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Morphological and chemical analysis of magic mushrooms in Japan

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Abstract

Morphological and toxicological analyses were performed on hallucinogenic mushrooms that are currently circulated in Japan. Scanning electron microscope (SEM) indicated a three-dimensional microstructures in the mushrooms. The complementary use of SEM with an optical microscope was effective for observing characteristic tissues, such as basidiomycetes, spores, cystidia and basidia. Hallucinogenic alkaloids were extracted with methanol and determined by high performance liquid chromatography (HPLC) with a UV detector set at 220 nm. The psilocin/psilocybin contents in *Psilocybe cubensis* were in the range of 0.14–0.42%/0.37–1.30% in the whole mushroom (0.17–0.78%/0.44–1.35% in the cap and 0.09–0.30%/0.05–1.27% in the stem), respectively. The hallucinogenic alkaloids in *Copelandia* were 0.43–0.76%/0.08–0.22% in the whole mushroom (0.64–0.74%/0.02–0.22% in the cap and 0.31–0.78%/0.01–0.39% in the stem). It thus appears that *P. cubensis* is psilocybin-rich, whereas *Copelandia* is psilocin-rich.

Keywords: *Psilocybe*; *Copelandia*; Psilocin; Psilocybin; Scanning electron microscope

1. Introduction

In recent years, the recreational use of the hallucinogenic mushroom, so-called ''magic mushroom'', has become an increasing social problem in several countries [1–3]. They are not only naturally occurring but also offered as a kits for cultivation, and contain the hallucinogenic indole derivatives, psilocin and psilocybin. The cultivation or possession of mushrooms containing psilocin and/or psilocybin have been controlled by the Narcotics and Psychotropics Control Law in Japan since 2002.

Mushroom is a vague and general term used to describe the fruit bodies of fungi, particularly all gill fungi, which have characteristic structures, cystidia and basidia. The observation of spores is essential for species identification, although they are not characteristic only to mushrooms. Such observations of spores, cystidia and basidia on the gills are usually done with an optical microscope. However, optical microscopic observation is not easy in the case of colorless or thick tissues. A scanning electron microscope (SEM) may compensate for the defects associated with an optical microscope because it is possible to observe colorless tissues well and it provides three-dimensional images.

There are several reports on the contents of psilocin and psilocybin in magic mushrooms [2,4–8]. Differences in the psilocin and psilocybin contents of the fruit bodies depends on factors such as species, developmental stages, climatic conditions and the availability of soluble nitrogen and phosphorous in the soil [9,10].

In this study, we report on the morphological characteristics of magic mushrooms that are circulated in Japan, under an optical microscope and a SEM. The contents of psilocin and psilocybin in the samples were determined using high performance liquid chromatography (HPLC).

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2. Materials and methods

2.1. Samples and chemicals

Eight dried magic mushroom samples, which were obtained in Japan, were used in this study; six were *Psilocybe cubensis* (see Fig. 1(A)) and two were *Copelandia* genus (see Fig. 1(B)). Psilocin and psilocybin were supplied by the Ministry of Health, Labor and Welfare of Japan. All other chemicals used in the experiments were of analytical grade.

2.2. Morphological examinations

2.2.1. Optical microscopic examination

A microscopic examination was performed using the method reported by Watling [11] with a minor modification. A 2.5% potassium hydroxide solution was used as a swelling agent and to return the dried tissues to their previous state. Small pieces of the gills were cut from the fruit body and mounted on a glass slide, while directly in the 2.5% potassium hydroxide solution. After covering with a glass coverslip, it was tapped with a rubber-tipped pencil to separate the

