Structural lipids and carbohydrates of the deep mycelium of phoma-like micromycetes, potential mycoherbicides

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Abstract. The work is devoted to the mycelium biochemical composition of *Stagonospora cirsii* C-211, *Calophoma complanata* 32.121, *Didymella macrostoma* 32.52. These phylogenetically distant species of phoma-like micromycetes are the potential mycoherbicides of *Cirsium arvense*, *Heracleum sosnowskyi*, and *Convolvulus arvensis*, respectively. The *S. cirsii* C-211, *C. complanata* 32.121, *D. macrostoma* 32.52 mycelium in the early stationary growth phase was obtained on sucrose-soybean nutrient medium. It was shown that the lipid and carbohydrate (polyols, sugars) profiles of these strains have much in common. We suppose that levels of arabitol and trehalose influence to the stress-resistant of phoma-like micromycetes. In particularly, these carbohydrates serve structural and protective roles in the cell walls during osmotic and temperatures stress. The ratio of phosphatidylcholine to phosphatidylethanolamine and the proportion of phosphatidylserine among structural lipids also determine the properties of mycelium, and can be used to assess its quality.

1 Introduction

Phytopathogenic phoma-like micromycetes can be used as a basis for the development of mycoherbicides for managing troublesome invasive grasses and worst weeds. An example is *Phoma macrostoma* (*=Didymella macrostoma*) strain 94-44B. Registered in Canada and the USA anti-broadleaf weeds bioherbicide were developed based on mycelium, spores and metabolites of this strain [1]. Phoma-like micromycetes are heterogeneous group of fungi formed by their morphological and cultural peculiarities. In this paper, we consider three phylogenetically distant species of phoma-like fungi belonging to different genera, including a strain of the same species *Phoma macrostoma* as in a commercial mycoherbicide. Two other strains of *S. cirsii* C-211, *C. complanata* 32.121, are positioned as potential

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mycoherbicides of *Cirsium arvense* and *Heracleum sosnowskyi* [2-3]. Recently, publications on the biochemistry of phytopathogenic micromycetes have reappeared. This is due to the fact that the quantitative and qualitative composition of structural lipids, free amino acids and carbohydrates (polyols and sugars) often allows us to predict the viability of pathogens (in the field conditions, during the stabilize organisms, distribution and storage), their resistance to stress-factors (drying up, UV, high and low temperatures), etc. For example, bioprotective effects of trehalose and polyols was shown [4]. In turn, structural lipids are responsible for fluidity, structure and durability membrane, cell signaling and adaptation process [5-7]. The aim of this work was a biochemical analysis of the deep mycelium *S. cirsii* C-211, *C. complanata* 32.121, *D. macrostoma* 32.52.

2 Materials and methods

Calophoma complanata 32.121 and *Didymella macrostoma* 32.52 strains from the Laboratory of Mycology and Phytopathology pure culture collection and *Stagonospora cirsii* C-211 from the Laboratory of Toxicology and Biotechnology collection of the All-Russian Institute of Plant Protection was used. The strains of *S. cirsii* C-211 was isolated from necrotic leaf lesions of *Cirsium arvense*, *C. complanata* 32.121 was isolated from *Heracleum sibiricum,* and *D. macrostoma* 32.52 was isolated from *Convolvulus arvensis.*

The sucrose-soybean growth medium contained sucrose 30 g/l, soy flour 14 g/l, KH_2PO_4 1 g/l, $MgSO_4 \times 7H_2O$ 0.5 g/l. The mycelium was obtained by submerged cultivation during 144 h on an orbital shaker at 180 rpm at 24°C. The deep mycelium was harvest in the early stationary growth phase.

The extraction of structural lipids was carried out according to Bligh, Dyer (1959) [8] with modifications [9]. High performance thin layer chromatography (HPTLC) was used to determine structural lipid profiles. The mobile phases were solutions containing chloroform: methanol: water: ammonia (24%) 60:34:4:2 w/w. The developed plates were dried Once the spots have been visualized by the copper sulfate solution (20 g $CuSO_4$, 200 ml methanol, 8 ml H_2SO_4, 8 ml H_3PO_4) with heating, the plates were scanned at 550 nm. Samples and standards were compared by Rf values and colors [10].

The extraction of carbohydrates mycelium was carried out by 80% ethanol at 80°C for 1 h. The alcohol extracts were concentrated in a rotary evaporator with vacuum controller at 40°C. The dried fractions were then redissolved in pyridine to obtain silyl derivatives which were analyzed by GC-MS according to Sokornova et al. [11].

The analysis of sugars in the ethanol extract of mycelium was performed by the HPTLC according to Sokornova et al. [12].

The experience was repeated 2 times, in at least 4 replications per variant. The data obtained was processed by analysis of variance. The difference significance assessment between the means was assessed by the smallest significant difference ($HCP_{0.05}$).

