Ethnomycology, biochemistry, and cultivation of *Psilocybe samuiensis* Guzmán, Bandala and Allen, a new psychoactive fungus from Koh Samui, Thailand

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Abstract

Several specimens of *Psilocybe* and *Copelandia* species in Koh Samui, Thailand were recently collected for herbarium deposit and scientific study. This paper presents an ethnomycological and biochemical study of one of the species; *P. samuiensis* Guzmán, Bandala and Allen, a new psychoactive gill fungus reported from Thailand. Mycelium for the cultivation of *P. samuiensis* was obtained on 6% malt agar from the spores of a dried specimen. The growth of *P. samuiensis* was similar to that of *P. tumanensis* Guzmán and Pollock, but more rapid than the mycelium of *P. semilanceata* (Fr.: Sacc.) Kumm. Laboratory analyses indicates that the alkaloid content in cultured fruit bodies of *P. samuiensis* is of the same order of magnitude as that found in naturally occurring mushrooms of this species. HPLC analyses of both naturally occurring and in vitro cultivated fruit bodies of *P. samuiensis* revealed high concentrations of psilocybin and psilocin. Small amounts of baeocystin were also detected. Psilocybin levels varied from 0.23% up to 0.90%. The psilocybin content was highest in the caps. Psilocybin was also found in the cultured non-bluing mycelia of *P. semilanceata* and varied from 0.24% to 0.32% dry weight. The relative alkaloidal content of psilocybin, psilocin, and baeocystin found in *P. samuiensis* was similar to that measured in many other psychoactive fungi species, but completely different from that found in *P. semilanceata*.

Keywords: *Psilocybe samuiensis*; Psilocybin; Psilocin; Cultivation; Koh Samui; *Psilocybe* spp.; Psychoactive fungi

1. Introduction

Recent ethnomycological investigations on Koh Samui Island in Thailand (Allen, 1991; Allen and Merlin, 1992a, b; Guzmán et al., 1993) confirm reports that certain species of psychoactive fungi are ingested for recreational purposes by foreign tourists and some indigenous people (Allen et al., 1992).

Koh Samui (280 km\(^2\) located at latitude 10° N, longitude 100° E), is a small tropical island in the Gulf of Siam where psychoactive fungi are
harvested by local farmers and their children. The psychoactive dung fungi (Psilocybe cubensis (Earle) Singer and/or Psilocybe subcubensis Guzmán) are known locally as 'hed keequai' (literally, 'mushrooms which appear after water buffalo defecates'). These mushrooms are sold by some farmers or their families directly to tourists and resort restaurants. At some resort restaurants the fungi are offered in a variety of meals (Allen and Merlin, 1992a).

Fungi specimens collected on Koh Samui Island (1991) for herbarium deposit and scientific study include the following species: P. cubensis, P. subcubensis, Copelandia cyanescens (Berkeley et Broome) Singer, and a previously unreported bluing Psilocybe species, P. samuiensis Guzmán, Bandala and Allen, sp. nov. (Fig. 1).

Chemical investigations of the psychoactive properties of some of these fungi include an unpublished study by Stijve (Nestec Laboratories, Vevey, Switzerland) who analysed Koh Samui collections of P. cubensis and P. samuiensis, as well as a Swiss collection of P. semilanceata (Fr.: Sacc.) Kumm. Tryptamine indoles were found in all three species. A second study by the authors was later conducted utilizing naturally occurring material and material grown only in vitro. Results of the HPLC and TLC analysis of both studies of P. samuiensis revealed high concentrations of tryptamine indole alkaloids. Furthermore, we were successful in isolating a pure strain of P. samuiensis on malt agar and grass seed.

2. Description of the cap, gills and stipe of P. samuiensis Guzmán, Bandala and Allen, sp., nov.

2.1. Cap

The cap is 7–15 mm in diameter, subconvex to conic-convex, conic umbonate or campanulate-umbonate, frequently with a small papilla. It is viscid with a separable pellicle, even and striate to sulcate at the margin, and hygrophanous. It is chestnut or reddish-brown to straw-color, becoming pale straw-color or brownish clay when dry.

2.2. Gills

The gills are adnate to adnexed, clay color, becoming violaceous brown or chocolate brown-violet when dry, with white edges.

2.3. Stipe

The stipe is 40–65 × 1.52 mm, equal or slightly subbulbous. It is hollow, white or whitish to pale straw color and covered with white fibrils. It is context concolorous with pileus, bluing with slightly farinaceous taste and odour.

