Title

Proteomics of Two Cultivated Mushrooms *Sparassis crispa* and *Hericium erinaceum*
Provides Insight into their Numerous Functional Protein Components and Diversity

Authors

Kiyotaka Horie¹, Randeep Rakwal²,³,*, Misato Hirano², Junko Shibato², Hyung Wook Nam⁴,
Yu Sam Kim⁴, Yoshiaki Kouzuma¹, Ganesh Kumar Agrawal³,⁵, and Masami Yonekura¹

¹Food Function Laboratory, School of Agriculture, Ibaraki University, Ami 300-0393, Japan,
²Human Stress Signal Research Center (HSS), National Institute of Advanced Industrial
Science and Technology (AIST), Tsukuba West, 16-1 Onogawa, Tsukuba 305-8569, Japan,
³Research Laboratory for Agricultural Biotechnology and Biochemistry (RLABB),
Kathmandu, Nepal, and ⁴Protein Network Research Center, Yonsei University, Seoul 120-749,
Korea

*To whom correspondence should be addressed. Dr. Randeep Rakwal, HSS, AIST, Tsukuba
West, 16-1 Onogawa, Tsukuba 305-8569, Japan. E-mail, rakwal-68@aist.go.jp; fax, +81-29-861-8508.

*Present address: University of Missouri-Columbia, Biochemistry Department, 204 Life
Sciences Center, Columbia, MO 65211, USA

Keywords: mushrooms • fungi • proteomic analysis • gel-based approach • mass
spectrometry • proteins
Abstract

Mushroom can be defined as a macrofungus with a distinctive fruiting body. Mushrooms of class Basidiomycete are primarily wood degradation fungi, but serve as food and a part of traditional medicine used by humans. Although their life cycle is fairly well established, the information on the molecular components, especially proteins, are very limited. Here we report proteomics analysis of two edible mushrooms (fruiting bodies) Sparassis crispa and Hericium erinaceum using one- and two-dimensional gel electrophoresis (1-DGE and 2-DGE) based complementary proteomics approaches. 1-DGE coupled to liquid chromatography with mass spectrometry identified 77 (60 nonredundant proteins) and 121 (88 nonredundant proteins) proteins from S. crispa and H. erinaceum, respectively. 2-DGE analysis revealed 480 and 570 protein spots stained with colloidal coomassie brilliant blue in S. crispa and H. erinaceum, respectively. Of the 71 and 115 selected protein spots from S. crispa and H. erinaceum 2-D gel blots on polyvinylidifluoride (PVDF) membranes, respectively, 29 and 35 nonredundant proteins were identified by N-terminal amino acid sequencing. Identified nonredundant proteins from 1- or 2-DGE belonged to 19 functional categories. Twenty-one proteins were found common in both S. crispa and H. erinaceum proteomes, including 14-3-3 proteins and septin; among these 21 proteins, 2 proteins were identified on 2-D gels. Together this study provides evidence for the presence of a large number of functionally diverse proteins, expressed in the fruiting body of two economically important mushrooms, S. crispa and H. erinaceum. Data obtained from 1-DGE and 2-DGE analyses is accessible through the mushroom proteomics portal – http://foodfunc.agr.ibaraki.ac.jp/mushprot.html.

1. Introduction

Mushrooms are defined as macrofungi with a distinctive fruiting body that may be above or below ground and can be seen with our naked eyes. According to this definition, a large percentage of the fungi belonging to class Basidiomycetes and some fungi of class Ascomycetes are classified as mushrooms. Mushrooms occupy an important position in the ecosystem formed on our earth as fungi. Fungi degrade organic matter in animal waste products and plant litter into inorganic matter. Through this process, fungi get energy for their survival, set up the carbon and nitrogen cycles, and help keep our environment (soil) clean. On the other hand, from ancient times, eating mushrooms has been considered healthy. This is due to the fact that mushrooms provide a low calorie diet and contain abundant dietary fibers, a combination of which helps prevent intestinal disorders and serve to lower the cholesterol in our blood. Moreover, mushrooms have been employed as a
“medicine”. In particular, polysaccharides derived from their cell wall have been reported to have anti-tumor activity and has been practically used as a form of medical treatment in Japan. Therefore, mushrooms strongly connect with our life and are valuable organisms for scientific study and research.

Academically, fungal research has focused on specific aspects of the organism. These include i) studies on fruiting body formation from mycelium for productivity advancement or expansion of edible species, ii) use of mushroom not only as nutritional food but as functional food for human health, and iii) use in degradation of xenobiotics for improvement of polluted environment or reuse of untapped resources. With this background, and our interest on the mushrooms from the food functionality and medicinal viewpoints, we have embarked on a molecular level investigation into two cultivated mushrooms, namely Sparassis crispa (S. crispa) and Hericium erinaceum (H. erinaceum). It should be mentioned here that compared to the popular edible mushroom Agaricus bisporus, S. crispa and H. erinaceum are the least studied. S. crispa and H. erinaceum are rare in nature, and only recently it has been possible to artificially cultivate these two edible medicinal mushrooms species in Japan. These two mushrooms are becoming popular among the Japanese consumers and can be found at major supermarkets around the country.

S. crispa is also called cauliflower mushroom and contains large amounts of β-glucan that shows anti-tumor activity on ICR (constructed type in Institute of Cancer Research) mice by intraperitonal or oral administration. H. erinaceum is called roe deer’s hip (norukungdaei) in Korea, and elsewhere is referred to as bear’s head, monkey’s head, and lion’s mane mushroom, etc., in relation to its shape. Kawagishi and co-workers identified the nerve growth factors hericenones, erinacines, and erinacol from its fruiting body. Two proteins, a sialic acid-binding lectin and a laccase have also been previously identified from H. erinaceum. Other than the polysaccharide β-glucan and 2 proteins identified from these mushrooms, there are no reports on other expressed protein components. Proteins are important to study as they have diverse functions in the cell and are essential components involved in life activity of an organism from the cradle to the grave. Moreover, proteins are dynamic, some are constitutively expressed, and others are only expressed at specific time or conditions during the life cycle. It may be suggested that total proteins profiles and their expression amount reflect on the life stage of the organism. Proteomics approaches such as one- and two-dimensional gel electrophoresis (1-DGE and 2-DGE) in combination with mass spectrometry (MS) have been widely applied to identify and profile proteins expressed in plant tissues or organelles. A number of studies have convincingly demonstrated that
1- and 2-DGE are complementary proteomics approaches, and application of both approaches provides a deeper insight into the proteome.\textsuperscript{16-23}

In the present study, we have conducted for the first time, to the best of our knowledge, a systematic proteomics analysis in two economically important mushrooms, \textit{S. crispa} and \textit{H. erinaceum}, to survey expressed proteins in their fruiting bodies. We also report the optimization of an extraction protocol and a gel-based methodology for studying mushroom proteomes. Both 1- and 2-DGE based proteomics approaches in combination with nanoelectrospray ionization liquid chromatography tandem MS (nESI-LC-MS/MS) and Edman \textit{N}-terminal amino acid sequencing, respectively, were applied on the fruiting bodies of \textit{S. crispa} and \textit{H. erinaceum} to obtain an in-depth insight into the proteome of these two mushrooms. A 2-D gel based high-resolution reference map of each mushroom was established.

\section*{2. Materials and Methods}

\subsection*{2.1. The Two Mushrooms used in this Study.} The \textit{S. crispa} and \textit{H. erinaceum} mature fruiting bodies were obtained from Harakin Corporation (Kashima, Ibaraki, Japan).

\subsubsection*{2.1.1. Culture of \textit{S. crispa}.} It has been known that \textit{S. crispa} and \textit{H. erinaceum} have saprotrophic ability and grow on a variety of woody substances. To prepare experimental substrates, the Japanese red pine sawdust as main component of the substrates was mixed with bran and its moisture content adjusted to 60\% with water (H\textsubscript{2}O). The mixed substrates were packed in mushroom culture bottles made of polypropylene resin, sterilized, and cooled to room temperature (RT). Pre-cultured \textit{S. crispa} KSC-03 and was inoculated onto the top of the substrates in culture bottles. To obtain a uniform spread of hypha in the substrates, bottles were kept at 22 \textdegree{}C, 75\% relative humidity (RH) for 4 weeks in the dark. After adequate hyphal spread, bottles were moved to growth phase at 12 \textdegree{}C, 90\% RH, and irradiation of 200 lux incandescent light for 3 weeks. The subsequently formed fruiting bodies were harvested and stored at \textdegree{}20 \textdegree{}C.

\subsubsection*{2.1.2. Culture of \textit{H. erinaceum}.} To prepare experimental substrates, corn cob meal as main component of substrates was mixed with bran, and its moisture content adjusted to 60\% with H\textsubscript{2}O. The mixed substrates were packed in mushroom culture bottles made of polypropylene resin, sterilized, and cooled to RT. Pre-cultured \textit{H. erinaceum} KX-YB044 was inoculated onto the top of the substrates in culture bottles. To obtain a uniform spread of
the hypha in the substrates, bottles were kept at 22 °C, 75% RH for 6 weeks in the dark. After adequate hyphal spread, bottles were moved to growth phase at 15 °C, 90% RH, irradiation of 200 lux incandescent light for 6 weeks. Subsequently formed fruiting bodies were harvested and stored at −20 °C.

2.2. Extraction of Total Protein. Frozen fruiting bodies (10 g) were ground to a fine powder in liquid nitrogen using pre-chilled ceramic mortar and pestle followed by transfer to a pre-chilled eppendorf tube. Total protein was extracted using phenol extraction protocol with some modifications. Proteins were extracted from tissue powder (200 mg) by addition of 500 µL Tris (pH 8.8) buffered phenol (hereafter referred to as TBP) and extraction media [0.9 M sucrose, 0.1 M Tris (pH 8.8), 10 mM EDTA, and 0.4% (v/v) 2-mercaptoethanol (2-ME) in MQ H2O], followed by mixing using an invert shaker at RT for 30 min. The suspensions were centrifuged at 15,000 rpm for 20 min at 4 °C. After centrifugation, top phenol phase was transferred to a new micro-tube, followed by addition of 500 µL TBP and same amount of extraction media to back extract aqueous phase, followed by centrifugation at 15,000 rpm for 20 min 4 °C. The top phenol phase was transferred into the first extraction and vortexed. Solubilized proteins in phenol were precipitated by addition of 5 volumes of ammonium acetate in 100% ethanol, vortexed, and incubated at −20 °C overnight. The suspension was centrifuged at 15,000 rpm for 20 min 4 °C to obtain the protein pellet. The pellet was washed twice with 5 volumes of ammonium acetate in 100% ethanol, with ice-cold 5 volumes of 80% acetone, and finally once with 70% ethanol. Following centrifugation (at 15,000 rpm for 20 min 4 °C), the supernatant was decanted and pellet was dried at 37 °C for 10 min. Proteins were solubilized in 200 µL of lysis buffer [7M urea, 2M thiourea, 4% (w/v) CHAPS, 18 mM Tris-HCl (pH 8.0), 14 mM trizma base, two EDTA-free proteinase inhibitor cocktail tablets in a final volume of 100 mL buffer, 0.2% (v/v) Triton X-100 (R), containing 50 mM dithiothreitol (DTT); hereafter called LB-TT], followed by centrifugation at 15,000 rpm for 20 min 4 °C. The supernatant was used for protein quantification by a Coomassie Plus™ (PIERCE, Rockford, IL) protein assay kit, and stored in aliquots at −80 °C.

2.3. One-Dimensional Gel Electrophoresis and Mass Spectrometry Analysis. The total protein obtained above was precipitated using a Protein Precipitation Kit (Calbiochem, Darmstadt, Germany). The pellet was resolubilized in homogenization buffer [0.2 M Tris-HCl buffer, pH 7.8, containing 5 mM EDTA.2Na, 14 mM 2-ME, 10% (v/v) glycerol, and 2
EDTA-free proteinase inhibitor tablets (Roche Diagnostics GmbH, Mannheim, Germany) per 100 mL buffer solution in MQ H$_2$O. To effectively solubilize the protein pellet, sodium dodecyl sulfate (SDS)-sample buffer [2.5 ×, 62 mM Tris (pH 6.8) containing 10% (v/v) glycerol, 2.5% (w/v) SDS, and 5% (v/v) 2-ME, pH 6.8] was added to the mixture, followed by vortexing, sonication (water bath), and centrifugation of the sample at 15,000 rpm for 15 min (4 ºC). The supernatant was used for protein quantification as described above. Just before electrophoresis, a drop of bromophenol blue (BPB) was added to the protein samples and boiled for 1 min at 95 ºC. Fifty µg of protein was loaded into three well replications for 1-DGE. 12.5% SDS-PAGE (4% T, 2.6% C stacking gels, pH 6.8 and 12.5% T, 2.6% C separating gels, pH 8.8) was carried out on a vertical electrophoresis unit at constant current of 35 mA for ca. 3 h. The running buffer was composed of 0.025 M Tris, 0.192 M glycine, and 0.2% (w/v) SDS. Five µL of the commercially available “ready-to-use” molecular mass standards (Precision Plus Protein Standards, Dual Color, Bio-Rad, Hercules, CA) were loaded in the well adjacent to the samples. The gel was stained with Coomassie brilliant blue (CBB) R-250.

