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DISTRIBUTION OF DIACYLGLYCEROTRIMETHYLHOMOSERINE AND PHOSPHATIDYLCHOLINE IN MUSHROOMS

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IN HONOUR OF THE RETIREMENT OF PROFESSOR ANDRÉ CAVÉ

Key Word Index—Basidiomycetes; mushrooms; lipids; betaine lipids; diacylglycero-*N*,*N*,*N*-trimethylhomoserine; phosphatidylcholine; chemotaxonomy.

Abstract—Fifty-eight species of mushrooms from different orders of Basidiomycetes were examined by HPTLC for the presence of 1,2-diacylglycero-*O*-4′-(*N*,*N*,*N*,-trimethyl)homoserine(DGTS) and phosphatidylcholine (PC). It was found that DGTS was one of the main polar lipids in all species investigated from the orders Boletales and Hygrophorales. This lipid was detected as a minor component in a few species from Aphyllophorales and the family Tricholomataceae. The presence of DGTS does not depend on the stage of the development of fruit bodies and place of collection of these mushrooms. Phosphatidylcholine was the major phospholipid in all species investigated except for *Leccinum scabrum*, *L. variocolar* and *Hygrophorus hypothejus* in which this lipid was virtually absent. Possible biosynthetic mechanisms resulting in absence of PC are discussed.

INTRODUCTION

The betaine lipid 1,2-diacylglycero-*O*-(*N*,*N*,*N*,-trimethyl)homoserine is produced by almost all cryptogamic plants [1, 2]. With regards mushrooms, DGTS was first isolated and identified from *Boletus edulis* [3] and in some other species [4]. A recent review devoted to betaine lipids has shown that the information on distribution of DGTS in mushrooms is very limited and that only a few mushroom species (less than 20) have been investigated [4]. Following the identification of DGTS [5], this lipid was thought to play a role similar to the main phospholipid, phosphatidylcholine, based on the zwitterionic structure which is characteristic of both of these lipids. Indeed, a number of plants seem to produce betaine lipids in place of PC [2].

A literature search has shown that PC is usually a major polar lipid in mushrooms [6–9]. However, in some species of mushrooms PC was not detected. Griffin *et al.* [10] and Byrne and Brennan [11] could not detect PC in the mycelial or sporophore tissue of the cultivated species *Agaricus bisporus*, while other authors describe this phospholipid as the major lipid component in the same species [12, 13]. Mlodeski *et al.* [7] did not find PC in fresh and dried mushrooms from the species *Leccinum scabrum*, *L. aurantiacum* and *A. bisporus*.

Thus the available information in the literature does not give a general view on the distribution of DGTS and PC in mushrooms. Therefore, we analysed members of the differeent orders of Basidiomycetes to obtain further information.

RESULTS AND DISCUSSION

Screening the polar lipid composition of mushrooms was performed by high performance thin-layer chromatography (HPTLC) [14]. Polar lipids were separated by 2D TLC using systems [15], in which DGTS, PC and all the other main phospholipids have distinctive positions on chromatograms. This is very important in obtaining reliable results, as another betaine lipid, diacylglycerylhydroxymethyltrimethyl-β-alanine (DGTA) gives the same colour reactions as DGTS and is not separated from it in certain solvent systems [16]. The polar lipids were identified by comparison with standards and with the use of specific spray-reagents: Dragendorff's reagent for betaine group-containing lipids [17], molybdenum blue for phospholipids [18], malachite green for phosphorus-containing substances [19], allows the detection even of traces of phospholipids. An interesting peculiarity

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of DGTS and DGTA is their ability to stain with the molybdate reagent as phospholipid [18], but they do not give a positive reaction with the more specific malachite-green reagent for phosphorus-containing substances [19].

