Title

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Short contribution

Analysis of expressed sequence tags (ESTs) from *Lentinula edodes*

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Running title: *Lentinula edodes* EST

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Abstract

The 1,031 expressed sequence tags (ESTs) from the basidiomycete *Lentinula edodes* were generated as a pilot experiment to see distribution of genes expressed in *L. edodes*. Among them, genes for hydrophobin, which are specifically found in filamentous fungi, were most frequently obtained (33 times), indicating they are highly expressed in *L. edodes*. In addition to known hydrophobin 1 and 2 types, our analysis revealed the existence of novel types of hydrophobin, which we named hydrophobin 3, 4, and 5. As secondly and thirdly expressed genes, genes for phosphatidylserine decarboxylase and formate dehydrogenase were obtained 8 times and 7 times, respectively. It should be noted that two important genes involved in the RNAi pathway were found, suggesting a future application for gene shut down. The 50 ESTs were identical with the sequences already reported in *L. edodes*. The 402 ESTs were found to show significant sequence similarity (E value $\leq 1 \times 10^{-5}$) with the proteins reported (or predicted) in other species. The 419 ESTs contained polyA. In total 387,952 bp sequences were obtained and registered in DDBJ/GenBank (accession number BJ998097-BJ999127).

Keywords *Lentinula edodes*, cDNA, EST

Introduction

Lentinula edodes belonging to Basidiomycota is the edible mushroom popularly known as Shiitake mushroom in Japan. There are 250 genomic DNA or cDNA sequence entries available in GenBank database (Jul, 2006 release). Analysis of about 60 different genes from *L. edodes* were reported in the literature including the CAP and 14-3-3 genes which we had analyzed (Kawamukai et al., 1992; Zhou et al., 1998; Zhou et al., 2000). Isolation of the genes that are specifically expressed in fruit body formation in *L. edodes* led to the isolation of the gene encoding a cytochrome P450 protein (Hirano et al., 2004). A representational difference analysis during fruiting body formation in *L. edodes* revealed 105 genes (Miyazaki et al., 2005). Up to now, genomes of several mushrooms have been determined, but the genome and cDNA sequences of *L. edodes* have not been thoroughly elucidated. Because knowledge of *L. edodes* chromosomes is very limited, even the genome size and the exact chromosome number sof *L. edodes* are not clear yet although there is a proposal that *L. edodes* has eight chromosomes (Tanaka R. and Koga I, 1972).

Expressed Sequence Tags (ESTs) were useful to know how many different genes were roughly expressed in a certain species. It will also help to know the sites of intron(s) by comparing EST with the genomic DNA sequences. Thus, EST provides a cost-effective approach for gene discovery. A large scale EST analyses were conducted in *Aspergillus niger* (Semova et al., 2006), *Neurosphora crassa* (Zhu et al., 2001), *Paracoccidioides brasiliensis*, and *Ustilago maydis* (Sacadura and Saville, 2003) among fungi apart from yeasts. Genome sequences of the lignocellulose degrading *fungus*

Phanerochaete chrysosporium (Martinez et al., 2004) human pathogenic basidiomycetous yeast *Cryptococcus neoformanswas* (Loftus et al., 2005), rice blast fungus *Magnaporthe grisea* (Dean et al., 2005) and *Aspergillus fumigatus* (Nierman et al., 2005) were completed. But, no such an approach was conducted in *L. edodes*.

In this study, we have determined 1,031 ESTs of *L. edodes* as a pilot experiment by random sequencing of the cDNA library.

Materials and Methods

Construction of *L. edodes* cDNA libraries

We adopted two strategies for constructing the cDNA libraries which was made in the two-hybrid vector for the other purpose. One strategy made use of a previously-constructed phage library. We amplified a phage population of around 1.2 x 10^6 plaque-forming units from a previously-constructed λgt 10 cDNA library and extracted the phage DNA from 1 liter of phage culture. The phage DNA was digested with *Eco* RI and inserts longer than 500 bp were recovered by sucrose-gradient centrifugation. These cDNAs were then inserted into *Eco* RI-digested, dephosphorylated pGAD GH (Clontech), introduced into *E. coli* and purified using Qiagen-500 tips (Qiagen). The library thus obtained consisted of plasmids prepared from about 2 x 10^6 *E. coli* colonies. The other strategy we used was more conventional. Total RNA was extracted from vegetative mycelia of strain FMC12 by the AGPC (acid guanidinium-phenol-chloroform) method. Poly (A)+ mRNA was purified with oligotex-dT 30 (TaKaRa) and cDNA was synthesized from poly (A)+ mRNA using a cDNA synthesis kit from Amersham according to the manufacturer's instructions. The cDNA fragments were purified using a spin column (Pharmacia) and those longer than 400 bp were collected. The library thus obtained consisted of about 5 x 10^6 independent clones.

DNA sequencing and analysis

For sequencing three primers, namely, M13 forward primer GTTTTCCCAGTCACGACGTTGTA, M13 reverse primer AGCGGATAACAATTTCACACAGGA and GAD primer CCGTCGACGTAATACGACTCACTATAG, were used. To determine the long DNA in some clones, oligonucleotides were synthesized. DNA sequences were determined by the dideoxynucleotide chain termination method using an ABI377 DNA sequencer. Similarity searches were performed by BLASTx, BLASTp and BLASTn against DNA/protein/EST data bases in DDBJ/GenBank.

Results

Sequence analysis

Random sequencing of cDNA from *L. edodes* was done in the library constructed in the vector pGAD GH. The library was originally constructed to find the proteins that interact with the bait protein by two hybrid screening (Zhou et al., 2000). But it was here used as an EST analysis. Because the cDNA library was constructed in the vector pGAD GH at the *Eco* RI sites, the fragments of cDNA frequently contained *Eco* RI sites in both ends. By randomly sequencing, 1,031 ESTs was constructed. In total 387,952 bp sequences were obtained and registered in GenBank/DDBJ (accession number BJ998097-BJ999127). Average EST length was calculated to be 376bp.

