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Full paper

Cloning of glyceraldehyde-3-phosphate dehydrogenase genes from the basidiomycete mushroom *Pleurotus ostreatus* and analysis of their expression during fruit-body development

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ABSTRACT

Full-length cDNAs of three genes encoding glyceraldehyde-3-phosphate dehydrogenases (GPDs), *PoGPD1*, *PoGPD2-1*, and *PoGPD2-2*, and their corresponding genomic DNA were isolated from the basidiomycete mushroom *Pleurotus ostreatus* strain H1. The deduced amino acid sequences of PoGPD1, PoGPD2-1, and PoGPD2-2 had a high degree of similarity to known GPDs of other organisms, especially basidiomycetes. Similar to GPDs of other organisms, the three PoGPDs consist of the GPD NAD^+-binding domain, the GPD C-terminal catalytic domain, and conserved amino acid domains responsible for catalysis, cofactor binding, and substrate binding. Genomic sequence analysis revealed that *PoGPD1*, *PoGPD2-1*, and *PoGPD2-2* contained seven, eight, and seven introns, respectively, with highly conserved positions. Comparison of the genomic sequences of *PoGPD2-1* and *PoGPD2-2* in the dikaryon of strain H1 and monokaryons of strain H1 revealed that *PoGPD2-1* and *PoGPD2-2* are different alleles of *PoGPD2*. Real-time quantitative reverse-transcription-PCR revealed that the expression patterns of *PoGPD1* and *PoGPD2* differed during fruit-body development. Specifically, transcript levels of *PoGPD1* were high in the primordial stage, while transcript levels of *PoGPD2* were high in the mycelial stage. Furthermore, levels of both transcripts were the highest in the base of mature fruit bodies.

1. Introduction

Edible mushrooms, the fruit bodies of basidiomycetes, are commercially cultivated in many countries and are used as food, food flavoring, and medicine (Kües and Liu 2000). The fruit bodies are the largest and most complex differentiated structures in the fungal kingdom (Deacon 2006). Therefore, their development is thought to be equally complex and accompanied by dynamic metabolic changes. During commercial production of mushrooms, environmental factors such as temperature, humidity, light, and carbon dioxide concentration are tightly controlled (Kües and Liu 2000).

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Nevertheless, the precise mechanism of fruit-body development remains unclear, and a better understanding could improve commercial production of various mushrooms. In order to elucidate the precise molecular mechanism of fruit-body development, previous studies have used various molecular techniques to identify and characterize a large number of genes that are differentially expressed at various developmental stages among several mushrooms. These mushrooms include *Agaricus bisporus* (Ospina-Giraldo et al. 2000), *Agrocybe aegerita* (Wang et al. 2013), *Antrodia cinnamomea* (Chu et al. 2008), *Coprinopsis cinerea* (Cheng et al. 2013), *Lentinula edodes* (Chum et al. 2011), *Ganoderma lucidum* (Luo et al. 2010), *Flammulina velutipes* (Yamada et al. 2006), *P. ostreatus* (Joh et al. 2007), and *Tuber borchii* (Gabella et al. 2005). The comprehensive analyses of these genes have provided valuable genetic and biochemical information for future studies on fruit-body differentiation in basidiomycetes.