Distribution of DGTS

After preliminary screening of a few species of mushrooms and the finding of DGTS-containing species among them, we examined the influence of stage of the development of fruit bodies and place of collection on the presence of DGTS in mushrooms. *Hygrophorus russula*, a widely distributed species which contains DGTS, was selected as the model for these purposes. We compared the polar lipid composition of *H. russula* of different sizes of fruit bodies, collected from different places. It was found that the caps and stems of all specimens were rich in DGTS independent of their size and place of harvest. Therefore, in further work the lipid composition of mushroom species from different taxa was analysed, continuing to collect samples of mushrooms of different sizes of fruit bodies and from various places. Fifty-eight species of mushrooms from five orders were investigated (Table 1). The systematic part of the table was constructed based on the taxonomic system of Moser [20] with two substantial differences. All pleurotoid genera were included into the family Pleurotaceae as described by Vassilieva [21] and Vasser [22]. We also recognized the order Hygrophorales [23].

Table 1 shows that there is a correlation between DGTS distribution and taxonomic position. All 14 species of the order Boletales had high DGTS content and none of the 11 species from the order Russulales contained it. All four representatives of Hygrophorales were rich in DGTS. No one species from the other orders contained DGTS as a major polar lipid. In three species of the family Tricholomataceae—*Calocybe gambose*, *Oudemansiella platyphylla* and *O. mucida* as well as in *Laetiporus sulphureus* and *Clavariadelphus pistillaris* from the order Aphyllophorales, DGTS was detected as a minor component. This lipid was not detected in the other six members of Tricholomataceae and in six species of the Aphyllophorales. This lipid was not found in the nine species from the families Rhodophyllaceae, Amanitaceae, Agaricaceae, Strophariaceae and Cortinariaceae examined.

The results obtained demonstrated that the distribution of DGTS in mushrooms differs greatly from the distribution of this lipid in higher plants. It was shown for different divisions of terrestrial plants [24, 25] and marine macrophytic algae [2, 16] that either all species of each order and even class or division contained or did not contain DGTS, although in some species its content may vary considerably. In mushrooms, DGTS may be present or absent in species of the same order or even family. However, as Table 1 shows, the distribution of DGTS in Basidiomycetes is also connected with taxonomy. All representatives of the orders Boletales and Hygrophorales have DGTS as one of the main polar lipids. This lipid was not found in any species of Russulales. In some species of other orders it was a minor component.

The results are of interest for fungal taxonomy. The distribution of DGTS in mushrooms shows that Basidiomycetes is a heterogeneous group. The orders Boletales and Russulales are the most distant. This is in accordance with the system of Moser [20] and Vasser [22]. The order Agaricales takes an intermediate position. Somewhat unexpected results were obtained for Hygrophorales. All four species of this order were rich in DGTS as well as members of Boletales. It may provide once more an argument in favour of the classifications by Vasser [22] and Kovalenko [23], who separated the Hygrophorales, as a new order, from the Tricholomatales. It is of interest that Hygrophorales appear related to Boletales with regard to the presence of DGTS.

Our results differ from those of Dembitski *et al.* [4]. They detected DGTS in all 10 mushroom species from different taxonomic orders and did not find a correlation between distribution of DGTS and taxonomy. Therefore, although this current work has analysed polar lipids of more than 50 species of mushrooms, the conclusions need more support. To obtain a wider and more exact description of the distribution of DGTS in mushrooms it will be necessary to increase the number of analysed species.

Distribution of phosphatidylcholine

The distribution of DGTS in plants show that some species which accumulate a high level DGTS, do not contain phosphatidylcholine (PC) [2, 25]. A similar tendency was shown for brown algae: PC was not found in those species containing another betaine lipid, DGTA [2, 16, 26]. Therefore, we paid attention to the distribution PC in various groups of mushrooms.

