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CULTIVATION AND ANALYSIS OF *PSILOCYBE* SPECIES AND AN INVESTIGATION OF *GALERINA STEGLICHII*

Abstract - JOCHEN GARTZ - Cultivation and analysis of *Psilocybe* species and an investigation of *Galerina steglichii*.

Cultivation and formation of sclerotia of *Psilocybe mexicana* could be demonstrated on various grain substrates.

Analysis of sclerotia from wet rice grain revealed the presence of psilocybin, in most cases psilocin and always low concentrations of baeocystin.

Psilocybin, psilocin and baeocystin levels varied in the blueing fruit bodies of the new species *Psilocybe natalensis* from South Africa.

The highest concentrations of these alkaloids were found in naturally grown and cultivated fruit bodies of *Psilocybe azurescens* which is an indigenous species of the Pacific Northwest, U.S.A.

The relative alkaloidal content of psilocybin, psilocin and baeocystin found in *Galerina steglichii* from Germany was similar to that measured in *Psilocybe natalensis*.

Psilocybin was also found in the cultured blueing mycelium of these species.

Key words: Cultivation, Analysis, *Psilocybe mexicana*, *Psilocybe natalensis*, *Psilocybe azurescens*, *Galerina steglichii*.

Riassunto - JOCHEN GARTZ - Coltivazione e analisi di specie di *Psilocybe* e un'investigazione su *Galerina steglichii*.

La coltivazione e la formazione di sclerosi di *Psilocybe mexicana* è stata dimostrata su vari substrati.

L'analisi di sclerosi provenienti da coltivazione su riso umido ha rivelato la presenza di psilocibina, psilocina (nella maggior parte dei campioni) e sempre basse concentrazioni di baeocistina.

I livelli di psilocibina, psilocina e baeocistina si sono dimostrati variabili nei corpi fruttiferi bluescenti della nuova specie sud africana *Psilocybe natalensis*.

Le concentrazioni più alte di questi alcaloidi sono state trovate in carpofori coltivati o sviluppati spontaneamente di *Psilocybe azurescens*, specie indigena del Nordest Pacifico (U.S.A.).

Il contenuto relativo di psilocibina, psilocina e baeocistina rinvenuto in *Galerina steglichii* proveniente dalla Germania si è rivelato simile a quello misurato in *Psilocybe natalensis*. La psilocibina è stata rinvenuta anche nel micelio bluescente di questa specie in cultura.

Parole chiave: Coltivazione, Analisi, *Psilocybe mexicana*, *Psilocybe natalensis*, *Psilocybe azurescens*, *Galerina steglichii*.

Recent ethnomycological and chemical investigations confirm earlier results that psychoactive species of various genera are growing wild in Europe, North- and South America, Australia and Asia (GARTZ, 1991, 1993, 1995; GUZMAN, 1983).

We described some ethnobotanical facts and cultivation results of a new fungus from Thailand, *Psilocybe samuiensis* GUZMAN, BANDALA & ALLEN in comparison with *Psilocybe tampanensis* GUZMAN & POLLOCK from Florida and *Psilocybe semilanceata* (Fr.) KUMM. from Europe (GARTZ *et al.*, 1994).

In January 1994 we also found the first indigenous blueing *Psilocybe* species from South Africa on a field trip in the province Natal. This overall whitish and large species without a ring or even a velum grows on cow pastures and is named *Psilocybe natalensis* nom. prov. here. (GARTZ *et al.*, 1995).

Since 1979 an interesting and large *Psilocybe* species was collected along the Columbia river network on the coastal regions of the Pacific Northwest, U.S.A. These mushrooms turn also bluish after bruising and live in soils enriched with deciduous wood-debris. Until now we describe these fungi as *Psilocybe azurescens* nom. prov. (STAMETS & GARTZ, 1995).

In continuation of these studies, in this paper some analysis and cultivation experiments of the species from America and South Africa including of some results of the «classic» fungus *Psilocybe mexicana* HEIM and a new *Galerina* (BESL, 1993) are described.

