Biotransformation of Tryptamine in Fruiting Mycelia of *Psilocybe cubensis*

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Abstract

Mycelial cultures of *Psilocybe cubensis*, with the ability to form psilocybin and psilocin *de-novo*, also hydroxylated and methylated fed tryptamine to give psilocin in up to 3.3% dry mass of the obtained fruit bodies. By using HPLC and TLC, it was found that these mushrooms contain only a small amount of psilocybin (0.01–0.2% dry mass). The values of psilocin are the highest described in any mushrooms.

Introduction

Psilocybe cubensis (Earle) Sing. is a subtropical mushroom and contains the indole alkaloid psilocybin and only small amounts of its dephosphorylated counterpart psilocin (1–4). Variations in these metabolites have been well demonstrated by investigations of fruit bodies cultivated under controlled conditions on a rye-grain medium (2) and rice substratum (3), respectively.

The study of psilocybin biosynthesis in submerged culture of *P. cubensis* showed that radioactive tryptamine functioned as a better precursor than tryptophan (5–7). It was found that not less than 22.4% of the psilocybin formed was derived from the labelled precursor tryptamine (5). The level of psilocin was generally zero in the mycelial tissue from these experiments (5–7).

In the present paper, the biotransformation of fed tryptamine in fruiting mycelia of *P. cubensis* is described.

Materials and Methods

Cultivation of Psilocybe cubensis

A dried cow dung/rice-grain mixture (2:1) with twice the amount of water was used to obtain fast fructifications without casing of a strain (3) of *P. cubensis*. A 25 mM concentration of tryptamine (as hydrochloride) was added to this medium. Cultivations without the addition of any tryptamine were also tested. The methods of cultivations were described in (3).

The first sporocarps were produced by cultures of *P. cubensis* in 3 to 4 weeks. The cultures continued to produce mushrooms in five flushes. Each flush was harvested as soon as the sporocarps were mature. The mushrooms were immediately freeze-dried, sealed in plastic, and stored at −10 °C until analysis.

Extraction and analysis

The extraction procedure and the analysis of the indole alkaloids by using HPLC and TLC were described in the previous papers (3, 8–10). The presence or absence of tryptamine was demonstrated by TLC as described by Stijve et al. (11).

Results and Discussion

The cow dung-rice mixture actually produced the first flush of mushrooms earlier than the cultivations on rye (with casing) (2) and rice (3), respectively. They yielded an average of 3 g dry mass per 10 g substratum.

Under the same culture conditions, the fructification times, the yields, and sizes of mushrooms as well as the blueing feature (3) were equal when the growth media also contained high concentrations of tryptamine. Initial experiments without the addition of tryptamine were performed to determine the content of psilocybin and psilocin in comparison with experiments using other culture conditions and/or media (2, 3).

The levels of psilocybin and psilocin varied from one flush to the next, but generally were much the same as those in the other experiments (2, 3) (Table 1). Consistently low levels of psilocin were found in the mushrooms without the addition of tryptamine to the substratum. Additionally, psilocin generally was absent in the first flush as was also observed in earlier investigations (2, 3). Table 1 shows that the fed tryptamine gives high values of psilocin in each flush from the cultures.

Table 1 Variation of psilocybin and psilocin levels in *P. cubensis* as a function of flush number from the cultivations with (a) and without (b) addition of tryptamine (25 mM concentration).

Flush No.	Psilocin (% dry mass)		Psilocybin (% dry mass)	
	a	b	a	b
1	2.1	–	0.01	0.55
2	3.3	0.01	0.02	0.48
3	2.8	0.02	0.20	0.51
4	3.1	0.09	0.07	0.46
5	2.9	0.15	0.13	0.61

These psilocin levels are uncommonly high (from 2.1 to 3.3%) since values reported for psilocin in dried mushrooms are always below 1% (1–4, 12, 13).

Inocybe aeruginascens Babos contains only traces of psilocin but high amounts of the incompletely methylated psilocybin (baeocystin) (9). In contrast to the initial experiments without an addition of tryptamine, the mushrooms generally contained only small amounts of psilocybin. The tryptamine level was always zero in each mushroom. In this case no tryptamine was additionally found in the methanolic extract of the vegetative mycelia from the substratum.

In a previous report, Gartz (3) was unable to detect baeocystin in *P. cubensis*. But Repke et al. (14) reported traces of baeocystin in other strains of *P. cubensis* about 10 years ago. They suggested that many non-specific enzyme systems exist in fungi which have the ability to oxidize exogenously added compounds, as well as normal, obligatory intermediates (14).

The results in Table 1 show that the enzyme systems in *P. cubensis* have a high hydroxylation and methylation capacity to convert added tryptamine to psilocin. It is possible that a reduced amount of phosphate in the culture media decreased the biosynthesis of psilocybin from psilocin in the mycelia.

P. cubensis also failed to produce detectable amounts of baeocystin under these culture conditions.

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