RESEARCH ARTICLE
INTERNATIONAL MICROBIOLOGY (2011) 14:111-120
DOI: 10.2436/20.1501.01.141 ISSN: 1139-6709 **www.im.microbios.org**

Genomics and transcriptomics characterization of genes expressed during postharvest at 4°C by the edible basidiomycete *Pleurotus ostreatus*

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Received 20 April 2011 · Accepted 15 May 2011

Summary. *Pleurotus ostreatus* is an industrially cultivated basidiomycete with nutritional and environmental applications. Its genome, which was sequenced by the Joint Genome Institute, has become a model for lignin degradation and for fungal genomics and transcriptomics studies. The complete *P. ostreatus* genome contains 35 Mbp organized in 11 chromosomes, and two different haploid genomes have been individually sequenced. In this work, genomics and transcriptomics approaches were employed in the study of *P. ostreatus* under different physiological conditions. Specifically, we analyzed a collection of expressed sequence tags (EST) obtained from cut fruit bodies that had been stored at 4°C for 7 days (postharvest conditions). Studies of the 253 expressed clones that had been automatically and manually annotated provided a detailed picture of the life characteristics of the self-sustained fruit bodies. The results suggested a complex metabolism in which autophagy, RNA metabolism, and protein and carbohydrate turnover are increased. Genes involved in environment sensing and morphogenesis were expressed under these conditions. The data improve our understanding of the decay process in postharvest mushrooms and highlight the use of high-throughput techniques to construct models of living organisms subjected to different environmental conditions. [**Int Microbiol** 2011; 14(2):111-120]

Keywords: *Pleurotus ostreatus* · edible mushroom · expressed sequence tag (EST) · postharvest · transcriptome profiling

Introduction

Pleurotus ostreatus (Jacq.: Fr) Kumm. (Dikarya, Basidiomycota, Agaricomycotina, Agaricales) [29] is a white-rot, edible fungus, and an active lignin degrader that lives as a saprophyte on dead or decaying wood. It has been industrially cultivated for food production [27], its health stimulating [6] properties, the production of secondary metabolites of medical interest [14] and enzymes for pulp bleaching [38], recycling of agricultural wastes [1], and bioremediation processes [7]. The genome of *P. ostreatus* was sequenced at the Joint Genome Institute (JGI, Walnut Creek, California, USA) [http://genome.jgi-psf.org/PleosPC15_1/PleosPC15_1.home.html]. The two genomes present in the dikaryotic strain N001 were sequenced individually, producing two genomes corresponding to the two haplotypes of this strain. These data, obtained after several preliminary studies of the genome's structure and the characteristics of this fungus [23,31,35], expand upon classical molecular techniques applied to the breeding of basidiomycetes [34].

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The identification of genes expressed under different physiological conditions offers insight into the responses of an organism to environmental stresses [32]. In the case of edible mushrooms, postharvest is in itself a stress condition of relevant economic impact [39]. These mushrooms are usually sold as living organisms but during the long postharvest period they must survive on the basis of their own accumulated resources. The molecular processes that take place in postharvest mushrooms were preliminarily studied in the button mushroom *Agaricus bisporus* [5,13,21] but before the advent of genomic and transcriptomic techniques.

In the present study, the available genomics and transcriptomics resources were used to identify and analyze genes enriched in 7-day-old *P. ostreatus* fruit bodies stored at 4°C. Specifically, the metabolism taking place in the fruit bodies, as deduced from the expressed genes, was investigated.

Materials and methods

Fungal strain and culture conditions. *Pleurotus ostreatus* dikaryotic strain N001 and the two monokaryotic protoclones PC9 (accession number CECT20311) and PC15 (accession number 20312) [24] were used in this study. The dikaryotic strain was cultured on wheat straw until the first fruit flush. Once the fruit bodies had acquired their typical commercial size (around 5 cm in diameter), the fruits were collected. Half of the harvested material was used for mRNA purification while the rest of the harvest was kept at 4°C in the dark with high humidity (80%) for 7 days and then processed for mRNA.