The list of the ESTs found in *L. edodes* that retain the sequence similarity at the amino acids (or DNA) level (E value $\leq 1 \times 10^{-5}$) with the proteins (or genes) from other species was shown in Table 1. Only one EST were listed in Table 1 even though more than two ESTs were obtained. The sequences of the genes homologous to uncharacterized genes were 98 and those are omitted from the list of Table 1. EST was first compared with the sequence registered in the NCBI data base by tBLASTx or BLASTn. The annotated web sites based on the characterized genes from two yeasts *S. cerevisiae* [http://www.yeastgenome.org/] and *S. pombe* [http://www.sanger.ac.uk/Projects/S_pombe/] gave us the most useful references for estimation of their functions. By classification, the genes for metabolic enzymes were most frequently obtained and then ones for transport, translation, and protein degradation followed (Table 1).

The number of the times in which the same clones were obtained was shown in Table 2. The 25 genes obtained two times were omitted from the list of Table2. Among 1,031 ESTs, hydrophobins were most frequently identified. They summed up to 33 times that means hydrophobins 1, 2, 3, 4, and 5 types are obtained 24, 2, 2, 2, 3 times, respectively. From the sequences we found the existence of hydrophobin 3, 4, and 5 types besides already known hydrophobin 1 and 2 (Ng et al., 2000; Nishizawa et al., 2002). Our result that hydrophobins are highly expressed in *L. edodes* support the results that hydrophobins are expressed at high levels in many filamentous fungi (Linder et al., 2005). The next frequently obtained ESTs are the ones that encode phosphatidylserine decarboxylase (8 times) and then formate dehydrogenase (7 times). Sometimes, two or more paralogous genes were obtained in ESTs such as the genes for D-arabinono-1,4-lactone oxidase, DAHP synthase, flavin-containing monooxygenase, superoxide dismutase, calmodulin, and metalloprotease. 21 different ribosome encoding ESTs were obtained, but no systematic naming of ribosomal proteins were performed here. 5.8 S, 25S and 18S ribosomal RNA sequences and their internal spacer sequences were included in ESTs, where 5.8S and 18S ribosomal RNA sequence have not been reported in *L. edodes*.

Discussion

In this study, we have determined the 1,031 ESTs from *Lentinula edodes* (Shiitake mushroom). The 50 ESTs were identical with the sequences already reported in *L. edodes*. The 402 ESTs were found to show significant sequence similarity (E value $\leq 1 \times 10^{-5}$) with the proteins reported (or predicted) in other species. Those ESTs include many interesting genes to study for application and basic microbiology.

Some ESTs for glycoside hydrolases including alfa-amylase, chitinase, alfa-galactosidase, beta-1,3-glucanase (Sakamoto et al., 2005), beta-glucosidase, mannase, alfa-mannosidase, and beta-mannosidase were obtained. Those genes are potentially useful as enzyme resources for searching new sugar hydrolytic way. The genes for P450 type and other oxygenases may contribute the understanding and application for none-degradable compound, which *L. edodes* naturally can degrade. To support the knowledge that *L. edodes* produces the precursor of vitamin D, several sterol biosynthetic genes were found. In addition to *fad1* encoding delta 9-fatty acid desaturase and *fad2* encoding delta 12-fatty acid desaturase (Sakai and Kajiwara, 2005), the gene for delta 6-fatty acid desaturase was obtained in ESTs. The *priA* gene which has been known to be developmentally regulated was obtained (Kajiwara et al., 1992). The second *ras* gene, which we named *ras2,* similar to *ras1* (Hori et al., 1991) was obtained. Those genes will probably play roles in development of *L. edodes*. Most interestingly, there exist the genes similar to argonaute and RNA dependent RNA polymerase in ESTs, that is the part of evidence that the RNAi mechanism is operating in *L. edodes*. The RNAi method for gene shut down will be extremely useful if it become possible, because otherwise it is extremely difficult at present to make gene disruption in *L. edodes*.

Our EST analysis proved that it is very efficient approach to find distributions of the expressed genes and the novels genes, which retain the potential source of beneficial enzymes for biotechnology. Continuation of the EST analysis will give us more information on the distributions of the genes in *L. edodes* and further contribute in the field of applied microbiology as well.

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References

Dean R A, Talbot N J, Ebbole D J, Farman M L, Mitchell T K, Orbach M J, Thon M, Kulkarni R, Xu J R, Pan H, Read N D, Lee Y H, Carbone I, Brown D, Oh Y Y, Donofrio N, Jeong J S, Soanes D M, Djonovic S, Kolomiets E, Rehmeyer C, Li W, Harding M, Kim S, Lebrun M H, Bohnert H, Coughlan S, Butler J, Calvo S, Ma L J, Nicol R, Purcell S, Nusbaum C, Galagan J E, Birren B W (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434: 980-986

Hirano T, Sato T, Enei H (2004) Isolation of genes specifically expressed in the fruit body of the edible basidiomycete *Lentinula edodes*. Biosci Biotechnol Biochem 68: 468-472

Hori K, Kajiwara S, Saito T, Miyazawa H, Katayose Y, Shishido K (1991) Cloning, sequence analysis and transcriptional expression of a *ras* gene of the edible basidiomycete *Lentinus edodes*. Gene 105: 91-96

Kajiwara S, Yamaoka K, Hori K, Miyazawa H, Saito T, Kanno T, Shishido K (1992) Isolation and sequence of a developmentally regulated putative novel gene, *priA*, from the basidiomycete *Lentinus edodes*. Gene 114: 173-178

Kawamukai M, Gerst J, Field J, Riggs M, Rodgers L, Wigler M, Young D (1992) Genetic and biochemical analysis of the adenylyl cyclase-associated protein, cap, in *Schizosaccharomyces pombe*. Mol Biol Cell 3: 167-180

Linder M B, Szilvay G R, Nakari-Setala T, Penttila M E (2005) Hydrophobins: the protein-amphiphiles of filamentous fungi. FEMS Microbiol Rev 29: 877-896