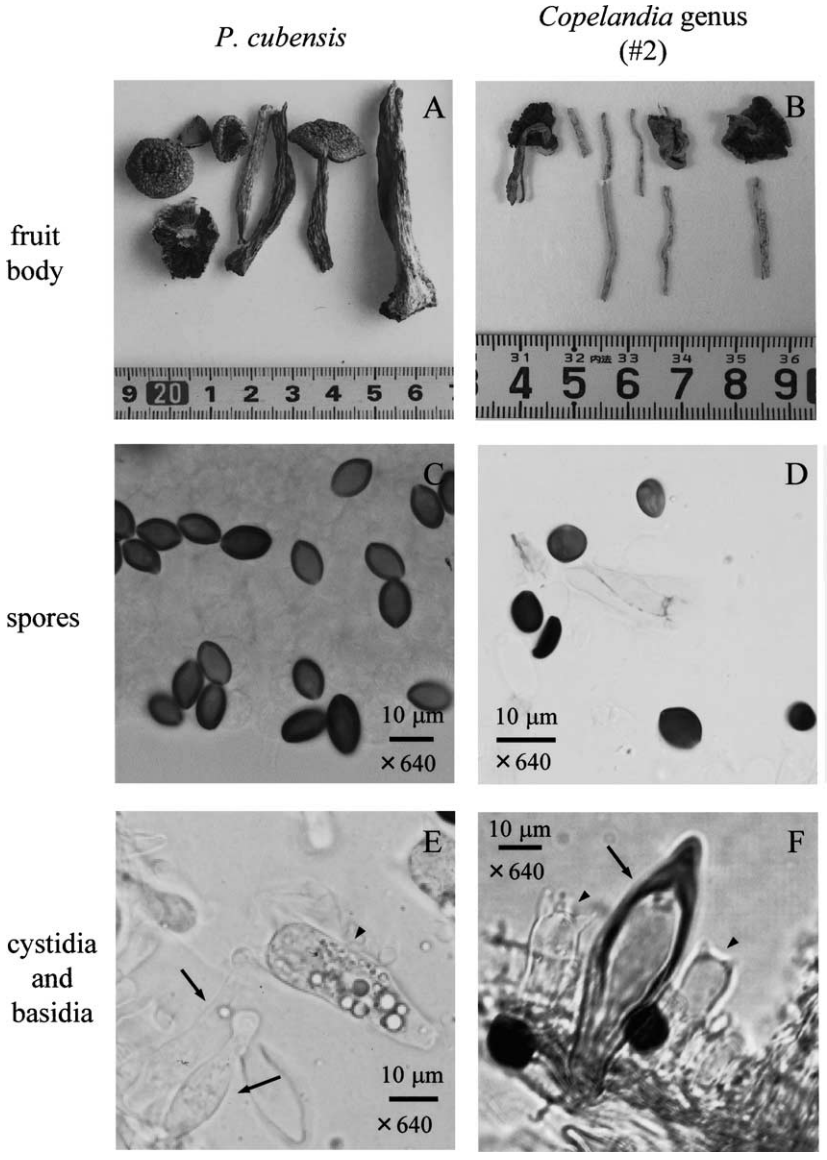

Fig. 1. Morphologic characteristics of *P. cubensis* and *Copelandia* (#2) macroscopically (A and B) and under an optical microscope (C–F). Arrows and arrowheads in E and F indicate cystidia and basidia, respectively.

tissues from each other. Observation was carried out using a biological microscope. The sizes of the spores were measured with Image J (Wayne Rasband, National Institute of Health, USA) and an average of 10 spores was measured for each sample.

2.2.2. Electron microscopic examination

Small pieces of dried gills were covered with a fine layer of gold by sputtering in a vacuum sputterer and observed on a JSM-5800LV SEM (JEOL, Japan) at an accelerating voltage of 15 kV.

2.3. Determination of the alkaloids

2.3.1. Extraction procedure

For the extraction of psilocin and psilocybin, the method described by Musshoff et al. [2] was used with minor modifications as follows. The dried samples of fruit bodies were cut into the sections of caps and stems. Each section was ground to a fine powder in a mortar. Ten milligram of the powdered sample was extracted twice with 1 ml of methanol in an ultrasonic bath for 30 min. After centrifugation at 3000 rpm for 2 min, the supernatant was placed in a separate vial. The supernatant was evaporated to dryness under a stream of nitrogen. A portion of the residue was dissolved in 100 μl of the mobile phase containing 25 μg/ml 4-hydroxyindole as an internal standard (IS), and a 10 μl aliquot was used for the HPLC analysis.

2.3.2. Apparatus and chromatographic conditions

The HPLC system consisted of a Shimadzu LC-10A Series liquid chromatograph equipped with an SPD-M10A diode array detector set at 220 nm. Chromatographic separation was performed with a Symmetry C18 column (2.1 mm × 150 mm, 5 μm, Waters Assoc., Milford, USA). The mobile phase was 10 mM ammonium formate buffer (pH 3.5) -acetonitrile (95:5, v/v) and pumped at a flow-rate of 0.2 ml/min.

3. Result

3.1. Morphological examination

The morphologic features of the two types of hallucinogenic mushroom are summarized in Table 1. Due to apparent macroscopic differences, two *Copelandia* samples are described individually.