Construction of cDNA library. Total RNA (10 μg) was extracted from the fruit bodies using a protocol described elsewhere [36]. The expressed sequence tag (EST) library enriched in postharvest clones (7-0 library) was produced by suppression subtractive hybridization as described by Diatchenko et al. [10]. mRNA collected from the cold-stored material was used as tester, and mRNA from the freshly cut fruit bodies as driver.

Genomic and transcriptomic analyses. The whole genome of the two *P. ostreastus* protoclones and the annotation tools used in this work are freely available at the Joint Genome Institute [http://genome.jgi-psf.org/PleosPC15_1/PleosPC15_1.home.html]. Transcriptomics analyses were carried out using a database of the transcriptome of *P. ostreatus* cultured as monokaryons or dikaryons in rich SMY culture medium [25], and the results analyzed with the SOLID platform (Applied Biosystems). For normalization of the transcriptomics data, normalized values from the reads per kilobase exon model per million mapped sequenced reads (RPKM) were used [4].

Results and Discussion

Isolation of genes expressed in postharvest stress. The subtracted mRNA was retrotranscribed into cDNA and then cloned and sequenced using the classical Sanger method with the M13D primer. From the 7-0 sample, 253 EST were recovered. These were sequenced and then used as the query in the identification of the corresponding transcripts. In a complementary control, mRNA from freshly cut fruit bodies was used as tester and mRNA from 7-day cold-stored fruit bodies as driver. The 253 EST from the 7-0 sample were sequenced; their average size was 323.27 nucleotides (SD 128.24).

Genomic analysis of the genes expressed in postharvest stress 7-0. The EST 7-0 sample was used first in a BlastN search against the complete nucleotide collection (nr/nt database) of the National Center for Biotechnology Information (NCBI), a division of the US National Library of Medicine. The search produced hits for 26 EST and permitted the identification of eight EST corresponding to ribosomal RNA genes mapping to scaffold (chromosome) 2. Among the 18 EST with BlastN matches other than rRNA, four were of particular interest. EST 70_363 and 70_489 were highly similar to genes coding for ubiquitin in *Coprinopsis cinerea* (see below), and EST 70_306 and 70_420 hit NCBI database genes identified as *P. ostreatus* mRNA regulated by blue-light stimulation (NCBI accession number AB551986). These sequences did not appear in any of the other basidiomycete sequenced genomes and thus could be considered as *P. ostreatus*-specific. They coded for gene model 167484, and mapped to scaffold 4.

The EST 7-0 sample was then used as query in a BlastN search against the complete collection of the *P. ostreatus* genome (PC15 v1.0) at the Joint Genome Institute portal [http://genome.jgi-psf.org/PleosPC15_1/PleosPC15_1.home.html]. With the 253 EST of the 7-0 sample used as query, 272 hits with E-values ranging from 2.53E-01 to 0.0 were recovered. This was due to the combined effect of some EST that failed to recover any PC15 hit (cloning contaminants) and to the occurrence of EST that recovered several genes (corresponding to members of complex gene families). As expected, none of the EST corresponding to rRNA recovered any automatically annotated protein. Most EST (176) retrieved only one PC15 v1.0 gene model, whereas two of them (EST number 70_363 and 70_489, corresponding to gene models 46579 and 185947, respectively) retrieved seven different polyubiquitin genes containing four repeats of the conserved ubiquitin domain.

