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Short Communication

Analysis and Cultivation of Fruit Bodies and Mycelia of *Psilocybe bohemica*

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Key Term Index: analysis, cultivation, fruit bodies, mycelia, indole alkaloids; *Psilocybe bohemica*

Summary

The analysis of fruit bodies of *Psilocybe bohemica* from a single location revealed psilocybin, baeocystin and in some cases psilocin. Psilocybin levels varied from 0.11 % up to 1.34 % by dry weight. The content of baeocystin and psilocybin was highest in the caps of the mushrooms. Psilocybin was also found to be contained in the cultured mycelia of this species. No other alkaloids were detected in the mycelial extracts.

A rhizomorphic to closely linear growth of the blueing mycelia was observed on soaked unsterilized cardboard.

For the first time, the fruiting of *Psilocybe bohemica* could be demonstrated.

When undertaking quantitative analysis of psilocybin, baeocystin and psilocin levels in species of *Psilocybe* and in *Inocybe aeruginascens* Babos, we generally found variations from one collection to another even within one species and in cultures (GARTZ 1987a, b; SEMERDŽIEVA et al. 1986).

We report here on the observed variation of the alkaloid levels in natural grown fruit bodies of *Psilocybe bohemica* SEBEK (KRIEGLSTEINER 1984) from a single location.

The cultivation of mycelia and fruit bodies of this species is also described.

Mushroom samples

The mushrooms were harvested for analysis from a location near Sazava (Czechoslovakia) in November 1986 where the species was already found in 1942 (KRIEGLSTEINER 1984).

23 fruit bodies were air dried at 25 °C, sealed in plastic and stored at −10 °C until analysis.

Analysis

The extraction procedure and the analysis of the indole alkaloids by using HPLC and TLC were described in previous papers (GARTZ 1987a, b; SEMERDŽIEVA et al. 1986).

Cultivation

Mycelium obtained from the spores of a mushroom (REPKE et al. 1977) was kept as a stock culture on 6 % malt agar (GARTZ 1987a). A rice grain/water mixture (1 : 2) ws used to get fruiting of the *Psilocybe* species at 23 °C (GARTZ 1987a).

We also tested the influence of low temperature (4 °C) on the fructification of the mycelia. Cultivation of the mycelium was even carried out on soaked unsterilized cardboard (STAMETS and CHILTON 1983).

Table 1. *Amount of indole alkaloids in Psilocybe bohemica.*
Analysis of methanolic extracts of dried fruit bodies (Fb) by using HPLC (standard deviation: ± 3,2 %, six analysis of each sample)

Sample	dry weight of the mushrooms (mg)	part of the mushrooms	psilocybin (% dry weigt)	psilocin	baeocystin
1	25	Fb	0.96	0.02	0.03
2	31	Fb	1.34	–	0.01
3	80	Fb	0.29	–	0.008
4	86	Fb	1.12	–	0.02
5	90	Fb	0.94	0.01	0.01
6	101	Fb	0.22	0.01	0.02
7	102	Fb	0.55	0.01	0.02
8	103	Fb	0.50	0.01	0.03
9	104	Fb	0.41	–	0.02
10	105	Fb	0.37	–	0.01
11	110	Fb	0.56	0.01	0.02
12	120	Fb	0.11	–	0.02
13	147	Fb	0.49	–	0.01
14	170	Fb	0.34	–	0.01
15	170	Fb	0.27	0.01	0.01
16	175	Fb	0.90	–	0.01
17	180	Fb	0.63	0.01	0.02
18	200	Fb	0.71	0.01	0.02
19	200	cap	0.39	0.02	0.02
		stem	0.14	–	0.02
20	220	Fb	0.37	–	0.01
21	222	cap	0.31	–	0.03
		stem	0.20	–	0.01
22	240	cap	1.02	–	0.03
		stem	0.50	–	0.01
23	385	cap	0.56	–	0.02
		stem	0.48	–	0.01

– psilocin was undetectable

We found variations in the levels of the alkaloids psilocybin, baeocystin and psilocin from one sample to another (Table 1). The observation of variable amounts was consistent with our findings with *Psilocybe cubensis* (Earle) Singer (GARTZ 1987a) and *Inocybe aeruginascens* (GARTZ 1987b).

It was found that the caps generally contained more psilocybin and baeocystin than the stems. In contrast to *Inocybe aeruginascens* (GARTZ 1987b), *Psilocybe cubensis* also accumulates higher concentrations of psilocybin and psilocin in the caps as compared to the other parts of the mushrooms (GARTZ 1987a). The amounts of psilocybin were in same order of magnitude as in other earlier analyzed fruit bodies of *Psilocybe bohemica* from various origin (SEMERDŽIEVA et al. 1986; STIJVE and KUYPER 1985; WURST et al. 1984).

