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Isolation of Genes Specifically Expressed in Different Developmental Stages of *Pleurotus ostreatus* Using Macroarray Analysis

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The oyster mushroom (*Pleurotus ostreatus*) is one of the most important edible mushrooms worldwide. The mechanism of *P. ostreatus* fruiting body development has been of interest both for the basic understanding of the phenotypic change of the mycelium-fruiting body and to improve breeding of the mushrooms. Based on our previous publication of *P. ostreatus* expressed sequence tag database, 1,528 unigene clones were used in macroarray analysis of mycelium, fruiting body and basidiospore developmental stages of *P. ostreatus*. Gene expression profile databases generated by evaluating expression levels showed that 33, 10, and 94 genes were abundantly expressed in mycelium, fruiting body and basidiospore developmental stages, respectively. Among them, the genes specifically expressed in the fruiting body stage were further analyzed by reverse transcription-polymerase chain reaction and Northern blot to investigate temporal and spatial expression patterns. These results provide useful information for future studies of edible mushroom development.

KEYWORDS : *Pleurotus ostreatus*, Fruiting body development, Oyster mushroom, Specific gene expression

The oyster mushroom (*Pleurotus ostreatus*) is commercially important in the worldwide mushroom market. In addition to its importance in food production, *P. ostreatus* exhibits vigorous lignin degradation activity, which is exploited in industrial applications for biobleaching and catalysis of difficult chemical conversion, and potent immunoactivation activity that is used in pharmaceutical products (Kues and Liu, 2000; Cohen *et al*., 2002). Basidiomycetes, which have fleshy fruiting bodies commonly known as edible mushrooms, are an important part of many diets worldwide. Understanding the mechanism of fruiting body formation is important because it not only provides basic knowledge of basidiomycetes sexual development but also facilitates improvement of industrial mushroom cultivation.

Coprinus cinereus and *Schizophyllum commune* are model organisms for studying basidiomycetes mating type factors. Molecular studies on both mushrooms have provided basic understanding about mating and dikaryon formation, which are necessary but not sufficient for fruiting body formation (Casselton and Olesnicky, 1998; Kamada, 2002; Fowler *et al*., 2004). In addition, particular environmental conditions such as temperature, humidity and nutrient deficiency are required to induce fruiting body formation. These physiological and environmental conditions have been well-characterized, but the molecular mechanisms of fruiting body formation remain unknown despite isolation of several abnormal fruiting body mutants and their complementing genes in *C. cinereus* (Muraguchi and Kamada, 1998; Kues, 2000; Terashima *et al*., 2005; Liu *et al*., 2006).

Recently, several different molecular biology methods including differential display reverse transcription polymerase chain reaction (DDRT-PCR), expressed sequence tag (EST) analysis and reverse Northern blot were used to isolate developmentally expressed genes from several commercial mushrooms including *Lentinus edodes* and *P. ostreatus*, as well as from model basidiomycetes such as *C. cinereus* and *S. commune* (Dons *et al*., 1984; Sunagawa and Magae, 2005; Yamada *et al*., 2006). In addition, transformation techniques were developed to identify the function of genes in several commercial mushroom species, although the transformation efficiency was lower than that in other model basidiomycetes (Kim *et al*., 1999, 2003; Kuo *et al*., 2004). We tried to profile quantitative gene expression patterns during fruiting body development using large scale macroarray analysis in *P. ostreatus*. From a *P. ostreatus* EST database of three different developmental stages (mycelium, fruiting body and basidiospore), 1528 genes were isolated as unigenes through sequence comparison (Lee

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et al., 2002; Joh *et al*., 2007). Presently, these cDNA clones were selected as unigenes and were used in macroarray analysis to generate a gene expression profile for fruiting body development.