To assign a single gene model to each EST when two or more gene models were retrieved with the same EST, the one with the lowest E-value and highest score was considered to be the gene corresponding to that EST. Accordingly, in all cases but one each EST could be assigned to a unique gene model. The exception was EST 70_576, whose sequence exactly matched that of four PC15 gene models (154568, 159877, 166420 and 168621) mapping to scaffolds 2, 7, 3, and 6, respectively. Note that these four models were missing

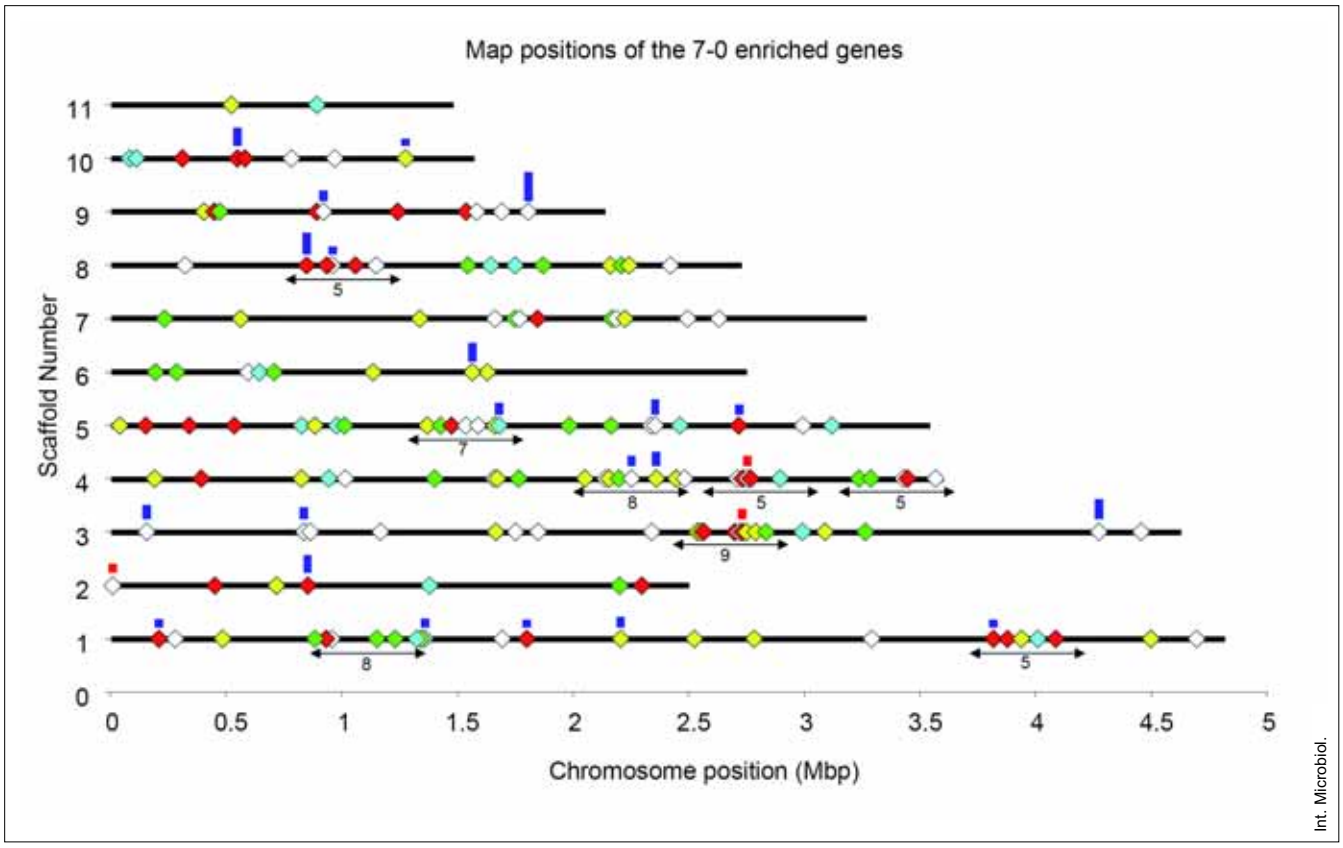

Fig. 1. Positions of the 7-0 enriched genes shown with diamonds on each of the 11 *Pleurotus ostreatus* scaffolds. KOG annotation: red, metabolism; yellow, cellular processes and signaling; green, information storage and processing; blue, poorly characterized genes; and white, genes without KOG annotation. The bars on top of the diamonds indicate the overexpression (log_2) of the corresponding gene in dikaryons (blue bars) and monokaryons (red bars). The clusters of genes are indicated by double arrowed lines. The numbers below these lines indicate the number of genes in the corresponding cluster.

in the genome of PC9 and in all other available basidiomycete genomes. Moreover, as this sequence did not retrieve any sequence either in BlastN or in BlastX searches in the NCBI gene database, it was considered exclusive for PC15. Since it was not possible to distinguish which of the three models was retrieved by EST 70_576, the four models were maintained as if they were expressed since none of them fell into the gene clusters described below. Taking into account the previous considerations, 212 different gene models were finally identified in this analysis.