Loftus B J, Fung E, Roncaglia P, Rowley D, Amedeo P, Bruno D, Vamathevan J, Miranda M, Anderson I J, Fraser J A, Allen J E, Bosdet I E, Brent M R, Chiu R, Doering T L, Donlin M J, D'Souza C A, Fox D S, Grinberg V, Fu J, Fukushima M, Haas B J, Huang J C, Janbon G, Jones S J, Koo H L, Krzywinski M I, Kwon-Chung J K, Lengeler K B, Maiti R, Marra M A, Marra R E, Mathewson C A, Mitchell T G, Pertea M, Riggs F R, Salzberg S L, Schein J E, Shvartsbeyn A, Shin H, Shumway M, Specht C A, Suh B B, Tenney A, Utterback T R, Wickes B L, Wortman J R, Wye N H, Kronstad J W, Lodge J K, Heitman J, Davis R W, Fraser C M, Hyman R W (2005) The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. Science 307: 1321-1324

Martinez D, Larrondo L F, Putnam N, Gelpke M D, Huang K, Chapman J, Helfenbein K G, Ramaiya P, Detter J C, Larimer F, Coutinho P M, Henrissat B, Berka R, Cullen D, Rokhsar D (2004) Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. Nat Biotechnol 22: 695-700

Miyazaki Y, Nakamura M, Babasaki K (2005) Molecular cloning of developmentally specific genes by representational difference analysis during the fruiting body formation in the basidiomycete *Lentinula edodes*. Fungal Genet Biol 42: 493-505

Ng W L, Ng T P, Kwan H S (2000) Cloning and characterization of two hydrophobin genes differentially expressed during fruit body development in *Lentinula edodes*. FEMS Microbiol Lett 185: 139-145

Nierman W C, Pain A, Anderson M J, Wortman J R, Kim H S, Arroyo J, Berriman M, Abe K, Archer D B, Bermejo C, Bennett J, Bowyer P, Chen D, Collins M, Coulsen R, Davies R, Dyer P S, Farman M, Fedorova N, Feldblyum T V, Fischer R, Fosker N, Fraser A, Garcia J L, Garcia M J, Goble A, Goldman G H, Gomi K, Griffith-Jones S, Gwilliam R, Haas B, Haas H, Harris D, Horiuchi H, Huang J, Humphray S, Jimenez J, Keller N, Khouri H, Kitamoto K, Kobayashi T, Konzack S, Kulkarni R, Kumagai T, Lafon A, Latge J P, Li W, Lord A, Lu C, Majoros W H, May G S, Miller B L, Mohamoud Y, Molina M, Monod M, Mouyna I, Mulligan S, Murphy L, O'Neil S, Paulsen I, Penalva M A, Pertea M, Price C, Pritchard B L, Quail M A, Rabbinowitsch E, Rawlins N, Rajandream M A, Reichard U, Renauld H, Robson G D, Rodriguez de Cordoba S, Rodriguez-Pena J M, Ronning C M, Rutter S, Salzberg S L, Sanchez M, Sanchez-Ferrero J C, Saunders D, Seeger K, Squares R, Squares S, Takeuchi M, Tekaia F, Turner G, Vazquez de Aldana C R, Weidman J, White O, Woodward J, Yu J H, Fraser C, Galagan J E, Asai K, Machida M, Hall N, Barrell B, Denning D W (2005) Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. Nature 438: 1151-6

Nishizawa H, Miyazaki Y, Kaneko S, Shishido K (2002) Distribution of hydrophobin 1 gene transcript in developing fruiting bodies of *Lentinula edodes*. Biosci Biotechnol Biochem 66: 1951-1954

Sacadura N T, Saville B J (2003) Gene expression and EST analyses of *Ustilago maydis* germinating teliospores. Fungal Genet Biol 40: 47-64

Sakai H, Kajiwara S (2005) Cloning and functional characterization of a Delta12 fatty acid desaturase gene from the basidiomycete *Lentinula edodes*. Mol Genet

Genomics 273: 336-341

Sakamoto Y, Irie T, Sato T (2005) Isolation and characterization of a fruiting body-specific exo-beta-1,3-glucanase-encoding gene, exg1, from *Lentinula edodes*. Curr Genet 47: 244-252

Semova N, Storms R, John T, Gaudet P, Ulycznyj P, Min X J, Sun J, Butler G, Tsang A (2006) Generation, annotation, and analysis of an extensive *Aspergillus niger* EST collection. BMC Microbiol 6: 7

Tanaka R, Koga I (1972) Karyological studies on *Lentinus edodes*, a basidiomycete. J Jpn Bot 7:289-296

Zhou G L, Miyazaki Y, Nakagawa T, Tanaka K, Shishido K, Matsuda H, Kawamukai M (1998) Identification of a CAP (adenylyl-cyclase-associated protein) homologous gene in *Lentinus edodes* and its functional complementation of yeast CAP mutants. Microbiology 144 (Pt 4): 1085-1093

Zhou G L, Yamamoto T, Ozoe F, Yano D, Tanaka K, Matsuda H, Kawamukai M (2000) Identification of a 14-3-3 protein from *Lentinus edodes* that interacts with CAP (adenylyl cyclase-associated protein), and conservation of this interaction in fission yeast. Biosci Biotechnol Biochem 64: 149-159

Zhu H, Nowrousian M, Kupfer D, Colot H V, Berrocal-Tito G, Lai H, Bell-Pedersen D, Roe B A, Loros J J, Dunlap J C (2001) Analysis of expressed sequence tags from two starvation, time-of-day-specific libraries of *Neurospora crassa* reveals novel clock-controlled genes. Genetics 157: 1057-1065

Table 1. footnotes

[a] The Accession number of DDBJ/GenBank

[b] The determined nucleotide base pair

[c] Circle indicates the clone that contained polyA and hyphen did not.

[d] The putative functions given by NCBI database are classified by a broad category.

[d] The gene name in NCBI data base. Bold ones indicate the gene naming in *L. edodes*.

