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# Carbohydrate changes during growth and fruiting in *Pleurotus ostreatus*

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ABSTRACT

The carbohydrate distribution in mushrooms is reported changing greatly in its different regions during growth and fruiting. In this study, the carbohydrate distribution in the compost and fruiting bodies of *Pleurotus ostreatus* was analysed. Sugar, polyol, polysaccharide, and chitin content during different growth phases and in different regions of the mushroom were determined. Results indicate that trehalose, mannitol, and glucose were first accumulated in the compost and then decreased during differentiation and growth of fruiting bodies. Meanwhile, trehalose, mannitol, and glucose also accumulated in the fruiting bodies and primarily distributed in the stipe, base, and pileus region, respectively. Polysaccharides mainly accumulated within the pileus and stipe regions, and chitin was mainly observed in the base region. These findings provide insights into carbohydrate function and utilisation during mushroom growth.



## Introduction

Mushrooms are well known for their nutritional value and unique flavour. Carbohydrates are one of the primary nutrients in mushrooms and constitute approximately 40–70 % of the dry weight (Crisan *et al.* 1978), including both low- and high-molecular-weight carbohydrates. The low-molecular-weight carbohydrates are primarily monosaccharides, disaccharides, and sugar alcohols (polyols), such as glucose, trehalose, mannitol, and arabitol. The high-molecular-weight carbohydrates are primarily polysaccharides, such as glucan and chitin (Kitamoto 2006). Some carbohydrates abundant in mushroom can be extracted for food, medical, and cosmetic uses: sugar alcohols contain relatively few calories and can be used as sugar substitutes in sweets and other foods (Zumbé *et al.* 2001). The hygroscopicity of trehalose makes it an ideal moisturiser for use in cosmetics (Schiraldi *et al.* 2002). Fungal chitin and its deacetylated derivative chitosan possess antimicrobial bioactivity and can be used as accelerators of wound healing (Cho *et al.* 1999). Finally, polysaccharides such as beta-glucan possess immunomodulatory activities by stimulating macrophages and other white blood cells (Brown & Gordon 2001).

Mushroom carbohydrates are derived from compost substrates, such as wood, sawdust, and straw, which contain approximately 60–70 % of cellulose and hemicelluloses

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(Cosgrove 1997). During mushroom growth, the cellulose, and hemicellulose are hydrolysed by enzymes from mycelium, transported into the mycelial cells, and then metabolised through a variety of carbohydrate pathways (Shoham *et al.* 1999; Martínez *et al.* 2010). Besides being degraded by glycolysis to produce energy for growth, metabolites can be synthesised into structural polysaccharides of the cell wall, such as glucan and chitin (Latgé 2007). The metabolites can also be synthesised into glycogen, arabitol, trehalose, or mannitol for use during development or later growth phases (Bidochka *et al.* 1990).

Significant changes in the levels of these carbohydrates have been associated with mushroom development. In *Agaricus bisporus*, it is found that the accumulation of mannitol during fruiting bodies growth is not accompanied by an increase in mycelial mannitol, and the primary site of mannitol accumulation is in the pileus and stipe of the fruiting bodies. By contrast, trehalose levels decrease in the fruiting bodies and mycelium during growth, and glucose, which is present prior to fruiting, decreases during fruiting in the mycelial samples (Hammond & Nichols 1976). Study in *Pleurotus ostreatus* also indicates that there were remarkable differences among pilei, stipe, and base of the fruiting bodies (Yoshida *et al.*, 1987). Glycogen levels are maximal during interflush periods (Hammond & Nichols 1979). And the translocation of glycogen from the mycelium to the fruiting bodies during growth in *Coprinus cinereus* is also suggested (Ji & Moore 1993).

