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Characterization and cultivation of *Psilocybe barrerae*

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ABSTRACT

A strain of *Psilocybe barrerae* (*Strophariaceae*) was isolated, characterized, and cultivated under laboratory conditions. Mycelial colonies were white to off-white, showing average growth rates of 3.9 mm/day on potato dextrose agar (PDA) and 3.6 mm/day on corn meal agar (CMA). The production of biomass varied from 0.2872 g dry weight/L/day (CMA) to 0.1353 g dry weight/L/day (PDA). One flush of fruit bodies, cultivated on a mixture of sand and compost as substrate, was produced reaching a biological efficiency of 28.9%. The morphology of cultivated fruit bodies was equivalent to that of wild mushrooms.

Key words: Cultivation, *Psilocybe barrerae*, substrates, traditional medicine.

INTRODUCTION

There are more than 100 species within the genus *Psilocybe* worldwide. Several species are known to have neurotropic properties, and are subject of increasing public concern[6].

Psilocybe barrerae Cifuentes & Guzmán

emend. Guzmán (*Strophariaceae*, Section *Zapotecorum*) grows wild on soil from Mexican temperate and subtropical forests (*Pinus*, *Quercus*), mainly in the States of Guerrero, Hidalgo, Mexico, Morelos, and Veracruz[5]. The ecological distribution of *P. barrerae* is limited, so far restricted to Mexico, and thus it has been considered as a species at risk and having priority for conservation[3]. Local names for *P. barrerae* are "santito" ("little saint") and "derrumbe" ("landslide"); however, its neurotropic or specific properties are unknown.

In the State of Morelos, *P. barrerae* is used in the traditional medicine, despite its neurotropic properties, associated with stomach-ache and toothache[4,7]. In this work, we isolated a wild strain of this species, which was characterized and cultivated on different substrates.

Fig. 1. Wild fruit bodies of *Psilocybe barrerae* gathered at the "Biological Corridor Chichinautzin" National Park, State of Morelos, Mexico.

MATERIALS AND METHODS

Strain. Fruit bodies of *Psilocybe barrerae*, found growing in the wild on sandy soil from *Pinus-Quercus* forest, were gathered during June-October (2001) at the "Biological Corridor Chichinautzin" National Park, State of Morelos, Mexico (**Fig. 1**). The strain isolated from those fruit bodies on potato dextrose agar (PDA, Bioxon) was recorded as COBIOCH-UAEMor-p31 at the University research culture collection. The macroscopic and microscopic morphology of a representative sample of fruit bodies was described, and dry specimens were deposited at the University Herbarium (HEMIM). Colors were recorded according to the Munsell soil charts[1].

Culture media. PDA and corn meal agar (CMA, Bioxon) were used for strain characterization. Culture media were prepared, sterilized (121 C for 15 min), and poured (30 ml/plate) in sterile petri dishes (90 mm diameter). After cooling, petri dishes were inoculated with the strain of *P. barrerae*. Inoculated petri dishes were incubated at 22 C, using three replicates per culture medium. Mycelial growth rate and colony morphology were assessed by simple observations recorded every two days.

Fungal biomass. After complete colonization by the fungal mycelium, petri dishes were put in the microwave oven for 15 s. When the culture medium melted, the biomass was separated by filtration (Whatman no. 1). Thereafter, the filter paper and the mycelium were dried at 65 C for 24 h, and the biomass determined as dry weight.

Mushroom cultivation. Sorghum grain spawn was prepared in glass jars according to standard methods[2]. Jars inoculated with *P. barrerae* were incubated at 26 C until complete mycelial colonization.