EXPERIMENTAL

The strain of *Psilocybe mexicana* was obtained from the «underground mushroom movement» of the U.S.A. A successful fruiting experiment on rye grass seed (*Lolium* sp.) water mixture by using a casing layer (POLLOCK, 1977; STAMETS & CHILTON, 1983) and the subsequent microscopic examination of the few fruit bodies (GUZMAN, 1983) confirmed the identity of the species. Mycelium was kept as a stock culture on 4% malt agar.

Cotton - plugged 500 ml Erlenmeyer flasks were filled with 100 g grain and 180 ml water, sterilized by autoclaving, cooled, inoculated from stock cultures and incubated at 23° C in darkness to promote the formation of sclerotia. Rye grain, soft rice grain and rye grass seed were used, respectively. Fruit bodies of *Psilocybe natalensis* (leg. O'Neill's cottage, Natal-South Africa, Jan. 22, 1994) were dried at 20-40°C.

Possible present residual water was removed from the mushrooms by freeze-drying. Mycelium obtained from spore prints (STAMETS & CHILTON, 1983) was also kept as a stock culture on 4% malt agar. The mycelium from a cultivation on 100 ml agar was analyzed after 4 weeks of cultivation.

Mycelium from *Psilocybe azurescens* (STAMETS & GARTZ, 1995) on 4% malt

agar was used to inoculate a rye grain/water mixture identically to the cultivation of *Psilocybe mexicana*. After 3 weeks cultivation 500 g of sawdust soaked with water in plastic bag was inoculated with the mycelia on grain (STAMETS & CHILTON, 1983). The duration of the spawn run was 4 weeks.

In March commercial garden mulch in a shady outdoor bed was spawned with the mycelia on sawdust. A weekly watering to keep the moisture content high. In September of the same year about 200 mushrooms appeared. Some were dried for analysis in the same way as *Psilocybe natalensis*.

Dried fruit bodies of *Galerina steglichii* from (BESL, 1993) were also analyzed. It was also possible to isolate a strain from spores on 6% malt agar.

The extraction procedures of mushrooms and mycelia as well as the analysis by using HPLC and TLC are described elsewhere (GARTZ, 1989, 1991b).

RESULTS

It was found *Psilocybe mexicana* soon formed yellowish to brownish sclerotia on rye grass seed/water. STAMETS & CHILTON (1983) reported similar growth

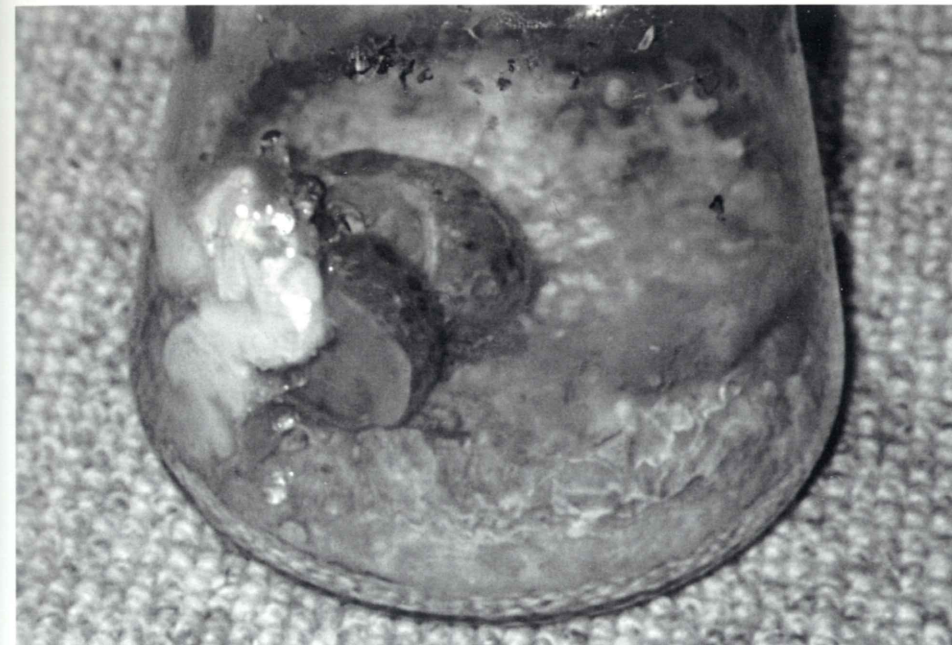


Fig. 1 - Sclerotia from *Psilocybe mexicana* Heim on rice grain 40 days after inoculation.

patterns of sclerotia after 3 weeks on the same substrate. Observations on the formation of sclerotia in the highly similar species *Psilocybe tampanensis* after a cultivation of 4-8 weeks were reported by STAMETS & CHILTON (1983) and by GARTZ *et al.* (1994). In contrast, *Psilocybe natalensis* and *Psilocybe azurescens* under any cultivation only formed whitish mycelium throughout various substrates but no sclerotia.

