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Several Genes Expression on Fructification of *Lentinula edodes*

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Lentinula edodes was cultivated in the artificial blocks of sawdust, and the fruiting body was induced on the surface of the sawdust blocks. The four different fungal stages; mycelia (D), primordia (C), stipes (B), and pileus (A) of fruiting bodies were collected for the extraction of mRNA, searching the genes related to morphogenesis. The three fragments of cDNA not showed in the stage (A) were picked up and displayed, to be two bands in the agarose gels. Total five bands were selected for analyzing the DNA sequences, using the pRIP–T™ vector system. The fragments of cDNA investigated in this work were not those related to the structural genes (exons), but to be related to the controlling genes (introns).

INTRODUCTION

The mushroom of *Lentinula edodes* (Berk.) Pegler (shiitake) is a wood rotting edible fungus belonging to the family of Tricholomataceae, and the most popular cultivated mushroom in the world (Chang and Miles, 1991). The traditional cultivation of *L. edodes* mainly uses natural logs, but its production depends on the climatic events, and a whole cycle of productions is very long for approximately five years. As the cultivation of other mushroom, the sawdust blended with the rice bran is developed for a new technique. After sterilization, each bags of substrate mentioned above is inoculated with the spawn and cultivated for 3–4 months (Diehle and Royse, 1991; Ohga *et al.*, 1992; Lee *et al.*, 2000). The mycelia of *L. edodes* form a compact mat on the sawdust solid substrate. In the presence of environmental induction (stress) such as temperature, humidity and light, which would often stress the mycelia, induce the hyphae to form primordium, which may subsequently develop into the fruiting bodies.

Understanding the control of fruit body initiation and maturation would be critical for the cultivation of *L. edodes* (Leung *et al.*, 2000 Ohga *et al.*, 2000b;). Studies on *L. edodes* have been focused mainly on the sawdust medium under the artificial cultivation (Kim *et al.*, 1987; Ohga *et al.*, 1992), the physiology and improvement of substrate (Park *et al.*, 1992; Ohga *et al.*, 2000b), selective breeding (Bak *et al.*, 1996), the cultivation in liquid media (Song *et al.*, 1987). Recently, molecular biological techniques have been applied to the study of *L. edodes* or other mushrooms, including with classification of isolate (Chiu *et al.*, 1996; Lee *et al.*, 1997; Park *et al.*, 1997; Lee *et al.*, 1999; Ohga *et al.*, 1999; Ohga *et al.*, 2000a) and isolations of the differentially expressed genes (Zhang *et al.*, 1998; Leung *et al.*, 2000). Molecular studies of *L. edodes* have been focused on

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identifying the genes involved in the regulation of fruit body development (Lee *et al.*, 1996; Ng *et al.*, 2000). The gene is known to express only in primordium and immature fruiting bodies. The novel *priA* gene is actively transcribed at the state of primordia/immature fruiting bodies in *L. edodes* (Kajiwara *et al.*, 1992). The novel *priBc* transcript is reported to be abundant in primordia, while pre-primordial mycelia and mature fruiting bodies contained lower levels of this gene transcript (Endo *et al.*, 1994). The expression of β subunit of mitochondrial processing peptidase (β-MPP) gene is higher during the development of the fruiting bodies, suggesting that higher mitochondrial activities may be required to meet the energy demand in the rapid growth of the fruiting bodies (Zhang *et al.*, 1998). Transcript level of *Le hyd1* is high in the primordium (Ng *et al.*, 2000). In other *L. edodes*, developmental genes are expressed specifically in the fruit body; The *mfbAc* transcript is abundant in mature fruiting bodies, detectable in immature fruiting bodies but absent in earlier developmental stages and in the vegetative mycelium (Kondoh *et al.*, 1995; Ng *et al.*, 2000).

The purposes of this study were subjected i) to identify, and ii) determine the differentially expressed genes in total RNA populations from three morphogenesis stages of *L. edodes* including mycelia, primordia and fruiting body, two tissues of stipe and pileus using the differential display reverse transcription–polymerase chain reaction (DDRT–PCR) (Liang and Pardee, 1992; Haag and Raman, 1994). Several genes differently expressed were detected and analyzed for further works.