The following substrates were studied: commercial peat moss from Canada (Premier

Horticulture, Ltd.), vermicompost from red wigglers (*Eisenia foetida* Savigny), compost, sand, and sand+compost (1:1 w/w). The vermicompost was made from cow manure, and contained 1.3% nitrogen, 0.034% phosphorus, 0.06% potassium, and 15.1% organic matter. The compost was made of by-products from domestic fruits and vegetables, and contained 0.3% nitrogen, 1% phosphorus, 0.5% potassium, and 8.3% organic matter. The sand was granular from a local river-bed, which is normally used as building material. The vermicompost, compost, and sand were provided by the Edaphology Department, UAMor. About a kilogram (870 g dry weight) of each substrate was placed within polypropilene plastic bags, adjusting moisture content to 50-60% with distilled water. These bags were then sterilized at 121 C for 1 h. Each bag was homogeneously inoculated with 150 g of spawn on the top of the substrate under sterile conditions. After substrate colonization, an independent portion of sterile sand to a depth of 3-4 cm was used for casing each bag. Cased bags were incubated at 26 C in the dark. Three replicate bags were done for each substrate.

When substrates and casing material were colonized by the mushroom mycelium, ventilation and watering were increased to promote fruiting. The yield was recorded, and fruit bodies were harvested for studying main morphological characters. Biological efficiency (BE) was determined by expressing the yield of fruit bodies (fresh weight) as a percentage of the dry weight of substrate at spawning[8]. The macroscopic and microscopic morphology of a representative sample of fruit bodies was described.

RESULTS AND DISCUSSION

Mycelial colonies of *P. barrerae* varied according to the culture medium (**Table 1**). They were off-white on PDA, showing regular density, velvety texture, and scarce aerial hyphae. The growth rate recorded on PDA was 3.9 mm/day, and the biomass produced was 0.1353 g/L/day. On CMA, mycelial colonies were white showing regular growth and aerial hyphae. The growth rate (3.6 mm/day) and biomass produced (0.2872 g/L/day) on CMA were lower than those on PDA.

Table 1. Colony morphology of *Psilocybe barrerae* (strain COBIOCH-UAEMor-p31) grown on culture media studied at 22 C.

Medium	Mycelial characteristics						
	Density	Texture	Color	Aerial hyphae	Colony growth	Growth rate (mm/day)	Biomass dry weight (g/L/day)
Potato dextrose agar (PDA)	Regular	Velvety	Off-white	Scarce	Regular	3.9	0.1353±0.0071
Corn meal agar (CMA)	Regular	Velvety	White	Regular	Regular	3.6	0.2872±0.0421

Table 2. Biological efficiency and mushroom yield from *Psilocybe barrerae* cultivated on substrates studied.

Substrate	Dry weight (g)	Mycelial colonization	1st flush	Mushroom yield (g)	Biological efficiency (%)
Peatmoss	870	Poor	-	-	-
Vermicompost	870	Poor	-	-	-
Compost	870	Poor	-	-	-
Sand	870	Poor	-	-	-
Sand+compost	870	Good	251.6	251.6±106.03	28.9

Table 3. Morphology of fruit bodies from *Psilocybe barrerae* cultivated on a mixture of sand and compost.

Level	Structure	Description
Macroscopic	Basidiome	All parts stained dark blue or dark blue-green, farinaceous odor, and slight bitter taste
	Pileus	10-170 mm in diameter, campanulate to subconvex, papillate (0.3mm), glabrous, irregular margin, hygrophanous, and brownish yellow (10YR/6/8)
	Gills	Lamellae subadnate, light gray (SYR/6/2)
	Stipe	30-230 x 5-60 mm, cylindric, whitish (5YR/8/1), white floccose scales
Microscopic	Spores	6.3-9.0 x 4.5-5.4 µm, elliptic to oblong-ellipsoid, thin wall, brown yellowish, broad germ pore, short appendage at the distal edge
	Basidia	9.0-18.0 x 4.5-9.0 µm, 4-spored, hyaline, cylindric-vesiculose, occasional central constriction, sterigmata up to 4.5 µm long
	Pleurocystidia	22.5-32.4 x 7.2-13.5 µm, hyaline, abundant, ventricose or ventricose rostrate, short or long apex, sometimes digitate irregularly
	Cheilocystidia	10.8-22.5 x 4.5-9.0 µm, hyaline, abundant, ventricose or ventricose rostrate, short neck, sometimes submoniliform or irregular shape
	Subhymenium	Subcellular, elements of 2.7-7.2 µm wide
	Gill trama	Regular, hyphae of 9.0-13.5 µm wide, hyaline, irregular yellow pigments at the hyphal wall
	Epicutis	Subgelatinized, hyaline hyphae, 2.7-18.0 µm, abundant clamp connections