Each lane was sliced in six pieces of gel matrix and digested with 1 µg trypsin at 37 ºC for 18 h. The tryptic peptides samples were separated by C-18 reverse-phase column and analyzed on a nano electro spray ionization mass spectrometer (nESI-LC-MS/MS). Ultimate nanoLC systems, combined with the FAMOS autosampler and Switchos column switching valve (LC-Packings, Amsterdam, Netherlands) was used. The samples were loaded onto precolumn (2 cm × 200 µM i.d.; Zorbax 300SB-C18, 5 µM, Agilent, CA), and washed with the loading solvent (H$_2$O/0.1% formic acid, flow rate: 4 µL/min.) for 10 min. to remove salts. Subsequently, a Switchos II column switching device transferred flow paths to the analytical column (15 cm × 75 µM i.d.; Zorbax 300SB-C18, 5 µM, Agilent). The nano-flow eluted at a flow rate of 200 nL/min. using a 110 min gradient elution from 0% solvent A to 32% solvent B, where solvent A was 0.1% formic acid with 5% acetonitrile and solvent B was 0.1% formic acid with 90% acetonitrile. The column outlet was coupled directly to the high voltage ESI source, which was interfaced to the QSTAR mass spectrometer (Applied Biosystems, Foster City, CA). The nanospray voltage was typically 2.3 kV in the nESI-LC-MS/MS mode. The nESI-LC-MS/MS running on the QSTAR instrument was acquired in `Information Dependent Acquisition` mode, which allows the user to acquire MS/MS spectra based on an inclusion mass list and dynamic assessment of relative ion intensity. The data acquisition time was set to 3 s per spectrum over m/z range of 400–1,500 Da.
Acquired data were searched against the National Center for Biotechnology Information (NCBI) nonredundant protein database using the MASCOT software package (Version 2.1, Matrix Sciences, UK; www.matrixscience.com). The peptide mass and MS/MS tolerance were 1.0 Da and 0.8 Da, respectively. The peptides have the allowance of two tryptic mis-
cleavages and also partially modified with oxidation (M) within two charge states (+2, +3).

2.4. Two-Dimensional Gel Electrophoresis. 2-DGE was carried out using pre-cast IPG strips (18 cm, pH 4-7) on an IPGphor unit (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) followed by 12.5% SDS-PAGE on a vertical electrophoresis unit.25,26 The volume carrying 750 µg total protein was mixed with LB-TT containing 0.5% (v/v) pH 4-7 IPG buffer to bring to a final volume of 340 µL. A trace of BPB was added and centrifuged at 15,000 rpm for 15 min followed by pipetting into 18 cm strip holder tray placed into the IPGphor unit. IPG strips were carefully placed onto the protein samples avoiding air bubbles between the sample and the gel strip. The IPG strips were allowed to passively rehydrate with the protein samples for 1.5 h, followed by overlaying the strips with cover fluid (mineral oil), and this was directly linked to a five-step active rehydration and focusing protocol (18 cm strip) as described previously.26 The whole procedure was controlled at 20 ºC, and a total of 68,902 Vh was used for the 18 cm strip. Following IEF, the IPG strips were immediately used for the second dimension. The IPG strips were incubated in equilibration buffer (50 mM Tris-HCl (pH 8.8), 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS) containing 2% (w/v) DTT for 10 min (twice) with gentle agitation, followed by incubation in the same equilibration buffer supplemented with 2.5% (w/v) iodoacetamide for the same time periods as above at RT. SDS-PAGE (35 mA/gel) was performed for 3.5 h. For each sample, a minimum of three IPG strips and corresponding SDS-PAGE was used under the same conditions.

To visualize the protein spots, the 2-D gels were stained with colloidal CBB G-250. Protein patterns in the gels were recorded as digitalized images using a digital scanner (CanoScan 8000F, resolution 300 dpi), and saved as TIFF files. ImageMaster 2D Platinum software ver. 5.0 (GE Healthcare) was used for downstream analysis of detected protein spots on 2-D gels.

2.5. Electroblotting of Proteins onto PVDF Membranes and N-Terminal Amino Acid Sequencing. Electrotransfer of proteins on gel to a polyvinylidifluoride (PVDF) membrane (NT-31, 0.45 µM pore size; Nihon Eido, Tokyo, Japan) was carried out at 1mA/cm² for 80 min
at RT using a semi-dry blotter (Nihon Eido). The transferred proteins were stained with CBB R-250 as described. The transfer efficiency is ca. 99% for almost all low molecular mass proteins below molecular masses of 100 kDa; the transfer efficiency was also checked by staining the gels after transfer with CBB, which revealed no proteins spots left on gel except for a slightly stained standard marker protein of 250 kDa. N-terminal amino acid sequencing of proteins on the PVDF membranes was carried out on an Applied Biosystems 494 protein sequencer (Perkin Elmer; Applied Biosystems) as described. The obtained sequences were used to interrogate databases (UniProtKB/Swiss-Prot and UniProtKB/TrEMBL) with Web accessible search programs like Fasta3, available online from EMBL Outstation of the European Bioinformatics Institute, to identify homology to proteins already present in the protein and nucleic acid databases.

3. Results and Discussion

Mushrooms are used as food resource, participate in material cycles, and have possibility of variable use in bio-industry. Therefore, mushrooms, as a bio-resource are becoming an essential part of our existence. So far, studies on mushrooms have dealt mostly with polysaccharides derived from cell wall and on specific proteins, but a proteomics-scale investigation aimed at creating a mushroom(s) proteome is completely lacking. We at the Food Function laboratory and HSS are interested in mushroom proteomics for the above mentioned reasons, including finding new and interesting proteins for human health. For this, we started the present study, with a aim to i) optimizing the protein extraction protocol, and ii) try to understand mushroom biology from the proteomics data.

3.1. Experimental Strategy. An overview of the experimental strategy for a systematic analysis of the mushrooms proteomes is schematically depicted in Figure 1. The phenol extraction method was used to extract and purify total protein from mature fruiting bodies of S. crispa and H. erinaceum, mainly due to high content of polysaccharides and other compounds. Total protein solubilized in LB-TT was used for 1-DGE (Figure 2) and 2-DGE (Figures 3 and 4) analyses. In case of 1-DGE, total protein was separated by 12.5% SDS-PAGE and the gel was divided into six sections (one through six), and the tryptic peptides derived from the gel bands was analyzed by nESI-LC-MS/MS. Acquired MS/MS data were searched against NCBI nonredundant protein database using the MASCOT search engine. However, 1-DGE analysis in combination of nESI-LC-MS/MS and database search resulted in identification of low number of unique peptide per protein making it difficult for confident
protein assignment. This might be in part due to lack of sequence information on mushroom and less similarity of proteins expressed in mushroom with other organisms. The 2-D gel spots after transfer to PVDF membrane were cut off and taken for Edman sequencing. N-terminal amino acid sequencing is a low-throughput analysis, but provides good sequence information on N-terminal amino acid sequence of a protein. As N-terminal amino acid sequences of proteins are usually not conserved, and considering the low number of unique peptides from 1-DGE, we used Edman sequencing for 2-D gel blotted protein spots for protein assignment.

1- and 2-DGE analyses together identified 89 and 123 proteins, representing 84 and 119 nonredundant proteins from S. crispa and H. erinaceum, respectively (Figure 5). As expected, 1-DGE analysis identified highly basic and acidic proteins, which are poorly represented on 2-D gel, especially the basic proteins. These proteomics data including the high-resolution 2-D gel reference maps have been posted to the website http://foodfunc.agr.ibaraki.ac.jp/mushprot.html to serve as a proteomics resource for the scientific community, especially the researchers working on mushrooms.

3.2. The S. crispa Proteome

3.2.1. Identification of 60 Non-Overlapping and Nonredundant Proteins by 1-DGE.

1-DGE analysis in combination with LC-MS/MS identified a total of 77 proteins containing 60 nonredundant proteins from the 6 excised fractions. Seven proteins were identified from fraction 1, 10 proteins from fraction 2, 21 proteins from fraction 3, 18 proteins from fraction 4, 9 proteins from fraction 5, and 12 proteins from fraction 6. Some of the proteins had similar protein identifications in more than one fraction, which may be due to fragmentation during the experiment process, extraction, and on SDS-PAGE. For example, heat shock protein (HSP) 70 was detected among 4 fractions.

3.2.2. 2-DGE and N-Terminal Amino Acid Sequencing Identified 29 Non-Overlapping and Nonredundant Proteins. Most of the visualized protein spots were concentrated in pI range 5 to 7 and between molecular mass 25 and 100 kDa. ImageMaster analysis revealed approximately 480 protein spots. We selected a total of 71 protein spots and analyzed those by N-terminal amino acid sequencing. Out of these, the N-terminals of 5 proteins were blocked. A similarity search on unblocked 66 protein sequences identified a total of 29 nonredundant proteins; the remainder of 37 proteins could not be identified.
3.3. The *H. erinaceum* Proteome

3.3.1. 1-DGE and LC-MS/MS Identified 88 Non-Overlapping and Nonredundant Proteins. 1-DGE analysis in combination with tandem MS identified a total of 121 proteins containing 88 nonredundant proteins from the 6 excised fractions. Thirteen proteins were identified from fraction 1, 16 proteins from fraction 2, 19 proteins from fraction 3, 24 proteins from fraction 4, 23 proteins from fraction 5, and 23 proteins from fraction 6. Interestingly, same protein identifications were detected in more than one fraction, which may be due to fragmentation during the experiment process, extraction, and on SDS-PAGE. For example, the translation elongation factor 1α was identified among 5 fractions.

3.3.2. 2-DGE and N-Terminal Amino Acid Sequencing Identified 36 Non-Overlapping and Nonredundant Proteins. Most of the protein spots were concentrated in pI range 5 to 7 and between molecular mass 30 and 100 kDa. ImageMaster analysis revealed approximately 570 protein spots. A total of 115 protein spots were selected and analyzed by N-terminal amino acid sequencing. Among these, the N-terminals of 46 proteins were blocked. Following a similarity search, 36 nonredundant proteins were identified from among the 69 unblocked proteins; 33 proteins could not be identified.

3.4. Identified Nonredundant Proteins in *S. crispa* and *H. erinaceum* belong to 19 Functional Categories. A total of 84 and 119 non-overlapping and nonredundant proteins were identified from *S. crispa* and *H. erinaceum* using 1-DGE and 2-DGE. Based on annotations from NCBI, Swiss-Prot and EMBL databases, these identified proteins were functionally categorized according to their biological process and are represented by pie diagrams in Figure 6. 1-DGE and 2-DGE analyses separately identified 60 and 29 proteins, respectively, where only 5 proteins were common in both datasets of *S. crispa*. From these result it is quite clear that different proteins are present and/or identified by 1-DGE and 2-DGE. In all 55 proteins were identified from 1-DGE analysis. It was seen that 15 of the 55 proteins have a pI value of over 7.0, which are basic in nature, and thus may not be represented on the 2-D gel. In *H. erinaceum*, 1-DGE and 2-DGE separately identified 88 and 35 proteins, respectively, where only 4 proteins were common in both datasets. In all 84 proteins were identified from 1-DGE analysis. As 34 of the 84 proteins have a pI value of over 7.0, these may not be represented on the 2-D gel. Interestingly, 21 identified proteins from 1-DGE overlapped between *S. crispa* and *H. erinaceum*. Surprisingly, 2 of these overlapped proteins were also found in 2-D gels of both mushrooms. These findings are in
line with previous reports that not one but multiple proteomics approaches are needed to dig deeper into proteome.\textsuperscript{16-23}

3.4.1. Functional Categorization of the Identified Proteins from \textit{S. crispa}. Proteins involved in three functional categories such as energy production and conversion (19\%), translation, ribosomal structure, and biogenesis (17\%), and carbohydrate transport and metabolism (15\%) together accounted for 51\% of the total protein identified from \textit{S. crispa} suggesting that proteins in these categories are constitutively expressed. Moreover, proteins involved in glycolytic pathway, citric acid cycle and glyconeogenesis were also identified. Other proteins account for small number of the total, proteins related to amino acid transport and metabolism (6\%), lipid metabolism (6\%), DNA replication, recombination and repair (5\%), transcription (4\%), posttranslational modification, protein turnover and chaperones (4\%), nucleotide transport and metabolism (4\%), cell envelope biogenesis, outer membrane (4\%), signal transduction (4\%), chromatin structure and dynamics (2\%), cell division and chromosome partitioning (2\%), inorganic ion transport and metabolism (2\%), intracellular trafficking and secretion (2\%), secondary metabolites biosynthesis, transport and catabolism (1\%), RNA processing and modification (1\%), cytoskeleton (1\%), and sporulation (1\%). Proteins categorized in RNA processing and modification and sporulation were only identified in this mushroom. We discuss below some of the noteworthy proteins.