2.4. Habitat

P. samuiensis was first collected in soil of mixed sand and clay, among fan palms in rice paddies situated 2 km west of the village of Ban Hua Thanon, Koh Samui, Thailand. Unlike Copelandia, and some species of Psilocybe which are coprophilous, P. samuiensis does not fruit directly in manure but appears scattered or gregarious in the manured soil of rice paddies. This fungus fruits from early July through late August.
3. Methodology

While foraging for psychoactive fungi on Koh Samui island in August of 1991, John W. Allen, accompanied by several Samui children, harvested *P. samuiensis* for herbarium deposit and scientific examination (see Guzmán et al., 1993). *P. samuiensis* first attracted the attention of the collector because of its macroscopic similarity to *P. semilanceata* (the 'liberty cap' mushroom). Both species can be found in similar environments (i.e., pasture lands, rice paddies, etc.), occurring in the manured soil of ruminants. Carpophores of *P. samuiensis*, *P. cubensis* (and/or *P. subcubensis* which is macroscopically indistinguishable from *P. subcubensis*), and *C. cyanescens* were photographed in situ, all growing in a single rice paddy.

Psychoactive fungi species described in this study were harvested from manured soil or decomposed manure of Asian water buffalo (*Bubalus bubalis*), and cattle (*Bos indicus* and *Bos suis*). All of the above mentioned fungi were collected (2-8 August 1991) in rice paddies at four different locations near the villages of Ban Saket, Ban Hua Thanon, Bo Phut and Ban Lipa Yai on Koh Samui Island.

Several collections of these fungi (including *P. samuiensis*, labeled as collection F), were sun dried and forwarded to Dr Gastón Guzmán of the Instituto de Ecología, in Xalapa, Mexico for botanical identification and to Dr T. Stijve of Nestec Ltd., Vevey, Switzerland for chemical analyses to determine the presence or absence of toxic and/or psychoactive alkaloids.

4. Experimental

Mycelium was obtained from the spores of a dried specimen of *P. samuiensis* by methods described by Stamets and Chilton (1983). It was then stored as stock culture on 6% malt agar. Strains of a related species *P. tampanensis* Guzmán and Pollock and *P. semilanceata* from Germany were also obtained on agar. In a ratio of 1-6% on malt agar, the whitish mycelium of *P. samuiensis* grew at a faster pace than that of similar mycelium of *P. semilanceata*. The rapid growth of *P. tampanensis* was similar to the growth of *P. samuien-

sais; however, the former species soon formed brownish sclerotia on the agar. Stamets and Chilton (1983) reported similar growth patterns of sclerotia on the agar of a related species *P. mex-icana* Heim. Even after a relatively long growth period (3 months), the mycelium of *P. samuiensis* formed only a few small brownish sclerotia (in the agar only, not in the culture).

Similar growth patterns were also observed while cultivating the three species on *Lolium* seed/water (1:1.5), and in complete darkness. Observations on the rapid formations of sclerotia in *P. tampanensis* after a few weeks of cultivation was first reported by Stamets and Chilton (1983). In contrast, *P. samuiensis* under cultivation only formed thick whitish mycelium throughout the media (rhizomorphs, diameter 2–3 mm), and produced no sclerotia. Under the same conditions of cultivation, *P. semilanceata* grew slowly, producing only a fine and whitish mycelium with no formation of sclerotia or rhizomorphs.

Psilocybin was found to be present in the cultured, non-bluing mycelium of *P. samuiensis* grown on 6% malt agar. Amounts of psilocybin, ranging from 0.24% to 0.32% dry weight, were analysed in 5 different batches of mycelium grown over a 4-week period. Analyses also revealed that these quantities of psilocybin were much lower than those detected in the naturally occurring fruit bodies obtained from the field. Interestingly, no other indole derivatives were detected in the extracts of the in vitro grown mycelium.

The alkaloidal levels obtained from the slightly bluing sclerotia of *P. tampanensis* were relatively high when compared with the sclerotia of other psychoactive fungi species. In addition, the amount of psilocybin obtained from five different cultivated samples of *P. tampanensis* grown on 6% malt agar and *Lolium* seed ranged from 0.34% to 0.68% by dry weight, and from 0.41% to 0.61% in three sections of sclerotia obtained from a single cultivation on *Lolium* seed. The sclerotia of *P. tampanensis* obtained from malt agar contained 0.21–0.52% psilocin, but no baeocystin was detected. The sclerotia obtained from *Lolium* had a concentration of psilocin of 0.11–0.32%. Until now, no cultivation of complete fruit bodies of *P. samuiensis* on either malt agar or *Lolium* seed has
been reported. In the experiments undertaken for this study, some small incomplete fruit bodies of *P. samuiensis* (up to 2 cm high) did appear, but failed to develop into normal sporulating mushrooms. These premature formations only occurred on agar with a low concentration of malt (0.5–1.5%). After their natural growth stopped, these incomplete fruit bodies began to exhibit a slight spontaneous bluing reaction.