Materials and Methods

Strains and culture conditions. *P. ostreatus* strain ASI 2029, a Korean wild type strain, was obtained from the National Institute of Agricultural Science and Technology (NIAST, Suwon, Korea). ASI 2029 was grown on MCM agar plate (0.2% yeast extract, 0.2% peptone, 2% glucose, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KH_2PO_4 and 0.1% K_2HPO_4) for 1 week at 28°C. The mycelia were homogenized using a blender, inoculated into liquid MCM, and then cultured vigorously by shaking (230 rpm) at 28°C for 1 week. For the production of fruiting bodies, ASI 2029 was inoculated in 570 g poplar sawdust 120 g rice bran and 65% water in a 1000 *ml* bottle. The cultures were incubated at 25°C in the dark for 25~40 days and then transferred to conditions that induced fruiting (12~15°C, greater than 85% humidity, and light). Liquid-cultured mycelia and fruiting bodies were frozen in liquid nitrogen and stored at −80°C.

RNA and plasmid DNA extraction. For macroarray analysis and Northern blot hybridization, samples were harvested in several different stages: mycelium grown in sawdust (45 days after incubation); mycelium (9 days after cold shock); primordia, young fruiting body, mature fruiting body and basidiospore. Total RNA was extracted using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's protocol. Plasmid DNAs were prepared using a plasmid purification kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol.

Macroarray analysis. Concentration-adjusted (100 ng) plasmids for 1528 unigene clones were manually deposited on positively charged nylon membranes (Amersham Biosciences, Piscataway, NJ, USA). Radiolabelled cDNA probes were synthesized by incorporating [α-^{33}P] dCTP into primary strand cDNA. Total RNA (50 μg) was mixed with oligo $(dT)_{18}$, heated to 70°C for 10 min and chilled on ice. It was then mixed with 20 μl 5 × first strand buffer, dATP, dGTP, dTTP (333 μM, each) and dCTP (10 μM), 70 μCi of [α-^{33}P]-dCTP and 1000 units Moloney murine leukemia virus (MMLV) reverse transcriptase (Promega, Madison, WI, USA). The reaction mixture was incubated at 37°C for 1 h. The blots were prehybridized in 15 *ml* hybridization solution at 65°C for 1 h. The entire collection of cDNA probes was added to the prehybridization solution and the filters were hybridized with probes for 20 h. The hybridized filters were washed consecutively with 2 × standard saline citrate (SSC)/0.1% sodium dodecyl sufate (SDS) for 20 min at 65°C, with 1 × SSC/0.1% SDS for 20 min at 65°C and with 0.5 × SSC/0.1% SDS for 20 min at 65°C. The filters were exposed to BioMax MR film (Kodak, Rochester, NY, USA) for 48 h.

Data analysis. The exposed BioMax MR films were scanned on an ARCUS II apparatus (Agfa-Gevaert, Mortsel, Belgium). The resulting TIFF image files were analyzed using TotalLab software (Nonlinear Dynamics, Newcastle upon Tyne, UK) to determine the pixel density (intensity) for each spot on the filter. β-actin clone spot intensity was calculated as 100 and was used as a scaling factor for the spot intensities of genes.

RT-PCR and RNA analysis. First-strand cDNA was synthesized using MMLV reverse transcriptase (Promega) with total RNA and oligo dT primer. Second-strand primers were designed based on EST sequences to produce 300~500 bp DNA fragments. PCR was performed using a PTC-200 thermocycler (MJ Research, Waltham, MA, USA). Total RNA was fractionated on a 1% denaturing agarose gel and transferred onto nylon membranes (Amersham Biosciences). Northern blots were hybridized with ^{32}P-labelled probe DNA, and washed consecutively with 2 × SSC/0.1% SDS for 20 min at 65°C, with 1 × SSC/0.1% SDS for 20 min at 65°C and with 0.5 × SSC/0.1% SDS for 20 min at 65°C. The filters were exposed to BioMax MR film (Kodak).