Several EST can hit the same gene model, giving a rough estimation of its expression frequency in the conditions under study. Using the EST of the 7-0 sample, 157 different PC15 gene models were identified. The three with the highest expression values were model 34610 (seven repetitions), encoding a putative acetyl ornithine aminotransferase mapping to scaffold 9, and models 175868 and 156226 (six repetitions each), encoding two proteins mapping to scaffold 3 that are highly conserved in the PC9 and PC15 genomes but not found in other available basidiomycete genomes. Model 175868 could be preliminarily annotated as a BEM46-like protein (see below, Table 1) whereas no annotation could be provided for model 156226.

The availability of the sequence of the whole *P. ostreatus* genome and the high quality of the assembly of the PC15 genome allowed the scaffold position of the genes expressed in the 7-0 stressed samples to be determined (Fig. 1). The 157 gene models in the 7-0 sample were distributed across 11 *P. ostreatus* PC15 v1.0 scaffolds. To determine whether this distribution was even across the genome, we calculated the number of 7-0 enriched genes per chromosome per Mbp, which yielded a global gene density of 4.12 genes per Mbp (S.D. 2.10). Scaffolds 4 and 5 appeared to be slightly enriched in 7-0 genes (27 and 23 gene models corresponding to densities of 7.51 and 6.50 7-0 enriched genes per Mbp, respectively). By contrast, scaffold 11 had a significantly lower 7-0 gene density (1.35 genes per Mbp) and no 7-0 enriched genes were found either in scaffold 12 or in the mitochondrial DNA.

For a finer analysis of the chromosome gene positions, a sliding window of 0.5 Mbp was used; also, genes were considered to cluster whenever five or more 7-0 enriched gene models fell into the window. Accordingly, eight gene clusters were identified that grouped 52 of the gene models (33.1% of the enriched models); these clusters could be placed in five of the scaffolds. The most prominent gene cluster was found on scaffold 3 and grouped nine gene models.

Functional analysis of the genes expressed in postharvest stress 7-0. To obtain functional information about the genes retrieved using the 7-0 EST sample, the 253 EST were submitted to a BLAST search against the NCBI database, with the significance threshold of the E-value set at < E-04. Under these search conditions, 156 hits were recovered (hits recovering rRNA were excluded from this study). The principal groups of genes identified with the 7-0 EST are listed in Table 1 (for complementary information, ask authors). The EST were classified according to the functions of their corresponding genes.

The largest EST class corresponded to genes involved in general and secondary metabolism (46 EST). Within this group, genes coding for carbohydrate active enzymes (CAZy) were predominant. These genes included members of different glycosyl hydrolase families, a carbohydrate binding module, and two genes involved in glucan metabolism. Three other CAZy genes (corresponding to four EST) were included in the cell structure and growth class since they are involved in chitin metabolism. In total, the CAZy genes and EST accounted for 15 EST and 14 gene models, indicating the high relevance of this family under the studied conditions.

Three gene models were identified as directly related to amino-acid and nitrogen metabolism. Given their recovery frequency (eight EST), they are likely important for postharvest mushroom metabolism. The metabolism of ornithine is especially relevant since a gene model for carbamoyl phosphate synthase was also found among the 7-0 enriched genes along with other gene models coding for ornithine aminotransferase. In addition to these genes, others, such as gene model 26312 (corresponding to a cleft lip and palate associated transmembrane protein), have been associated with the metabolism of arginine [15]. Five other EST retrieved a gene containing the DUF323 domain (gene model 40078), which has been associated with formylglycine-generating enzymes [11].