[f] Abbreviations of organisms are followings:

Agaricus bisporus Agaricus xanthodermus Agrobacterium tumefaciens Amanita muscaria Antrodia camphorata Arabidopsis thaliana Arthroderma benhamiae Aspergillus flavus Aspergillus fumigatus Aspergillus nidulans Aspergillus niger Aspergillus oryzae Botryotinia fuckeliana Caenorhabditis elegans Candida albicans Chlamydomonas reinhardtii Chloroflexus aurantiacus Coprinus cinereus Cryptococcus neoformans Danio rerio Dictyostelium discoideum Emericella nidulans Ganoderma microsporum Homo sapiens Laccaria bicolor Lentinula edodes Mus musculus Neurospora crassa Nicotiana sylvestris Paracoccidioides brasiliensis Paxillus involutus Pleurotus djamor Pleurotus sapidus Rhizopus stolonifer Russula olivacea Saccharomyces cerevisiae Schizophyllum commune Schizosaccharomyces pombe Thermoascus aurantiacus Thermotoga maritima Ustilago maydis Xenopus tropicalis

[g] The *E* score corresponds to the tBLASTX expected value obtained from homology analysis, except scores donated by “* “ where the BLASTn value was indicated.

Table 1 Putative functions of *L.edodes* ESTs

[a]DDBJ/GenBank	[a]bp	[c]polyA	[d]**Category** / Putative Function	[e]gene	[f]organisms	[g]score
Metabolism						
BJ998177	557	-	17-beta-hydroxysteroid dehydrogenase type 4	*mfp2*	*D. rerio*	2.00E-21
BJ998991	497	-	2-methyl citrate dehydratase	*PDH1*	*S. cerevisiae*	5.00E-66
BJ998475	568	○	2-nitropropanedioxygenase	*Afu2g09850*	*A. fumigatus*	1.00E-27
BJ998225	577	-	2,6-dihydroxybenzoic acid decarboxylase	*rdc*	*A. tumefaciens*	1.00E-23
BJ998281	552	○	3'(2'),5'-bisphosphate nucleotidase	*tol1*	*S. pombe*	3.00E-29
BJ998531	507	○	6-phosphofructokinase	*pfk1*	*S. pombe*	6.00E-06
BJ998600	226	-	6-phosphogluconate dehydrogenase	*GND2*	*S. cerevisiae*	3.00E-25
BJ998416	296	-	acetyl-CoA acetyltransferase	*aat*	*L. bicolor*	2.00E-21
BJ998465	614	-	acyl-Coenzyme A dehydrogenase, short/branched chain	*ACADSB*	*H. sapiens*	1.00E-53
BJ998238	569	-	alcohol dehydrogenase	*ADH1*	*S. pombe*	3.00E-52
BJ999047	525	-	aldehyde dehydrogenase 1	*aldA*	*A. nidulans*	3.00E-57
BJ998838	568	-	aldehyde dehydrogenase 2	*ALD2*	*S. cerevisiae*	2.00E-24
BJ998770	525	-	alpha-amylase	*amyA*	*A. niger*	3.00E-29
BJ998671	481	-	alpha-galactosidase	*agl3*	*T. reesei*	3.00E-41
BJ998955	369	-	alpha-glucan synthase	*mok1*	*S. pombe*	8.00E-08
BJ998453	632	-	alpha-mannosidase 1A class I	*mns1A*	*E. nidulans*	4.00E-24
BJ998729	444	-	alpha-mannosidase	*MAN1B1*	*H. sapiens*	3.00E-21
BJ998414	630	-	aminolevulinic acid synthetase	*hem1*	*A. bisporus*	2.00E-24
BJ998205	860	-	aminotransferase	*YJL060w*	*S. cerevisiae*	2.00E-44
BJ999076	638	-	beta-1,3-glucan binding protein	*NCU04168.1*	*N. crassa*	3.00E-32
BJ999109	333	-	beta-1,3-glucan synthase	*bgs4*	*S. pombe*	4.00E-17
BJ998774	448	○	beta-1,3-glucanase	***exg1***	*L. edodes*	1.00E-71
BJ998278	400	-	beta-glucosidase	*BGL2*	*T. aurantiacus*	3.00E-13
BJ998470	676	-	beta-mannosidase	*manB*	*T. maritima*	7.00E-08
BJ998456	562	-	C-4 methyl sterol oxidase	*erg25*	*S. pombe*	5.00E-55