MATERIALS AND METHODS

Organism and culture

A line of *L. edodes* (ImHyup–1; very commonly produced in the oak logs in Korea) was obtained from National Forestry Cooperatives Federation (NFCF) in Korea used for this purpose. The cultures of *L. edodes* were maintained on PDA (Potato Dextrose; Difco) at 25 °C. The blocks of PDA grown by *L. edodes* were cultured in synthetic liquid medium (glucose 20 g, yeast extracts 2 g, tryptone 2 g, KH_2PO_4 1 g, $MgSO_4$ 0.75 g, $FeSO_4$ 0.02 g, $ZnSO_4$ 0.02 g, H_2O 1 L) at 25 °C by shaking incubator for the liquid spawn. The mycelia cultured in the liquid broth were inoculated to the 1–1.5 kg of the sterilized oak sawdust (with rice bran by the ratio 80:20) moistured by the approximately 60% before sterilization. The *L. edodes* was grown on sawdust substrate at 25 °C in the dark rooms for three months until the white mycelia was covered in whole surface. To induce fruiting, the substrate blocks were kept in moist and cool room (at 15 °C, for 2 days) under the dim light (Lee *et al.*, 2000). Four stages of mycelial growth were collected for this work; (A): Pileus of fruiting body, (B): Stipe of fruiting body, (C): Primordium from the surface of the sawdust, and (D): Mycelia colonized in the sawdust media shown in Fig. 1.

RNA isolation

The total RNA's from the fungal tissues mentioned above were extracted by the based SDS–Phenol extraction method (Ausbel *et al.*, 1999); all samples (5 g of the fungal tissue and 15 g of the mycelia mixed with the sawdust) were ground in liquid nitrogen using mortars and pestles. The grounded tissues were transferred into the 50 Mℓ centrifuge tube, and the extraction buffer (0.18 M *tris*, pH 8.7, 90 mM LiCl, 4.5 mM EDTA, 1% SDS) were added to the tube. An equal volume of acidic phenol (pH 4.3) were added to and mixed into the homogenized powder. After centrifugation at 12,000 rpm at 4 °C for 10 min, the 5 Mℓ of the top phase was transferred to a new 50 Mℓ centrifuge tube and was added with an equal volume of chloroform. The mixture was shaken vigorously and was centrifuged at 12,000 rpm at 4 °C for 10 min. The 800 $\mu\ell$ of the top phase was, again, transferred to a new eppendorf tube (E-tube) and an equal volume of 4 M LiCl was added to. The tube was placed overnight at -20 °C to precipitate RNA, and centrifuged at 12,000 rpm at 4 °C for 10 min. The supernatant was decanted and the pellet was rinsed with the 2 M LiCl solution and the tube was centrifuged, again. The pellet was washed with the 70% ethanol and dried, dissolved in 50 $\mu\ell$ DEPC-treated water. The RNA sample was treated with the RNA PrepMateTM Lysis buffer and an equal volume of chloroform was added to, again. The same procedure was duplicated with the isopropanol and 70% ethanol, as described, until the pure RNA sample was obtained. The pellet was dissolved in the 100 $\mu\ell$ DEPC-treated water, and was measured by spectrophotometer for RNA concentration. Finally, the bands of RNA's were confirmed by the electrophoresis method, using the formaldehyde agarose gel (Ausbel *et al.*, 1999; Sambrook *et al.*, 1989).

DDRT-PCR

The cDNA corresponding to the RNA's extracted was synthesized from by a reverse transcriptase reaction by using the AccuPowerTM RT PreMix (Bioneer). Each 20 $\mu\ell$ mixture contained 1 μg of total RNA, 1,2,3 M-MLV reverse transcriptase 20 U, RNasin 10 U, stablizer, tracking dye and 20 pmol 3' one anchored oligo-dT primers (Table 1). The reverse transcription was performed at 42 °C for 1 h and the reaction was stopped by incubation at 94 °C for 5 min. The cDNA synthesized was stored at -20 °C for a subsequent PCR reaction. One tenth of the cDNA was then amplified in a total volume of the 20 $\mu\ell$ the PCR mixtures contained 1 U Taq DNA polymerase, the 250 μM dNTP of each, 10 mM Tris-HCl (pH 9.0), 40 mM KCl, 1.5 mM $MgCl_2$, stabilizer, tracking dye (Bioneer), 20 pmol 3' one anchored oligo-dT primers and 20 pmol arbitrary primers. The amplification reaction was done for 1 cycle with 94 °C for 5 min, 40 °C for 30 sec, 72 °C for 30 sec; 30 cycles with 94 °C for 30 sec, 40 °C for 30 sec, 72 °C for 1 min, and an additional extension cycle with 94 °C for 30 sec, 40 °C for 30 sec, 72 °C for 5 min. The 5 $\mu\ell$ of the PCR products were resolved on a 1.5% agarose gel and stained with ethidium bromide for confirmation. The band was photographed with Digital Camera (Kodak).