Three gene models, encoding a catalase (four EST), an aryl alcohol oxidase (one EST), and a copper radical oxidase (one EST), were also retrieved. These genes are presumably involved in the fungus' ligninolytic activity [18,19,40], which remains active during postharvest. Fourteen EST were classified in the protein metabolism (synthesis and degradation) group, which contained eight EST retrieving genes encoding proteases and protease-related enzymes.

In the nucleic acid metabolism group there were 11 EST. Among them, gene models 44554 (coding for an argonaute-like protein) and 44257 (coding for a pentatricopeptide repeat protein) were of particular interest. Members of the argonaute gene protein family interact with small non-coding RNAs involved in RNAi gene silencing [20,30,41], while the protein encoded by gene model 44257 belongs to the pentatricopeptide repeat-containing proteins involved in the maintenance of ribosomal RNA in yeasts [33]. These proteins are probably related to different steps in RNA metabolism and to the maintenance of intracellular organelles [37], especially mitochondria [42]. The relevance of RNA metabolism under the conditions studied in this work is further emphasized by the presence of an EST corresponding to gene model 24106, which encodes a member of the DEAD/DEAH box helicase family implicated in most RNA metabolic processes [8,9,26], and of another EST corresponding to model 186100, which encodes another RNA helicase.

Within the group of EST classified as related to cell structure and growth, and in addition to the CAZy enzymes described above, EST 70_68, corresponding to gene model 175868 and preliminarily identified as a BEM46 family protein, merits attention. This family of plant proteins includes signal transducer/receptor proteins involved in the response to stimuli such as gravity and light [28]. Note that EST 70_306 and 70_420 were annotated as corresponding to mRNA regulated by blue light, which is considered to be essential for fruit body development in these fungi [2]. A high rate of recovery of fungal lectins was also obtained in this group of genes.

The group of genes involved in cellular signaling contained 11 EST. The more abundant group corresponded to Ras-like proteins, which are involved in cell polarity [17,22]. It was recently reported that a small Ras-like protein is involved in the autophagy processes induced by starvation in mammals [3]; an analogous response could be expected in cut and stored fungal fruit bodies. Another gene of interest within this group was retrieved by EST 70_190 (gene model 172262). It was preliminarily annotated as a TNFR/NGFR gene encoding a member laminin of the cysteine-rich region family protein. Interestingly, a similar gene, identified in rice, is responsible for a major quantitative trait locus (QTL) involved in grain characteristics [12].

The second largest group (23 members) of EST were those classified as belonging to transport-related genes. The

Table 1. Preliminary functional annotation based on the BlastX hits in the NCBI gene database

Gene ID	Preliminary functional annotation
	General and secondary metabolism (46 ESTs)
185768	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase
42689	1,3-beta-glucan synthase
19778	Alanine aminotransferase
164890	Aryl alcohol oxidase
43812	ATPase
61627	Carbamoyl-phosphate synthase
32832	Carbohydrate-binding module family 13 protein
62309	Catalase
170071	Cephalosporin esterase
26312	Cleft lip and palate associated transmembrane protein
186162	Copper radical oxidase variant A glyoxal oxidase
162240	Cyclohexanone monooxygenase
41780	Dioxygenase
40078	DUF323 domain-containing protein
173106	Epoxide hydrolase 2
173859	FAD binding domain-containing protein
39461	Glucan 1,3 β-glucosidase
154880	GLycoside hydrolase family 15 protein
41613	Glycoside hydrolase family 3 protein
175036	Glycoside hydrolase family 31 protein
43197	Glycoside hydrolase family 47 protein α-mannosidase 1
175899	Glycosyltransferase family 15 protein
164450	Glycosyltransferase family 22 protein
30539	Glycosyltransferase family 39 protein
41463	IMP dehydrogenase
159066	NUXM, NADH-ubiquinone oxidoreductase subunit
34610	Ornithine aminotransferase
34610	Ornithine-oxo-acid aminotransferase
12746	Phosphatidylinositol phosphate phosphatase
170268	Phosphatidylinositol transfer protein
185961	Phosphoenolpyruvate carboxykinase