BJ998448	633	-	chitinase	*chi*	*Aeromonas*	4.00E-07
BJ998903	354	-	chitin deacetylase-like mannoprotein MP98	*CND03490*	*C. neoformans*	6.00E-06
BJ998551	473	-	citrate synthase	*cit1*	*S. pombe*	2.00E-33
BJ998189	194	-	cyclohexanone 1,2-monooxygenase	*Afu8g00440*	*A. fumigatus*	6.00E-05
BJ999008	487	-	DAHP-synthase 1	*aro3*	*S. cerevisiae*	6.00E-37
BJ998821	379	○	DAHP-synthase 2	*aro4*	*S. cerevisiae*	1.00E-09
BJ998341	606	-	D-arabinitol dehydrogenase	*arbD*	*E. nidulans*	7.00E-15
BJ998665	266	○	D-arabinono-1,4-lactone oxidase	*CNN01640*	*C. neoformans*	5.00E-07
BJ999025	167	-	delta 6 fatty acid desaturase	*RnD6D*	*R. stolonifer*	3.00E-08
BJ999042	535	-	delta 9-fatty acid desaturase	***fad1***	*L. edodes*	2.00E-117
BJ999088	291	○	delta 12-fatty acid desaturase	***fad2***	*L. edodes*	2.00E-06
BJ998312	352	-	dolichyl-diphosphooligosaccharide protein glycotransferas	*CNB05600*	*C. neoformans*	4.00E-21
BJ998272	398	○	dolichyl-diphosphooligosaccharide protein glycotransferas	*CNJ01740*	*C. neoformans*	3.00E-22
BJ998994	483	-	ectonucleotide pyrophosphatase	*ENPP3*	*H. sapiens*	9.00E-20
BJ998889	602	○	endoglucanase	*Afu7g06740*	*A. fumigatus*	9.00E-05
BJ998998	522	-	flavin-containing monooxygenase 1	*Afu5g00310*	*A. fumigatus*	4.00E-21
BJ998331	566	○	flavin-containing monooxygenase 2	*Afu5g00310*	*A. fumigatus*	1.00E-33
BJ998395	516	-	fructose 1,6-bisphosphate aldolase	*FBA1*	*S. cerevisiae*	2.00E-46
BJ999028	497	-	GDP-mannase 4,6-dehydratase	*gmd*	*X. tropicalis*	2.00E-63
BJ998656	514	-	GTP cyclohydrolase	*SPAC1002.19*	*S. pombe*	2.00E-54
BJ998323	529	-	glucose oxidase	*god1*	*B. fuckeliana*	6.00E-09
BJ998180	540	○	glutamate decarboxylase	*GAD1*	*S. cerevisiae*	9.00E-49
BJ999111	378	○	glutamine dependent NAD+ synthase	*QNS1*	*S. cerevisiae*	2.00E-17
BJ998863	581	-	glutaminyl cyclase	*QPCT*	*H. sapiens*	9.00E-12
BJ998839	614	○	glycerol-1-phosphatase	*CNM01280*	*C. neoformans*	5.00E-20
BJ998383	697	○	glyoxysomal citrate synthase	*At3g58750*	*A. thaliana*	2.00E-49
BJ999023	410	-	guanosine-diphosphatase	*gda1*	*S. pombe*	2.00E-14
BJ998420	446	○	homoserine dehydrogenase	*SPBC776.03*	*S. pombe*	1.00E-15
BJ998680	450	○	inorganic pyrophosphatase	*ppa1*	*S. pombe*	3.00E-27
BJ998918	348	-	isocitrate lyase I	*ICL2*	*S. cerevisiae*	4.00E-15
BJ998537	589	-	mannanase	*man*	*A. bisporus*	6.00E-27
BJ998354	503	-	monocarboxylate permease 1	*MCH4*	*S. cerevisiae*	5.00E-15
BJ998509	504	-	monocarboxylate permease 2	*MCH5*	*S. cerevisiae*	4.00E-13
BJ998618	568	-	N-acetylglucosamine-phosphate deacetylase	*CaNAG2*	*C. albicans*	2.00E-17
BJ998329	310	-	N-acetylglucosamine-phosphate mutase	*SPAC13C5.05c*	*S. pombe*	2.00E-20
BJ998999	517	-	NAD-dependent formate dehydrogenase 1	*fdh1*	*S. cerevisiae*	3.00E-44
BJ998387	317	-	NAD-dependent formate dehydrogenase 2	*fdh2*	*C. boidinii*	3.00E-23
BJ998661	499	-	NADH dehydrogenase Fe-S protein	*NDUFS2*	*H. sapiens*	3.00E-83
BJ998349	576	○	N-oligosaccharyltransferase (gamma subunit)	*SPAPB17E12.11*	*S. pombe*	2.00E-22