Table 1. Primers used in the experiment.

Primer	Length	Nucletide sequences
oligo dT/G	16 mer	5' - AAGCTTTTTTTTTTTG - 3'
oligo dT/C	16 mer	5' - AAGCTTTTTTTTTTTC - 3'
oligo dT/A	16 mer	5' - AAGCTTTTTTTTTTTA - 3'
AP 1	13 mer	5' - AAGCTTGATTGCC - 3'
AP 2	13 mer	5' - AAGCTTCGACTGT - 3'
AP 3	13 mer	5' - AAGCTTTGGTCAG - 3'
AP 4	13 mer	5' - AAGCTTCTCAACG - 3'

Electrophoresis

Electrophoresis was performed on the Econo Sequencer I™ Electrophoresis System (Bioneer). The 6$\mu\ell$ of loading dye was added to 15$\mu\ell$ of each DDRT–PCR products and the mixtures were heated for 5 min at 100 °C; the 3$\mu\ell$ mixture was loaded on the sequencing gel. The sequencing gel was prepared, and pre–run at 1600 V until the xylen cyanol dye was reached the bottom of the gel. The gel was stained by silver staining method using a Silverstar™ staining kit (Bioneer) by followed with the supplier's specifications. After electrophoresis, the plate was separated using the plastic wedge. The gel (plate) was placed in a shallow plastic tray, covered with fix/stop solution (10% glacial acetic acid) and agitated for 30 min. The gel was rinsed three times (2 min each) with the ultra pure water using agitation. The gel was transferred to staining solution (0.1% $AgNO_3$, 0.15% formaldehyde) and agitated well for 30 min. The gel was also briefly dipped into the tray containing the ultra pure water. The solution in the tray was drained, and placed immediately into the tray of chilled developing solution. The gel was agitated well until the template band starts to be developed or until the first bands was visible and continued developing for an additional 2–3 min. To terminate the developing reaction and fix the gel, the fix/stop solution was directly added to the developing solution and incu–bated with shaking for 2–3 min. The gel was rinsed twice for 2 min with the ultra pure water and placed on the dried at the room temperature. The PCR–amplified cDNA products were resolved on a 6% poly– acrylamide sequencing gel.

cDNA fragment

The bands representing the cDNA differentially expressed were excised with a scalpel blade. The cDNA fragments were soaked in 100$\mu\ell$ of distilled H_2O (dH_2O) for 10 min, boiled for 15 min and centrifuged for 2 min, and clarified by precipitation (Reeves *et al.*, 1994). The cDNA precipitated were diluted with the buffer in the rate of 1/20. For PCR reactions, the 2$\mu\ell$ dilution cDNA products were used in 20$\mu\ell$ PCR mixtures contained 1 U Taq DNA polymerase, each 250μM dNTP, 10 mM *tris*–HCl (pH 9.0), 40 mM KCl, 1.5 mM $MgCl_2$, stabilizer, tracking dye (Bioneer). Using the same set of 3' one anchored oligo–dT primers in combinations with the arbitrary primer (AP). The amplification was made for 1 cycle with 94 °C for 5 min, 40 °C for 30 sec, and 72 °C for 30 sec; 30 cycle with 94 °C for 30 sec, 40 °C for 30 sec, 72 °C for 1 min and an additional extension cycle with 94 °C for 30 sec, 40 °C for 30 sec, 72 °C for 5 min. The re–amplified cDNA fragments were resolved on a 1.5% agarose gel and stained with ethidium bromide. The bands were photographed with Digital Camera (Kodak) and estimated their sizes with the standard markers.

