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VARIATION OF PSILOCYBIN AND PSILOCIN LEVELS WITH REPEATED FLUSHES (HARVESTS) OF MATURE SPOROCARPS OF *PSILOCYBE CUBENSIS* (EARLE) SINGER

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Summary

Analysis of *Psilocybe cubensis* (Earle) Singer grown in controlled culture showed that the level of psilocin was generally zero in the first (or sometimes even the second) fruiting of the mushroom from a given culture and that the level reached a maximum by the fourth flush. The level of psilocybin, which was nearly always at least twice the level of psilocin, showed no upward or downward trend as fruiting progressed, but was variable over a factor of four. Samples obtained from outside sources had psilocybin levels varying by over a factor of ten from one collection to the next.

Introduction

When undertaking quantitative analysis of psilocybin and psilocin levels in Pacific Northwest species, we generally found large variations from one collection to another even within one species and even when all collections were made over time from a single location (Beug and Bigwood, 1982). In investigating biosynthetic pathways in the formation of psilocin and psilocybin in *Psilocybe cubensis* (Earle) Singer, we also observed variations in psilocybin and psilocin levels from one fruiting to the next (Chilton *et al.*, 1979). We therefore set out to grow a selected Amazonian strain of *Psilocybe cubensis* (Earle) Singer in carefully controlled cultures and study the variation of psilocybin and psilocin levels with time. We also analyzed psilocybin and psilocin levels in other strains of *Psilocybe cubensis*. We report here on the observed variation of psilocybin and psilocin levels with repeated flushes from a single culture and the variation observed in other strains.

Experimental

The strain of *Psilocybe cubensis* cultivated in this study originated from a spore print taken in the Amazon basin area near Pucalpa, Peru (Repke *et*

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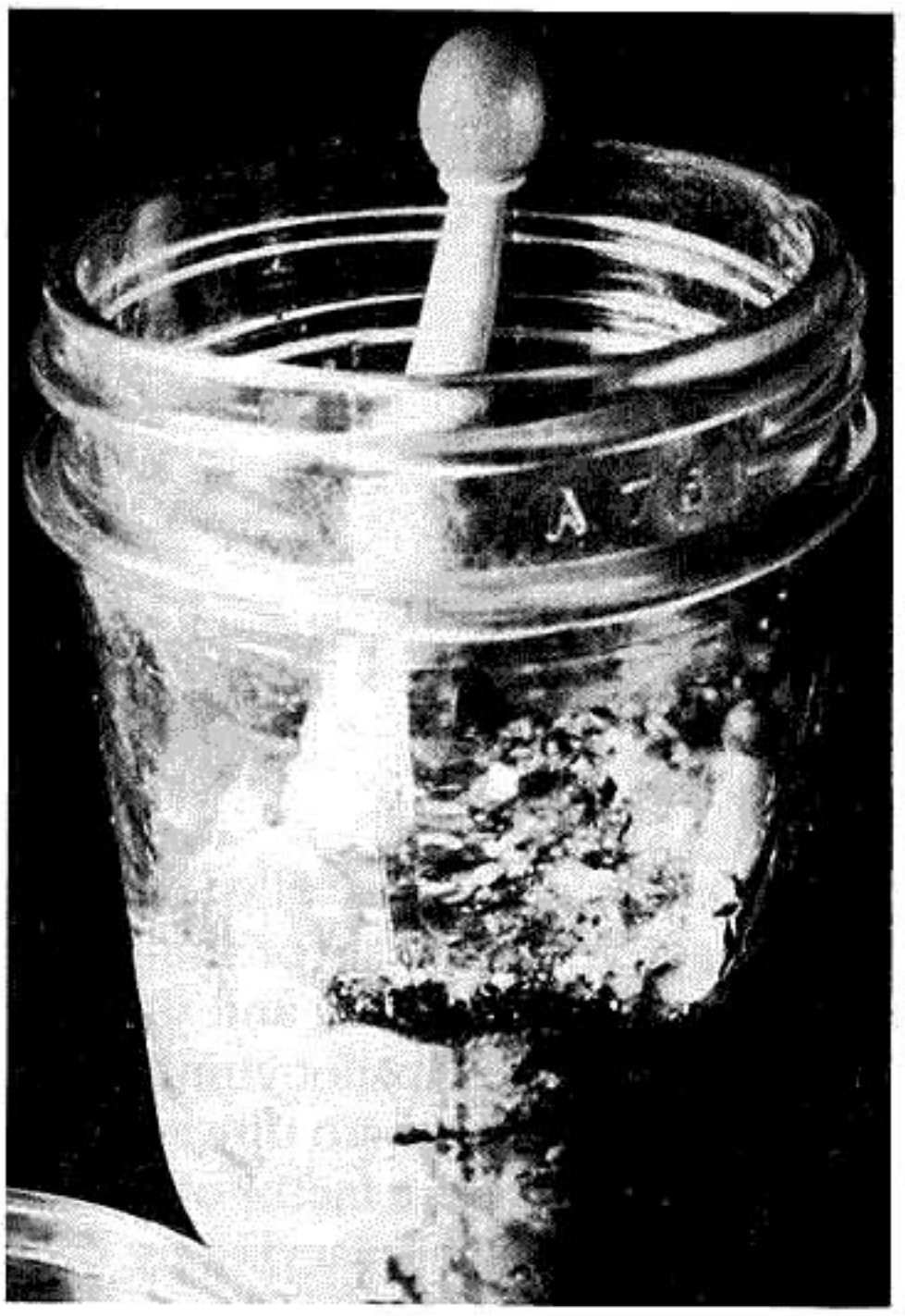