BJ998696	481	-	OMST-oxidoreductase	*ord1*	*A. flavus*	2.00E-31
BJ998337	266	-	oxalate decarboxylase	*TOXDC*	*T. versicolor*	2.00E-38
BJ998428	708	-	oxysterol binding protein	*SPBC646.08c*	*S. pombe*	3.00E-35
BJ999038	532	○	para-aminobenzoic acid synthetase	*pab1*	*C. cinereus*	4.00E-46
BJ998176	573	○	phosphatidylserine decarboxylase	*CNA08120*	*C. neoformans*	5.00E-18
BJ998676	411	-	phosphoglucomutase A	*pgmA*	*D. discoideum*	7.00E-24
BJ998486	326	○	pyranose dehydrogenase	*pdh1*	*A. xanthodermus*	5.00E-15
BJ998943	240	-	pyruvate decarboxylase	*pdcA*	*A. fumigatus*	6.00E-23
BJ998228	788	○	riboflavin aldehyde-forming enzyme	***fbg16***	*L. edodes*	2.00E-121
BJ998683	449	○	short chain dehydrogenase	*SPAC4H3.08*	*S. pombe*	1.00E-06
BJ998472	642	-	sphinganine C4-hydroxylase	*Sur2p*	*S. cerevisiae*	1.00E-29
BJ998487	486	-	sterol 14 alpha-demethylase	*erg11*	*U. maydis*	8.00E-40
BJ998824	532	○	succinate dehydrogenase	*Sdh3*	*S. pombe*	1.00E-28
BJ998561	196	-	succinyl-CoA synthetase	*LSC2*	*S. cerevisiae*	7.00E-08
BJ998651	127	-	succinyl-CoA:L-malate CoA transferase subunit A	*SmtA*	*C. aurantiacus*	2.00E-08
BJ998978	499	○	sulfite reductase	*cys-4*	*N. crassa*	3.00E-27
BJ998300	409	○	thiazole biosynthetic enzyme	*thi2*	*S. pombe*	3.00E-13
BJ998787	459	-	thiazole biosynthetic enzyme	*nmt2*	*S. pombe*	6.00E-45
BJ998249	762	○	trehalose-6-phosphate synthase	*tps1*	*P. ostreatus*	3.00E-09
BJ998108	738	-	UDP-glucose-glycoprotein glucosyltransferase	*gpt1*	*S. pombe*	2.00E-58
BJ998284	222	○	uricase	*AN9470.2*	*A. nidulans*	1.00E-05
BJ998879	528	○	xylitol dehydrogenase	*XYL2*	*S. cerevisiae*	5.00E-05
BJ998151	566	-	zinc-binding dehydrogenase	*YML131W*	*S. cerevisiae*	8.00E-09
Energy production						
BJ998479	654	-	ATP synthase subunit 4	*ATP4*	*S. cerevisiae*	7.00E-36
BJ998650	505	○	ATP synthase subunit delta	*ATP7*	*S. cerevisiae*	6.00E-45
BJ998170	397	-	ATP synthase subunit gamma	*ATP3*	*S. pombe*	5.00E-14
BJ998165	409	○	cytochrome c oxidase subunit VIa	*COX13*	*S. cerevisiae*	2.00E-06
BJ998565	570	-	cytochrome b5 reductase	*CBR1*	*S. cerevisiae*	3.00E-27
BJ998741	424	-	mitochondrial ADP/ATP carrier protein	*ANC1*	*S. pombe*	2.00E-49
BJ998330	602	-	NADH:ubiquinone dehydrogenase 51 kDa subunit	*NDUFV1*	*H. sapiens*	2.00E-77
BJ998645	136	-	NADPH oxidase	*Nox2*	*P. anserina*	3.00E-07
Cell defence, Stress						
BJ998196	304	-	catalase	*CTT1*	*S. pombe*	8.00E-27
BJ998815	508	○	cyclophilin	*cpa1*	*P. ostreatus*	4.00E-45
BJ998959	146	-	cytochrome P450-1	*cyp450*	*P. sapidus*	5.00E-05
BJ998306	606	-	cytochrome P450-2	*cyp450*	*P. sapidus*	3.00E-26
BJ998385	683	-	cytochrome P450-3	*cypA*	*A. bisporus*	1.00E-24
BJ998282	349	-	glutaredoxin	*grx2*	*S. pombe*	7.00E-12
BJ998259	335	-	glutathione peroxidase	*gpx1*	*S. pombe*	2.00E-27
BJ998800	102	-	glutathione S-transferase	*ECM4*	*S. cerevisiae*	5.00E-08
BJ998355	256	○	glyoxalase I	*GLO1*	*H. sapiens*	1.00E-11
BJ998444	238	-	heat shock protein 104	*HSP104*	*S. cerevisiae*	2.00E-12
BJ999007	413	○	heat shock protein 12	*CNG04220*	*C. neoformans*	5.00E-17
BJ998313	362	○	heat shock protein 70-1	*SSB1*	*S. cerevisiae*	5.00E-12
BJ998621	555	-	heat shock protein 70-2	*SSC1*	*S. cerevisiae*	5.00E-80
BJ998702	561	-	heat shock protein 90	*HSP90*	*C. neoformans*	1.00E-60
BJ998788	412	○	heat shock protein12	*Afu6g12450*	*A. fumigatu*	2.00E-06
BJ998206	382	○	manganese superoxide dismutase 1	*sod1*	*A. bisporus*	2.00E-46
BJ998961	503	-	manganese-superoxide dismutase 2	*sod2*	*G. microsporum*	8.00E-66
BJ998430	346	○	metallothionein	*MT1*	*P. involutus*	6.00E-07
BJ998930	448	○	macrolide-binding protein FKBP12	*frr1*	*C. neoformans*	4.00E-30
BJ998882	102	-	peroxisomal Omega-class glutathione transferase	*GTO1*	*S. cerevisiae*	6.00E-07
Cell growth						
BJ998795	593	○	calmodulin 1	*cmd1*	*N. crassa*	2.00E-69
BJ998608	396	-	calmodulin 2	*cmd2*	*N. crassa*	2.00E-69
BJ998634	592	-	developmentally regulated gene	***priA***	*L. edodes*	2.00E-112
BJ999003	458	○	filament-specific protein	*UFU57*	*U. maydis*	4.00E-09
BJ998762	160	-	hydrophobin 1	***hyd1***	*L. edodes*	3.00E-73
BJ998866	534	○	hydrophobin 2	***hyd2***	*L. edodes*	7.00E-14
BJ999085	581	-	hydrophobin 3	***hyd3***	*L. edodes*	2.00E-12
BJ998875	515	○	hydrophobin 4	***hyd4***	*L. edodes*	2.00E-09
BJ998526	524	○	hydrophobin 5	***hyd5***	*L. edodes*	4.00E-18
BJ998436	465	○	highdosage growth inhibitor	*HMF1*	*S. cerevisiae*	2.00E-27
BJ998418	528	-	opioid growth factor receptor-like 1	*OGFRL1*	*H. sapiens*	2.00E-31
BJ999069	322	-	septin	*aspA*	*A. nidulans*	6.00E-16
BJ999119	462	-	zygote-specific class V copy B	*AAB21907.1*	*C. reinhardtii*	7.00E-14
Cytoskeleton						

