Cloning and molecular characterization of glyceraldehyde-3-phosphate dehydrogenase gene from thermotolerant mushroom, *Lentinus polychrous*

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This study describes the cloning and expression analysis of the heat shock protein gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in thermotolerant mushroom *Lentinus polychrous*, one of the most widely cultivated commercial edible mushroom in Thailand. The complete GAPDH coding sequence contained 1,011 bp, encoding for a polypeptide of 337 amino acid residues with the calculated molecular mass of 36.2 kDa and pI of 5.64. Southern blot analysis revealed the presence of a single copy of the GAPDH gene in the *L. polychrous* genome. The putative amino acid sequence of the *L. polychrous* GAPDH-encoded protein shared significant sequence identity with other GAPDH proteins from basidiomycetes. Phylogenetic analysis clustered the *L. polychrous* GAPDH protein with other homobasidiomycetes. Expression analysis of the GAPDH gene by RT-PCR showed that this gene was highly induced not only by heat, but also by cold, ethanol and salt stresses. The GAPDH gene was expressed in both mycelia and fruiting bodies, suggesting that the GAPDH gene product is a heat shock protein which might be involved in the developmental phase of the *L. polychrous*.

Key words: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), *Lentinus polychrous*, heat shock protein gene, basidiomycetes, gene expression.

INTRODUCTION

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) is one of the key enzymes in glycolysis and gluconeogenesis. In the former pathway, it catalyses the oxidative phosphorylation of glyceraldehydes-3-phosphate into 1,3-biphosphoglycerate in the presence of nicotinamide adenine dinucleotide and inorganic phosphate, and in the latter pathway, it catalyses the reverse reaction (Niu et al., 1994; Hirano et al., 1999). In addition to these long established metabolic function, GAPDH has recently been implicated in several non-metabolic processes such as surface antigen (Goudout-Crozel et al., 1989), membrane transport and fusion, nuclear RNA transport (McDonald and Moss, 1993; Singh and Green, 1993; Sirover, 1999), transferrin-binding protein (Modun and Williams, 1999), transcription activation, initiation of apoptosis (Tarze et al., 2007) and cell surface protein to assist the adhesion of pathogen on host tissue (Barbosa et al., 2006; Egea et al., 2007). The GAPDH is a tetramer composed of identical subunits.

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Abbreviations: GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction; HSPs, heat shock proteins; PDA, potato dextrose agar; CTAB, cetyl trimethyl ammonium bromide; ORF, open reading frame; PSD, phosphatidylserine decarboxylase.
cellular proteins (Redkar et al., 1998; Hirano et al., 1999). These observations suggest that the GAPDH gene is controlled by a highly active promoter. The promoter sequences of native GAPDH-encoding genes have proven useful for efficient expression of heterologous genes in several yeasts and fungi such as \textit{Pichia pastoris} (Vassileva et al., 2001; Menendez et al., 2004), \textit{Mucor circinelloides} (Wolff and Arnau, 2002), \textit{Lentinus edodes} (Nitta et al., 2004) and \textit{Aspergillus niger} (Halaouli et al., 2006).

\textit{Lentinus polychrous}, commonly known as hed kradang, is one of the most widely commercial cultivated edible mushrooms of the Northeast and Northern part of Thailand. This basidiomycetous mushroom is classified in the order Poriales, family Lentinaceae (Rusakawong and Fliegel, 2001). In addition to high concentration of protein, carbohydrate, calcium and phosphorus, this mushroom also contains high quantities of special substances, referred to as nutriceuticals, such as eritadenine, germanium and ergosterol. These nutriceutical compounds can modulate the immune system, lower blood pressure, reduce cholesterol and act as antithrombotic (Pegler, 1983; Waser and Weis, 1997).