About the roles, carbohydrates play during mushroom growth and fruiting. Glucose, a basic carbohydrate, was hydrolysed from compost cellulose, utilised or accumulated in the mycelium, and then transported to the fruiting body for developmental growth. In the compost mycelium, some portion of glucose was synthesised and accumulated as trehalose and mannitol. Trehalose acts as an energy source for reproduction and development in fungi and can protect cells from environmental stresses such as dehydration, temperature, nutrient limitation, and oxygen radicals (Elbein *et al.* 2003; Al-Bader *et al.* 2010). It is also suggested that trehalose is synthesised and stored as either a carbon or energy source for further hyphal aggregate growth or for the synthesis of the osmolyte mannitol (Wannet *et al.* 1999). Mannitol is thought to function as an osmoregulator that encourages the influx of water from the environment to maintain turgor pressure during fruiting bodies development (Chakraborty *et al.* 2004). Another portion of glucose was synthesised into polysaccharides and chitin. There was a study suggested that glycogen of the polysaccharides is the main storage carbohydrate in the mushroom mycelium and young fruiting bodies (Kitamoto & Gruen 1976). The insoluble polysaccharide, mostly were the structural polysaccharide, such as chitin and beta-glucan composed the majority of the fungal cell wall, which was used to support cells and protect them from external damage (Muzzarelli *et al.* 2012).

Studies above have indicated that carbohydrates accumulate in different regions of the mushroom and the distribution of them also changes during growth and fruiting. Some of them play important roles during the mushroom growth. However, structural carbohydrates, such as chitin and other polysaccharides, have not been well characterised. It is uncertain that whether the distribution changes of trehalose, sugar alcohol, and glucose during growth are the same in different mushroom species. The carbohydrate changes in the compost have seldom been analysed. In addition, analyses of carbohydrate distributions during mushroom growth could suggest which growth phase and region of the organism are best suited for carbohydrate extraction and utilisation.

In this study, we analysed the distributions of structural and nonstructural carbohydrates in the fruiting bodies and compost during different growth phases of the mushroom *P. ostreatus*. Based on the results, we analysed how carbohydrates are accumulated and changed within mushrooms. The most suitable growth stages and fruiting bodies regions for different carbohydrate utilisation were discussed.

## Materials and methods

### *Organism, culture conditions, and sample collection*

*Pleurotus ostreatus* was obtained from the College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China in 2012. The strain was cultured on potato-dextrose agar medium, and fruiting bodies were produced in compost containing 78 % mixed sawdust, 13 % wheat bran, 7 % corn flour, 1 % sucrose, and 1 % gypsum powder with 60 % moisture. In each plastic bag, 600 g wet weight of the substrate was filled in. The diameter and the height of each compost bag were about 9 cm and 15 cm, respectively. The temperature of myceilum growth and fruiting were 22–25 °C and 15–17 °C, respectively. The humidity of myceilum growth and fruiting were 65 % and 75 %, respectively.

The growth and fruiting process was divided into four phases: phase I, fully grown mycelium (the mycelium spreading to the bottom of the compost); phase II, primordium (the primordium developing into a group of coral like button); phase III, young fruiting bodies (the pileus and stipe of fruiting bodies forming); and phase IV, mature fruiting bodies (the gill forming and the pileus fully extended). The morphology of four phases was shown in Fig 1. At each phase, three samples were collected from the upper, middle, and lower layers (the distance of three layers were 0–5 cm, 5–10 cm, and 10–15 cm from the top) of the compost, respectively. The initial growth substrate prior to inoculation was collected as a control sample. Fruiting bodies samples were collected from the pileus, stipe, and base (region between the stipe and upper compost layer) in triplicate. All samples were frozen dried and crushed into powder before test.

### *Sugar and sugar alcohol test*

Samples (100 mg) were extracted in 50 mL of water at 100 °C for 2 h, passed through a 0.45-μm filter (Millipore, Bedford, MA, USA), and then analysed using an IC2500 HPAEC-PAD system with a GP50 quaternary gradient pump, a LC30 column oven, an EG50 Automatic eluent generator, an ED50 Electrochemical detector and a Dionex CarboPac MA1 column (Dionex, Sunnyvale, CA, USA). The column temperature was 30 °C, and the mobile phase was 480 mM NaOH solution at a flow rate of 0.4 mL $min^{-1}$. Four external standard substances were used including arabitol, trehalose, mannitol, and glucose