L-seryl tRNA selenium transferase was identified from spot 59 of \textit{S. crispa} 2-D gel. Selenium is an essentially required element for synthesis of selenoproteins including glutathione peroxidase (GPX) that is an important antioxidant enzyme; L-seryl tRNA selenium transferase is involved in the biosynthesis process.\textsuperscript{29} Amazingly, it was shown that brown colored \textit{A. bisporus} fruiting bodies contain selenium at the rate of 3.2 mg/kg dry weight (the recommended daily allowance for women and men are 55 and 70 µg, respectively.), thus this enzyme also relates to the selenium accumulation mechanism.\textsuperscript{30} Polygaraturonase 1, trehalose phosphorylase and α-α-trehalase were found by 1-DGE analysis. It is a well known fact that degradation of cell wall constructing pectin occurs during fruit ripening, and polygaracturonase participates in this process.\textsuperscript{31} As mature fruiting bodies were used for the present study, it is not surprising to see this enzyme, which is suggestive of cell wall degradation. Trehalose phosphorylase and α-α-trehalase are involved in trehalose synthesis and decomposition respectively. Trehalose is a α-α-1,1-glycosidic linked disaccharide and present in a wide variety of organisms, including bacteria, fungi, insect, and
plant where it serves as source of energy and carbon. In addition, it has been shown that trehalose can protect proteins and cellular membranes from inactivation and denaturation caused by a variety of stress condition, including desiccation, dehydration, heat, cold and oxidation. Due to these reasons, it can be suggested that a stress tolerance system exists in this mushroom.

Aspartil proteinase and aminopeptidase were found from 1-DGE analysis, and ketol acid reductoisomerase was identified from spot 35 of the 2-D gel. Proteinases can be used for amino acid production in food-industrial bioprocesses. So far ketol acid reductoisomerase was found in bacteria, fungi, and plant, and is known to catalyze two steps in the biosynthesis of branched-chain amino acids (BCAA). BCAA is composed of leucine, isoleucine and valine and must be taken from diet, because these amino acids cannot be synthesized in our bodies. Due to BCAA involvement in muscle protein anabolism and healing of wound muscle, it is widely used in athletic drinks as a supplement. Therefore, enzyme(s) related to BCAA synthesis may serve well in drink manufacturing. Three carboxy terminal domain (CTD) kinase isoforms were identified from 2-D gel protein spots 9, 10, and 11. Through the phosphorylation of a serine residue located in RNA polymerase CTD, CTD kinase stimulates efficient elongation by RNA polymerase 2. Because N-terminal amino acid sequences and molecular mass of these three proteins are similar, but their pIs are different, it can be speculated that these proteins may be phosphorylated. It was found that CTD kinase includes 12 serine, 11 threonine and 3 tyrosine residues on its internal sequence by phosphorylation search using NetPhos 2.0, thus supporting our above statement; the phosphorylation state of CTD kinase however needs to be experimentally proved in future studies.

Catalase (CAT) was found by 1-DGE analysis, whereas mercuric transporter protein precursor and laccase were identified from spot 40 and 57, respectively, in 2-D gels. These enzymes play important roles in detoxification. Mercury mainly exerts toxicity such as mutagenicity, carcinogenesis, and cell death through DNA damage. Mercury-induced production reactive oxygen species (ROS) occurs via Fenton like reaction. Mercuric transporter proteins are involved in clearance of mercury, whereas CAT works as an antioxidant enzyme for degradation of cytotoxic ROS. Laccase belongs to a group of polyphenol oxidases typically found in plants and fungi. Plant laccase participate in the radical-based lignin polymer formation, whereas in fungal laccases have additional roles such as morphogenesis, fungal plant pathogen/host interaction, stress, defense, and lignin degradation. Laccase, unlike other lignin peroxidases, does not need the addition or
synthesis of a low molecular weight cofactor like hydrogen peroxide as its co-substrate. Added to this, most laccases are extracellular enzymes, making their collection very easy and laccases generally exhibit a considerable stability in the extracellular environment. Because of these properties, laccase have potential in various applications such as bioremediation of polluted environment and ecological decolorization of textile dye.\textsuperscript{37}

### 3.4.2. Functional Categorization of the Identified Proteins from \textit{H. erinaceum}.

Proteins related to translation, ribosomal structure and biogenesis and energy production and conversion account for large proportion of the identified proteins in \textit{H. erinaceum}. Compared to \textit{S. crispa} protein categories, \textit{H. erinaceum} proteome largely contained proteins related to protein turnover and chaperones, modification and signal transduction. Among these identified proteins, 23\% of the unique proteins were involved in translation, ribosomal structure and biogenesis, 16\% of these were in energy production and conversion, 12\% were in posttranslational modification, protein turnover and chaperones and 8\% were in signal transduction. Other proteins account for small number of the total, proteins related to carbohydrate transport and metabolism (8\%), amino acid transport and metabolism (6\%), intracellular trafficking and secretion (4\%), DNA replication, recombination and repair (3\%), inorganic ion transport and metabolism (3\%), chromatin structure and dynamics (3\%), cell division and chromosome partitioning (3\%), transcription (3\%), cell envelop biogenesis, outer membrane (3\%), nucleotide transport and metabolism (2\%), secondary metabolites biosynthesis, transport and catabolism (2\%) lipid metabolism (1\%), coenzyme metabolism (1\%), cytoskeleton (1\%), and defense(1\%). Added to this, some of antioxidant and detoxification enzymes, protein categorized in coenzyme metabolism and defense were identified from only \textit{H. erinaceum}. Some of the identified proteins have been discussed below.

Two HSP 70 isoforms, one HSP 90, and two cyclophilins were identified from 1-DGE analysis. HSP 60 was identified from spot 93 of 2-D gel. All these proteins are known to play a role in protein rescue as molecular chaperons.\textsuperscript{38,39} Cyclophilin has been recently shown to be up-regulated in yeast cells under stress condition.\textsuperscript{40} Two ubiquitins, 1 ubiquitin fusion degradation protein and 4 proteins related to proteasome structure were found by 1-DGE analysis; the ubiquitin fusion degradation protein and proteasome structure protein was identified from spot 13 and 31 respectively, on the 2-D gel. The ubiquitin-proteasome system is a well known protein degradation system.

Other stress and related proteins, CAT, GPX, and cytochrome P450 were also identified.
from 1-DGE analysis. Two superoxide dismutase (SOD) isoforms and a glutathione-S-
transferase (GST) were identified from spots 21, 22, and 23, respectively, on 2-D gels. The
CAT, GPX, and SOD are oxidative stress-related proteins and reduce cytotoxic ROS such as
hydrogen peroxide and lipid peroxide. Cytochrome P450 and GST are involved in
detoxification of toxic organic materials or degradation of steroids; interestingly it was
reported that cytochrome P450s catalyze xenobiotics such as benzopyrene.\textsuperscript{41} Existence of
these stress-related enzymes ensures that a stress response mechanism is present in the
maturing fruiting bodies. Many proteins concerned with vesicular transport and modulation
such as Rab7, clathrin-associated protein, Ran, ADP ribosylation factor, calmodulin binding
protein and serine threonine kinase were identified from 1-DGE analysis, while a G protein
gamma subunit (spot 1) was identified from 2-DGE analysis.

Endo-\textbeta-1,4-glucanase and a putative chitinase (spot 69) were identified by 1- and 2-DGE
analysis, respectively. Both enzymes have been previously shown to be involved in cell wall
degradation.\textsuperscript{42,43} Xylose reductase, glutamine synthase, and endopeptidase were identified
from 1-DGE analysis, and serine proteinase, cathepsin B like protein and ribonuclease H2
were identified from 2-D gels spots 5, 51, and 6 respectively. These enzymes can be applied
for use in xylitol, amino acid, and nucleotide production in food-industrial bioprocess. Blue
light photoreceptor (phototropin) was identified from spot 4 of 2-D gel. Plant phototropin is
light-regulated protein kinases and mediates phototropism and other processes in plants.
Phototropin has also been reported in the filamentous fungi, \textit{Neurospora crassa}, and shown to
be involved in circadian clock or other light response.\textsuperscript{44} Therefore it can be suggested that
the phototropin identified in this study might play an important role in light-dependent
signaling in the fruiting body. Plasminostreptin homolog was identified from spot 101 of 2-
D gel. So far, it is clear that plasminostreptin inhibits some kind of proteinase, prasmin,
trypsin and subtilisin.\textsuperscript{45} It is likely that plasminostreptin is involved in a defense mechanism
upon invasion by pathogenic organisms or modulation of intracellular proteolysis. Two
translationally controlled tumour protein (TCTP) homolog isoforms were identified from
spots 19 and 20. TCTP was named due to the fact that the cDNA was cloned from human
tumour, and on the observation that TCTP is regulated at the translational level. Recent
research has shown that TCTP forms small chaperone-like structure and has diverse functions
including cell progression, malignant transformation, histamine releasing factor, and anti-
apoptotic activity.\textsuperscript{46}

\textbf{3.5. Identification of 14-3-3 Proteins and Septin from Both Mushrooms.} Twenty-one
identified proteins from 1-DGE overlapped between *S. crispa* and *H. erinaceum*. Among these, actin and 14-3-3 proteins were found by both 1-DGE and 2-DGE. Almost all of these proteins were constitutively expressed and have been already discussed in section 3.4.

The 14-3-3 proteins were identified from spots 25 and 37 of 2-D gels of *S. crispa* and *H. erinaceum*, respectively. Location of 14-3-3 proteins on the 2-D maps is strictly coincident between two mushrooms; these proteins have pI of about 4.7 and molecular mass of 28.8 kDa. The 14-3-3 protein was first named in 1967 during a systematic classification of brain proteins, and the numbers in the name are based on a fraction number after DEAE-cellulose chromatography and the position after subsequent gel electrophoresis. So far, 14-3-3 proteins have been identified in eukaryotic organisms ranging from yeast to mammals. The function of 14-3-3 proteins was originally described as “activator of neurotransmitter”. The 14-3-3 proteins undergo phosphorylation, and thus play a role in various cellular processes like signal transduction, cell cycle regulation, apoptosis, stress response, cytoskeleton organization, and malignant transformation.

For a long time mushrooms have been classified based on morphological properties of fruiting body, but several defects were included in this method: i) mushroom spend a large part of own life as mycelia, fruiting body forming stage is a specific part of the life cycle, and not all species can form fruiting body, ii) it is sometimes difficult to distinguish the different mushrooms based on fruit body appearances alone, for non-professionals, and iii) it is easy to include subjective judgment while discussing the phylogenetic relationships. Due to these reasons, molecular genetic approaches are currently gaining attention in the classification process. By using molecular phylogenetics new insight into the evolution and taxonomy of these organisms is emerging, but uncertainties remain regarding the position and status of numerous fungal species, due to lack of sufficient properties in small subunit rDNA (SSU rDNA). Therefore it is essential that classification of fungi is based on multiple molecular species, including SSU rDNA. The 14-3-3 proteins are highly conserved, and easy to purify because of common acidic pIs (at least for the two mushrooms studied here) and molecular mass. Additionally 14-3-3 proteins participate in many biological processes and its deletion is lethal. Therefore, the 14-3-3 proteins have a possibility to serve as a molecular marker.

Additionally, septin was commonly identified from *S. crispa* and *H. erinaceum* fruiting bodies by 1-DGE. The septin genes were originally discovered through genetic screening for budding yeast mutants defective in the cell-cycle progression. Septins are ubiquitous GTP-binding proteins generally regarded as cytoskeletal components. Most of them also contain a coiled-coil domain that could be involved in their assembly into filaments. The functions
of septins are best known for their role in cytokinesis. For instance, budding yeast has septins specific for sporulation, fly septins are associated with development of germ cells, photoreceptor cells, and nervous system, and mammalian septins are implicated in exocytosis, tumorigenesis, apoptosis, synaptogenesis, and neurodegeneration. Both yeast and mushroom belong to “fungi” and are located close on the phylogenetic tree. Spore forming mechanism in these two fungi is also similar. Therefore it is likely that septin is involved in spore formation in mushroom.

4. Conclusion and Future Prospects

This is a first proteomics research on two edible medicinal mushrooms. Using 1-DGE and 2-DGE in conjunction with LC-MS/MS and N-terminal amino acid sequencing, we identified 115 and 172 from *S. crispa* and *H. erinaceum* proteins, identifying 84 and 119 unique proteins respectively. Two proteins, namely, septin involved in sporulation and 14-3-3 protein that functions in cell progression, signal transduction, etc., were commonly found in both the mushrooms. Furthermore, numerous proteins of interest and of potential use to the food industry were identified from this study, including laccase, polygalacturonase, xylose reductase, trehalose phosphorylase, glutamine synthase, and some restriction enzymes. The proteomics database and 2-D gel reference maps established for these two mushrooms will be helpful in profiling protein changes during the growth of fruiting bodies and against diverse environmental factors in comparative proteomics studies. The latter may involve comparative studies in control vs. cultivated mushrooms in stress environment and artificial culture vs. natural growth. This study presents only a snapshot of mushroom life cycle at the fruiting body stage, and therefore we have to investigate mushrooms at other developmental stages such as mycelia, etc., to complete the whole album. We believe that these studies are connected to the expansion of food resource, improvement of environment and human wellness via optimized production of mushroom fruiting bodies, and collection of biodegradation enzymes and bioactive proteins for human health.

**Acknowledgements.** During his study at HSS, AIST, KH greatly appreciates the support and encouragement from Drs. Hitoshi Iwahashi, Yoshinori Masuo, and Yasukazu Yoshida. This work was partly supported by a grant from the Korea Science and Engineering Foundation through Protein Network Research Center at Yonsei University (Grant No. R112000078010010).
References


**Figure Legends**

**Figure 1.** The experimental strategy and proteomics workflow. The mature fruiting bodies of *Sparassis crispa* and *Hericium erinaceum* were used as source material for total protein extraction using a phenol extraction protocol. The pellet was solubilized in LB-TT and used for 1-DGE and 2-DGE analysis. The separated proteins on 1-D and 2-D gels were stained with CBB R-250 and colloidal CBB G-250, respectively. Proteins separated on 1-D gels were excised from gels, in-gel tryptic digested for analyses by tandem MS (nESI-LC-MS/MS), and identified using MASCOT search engine and NCBI nonredundant protein database. Protein spots on 2-D gel were transferred onto PVDF membrane, cut-off and processed for analysis by N-terminal amino acid sequencing and identification using Swiss-Prot and TrEMBL databases.