Unlike \textit{Lentinula edodes} (formally known as \textit{L. edodes}) or shiitake mushroom in which fruiting body is typically induced by cold shock at 4°C, the formation of fruiting body in \textit{L. polychrous} is activated by heat shock at 40-45°C (Nariso, 2004). It is well known in a wide variety of organisms that this circumstance favors the synthesis of a specific set of proteins, known as heat shock proteins (HSPs) (Lindquist and Craig, 1988). In a previous study, we found that heat shock conditions induce the synthesis of several heat shock proteins in this mushroom (unpublished results). Among them, a protein with the apparent molecular mass of 36 kDa was chosen and analyzed. N-terminal amino acid sequences analysis revealed that this protein is GAPDH which has been previously reported as a heat shock protein in \textit{Saccharomyces cerevisiae} (Thanonkeo et al., 2003). Although gene encoding this enzyme has been cloned and characterized in several filamentous fungi such as \textit{Cryphonectria parasitica} (Choi and Nuss, 1990), \textit{L. edodes} (Hirano et al., 1999), \textit{M. circinelloides} (Wolff and Arnau, 2002), \textit{Phaeosphaeria nodorum} (Ueng et al., 2003), \textit{Pleurotus sajor-caju} (Jeong et al., 2000), \textit{Rhizomucor miehei} (Vastag et al., 2004), \textit{Ganoderma lucidum} (Fei et al., 2006), \textit{Penicillium marneffei} (Thirach et al., 2008), \textit{Moniliophthora perniciosa} (Lima et al., 2009) and \textit{Trichoderma virens} (Oh et al., 2009), to our knowledge this is the first study to isolate and characterize the GAPDH gene in \textit{L. polychrous}. We report here that GAPDH is a heat shock protein gene that might be involved in the developmental phase of this mushroom.

\section*{MATERIALS AND METHODS}

\textbf{Strain, culture conditions and plasmid}

\textit{L. polychrous} KPM3 isolated from national park at Puvieeng, Khon Kaen (Nariso, 2004) was used in this study. The culture was maintained on potato dextrose agar (PDA) at 4°C and subculturing was performed every 4 weeks. \textit{Escherichia coli} DH5α was used for gene transformation. It was grown in LB medium at 37°C. Vector used for gene transformation was pGEM T-easy vector (Promega, Madison, WI, USA). All molecular cloning techniques were carried out as described by Sambrook and Russell (2001).

\section*{Genomic DNA and total RNA isolation}

Mycelium from \textit{L. polychrous} cultures grown in potato dextrose broth at 30°C for 7 days was harvested by filtration through four layers of cheesecloth, immediately frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. Genomic DNA was isolated from the fungal mycelia using the CTAB method (Russo et al., 1992), whereas total RNA was extracted from this fungal mycelia using RNeasy Plant Mini Kit (QIAGEN, Germany). The extraction of total RNA was carried out essentially as recommended by the manufacturer's instruction except that RNase-free DNase I was added to a final concentration of 0.1 mg/ml to remove any contamination of genomic DNA.

\section*{Cloning and sequencing of GAPDH gene}

An internal fragment encoding the amino acid sequence of the GAPDH gene product was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using total RNA isolated from \textit{L. polychrous} as a template and degenerated oligonucleotide primers synthesized based on the GAPDH gene from other basidiomycetous fungi in the GenBank database. The sequences of the two degenerated oligonucleotide primers were forward GAPDH-Flen (5'-TACATGGTGYTACATGGTCAA-3') and reverse GAPDH-Rlen (5'-CTTTGTCGTACCAGTCAA-3'). The RT-PCR reaction was carried out using OneStep RT-PCR Kit (QIAGEN, Germany).

The reaction mixture (50 μl) consisted of 10 μl of 5xQIAGEN OneStep RT-PCR buffer, 400 μM dNTP, 0.6 μM of each forward and reverse primer, 2 μl of QIAGEN OneStep RT-PCR enzyme mix and 1 μg of RNA template. The RT reaction was carried out at 45°C for 45 min. Following an initial denaturation of the template cDNA at 95°C for 15 min, 35 cycles of the following temperatures were used: denaturation at 94°C for 1 min, annealing at 48°C for 2 min and extension at 72°C for 5 min. The amplified product was separated on 0.7% agarose gel and target DNA was excised from the gel and purified using the NucleoSpin Extract II Kit (Macherey-Nagel, Germany).

