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Characterisation of the large-scale production process of oyster mushroom (*Pleurotus ostreatus*) with the analysis of succession and spatial heterogeneity of lignocellulolytic enzyme activities

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ABSTRACT

Oyster mushroom (*Pleurotus ostreatus*) lignocellulolytic enzyme activity pattern and variation was investigated in a large-scale facility from spawning until the end of the second flush. In the first cultivation cycle laccase production reached its peak during vegetative growth stage, while manganese-peroxidase showed the highest activity during fruiting body induction. Cellulose and hemicellulose degrading enzymes had maximal activity at the beginning of flush and harvest stage. The enzyme activities showed similar tendencies among five different mushroom substrate blocks representing a production house. The spatial variability analysis of enzyme activities pointed out the within substrate block heterogeneity as the main source if variation. This result was confirmed by Combined Cluster and Discriminant Analysis (CCDA) method showing minimal among block heterogeneity considering the whole investigation period; furthermore in the first cultivation cycle all blocks were grouped into one cluster.

Introduction

Pleurotus ostreatus (oyster mushroom or hiratake) is a saprotrophic white rot fungus. It has been described as selective (Martínez *et al.* 2005; Taniguchi *et al.* 2010) and simultaneous (Zhang *et al.* 2007; Bari *et al.* 2015) lignocellulose degrader, as well. First, the growth of the oyster mushroom is supported by the easier degradable soluble materials from intra- and intercellular spaces from the plant substrate tissues. After their depletion the resulted fungal biomass starts to degrade the plant cellulose and lignin polymers (Schiesser *et al.* 1989). For this degradation of the substrate a cooperation and subsequent activity of oxidative and hydrolytic enzymes is needed.

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In lignin degradation by *P. ostreatus* laccases (EC 1.10.3.2) and manganese peroxidases (MnP) have a substantial role. Laccases catalyse the oxidation of various aromatic compounds along with the reduction of oxygen to water (Baldrian 2006). MnPs (EC 1.11.1.13) oxidise Mn^{2+} ion by reducing H_2O_2. The produced Mn^{3+} has high redox potential and is chelated by organic acid anions. These complexes oxidise the phenolic structures of lignin, creating free radicals which cause spontaneous disintegration of the macromolecule (Hofrichter 2002). Due to its ligninolytic enzymes *P. ostreatus* has large potential in bioremediation, in upgrading lignocellulosic agricultural wastes for animal feed and in pulp and paper industry (Cohen *et al.* 2002).

The degradation of cellulose is carried out by a group of hydrolytic enzymes. Endoglucanases (EC 3.2.1.4) hydrolyse (1 → 4)-β-D-glucosidic linkages randomly in the cellulose chain. The produced shorter chains serve as substrates for cellobiohydrolases I (EC 3.2.1.91), which hydrolyse (1 → 4)-β-D-glucosidic bonds on the non-reducing ends of cellulose chains removing cellobiose units. β-Glucosidases (3.2.1.21) are responsible for the release of β-D-glucose molecules form the non-reducing β-D-glucosyl residues (Baldrian & Valášková 2008). The hemicellulose component in some type of plant cell wall is rich in glucuronoxylan (Faik 2010). Endo-1,4-β-xylanases (EC 3.2.1.8) hydrolyse (1 → 4)-β-D-xylosidic bonds in the chains of xylans, whereas 1,4-β-xylosidases (EC 3.2.1.37) remove D-xylose residues from the non-reducing ends of the generated oligosaccharides (Pérez *et al.* 2002).