**Figure 2.** 1-D gel profile of *S. crispa* and *H. erinaceum* fruiting body proteins. Total protein was separated on 12.5% SDS-PAGE followed by excision of gel into six gel sections. In-gel trypsin-digested peptides were subjected to nESI-LC-MS/MS. Proteins were identified using the MASCOT search engine and NCBI nonredundant protein database.

**Figure 3.** Development of 2-D gel reference map of fruiting body in *S. crispa*. The total soluble proteins were separated on pre-cast IPG strips (18 cm, pH 4-7) in the first dimension followed by 12.5% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5 µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250. Total spot numbers (ImageMaster 2D platinum software 5.0) detected on gel is given at the bottom right-hand corner. The electroblotted proteins onto PVDF membrane were sequenced on an Applied Biosystems 494 protein sequencer. The obtained sequences were used to interrogate databases with Web accessible search programs Fasta3 (EMBL Outstation of the European Bioinformatics Institute) to identify homology to proteins already present in the protein and nucleic acid databases.

**Figure 4.** Development of 2-D gel map of *H. erinaceum* fruiting body proteins. 2-DGE was carried out as described in Figure 3.

**Figure 5.** Distribution of the identified nonredundant proteins in *S. crispa* and *H. erinaceum*. Numbers circled black lines and gray lines indicate proteins identified using 1-
DGE coupled to tandem MS and 2-DGE in conjunction with Edman sequencing, respectively. 1-DGE and 2-DGE separately identified 60 and 29 proteins, respectively, where only 5 proteins were common in both datasets of *S. crispa*. In *H. erinaceum*, 1-DGE and 2-DGE separately identified 88 and 35 proteins, respectively, where only 4 proteins were common in both datasets. A total of 84 and 119 nonredundant and non-overlapped proteins were identified from *S. crispa* and *H. erinaceum* fruiting body respectively. A total of 21 proteins were common between the two mushrooms. The number in parenthesis indicates overlapped proteins identified by 2-DGE.

Grinding
Liquid nitrogen
Extraction
Tris buffered phenol
Purification
Methanol, Acetone, Ethanol
Solubilization

Sparassis crispa
Hericium erinaceum

SDS-PAGE
Trypsin digestion
nESI-LC-MS/MS
SDS-PAGE
Isoelectric focusing
Edman sequencing

Peptide matching/N-terminal sequence homology

MASCOT SEARCH ENGINE & NCBI/Swiss-Prot Protein Database
Inventory of Mushroom Proteins [http://foodfunc.agr.ibaraki.ac.jp/mushprot.html]

Figure 1
Horie et al.
Figure 2
Horie et al.
Figure 4
Horie et al.
Figure 5
Horie et al.
Figure 6
Horie et al.

Sparassis crispa
Hericium erinaceum

Figure 6 contd…
Horie et al.
The table lists indicated fraction numbers, matching peptides, scores, accession numbers, protein names, theoretical mass (kDa), theoretical pH left to right.

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Matching Peptide number</th>
<th>accession number</th>
<th>protein name</th>
<th>analytical MW</th>
<th>theoretical pI</th>
<th>peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatin structure and dynamics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>92</td>
<td>gi</td>
<td>1742929</td>
<td>Histone H2B *</td>
<td>15165.40</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>67</td>
<td>gi</td>
<td>3142</td>
<td>Histone H4*</td>
<td>11366.34</td>
</tr>
<tr>
<td>DNA replication, recombination, and repair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>57</td>
<td>gi</td>
<td>14245691</td>
<td>Reverse transcriptase *</td>
<td>40781.01</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>69</td>
<td>gi</td>
<td>908896</td>
<td>DNA repair protein rad32*</td>
<td>73688.98</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>47</td>
<td>gi</td>
<td>47132515</td>
<td>recQ family Helicase*</td>
<td>169673.76</td>
</tr>
<tr>
<td>Cell division and chromosome partitioning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>102</td>
<td>gi</td>
<td>10241488</td>
<td>Cell division control protein 10 *</td>
<td>38657.99</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>51</td>
<td>gi</td>
<td>6977953</td>
<td>Septin*</td>
<td>53737.59</td>
</tr>
<tr>
<td>Transcription</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>49</td>
<td>gi</td>
<td>57228114</td>
<td>Transcriptional activator*</td>
<td>13338.53</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>47</td>
<td>gi</td>
<td>19068759</td>
<td>Heat shock transcription factor *</td>
<td>33685.36</td>
</tr>
<tr>
<td>Translation, ribosomal structure and biogenesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>208</td>
<td>gi</td>
<td>58758727</td>
<td>Translation elongation factor EF1-α *</td>
<td>44184.90</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>193</td>
<td>gi</td>
<td>11078214</td>
<td>Translation elongation factor EF1-α</td>
<td>46634.83</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>263</td>
<td>gi</td>
<td>2897607</td>
<td>Translation elongation factor EF1-α</td>
<td>50133.84</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>259</td>
<td>gi</td>
<td>58758727</td>
<td>Translation elongation factor EF1-α</td>
<td>44184.90</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>237</td>
<td>gi</td>
<td>2313</td>
<td>Translation elongation factor EF1-α</td>
<td>49828.51</td>
</tr>
</tbody>
</table>

Table 1.

A functional category list of proteins (*Sparassis crispa*) identified by 1-DGE and nESI-LC-MS/MS

The table lists indicated fraction numbers, matching peptides, scores, accession numbers, protein names, theoretical mass (kDa), theoretical pH left to right.
<table>
<thead>
<tr>
<th>No.</th>
<th>Gene ID</th>
<th>Description</th>
<th>Molecular Weight (kDa)</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>gi</td>
<td>32567511</td>
<td>Translation elongation factor EF1-α</td>
<td>14692.27</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>8927046</td>
<td>Translation elongation factor EF2*</td>
<td>89733.93</td>
</tr>
<tr>
<td>5</td>
<td>gi</td>
<td>46339196</td>
<td>Ribosomal protein L3*</td>
<td>43948.70</td>
</tr>
<tr>
<td>5</td>
<td>gi</td>
<td>3766376</td>
<td>Ribosomal protein L10 *</td>
<td>33565.30</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>16943769</td>
<td>Ribosomal protein S19*</td>
<td>14522.61</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>3806</td>
<td>Translation initiation factor 5A-2*</td>
<td>17114.28</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>3560198</td>
<td>Ribosomal protein L18E *</td>
<td>21189.79</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>115955</td>
<td>Ribosomal protein S28*</td>
<td>15909.58</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>1164943</td>
<td>Ribosomal protein S7e*</td>
<td>21987.65</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>1850540</td>
<td>Ribosomal P2 phosphoprotein*</td>
<td>11145.20</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>7521943</td>
<td>Heat shock protein 70 *</td>
<td>71055.79</td>
</tr>
<tr>
<td>2</td>
<td>gi</td>
<td>7521943</td>
<td>Heat shock protein 70</td>
<td>71055.79</td>
</tr>
<tr>
<td>2</td>
<td>gi</td>
<td>7521943</td>
<td>Heat shock protein 70</td>
<td>71055.79</td>
</tr>
<tr>
<td>4</td>
<td>gi</td>
<td>7521943</td>
<td>Heat shock protein 70</td>
<td>71055.79</td>
</tr>
<tr>
<td>5</td>
<td>gi</td>
<td>172714</td>
<td>Heat shock protein 70</td>
<td>16141.27</td>
</tr>
<tr>
<td>2</td>
<td>gi</td>
<td>4376093</td>
<td>Pyruvate kinase *</td>
<td>58248.74</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>4376093</td>
<td>Pyruvate kinase</td>
<td>58248.74</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>584806</td>
<td>ATP synthase alpha chain*</td>
<td>59522.39</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>44985523</td>
<td>F0F1-type ATP synthase*</td>
<td>58941.62</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>5190</td>
<td>Phosphoglycerate kinase *</td>
<td>44406.05</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>2654</td>
<td>Vacuolar ATPase subunit b *</td>
<td>57199.76</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>469103</td>
<td>Fumarase *</td>
<td>53156.73</td>
</tr>
</tbody>
</table>

**Posttranslational modification, protein turnover, chaperones**

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene ID</th>
<th>Description</th>
<th>Molecular Weight (kDa)</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>gi</td>
<td>7521943</td>
<td>Heat shock protein 70</td>
<td>71055.79</td>
</tr>
<tr>
<td>2</td>
<td>gi</td>
<td>7521943</td>
<td>Heat shock protein 70</td>
<td>71055.79</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>7521943</td>
<td>Heat shock protein 70</td>
<td>71055.79</td>
</tr>
<tr>
<td>4</td>
<td>gi</td>
<td>7521943</td>
<td>Heat shock protein 70</td>
<td>71055.79</td>
</tr>
<tr>
<td>5</td>
<td>gi</td>
<td>172714</td>
<td>Heat shock protein 70</td>
<td>16141.27</td>
</tr>
</tbody>
</table>

**Energy production and conversion**

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene ID</th>
<th>Description</th>
<th>Molecular Weight (kDa)</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>gi</td>
<td>4376093</td>
<td>Pyruvate kinase</td>
<td>58248.74</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>4376093</td>
<td>Pyruvate kinase</td>
<td>58248.74</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>584806</td>
<td>ATP synthase alpha chain</td>
<td>59522.39</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>44985523</td>
<td>F0F1-type ATP synthase</td>
<td>58941.62</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>5190</td>
<td>Phosphoglycerate kinase</td>
<td>44406.05</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>2654</td>
<td>Vacuolar ATPase subunit b</td>
<td>57199.76</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>469103</td>
<td>Fumarase</td>
<td>53156.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>gi</td>
<td>Description</td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>130</td>
<td>gi</td>
<td>46444820 Malate dehydrogenase*</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>77</td>
<td>gi</td>
<td>4029338 Malate dehydrogenase*</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>81</td>
<td>gi</td>
<td>46433360 NAD-formate dehydrogenase*</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>74</td>
<td>gi</td>
<td>13785197 Inorganic pyrophosphatase*</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>45</td>
<td>gi</td>
<td>218041 Phosphoglycerate kinase*</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>44</td>
<td>gi</td>
<td>929983 Glycerol-3-aldehyde dehydrogenase*</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>56</td>
<td>gi</td>
<td>15808971 Glycogen synthase*</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>88</td>
<td>gi</td>
<td>6651233 Trehalose phosphorylase*</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>51</td>
<td>gi</td>
<td>171569 1,4-glucan-6-(1,4-glucano)-transferase*</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>95</td>
<td>gi</td>
<td>169852 Glyceraldehyde-3-phosphate dehydrogenase*</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>74</td>
<td>gi</td>
<td>3367647 Enolase*</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>47</td>
<td>gi</td>
<td>40713647 Glucose-6-phosphate isomerase*</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>15</td>
<td>gi</td>
<td>30038515 Glyceraldehyde-3-phosphate dehydrogenase*</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>152</td>
<td>gi</td>
<td>929979 Glycerol-3-phosphate dehydrogenase*</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>59</td>
<td>gi</td>
<td>3289019 Xylose reductase*</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>44</td>
<td>gi</td>
<td>19068968 Alpha alpha trehalase*</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>65</td>
<td>gi</td>
<td>68429 Triose-phosphate isomerase*</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>42</td>
<td>gi</td>
<td>19068968 Alpha alpha trehalase</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>74</td>
<td>gi</td>
<td>169852 Glyceraldehyde-3-phosphate dehydrogenase*</td>
</tr>
</tbody>
</table>

### Carbohydrate transport and metabolism

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>gi</th>
<th>Description</th>
<th>E.C.</th>
<th>PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>44</td>
<td>gi</td>
<td>929983 Glycerol-3-aldehyde dehydrogenase*</td>
<td>30768.23</td>
<td>6.13</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>74</td>
<td>gi</td>
<td>3367647 Enolase*</td>
<td>46877.80</td>
<td>5.15</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>47</td>
<td>gi</td>
<td>40713647 Glucose-6-phosphate isomerase*</td>
<td>61445.79</td>
<td>6.38</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>15</td>
<td>gi</td>
<td>30038515 Glyceraldehyde-3-phosphate dehydrogenase*</td>
<td>36607.76</td>
<td>7.67</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>152</td>
<td>gi</td>
<td>929979 Glycerol-3-phosphate dehydrogenase*</td>
<td>30895.28</td>
<td>5.71</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>59</td>
<td>gi</td>
<td>3289019 Xylose reductase*</td>
<td>30621.33</td>
<td>5.77</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>44</td>
<td>gi</td>
<td>19068968 Alpha alpha trehalase*</td>
<td>76692.14</td>
<td>5.92</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>65</td>
<td>gi</td>
<td>68429 Triose-phosphate isomerase*</td>
<td>27234.99</td>
<td>6.13</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>42</td>
<td>gi</td>
<td>19068968 Alpha alpha trehalase</td>
<td>76692.14</td>
<td>5.92</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>74</td>
<td>gi</td>
<td>169852 Glyceraldehyde-3-phosphate dehydrogenase*</td>
<td>36055.25</td>
<td>7.01</td>
</tr>
</tbody>
</table>