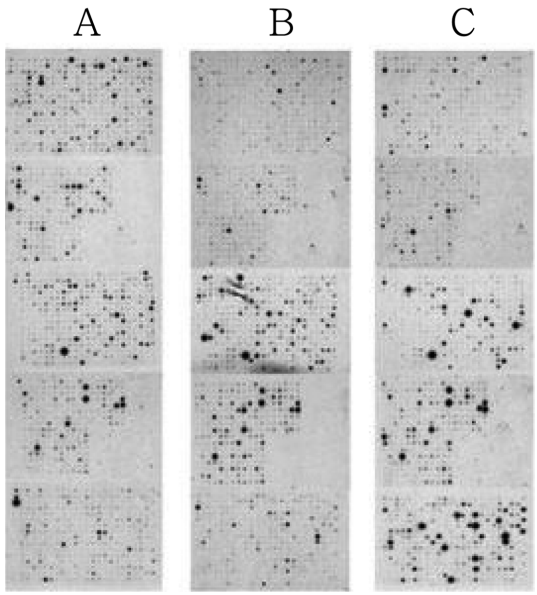

Fig. 1. cDNA macroarrays of 1594 unigenes of *P. ostreatus* hybridized with probes generated from RNA extracted from A, liquid-cultured mycelia; B, mature fruiting body; C, basidiospore.

Results

Gene expression profiles in different developmental stages of *P. ostreatus*. A total of 4,060 different genes (PoUnigenes) were already identified in *P. ostreatus* by EST studies (Lee *et al*., 2002; Joh *et al*., 2007). Of these unigene clones, 581, 592 and 355 unigenes were selected from three different cDNA libraries of liquid-cultured mycelia, fruiting bodies, and basidiospores, respectively, for gene expression profiling. Plasmids isolated from these clones were blotted on nylon membranes for macroarray analysis. Meanwhile, total RNA was isolated from three different developmental stages [mycelia cultured for 1 week after 1 week preculture in MCM liquid media (Mc) fruiting bodies sampled 20 days after cold shock (Fb) and basidiospores (Sp)]. ^{33}P-labelled cDNA probes generated from total RNAs were hybridized with the clones fixed on membranes. All photographic values of hybridization signals were quantified. Of 1,528 genes screened, 1,036 (68%) genes showed clearly readable expression levels (expression value ≥ 50) in at least one of the three developmental stages (Fig. 1). Genes with known patterns of expression, such as *HYDROPHOBIN* and *PRIA*, served as a quality control and showed that this macroarray analysis produced reasonable gene expression data.

Table 1. Genes specifically expressed in liquid cultured mycelia

Clone #	LCM Expression value	MFB Expression value	Spo Expression vaule	Blastx homology description	E Value
01893LM	1403	30	15	–	–
00971LM	1348	59	22	hypothetical protein An07g09050 (*Aspergillus niger*), XP_001391979	8.00E-09
01719LM	1328	35	13	predicted protein (*Laccaria bicolor*), XP_001880508	3.00E-28
00131LM	913	4	4	Epl1 protein (*Trichoderma atroviride*), CAL80754	8.00E-35
00703LM	858	33	41	–	
00217LM	812	12	14	–	–
01846LM	766	8	10	predicted protein (*Laccaria bicolor*) XP_001874296	2.00E-24
01725LM	698	48	10	–	–
00928LM	685	21	18	–	–
01696LM	643	90	12	–	–
01934LM	575	36	18	Hydrophobin (*Pleurotus ostreatus*) CAA76494	2.00E-34
00511LM	545	2	1	predicted protein (Laccaria bicolor) XP_001888977	2.00E-42
01940LM	522	40	13	–	
00972LM	495	83	31	glycoside hydrolase family 5 protein (*Laccaria bicolor*) XP_001874853	3.00E-47-
00316LM	453	4	5	–	–
00526LM	445	1	1	–	–
00283LM	396	7	26	short chain dehydrogenase - fission yeast (*Schizosaccharomyces pombe*) NP_588241	7.00E-21
00403LM	387	20	6	–	–
00371LM	376	0	1	predicted protein (*Laccaria bicolor*) XP_001878156	3.00E-30
00664LM	328	15	8	–	–
01654LM	317	29	10	hypothetical protein (*Botryotinia fuckeliana*) XP_001555845	2.00E-04
01852LM	302	57	16	predicted protein (*Laccaria bicolor*) XP_001877401	1.00E-10
00333LM	300	11	17	predicted protein (*Laccaria bicolor*) XP_001880956	1.00E-30-
00946LM	282	4	3	hypothetical protein CC1G_12072 (*Coprinopsis cinerea*) XP_001828342	1.00E-46
01004LM	270	47	17	uricase (Urate oxidase) (*Cryptococcus neoformans*) XP_572701	4.00E-11
01873LM	265	155	18	predicted protein (*Laccaria bicolor*) XP_001876153	4.00E-58
00468LM	261	15	9	predicted protein (Laccaria bicolor) XP_001879765	5.00E-30-
00547LM	256	2	0	OPT oligopeptide transporter (Laccaria bicolor) XP_001874246	6.00E-65
00133LM	253	0	1	–	–
00350LM	238	0	13	C2H2 type zinc finger domain protein (*Neosartorya fischeri*) XP_001264975	9.00E-40
01922LM	236	16	11	hypothetical protein CC1G_02714 (*Coprinopsis cinerea*) XP_001840251	4.00E-08-
00947LM	233	3	0	TAP domain protein (*Kineococcus radiotolerans*) YP_001360245	5.00E-06