DNA sequencing

The re–amplified cDNA were cloned into the pRIP–T™ vector system and transformed in to *E. coli* DH 12S (Bioneer). DNA sequencing was performed by the dideoxy sequencing method (Ausbel *et al.*, 1998). The date for the cDNA sequence were edited and used to searching the homology BLAST X (URL: http://www.ncbi.nlm.nih.gov; Altschul *et al.*, 1990). The sequence of DNA fractions was confirmed with the modified Northern hybridization using unfractionated RNA immobilized by the slot blotting (Sambrook *et al.*, 1989)

RESULTS

Growth

The ImHyup-1 line of *L. edodes*, were selected from more than 20 lines known and being commercially sold in Korea. This line was developed for cultivation of *L. edodes*, a long time ago, because the rapid growth takes place in the log cultivation and morphogenesis at the high temperate as compared with other lines of *L. edodes*. Also, this line was known to be traditionally cultivated in the oak or other tree logs. The liquid spawns grown were inoculated in the sawdust solid blocks and the cultivation was made at 25 °C under a laboratory ways. The white mycelia grew for 1 or 1.5 months in the dark room and covered the whole surface of sawdust blocks (D in Fig. 1). To induce fruiting body (primordium), the sawdust substrate was kept in the moist and cool room under dim light. The white mycelia turned to the black brown color (C in Fig. 1) and made to produce several harden mass in the surfaces. This mass was projected out for a week in the cooled room, showing B in Fig. 1. Finally, more than ten basidiocarps were protruded from the mass on the surfaces of the black brown block (A in Fig. 1).

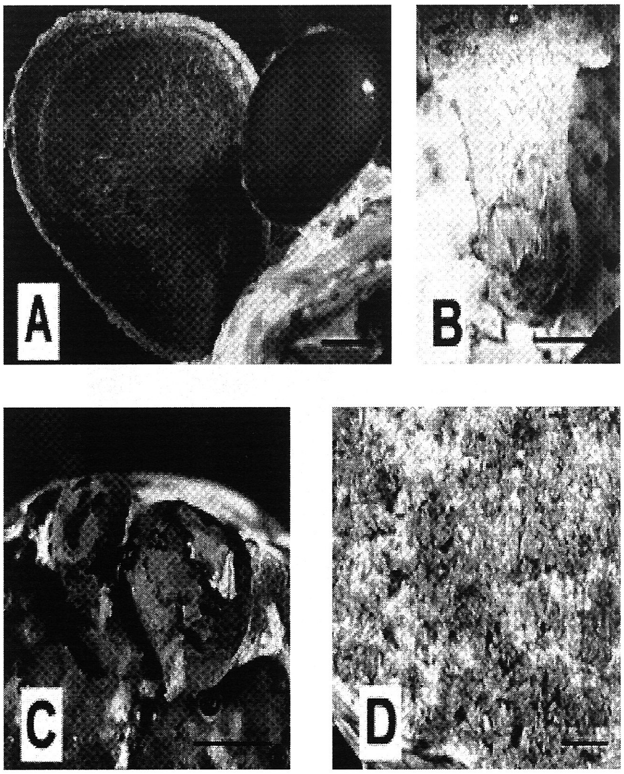

Fig. 1. The different stages of *L. edodes* growth; A: Formation of fruiting body pileus, B: Fruiting body stipe, C: Primordia formations from the surface of the sawdust, D: Mycelia colonized in the sawdust media (Scale bars=1 cm).

RNA bands

The DDRT–PCR was displayed after reacted with the equal amounts of RNA (1 μg) extracted from each of the three developmental stages and different tissue types using three 3' one anchored oligo–dT primer combinations with the four arbitrary primers (Figs. 2 and 3). To distinguish the different genes expressed, the detail bands of the DDRT–PCR products shown in Figs. 2 and 3 were run on the 1.5% agarose gel and stained with ethidium bromide (Fig. 4). Several times, these products were displayed on the agarose gel, but the bands were difficultly distinguished as compared with those shown in the 6.0% polyacrylamide gels (Figs. 2 and 3). The four differential display fragments (F series; the bands marked in the left of Fig. 2) were distinguished, when the primer of AP # 1 was reacted with the three different anchored oligo–dT primers; The fine bands of the fragments indicated above were also displayed on the 6.0% polyacrylamide gels shown in both Figs. 2 and 3. The band or fragment of F–1 originated from the tissues of primordi-