### Amino acid transport and metabolism

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>gi</th>
<th>Description</th>
<th>E.C.</th>
<th>PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>97</td>
<td>gi</td>
<td>16415894 Aspartyl-proteinase*</td>
<td>18517.90</td>
<td>5.77</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>51</td>
<td>gi</td>
<td>44980853 Aminopeptidase I*</td>
<td>53590.96</td>
<td>6.08</td>
</tr>
</tbody>
</table>

### Nucleotide transport and metabolism

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>gi</th>
<th>Description</th>
<th>E.C.</th>
<th>PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>49</td>
<td>gi</td>
<td>44981739 Carbamoylphosphate synthase*</td>
<td>248131.78</td>
<td>5.48</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>45</td>
<td>gi</td>
<td>22095310 C1-tetrahydrofolate synthase*</td>
<td>67745.49</td>
<td>6.28</td>
</tr>
</tbody>
</table>

### Lipid metabolism
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>GI</th>
<th>Protein Name</th>
<th>Score</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>43</td>
<td>gi4959943</td>
<td>Esterase *</td>
<td>170506.56</td>
<td>4.67</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>43</td>
<td>gi12718322</td>
<td>C-8, 7 sterol isomerase*</td>
<td>30464.65</td>
<td>6.51</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>47</td>
<td>gi44986286</td>
<td>Diacylglycerol kinase *</td>
<td>63153.68</td>
<td>5.37</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>42</td>
<td>gi5725410</td>
<td>Phosphocholine cytidylyltransferase *</td>
<td>41556.61</td>
<td>5.39</td>
</tr>
</tbody>
</table>

**Inorganic ion transport and metabolism**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>GI</th>
<th>Protein Name</th>
<th>Score</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>44</td>
<td>gi2776</td>
<td>Catalase *</td>
<td>57848.89</td>
<td>6.42</td>
</tr>
</tbody>
</table>

**Cytoskeleton**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>GI</th>
<th>Protein Name</th>
<th>Score</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
<td>137</td>
<td>gi508701</td>
<td>Actin *</td>
<td>41736.68</td>
<td>5.17</td>
</tr>
</tbody>
</table>

**Cell envelope biogenesis, outer membrane**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>GI</th>
<th>Protein Name</th>
<th>Score</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1</td>
<td>60</td>
<td>gi46442658</td>
<td>GPI-protein transamidase *</td>
<td>55466.46</td>
<td>5.40</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>44</td>
<td>gi51215619</td>
<td>Chitin biosynthesis protein *</td>
<td>27327.64</td>
<td>5.31</td>
</tr>
</tbody>
</table>

**Intracellular trafficking and secretion**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>GI</th>
<th>Protein Name</th>
<th>Score</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5</td>
<td>314</td>
<td>gi11262436</td>
<td>14-3-3 protein homolog *</td>
<td>28913.33</td>
<td>4.67</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>53</td>
<td>gi3218407</td>
<td>Adenylate cyclase*</td>
<td>190334.33</td>
<td>5.86</td>
</tr>
</tbody>
</table>

**Signal transduction**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>GI</th>
<th>Protein Name</th>
<th>Score</th>
<th>E Value</th>
</tr>
</thead>
</table>

**Unclassified**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>GI</th>
<th>Protein Name</th>
<th>Score</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>53</td>
<td>gi6321879</td>
<td>Protein involved in RNA metabolism</td>
<td>12009.47</td>
<td>5.05</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>52</td>
<td>gi6321879</td>
<td>Protein involved in RNA metabolism</td>
<td>12009.47</td>
<td>5.05</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>179</td>
<td>gi30024660</td>
<td>guanine nucleotide binding protein</td>
<td>34556.86</td>
<td>5.98</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>44</td>
<td>gi28564107</td>
<td>SOL1</td>
<td>16776.24</td>
<td>5.58</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>67</td>
<td>gi46438514</td>
<td>ATP/ADP translocator</td>
<td>32754.91</td>
<td>9.62</td>
</tr>
</tbody>
</table>

Asterisks indicate unique proteins identified from *S. crispa* fruiting body using LC-MS/MS and Edman sequencing. Commonly identified proteins in *S. crispa* and *H. erinaceum* are shown in red letters.
<table>
<thead>
<tr>
<th>spot number</th>
<th>amino acid sequence</th>
<th>score</th>
<th>accession number</th>
<th>protein name</th>
<th>identity/similarity</th>
<th>analytical MW</th>
<th>theoretical pI</th>
<th>organism</th>
<th>functional category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>gefsddsdqagaydq</td>
<td>74</td>
<td>A1CE10</td>
<td>Phosphoglycerate mutase*</td>
<td>73 / 87</td>
<td>12190.12</td>
<td>4.81</td>
<td>Fungi</td>
<td>Carbohydrate transport and metabolism</td>
</tr>
<tr>
<td>2</td>
<td>sntgvvaatv</td>
<td></td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>sptvxvndvpsgftflyvpy</td>
<td>74</td>
<td>Q2PCV2</td>
<td>Laccase*</td>
<td>69 / 81</td>
<td>5404.94</td>
<td>6.26</td>
<td>Fungi</td>
<td>Secondary metabolites biosynthesis, transport, and catabolism</td>
</tr>
<tr>
<td>4</td>
<td>sxraaxgsgavnagsraaksgavnaqstqsfvnlsefn</td>
<td></td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>raakgsgavnagstqsfvgnlsefn</td>
<td></td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>rgkgsrngvngtqffv</td>
<td>65</td>
<td>Q6ZXX3</td>
<td>Histone H4</td>
<td>53 / 100</td>
<td>11382.34</td>
<td>11.36</td>
<td>Fungi</td>
<td>Chromatin structure and dynamics</td>
</tr>
<tr>
<td>7</td>
<td>aplgievadapstaafgvv</td>
<td>74</td>
<td>Q1IL41</td>
<td>Hypothetical protein</td>
<td>53 / 88</td>
<td>32116.70</td>
<td>5.64</td>
<td>Bacteria</td>
<td>Unclassified</td>
</tr>
<tr>
<td>8</td>
<td>aplsievadapstiafgv</td>
<td>61</td>
<td>Q9FAX2</td>
<td>DNA gyrase subunit B*</td>
<td>56 / 78</td>
<td>53556.69</td>
<td>6.33</td>
<td>Bacteria</td>
<td>DNA replication, recombination, and repair</td>
</tr>
<tr>
<td>9</td>
<td>sdseairhlflhfln</td>
<td>56</td>
<td>P46963</td>
<td>CTD kinase subunit gamma*</td>
<td>69 / 92</td>
<td>34809.18</td>
<td>5.88</td>
<td>Fungi</td>
<td>Transcription</td>
</tr>
<tr>
<td>10</td>
<td>sdseairhlflhfln</td>
<td>56</td>
<td>P46963</td>
<td>CTD kinase subunit gamma</td>
<td>69 / 92</td>
<td>34809.18</td>
<td>5.88</td>
<td>Fungi</td>
<td>Transcription</td>
</tr>
<tr>
<td>11</td>
<td>sdseairhlflhfln</td>
<td>56</td>
<td>P46963</td>
<td>CTD kinase subunit gamma</td>
<td>69 / 92</td>
<td>34809.18</td>
<td>5.88</td>
<td>Fungi</td>
<td>Transcription</td>
</tr>
<tr>
<td>12</td>
<td>sdseairhlflhfln</td>
<td>54</td>
<td>Q753W3</td>
<td>Vacuolar sorting protein SNF7*</td>
<td>64 / 79</td>
<td>26685.19</td>
<td>4.84</td>
<td>Fungi</td>
<td>Intracellular trafficking and secretion</td>
</tr>
<tr>
<td>13</td>
<td>speqelaavngrlqssvglpdvv</td>
<td>79</td>
<td>A0V74</td>
<td>Phosphatidylycerine decarboxylase*</td>
<td>54 / 75</td>
<td>30888.40</td>
<td>9.09</td>
<td>Bacteria</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>14</td>
<td>speqelaavngrlqssvgl</td>
<td></td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>sldvearlyiesldifg</td>
<td>59</td>
<td>Q3IYU1</td>
<td>tRNA pseudouridine synthase B</td>
<td>56 / 88</td>
<td>32194.57</td>
<td>5.20</td>
<td>Bacteria</td>
<td>Translation, ribosomal structure and biogenesis</td>
</tr>
<tr>
<td>16</td>
<td>rvpketidqlakay</td>
<td></td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>gldklvnvnqlqdmvglgd</td>
<td>68</td>
<td>Q8ZVM9</td>
<td>Probable exosome complex exonuclease 1*</td>
<td>56 / 94</td>
<td>27148.26</td>
<td>6.86</td>
<td>Bacteria</td>
<td>Amino acid transport and metabolism</td>
</tr>
<tr>
<td>18</td>
<td>rlgkvgmvrgagg</td>
<td></td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.
Proteins (*Sparassis crispa*) identified by 2-DGE and N-terminal amino acid sequencing

The table lists indicated spot numbers, amino acid sequences, scores, accession numbers, protein names, identity/similarity, theoretical mass (kDa), theoretical pH, organism and function left to right.
<table>
<thead>
<tr>
<th>i</th>
<th>Description</th>
<th>Accession</th>
<th>Start-Stop</th>
<th>Score</th>
<th>E-value</th>
<th>Organism</th>
<th>Functional Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Chloroplast 3OS ribosomal protein S8*</td>
<td>Q9MUU6</td>
<td>75 / 100</td>
<td>14764.25</td>
<td>9.73</td>
<td>Plant</td>
<td>Translation, ribosomal structure and biogenesis</td>
</tr>
<tr>
<td>25</td>
<td>14-3-3 protein</td>
<td>Q562H7</td>
<td>92 / 92</td>
<td>28815.19</td>
<td>4.76</td>
<td>Fungi</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>30</td>
<td>Elongation factor 2</td>
<td>O14460</td>
<td>73 / 93</td>
<td>93230.90</td>
<td>6.02</td>
<td>Fungi</td>
<td>Translation, ribosomal structure and biogenesis</td>
</tr>
<tr>
<td>33</td>
<td>Splicing factor 3B subunit 1*</td>
<td>O57683</td>
<td>57 / 100</td>
<td>146214.52</td>
<td>6.48</td>
<td>Insect</td>
<td>RNA processing and modification</td>
</tr>
<tr>
<td>34</td>
<td>Cytochrome c oxidase subunit 3*</td>
<td>Q9B6D8</td>
<td>73 / 91</td>
<td>30467.04</td>
<td>6.32</td>
<td>Fungi</td>
<td>Energy production and conversion</td>
</tr>
<tr>
<td>37</td>
<td>Putative cytochrome c oxidase polypeptide Vc-4*</td>
<td>Q9FNE0</td>
<td>43 / 86</td>
<td>7421.61</td>
<td>8.03</td>
<td>Plant</td>
<td>Energy production and conversion</td>
</tr>
<tr>
<td>39</td>
<td>Urease accessory protein ureE*</td>
<td>Q8DI62</td>
<td>89 / 100</td>
<td>16285.70</td>
<td>6.49</td>
<td>Bacteria</td>
<td>Amino acid transport and metabolism</td>
</tr>
<tr>
<td>41</td>
<td>UPF0061 protein MAP 3154</td>
<td>Q73V62</td>
<td>67 / 87</td>
<td>53324.47</td>
<td>5.07</td>
<td>Bacteria</td>
<td>Unclassified</td>
</tr>
<tr>
<td>42</td>
<td>S-locus-specific glycoprotein BS29-2 precursor*</td>
<td>P22553</td>
<td>70 / 100</td>
<td>49543.04</td>
<td>8.48</td>
<td>Plant</td>
<td>Sporulation</td>
</tr>
<tr>
<td>43</td>
<td>Actin</td>
<td>P48465</td>
<td>82 / 100</td>
<td>41736.68</td>
<td>5.17</td>
<td>Bacteria</td>
<td>Cytoskeleton</td>
</tr>
<tr>
<td>Position</td>
<td>Sequence</td>
<td>Accession</td>
<td>Description</td>
<td>E-value</td>
<td>Score</td>
<td>Bit Score</td>
<td>Organism</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>-----------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------</td>
<td>--------</td>
<td>-----------</td>
<td>-------------------</td>
</tr>
<tr>
<td>47</td>
<td>rvtglklgavairv</td>
<td>Q8NSS8</td>
<td>Hypothetical transport protein Cgl0590/cg0683</td>
<td>67 / 87</td>
<td>55665.64</td>
<td>5.78</td>
<td>Bacteria</td>
</tr>
<tr>
<td>48</td>
<td>kgnggllgqndadlgr</td>
<td>P43852</td>
<td>Bifunctional purine biosynthesis protein purH*</td>
<td>83 / 92</td>
<td>58349.59</td>
<td>5.75</td>
<td>Bacteria</td>
</tr>
<tr>
<td>49</td>
<td>qiggasggr</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>nvlveelnvlva</td>
<td>Q83PY1</td>
<td>Protein slyX</td>
<td>64 / 82</td>
<td>8184.30</td>
<td>4.89</td>
<td>Bacteria</td>
</tr>
<tr>
<td>51</td>
<td>kgyatpiftmdateatgtgqkvtigavvd</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>kgnggllgqndadlgr</td>
<td>Q98EV8</td>
<td>ATP synthase subunit beta*</td>
<td>65 / 95</td>
<td>50775.77</td>
<td>4.97</td>
<td>Bacteria</td>
</tr>
<tr>
<td>53</td>
<td>slgahgakry</td>
<td>Q00094</td>
<td>Gene 73 protein kinase*</td>
<td>80 / 100</td>
<td>106567.73</td>
<td>8.80</td>
<td>Viruses</td>
</tr>
<tr>
<td>54</td>
<td>plftsqqfnstsv</td>
<td>Q8CWA2</td>
<td>Cation efflux system protein cusB precursor*</td>
<td>67 / 92</td>
<td>44409.95</td>
<td>5.70</td>
<td>Bacteria</td>
</tr>
<tr>
<td>55</td>
<td>rtagepydtv</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>paaiakvpathkti</td>
<td>Q4WGM4</td>
<td>Ubiquinol-cytochrome c reductase complex*</td>
<td>47 / 80</td>
<td>14662.88</td>
<td>5.55</td>
<td>Fungi</td>
</tr>
<tr>
<td>57</td>
<td>apqtklqatdavaay</td>
<td>P94186</td>
<td>Mercuric transport protein periplasmatic component precursor*</td>
<td>73 / 91</td>
<td>9505.12</td>
<td>9.26</td>
<td>Bacteria</td>
</tr>
<tr>
<td>58</td>
<td>ahkdikfsnegrasi</td>
<td>Q0H0L2</td>
<td>Heat shock protein 60</td>
<td>71 / 83</td>
<td>63039.38</td>
<td>5.91</td>
<td>Fungi</td>
</tr>
<tr>
<td>59</td>
<td>slqvaliyqkndyh</td>
<td>Q7V168</td>
<td>L-seryl-tRNA(Sec) selenium transferase*</td>
<td>53 / 93</td>
<td>50580.15</td>
<td>8.50</td>
<td>Bacteria</td>
</tr>
<tr>
<td>60</td>
<td>kldgklkrgkg</td>
<td>Q0K852</td>
<td>NADH dehydrogenase type2 *</td>
<td>100 / 100</td>
<td>47467.77</td>
<td>8.46</td>
<td>Bacteria</td>
</tr>
<tr>
<td>61</td>
<td>glearvptdsqpr</td>
<td>Q2H0Z4</td>
<td>Hypothetical protein</td>
<td>75 / 100</td>
<td>51089.66</td>
<td>4.91</td>
<td>Fungi</td>
</tr>
<tr>
<td>62</td>
<td>qteeyvitlqlfhhv</td>
<td>P37485</td>
<td>Hypothetical protein yybS</td>
<td>64 / 91</td>
<td>34534.02</td>
<td>9.65</td>
<td>Bacteria</td>
</tr>
<tr>
<td>63</td>
<td>sptdlprkhhrgmsg</td>
<td>Q2LEM4</td>
<td>Hypothetical protein</td>
<td>71 / 86</td>
<td>25414.61</td>
<td>5.89</td>
<td>Bacteria</td>
</tr>
<tr>
<td>64</td>
<td>sageglgtntier</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>atvsegnigaty</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>alvgeggrgt</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>alvgeggrgt</td>
<td></td>
<td>N-terminal amino acid blocked</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>slgrrdlrlrt</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>slvvggyghth</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>kraenlen</td>
<td></td>
<td>No significant hits found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Asterisks indicate unique proteins identified from *S. crispa* fruiting body using LC-MS/MS and Edman sequencing. Commonly identified proteins in *S. crispa* and *H. erinaceum* are shown in red letters.
Table 3.