The mixture of sand and compost supported good mycelial growth and colonization, whereas poor mycelial growth was observed in the other substrates (peatmoss, vermicompost, compost, sand). Environmental temperature ranged from 15-24 C during fruiting, while the relative humidity was maintained at about 90%. Fruit bodies of *P. barrerae* were only developed in the mixture of sand and compost, one flush 78 days after spawning (**Fig. 2**), reaching a biological efficiency of 28.9% (**Table 2**). These fruit bodies were equivalent to wild mushrooms, and their main morphological characteristics are shown in **Table 3**. In comparison with previous descriptions of *P. barrerae* (Guzmán *et al*., 1999), cultivated fruit bodies showed variations in the size of pileus, stipe, basidia, sterigmata, pleurocystidia and cheilocystidia, as well as the fact that gills stained dark blue when bruised.

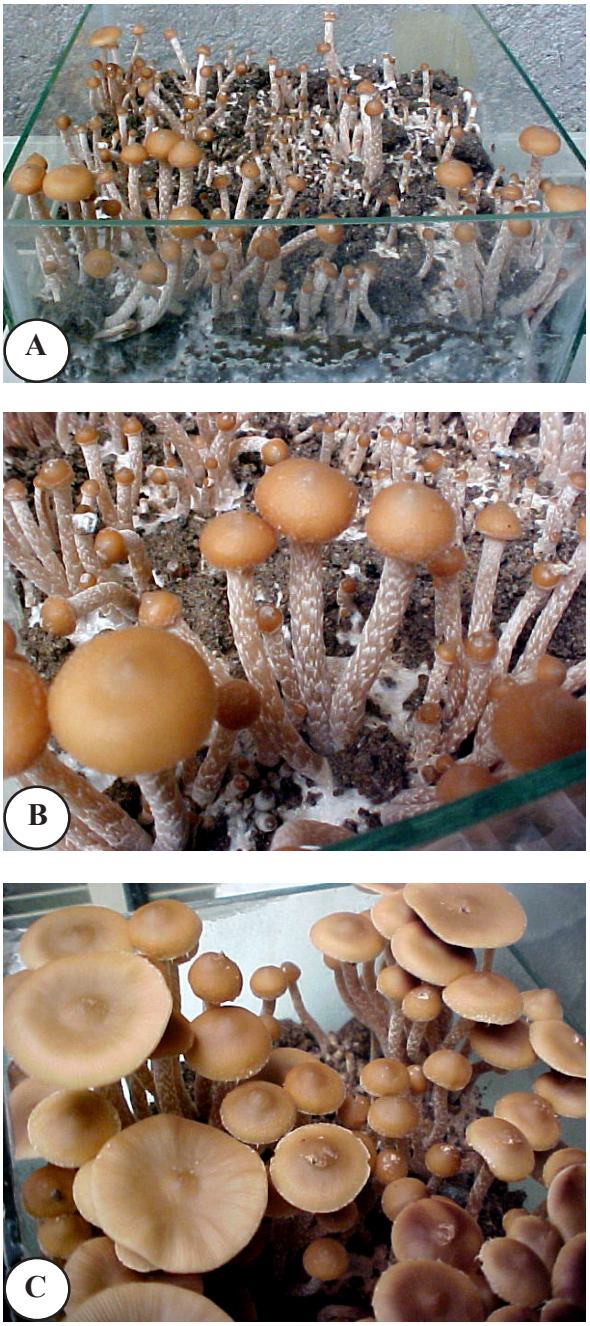

Fig. 2. Different stages of development from fruit bodies of *Psilocybe barrerae* cultivated on a mixture of sand and compost. A: Glass chamber to promote fruiting. B-C: Young and mature spore-bearing fruit bodies with normal morphology.

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