Attempts to cultivate fruiting bodies on a *Lolium* seed/water mixture (Stamets and Chilton, 1983) were not successful. However, *P. samuiensis* does grow well on grains such as rye or rice. A mixture of rye/horse dung/water (2:1:2) did produce fruit bodies of *P. samuiensis* after 4 months of cultivation, and 3 weeks after casing with peat/chalk (2:1) (Stamets and Chilton, 1983, see Fig. 2). Two flushings that produced a total of eight mushrooms were observed; six of the mushrooms were analysed (see Fig. 2 and Table 1).

### 5. Results

Two separate chemical studies were undertaken to determine the tryptamine alkaloid content of *P. samuiensis*. The first involved naturally occurring field specimens; the second analysed material cultivated in the laboratory.

In the first study, 15 specimens of naturally occurring fruit bodies of *P. samuiensis* were analysed by HPLC and TLC techniques (Gartz, 1987). High amounts of psilocybin were detected (0.23–0.90% dry wt.); and a few specimens contained similar amounts of psilocin (0.05–0.81% dry wt.). Baeocystin, a precursor to psilocybin, was also detected (0.01–0.5% dry wt.) in all naturally occurring specimens of *P. semilanceata*, both in naturally occurring field specimens from various origin (Gartz, 1993) and in vitro cultivated fruit bodies (Gartz, 1991a, b).

In contrast to cultivated *P. cubensis* (Gartz, 1987), where the accumulation of psilocin is often higher in the stems than in the caps, analyses of *P. samuiensis* revealed that the caps contained more psilocybin than the stems. Identical concentrations of the alkaloids (psilocybin, psilocin, and baeocystin) were found in cultivated fruit bodies.

<table>
<thead>
<tr>
<th>Fruit body</th>
<th>Psilocybin</th>
<th>Psilocin</th>
<th>Baeocystin</th>
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<tr>
<td>1</td>
<td>0.58</td>
<td>0.34</td>
<td>0.02</td>
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<tr>
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<td>0.21</td>
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of \textit{P. samuiensis} and \textit{P. semilanceata} grown in rye/horse dung (Gartz, 1991a, b). Stijve also found similar concentrations of psilocin and psilocybin in 5 naturally occurring fruit bodies of \textit{P. samuiensis} (collection F, 8 August, 1991, psilocybin, 0.14%; psilocin, 50%; baeocystin, <0.01%).

Fig. 3 reveals the qualitative results of Stijve's analyses of \textit{P. samuiensis} (collection F, 8 August 1991), comparative study of two Thai collections of \textit{P. cubensis} (collections C and G, 3 August 1991 and 8 August 1991), and analysis of a Swiss collection of \textit{P. semilanceata}. Analyses were performed by thin layer chromatography (TLC) on cellulose 10 x 20 cm Nano plates, butanol/acetic acid/water (60:15:25 v/v); pDMCA reagent.

Fig. 4 reveals the qualitative results of Stijve's analyses of \textit{P. samuiensis} (collection F, 8 August 1991), a Swiss collection of \textit{P. semilanceata}, and a Thai collection of \textit{P. cubensis} (collection G, 8 August 1991). Analyses were performed by TLC on NANO-cellulose 10 x 10 cm, \textit{N}-propanol/10% ammonia (5:2 v/v); pDMCA reagent.

6. Discussion

Previous studies by Allen and Merlin (1992a, b) and Guzmán et al. (1993) confirm reports that several species of psychoactive fungi are used in Thailand for non-traditional recreational purposes. The most commonly used species is \textit{P. cubensis} (and/or \textit{P. subcubensis}). Although psychoactive fungi are currently illegal in Thailand, such use is still common at many resorts, including some on the tropical islands of Koh Samui, Koh Pha-Ngan, Koh Samet, and Phuket. Allen and Merlin (1992) also reported that some adults and children have eaten (or attempted to smoke) psychoactive fungi species for recrea-
tion. Furthermore, some tourists have apparently encouraged a small segment of the native inhabitants to consume such fungi. Foreign visitors may have been responsible for introducing the use of psychoactive fungi to Koh Samui and other resort areas in Thailand.

During the collection of field specimens, we questioned several native children and adults concerning their relationship with 'hed keequai' and other fungi found in their environment. Some children were aware of numerous varieties of edible fungi as well as several poisonous and psychoactive fungi species occurring on Koh Samui. On one occasion, several children warned us not to eat *Panaeolus antillarum* (Fr.) Dennis, explaining that it was 'antaray' (dangerous). A toxin is not known from this species at present.