A functional category list of proteins (*Hericiurn erinaceum*) identified by 1-DGE and nESI-LC-MS/MS

The table lists indicated fraction numbers, matching peptides, scores, accession numbers, protein names, theoretical mass (kDa), theoretical pH left to right.

<table>
<thead>
<tr>
<th>fraction number</th>
<th>matching peptide</th>
<th>score</th>
<th>accession number</th>
<th>protein name</th>
<th>analytical MW</th>
<th>theoretical pI</th>
<th>peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>44</td>
<td>gi</td>
<td>44982537</td>
<td>Chromatin remodeling complex subunit RSC8*</td>
<td>64437.55</td>
<td>5.46</td>
<td>DLAQLNISKK</td>
</tr>
<tr>
<td>6</td>
<td>91</td>
<td>gi</td>
<td>1742929</td>
<td>Histone H2B *</td>
<td>15165.40</td>
<td>10.16</td>
<td>AMAILNSFVNDIFER</td>
</tr>
<tr>
<td>6</td>
<td>77</td>
<td>gi</td>
<td>3142</td>
<td>Histone H4.2 *</td>
<td>11366.34</td>
<td>11.36</td>
<td>TVTALDVVYALKR</td>
</tr>
</tbody>
</table>

**Chromatin structure and dynamics**

<table>
<thead>
<tr>
<th>fraction number</th>
<th>matching peptide</th>
<th>score</th>
<th>accession number</th>
<th>protein name</th>
<th>analytical MW</th>
<th>theoretical pI</th>
<th>peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>gi</td>
<td>57898980</td>
<td>DNA topoisomerase II *</td>
<td>176899.32</td>
<td>7.14</td>
<td>SIGMGRGK</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>gi</td>
<td>550429</td>
<td>Helicases*</td>
<td>124537.62</td>
<td>6.82</td>
<td>EINSNTKLENI</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>gi</td>
<td>2213553</td>
<td>DNA repair protein RAD4 *</td>
<td>79023.81</td>
<td>8.76</td>
<td>EEALLPNAPK</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>gi</td>
<td>2340169</td>
<td>Telomerase reverse transcriptase 1 *</td>
<td>116457.25</td>
<td>9.75</td>
<td>KFLNLSLR</td>
</tr>
</tbody>
</table>

**DNA replication, recombination, and repair**

<table>
<thead>
<tr>
<th>fraction number</th>
<th>matching peptide</th>
<th>score</th>
<th>accession number</th>
<th>protein name</th>
<th>analytical MW</th>
<th>theoretical pI</th>
<th>peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>73</td>
<td>gi</td>
<td>5725417</td>
<td>Septin *</td>
<td>40119.94</td>
<td>5.01</td>
<td>VNVIPVIGK</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>gi</td>
<td>38704270</td>
<td>Beta-tubulin</td>
<td>25238.83</td>
<td>5.07</td>
<td>AVLNDLPEGTM</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>gi</td>
<td>10241488</td>
<td>Cell division control protein 10 *</td>
<td>38657.99</td>
<td>7.20</td>
<td>STLINTFASHL</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>gi</td>
<td>984572</td>
<td>Beta-tubulin*</td>
<td>48984.54</td>
<td>4.92</td>
<td>AVLIDLEPGTM</td>
</tr>
</tbody>
</table>

**Cell division and chromosome partitioning**

<table>
<thead>
<tr>
<th>fraction number</th>
<th>matching peptide</th>
<th>score</th>
<th>accession number</th>
<th>protein name</th>
<th>analytical MW</th>
<th>theoretical pI</th>
<th>peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>45</td>
<td>gi</td>
<td>21745321</td>
<td>Transcription factor *</td>
<td>56479.77</td>
<td>9.38</td>
<td>LPPITNHSEK</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>gi</td>
<td>5055</td>
<td>RNA polymerase II large subunit *</td>
<td>194162.51</td>
<td>5.62</td>
<td>QILSLIIPK</td>
</tr>
</tbody>
</table>

**Transcription**

<table>
<thead>
<tr>
<th>fraction number</th>
<th>matching peptide</th>
<th>score</th>
<th>accession number</th>
<th>protein name</th>
<th>analytical MW</th>
<th>theoretical pI</th>
<th>peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>151</td>
<td>gi</td>
<td>11078222</td>
<td>Translation elongation factor EF1-α</td>
<td>40282.27</td>
<td>8.71</td>
<td>TLLAEIADIEPPSRPSDKPLR LPLQDVYK, TLLAEIADIEPPSRPSDKPLRLPLQDVYK</td>
</tr>
<tr>
<td>1</td>
<td>119</td>
<td>gi</td>
<td>27960789</td>
<td>Translation elongation factor EF1-α</td>
<td>36015.52</td>
<td>8.81</td>
<td>SVEMHHEQLQEGLPGDNGFNVK , QLIAJNK</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>gi</td>
<td>42547595</td>
<td>60S ribosomal protein L15 *</td>
<td>24017.93</td>
<td>11.27</td>
<td>YYEVILVPQIK</td>
</tr>
<tr>
<td>2</td>
<td>113</td>
<td>gi</td>
<td>10637881</td>
<td>Translation elongation factor EF1-α</td>
<td>50051.61</td>
<td>9.15</td>
<td>GDVASDSKNDPAK, YMVTVIDAP</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>gi</td>
<td>1364060</td>
<td>Translation elongation factor EF1-α</td>
<td>49663.33</td>
<td>9.22</td>
<td>DDLFTNTNASIVR, IQFGGDEVVK</td>
</tr>
</tbody>
</table>

**Translation, ribosomal structure and biogenesis**

<table>
<thead>
<tr>
<th>fraction number</th>
<th>matching peptide</th>
<th>score</th>
<th>accession number</th>
<th>protein name</th>
<th>analytical MW</th>
<th>theoretical pI</th>
<th>peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>gi</td>
<td>11078222</td>
<td>Translation elongation factor EF1-α</td>
<td>40282.27</td>
<td>8.71</td>
<td>TLLAEIADIEPPSRPSDKPLR LPLQDVYK, TLLAEIADIEPPSRPSDKPLRLPLQDVYK</td>
</tr>
<tr>
<td>1</td>
<td>119</td>
<td>gi</td>
<td>27960789</td>
<td>Translation elongation factor EF1-α</td>
<td>36015.52</td>
<td>8.81</td>
<td>SVEMHHEQLQEGLPGDNGFNVK , QLIAJNK</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>gi</td>
<td>42547595</td>
<td>60S ribosomal protein L15 *</td>
<td>24017.93</td>
<td>11.27</td>
<td>YYEVILVPQIK</td>
</tr>
<tr>
<td>2</td>
<td>113</td>
<td>gi</td>
<td>10637881</td>
<td>Translation elongation factor EF1-α</td>
<td>50051.61</td>
<td>9.15</td>
<td>GDVASDSKNDPAK, YMVTVIDAP</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>gi</td>
<td>1364060</td>
<td>Translation elongation factor EF1-α</td>
<td>49663.33</td>
<td>9.22</td>
<td>DDLFTNTNASIVR, IQFGGDEVVK</td>
</tr>
<tr>
<td>GI</td>
<td>Description</td>
<td>Score 1</td>
<td>Score 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------</td>
<td>---------</td>
<td>---------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>4056551</td>
<td>Translation initiation factor 3 subunit*</td>
<td>6236.95</td>
<td>6.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>58618695</td>
<td>Translation elongation factor EF1-α</td>
<td>46048.02</td>
<td>8.79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>58758729</td>
<td>Translation elongation factor EF1-α</td>
<td>34283.63</td>
<td>8.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>11078222</td>
<td>Translation elongation factor EF1-α</td>
<td>40282.27</td>
<td>8.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>542225</td>
<td>Ribosomal protein L4.e *</td>
<td>39005.13</td>
<td>10.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>32810507</td>
<td>Translation elongation factor EF1-α</td>
<td>20360.35</td>
<td>8.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>3766376</td>
<td>Ribosomal protein L10*</td>
<td>33565.30</td>
<td>4.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>1039443</td>
<td>Ribosome-associated protein*</td>
<td>31684.85</td>
<td>4.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>57225965</td>
<td>60S Ribosomal protein 15-b*</td>
<td>34650.37</td>
<td>8.79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>2104451</td>
<td>Threonyl-tRNA synthetase *</td>
<td>80138.01</td>
<td>6.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>1364060</td>
<td>Translation elongation factor EF1-α</td>
<td>49663.33</td>
<td>9.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>1164943</td>
<td>Ribosomal protein S7e*</td>
<td>21987.65</td>
<td>9.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>46098158</td>
<td>40S Ribosomal protein S9 *</td>
<td>22237.86</td>
<td>10.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>3256304</td>
<td>Translation elongation factor EF1-α</td>
<td>30650.33</td>
<td>6.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>1039443</td>
<td>Ribosome-associated protein</td>
<td>31684.85</td>
<td>4.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>12329975</td>
<td>Ribosomal protein S2 *</td>
<td>28310.92</td>
<td>10.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>3589691</td>
<td>Ribosomal protein L13e *</td>
<td>23053.64</td>
<td>10.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>44885669</td>
<td>S-phase specific ribosomal protein</td>
<td>29423.16</td>
<td>9.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>16944409</td>
<td>related to GTPase MSS1*</td>
<td>56936.39</td>
<td>4.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>2414648</td>
<td>60S ribosomal protein L7*</td>
<td>28449.26</td>
<td>10.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>3766376</td>
<td>Ribosomal protein L10</td>
<td>33565.30</td>
<td>4.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>14994314</td>
<td>40S Ribosomal protein S8*</td>
<td>23557.83</td>
<td>10.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>19571756</td>
<td>Ribosomal protein L18E *</td>
<td>11145.20</td>
<td>4.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>1742935</td>
<td>40S Ribosomal protein S13 *</td>
<td>17080.03</td>
<td>10.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gi</td>
<td>46098158</td>
<td>40S Ribosomal protein S9</td>
<td>22237.86</td>
<td>10.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>Gene Name</td>
<td>Description</td>
<td>Mw</td>
<td>PI</td>
<td>Modification Details</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-----------</td>
<td>-------------</td>
<td>----</td>
<td>----</td>
<td>----------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ribosomal protein S28</td>
<td>*</td>
<td>15909.58</td>
<td>10.60</td>
<td>VSVGVGLLALWK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ribosome-associated protein</td>
<td></td>
<td>31684.85</td>
<td>4.91</td>
<td>FTPGSFNTYTR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>40S Ribosomal protein S5*</td>
<td></td>
<td>23680.17</td>
<td>9.30</td>
<td>DILTDIYQR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ribosomal protein L29*</td>
<td></td>
<td>16721.55</td>
<td>10.62</td>
<td>INMDKYHPGYFGK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>40S Ribosomal protein rps16*</td>
<td></td>
<td>17114.28</td>
<td>4.81</td>
<td>VHLYAIDIFTGK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ribosomal protein L6*</td>
<td></td>
<td>95480.78</td>
<td>6.15</td>
<td>AFKQGNIDAGVAGDIYFQMNY</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Posttranslational modification, protein turnover, chaperones**