Optical microscopic examination revealed the spores, cystidia and basidia on the gills, as shown in Fig. 1(C–F). *P. cubensis* had dark brown spores, hyaline cystidia, and hyaline 4-spored basidia. The *Copelandia* genus had dark

Table 1
Morphologic characteristics of hallucinogenic mushrooms used in this study

Species	Description
P. cubensis	Macroscopic feature Cap: 0.3–2.2 cm broad, conic-campanulate shape, whitish to yellow color, smooth surface Stem: 2.6–11.5 cm × 3–14 mm, whitish to yellow color Gill: nearly black color
	Microscopic feature Spores: 10.2–16.5 μm × 5.9–10.0 μm, subellipsoid, dark brown color, a distinct apical germ pore, smooth surface Cystidia: subcapitate, hyaline Basidia: hyaline, 4-spored
Copelandia #1	Macroscopic feature Cap: 0.1–0.3 cm broad, conic shape, dark brown to black color, smooth surface Stem: 0.4–1.4 cm × 0.5–1 mm, whitish to yellow color Gill: nearly black color
	Microscopic feature Spores: 15.1–19.3 μm × 12.2–14.7 μm, ellipsoid, dark brown color, a distinct apical germ pore, smooth surface Cystidia: fusiform, yellow color, thick wall Basidia: hyaline, 4-spored
Copelandia #2	Macroscopic feature Cap: 0.3–1.2 cm broad, conic shape, brown to yellow color, smooth surface Stem: 1.3–2.2 cm × 4–5 mm, whitish gray color Gill: nearly black color
	Microscopic feature Spores: 8.8–12.0 μm × 8.5–10.0 μm, ellipsoid, dark brown color, a distinct apical germ pore, smooth surface Cystidia: fusiform, yellow color, thick wall Basidia: hyaline, 4-spored