Gene ID	Preliminary functional annotation
	Protein metabolism (synthesis and degradation) (14 ESTs)
31826	26S protease regulatory subunit 6B
185944	60S ribosomal protein L14
26193	EF Tu GTP binding domain-containing protein
186121	Eukaryotic translation initiation factor 5A
35487	Peptidase M14
185945	40S ribosomal protein S12
185945	40S ribosomal protein S13
153610	Proline-specific peptidase
52559	ATP-dependent protease La
42347	Translation release factor
155735	Mitochondrial intermediate peptidase
156001	Polyubiquitin containing 7 ubiquitin monomers
42347	Translation release factor
	Transport (23 ESTs)
185815	ADP-ATP carrier protein 2, mitochondrial precursor
41688	APC Amino-acid permease
50967	Arabinose-proton symporter
14522	ATP-binding cassette transporter
44692	Cation/H^+ exchanger
50923	Coenzyme A transporter
185815	Eukaryotic ADP/ATP carrier
48688	Glyceroaquaporin
155224	Major facilitator superfamily transporter
50790	Mitochondrial import inner membrane translocase
47115	Multidrug/Oligosaccharidyl-lipid/Polysaccharide flippase
158619	Phthalate transporter
22455	Plasma membrane H^+-transporting ATPase
25942	RTA1-like protein
39521	Stomatin family protein
28225	Succinate/fumarate mitochondrial transporter

Continued on next page

Table 1. *Continued*

Gene ID	Preliminary functional annotation
	Cell structure and growth (15 ESTs)
160531	Actinin-like protein
175868	BEM46 family protein
20480	Cell division cycle protein
161688	Chitin deacetylase partial sequence
21633	Chitin synthase 8 (glycosyltransferase family 2 protein)
35056	Endochitinase (Glycoside hydrolase family 18 protein)
153813	Fungal Lectin
27082	Peroxisomal biogenesis factor 2
49499	Peroxisomal membrane protein PEX16
42414	Tubulin gamma chain
173323	Unc104-type kinesin
185814	Vesicular-fusion protein SEC17
	Nucleic acid metabolism (11 ESTs)
158410	Chromodomain-helicase DNA-binding protein
51943	DNA repair protein rhp42
174532	Histone acetyltransferase mst2
51378	pre-rRNA-processing protein IPI3
52551	DNA clamp loader
44554	Argonaute-like protein
44257	Pentatricopeptide repeat protein
24106	DEAD/DEAH box helicase
52061	pre-mRNA-processing-splicing factor
186100	RNA helicase
51393	tRNA-intron endonuclease
	Cellular signalling (11 ESTs)
29587	CAMK/CAMK-Unique protein kinase
165985	COP9 signalosome subunit 6
46599	MAP kinase phosphatase
169392	Protein kinase C
46433	Ras-like protein
61429	Ser/Thr protein kinase
172262	TNFR/NGFR cysteine-rich region family protein
25694	Transcription factor PacC
159408	Protein typrosin kinase

Hypothetical proteins (37 ESTs)[a]		
11121	43691	158093
12374	44536	160378
13580	48688	160783
15621	48688	162145
20345	50936	162867
21997	51247	167493
23844	52495	172796
23906	61655	173706
27289	62016	174283
28111	155224	175114
30743	157567	176014

[a]The hypothetical protein models correspond to hits with *Coprinopsis cinerea* (five models/ESTs), *Laccaria bicolor* (five models/ESTs), *Moniliophthora perniciosa* (three models/four ESTs), *Schizophyllum commune* (six models/ESTs) and *Serpula lacrymans* (14 models/17 ESTs).