The purified PCR product was cloned into pGEM T-easy vector (Promega, Madison, WI, USA) and the resulting plasmid was transformed into the competent \textit{E. coli} JM109 using the Rapid DNA Ligation and Transformation Kit (Fermentas, USA). The plasmid DNA was isolated from a positive clone and target DNA was sequenced by the dideoxy chain termination methods using the DNA was isolated from the fungal mycelia using the CTAB method (Russo et al., 1992), whereas total RNA was extracted from this fungal mycelia using RNeasy Plant Mini Kit (QIAGEN, Germany). The extraction of total RNA was carried out essentially as recommended by the manufacturer's instruction except that RNase-free DNase I was added to a final concentration of 0.1 mg/ml to remove any contamination of genomic DNA.

The complete full length open reading frame (ORF) of the GAPDH gene was confirmed by RT-PCR using the forward and reverse primers synthesized based on the known sequences of the 5'- and 3'-end of the GAPDH gene. These two primers were forward GAPDH-Flen (5'-ATGCGCGTCAAAATGCGTATC-3') and reverse GAPDH-Rlen (5'-TTTTGTCTACCCCTTGAGATT-3'). After RT-PCR reaction, the amplified product was separated on a 0.7% agarose gel, purified, cloned and sequenced as previously described. The sequence of the GAPDH gene and deduced amino acid sequence was analyzed using GENETYX (Software Development,
Tokyo, Japan), while homology searching was performed using FASTA and BLAST program in the GenBank and DDBJ databases. The nucleotide sequence reported in this study was deposited in GenBank under the accession number GU356537.

Southern hybridization analysis

The GAPDH gene in the L. polychrous genome was investigated by Southern blotting. Genomic DNA (20 μg) was digested with SalI, PstI, HindIII, BanHI and EcoRI, electrophoresed on 0.7% agarose gel and transferred to Hybond N+ positively charged nylon membrane (Schleicher and Schuell) by overnight capillary transfer. The DNA was cross-linked to the membrane by UV irradiation and baked at 80°C for 2 h. All procedures were performed according to the standard methods as described by Sambrook and Russell (2001).

To prepare a probe for Southern hybridization analysis, an internal fragment encoding the amino acid sequence of the GAPDH gene product was amplified by RT-PCR using total RNA isolated from L. polychrous as a template and forward GAPDH-Flen and reverse GAPDH-Rlen primers. The GAPDH PCR fragment was alkaline phosphatase labeled using Gene Images AlkPhos Direct Labeling and Detection System (AlkPhos DirectTM, Amersham). All procedures for DNA labeling were carried out as recommended by the manufacturer.

The Hybond N+ positively charged nylon membrane with cross-linked DNA was prehybridized at 50°C for 2 h in hybridization oven (Hybrid limited equipment class I) with gentle rotation. After incubation, the labeled-DNA probe was added and subsequently incubated at 55°C an overnight. The membrane was washed according to the standard procedure for Southern blot analysis. After washing, the CDP-Star detection reagent (AlkPhos DirectTM, Amersham) was added onto the membrane and left at room temperature for 5 min. The membrane was then exposed to X-ray film (Hyper film, Amersham) for 1 h, thereafter it was developed and fixed with developer and fixer solution (Kodak), respectively.