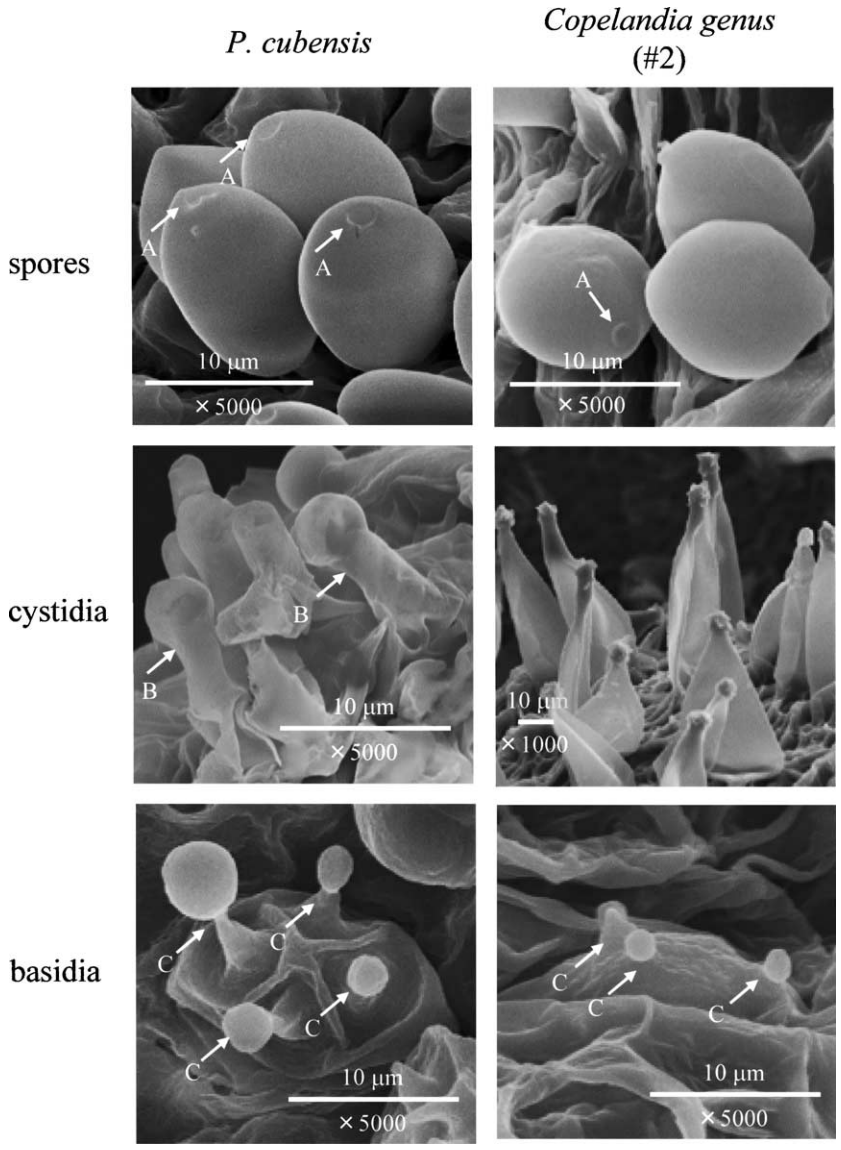

Fig. 2. Morphologic characteristics of *P. cubensis* and *Copelandia* (#2) observed by SEM. Arrows indicate germ pores (A) on the spores, cystidia (B), and sterigmata (C) on the basidia.

brown spores, yellow thick-walled cystidia, and hyaline 4-spored basidia. Spore sizes were different between the two *Copelandia* samples. Colored tissue such as spores of *P. cubensis* or spores and cystidia of the *Copelandia* genus were easily observed. However, hyaline tissues such as the cystidia and the basidia of *P. cubensis* and basidia of Copelandia could not be observed clearly under the optical microscope. The characteristic structure of 4-sterigmata on the basidia could not be observed at the same time.

In contrast to the optical microscope, a SEM revealed images of the tissues clearly and stereoscopically, as shown in Fig. 2. Hyaline tissues could be observed clearly regardless of their colors. Three-dimensional details in the structures, such as a smooth surface and the germ pores of the spores, could be observed clearly. Differing from an optical microscope, the 4-spored structure of basidia was observed clearly. Meanwhile, cystidia of *P. cubensis* were crushed under vacuum and the shapes were different from those observed with the optical microscope.

3.2. Determination of the alkaloid

Fig. 3 illustrates a typical chromatogram obtained from the extract of a *P. cubensis*. Peaks corresponding to psilocybin, psilocin and 4-hydroxyindole (IS) were well separated from each other without any interfering peaks.

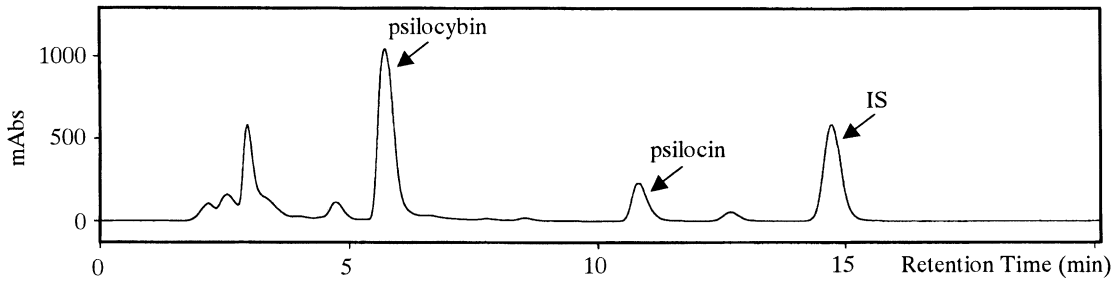

Fig. 3. Chromatogram of the extract of *P. cubensis* (detection: 220 nm).

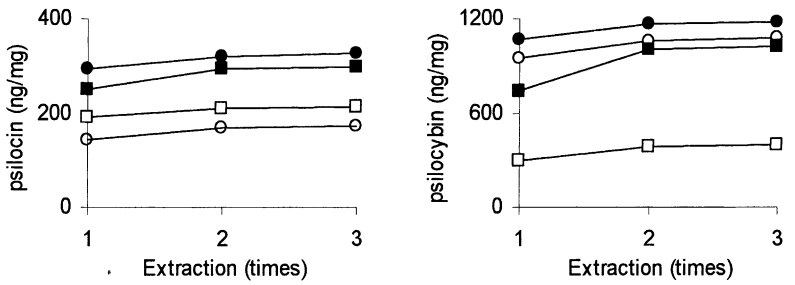

Fig. 4. Recovery of psilocin and psilocybin in *P. cubensis* after repeated extraction. (●) Caps of sample 1, (○) caps of sample 2, (■) stems of sample 1, (□) stems of sample 2.

The calibration curves were linear in the range 10 ng–1 μg psilocin and psilocybin on column with correlation coefficients of 0.999 and 0.994, respectively.