LCM (liquid cultured mycelia), SPO (basidiospores), MFB (mature fruiting body)

Table 2. Genes specifically expressed in basidiospores

Clone #	LCM Expression value	MFB Expression value	SPO Expression value	Blast homology	E Value
P03-E11	13	74	504	–	–
P01-E06	9	86	439	–	–
P03-B09	4	54	397	hypothetical protein CC1G_01648 (*Coprinopsis cinerea*) XP_001833971	7.00E-20
P09-E07	12	73	386	–	–
P02-B08	5	95	373	predicted protein (*Laccaria bicolor*) XP_001873869	4.00E-08
P02-E12	4	91	291	–	–
P11-F09	6	66	240	hypothetical protein CC1G_02968 (Coprinopsis cinerea) XP_001835880	3.00E-57
P03-G11	10	80	233	–	–
P11-F08	11	87	201	Acyl-CoA dehydrogenase (*Cryptococcus neoformans*) XP_568834	4.00E-34
P01-D03	7	31	200	predicted protein (*Coprinopsis cinerea*)XP_001831584	1.00E-05

LCM (liquid cultured mycelia), SPO (basidiospores), MFB (mature fruiting body)

Isolation of specifically expressed genes in mycelia and basidiospores stages. Gene expression profiles were analyzed in order to isolate genes specifically expressed at the fruiting body stage of developmental stages. Genes were selected when the expression value was above 200 in only one developmental stage and below 100 in the other developmental stages. We identified 33 genes that were expressed specifically in liquid-cultured mycelia (Table 1). Among them, the 01893LM clone encoding an unidentified protein showed the highest expression value. The hydrophobin gene (1934LM) showed specific expression in liquid-cultured mycelia, as previously reported. However, most of the genes specifically expressed in mycelia did not show significant homology with functionally characterized genes (E value < 1E-10). Ten genes were identified as genes specifically expressed in basidiospores (Table 2). Among them, two genes showed high homology with secreted glycosyl hydrolase and acyl-CoA dehydrogenase. Genes P03-E11 and P01-E06 exhibited the two highest expression values.

Specific gene expression related to fruiting body formation. Using the criteria described above, we isolated 94 genes that were expressed specifically during the fruiting body stage (Table 3). Of these, 14 genes were highly expressed, exhibiting expression values > 1,000. Of these 14 genes, AaPri1 was the only gene previously reported, while the other 13 did not correspond to any

Table 3. Genes abundantly expressed in mature fruiting bodies

clone #	LCM Expression value	MFB Expressio value	SPO Expression value	Blast homology	E Value
MFB01-B08	9	3637	84	predicted protein (*Laccaria bicolor)* XP_001874669	2.00E-43-
MFB02-E09	5	3178	63	12 kDa heat shock protein (*Cryptococcus neoformans*) XP_572088	8.00E-21
MFB06-B06	3	2432	57	–	–
MFB01-F07	6	2232	65	–	–
MFB01-A01	1	1579	33	symbiosis-related protein [*Laccaria bicolor*] AAB53650	6.00E-67
MFB12-B10	14	1566	8	–	–
MFB01-E08	3	1452	45	predicted protein (Coprinopsis cinerea) XP_001836977	2E-17-
MFB03-B05	1	1381	27	PriA (*Pleurotus ostreatus*) AAL57035	4.00E-78
MFB07-F11	13	1227	85	–	–
MFB05-A01	0	1131	35	–	–
MFB09-A07	2	1028	32	–	–
MFB08-D01	2	1025	58	predicted protein (Coprinopsis cinerea) XP_001837184	4.00E-04
MFB08-B09	24	1017	60	–	–
MFB06-D06	2	1005	35	–	–
MFB09-B01	35	994	57	transketolase (*Laccaria bicolor*) XP_001885329	3.00E-28
MFB06-B07	0	938	31	nam9 protein, mitochondrial precursor (*Cryptococcus neoformans*) XP_568963	6.00E-33
MFB08-F02	6	928	54	–	–