The authors were unable to confirm if the gathering and marketing of psychoactive fungi by Samui farmers and their families had caused any serious poisonings due to the possible misidentification of species. However, the native farmers and their children are very knowledgeable regarding the natural flora of their environment.

Some local residents are able to differentiate *P. samuiensis* from other mind-altering mushrooms. However, it is not known if this species is harvested for human consumption by foreign tourists or immigrants living on Koh Samui Island. It is possible that some European mycophagists have collected and experimented with this species after noticing its macroscopic similarity to *P. semilanceata*. A few children and one adult informant apparently recognized fresh
carpophores of *P. sumuiensis* (harvested by J.W.A.) as a unique type of fungus. At least some Samui farmers and their children are aware that the effects resulting from the consumption of *P. sumuiensis* are similar to the mind-altering effects of the larger specimens of 'hed kequai' (i.e. *P. cubensis*) which they often gathered.

After noticing that several carpophores of *P. sumuiensis* exhibited a slight bluing reaction after handling, we bioassayed 25 fresh specimens (weighing approximately 6 g wet wt.); this resulted in an intensely visual experience, quantitatively similar to the effects produced by the consumption of equal amounts of specimens of *P. semilanceata* collected in Germany.

Prior to the botanical identification of *P. sumuiensis* by Guzmán et al. (1993), a small collection of *P. sumuiensis* was sent to Dr T. Stijve of Nestec Ltd., Vevey, Switzerland for botanical identification. Unable to properly identify the fungi, Stijve (pers. commun., 1992) forwarded several carpophores of *P. sumuiensis* to Klaus Høiland of the Botanical Garden and Museum in Oslo, Norway. Høiland, in a personal communication to Stijve (11 June 1992), reported that he 'examined the dried specimens according to Guzmán's taxonomic key of the genus Psilocybe,' suggesting that the dried material 'corresponded to Psilocybe mexicana Heim or a very closely related species'. Furthermore, Høiland suggested that 'since it [P. mexicana] is only known [of] from [the North] America[n] [continent], care should be undertaken to accept the species from Thailand. It may occur there naturally [Koh Samui], or it may have been introduced by people from [North] American samples, or it is a close, but undescribed species' (Stijve, pers. commun. 12 June 1992).

*P. sumuiensis* is microscopically similar to *P. mexicana*, but the form and size of the spores, as well as the presence of pleurocystidia, its macroscopic features, and the habitat are very similar to *P. semilanceata*. Guzmán et al. (1993) placed this species in the section Mexicanae because of the big rhomboid or subrhomboid spores which separate this species from *P. mexicana* and other species in the *Psilocybe* section *Mexicanae*; it is the first species of that section to be found outside of the New World.

Although *P. sumuiensis* is microscopically similar to *P. mexicana*, it macroscopically resembles *P. semilanceata*. However, the latter species differs macroscopically from *P. sumuiensis* by the height or length of their respective stipes and the color of the fruit bodies. *P. sumuiensis* and *P. semilanceata* attain heights of 40–65 × 1.52 mm and 70–110 × 1.52 mm respectively. Since *P. sumuiensis* is a small inconspicuous fungus not more than 2–3 inches in height, it could be easily overlooked by both tourists and native collectors seeking the larger specimens of *P. cubensis*.

The chemical composition of *P. sumuiensis* is also quite different than that of *P. semilanceata* which contains much more baeocystin than *P. sumuiensis* (Gartz, 1991a, b, 1993). During crossing experiments, complete reproductive barriers have been found between four mono karyons of *P. semilanceata*, two from Germany and two from Austria, and in three strains of *P. sumuiensis* from Thailand. It is clear that both are autonomous species which do not form hybrid dikaryons.

Recent chemical analyses of both naturally occurring and cultivated specimens of *P. sumuiensis* by the authors, as well as analysis of five naturally occurring fruit bodies by Stijve, indicate that this species is relatively potent, containing high concentrations of both psilocybin and psilocin.

### 7. Herbarium deposits

Duplicate collections of fungi specimens referred to in this study (collected 2–11 August 1991) have been deposited at the Instituto de Ecología in Xalapa, Veracruz, México (including *P. sumuiensis*, holotype XAL, Allen F, 1991) and at the Pacificum Herbarium in the Bernice P. Bishop Museum in Honolulu, Hawaii (including *P. sumuiensis*, isotypes in BISH and 0, Allen F, 626452, Allen F1, 626825). Additional specimens of *P. sumuiensis* were sent to Dr Rolf Singer, Field Museum of Natural History, Chicago, IL, and to Dr Prakitsin Sihanonth, Chulalongkorn University, Bangkok, Thailand.

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