The dry weights of the slightly blueing sclerotia of *Psilocybe mexicana* varied significantly during cultivation on rye grass seed/water. It seems that the tendency to lose its moisture is the reason for such a variation.

In addition, the cultivation on soft rice grain/water also soon formed sclerotia after only 2 weeks (Fig. 1). It was found that total darkness is not necessary for the formation of sclerotia on this substrate. Incubation of *Psilocybe mexicana* in diffuse daylight but without direct sunlight also promote this vegetative form.

Sclerotia formation on rice grain (100 g)/water after 2 months is 15-20 grams dry weight. The alkaloidal levels obtained from these sclerotia were relatively high but varied even in the same batch (Table 1). Larger sclerotia contained more psilocin than smaller agglomerations. It seems that during the growing process of the sclerotia a significant enzymatic decomposition of psilocybin to psilocin (BOCKS, 1968) occurred (Table 1). In own investigations the same reaction was found in old fruit bodies of *Psilocybe cubensis* (EARLE) SING. (GARTZ, 1989). Only a few sclerotia were obtained on rye grain/water mixture after prolonged incubation (up to 12 weeks). It seems that the substrate based on soft rice grain was the best to promote a regular formation of sclerotia in *Psilocybe mexicana*.

INDOLE ALKALOIDS IN SCLEROTIA OF *Psilocybe mexicana* FROM A SINGLE RICE GRAIN MEDIUM 60 DAYS AFTER INOCULATION (% dry weight)

Table 1

Sample	Dry weight (g)	Psilocybin	Baeocystin	Psilocin
1	0.341	0.65	0.02	-
2	0.712	0.42	0.01	0.21
3	0.210	0.36	0.02	0.23
4	1.542	0.33	0.01	0.33
5	2.678	0.28	0.02	0.30
6	3.524	0.25	0.01	0.31
7	3.921	0.18	0.02	0.39

For the first time, BRODIE (1935) observed a formation of sclerotia in a psilocybian mushroom which is now known as *Panaeolus subbalteatus* (BERK. & Tz, BR.) SACC. (GARTZ, 1993).

Psilocybin and its precursor baeocystin as well as psilocin were found in every fruit body of *Psilocybe natalensis* (Table 2). The highest concentrations were found in the smallest basidiocarps, as early was the case in *Psilocybe cubensis* (GARTZ, 1989) and other species (GARTZ, 1995).

In *Psilocybe natalensis* psilocybin was found to be accompanied by only slight amounts of baeocystin. Other species like *Inocybe aeruginascens* BABOS (GARTZ, 1993) or *Psilocybe semilanceata* (GARTZ, 1991, a, b) contain much higher concentrations of this substance.

The alkaloidal concentrations in *Psilocybe natalensis* were very similar to the amounts in the subtropical and somewhat similar (Fig. 2 and GARTZ, 1989) *Psilocybe cubensis* (GARTZ, 1991b) and in *Psilocybe samuiensis* from Thailand (GARTZ *et al.*, 1994)



Fig. 2 - *Psilocybe natalensis* from a cow pasture in Natal (South Africa).

CONTENT OF INDOLE DERIVATIVES IN DRIED MUSHROOMS OF
Psilocybe natalensis (%)

Table 2

Sample	Dry weight (g)	Psilocybin	Baeocystin	Psilocin
1	0.058	0.60	0.04	0.21
2	0.102	0.52	0.03	0.20
3	0.152	0.46	0.02	0.18
4	0.268	0.38	0.02	0.20
5	0.251	0.39	0.03	0.18
6	0.348	0.29	0.01	0.17
7	0.392	0.25	0.01	0.15
8	0.421	0.18	0.01	0.10

Only psilocybin was found to be present in the cultured, even blueing mycelium grown on malt agar. Amounts of psilocybin, ranging from 0.13 to 0.28% dry weight, were analyzed in 4 different batches grown over a 4 week period.