Table 3. Continued

clone #	LCM Expression value	MFB Expressio value	SPO Expression value	Blast homology	E Value
MFB05-H04	41	840	83	–	–
MFB05-H01	2	764	40	DNA repair helicase RAD3 (*Aspergillus terreus*) XP_001210680	3.00E-45
MFB28-A04	41	754	42	ATP-synthase delta-subunit (*Agaricus bisporus*) CAB04785	3.00E-58
MFB29-D04	37	742	58	predicted protein (*Laccaria bicolor*) XP_001873162	2.00E-37
MFB08-G11	3	716	42	–	–
MFB05-G07	83	692	78	predicted protein (*Laccaria bicolor*) XP_001874279	1.00E-75
MFB06-A06	7	669	48	–	–
MFB28-A06	18	666	13	predicted protein (*Laccaria bicolor*) XP_001876525	7.00E-17-
MFB04-C09	14	605	33	predicted protein (*Coprinopsis cinerea*) XP_001836046	9.00E-24
MFB04-F10	11	603	82	–	–
MFB29-C05	44	581	67	pre-mRNA splicing factor (*Cryptococcus neoformans*) XP_569020	7.00E-50
MFB12-A02	16	569	15	predicted protein [*Laccaria bicolor* S238N-H82] XP_001880714	1.00E-09-
MFB04-C07	29	529	52	–	–
MFB05-F02	47	527	75	exo-beta-1,3-glucanase [*Cryptococcus neoformans*] XP_568026	2.00E-09
MFB04-E04	6	510	72	–	–
MFB03-D03	65	505	75	predicted protein [*Laccaria bicolor* S238N-H82] XP_001878578	1.00E-29
MFB06-H12	0	505	22	–	–
MFB09-C02	16	494	60	hypothetical protein CC1G_05518 (Coprinopsis cinerea) XP_001838965	3.00E-11
MFB05-A05	38	477	86	60S ribosomal protein L7/L12 [*Pichia stipitis* CBS 6054] XP_001385643	1.00E-16
MFB06-B12	19	465	58	calcium sensor NCS-1 [*Magnaporthe grisea* 70-15] XP_363624	6.00E-81
MFB10-A10	63	458	59	histone H4 [*Coprinopsis cinerea* XP_001831685	3.00E-38
MFB10-A07	1	453	91	–	–
MFB10-F08	12	437	11	–	–
MFB12-D07	61	431	96	–	–
MFB28-D06	11	426	7	–	–
MFB02-A07	18	419	79	predicted protein [*Laccaria bicolor* S238N-H82] XP_001880346	5.00E-42
MFB04-F09	7	416	62	oxidoreductase, short chain dehydrogenase/reductase family [*Aspergillus clavatus*] XP_001276144	5.00E-18
MFB05-A07	6	405	30	predicted protein [*Laccaria bicolor* S238N-H82] XP_001880399	1.00E-54
MFB05-D06	27	398	70	–	–
MFB04-F01	1	388	26	linoleate diol synthase (*Laccaria bicolor*) XP_001874536	5.00E-35
MFB01-G09	4	377	57	hypothetical protein CC1G_07641 [Coprinopsis cinerea] XP_001832381	3.00E-11-
MFB01-E10	20	371	83	ATP synthase beta chain [*Coprinopsis cinerea* okayama7#130] XP_001833020	8.00E-80
MFB09-F01	36	371	77	–	–
MFB05-A11	77	356	48	eukaryotic translation initiation factor eIF-1A subunit, putative [*Neosartorya fischeri*] XP_001257440	5.00E-41
MFB09-H02	40	340	87	Kin17 gene product [*Drosophila melanogaster*] AAF51578	4.00E-61
MFB09-A11	4	319	61	–	–
MFB06-H09	53	307	52	ubiquinol-cytochrome c reductase iron-sulfur subunit, mitochondrial precursor [*Cryptococcus neoformans* var. neoformans JEC21] XP_775517	1.00E-62
MFB07-E11	38	307	27	UDP-glucuronic acid decarboxylase Uxs1p [*Cryptococcus neoformans* var. neoformans JEC21] XP_572003	5.00E-58
MFB04-D08	1	302	51	–	–
MFB05-A08	11	302	34	50S ribosomal protein L17 [*Aspergillus clavatus* NRRL 1] XP_001269318	1.00E-34
MFB03-C06	48	298	77	electron transporter, transferring electrons within CoQH2-cytochrome c reductase complex [*Cryptococcus neoformans* var. neoformans XP_571714	2.00E-57
MFB28-C05	19	297	14	–	–
MFB05-A03	69	292	54	40s ribosomal protein s5-1 [*Cryptococcus neoformans* XP_567915	4.00E-70