RT-PCR analysis

The expression level of the L. polychrous GAPDH gene was determined by RT-PCR. Total RNA was isolated from fungal mycelia grown under various stress conditions including heat shock (45°C), cold shock (10°C), ethanol shock (6% v/v) and salt stress (1 M NaCl) for 6 h using RNeasy Plant Mini Kit (QIAGEN, Germany). In order to determined the possible physiological role of GAPDH gene in the formation of fruiting bodies of L. polychrous, total RNA was extracted from fungal fruiting bodies and used as template in RT-PCR analysis. RT-PCR was carried out using OneStep RT-PCR Kit (QIAGEN, Germany) with the forward GAPDH-F1len and reverse GAPDH-R1len primers synthesized based on the 5’- and 3’-region of the L. polychrous GAPDH gene. The reaction mixture (50 μl) consisted of 10 μl of 5xQIAGEN OneStep RT-PCR buffer, 400 μM dNTP, 0.6 μM of each forward and reverse primer, 2 μl of QIAGEN OneStep RT-PCR enzyme mix and 1 μg of RNA template. As a control, 10 μl samples of total RNA were subjected to agarose gel electrophoresis (0.9% agarose) and stained with ethidium bromide. Actin gene was used as an internal control. A thermocycler (Hybrid limited equipment class I) with gentle rotation. After staining with ethidium bromide, the relative amounts of the products were compared using the Gel Image Master (Pharmacia Biotech). The experiment was repeated at least twice. Under these conditions, the OneStep RT-PCR was able to specifically detect mRNA because no band was observed when reverse transcriptase was omitted.

RESULTS AND DISCUSSION

Cloning of the L. polychrous GAPDH gene

Using degenerated oligonucleotide primer GAPDH-Flen (coding for YMVYMFK) and GAPDH-Rlen (coding for IAWYDNE), a 0.8-kb internal fragment of the GAPDH gene was obtained from L. polychrous. This fragment was purified and ligated into pGEM T-easy vector for nucleotide sequencing analysis. DNA sequence of this fragment showed high homology to other GAPDH genes of basidiomycetous fungi (data not shown). To isolate full-length GAPDH cDNA, RT-PCR amplification using the SMART™ RACE cDNA Amplification Kit (Clontech, USA) was performed using total RNA isolated from mycelia of L. polychrous as a template. Nucleotide sequencing analysis revealed that the resulting 1.0-kb PCR product was full-length GAPDH cDNA of L. polychrous.

Sequence analysis of the L. polychrous GAPDH gene

The L. polychrous GAPDH cDNA contained 1,011 bp of the coding sequence, encoded a protein of 337 amino acid residues (Figure 1). A typical translation initiation codon (ATG) and translation termination codon (TTA), the most frequently found codon in filamentous fungi, were identified in the GAPDH cDNA, indicating a full-length coding sequence of the gene. The calculated molecular mass of the deduced polypeptide is 36.2 kDa and the predicted isoelectric point is 5.64. Sequence analysis revealed the presence of a potential site for substrate binding (ASCTTNCF), as described for GAPDH sequence (Goudot-Crozat et al., 1989), at position 149-156. Amino acids potentially associated with catalysis were found at amino acid positions 151 (C) and 178 (H). Potential phosphorylation sites were located at positions 102-106, 183-185, 191-194, 240-242, 245-250, 292-295, 311-314 and 321-329. The amino acid residues at positions 34 (D) and 315 (N) corresponded to the putative nicotinamide adenine dinucleotide binding sites. Amino acids at positions 150 (S), 152 (T), 196 (R), 210 (T) and 225 (G) were found to be probable sites for inorganic phosphate binding. Positions 181, 233 and 247 were found to be amino acid residues that putatively related to the binding of the phosphate from the substrate (T, R and R) (Thirach et al., 2008).