Glyceraldehyde-3-phosphate dehydrogenase (GPD, EC 1.2.1.12.) is a key enzyme in both glycolysis and gluconeogenesis (Harris and Waters 1976). It catalyzes the oxidation and phosphorylation of glyceraldehyde-3-phosphate to 1,3-bis-phosphoglycerate. The GPD gene is usually expressed at a high level. In *Saccharomyces cerevisiae* and other higher eukaryotes, the GPD protein comprises up to 5% of the total soluble cellular protein (Krebs 1953; Piechaczyk et al. 1984). In *S. cerevisiae*, GPD mRNA comprises 2–5% of the total poly(A)$^+$ RNA (Holland and Holland 1978). Furthermore, the GPD gene is expressed constitutively. Hence, the GPD gene is thought to be a housekeeping gene and has been used as an internal reference gene for real-time quantitative reverse-transcription (RT)-PCR (Banerjee et al. 2008; Kamei et al. 2008; Kittl et al. 2008). However, previous studies have demonstrated that the expression of GPD genes is regulated by several cellular conditions in some fungi. For instance, under conditions of environmental stress such as high salt content and cold, GPD gene expression is up-regulated in *Lentinus sajor-caju* (Jeong et al. 2000) and *Rhodosporidium toruloides* (Liu et al. 2013). During cellular growth and differentiation, GPD gene expression is induced during the transition from mycelium to yeast in *Paracoccidioides brasiliensis* (Barbosa et al. 2004), while GPD gene expression is repressed during conidiation and mycoparasitism in *Trichoderma harzianum* (Puyesky et al. 1997). In addition, the GPD gene is specifically expressed in asexual reproductive organs of *Pilobolus crystallinus* (Kubo 2012). These findings raise the question of whether GPD gene expression is regulated during the developmental process in basidiomycetous fruit bodies. To our knowledge, only one study has investigated GPD gene expression in mushrooms during fruit-body development; that study investigated the *L. edodes* GPD gene (Hirano et al. 1999), and little is known about its function.

Pleurotus ostreatus, commonly known as the oyster mushroom, is a wood-rotting basidiomycete that is a widely cultivated edible mushroom, similar to the button mushroom *A. bisporus* (Kües and Liu 2000). Because *P. ostreatus* can be easily cultivated in laboratories, this mushroom is a useful model for investigating gene expression during fruit-body development. In the present study, three GPD genes, designated *PoGPD1*, *PoGPD2-1*, and *PoGPD2-2*, were isolated from *P. ostreatus* strain H1 in order to better elucidate fruit-body formation in the mushroom. Here, we report the genomic structures of three PoGPD genes, their deduced amino acid sequences, and their expression profiles during fruit-body development.

2. Materials and methods

2.1. *Fungal strain and culture conditions*

A commercial dikaryotic strain of *Pleurotus ostreatus* strain H1 (Onuki Kinjin, Utsunomiya, Japan) was used in this study. In

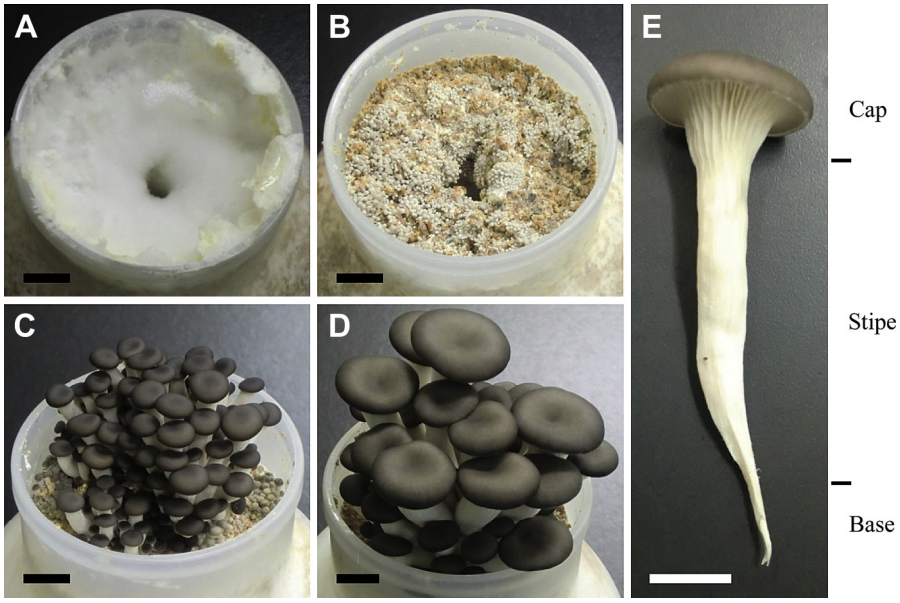

Fig. 1 – Four stages of fruit-body development of *Pleurotus ostreatus* strain H1 in culture bottles containing sawdust medium and the three parts of the mature fruit body. A: Mycelia. B: Primordia. C: Young fruit bodies (3–6 mm in cap diameter). D: Mature fruit bodies (15–20 mm). E: Cap, stipe, and base of the mature fruit body. *Bars*: 1 cm.