Oyster mushroom can also be appreciated for its good nutritional role as it contains dietary fibre, proteins, vitamins, minerals and has a variety of medicinal properties. Its production was described for first time at the beginning of the twentieth century by Richard Falck, and during the last decades it has become the second most popular cultivated edible mushroom all over the world (Rühl & Kües 2007). As a white rot fungus it can be cultivated on a wide variety of agro-industrial lignocellulosic wastes (Sánchez 2010). One of the most widely used substrate for oyster mushroom cultivation is wheat straw, especially in Europe (Rühl & Kües 2007). There are several methods for substrate preparation including sterilisation, partial sterilisation, pasteurisation, aerobic or anaerobic 'fermentation', and composting with or without subsequent conditioning (Ororbia & Núñez 2001). In Europe partial composting, pasteurisation and subsequent condition is used (Rühl & Kües 2007). After spawning of the substrate the applied growth conditions for *P. ostreatus* show much less variability.

It should be noted that even using the same type of raw material (e.g. wheat straw) the quality and composition of the mushroom substrate is highly variable. Therefore the colonisation of mushroom substrates can be inconsistent resulting in fluctuating mushroom yield. This case of variability can be also a problem in other applications (e.g. bioremediation), therefore a detailed analysis of mushroom growth indicators, as lignocellulolytic activities is needed.

There are few studies on monitoring lignocellulolytic enzyme activities of *P. ostreatus* during its cultivation process. Moreover most of these studies were carried out in laboratory-scale experiments using small amounts of substrate. These investigations applied various growing conditions, e.g. several types of substrate (wheat or rice straw, saw dust, cotton production and other agricultural lignocellulosic wastes, etc.) with different pre-treatments: pasteurisation, sterilisation, nitrogen-source supplement, etc. (Elisashvili *et al.* 2003; Velázquez-Cedeño *et al.* 2004; Savoie *et al.* 2007; Isikhuemhen & Mikiashvilli 2009; Kurt & Buyukalaca 2010; Rodrigues da Luz *et al.* 2012). Rühl *et al.* (2008) presented the lignocellulolytic enzyme activity pattern of oyster mushroom using commercial substrate blocks but did not cover the whole cultivation period and monitored only a few enzymes. But none of these studies carried out a comprehensive analysis of colonisation heterogeneity.

The aim of this study was to investigate the lignocellulose degrading enzyme activities of *P. ostreatus* in a large-scale mushroom production facility during the entire period of the cultivation process over two flushes. These activities were

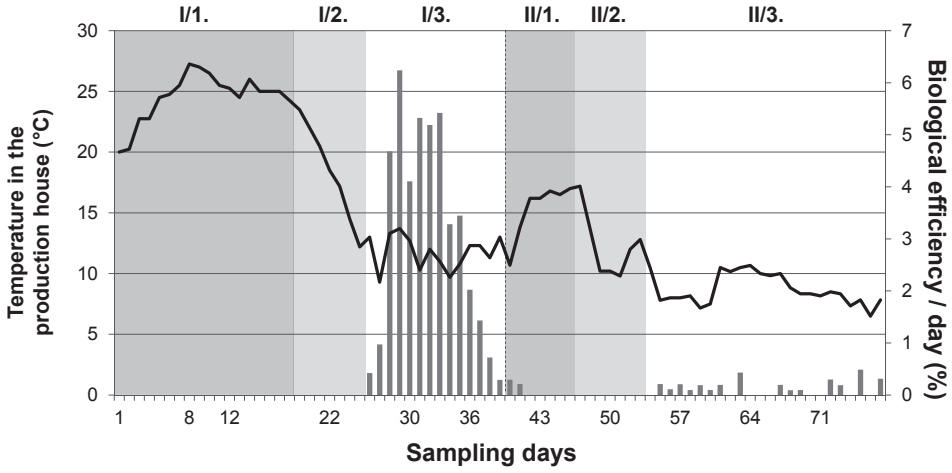

Fig 1 – Stages of the oyster mushroom cultivation process and the 11 sampling days. I/1. vegetative growth; I/2. fruiting body induction; I/3. first flush and harvest; II/1. second vegetative growth; II/2. second fruiting body induction; II/3. second flush and harvest; columns: biological efficiency/day in the production house; black line: temperature during the investigation period.