In the early stage of the experiment, the hallucinogenic alkaloids in magic mushrooms were extracted three times with methanol to investigate the efficiency of the extraction. As shown in Fig. 4, nearly all alkaloids were recovered from the mushrooms by two extractions with methanol. Hereafter, a two-time extraction was used for the quantitative analysis of the hallucinogenic alkaloids.

Table 2
Psilocin and psilocybin contents of hallucinogenic mushrooms

Sample	Psilocin (%)			Psilocybin (%)		
	Cap	Stem	Whole body	Cap	Stem	Whole body
Psilocybe cubensis						
#1	0.17	0.30	0.22	1.17	1.01	1.10
#2	0.32	0.21	0.25	1.05	0.58	0.75
#3	0.78	0.18	0.42	0.76	0.64	0.69
#4	0.26	0.09	0.18	0.44	0.29	0.37
#5	0.21	0.09	0.15	0.85	0.05	0.47
#6	0.19	0.11	0.14	1.35	1.27	1.30
Copelandia genus						
#1	0.64	0.31	0.43	0.22	0.01	0.08
#2	0.74	0.78	0.76	0.02	0.39	0.22

Table 2 summarizes the contents of the hallucinogenic alkaloids in magic mushrooms. The total contents of alkaloids (psilocin and psilocybin) ranged from 0.51 to 1.44% per dry mass of the whole mushrooms.

4. Discussion

Most magic mushrooms circulated in Japan appear to be *P. cubensis* and/or *P. subcubensis* and *Copelandia* genus. Although it has been confirmed that *P. cubensis* grows wild in certain regions of Japan, illicitly sold mushrooms are thought to be imported in the form of dried mushrooms, spores or kits consisting of mycelium on a growing medium.

P. subcubensis was classified as a different species from *P. cubensis* by Guzmán [12]. These two species are macroscopically and even microscopically very similar, with a slight difference in spore sizes. Since the diagnosis of species of dried hallucinogenic mushrooms is extremely difficult, we describe them as *P. cubensis* without discrimination between the two species.

Similarly, we describe our two samples of *Copelandia* genus as simply *Copelandia* without description of the species. The *Copelandia* species grows in tropical regions such as Thailand [13] and Hawaii [14], and most species have not been intensively investigated. However, the two *Copelandia* samples used in this study may be different species because their appearance and scale of spores were apparently different.

An optical microscope is generally used for the observation of magic mushrooms. After swelling, it gives living-like colored images, which are important in species identification. However, it is difficult to observe hyaline tissues such as cystidia and basidia of *P. cubensis* and basidia of *Copelandia,* and three-dimensional structures such as smooth faces and the germ pores of the spores.

An SEM gives three-dimensional images stereoscopically regardless of the clarity of the tissues. Meanwhile, SEM can not be used to identify the color and considerable amount of tissue must be crushed under high vacuum. Thus, the complementary use of SEM with an optical microscope is effective for the observation of magic mushrooms.

The extraction of psilocin and psilocybin in magic mushrooms has been reported previously. Gartz [15] used aqueous alcohol and dilute acetic acid as extraction solvents, and Lee [16] employed butyl chloride. However, an aqueous solution of alcohol and dilute acetic acid resulted in the dephosphorylation of psilocybin, and butyl chloride did not completely extract psilocybin. In the present study, two extractions with methanol permitted both psilocin and psilocybin to be recovered quantitatively.

The psilocin/psilocybin contents in *P. cubensis* were in the range of 0.17–0.78%/0.44–1.35% in the cap and 0.09–0.30%/0.05–1.27% in the stem. Hallucinogenic alkaloids (psilocin and psilocybin) have a tendency to be contained in the cap more than the stem. Our results are in agreement with the findings of Wurst et al. [8] who found lower concentrations of psilocybin in the stem than in the cap of the hallucinogenic fungus, *Psilocybe bohemica*. *P. cubensis* samples also had a tendency to contain larger amounts of psilocybin than psilocin. This tendency is consistent with results reported by Bigwood and Beug [4]. On the other hand, the contents of hallucinogenic alkaloid (psilocin/psilocybin) in *Copelandia* were 0.43–0.76%/0.08–0.22% in the whole body. *Copelandia* samples contained larger amounts of psilocin than psilocybin, although only two samples were analyzed.

5. Conclusion

The purpose of this study was to conduct a morphological and toxicological examination of magic mushrooms that are circulated in Japan. The complementary use of an optical microscope in conjunction with SEM was effective for these the examinations. The contents of hallucinogenic alkaloids in magic mushrooms were quantitatively determined by HPLC after extraction with methanol.

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