3 Results and discussion

A biochemical analysis of the *S. cirsii* C-211, *C. complanata* 32.121, *D. macrostoma* 32.52 mycelium at the beginning of the stationary growth phase was carried out. It has been shown previously that the similar mycelium of *S. cirsii* is most resistant to drying [3].

The mycopesticides disadvantages include instability field efficacy, the difficulty of stabilizing propagules and the limited shelf life of the formulations. It is known that sugars, in particular trehalose, are the most effective cryoprotectants during drying or freezing. Their action is due to a slowdown in the rate of change of the lipid phase under extreme conditions, which maintains the integrity and function of the membrane [13]. In turn, the protective effect

of polyols is associated with a decrease the temperature of freezing, support for osmotic pressure and protection of enzymes from adverse effects [14]. In the study we analyzed the content of lipids and carbohydrates in the mycelium of phoma-like fungi.

3.1 Phospholipids profiles of phoma-like micromycetes

The mycelium of phoma-like fungi had, in almost all cases, a typical for mycromicetes phospholipid profile [15]. The main lipid classes were phosphatidylcholine **(PC)** and phosphatidylethanolamine **(PE)**, whose percentage of the mycelium structural lipids was 58.0 ± 3.2, 64.5 ± 4.0 and 65.8 ± 3.8 for *S. cirsii* C-211, *C. complanata* 32.121, *D. macrostoma* 32.52, respectively (Fig. 1). According to available data, that the ratio of these phospholipids, along with phosphatidylserine **(PS)** and ergosterol determines the adaptive properties of fungal membranes [7]. For *S. cirsii* C-211, *C. complanata* 32.121, *D. macrostoma* 32.52 mycelium of the same age, there was no significant difference in the ratio of **PC** to **PE**, it was 1.4, 1.5, and 1.5, respectively. In the same variants there were significant differences, however, between **PS** level (10.0 ± 2.8, 3.7 ± 2.2, and 7.5 ± 2.6 %) (Fig. 1). A sufficiently high level of phosphatidic acid (**PA**) was found in the *S. cirsii* C-211 and *C. complanata* 32.121. It should be noted that **PA** is a secondary messenger of eukaryotic cell processes. The strains were not different about phosphatidylinositol (**PI**) and glycoceramides (**GlCer**) levels.

Thus, we suppose, an evaluation of PS level makes it possible to characterization mycelium and thus its environmental impact stability.

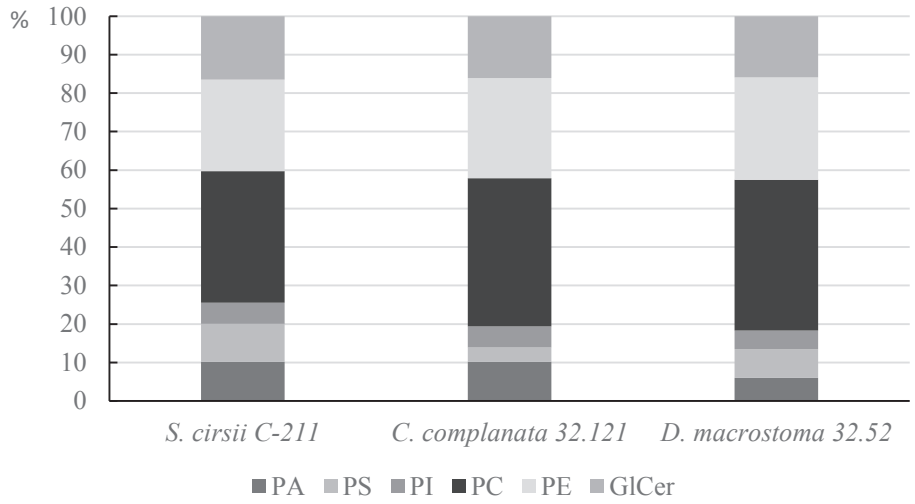

Fig. 1. The content of structural lipids in the mycelium of phoma-like micromycetes, $LSD_{0.05}$=2.8

3.2 Carbohydrate profiles of phoma-like micromycetes

Disaccharides (trehalose, sucrose, mannose) and polyols (mannitol, arabitol, glycerol) accumulation is one of the several mechanisms employed by phoma-like fungi to cope with biotic and abiotic stress [16]. Often, the accumulation of intracellular polyols occurs in the stationary phase of growth. Also, carbohydrates are reserves of carbon and energy, mobilized during regrowth [17].