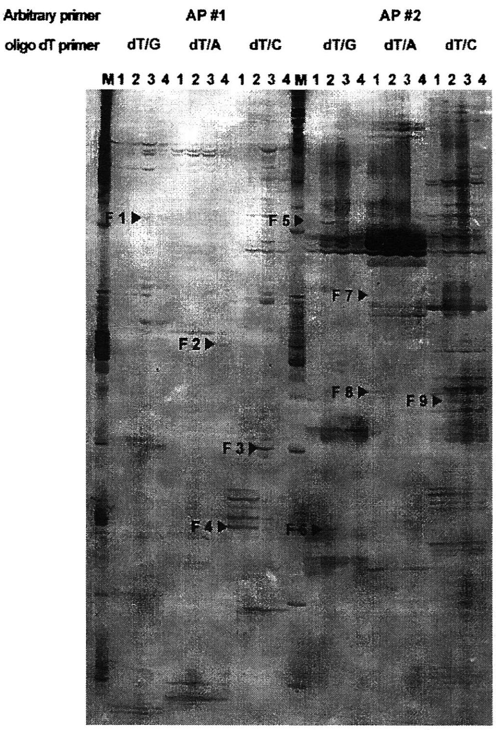

Fig. 2. Differential display of total RNA from the three development stages and different tissue types of *L. edodes* using the arbitrary primers AP # 1 and AP # 2. The DDR-PCR bands were displayed on 6 % polyacrylamide gel stained with the silver staining method. Marked (M): marker (100 bp ladder), 1: Fruiting body pileus, 2: Fruiting body stipe, 3: Primordia, 4: Mycelia.

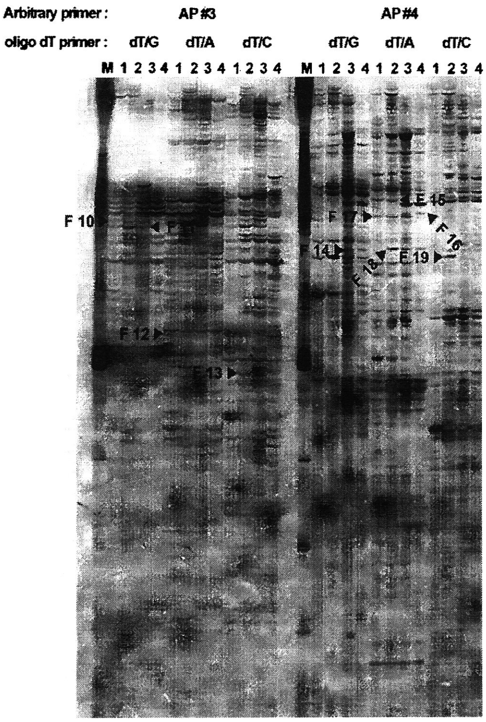

Fig. 3. Differential display of total RNA from the three development stages and different tissue types of *L. edodes* using the arbitrary primers AP # 3 and AP # 4. The DDRT-PCR products were resolved on 6% polyacrylamide gel stained with silver staining method. Marked M: the marker (100 bp ladder), 1: Fruiting body pileus, 2: Fruiting body stipe, 3: Primordia, 4: Mycelia.

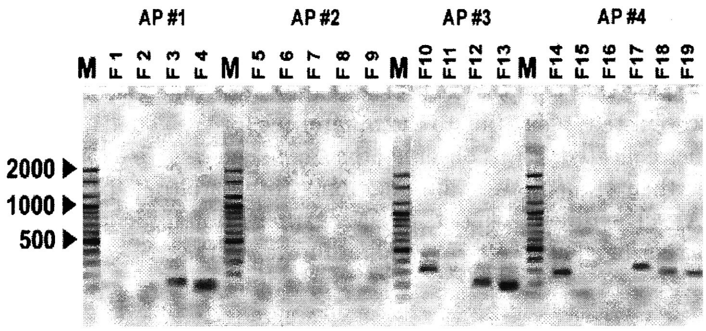

Fig. 4. The reamplified bandproducts differentially expressed by *L. edodes* on 1.5% agarose gel. The differentially expressed bands were picked up from the polyacrylamide gel. Marked M: marker (100 bp ladder).

Table 2. The fragments of gene differentially expressed and determinated.

Fragments	Primer combination		Molecular weight[a] (bp)	Expression stage and tissue			
	Arbitrary primer	Oligo–dT primer		Pileus	Stipe	Primordia	Mycelia
F 1	AP 1	dT/G	–[b]			○	○
F 2	AP 1	dT/A	–				○
F 3	AP 1	dT/C	150			○	
F 4	AP 1	dT/C	110	○	○		
F 5	AP 2	dT/G	–	○	○	○	
F 6	AP 2	dT/G	–		○		
F 7	AP 2	dT/A	–	○			
F 8	AP 2	dT/A	–	○	○		
F 9	AP 2	dT/C	160	○	○	○	
F 10	AP 3	dT/G	320	○	○	○	
F 11	AP 3	dT/G	–	○	○	○	
F 12	AP 3	dT/A	240	○	○		
F 13	AP 3	dT/C	230		○		
F 14	AP 4	dT/G	300			○	○
F 15	AP 4	dT/A	–	○	○		
F 16	AP 4	dT/A	160				○
F 17	AP 4	dT/A	330	○	○	○	
F 18	AP 4	dT/A	300		○	○	○
F 19	AP 4	dT/C	300		○		

a) Re–amplified molecular weight
b) Re–amplification band was not shown.