APC amino-acid permease family and the major facilitator superfamily of transporters are two of the largest transporter families described thus far [16,35]. The high recovery rates of these two types of transporters, as well as the overall large number of genes belonging to this class in the 7-0 sample, underline the importance of these processes in the postharvest life of *P. ostreatus*.

For 37 EST retrieving genes, no preliminary annotation could be provided.

KOG annotation. The EuKaryotic Orthologous Groups (KOG) of proteins is a eukaryote-specific version of the NCBI's Clusters of Orthologous Groups (COG) tool for identifying ortholog and paralog proteins [http://www.ncbi.nlm.nih.gov/COG/]. Provided by the JGI for JGI-sequenced organisms, the KOG tool offers a complementary approach to assessing the integrated function of the genes retrieved in the 7-0 database. Table 2 summarizes the KOG annotation of the genes identified, grouping KOG hits within each catego-

ry for purposes of simplification. Each retrieved KOG term was weighted by the number of times that each EST or gene was recovered in the general analysis. Of the 155 queried KOG terms identified, those with the highest values in each functional category were: post-translational modification, protein turnover, and chaperones, in the class of cellular processes and signaling; and translation, ribosomal structure, and biogenesis. in the class of information storage and processing. In the category of metabolism, the processes involved in protein and carbohydrate transport and in metabolism were predominant. These results confirmed an active metabolism in the 7-day harvested fruit bodies that was centered on carbohydrate and protein turnover.

Table 2. KOG-term frequency

KOG term	Frequency
Cellular Processes and Signaling	48
Posttranslational modification, protein turnover, chaperones	15
Cytoskeleton	8
Intracellular trafficking, secretion, and vesicular transport	6
Signal transduction mechanisms	6
Cell wall/membrane/envelope biogenesis	4
Defense mechanisms	4
Extracellular structures	4
Nuclear structure	1
Information Storage and Processing	29
Translation, ribosomal structure, and biogenesis	11
Transcription	7
RNA processing and modification	6
Chromatin structure and dynamics	3
Replication, recombination and repair	2
Metabolism	52
Amino acid transport and metabolism	12
Carbohydrate transport and metabolism	12
Energy production and conversion	8
Lipid transport and metabolism	8
Inorganic ion transport and metabolism	7
Cell cycle control, cell division, chromosome partitioning	2
Secondary metabolites biosynthesis, transport, and catabolism	2
Nucleotide transport, and metabolism	1
Poorly Characterized	26
General function prediction only	20
Function unknown	6
Total	155

Transcriptomics analysis of the retrieved genes.

To complement the annotation data discussed above, we scored the transcription values of the 7-0 enriched genes in the general transcriptomics database for *P. ostreatus*. Only the data from static cultures were used, since compared to shaken cultures this condition more closely resembles that expected for mature fruit bodies. For each of the retrieved genes, the corresponding expression value in monokaryons and dikaryons was calculated. The obtained expression levels (RPKM) ranged from 0.0 to 1433.65 in monokaryons, and from 0.0 to 1416.73 in dikaryons, indicating that the 7-0 enriched genes are those with low expression levels in liquid cultures of *P. ostreatus* (data not shown). The transcription levels of each one of the 7-0 enriched genes in monokaryons and dikaryons were compared by using the base-2 logarithm of the ratio between the expression of each gene in monokaryons vs. dikaryons, with a threshold of 1.5 used to determine whether a gene was overexpressed. As shown in Table 3, 21 gene models appeared to be overexpressed in dikaryons. This result was expected, since the EST came from a dikaryon fruit body. The degree of dikaryotic overexpression was, in some cases, very high: 29.08-fold for gene model 162333 (coding for a peptidase S33, mapping to chromosome 9) and > 9-fold for model 160783 (codin for a major intrinsic protein, aquaglyceroporin). Model 175868, encoding the above-mentioned BEM46 protein, was also overexpressed in dikaryons; this gene likewise showed a high recovery frequency as an EST.

Conversely, three 7-0 enriched gene models were overexpressed in monokaryons. Two of them showed minimal expression levels (models 153859 and 173106) whereas the other was model 167484, encoding an epoxide hydrolase and recovered twice as an EST in the 7-0 sample.

