J. Basic Microbiol. **29** (1989) 6, 347—352

(Institut für Biotechnologie der AdW, DDR-7050 Leipzig)

Biotransformation of tryptamine derivatives in mycelial cultures of *Psilocybe*[1]

JOCHEN GARTZ

(Received 14 September 1988/Accepted 25 October 1988)

Mycelial cultures of *Psilocybe cubensis* capable of forming psilocybin and psilocin *de novo* display a high capacity for hydroxylation of tryptamine derivatives at the 4-position. A specific biotransformation of added synthetic N,N-diethyl-tryptamine was found. Thus high amounts of 4-hydroxy-N,N-diethyltryptamine (up to 3.3%) and a minor quantity of 4-phosphoryloxy-N,N-diethyltryptamine (0.01—0.8%) were isolated from fruiting bodies of *Psilocybe cubensis* in corresponding experiments. This is the first example of a directed biosynthesis of tryptamine substances by fungi.

An effective biotransformation of N-methyltryptamine was also demonstrated with surface cultures of *Psilocybe semilanceata*. Baeocystin, a possible natural precursor of psilocybin, was detected and quantified in the biomasses.

No alkaloids could be found in the culture medium.

Various secondary metabolites from basidiomycetes are known which have interesting features for medical and biological purposes. But there is a great lack of basis knowledge which would enable strain improvement the basidiomycetes in such a manner as demonstrated for antibiotic-producing actinomycetes or various ascomycetes. By performing a quantitative analysis of the indole alkaloids psilocybin and psilocin in some species of *Psilocybe* and *Inocybe*, we found considerable variations even within one species (SEMERDŽIEVA *et al.* 1986) as well as in cultures (GARTZ 1987). Moreover, fruiting mycelia of *Psilocybe cubensis* (EARLE) SINGER possess a high capacitiy to transform fed tryptamine to psilocin in a methylation and hydroxylation reacti GARTZ 1988). Earlier studies of psilocybin biosynthesis in submerged culture of *Psilocybe cubensis* revealed that tryptophan and tryptamine are precursors of the alkaloids (AGURELL *et al.* 1966, AGURELL and NILSSON 1968a, b).

In the present paper, the biotransformation of fed synthetic tryptamine derivatives by mycelial cultures of *Psilocybe cubensis* and *P. semilanceata* (FR.) KUMM. (MICHAELIS 1977) is described.

Materials and methods

Cultivation of *P. cubensis*: A dried cow dung/rice grain mixture (2:1) suspended into the double amount of water was used to obtain fast fruiting without casing of a *P. cubensis* strain (GARTZ 1987). 0.25 mM N,N-diethyltryptaminehydrochlorid (synthesis: NOGRADI 1957) were added to 13.5 g of this medium. Cultivation without addition of any indole derivative was also tested. The methods for sterile cultivation are described elsewhere (GARTZ 1987). The first mushrooms were produced by the *P. cubensis* cultures with in 4 weeks. The cultures continued to produce sporocarps in five flushes. Each flush was analysed after freeze-drying and storage at −10 °C.

Naturally grown mushrooms: Fruiting bodies of *P. semilanceata* (leg. Dübener Heide, 21. 9. 1985) were analyzed to determine the average alkaloid level in 10 mushrooms. Mycelium obtained from the spores of

[1]) Paper presented af the 2nd International Symposium on "New Bioactive Metabolites from Microorganisms", Gera (GDR), May 2—7, 1988

one mushroom (GARTZ 1987) was kept as a stock culture on 6% malt agar.

Surface culture of *P. semilanceata*: Mycelia of *P. semilanceata* were grown in steady culture (50d) on a synthetic medium with 8% glucose (GARTZ 1986). Concentrations up to 10 mM/l of N-methyltryptamine (SIGMA) were added as hydrochloride in to the medium.

Extraction and analysis: The extraction procedures for dried mushrooms and mycelia as well as the analysis of indole alkaloids by using HPLC and TLC are described elsewhere (GARTZ 1985a, b, 1987, SEMERDZIEVA *et al.* 1986). Another mobile phase was also used in the TLC (VANHAELEN-FASTRE and VANHAELEN 1984).