Phosphatidylcholine was the major polar lipd in all the species investigated, except *Leccinum scabrum* and *L. variocolor*, in which it was absent (Table 1). However PC was one of the main lipids in two other representatives of the genus *Leccinum*—*L. chromapes* and *L. testaceoscabrum*. No PC was found in *Hygrophorus hypothejus*. Other members from the same genus contained PC. No specimen of these three species collected from different places and different periods contained PC. However, this phospholipid was present in high amounts in the cultivated mushroom *Agaricus bisporus* and in the related wild species, *A. campestris*. The first species is of especial interest because its lipids have been investigated many times and the results obtained for the presence of PC are contradictory. Phosphatidylcholine was not found in mushrooms, collected from natural habitats [7] or in cultivated ones [10, 11]. But PC was a major phospholipid in sporophores and mycelium of four strains of *A.*

Table 1. Distribution of diacylglycero-*N*,*N*,*N*-trimethylhomoserine (DGTS) and phosphatidylcholine (PC) in Basidiomycete fruit bodies

ORDER **Family** Species	DGTS	PC
APHYLLOPHORALES		
Polyporaceae		
Laetiporus sulphureus (Fr.) Bond. *et* Sing	+	++
Grifolia frondosa (Fr.) S. F. Gray	–	++
Clavariaceae		
Ramaria botrytoides (Pk.) Corner	–	++
R. gracilis (Fr.) Quel.	–	++
R. aurea (Fr.) Quel.	–	++
Clavariadelphus pistillaris (Fr.) Donk	+	++
Cantharellaceae		
Cantharellus cibarius Fr.	–	++
Hydnaceae		
Hydnum repandum Fr.	–	++
BOLETALES		
Strobilomycetaceae		
Strobilomyces floccopus (Fr.) Karst.	++	++
Porhhyrellus atrobrunneus L. Vass.	++	++
Boletaceae		
Gyroporus castaneus (Fr.) Quel.	++	++
Suillus luteus (Fr.) S. F. Gray	++	++
Xerocomus subtomentosus (Fr.) Quel.	++	++
Boletus calopus Fr.	++	++
B. dupaini Boud.	++	++
B. edulis Bull. ex Fr.	++	++
B. regius Krbh.	++	++
B. rhodoxanthus (Krobh.) Kallenb.	++	++
Leccinum chromapes (Frost.) Sing.	++	++
L. scabrum Fr.	++	–
L. testaceascabrum (Secr.) Sing.	++	++
L. variicolor Watl.	++	–
HYGROPHORALES		
Hygrophoraceae		
Hygrophorus russula (Fr.) Quel.	– –	– –
H. olivaceoalbus (Fr.: Fr.) Fr.	++	++
H. hypothejus (Fr.: Fr.) Fr.	++	–
Hygrocybe sp.	++	++
AGARICALES		
Pleurotaceae		
Pleurotus pulmonarius Fr.		
Tricholomataceae		
Clitocybe gibba (Fr.) Kumm.	–	++
Lepista nuda (Fr.) Cke.	–	++
Armillariella mellea (Fr.) Karst.	–	++
Calocybe constricta (Fr.) Kuhn.	–	++
C. gambosa (Fr.) Donk.	+	++
Collybia Dryophyla (Fr.) Kumm.		++
Melanoleuca grammopodia (Fr.) Pat.	–	++
Oudemansiella mucida (Fr.) v. Hoehn.	+	++
O. platyphylla (Fr.) Mos.	+	++
Flammulina velutipes (Fr.) Sing.	–	++
Rhodophyllaceae		
Rhodophyllus rhodopolius (Fr.) Quel.	–	++

Table 1.—*Continued.*

ORDER **Family** Species	DGTS	PC
Amanitaceae		
Amanita caesareaoides L. Vass.	–	++
A. pantherina (Fr.) Secr.	–	++
Agaricaceae		
Agaricus campestris (l.) Fr.	–	++
Agaricus bisporus (J. Lge) Imbach	–	++
Macrolepiota gracilenta (Fr.) Zitzenschirmling	–	++
M. procera (Fr.) Sing.	–	++
Strophariaceae		
Stropharia albocrenulata (Pk.) Kreisel	–	++
Pholiota aurivella (Fr.) Kumm.	–	++
Cortinariaceae		
Inocybe fastigiata (Fr.) Quel.	–	++
RUSSULALES		
Russulaceae		
Russula aurata (With.) Fr.	–	++
R. bell Hongo	–	++
R. brunneola Burl.	–	++
R. foetens Fr.	–	++
R. lactea (Pers.) Fr.	–	++
R. pseudodelica Lge.	–	++
R. sanguinea Quel.	–	++
R. xerampelina (Secr.) Fr.	–	++
Lactarius piperatus (Fr.) S. F. Gray	–	++
L. vellereus (Fr.) Fr.	–	++
L. volemus (Fr.) Fr.	–	++

++, intensity of the spot of lipid on chromatogram is equal to that of the spot of major lipids.