<table>
<thead>
<tr>
<th>ID</th>
<th>Gene Name</th>
<th>Description</th>
<th>Mw</th>
<th>PI</th>
<th>Modification Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ubiquitin</td>
<td>*</td>
<td>8540.78</td>
<td>6.56</td>
<td>IQDKEGIPPDQR</td>
</tr>
<tr>
<td>1</td>
<td>Heat shock protein 70</td>
<td></td>
<td>25092.47</td>
<td>5.52</td>
<td>DAVGIAGNVLR</td>
</tr>
<tr>
<td>2</td>
<td>Heat shock protein 70</td>
<td></td>
<td>71055.79</td>
<td>5.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Heat shock protein 70</td>
<td></td>
<td>70303.76</td>
<td>5.12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Heat shock protein 90</td>
<td></td>
<td>79295.85</td>
<td>4.92</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Heat shock protein 70</td>
<td></td>
<td>73898.47</td>
<td>4.91</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Proteasome regulatory subunit 4</td>
<td></td>
<td>47377.75</td>
<td>6.39</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Proteasome regulatory subunit</td>
<td></td>
<td>43552.98</td>
<td>5.28</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Proteasome alpha type 1</td>
<td></td>
<td>25604.02</td>
<td>6.90</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cyclophilin</td>
<td></td>
<td>17614.90</td>
<td>9.36</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cyclophilin B</td>
<td></td>
<td>21969.06</td>
<td>8.59</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ubiquitin</td>
<td></td>
<td>95480.78</td>
<td>6.15</td>
<td>AFKQGNIDAGVAGDIYFQMNY</td>
</tr>
</tbody>
</table>

**Energy production and conversion**

<table>
<thead>
<tr>
<th>ID</th>
<th>Gene Name</th>
<th>Description</th>
<th>Mw</th>
<th>PI</th>
<th>Modification Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyruvate carboxylase*</td>
<td></td>
<td>121206.58</td>
<td>6.27</td>
<td>NIIVEQGPEAFAK, TWTFTIFDDTMPEL, SAFGDGT, LVNPIPLLQALV, HVEINEFPR</td>
</tr>
<tr>
<td>1</td>
<td>Pyruvate carboxylase*</td>
<td></td>
<td>130907.76</td>
<td>6.25</td>
<td>QENGFTLMTTWR, HVEINEFPR</td>
</tr>
<tr>
<td>1</td>
<td>NAD-formate dehydrogenase*</td>
<td></td>
<td>41801.55</td>
<td>5.51</td>
<td>LGTVENELGI</td>
</tr>
<tr>
<td>1</td>
<td>Pyruvate carboxylase</td>
<td></td>
<td>43215.42</td>
<td>5.57</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Succinate dehydrogenase*</td>
<td></td>
<td>53767.12</td>
<td>7.71</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ATP synthase alpha chain*</td>
<td></td>
<td>59522.39</td>
<td>9.07</td>
<td>STVACLTVKLEAMITG, TEGIVD VPGPELELGR</td>
</tr>
<tr>
<td>No.</td>
<td>GI</td>
<td>Protein Name</td>
<td>MW</td>
<td>PI</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>--------------------------------------</td>
<td>------</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>46433360</td>
<td>NAD-formate dehydrogenase *</td>
<td>41801.55</td>
<td>5.51</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4376093</td>
<td>Pyruvate kinase *</td>
<td>58248.74</td>
<td>5.88</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1420736</td>
<td>PYK2 *</td>
<td>55195.22</td>
<td>6.43</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>59803007</td>
<td>Malate dehydrogenase *</td>
<td>35191.31</td>
<td>8.26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40929338</td>
<td>Malate dehydrogenase *</td>
<td>33029.29</td>
<td>6.13</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4376093</td>
<td>Pyruvate kinase *</td>
<td>58248.74</td>
<td>5.88</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1420736</td>
<td>PYK2 *</td>
<td>55195.22</td>
<td>6.43</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>34765759</td>
<td>NADH-ubiquinone oxidoreductase*</td>
<td>30373.33</td>
<td>8.83</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>29409959</td>
<td>Transketolase *</td>
<td>74759.56</td>
<td>5.98</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>21264637</td>
<td>Endo beta 1,4 glucanase *</td>
<td>36395.44</td>
<td>4.40</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>870831</td>
<td>Glucose-6-phosphate 1-dehydrogenase</td>
<td>58950.31</td>
<td>6.17</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>929983</td>
<td>Glucose-6-phosphate 1-dehydrogenase</td>
<td>30768.23</td>
<td>6.13</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>40731647</td>
<td>Glucose-6-phosphate isomerase *</td>
<td>61445.79</td>
<td>6.38</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>57232460</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase*</td>
<td>25354.62</td>
<td>8.81</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>30525994</td>
<td>Glycerol-3-phosphate dehydrogenase</td>
<td>11364.02</td>
<td>9.10</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3289019</td>
<td>Xylose reductase *</td>
<td>36021.33</td>
<td>5.77</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>929983</td>
<td>Glycerol-3-phosphate dehydrogenase</td>
<td>30768.23</td>
<td>6.13</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>40731647</td>
<td>Glucose-6-phosphate isomerase</td>
<td>61445.79</td>
<td>6.38</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>38306.81</td>
<td>Glutamine synthetase*</td>
<td>38306.81</td>
<td>5.88</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>47547.37</td>
<td>Endopeptidase*</td>
<td>47547.37</td>
<td>4.77</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>248131.78</td>
<td>Carbamoyl-phosphate synthase *</td>
<td>248131.78</td>
<td>5.48</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>44981739</td>
<td>Carbamoyl-phosphate synthase *</td>
<td>248131.78</td>
<td>5.48</td>
<td></td>
</tr>
</tbody>
</table>

**Carbohydrate transport and metabolism**

<table>
<thead>
<tr>
<th>No.</th>
<th>GI</th>
<th>Protein Name</th>
<th>MW</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>929983</td>
<td>Glycerol-3-phosphate dehydrogenase *</td>
<td>30768.23</td>
<td>6.13</td>
</tr>
<tr>
<td>2</td>
<td>29409959</td>
<td>Transketolase *</td>
<td>74759.56</td>
<td>5.98</td>
</tr>
<tr>
<td>2</td>
<td>3289019</td>
<td>Xylose reductase *</td>
<td>36021.33</td>
<td>5.77</td>
</tr>
<tr>
<td>3</td>
<td>30525994</td>
<td>Glycerol-3-phosphate dehydrogenase</td>
<td>11364.02</td>
<td>9.10</td>
</tr>
<tr>
<td>3</td>
<td>30768.23</td>
<td>Glycerol-3-phosphate dehydrogenase</td>
<td>30768.23</td>
<td>6.13</td>
</tr>
<tr>
<td>3</td>
<td>25354.62</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase*</td>
<td>25354.62</td>
<td>8.81</td>
</tr>
<tr>
<td>4</td>
<td>38306.81</td>
<td>Glutamine synthetase*</td>
<td>38306.81</td>
<td>5.88</td>
</tr>
<tr>
<td>4</td>
<td>47547.37</td>
<td>Endopeptidase*</td>
<td>47547.37</td>
<td>4.77</td>
</tr>
</tbody>
</table>

**Amino acid transport and metabolism**

<table>
<thead>
<tr>
<th>No.</th>
<th>GI</th>
<th>Protein Name</th>
<th>MW</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6746633</td>
<td>Glutamine synthetase*</td>
<td>38306.81</td>
<td>5.88</td>
</tr>
<tr>
<td>4</td>
<td>57223024</td>
<td>Endopeptidase*</td>
<td>47547.37</td>
<td>4.77</td>
</tr>
</tbody>
</table>

**Nucleotide transport and metabolism**

<table>
<thead>
<tr>
<th>No.</th>
<th>GI</th>
<th>Protein Name</th>
<th>MW</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>44981739</td>
<td>Carbamoyl-phosphate synthase *</td>
<td>248131.78</td>
<td>5.48</td>
</tr>
</tbody>
</table>

**Lipid metabolism**

<table>
<thead>
<tr>
<th>No.</th>
<th>GI</th>
<th>Protein Name</th>
<th>MW</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>44981739</td>
<td>Carbamoyl-phosphate synthase *</td>
<td>248131.78</td>
<td>5.48</td>
</tr>
<tr>
<td>No.</td>
<td>Type</td>
<td>Gene ID</td>
<td>Description</td>
<td>E Value</td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>---------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>gi</td>
<td>1907190</td>
<td>Acyl-CoA sterol acyltransferase *</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>gi</td>
<td>30580366</td>
<td>Catalase 3*</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>gi</td>
<td>57226036</td>
<td>Voltage-dependent ion-selective channel*</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>gi</td>
<td>38566870</td>
<td>Glutathione peroxidase *</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>gi</td>
<td>44982853</td>
<td>Cytochrome P450 *</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>gi</td>
<td>5053107</td>
<td>Actin 1 *</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>gi</td>
<td>31581446</td>
<td>Actin 1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>gi</td>
<td>31581482</td>
<td>Actin 1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>gi</td>
<td>15321714</td>
<td>UDP-glucose dehydrogenase *</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>gi</td>
<td>460999486</td>
<td>GTP-binding protein ypt1*</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>gi</td>
<td>46444053</td>
<td>Clathrin-associated protein AP-1 complex *</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>gi</td>
<td>30024664</td>
<td>Ras-related protein Rab7 *</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>gi</td>
<td>111752</td>
<td>Ran*</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>gi</td>
<td>461532</td>
<td>ADP-ribosylation factor*</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>gi</td>
<td>460999486</td>
<td>GTP-binding protein ypt1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>gi</td>
<td>3560251</td>
<td>Serine/Threonine protein kinases*</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>gi</td>
<td>3560</td>
<td>Calmodulin-binding protein 2*</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>gi</td>
<td>44985926</td>
<td>Serine/Threonine protein kinases*</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>gi</td>
<td>474400</td>
<td>Serine/Threonine protein kinases*</td>
</tr>
</tbody>
</table>