um and mycelia were distinguished from those originated from the others. The fragment of F–2 was expressed on only the tissue of mycelium, when the mRNA was reacted with the primer of AP #1 and the anchored oligo–dT/A primers. The fragment of F–5, F–9 to 11, or F–17 was found in the fungal tissues of pileus, stipe, and primordium, but not in the fungal tissues of mycelia. Many fragments of F–series were displayed on the mRNA originated from the fungal tissues of stipe, as compared with those reactions originated from the other tissues. The two fragments of F–14 and F–18 were selected with F–3 for further works. The bands or fragments synthesized and displayed, after many reactions of mRNA with the three arbitrary primers with three 3' one anchored oligo–dT primers, were listed in Table 2 and compared with each other. The nineteen fragments marked in Figs. 2 and 3 were listed, again, due to the differences for the genetic genes expressed to the fungal tissues collected; It was observed that more genes were expressed in the fungal tissues of stipe than other tissues, although only three arbitrary primers were reacted with the mRNA's. The three fragments of DNA expressed in three fungal tissues, but not in the fungal tissues of pileus, were selected for further works.

Sequence

The nineteen fragments of DDRT–PCR were selected, reacted with the two different primers mentioned above, and confirmed with the re–amplification on the 1.5% agarose gels (Fig. 4). Several fragments were showed to be stained with the darken bands in the

Table 3. The DNA sequences of the bands obtained from DDRT–PCR.

Clones	Sequences Length (bps)	The sequences determined
F 3–1	140	Aagcttgattgccaatgtgaggaagggtttcctggcaattgtattaatccaggagtaagtcg atcttaaaaccagtcctaaagggtcaataaggccatatattattaaatttatatattcctgatg gttgaaaaaaaaaa
F 3–2	121	Aagcttttttttttttcaaccatcaggaatatataaatttaataatatatttccttattgacccttt aggactggttttaagatcgacctactcctggattaatacaattgccaggaaaccc
F 14–1	143	Aagcttttttttttttggcatacatcaggagtaatttattgacaagtagtgtccaaatttggataaa tatggtcaagcaatatttatgcattaagtatacggttagcatatgtgcttgaaacaagagaaat agctctcatcaacc
F 14–2	165	Aaagaagctatggatgaaggggaattcgatgaagatgagcatgacgaatatgactatgacg acgacgacgatgagcggtgtccggaggtggacacaaggttgatgagagctatttctcttgtt tccaacacatattctaaccgtatacttaaatgcataaatatt
F 18	199	Gaattcactagtgattaagcttctcaacgtgctgcctcagctttcgttatccttacaggaact ctcctatacacttatgtcaaatctcgggagagtggcccatccaacccctcatcaggaaccaa ttctgcacctttgaaggatttggaatctcagtctacaaaacaggaacaggtggatgaaaagc cgctcacttaag

The poly A tails underlined. The black box represented 3' UTR region. Direction 5'→3'.

staining dyes. Out of the nineteen fragments, only three fragments made from the DDRT–PCR fingerprints (F–3, F–14 and F–18) were selected for determining the DNA sequences. The re–amplified cDNAs of the fragments sized about 110 to 330 bps in the length of DNA were shown to be a single band or double band, except that some were not showed nor clearly (Fig. 4). The two fragments (darken and dim bands) of F–3 and F–14 were shown and obtained for the DNA sequences. The fragment of F–18 was also selected for this further work. The five fragments were cloned to the pRIP–T™ vector known and determined for the DNA sequences. The fragments picked up from the polyacrylamide gels (Figs. 2 and 3) were loaded in the agarose gel, again, and showed to be separated to two kinds of band shown in Fig. 4. The two bands shown in the fragments of F–3 and F–14 (Fig. 4) were also picked up, being determined by the DNA sequences. The DNA sequences were determined under a range of 200 bps and showed in Table 3. The two fragments of F–3 were determined to be a DNA sequences of A/T rich 140 bps, whereas the fragments of F–18 of C/T rich sequence with 190 bps. The nucleotides of A, T, C, and G were observed be evenly distributed in the fragment of F–18 rather than the others. The F–3 clone was identified by hybridization to the 18sRNA of primordium (Fig. 5). The five DNA sequences were searched for further information by BLAST search program. However, the 15 to 20 bps, the partial region, of fragment were completely matched with those of several genes showed in Table 4, indicating the control gene (the 'intron' gene called in searching the Blast X). The partial region of DNA sequence