The fungal GAPDH genes contained diverse number of introns and the average number of introns in GAPDH genes of Basidiomycetes is higher than that of Ascomycetes (Harmsen et al., 1992). It has been reported that Agaricus bisporus GP1, A. bisporus GP2, L. edodes GPD, M. pernicioso GAPDH, Panerchaeta chrysosporium GPD, G. lucidum GPD and Schizophyllum
ATGGCCGTCAAAAGTCCGTATACACCGGAATTCGGGTATCGGACGTACCGTCCTCAGGAAT
M A V K G I N G F G R I G R T V L R N
GCTCTCTCCACACGGAACATCAACGTGTCGGCGGTATGACGCCCTTTCTGACCTCGAAT
Y M V Y M F Y D S V H R F K G T V
GCTAAGGAAGCCGACCTGTGTCGGAGAAAGCCCATCTCCGTCTCAGGAGGAAGAT
A K D G K L V L V G K P I S F E E K D
CCGCCAACAAATCAACTGGGCTCTTCTGGCGGTAGTACATCTGACGTCACGCGGTGTC
P A N I W S A G E Y I V E S T G V
TTCACCACACAGAAAAGCTCTTCGATTTGGAAGGCGGCTCCAAGAGGTCTAATCAT
F T T E K A S A H L K G G K K V I I
TCTGGCCCTCCGTATGCGCCAAATTCTGCTGCGTGCTGCAACTTGGAAGCTGTCGAG
S A P A D A M F V C G V N L E A Y D
CCGGAAGGCAAGCTATGCGGCACTTTCTGACCCACCAACTGCTGCGGCCCTCGCA
P K Y D V I S N (A T C N F) A P L A
AAGCTCATACAGAIAATCCTGGCAAGGCGCTCTAGACCCGCGCATCGCCCTCGCA
K V I H D N F G I V E G L M T T V R A T
ACCGCCAACAGCAGGCGACGTCGTCGGCGCTGACAAGGACTGGGCTGGAGGCGGTCGC
T A T Q R T V D G S P H K D W F G G R
GTTGGCAGAACACATATCCCTCCGTCTGACGTCGCGCGCAAGCGCGCGAAGTCATC
V G N N I I F S G A A A K A V G K V I
CCCGACTGACAGCGGCTACGGGAGCTCGGCGCTGACGTCGACGGGTGACTCC
P S L N G K L T G M S F R V P T I D V S
GTCGTCGACCTGCTGTCGCGCTCGAGAGGCTCGACGAGATCGAGAGAGCGGCCTCC
V V D L V V R L E K P A T Y D E I K D A
ATCAAGGCAGCGCCGCGCGCCTTTCAGGGGATTCCTGACTACCCGAGGGAAGGTC
I A A A A G P F K G I L D Y T E E K V
GTGTCGAGCAGCCTACCCGGCAACGACGGACATCTTGCTGATGCTGAGCCCGAATC
V S T D F T G N D A S S I F D A E A G I
GCCGCTAACAACAACATTTGTCAAGCTGATTGCGAATGAATGGGATATCC
A L N N N F V K L I A W Y D N E W G Y S
CAGCGTCGTCGACCTGTCGACTACGCTGCGAAACAGGATGGGAAACTCTAA
H R V C D L V Y A A Q D G N L *

Figure 1. Nucleotide and deduced amino acid sequences of the L. polychrous GAPDH gene. Nucleotides are numbered from the first nucleotide from 5' end of the sequence. Amino acids are indicated below the nucleotide sequence in single-letter codes. Translation stop codon is indicated by an asterisk. The sites putatively related to inorganic phosphate binding are indicated by boxes around the amino acids. Amino acids potentially associated with catalysis are in shaded boxes. Amino acid residues related to the binding of phosphate from substrate are in bold. Amino acid putatively related to the NAD$^+$ binding is in bold italic. The substrate-binding site is marked with a bracket. The putative phosphorylation sites are indicated by underlines.

commune GPD contained nine, nine, eight, eight, six, six and five introns, respectively (Smith and Leong, 1990; Harmsen et al., 1992; Fei et al., 2006; Thirach et al., 2008; Lima et al., 2009). However, a rare intron organization has also been reported for the GAPDH genes of A. nidulans, Claviceps purpurea, Blumeria graminis f. sp. hordei and Pseudozyma flocculosa, with one intron positioned outside the structural region (Punt et al., 1988; Christiansen et al., 1997; Neveu et al., 2007). Although the number of introns may vary in different GAPDH genes, some of the intron positions seem to be strongly conserved between the Basidiomycetes and Ascomycetes GAPDH genes (Harmsen et al., 1992).