Table 3. Continued

clone #	LCM Expression value	MFB Expressio value	SPO Expression value	Blast homology	E Value
MFB01-D04	80	288	56	CCR4-Not complex subunit Mot2 (predicted) [*Schizosaccharomyces pombe* NP_593078	2.00E-40
MFB35-B06	38	285	59	Succinyl-CoA-ligase [*Laccaria bicolor* S238N-H82] XP_001873761	6.00E-51
MFB09-F02	13	282	66	protein phophatase 2C family protein [*Neosartorya fischeri* NRRL XP_001264979181]	2.00E-28
MFB10-H02	86	281	52	Calmodulin [*Pleurotus ostreatus*] AAD17455	1.00E-80
MFB03-F01	1	278	26	Hydrophobin [*Paxillus involutus*] ABG66317	8.00E-04
MFB02-A08	1	274	25		2e-12
MFB04-F07	7	274	69	conserved hypothetical protein [*Coxiella burnetii*] ZP_ 02220074	2.00E-12
MFB05-G06	32	265	30	translocon protein Sec61beta, putative [*Neosartorya fischeri* NRRL 181] XP_001265176	2.00E-13
MFB28-G11	10	265	9	histidine kinase Mak2 (PMID 11758939) [*Schizosaccharomyces pombe* 972h-] NP_594410	8.00E-16
MFB07-G12	1	259	64	copper radical oxidase [*Phanerochaete chrysosporium* ABD61577	8.00E-14
MFB02-C03	0	258	41	–	–
MFB04-D06	29	253	93	NADH-ubiquinone oxidoreductase subunit B17.2 [*Ajellomyces capsulatus* NAm1]. XP_001536006	2e-30
MFB06-F06	7	253	22	–	–
MFB29-C06	11	249	10	zinc finger protein, putative [*Aspergillus clavatus* NRRL 1] XP_001276081	9.00E-16
MFB08-C11	67	248	45	hypothetical protein SNOG_10974 [*Phaeosphaeria nodorum* SN15] XP_001801230	9.00E-34
				Continued	
MFB05-B09	78	244	73	RAB18, member RAS oncogene family [*Xenopus tropicalis*] NP_001017281	1.00E-50
MFB10-D07	10	242	17	B-(1-6) glucan synthase [*Agaricus bisporus*]	4.00E-42
MFB06-E11	2	242	38	ribosomal protein L12 [*Homo sapiens*]	2.00E-35
MFB28-B08	23	241	35	LAS seventeen-binding protein 3 (LAS17-binding protein 3) NP_219497 *Saccharomyces cerevisiae*	5.00E-16
MFB06-C07	1	239	89	GH16 beta-1,3-glucan recognition protein [*Laccaria bicolor* S238N-H82] XP_001883344	6.00E-28
MFB06-H05	4	231	36	–	–
MFB08-C10	56	228	56	monothiol glutaredoxin-5, mitochondrial precursor [*Ajellomyces capsulatus* NAm1] XP_001541789	5.00E-37
MFB04-C06	81	227	45	mitochondrial pyruvate dehydrogenase E1 component beta subunit [*Laccaria bicolor* S238N-H82] XP_001881742	1.00E-61
MFB01-F11	29	226	50	60S ribosomal protein L28 [*Aspergillus fumigatus* Af293] XP_747557	3.00E-21
MFB04-G08	3	226	49	–	–
MFB03-B09	20	219	83	predicted protein [*Coprinopsis cinerea* okayama7#130] XP_001837093	2.00E-10
MFB29-D09	23	216	53	glycoside hydrolase family 16 protein [*Laccaria bicolor* S238N-H82] XP_001876832	2.00E-57
MFB04-H10	1	214	29	60s ribosomal protein l33-b [*Cryptococcus neoformans* var. neoformans XP_571529	6.00E-38
MFB05-A10	9	213	23	dihydropteroatesynthase/2-amino-4-hydroxy-6-hydroxymethyldihydropteridinediphosphokinase/dihydroneopterin aldolase [*Schizosaccharomyces pombe* 972h-] NP_595420	2.00E-13
MFB07-A09	6	212	37	–	–
MFB05-E12	29	211	53	predicted protein [*Laccaria bicolor* S238N-H82]XP_001879848	2.00E-17
MFB11-D11	44	210	78	ATP dependent RNA helicase [*Cryptococcus neoformans* var. neoformans JEC21] XP_572072	5.00E-07
MFB10-B04	40	203	78	asparaginyl-tRNA synthetase [*Pichia guilliermondii* ATCC 6260] XP_001482609	5.00E-09
MFB09-E08	3	200	45	–	–