**Inorganic ion transport and metabolism**

**Secondary metabolites biosynthesis, transport, and catabolism**

**Cytoskeleton**

**Cell envelope biogenesis, outer membrane**

**Intracellular trafficking and secretion**

**Signal transduction**
Asterisks indicate unique proteins identified from *H. erinaceum* fruiting body using LC-MS/MS and Edman sequencing. Commonly identified proteins in *S. crispa* and *H. erinaceum* are shown in red letters.
<table>
<thead>
<tr>
<th>Spot Number</th>
<th>Amino Acid Sequence</th>
<th>Score</th>
<th>Accession Number</th>
<th>Protein Name</th>
<th>Identity/Similarity</th>
<th>Analytical MW</th>
<th>Theoretical MW</th>
<th>pI</th>
<th>Organism</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>qlttkvrvlrdkrin</td>
<td>51</td>
<td>Q6FJ50</td>
<td>Guanine nucleotide-binding protein subunit gamma*</td>
<td>53 / 73</td>
<td>10251.63</td>
<td>6.05</td>
<td>Fungi</td>
<td>Signal transduction</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>gwfhddsdeaqaysq</td>
<td>118</td>
<td>Q4IID0</td>
<td>Hypothetical protein</td>
<td>67 / 83</td>
<td>11878.88</td>
<td>4.88</td>
<td>Fungi</td>
<td>Unclassified</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>anpggdyamn</td>
<td>44</td>
<td>P58724</td>
<td>No significant hit found</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>qftvvlkaan</td>
<td>59</td>
<td>Q9LA06</td>
<td>Serine protease do-like htrA*</td>
<td>62 / 92</td>
<td>41647.97</td>
<td>5.17</td>
<td>Bacteria</td>
<td>Amino acid transport and metabolism</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>hgeaeefigl</td>
<td>49</td>
<td>Q31G56</td>
<td>Ribonuclease HII*</td>
<td>70 / 90</td>
<td>22808.57</td>
<td>6.30</td>
<td>Bacteria</td>
<td>Nucleotide transport and metabolism</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>rakvyyvnls</td>
<td>40</td>
<td>P61174</td>
<td>50S ribosomal protein L36*</td>
<td>75 / 100</td>
<td>4694.62</td>
<td>12.14</td>
<td>Bacteria</td>
<td>Translation, ribosomal structure biogenesis</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>txgdpsaavgflray</td>
<td>57</td>
<td>Q9YAD8</td>
<td>No significant hit found</td>
<td></td>
<td></td>
<td></td>
<td>Bacteria</td>
<td>Unclassified</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>txgdpsaavgflraylfe</td>
<td>57</td>
<td>Q9YAD8</td>
<td>Hypothetical protein APE2001</td>
<td>57 / 86</td>
<td>15980.57</td>
<td>5.40</td>
<td>Bacteria</td>
<td>Unclassified</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>txgdpsaavgflraylfe</td>
<td>57</td>
<td>Q9YAD8</td>
<td>Hypothetical protein APE2001</td>
<td>57 / 86</td>
<td>15980.57</td>
<td>5.40</td>
<td>Bacteria</td>
<td>Unclassified</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>flekaqikvgekvsletvk</td>
<td>57</td>
<td>Q88QM6</td>
<td>30S ribosomal protein S17*</td>
<td>56 / 75</td>
<td>10057.76</td>
<td>9.81</td>
<td>Bacteria</td>
<td>Translation, ribosomal structure biogenesis</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>vektahaarsflekaqi</td>
<td>67</td>
<td>Q6H806</td>
<td>N-terminal amino acid blocked Putative ubiquitin fusion degradation protein*</td>
<td>50 / 94</td>
<td>35143.74</td>
<td>5.77</td>
<td>Plant</td>
<td>Posttranslational modification, turnover, chaperones</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>vektahaarsflekaqi</td>
<td>68</td>
<td>Q8U778</td>
<td>Transcriptional regulator, ROK family*</td>
<td>58 / 89</td>
<td>42235.10</td>
<td>5.97</td>
<td>Bacteria</td>
<td>Transcription</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>qplifiaddgyafy</td>
<td>62</td>
<td>Q9AJ64</td>
<td>Antigenic heat-stable 120 kDa protein</td>
<td>64 / 79</td>
<td>108518.46</td>
<td>5.08</td>
<td>Bacteria</td>
<td>Unclassified</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>mlysiitdemfsdafpv</td>
<td>101</td>
<td>Q5K7S2</td>
<td>N-terminal amino acid blocked N-terminal amino acid blocked N-terminal amino acid blocked Translationally-controlled tumor protein homolog*</td>
<td>65 / 95</td>
<td>18763.25</td>
<td>4.55</td>
<td>Fungi</td>
<td>Signal transduction</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Proteins (*Hericium erinaceum*) identified by 2-DGE and N-terminal amino acid sequencing

The table lists indicated spot numbers, amino acid sequences, scores, accession numbers, protein names, identity/similarity, theoretical mass (kDa), theoretical pI, organism and function left to right.
<table>
<thead>
<tr>
<th>No.</th>
<th>Protein Name</th>
<th>Accession</th>
<th>Score</th>
<th>E-Value</th>
<th>Description</th>
<th>Organism</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>mlysiitdemfsda</td>
<td>Q5K7S2</td>
<td>79</td>
<td>65 / 94</td>
<td>Translationally-controlled tumor protein homolog</td>
<td>Fungi</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>21</td>
<td>vhtlqpyaydalepysfr</td>
<td>Q9P4T6</td>
<td>123</td>
<td>90 / 100</td>
<td>Superoxide dismutase [Mn]</td>
<td>Fungi</td>
<td>Inorganic ion transport and metabolism</td>
</tr>
<tr>
<td>22</td>
<td>vhtlqpyayd</td>
<td>Q9P4T6</td>
<td>77</td>
<td>80 / 90</td>
<td>Superoxide dismutase [Mn]</td>
<td>Fungi</td>
<td>Inorganic ion transport and metabolism</td>
</tr>
<tr>
<td>23</td>
<td>viklghspstgttvvvl</td>
<td>Q1EBD9</td>
<td>84</td>
<td>75 / 85</td>
<td>Glutathione S-transferase*</td>
<td>Fungi</td>
<td>Secondary metabolites biosynthesis, transport, and catabolism</td>
</tr>
<tr>
<td>24</td>
<td>snavkeglftta</td>
<td></td>
<td></td>
<td></td>
<td>No significant hit found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>tlggrgafir</td>
<td>Q1CX38</td>
<td>56</td>
<td>78 / 89</td>
<td>ATP synthase epsilon chain*</td>
<td>Bacteria</td>
<td>Energy production and conversion</td>
</tr>
<tr>
<td>26</td>
<td>rplaglyrga</td>
<td></td>
<td></td>
<td></td>
<td>No significant hit found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>tgpivtgsilalk</td>
<td>A1CE34</td>
<td>71</td>
<td>71 / 94</td>
<td>Triosephosphate isomerase</td>
<td>Fungi</td>
<td>Carbohydrate transport and metabolism</td>
</tr>
<tr>
<td>28</td>
<td>rplaglyrga</td>
<td></td>
<td></td>
<td></td>
<td>No significant hit found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>mgngqglpganyitkvggges</td>
<td></td>
<td></td>
<td></td>
<td>No significant hit found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>pesredsvylakeqery</td>
<td>Q4G218</td>
<td>125</td>
<td>67 / 100</td>
<td>Proteasome component Pre4*</td>
<td>Fungi</td>
<td>Posttranslational modification, protein turnover, chaperones</td>
</tr>
<tr>
<td>31</td>
<td>mlpvrlgqmrliirddktavgdy</td>
<td>Q4PDU5</td>
<td>73</td>
<td>73 / 86</td>
<td>14-3-3 protein</td>
<td>Fungi</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>32</td>
<td>nkynagkygl</td>
<td>A1FRZ0</td>
<td>58</td>
<td>80 / 90</td>
<td>Lytic transglycosylase*</td>
<td>Bacteria</td>
<td>Cell envelope biogenesis, outer membrane assembly</td>
</tr>
<tr>
<td>33</td>
<td>tkvavlpagggigqpspyrilatdq</td>
<td>P17505</td>
<td>113</td>
<td>95 / 100</td>
<td>Malate dehydrogenase*</td>
<td>Fungi</td>
<td>Energy production and conversion</td>
</tr>
<tr>
<td>34</td>
<td>pkavlgagaqgqp</td>
<td>P17505</td>
<td>75</td>
<td>79 / 100</td>
<td>Malate dehydrogenase</td>
<td>Fungi</td>
<td>Energy production and conversion</td>
</tr>
<tr>
<td>35</td>
<td>skavvlgaaggigqps</td>
<td>P17505</td>
<td>75</td>
<td>79 / 100</td>
<td>Malate dehydrogenase</td>
<td>Fungi</td>
<td>Energy production and conversion</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Accession</td>
<td>PDB ID</td>
<td>Score</td>
<td>p-value</td>
<td>Description</td>
<td>Species</td>
<td>Function</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------</td>
<td>--------</td>
<td>-------</td>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------</td>
<td>----------------------------------------------</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>dyqta</td>
<td>49</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Transport protein comB*</td>
<td>Bacteria</td>
<td>Cell envelope biogenesis, outer</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>paltv</td>
<td>51</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Cathepsin B-like CP3 precursor</td>
<td>Insect</td>
<td>Amino acid transport and metabolism</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>dillf</td>
<td>53</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Uncharacterized protein</td>
<td>Human</td>
<td>Unclassified</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>vvkv</td>
<td>57</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>Fungi</td>
<td>Carbohydrate transport and metabolism</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>ivgrp</td>
<td>59</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Actin-1</td>
<td>Human</td>
<td>Cytoskeleton</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>mltele</td>
<td>61</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Protein S100-A8*</td>
<td>Human</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>lpihe</td>
<td>63</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>S100-A8</td>
<td>Human</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>vykvad</td>
<td>65</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Succinate-CoA ligase*</td>
<td>Fungi</td>
<td>Energy production and conversion</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>vaywep</td>
<td>67</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Adenosylhomocysteinase*</td>
<td>Fungi</td>
<td>Amino acid transport and metabolism</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>vaydep</td>
<td>69</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Hypothetical 11.7 kDa protein in IDS2-MPI2 intergenic region</td>
<td>Bacteria</td>
<td>Carbohydrate transport and metabolism</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>ginkg</td>
<td>71</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Probable inorganic polyphosphate /ATP-NAD kinase*</td>
<td>Fungi</td>
<td>Unclassified</td>
</tr>
<tr>
<td>N-terminal amino acid blocked</td>
<td>Q5KN95</td>
<td>73</td>
<td>N-terminal amino acid blocked</td>
<td>N-terminal amino acid blocked</td>
<td>Probable inorganic polyphosphate /ATP-NAD kinase*</td>
<td>Bacteria</td>
<td>Coenzyme metabolism</td>
</tr>
<tr>
<td>Position</td>
<td>Amino Acid Sequence</td>
<td>Accession</td>
<td>Score</td>
<td>E-value</td>
<td>Classification</td>
<td>Function</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>---------------------</td>
<td>-----------</td>
<td>-------</td>
<td>---------</td>
<td>----------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>76-79</td>
<td>N-terminal amino acid blocked</td>
<td>Q9Y8B5</td>
<td>51162.66</td>
<td>5.69</td>
<td>Fungi</td>
<td>Amino acid transport and metabolism</td>
<td></td>
</tr>
<tr>
<td>80-87</td>
<td>atavtqgpftevttl</td>
<td>61</td>
<td>57135.98</td>
<td>5.61</td>
<td>Bacteria</td>
<td>Energy production and conversion</td>
<td></td>
</tr>
<tr>
<td>88-95</td>
<td>ateakgaigakvgtiga</td>
<td>Q6FYM3</td>
<td>37143.22</td>
<td>6.14</td>
<td>Bacteria</td>
<td>Energy production and conversion</td>
<td></td>
</tr>
<tr>
<td>96-99</td>
<td>papadflkgysneaf</td>
<td>66</td>
<td>19342.51</td>
<td>8.34</td>
<td>Fungi</td>
<td>Signal transduction</td>
<td></td>
</tr>
<tr>
<td>100-103</td>
<td>qglpnprlaed</td>
<td>60</td>
<td>19342.51</td>
<td>8.34</td>
<td>Fungi</td>
<td>Signal transduction</td>
<td></td>
</tr>
<tr>
<td>104-105</td>
<td>qglngq</td>
<td>50</td>
<td>11438.28</td>
<td>4.59</td>
<td>Bacteria</td>
<td>Unclassified</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>------</td>
<td>-----</td>
<td>-------------------------</td>
<td>-------</td>
<td>--------</td>
<td>------------------</td>
<td>-----</td>
</tr>
<tr>
<td>106</td>
<td>dasnkseygtvigig</td>
<td>60</td>
<td>Q24895</td>
<td>78 kDa glucose-regulated protein precursor*</td>
<td>83 / 92</td>
<td>71675.38</td>
<td>5.09</td>
</tr>
<tr>
<td>107</td>
<td>pgqlaqaqphgdsg</td>
<td></td>
<td></td>
<td>No significant hit found</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>jqqlaqaqphg</td>
<td></td>
<td></td>
<td>No significant hit found</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>109</td>
<td></td>
<td></td>
<td></td>
<td>N-terminal amino acid blocked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td></td>
<td></td>
<td></td>
<td>N-terminal amino acid blocked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>spglnldgdarg</td>
<td>61</td>
<td>P04922</td>
<td>Circumsporozoite protein precursor</td>
<td>67 / 83</td>
<td>34782.33</td>
<td>5.38</td>
</tr>
<tr>
<td>112</td>
<td>fpaqgeqvkgvis</td>
<td></td>
<td></td>
<td>No significant hit found</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>113</td>
<td>aapvstndrfypsaa</td>
<td>57</td>
<td>P78972</td>
<td>WD repeat containing protein</td>
<td>64 / 91</td>
<td>53417.86</td>
<td>9.30</td>
</tr>
<tr>
<td>114</td>
<td>aapvslndqgpsaa</td>
<td>60</td>
<td>Q5A387</td>
<td>Hypothetical Protein MSS1</td>
<td>73 / 91</td>
<td>42895.97</td>
<td>5.98</td>
</tr>
<tr>
<td>115</td>
<td>gghsvpfltfnf</td>
<td>65</td>
<td>P07267</td>
<td>Saccharopepsin precursor*</td>
<td>73 / 100</td>
<td>44499.00</td>
<td>4.70</td>
</tr>
</tbody>
</table>

Asterisks indicate unique proteins identified from *S. crispa* fruiting body using LC-MS/MS and Edman sequencing. Commonly identified proteins in *H. erinaceum* are shown in red letters.
We conducted proteomics on two mushroom, *Sparassis crispa* and *Hericium erinaceum* fruiting body using phenol extraction, subsequent one- and two-dimensional (2-D) gel electrophoresis coupled to tandem mass spectrometry analysis and Edman sequencing for protein identification. Though this study, we could establish and optimize protein extraction protocols, obtain 2-D gel reference maps of two mushroom, identify numerous proteins and clarify the protein components. In particular, 14-3-3 proteins and septin were identified from both mushrooms. This is a first systematic study towards establishing mushroom proteomics.