Apart from their evolutionary significance, introns from GAPDH genes may have specific functions, e.g., involvement in the protein expression (Kuo and Huang, 2008) or important for identification of some ectomycorrhizal Basidiomycetes (Kreuzinger et al., 1996). With respect to the number and possible physiological roles of intron in the L. polychrous GAPDH genes, further studies such as cloning and sequencing of the genomic DNA and target disruption of the GAPDH gene have to be carried out in order to clarify these information and these are now under our investigation.

Southern blot analysis

Southern hybridization of digested L. polychrous genomic DNA with the PCR fragment of the GAPDH gene revealed
like that found in the GAPDH proteins of sporium region was presented in this putative protein (Figure 3),


functions as the binding site of the enzyme in the catalytic comparison of the amino acid sequence of the copy of the 2009) and (Hirano et al., 1999), (Neveu et al., 2007), (Kuo et al., 2007), Harmsen et al., 1992), also suggesting that the relationship between filamentous Ascomycetes and Basidiomycetes share a relatively recent common ancestor (Smith, 1989; Lima et al., 2009; Oh et al., 2009). Studies by Harmsen et al. (1992) also suggesting that the relationship between GAPDH of filamentous Ascomycetes and Basidiomycetes is higher and quite distinct from Ascomycetes yeasts.

a single band in each digestion (Sal, HindIII, BamHI and EcoRI), except that for PstI digestion in which two sites of restriction enzyme might be present, suggesting that the GAPDH gene is present as a single copy in the genome of L. polychrous (Figure 2). This result agrees with that of A. nidulans (Punt et al., 1988), S. commune, P. chrysosporium, A. bisporus (Harmsen et al., 1992), L. edodes (Hirano et al., 1999), Flammulina velutipes (Kuo et al., 2004), P. flocculosa (Neveu et al., 2007), Beauveria bassiana (Liao et al., 2008), M. perniciosa (Lima et al., 2009) and T. virens (Oh et al., 2009) in which a single copy of the GAPDH gene has been reported.

Comparison of the amino acid sequence of the L. polychrous GAPDH gene with GAPDH genes of other fungi

The L. polychrous GAPDH protein showed high sequence identity to the known fungal GAPDH sequences from Basidiomycetes: 88% identity with the GAPDH of P. chrysosporium and G. lucidum, 82% with Volvariella volvacea, 81% with L. edodes, 78% with S. commune and 77% with P. sajor-caju. The L. polychrous GAPDH protein was also highly similar to the GAPDH protein of some plant and animals (data not shown). The main conserved amino acid residues, that is, Cys-151, which functions as the binding site of the enzyme in the catalytic region was presented in this putative protein (Figure 3), like that found in the GAPDH proteins of P. chrysosporium, G. lucidum, S. commune, L. edodes and V. volvacea. However, this catalytic amino acid residue was changed in P. sajor-caju, Ser instead of Cys. This difference might be due to strain variation. The residues surrounding the Cys-151 active site, Ala-Ser-Cys151-Thr-Thr-Asn-Cys, also matched the consensus sequence that is conserved in prokaryotic and eukaryotic GAPDH proteins (Olsen et al., 1975).