LCM (liquid cultured mycelia), SPO (basidiospores), MFB (mature fruiting body)

previously reported gene. To confirm expression patterns of these 13 genes, RT-PCR was performed using total RNA isolated from four different developmental stages. Seven genes were successfully amplified (Fig. 2). Two genes (MFB01-B08 and MFB08-B09) were overexpressed abundantly in the fruiting body stage, although they were also expressed at much lower levels in mycelia and basidiospores. Two other genes were expressed in the fruiting body stage but also in basidiospores (MFB02-E09) or mycelia (MFB08-D01). Three genes (MFB12-B10, MFB9-A07 and MFB6-B06) were expressed only in the fruiting body stage. To investigate the temporal and spatial expression of these genes during fruiting body development, Northern blot analysis were performed using total RNA isolated from 17 dissected developmental stages and tissues. Three genes showed clear Northern hybridization signals corresponding to RT-PCR results. However MFB02-E09 showed fruiting body stage-specific expression but not fruiting body tissue-specific expression.

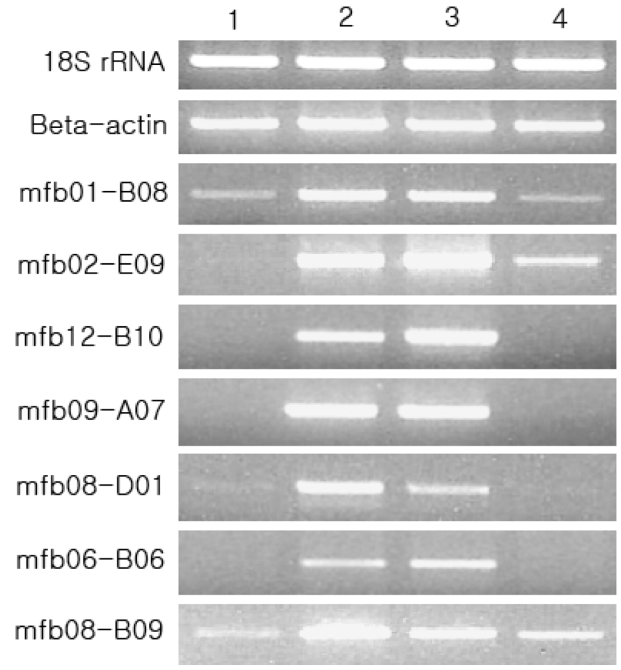

Fig. 2. RT-PCR analysis of genes specifically expressed in the fruiting body stage. Lane 1, liquid cultured mycelia; Lane 2, primordia; Lane 3, fruiting bodies; Lane 4, basidiospores.