Isolation of indole derivatives: The products of the N,N-diethyltryptamine[1]) biotransformation were isolated from methanolic extracts of the mushrooms by cellulose column chromatography as described for the isolation of psilocybin (KOIKE *et al.* 1981).

Mass spectrometry: Mass spectra were measured in the Varian MAT CH 6 apparatus (ionization energy: 70 eV).

Phosphatase reaction: An alkaline phosphatase from FLUKA was used as described (MICHAELIS 1977).

Results

It was found that in the fruiting mycelia of *P. cubensis* a specific hydroxylation of added synthetic DT in the 4-position of the indole nucleus occured (Fig. 1). 4-Phosphoryloxy-N,N-diethyltryptamine (PT), mp 261—263 °C $C_{14}H_{21}O_4N_2P$, was obtained from mushrooms (0.2% per dry weight) by cellulose column chromatography. Microanalysis suggested the molecular formula for the compound, while the mass spectrum showed fragment ions at m/e 232 (f_1, M^+-HPO_3), 188 (f_1-N $(C_2H_5)_2$, 187 (f_1-NH $(C_2H_5)_2$), 174 (f_1-CH_2N $(C_2H_5)_2$).

The measured UV maxima in methanol at 221, 267, 280 and 290 nm were very similar to reported data of psilocybin and could be attributed to a 4-substituted tryptamine derivative (KOIKE *et al.* 1981, REPKE and LESLIE 1977, WURST *et al.* 1984). 4-Hydroxy-N,N-diethyltryptamine (HT), mp 105 °C, $C_{14}H_{20}ON_2$, was isolated in high amounts (1.5% per

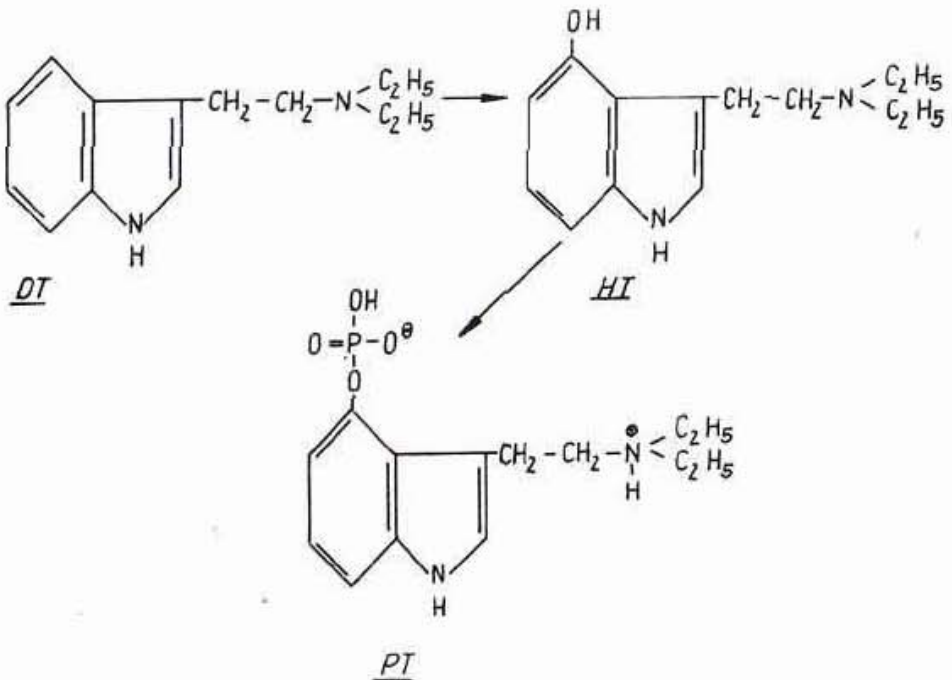

Fig. 1
Biotransformation of DT to HT and PT in fruiting mycelia of *P. cubensis*

[1]) Abbreviations: DT = N,N-diethyltryptamine, HT = 4-hydroxy-N,N-diethyltryptamine, PT = 4-phosphoryloxy-N,N-diethyltryptamine, MT = N-methyltryptamine