Fig. 1. *Psilocybe cubensis* (Earle) Singer. Immature sporocarp fruiting from "miniculture" (described in text). Photo: Bigwood, Washington, U.S.A.

Fig. 2. *Psilocybe cubensis* (Earle) Singer. Carpophores from compost substrate are more robust in appearance than those cultivated on rye. Photo: Bigwood, Washington, U.S.A.

al., 1977). Mycelium obtained from the spore print was kept as a stock culture on various agars. Since only one flush (fruiting) could be obtained from agar plates, we used a rye-grain medium described initially by San Antonio (1971), refined by Oss and Oeric (1976), and adapted to "miniculture" by us (Fig. 1). A wide-mouth half-pint jar (~250 ml) was charged with 10 g of rye grain and 15 ml of water and autoclaved. It was then inoculated under sterile conditions with a mycelium culture on agar. Every four days for a period of 28 days, the jars were shaken to distribute the growing mycelium evenly on the grain. In 28 days, the mycelium had covered the grain and the jars were then opened and the grain was cased (covered with a layer about 2 cm deep) with 2 parts peat : 1 part calcium carbonate : 2 parts perlite and/or vermiculite (Fig. 1). The mushrooms were "watered" once every two days with 1 ml of sterile water via syringe. The first flush (fruiting) occurred four to five weeks after inoculation (about two weeks after casing). The minicultures continued to produce mushrooms for at least 20 weeks provided they remained uncontaminated. They yielded an average of 2.7 g dry weight per miniculture. Each flush was harvested as soon as the sporocarps were mature. The mushrooms were immediately freeze-dried, sealed in plastic and stored at −5 °C until analysis. Voucher specimens were prepared for deposit in the University of Washington Herbarium (WTU).

The extraction procedure and analysis was described in the previous paper. The reversed-phase high performance liquid chromatograms were quantified with a Hewlett-Packard 3380A reporting integrator–plotter and calibrated against standards from the National Institute on Drug Abuse. We found a linear relationship (±10% repeatability) between concentration and peak area from 0.2 to 3 μg total psilocybin or psilocin. The detection limit was about 0.01 μg psilocybin or psilocin. The HPLC results were qualitatively confirmed by TLC using butanol–acetic acid–water (12:3:5).

Results

We found that the levels of psilocybin varied somewhat unpredictably from one flush to the next, but generally were much the same on the last flush as they were on the first flush (Table 1). Psilocin, on the other hand, generally was absent in the first one or two flushes, reached maximum by the fourth flush, and then appeared to start to decline (Table 1). Unfortunately, we could generally not follow the decline appreciably since five flushes is normally the maximum we can get before the mycelium stops fruiting. (With miniculture 1, we obtained a sixth flush but the fifth flush was totally consumed in another experiment and so is not reported here.)

