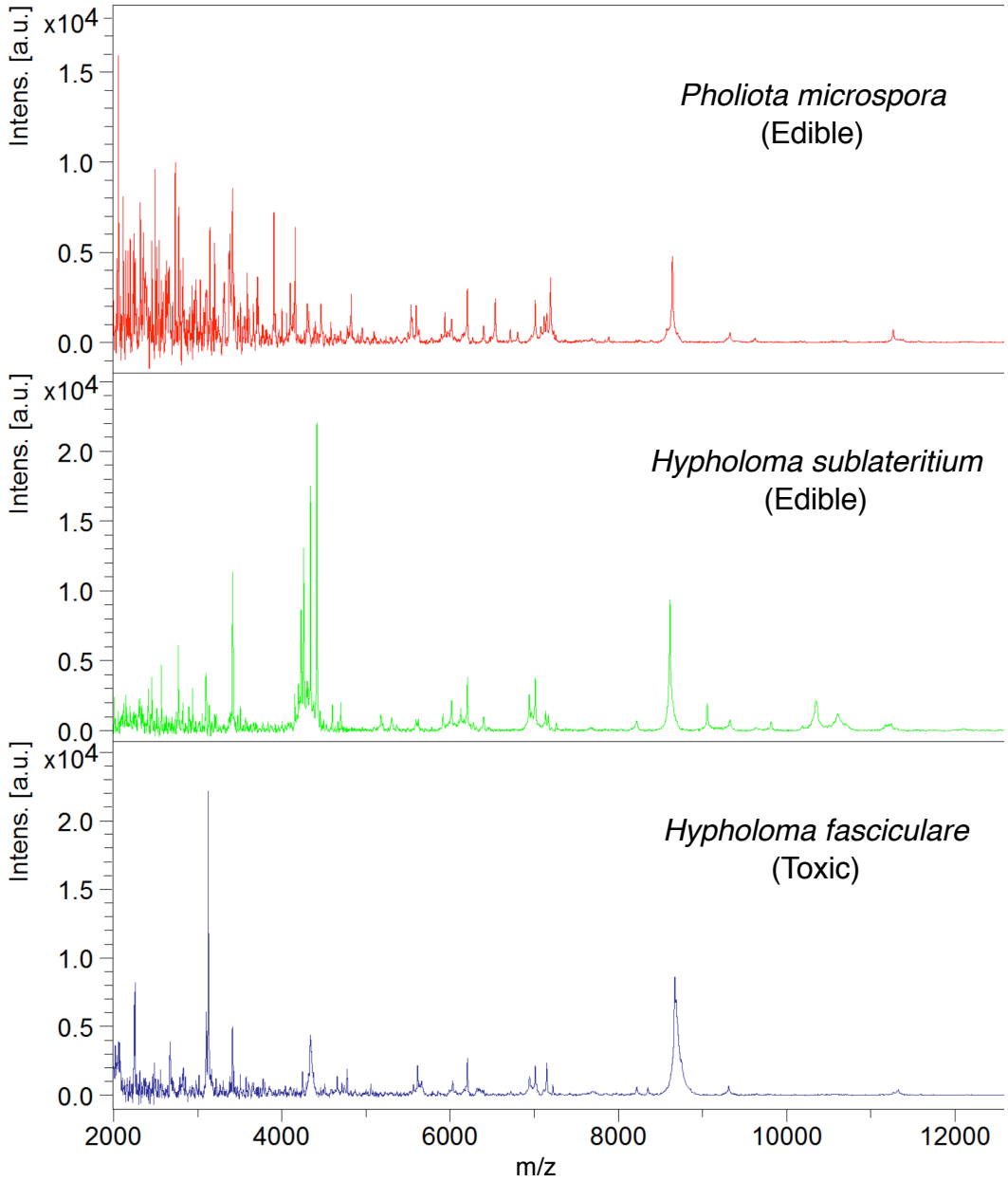
Figure captions

Fig. 1. Comparison of spectra obtained by MALDI-TOF MS for cap- and stem-portions of cultivated mushroom samples.

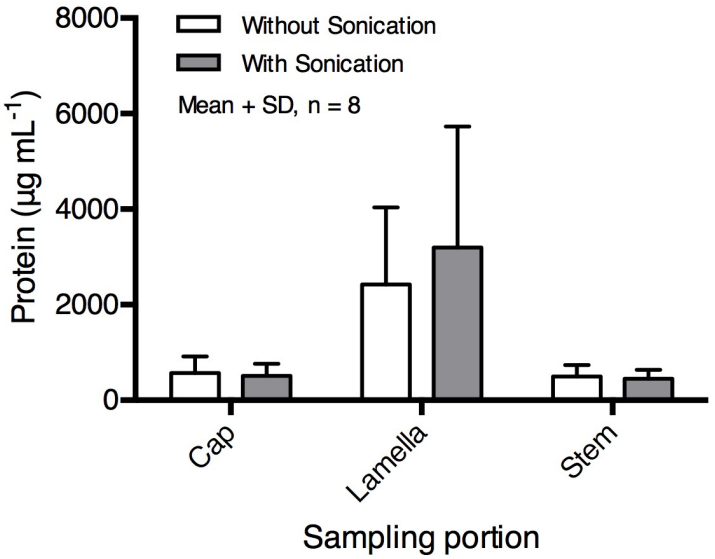
Fig. 2. Discrimination of a toxic mushroom from similar edible mushrooms by MALDI-TOF MS. The matching score was less than 1.69 in each combination.

Fig. 3. Comparison of protein concentration in the extracts of sampling portions of mushroom with or without sonication.


Hypsizygus marmoreus
Pleurotus eryngii
Stem
Cap
Intens. [a.u.]
x10^4
m/z
2000
4000
6000
8000
10000
12000


Intens. [a.u.]
x10^4
1.5
1.0
0.5
0.0
Pholiota microspora
(Edible)
Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
0.0
Hypholoma sublateritium
(Edible)
Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
0.0
Hypholoma fasciculare
(Toxic)
2000
4000
6000
8000
10000
12000
m/z

(Fig.3)


8000
6000
4000
2000
0
Protein (μg mL-1)
Without Sonication
With Sonication
Mean + SD, n = 8
Cap
Lamella
Stem
Sampling portion