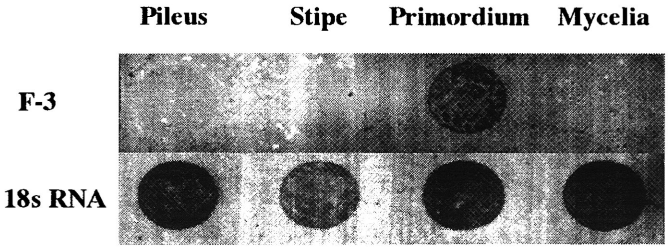

Fig. 5. Dot blotting of the unfractionated RNA of fragment–3 immobilized by the slot blotting.

Table 4. The DNA sequences of the five fragments compared with the other genomic sequences obtained from the the BLAST searchings.

	Code number	The organisms and some descriptions	Homology sequences
F3–1	gb|U69572.1|CPU6957	Mitochondrion Culex pipiens A+T rich m	97→119: 636 atatattattaaatttatatatt 658
	gb|U41277.1|CELC06E4	Caenorhabditis elegans cosmid C06E4	99→119: 15414 atattattaaatttatatatt 15394
	gb|AE003442.1|AE003442	Drosophila melanogaster genomic scaf	97→116: 167763 atatattattaaatttatat 167782
	gb|AC004845.2|AC004845	Homo sapiens clone RP4–635O5, comple	97→120: 50459 atatattatttaatttatatattc 5048
	gb|AC004749.1|AC004749	Homo sapiens chromosome 5, P1 clone	96→115: 36497 catatattattaaatttata 36478
F 3–2	gb|U69572.1|CPU69572	Mitochondrion Culex pipiens A+T	27→49: 658 aatatataaatttaatatatat 636
	ref|NC_001993.1	| Melanoplus sanguinipes entomopoxvirus	27→51: 24133 aatatataaagttaataatatattt 24109
	gb|AF063866.1|AF063866	Melanoplus sanguinipes entomopoxviru	27→51: 24133 aatatataaagttaataatatattt 24109
	gb|U41277.1|CELC06E4	Caenorhabditis elegans cosmid C06E4	27→47: 15394 aatatataaatttaataatat 15414
	gb|AE003442.1|AE003442	Drosophila melanogaster genomic scaf	30→49: 167782 atataaatttaataatatat 167763
F 14–1	gb|AE003528.1|AE00319528	Drosophila melanogaster genomic	75→95: 281829 gcaatatttatgcattaagta 281849
	gb|AC006075.1|AC006075	Homo sapiens chromosome 16	108→128: 155819 atgtgcttgaaacaagagaaa 155799
	gb|U73649.1|U73649	Human Chromosome 11 Cosmid	103→123: 21689 agcatatgtgcttgaaacaag 21669
	gb|U61947.1|CELC06G3	Caenorhabditis elegans cosmid	25→43: 1920 aggagtaatttattgacaa 1938
	gb|AC012446.2|AC012446	Homo sapiens clone RP11–101A12	64→85: 71654 aatatggtcaagcagtatttat 71633
F 14–2	gb|J05161.1|LUMHBC	Earthworm (L.terrestris) extracellular	50→74: 1111 atgactatgacgacgacgacgatga 1135
	emb|AJ249549.1|EMU249549	Echinococcus multilocularis	12→39: 2295 ggatgaaggggaattcgatgaggatgag 23
	ref|NC_001139.1	Saccharomyces cerevisiae chromosome VII	50→74: 70202 atgacgatgacgacgacgacgatga 70226
	emb|Z72749.1|SCYGL227W	S. cerevisiae chromosome VII	50→74: 872 atgacgatgacgacgacgacgatga 896
	dbj|AB025323.1|AB025323	Eptatretus burgeri mRNA	36→68: 862 tgaggatgacgaagatgacgatgacgacgacga 894
F 18	emb|AL365234.1|ATT30N20	Arabidopsis thaliana DNA chromosome	68→87: 16388 tatacacttatgtcaaatct 16407
	emb|AL121776. |HSJ1050K3	Human DNA sequence	170→189: 74436 aacaggtggatgaaaagccg 74455
	gb|AC009541.16|AC009541	Human Chromosome 7 clone	147→165: 80344 ggaatctcagtctacaaaa 80362
	gb|AC002352.1|AC002352	Homo sapiens 12q24 PAC P256D10	129→147: 6002 tgcacctttgaaggatttg 6020
	gb|AC005255.1|AC005255	Homo sapiens chromosome 19	138→160: 92056 gaaggatttggaatcacagtcta 92034