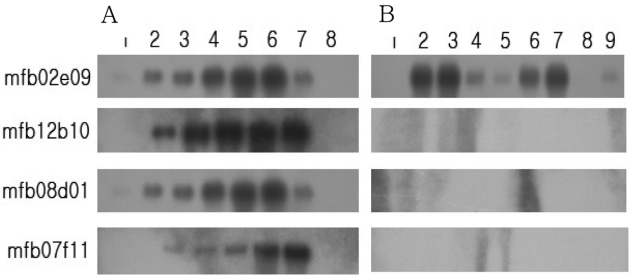

Fig. 3. Northern blot analysis of genes expressed during specific developmental stages and in specific tissues. (A) Lane 1, mycelium (45 days after incubation); Lane 2, mycelium (9 days after cold shock); Lane 3, primordia; Lane 4, young fruiting body 1; Lane 5, young fruiting body 2; Lane 6, young fruiting body 3; Lane 7, mature fruiting body; Lane 8, basidiospore. (B) Lane 1, mycelium (45 days after incubation); Lane 2, mycelium (1 day after cold shock); Lane 3, mycelium (4 days after cold shock); Lane 4, mycelium (9 days after cold shock); Lane 5, mycelium of primordia; Lane 6, mycelium of young fruiting body 1; Lane 7, mycelium of young fruiting body 2; Lane 8, mycelium of young fruiting body 3; Lane 9, mycelium of mature fruiting body.

Discussion

Fruiting body development from vegetative mycelia has attracted much scientific and economic interest. Many environmental factors (i.e., temperature, light, nutrients and fruiting inducing substances) and genetic factors (i.e., mating type genes and developmentally regulated genes) influence fruiting body formation (Kues and Liu, 2000; Wessels, 1993). These factors are well-characterized. However, how gene expression is regulated and how environmental signals are interpreted during fruiting body formation in the edible mushroom remains elusive. To study gene expression during fruiting body formation of *P.ostreatus*, expression profiles were established in three different developmental stages of the edible mushroom, *Pleurotus ostreatus,* using macroarray analysis. A total of 137 genes were identified as being specifically expressed in three different developmental stages. Among them, 94 genes were abundantly expressed in fruiting bodies, perhaps as part of fruiting body formation. Several of the genes encoded proteins of known function, including Aa-Pril, Hsp12, histidine kinase and glyoxal oxidase. Aa-Pri1 was previously reported to be highly expressed only in the primordial and fruiting body stages of *Agrocybe aegerita* (Fernandez and Labarere, 1997). Hsp12 gene was postulated to be highly expressed in fruiting bodies based on its high redundancy observed in the fruiting body EST database (Lee *et al*., 2002). Mutants of histidine kinase and glyoxal oxidase genes were reported to impair sexual development of *Dictyostelium* and *Ustilago maydis*, which suggests that they may play a critical role in fruiting body development in homobasidiomycetes (Leuthner *et al*., 2005; Thomason *et al*., 2006).

Fruiting body formation is induced in stress conditions such as nutrient deficiency and cold shock. Hence, it is reasonable to speculate that stress-related genes such as Hsp12, Nam9 and DNA repair helicase RAD15 are

expressed specifically in fruiting bodies. These genes were not found to be directly related to fruiting body induction, but they may be necessary components in the fruiting development processes. Complex signal transduction mechanisms are required in cells to recognize, transduce and respond to environmental signals. Components of signal transduction such as calmodulin, phosphatase 2C and RAB18 were developmentally expressed during fruiting body formation, and the function of these genes would be deduced by these expression profiles even if this is a primitive clue that these genes function in fruiting body formation.

Among the 95 genes expressed abundantly in fruiting body, more than half encoded proteins of unidentified function. These functions, and their relevance to fruiting body formation, await further studies.

The expression profile data presented here represents an important contribution to the elucidation of molecular mechanisms of fruiting body development of *P. ostreatus*.

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