BJ998417	279	○	beta-tubulin	*TUB2*	*S. cerevisiae*	3.00E-07
BJ999019	397	-	cofilin	*adf1*	*S. pombe*	3.00E-17
DNA, RNA, Chromatin						
BJ998552	518	-	centromere-binding factor 5	*cbf5*	*S. cerevisiae*	4.00E-88
BJ998549	691	-	DNA repair protein/DNA helicase	*rhp3*	*S. pombe*	4.00E-91
BJ999018	213	-	histone acetyltransferase	*HAT1*	*S. cerevisiae*	1.00E-05
BJ998909	474	-	histone H4	*hhf*	*A. bisporus*	1.00E-46
BJ998785	459	-	histone methyltransferase	*symd1*	*D. rerio*	4.00E-12
BJ999086	559	-	RNA-binding protein	*SPAC328.05*	*S. pombe*	1.00E-08
BJ998694	554	-	RNA-binding protein	*SND1*	*S. pombe*	6.00E-10
BJ998922	322	-	glycine-rich RNA binding protein	*rgp2*	*N.sylvestris*	5.00E-13
BJ998591	471	-	ATP-dependent RNA helicase (DEAD)	*BAD92619.1*	*H. sapiens*	8.00E-06
BJ998906	323	○	argonaute	*alg-1*	*C. elegans*	3.00E-06
BJ999065	605	-	RNA-dependent RNA polymerase	*rrf-2*	*C. elegans*	9.00E-18
Transcription, Splicing						
BJ998212	684	-	transcription factor	*prr1*	*S. pombe*	5.00E-14
BJ999092	511	-	transcriptional coactivator	*MBF1*	*S. cerevisiae*	3.00E-33
BJ998402	588	-	transformation/transcription domain-associated protein	*TRA1*	*S. cerevisiae*	8.00E-78
BJ998751	223	-	prp5	*prp5*	*S. pombe*	3.00E-08
BJ998914	273	-	U2 small nuclear RNA auxiliary factor 2	*U2AF2*	*H. sapiens*	4.00E-10
Translation						
BJ998635	155	-	alanine racemase	*SPAC1039.06*	*S. pombe*	3.00E-07
BJ998797	578	-	EF 1 beta	*eEF1b*	*H. sapiens*	1.00E-33
BJ998877	574	-	EF-1 delta	*EEF1D*	*H. sapiens*	6.00E-20
BJ999006	573	○	EF-1 gamma	*CAM1*	*S. cerevisiae*	3.00E-40
BJ998226	397	-	eIF4A	*tif1*	*S. pombe*	4.00E-57
BJ998633	448	-	alanine-tRNA synthetases	*SPAC23C11.09*	*S. pombe*	8.00E-43
BJ998164	319	-	mitochondrial aspartyl-tRNA synthetase	*MSD1*	*S. cerevisiae*	8.00E-19
BJ998462	729	-	tyrosyl-tRNA synthetase	*MSY1*	*S. cerevisiae*	8.00E-16
BJ998976	492	○	ribosomal protein (acidic) P2	*rpp203*	*S. pombe*	8.00E-17
BJ998981	498	-	ribosomal protein L2	*rpl402*	*S. pombe*	1.00E-65
BJ998807	425	○	ribosomal protein L2B	*RPL2B*	*S. cerevisiae*	2.00E-49
BJ998992	517	○	ribosomal protein L3	*RPL3*	*S. cerevisiae*	2.00E-30
BJ998997	521	○	ribosomal protein L12	*rpl1201*	*S. pombe*	8.00E-64
BJ998126	459	-	ribosomal protein L16	*RPL16A*	*S. cerevisiae*	4.00E-53
BJ998301	391	-	ribosomal protein L19	*rpl1902*	*S. pombe*	9.00E-24
BJ999050	502	○	ribosomal protein L24	*rpl24-2*	*S. pombe*	5.00E-23
BJ998283	282	○	ribosomal protein L34-like protein	*RPL34B*	*S. cerevisiae*	4.00E-15
BJ998856	421	○	ribosomal protein L37, mitochondrial precursor	*yml37*	*C. neoformans*	2.00E-06
BJ998339	494	-	ribosomal protein L38	*rpl38-1*	*S. pombe*	2.00E-16
BJ998478	361	○	ribosomal protein L41	*RPL41A*	*S. cerevisiae*	4.00E-46
BJ998494	242	○	ribosomal protein S3a	**cyc07**	*L. edodes*	3.00E-46
BJ998570	404	-	ribosomal protein S10	*rps1002*	*S. pombe*	1.00E-40
BJ998646	332	○	ribosomal protein S11	*rps1102*	*S. pombe*	2.00E-24
BJ998363	392	○	ribosomal protein S12	*rps12*	*S. pombe*	1.00E-13
BJ998266	431	-	ribosomal protein S13	*rps13*	*A. bisporus*	9.00E-70
BJ998728	306	○	ribosomal protein S15	*rps15-2*	*S. pombe*	6.00E-12
BJ998653	544	○	ribosomal protein S22	*rps2202*	*S. pombe*	2.00E-60
BJ998705	417	-	ribosomal protein S25	*RPS25B*	*S. cerevisiae*	7.00E-20
BJ998956	349	○	ribosomal protein S27	*CNG02740*	*C. neoformans*	1.00E-05
BJ998982	504	-	Yst2	*Yst2*	*S. cerevisiae*	2.00E-78
Protein degradation						
BJ998936	522	-	aminopeptidase	*ape3*	*A. bisporus*	3.00E-32
BJ998449	517	-	aspartyl proteinase	*AAP32823.1*	*P. brasiliensis*	8.00E-09
BJ998782	435	-	ATPase in proteasome	*RPT1*	*S. cerevisiae*	7.00E-30
BJ998745	455	○	endopeptidase 1	*CNC04480*	*C. neoformans*	3.00E-18
BJ998830	504	○	endopeptidase 2	*CNJ00730*	*C. neoformans*	6.00E-05
BJ998692	302	-	gamma glutamyl-transpeptidase	*GGT2*	*S. pombe*	9.00E-22
BJ998831	214	-	metalloprotease 1	*MEP2*	*A. benhamiae*	1.00E-29
BJ998254	291	-	metalloprotease 2	*MEP4*	*A. benhamiae*	8.00E-06
BJ998245	572	○	polyubiquitin	*ubi4*	*S. cerevisiae*	3.00E-87
BJ998296	329	-	protease B precursor	*PRB1*	*S. cerevisiae*	1.00E-33
BJ998910	665	-	protease	*prot1*	*A. muscaria*	7.00E-39
BJ998899	499	○	proteasome component beta 1 (20S)	DAPS-1	*D. discoideum*	8.00E-21
BJ998754	219	-	proteasome component beta 7 (20S)	*SPBC577.10*	*S. pombe*	3.00E-05
BJ998463	519	-	proteasome component beta 4 (20S)	*PSMB4*	*H. sapiens*	2.00E-57
BJ998356	596	-	tripeptidyl aminopeptidase	*TPAP1*	*A. oryzae*	1.00E-23
BJ998535	578	○	tripeptidyl peptidase A	*tppA*	*A. oryzae*	7.00E-33