Phylogenetic analysis

An evolutionary tree based on the GAPDH amino acid sequence of 45 species representative of Ascomycetes yeasts, filamentous Ascomycetes, Zygomycetes and Basidiomycetes was performed using the neighbor-joining method (Saitou and Nei, 1987). The results revealed a separation of the Basidiomycetes, Zygomycetes and Ascomycetes, and confirmed that the L. polychrous GAPDH clustered with other homobasidiomycetes (Figure 4). The close relationship between filamentous Ascomycetes and Basidiomycetes GAPDH proteins in comparison to those of Ascomycetes yeasts was in agreement with earlier studies and may be due to the fact that both filamentous Ascomycetes and Basidiomycetes share a relatively recent common ancestor (Smith, 1989; Lima et al., 2009; Oh et al., 2009). Studies by Harmsen et al. (1992) also suggesting that the relationship between GAPDH of filamentous Ascomycetes and Basidiomycetes is higher and quite distinct from Ascomycetes yeasts.

Differential expression of the L. polychrous GAPDH gene

To investigate whether or not the GAPDH gene becomes activated by environmental stresses such as heat, cold, ethanol and salt stresses, L. polychrous mycelia that had been cultured in PD broth for 7 days at 30°C were transferred to high temperature (45°C), low temperature (10°C), ethanol (6% v/v) and NaCl solution (1 M) for 6 h. The change of GAPDH expression was detected by RT-PCR using RNA isolated from various stress-treated L. polychrous mycelia. All four stress conditions increased the expression level of GAPDH gene (Figure 5a). The heat and cold treatments increased the expression level of GAPDH gene approximately six-fold, whereas ethanol and salt treatments increased the expression of the gene about three-fold, as compared to the control condition. This result agrees with that of P. sajor-caju in which the expression of GAPDH gene is highly induced not only by heat but also by salt, dehydration and cold stresses (Jeong et al., 2000), suggested that the GAPDH is stress-responsive gene. Heat shock is known to alter expression of glycolytic genes, suggesting that primary carbon metabolism is one of the pathways that respond to changing environmental condition (Yang et al., 1993; Laxalt et al., 1996). These findings suggested that the
Figure 3. Multiple alignment of the deduced amino acid sequence of the \textit{L. polychrous} GAPDH and GAPDH protein from \textit{G. lucidum} (DQ404343), \textit{P. chrysosporium} (AB272086), \textit{S. commune} (M81724), \textit{L. edodes} (AB012862), \textit{V. volvacea} (DQ140384) and \textit{P. sajor-caju} (AF087676). Amino acid residues identical and similar between each other are shown by asterisks and dots, respectively. Gaps introduced to maximize the alignment are indicated by a horizontal dash. The conserved active site cysteine residue that is important for the binding of glyceraldehydes-3-phosphate dehydrogenase in the catalytic site is shown by bold letter.
gene is strongly expressed in both mycelia and fruiting bodies (Hirano et al., 1999). Similar experiment was performed in this study using RNA isolated from mycelia and fruiting bodies of *L. polychrous* cultured in PD broth as described by Nariso (2004), as template for RT-PCR analysis. As shown in Figure 5b, the GAPDH gene was expressed in both mycelia and fruiting bodies of *L. polychrous*, suggesting that the GAPDH gene product is a heat shock protein involved in the developmental phase of the fungus.
The formation of fungal fruiting bodies is complex, and several candidate genes such as alcohol dehydrogenase, pyruvate decarboxylase, glucose-6-phosphate isomerase, citrate synthase, serine/threonine protein kinase, riboflavin aldehyde-forming enzyme gene and phosphatidylserine decarboxylase (PSD) genes have been proposed to be involved in this process, since their expressions are specifically expressed in primodia or fruiting bodies of the fungi (Hirano et al., 2004; Otto et al., 2004; Miyazaki et al., 2005). Some of them have been reported as the heat shock protein genes such as alcohol dehydrogenase and pyruvate decarboxylase (Miyazaki et al., 2005). Since the GAPDH gene is expressed in fruiting bodies of the *L. polychrous*, it might play specific roles in the course of fruiting bodies development. We can not determine the biological role of this gene at the present, but the direct involvement of the GAPDH gene in fruiting bodies formation could be elucidated by gene disruption experiments or the introduction of a promoter which enables the over-expression of the GAPDH gene in this mushroom.

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