Fig. 3 - *Psilocybe azurescens* on mulch in germany.

Psilocybe azurescens (Fig. 3) is one of the most potent hallucinogenic mushrooms ever found. Very high concentrations of psilocybin were detected in all extracts of this species (Table 3). Such substantial amounts of baeocystin have also been found in collections of *Inocybe aeruginascens* BABOS and *Psilocybe semilanceata* from Europe (GARTZ, 1991a, b; 1993).

INDOLE ALKALOIDS IN *Psilocybe azurescens* FROM THE PACIFIC NORTHWEST
(SAMPLE 1, 2) AND GERMANY (Dry weight %)

Table 3

Sample	Psilocybin	Baeocystin	Psilocin
1	1.62	0.34	0.24
2	1.54	0.28	0.21
3	1.42	0.37	0.32
4	1.35	0.32	0.28
5	1.29	0.25	0.28
6	1.56	0.34	0.27
7	1.39	0.25	0.01
8	1.33	0.18	0.29
9	1.55	0.34	0.50

Relative high levels of psilocin were even detected in every fruit of *Psilocybe azurescens* (Table 3). The mushrooms are characterized by a typical blue colouration of the stipe caused by handling of the mushrooms. Very old fruit bodies can possess blue flecks on the pileus and a bluish stipe too.

The alkaloidal levels of the 2 analyzed fresh mushrooms from the Pacific Northwest were in the same order of magnitude as that found earlier in dried mushrooms from the same area (STAMETS & GARTZ, 1995) and recently in «naturally» cultivated fruit bodies from Germany (Table 3). We also detected psilocybin and in some cases baeocystin and psilocin in the mycelium from agar depending on the concentration of malt extract (STAMETS & GARTZ, 1995). *Psilocybe azurescens* is a lignicolous species utilizing a number of wood types and is able to grow on a wide variety of wastes including garden mulch, newspaper and cardboard. As a primary composer like *Psilocybe bohémica* SEBEK (GARTZ, 1993) *Psilocybe azurescens* is more robust and contains higher amounts of indole derivatives than this Czech species. Additionally, *Psilocybe azurescens* shows a more aggressive growth of the mycelia in various substrates and a higher fruiting potential than *Psilocybe bohémica* and even its relative *Psilocybe cyanescens* WAKEFIELD (STAMETS & CHILTON, 1983; STAMETS, 1993).

Recently, the qualitative detection of psilocybin, psilocin and baeocystin in extracts of *Galerina steglichii* BESL has been described (BESL, 1993). This detection was the first evidence of the occurrence of the alkaloids in the genus *Galerina*. These tiny blueing mushrooms were described as a new species from the botanical garden of Regensburg, Germany (BESL, 1993). In early 1993 they grew on an acidic substrate in a warm house there. The origin of this species is unclear.

My own analysis revealed alkaloidal concentrations very similar to *Psilocybe natalensis* (Table 4). In the mushroom extracts psilocybin and psilocin were found to be accompanied by only slight amounts of baeocystin. There was also a variation of the content of these indole derivatives in single mushrooms from the same location.

CONCENTRATION OF ALKALOIDS IN DRIED FRUIT BODIES
OF *Galerina steglichii* (%)

Table 4

Sample	Psilocybin	Baeocystin	Psilocin
1	0.34	0.03	0.12
2	0.21	0.02	0.21
3	0.48	0.05	0.12
4	0.51	0.04	0.08
5	0.29	0.03	0.09
6	0.33	0.05	0.18
7	0.38	0.07	0.17
8	0.50	0.06	0.10

In contrast to earlier experiments (BESL, 1993) the own isolated mycelia grew at a fast pace on 6% malt agar. Psilocybin was also found to be present in the blueing mycelium of *Galerina steglichii* grown on agar over a 4 week period. Amounts of this alkaloids, ranging from 0.05% to 0.14% dry weight, were analyzed in 5 different batches of mycelium.

Interestingly, no other indole derivatives were detected in the extracts of the in vitro grown mycelium.

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