+, the spot of DGTS is a minor lipid.

–, lipid is absent.

bisporus, grown on compost [12] or from mycelium of *A. bisporus* grown in submerged culture [13]. Weete *et al.* [13] suggested that these differences can be explained by differences in the isolates.

Usually PC is the main phospholipid in animals from all phyla and plants from many taxa. In some groups of organisms this lipid is absent [16, 27–29]. Most commonly PC is absent from all representatives within a large taxon, e.g. green or brown algae [16]. However, a green unicellular alga *Chlamydomonas reinhardtii* which does not usually contain PC [29–32], sometimes produces this phospholipid in minute quantities [33, 34]. The situation with P C in mushrooms is distinct from both those situations as this lipid was present or absent in different species belonging to the same genus.

These results raise the question as to what changes in the mechanism of PC synthesis lead to its absence. Nothing is yet known on the pathway of PC biosynthesis in mushrooms, although the incorporation of radioactivity from both sodium [^{14}C]-acetate and [^{14}C]-linoleic acid into lipids of *Agaricus bisporus* has been shown [35]. In the related organisms, yeasts, two pathways of PC synthesis are found: *de novo* through cytidyldiphosphate-choline and by the methylation of phosphatidylethanolamine (PE), the contribution of the second pathway for PC synthesis being much more essential [36, 37]. Yeast mutants defective in PE methylation are known; however, they contain a reduced, but appreciable quantity of PC, which is synthesized from choline [38, 39]. Possibly, mushrooms utilize only the methylation pathway for PC biosynthesis, and several species are defective in this mechanism. It is suggested that in a small group of bacteria containing PC, this lipid was only synthesized by this route [36].

It is of interest that there are no differences in the appearance or any other visible characteristics of species from the genus *Leccinum* with and without PC. However, the yeast mutants, unable to methylate PE, need choline for normal growth [39]. It was shown, that the inhibition of PE methylation to PC is

the mechanism of the site of action of certain fungicides on the fungus *Pyricularia oryzae* [40, 41]. Therefore, mushroom species without PC are potentially interesting models for the study of PC biosynthesis and its role in biomembranes.

EXPERIMENTAL

Mushrooms were harvested in summer and autumn during the years 1990–1994, near Vladivostok, on islands close to it and at the seashore of the Sea of Japan. Cultivated *Agaricus bisporus* were purchased at local markets. Each sample consisted of 3–5 fruit bodies of the same species. Mushrooms were thoroughly cleaned of soil and animal and plant contamination. Small pieces were cut from each cap and stem a total weight of 5 g, and then heated in boiling H_2O 2–3 min to inactivate lipases [42]. Samples were then ground in a mortar with sand (*ca* 10:1).

Lipids were extracted with $CHCl_3$–MeOH (1:2) according to [43]. Polar lipids were sepd by 2D micro-TLC on 6 × 6 cm silica gel plates [14] in the solvent systems for the sepn of phospholipids [15]. The spots of lipids were detected with 10% H_2SO_4 in MeOH followed by charring and the following specific spray reagents: Dragendorf reagent for choline lipids [17], molybdate reagent for phospholipids [18], malachite-green reagent for phosphorus-containing substances [19], ninhydrin in Me_2CO for amine-containing lipids [44] and modified anthrone reagent for glycolipids [45]. Lipids were identified by co-chromatography on silica gel plates with authentic standards. PC was isolated from egg yolk by CC and TLC [46]. Authentic samples of DGTS were isolated by chromatography from the mushroom *Boletus edulis* and green alga *Ulva fenestrata* as described [3, 47].

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