The amount of total polyol pool of *S. cirsii* C-211, *C. complanata* 32.121, *D. macrostoma* 32.52 mycelium was 9.4 ± 0.8, 9.2 ± 0.9, 10.8 ± 0.9 mg / g. For all species

the major polyols were arabitol and mannitol. The *S. cirsii* C-211 and *C. complanata* 32.121 mycelium contained much more myo-inositol and erythritol than *D. macrostoma* 32.52 mycelium (Fig. 2). It is important to emphasize that phosphorylated myo-inositol is actively involved in the regulation of fungal development in response to stress [18]. In turn *D. macrostoma* 32.52 differed from other analyzed species in a rather high glycerol and sorbitol levels (1.2 ± 0.8 mg / g and 2.0 ± 0.8 mg / g, respectively).

The sugar profile of *S. cirsii* C-211, *C. complanata* 32.121 and *D. macrostoma* 32.52 mycelium showed that the major sugars are disaccharide trehalose and monosaccharides glucose and fructose. As in the case of polyols, the sugar pool of *D. macrostoma* 32.52 mycelium had some features. However, the trehalose and glucose levels did not differ significantly (Fig. 3).

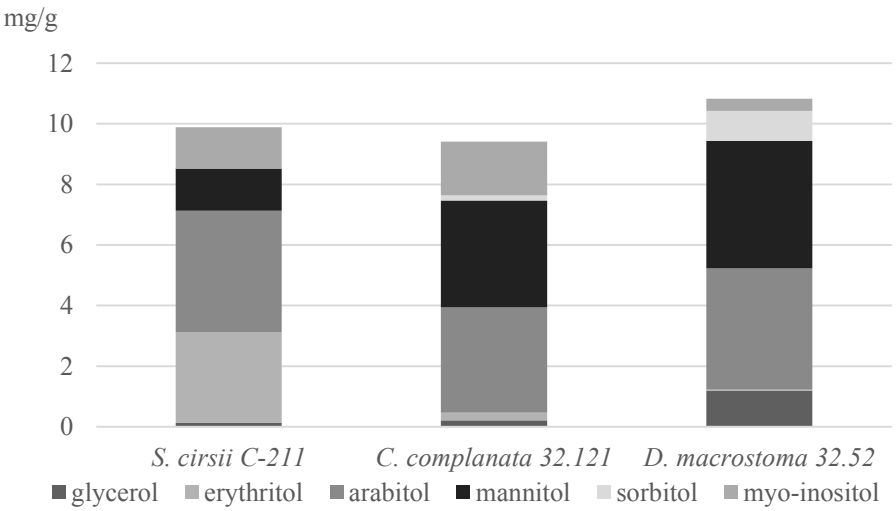

Fig. 2. Polyols contents in the mycelium of phoma-like micromycetes, $LSD_{0.05}$=0.9

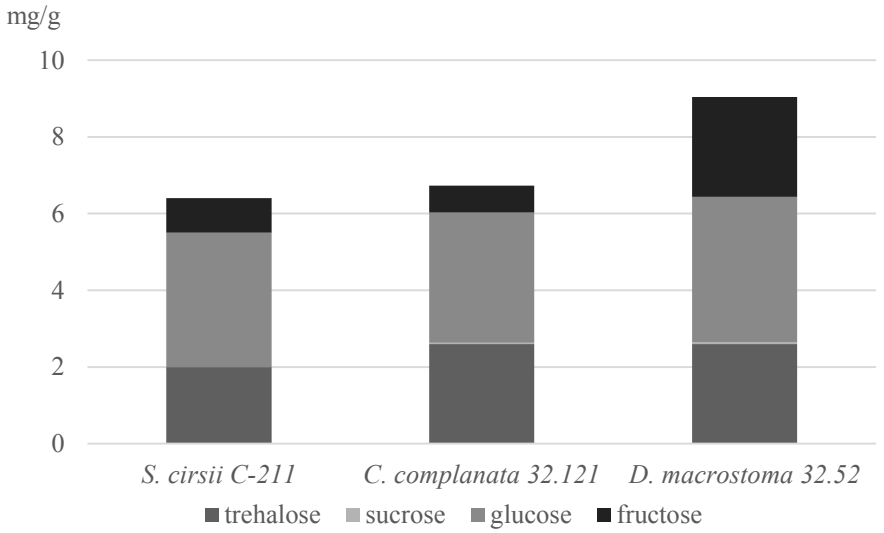

Fig. 3. Sugar contents in the mycelium of phoma-like micromycetes, $LSD_{0.05}$=1.2

4 Conclusion

Thus, the phoma-like fungi had a very similar phospholipids and carbohydrates pools. It was previously shown that the accumulation of arabitol and trehalose correlates with the development of *S. cirsii* C-163 mycelium [11-12]. We suppose that levels of arabitol and trehalose influence to the stress-resistant of phoma-like micromycetes. In particularly, these carbohydrates serve structural and protective roles in the cell walls during osmotic and temperatures stress. The ratio of phosphatidylcholine to phosphatidylethanolamine and the proportion of phosphatidylserine among structural lipids also determine the properties of mycelium, and can be used to assess its quality.

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