Our investigation showed the occurrence of very low levels of the incompletely methylated psilocybin (baeocystin) in every fruit body. *Inocybe aeruginiascens* contained much higher

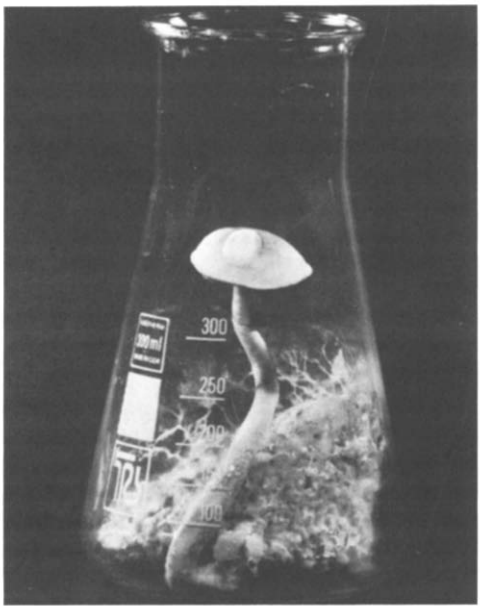

Fig. 1. *Fruit body of Psilocybe bohemica.* Fruiting after cultivation of the mycelia (12 weeks) on rice grain/water mixture (1:2).

amounts of this compound (up to 0.53 %) as the *Psilocybe* species (GARTZ 1987b). We also detected very low amounts of psilocin in only 10 of the 23 analyzed mushrooms (Table 1).

In contrast to *Psilocybe cubensis* (GARTZ 1987a), no relation between mass of the mushrooms and the alkaloid levels was detected in fruit bodies of *Psilocybe bohemica* (Table 1).

The amount of psilocybin ranged from 0.15 % to 0.21 % by dry weight in 6 different mycelia grown on 6 % malt agar over 4 weeks and was lower than in the natural grown fruit bodies. We detected no other indole derivatives in the mycelial extracts. The mycelia blues consistently after bruising and spontaneously in the age like the natural mushrooms and as other psilocybian species (BEUG and BIGWOOD 1982; KRIEGLSTEINER 1984, 1986; STAMETS 1982).

Fruiting of the mycelia on rice grain/water mixture occurred without casing 12 weeks after inoculation (Fig. 1), but only if a temperature of 4 °C was maintained for 3 days at the end of cultivation. This observation is in agreement with the occurrence of the natural grown fruit bodies in the late autumn and early winter (KRIEGLSTEINER 1984). No primordia could be observed in 20 mycelial cultures on rice grain/water mixture without cool conditions.

Wild mushrooms of *Psilocybe bohemica* differing from the cultivated mushrooms mainly by the absence of the 2 rings and the less robust habit have other features very similar, the microscopic and the blueing in particular.

The species required diffuse day light for pinhead initiation. Growth of the vegetative mycelia was even observed on malt agar at 4 °C.

Psilocybe bohemica also colonized soaked cardboard with a rhizomorphic to closely linear white mycelium (Fig. 2) as *Psilocybe cyanescens* Wakefield from the U.S.A. (STAMETS and CHILTON 1983).

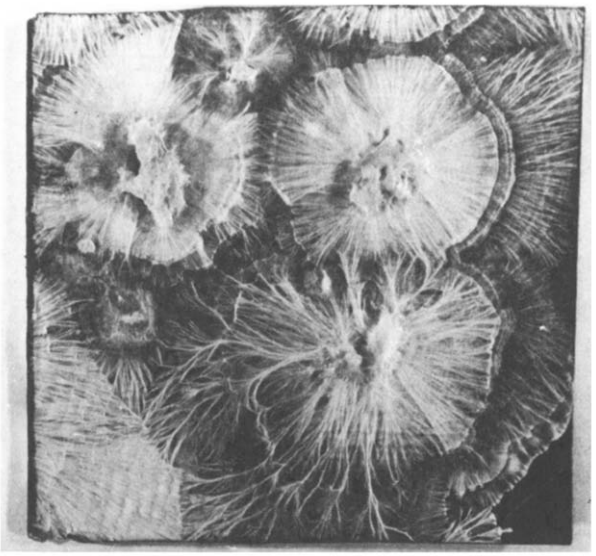

Fig. 2. *Mycelial culture of Psilocybe bohemica.* Growth on soaked cardboard (30 d).

KRIEGLSTEINER (1984) had been classified *Psilocybe bohemica* as *Psilocybe cyanescens.* But some doubt still exists about the taxonomic conformity of different collections. For example, we have reported about a collection of *Psilocybe cyanescens* with a high content of baeocystin from the G.D.R. (MÜLLER and GARTZ 1986). Fruit bodies of this species from the U.S.A. generally contained high amounts of psilocin (up to 1 % dry weight) and very low levels of baeocystin (BEUG and BIGWOOD 1982; KRIEGLSTEINER 1986; STIJVE and KUYPER 1985).

These various collections need to be further investigated.

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