BJ998350	361	○	ubiquitin carboxyl-terminal hydrolase5	*CNH02890*	*C. neoformans*	1.00E-15
BJ998971	499	-	ubiquitin conjugating enzyme E2	*UBC4*	*S. pombe*	9.00E-77
BJ998891	606	-	ubiquitin conjugating enzyme E2	*Xubc14*	*X. laevis*	8.00E-28
BJ999056	228	○	ubiquitin conjugating enzyme E2	*UBC5*	*S. cerevisiae*	2.00E-09
BJ999082	430	-	ubiquitin ligase E3	*CUL1*	*H. sapiens*	7.00E-43
BJ999108	319	○	vacuolar aspartyl protease	*PEP4*	*S. cerevisiae*	5.00E-11
BJ998644	259	-	vacuolar carboxy peptidase	*SPAC24C9.08*	*S. pombe*	2.00E-07
Transport, Secretion						
BJ998929	337	○	ABC transporter	*Afu3g09680*	*A. fumigatus*	3.00E-12
BJ998931	398	○	ABC transporter	*YOR1*	*S. cerevisiae*	6.00E-15
BJ998540	558	-	amino acid permease	*NAAP1*	*N. crassa*	1.00E-25
BJ998318	255	-	ADP/ATP translocator	*AAC1*	*S. cerevisiae*	2.00E-06
BJ999080	613	-	clathrin-associated adaptor complex AP-1 medium	*APM1*	*S. cerevisiae*	4.00E-52
BJ999103	651	○	hexose transporter	*HXT1*	*S. cerevisiae*	4.00E-13
BJ998347	136	-	carnitine/acyl carnitine carrier	*CNA04780*	*C. neoformans*	2.00E-06
BJ998763	215	-	coatomer gamma subunit	*sec21*	*S. pombe*	7.00E-27
BJ998293	486	-	coatomer zeta subunit	*ret3*	*S. pombe*	2.00E-17
BJ999126	478	-	COP9 signalosome subunit 6	*CNF03400*	*C. neoformans*	2.00E-14
BJ998678	422	○	ER to Golgi transport-related protein	*CNC01500*	*C. neoformans*	3.00E-08
BJ998568	241	-	golgi matrix protein	*SPBC119.12*	*S. pombe*	3.00E-06
BJ998289	649	-	GTPase activating protein of Rab-like GTPase	*GYP1*	*S. pombe*	1.00E-69
BJ998195	161	-	GDP-fucose transporter-like protein	*GDPF*	*P. djamor*	2.00E-11
BJ999057	372	○	importin alpha subunit	CND04770	*C. neoformans*	3.00E-12
BJ998967	275	-	mitochondrial import motor	*PAM18*	*S. cerevisiae*	4.00E-16
BJ998239	943	-	mitochondrial phosphate translocator	*micB*	*P. involutus*	1.00E-54
BJ998308	624	○	monocarboxylate transporter	*Afu3g03320*	*A. fumigatus*	2.00E-23
BJ998427	689	○	monosaccharide transporter	*mstB*	*A. niger*	3.00E-33
BJ998105	704	-	potassium channel beta subunit	*At1g04690*	*A. thaliana*	3.00E-42
BJ998501	505	-	potassium channel	*KAB1*	*A. thaliana*	7.00E-21
BJ998102	656	-	sodium/calcium exchanger	*SPAC3A12.06c*	*S. pombe*	1.00E-14
BJ998389	671	○	Rab7	***rab7***	*L. edodes*	3.00E-126
BJ998216	884	○	vacuolar H+/Ca(2+) exchanger	*VCX1*	*S. cerevisiae*	9.00E-09
BJ998320	369	-	v-SNARE	*ykt6*	*S. cerevisiae*	1.00E-21
BJ998805	423	-	v-SNARE	*VTI1*	*S. cerevisiae*	4.00E-10
BJ998209	622	○	Ypt interacting protein	*YIP3*	*S. cerevisiae*	1.00E-24
Signaling						
BJ998990	379	○	GDP dissociation inhibitor	*GDI1*	*S. cerevisiae*	1.00E-25
BJ998799	498	○	Ras2 protein	***ras2***	*S. commune*	6.00E-09
BJ998755	391	-	Ras-related protein Rap2A	*RAP2A*	*M. musculus*	3.00E-05
BJ998351	136	-	MAP kinase kinase Byr1	*CNC02350*	*C. neoformans*	4.00E-04
BJ998682	111	-	serine/threonine protein kinase	*CKI3*	*S. pombe*	4.00E-15
BJ998235	438	-	protein kinase	*dsk1*	*S. pombe*	1.00E-04
BJ998260	627	-	GTP binding protein	*VPS1*	*S. cerevisiae*	2.00E-32
BJ998995	528	-	SRPK1-like protein kinase	*sky2*	*C. albicans*	3.00E-11
BJ998613	504	-	zinc finger protein	*GIS2*	*S. cerevisiae*	2.00E-23
Unclassified						
BJ998454	157	-	armadillo repeat protein	*vac8*	*S. pombe*	3.00E-16
BJ998772	451	-	chaperonin-containing T-complex	*cct4*	*S. pombe*	1.00E-52
BJ999096	360	-	cleavage stimulation factor subunit3	*CSTF3*	*H. sapiens*	1.00E-05
BJ999124	464	-	immunomodulatory protein	*Aca1*	*A. camphorata*	1.00E-17
BJ998960	406	-	immunoreactive mannoprotein MP88	*CNA07540*	*C. neoformans*	2.00E-09
BJ998115	503	-	nucleoside diphosphate kinase I	*ndkA*	*P. involutus*	6.00E-66
BJ998382	450	-	phosphatidylinositol 3-phosphate binding protein	*SPAC6F6.12*	*S. pombe*	5.00E-08
BJ998112	676	-	plasma membrane protein	*SFK1*	*S. cerevisiae*	5.00E-07
BJ999026	300	-	P-type ATPase	*SPBC887.12*	*S. pombe*	6.00E-09
BJ999012	448	○	SnodProt1 precursor probable	*NCU07787.1*	*N. crassa*	7.00E-10
BJ998202	548	-	THIJ/PFPI family protein	*SPAC22E12.03c*	*S. pombe*	1.00E-16
rRNA, IGR						
BJ998114	103	-	18S ribosomal RNA	-	*A. lycoperdoides*	1E-51*
BJ998759	265	○	18S rRNA internal transcribed spacer	-	*R. olivacea*	3.00E-13
BJ998731	151	-	25S ribosomal RNA	-	*L. edodes*	9E-41*
BJ998988	131	-	25S rRNA internal transcribed spacer	-	*S. commune*	2.00E-13
BJ998870	97	○	5.8S ribosomal RNA	-	*Basidiomycete sp*	9E-37*
BJ998123	521	-	5S rRNA IGR2	-	*L. edodes*	5E-92*

Table 2. Numbers of times ESTs were obtained

Name	Times
Hydrophobin 1	24
Phosphatidylserine decarboxylase	8
Aldehyde dehydrogenase	6
NAD-dependent formate dehydrogenase 1	4
Metallothionein	4
Riboflavin aldehyde-forming enzyme	4
NAD-dependent formate dehydrogenase 2	3
Manganese superoxide dismutase 1	3
Polyubiquitin	3
Catalase	3
C-4 methyl sterol oxidase	3
Hydrophobin 5	3
Ras2	3
Succinate dehydrogenase	3
Ubiqutin conjugating enzyme Ubc4	3