Taken together, the genomics and transcriptomics data presented herein provide a detailed picture of the fruit bodies stored for 7 days at 4ºC, revealing an active metabolism that mobilized proteins and carbohydrates. RNA metabolism was also highly active during storage. The importance of nitrogen metabolism under these conditions was evidenced by the level of recovery of arginine and ornithine transaminases. As stored fruit bodies must maintain their biological activity using their own resources, the expression of autophagy-related genes to mobilize nutrients and responsible for the overall decay of the fruit bodies is not surprising. Genes enriched

Table 3. Comparative expression of the 7-0 enriched genes in monokaryotic (mk) and dikaryotic (dk) submerged cultures

EST number	ID PC15	ID PC9	Frequency	mk (sta)	dk (sta)	mk/dk
70_390	153610	116542	1	29.08	816.75	–5.81
70_570	160783	72552	2	18.60	173.41	–4.22
70_68	175868	90475	7	3.45	26.56	–3.95
70_264	159408	#N/A	1	4.99	33.18	–3.73
70_207	35056	99171	2	5.80	34.93	–3.59
70_22	43197	88568	1	33.61	189.70	–3.50
70_226	173859	84411	1	0.52	2.14	–3.03
70_212	185921	76372	1	88.14	305.95	–2.80
70_511	165648	101130	1	9.16	28.77	–2.65
70_136	154880	65412	1	17.93	43.56	–2.28
70_95	50967	94767	1	78.00	185.08	–2.25
70_180	174649	91404	2	2.85	6.27	–2.14
70_569	164450	121339	1	20.65	44.60	–2.11
70_393	158619	132960	1	26.98	52.25	–1.95
70_12	25942	83360	1	8.58	15.69	–1.87
70_451	175114	114131	1	33.49	59.08	–1.82
70_258	175036	91487	1	34.37	52.49	–1.61
70_402	20480	81919	1	44.71	65.42	–1.55
70_380	32832	116255	1	382.06	557.22	–1.54
70_328	48678	92716	1	11.01	15.65	–1.51
70_577	162867	21676	1	3.31	4.70	–1.51
70_401	153859	116979	1	7.76	1.10	1.83
70_420	167484	132620	2	1433.65	185.42	1.95
70_77	173106	115848	1	54.09	6.53	2.05

under cold-storage conditions were those with medium- to low-level expression in static liquid cultures. Note that the method used to construct the enriched library should have removed most housekeeping genes. Notwithstanding, some of the enriched genes appeared to be highly overexpressed in dikaryons vs. monokaryons. The enriched genes mapped throughout the *P. ostreatus* genome, although some gene clusters had specific chromosomal locations. In any case, many gene models must code for still unknown functions. Clearly, much work remains before the living conditions of an organism can be explained by its genomic and transcriptomic data.

Acknowledgements. This work was supported by funds from the projects AGL2008-05608-C02-01 of the Spanish National Research Plan; Bioethanol-Euroinnova, from the Autonomous Government of Navarre; and by additional institutional support from the Public University of Navarre. Some of the sequence data were produced in the *P. ostreatus* genome sequence project developed at the JGI within the community sequence program under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Livermore National Laboratory under contract no. W-7405-Eng-48, Lawrence Berkeley National Laboratory under contract no. DE-AC02-05CH11231, and Los Alamos National Laboratory under contract no. DE-AC02-06NA25396. LR leads the project and the research group; JAO, JLL and FS work in bioinformatics handling of data; GP and RC, in genomics quantitative RNA measurements; MA, in pro-

teomics analyses; AO, AP and EM, in enzymatic analyses; AGP coordinated and prepared the manuscript. JLL is supported by a postdoctoral fellowship from the Public University of Navarre; AO has been supported by a fellowship from the Spanish Agency of International Cooperation; MA and RC by funds of the Euroinnova project; and AP and EM have been partially supported by funds of the Public University of Navarre.

Competing interest. None declared.

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