The fragments synthesized were compared with the genes informed from the GeneBank matched above was searched to originate from the eucaryotic cells but not from the prokaryotic cells. The fragments selected from this work, especially A+T rich DNA sequence in F3–1 and F3–2, were considered to be a control gene (intron), but not a structural genes (exon; see the literatures of gb|U69572.1|CPU69572 Mitochondrion *Culex pipiens* A+T rich or gb|U41277.1 |CELC06E4 *Caenorhabditis elegans*). Some regions of the fragment F–18 was also searched to be matched with the intron genes (repeating regions) and the structural genes (exon) of DNA sequences.

DISCUSSION

The line of ImHyeop–1 *L. edodes* were known to be the fungus grown rapidly in the high temperature. The liquid spawn was employed for rapid growth in the common sawdust blocks. The morphognesis were induced under several known conditions. The mycelia was inoculated by the liquid spawn and cultured in the sawdust blocks for 1 or 1.5 month (Fig. 1). After then, the mycelia were observed to turn to dark brown color and, after then, to several protruding mass (2 or 3 cm diameters) in the surfaces (C in Fig. 1). Morphogenesis of *L. edodes* was observed to take place under certain conditions, although it was concerned under the conditions of our laboratory. The mRNA's induced by the four different stages of *L. edodes* morphogenesis were collected, reacted with the arbitrary primers and synthesized to the genes of cDNA's. The genes related to morphogenesis would be considered to be very important for commercial production of *L. edodes* in Eastern Asia. Thus, the genes inducing the morphogenesis of basidiocarp were considered to be critical for the production of commercial mushroom in the agricultures.

Many genes were expressed in the DDRT–PCR, when the three arbitrary primers of AP 2, 3 and 4 were reacted with mRNA rather than the other arbitrary primer # 1. Particularly, the many genes were detected when the AP primers were reacted with the end primers of the oligo primers T/C; the many bands or fragments were observed on the electrophoresis gels when the arbitrary primers # 2 or # 4 reacted with the oligo primers T/C. This would be helpful, especially, for further work of *L. edodes* or its related species. The gene fragments not expressed in only a fungal tissue were observed in the preparations of stipe more than the others, so less expressed in the preparations of mycelia. (Table 2, and Figs. 2 and 3). Generally, so many genes expected to be expressed in the fungal tissues of basidiocarps (pileus and stipe), because more complexity of structure was found in the basidiocarps than in the mycelia of the fungus. The fragments (F–3, F–14, and F–18) observed in preparations of the three fungal tissues, excluding the tissue of pileus, were selected for the analyses of DNA sequence. In other words, the possible genes related to the morphogenesis of basdiocarp should be expected to exist in the fungal tissues of mycelia, primordia and stipe (Table 2). The two fragments of F–3 and F–14 picked up from the agarose gels were showed to be two bands, again, and total five bands selected were determined to the DNA sequence shown in Table 3. The two fragments darken (F3–1: 140 bp) and the dim bands (F3–2: 121 bp) in Fig. 4, picked up from the one fragment (F–3 fragment in Fig. 2) were quite different each other's in the analysis of DNA sequences (Table 3).

Our works for the genes induced for morphogenesis were very important in the commercial production of *L. edodes*, even though the five fragments selected above should be confirmed by Northern blot or other PCR methods. All fragments analyzed by DNA sequence (Table 3) were searched to match with other DNA sequences of gene provided from Blast X. The analyses of the protein for the DNA sequences of the fragment selected were not conducted because finding the genes related to morphogenesis of basidiocarp were aimed by using the methology of DDRT–PCR. The minute differences between the fragments shown in the polyacryl–amide gels were tried to be detected for the mRNA originated from the different fungal tissues and also to be related to the DNA sequences. Our work was subjected to detect the differences of fragment shown in the polyacrylamide gels for the further work of *L. edodes*.

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