In two other strains grown by other sources, we also observed nearly complete absence of psilocin in the first flush (Fig. 2). In these, we analyzed the caps and stems separately and found that the caps generally contained twice as much psilocybin as the stems, but that the small amount of psilocin present was entirely in the stems (Table 2). In contrast, our Amazon strain had a trace of psilocin in the cap but not in the stem. The cap and stem contained equal amounts of psilocybin.

Finally, we analyzed five street samples of *Psilocybe cubensis* (Fig. 3) for which we did not know the flush number or the precise growing conditions (Table 3). We found highly variable levels of psilocybin and consistently low levels of psilocin.

TABLE 1

The dry weight variation of psilocybin and psilocin levels in *Psilocybe cubensis* as a function of flush number (quantified by HPLC)

Flush No.	Miniculture No. 1		Miniculture No. 2		Miniculture No. 3	
	Psilocybin (mg/g)	Psilocin (mg/g)	Psilocybin (mg/g)	Psilocin (mg/g)	Psilocybin (mg/g)	Psilocin (mg/g)
1	8.3	0.5	5.1	0	7.6	0
2	6.5	1.5	7.3	0	6.2	0
3	13.3	1.0	4.7	1.7	5.3	0.9
4	4.8	2.6	3.7	2.9	3.2	1.8
5	—	—	5.2	2.2	6.7	1.7
6	6.8	0.5	—	—	—	—

TABLE 2

Distribution of psilocybin and psilocin in the cap versus the stem in three strains of *Psilocybe cubensis* cultivated on rye-grain substrate

	M.R. strain 1st flush		Equadorian strain 1st flush		Amazon strain 1st flush	
	Psilocybin (mg/g)	Psilocin (mg/g)	Psilocybin (mg/g)	Psilocin (mg/g)	Psilocybin (mg/g)	Psilocin (mg/g)
Caps	9.7	0	7.6	0	5.7	0.1
Stems	4.2	0.35	4.7	0.4	5.7	0

Fig. 3. *Psilocybe cubensis* (Earle) Singer. Cultivated air-dried rye-grown mushrooms packaged by the ounce in sealed plastic bags — kept frozen until use. Photo: Bigwood, Washington, U.S.A.

TABLE 3

Psilocybin and psilocin levels in dried *Psilocybe cubensis* "street samples" (all samples were from material cultivated on a rye-grain substrate)

Sample No.	Psilocybin (mg/g)	Psilocin (mg/g)
1	5.6	0
2	6.2	0
3	0.7	0.3
4	0.7	0.3
5	1.3	0.3

Conclusions

We found that the level of psilocybin and psilocin varies by over a factor of four among various cultures of *Psilocybe cubensis* grown under rigidly controlled conditions, while specimens from outside sources varied tenfold. It is clear that entheogenic (Ruck *et al.*, 1979) and recreational users of this species have no way of predicting the amount of psilocybin and psilocin they are ingesting with a given dry weight of the mushroom. It thus seems likely that variations in the subjective experience will not only come from the effects of set and setting but will also stem in very real measure from large dosage differences.

References

Beug, M. W. and Bigwood, J., Psilocybin and psilocin levels in twenty species from seven genera of wild mushrooms in the Pacific Northwest, U.S.A. *Journal of Ethnopharmacology, 5* (1982) 271 - 285.

Chilton, W. S., Bigwood, J. and Jensen, R. E., Psilocin, bufotenine and serotonin: Historical and biosynthetic observations. *Journal of Psychedelic Drugs, 11* (1979) 61 - 69.

Oss, O. T. and Oeric, O. N., *Psilocybin Magic Mushroom Grower's Guide,* And/Or Press, Berkeley, California, 1976.

Repke, D. B., Leslie, D. T. and Guzmán, G., Baeocystin in *Psilocybe, Conocybe* and *Panaeolus. Lloydia, 40* (1977) 566 - 578.

Ruck, C. A. P., Bigwood, J., Staples, D., Ott, J. and Wasson, R. G., Entheogens. *Journal of Psychedelic Drugs, 11* (1979) 145 - 147.

San Antonio, J. P., A laboratory method to obtain fruit from cased grain spawn of the cultivated mushroom: *Agaricus bisporus. Mycologia, 63* (1971) 16 - 21.