Title

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

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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 polyvinyldifluoride (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 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.[1] 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.[2] 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.[3] 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 recourses.[4-8] 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*[9], *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 intraperitonial or oral administration.[10] *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.[11-13] Two proteins, a sialic acid-binding lectin[14] and a laccase[15] 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.[16-20] 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.[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, *S. crispa* and *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 *N*-terminal amino acid sequencing, respectively, were applied on the fruiting bodies of *S. crispa* and *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.

2. Materials and Methods

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

2.1.1. Culture of *S. crispa*. It has been known that *S. crispa* and *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_2O). The mixed substrates were packed in mushroom culture bottles made of polypropylene resin, sterilized, and cooled to room temperature (RT). Pre-cultured *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 ºC, 75% relative humidity (RH) for 4 weeks in the dark. After adequate hyphal spread, bottles were moved to growth phase at 12 ºC, 90% RH, and irradiation of 200 lux incandescent light for 3 weeks. The subsequently formed fruiting bodies were harvested and stored at –20 ºC.

2.1.2. Culture of *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_2O. The mixed substrates were packed in mushroom culture bottles made of polypropylene resin, sterilized, and cooled to RT. Pre-cultured *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 ºC, 75% RH for 6 weeks in the dark. After adequate hyphal spread, bottles were moved to growth phase at 15 ºC, 90% RH, irradiation of 200 lux incandescent light for 6 weeks. Subsequently formed fruiting bodies were harvested and stored at –20 ºC.

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[24] 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 H_2O], 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 ºC. 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 ºC. 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 ºC overnight. The suspension was centrifuged at 15,000 rpm for 20 min 4 ºC 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 ºC), the supernatant was decanted and pellet was dried at 37 ºC 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 ºC. The supernatant was used for protein quantification by a Coomassie Plus™ (PIERCE, Rockford, IL) protein assay kit, and stored in aliquots at –80 ºC.

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_2O]. 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 °C. 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 °C for 18 h. The tryptic peptides samples were separated by C-18 reverse-phase column and analyzed on a nano electrospray 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_2O/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 300SBC18, 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 polyvinyldifluoride (PVDF) membrane (NT-31, 0.45 μM pore size; Nihon Eido, Tokyo, Japan) was carried out at 1mA/cm^2 for 80 min

at RT using a semi-dry blotter (Nihon Eido). The transferred proteins were stained with CBB R-250 as described.[27] 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.[28] 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[3,7,8], 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 wad 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 p*I* 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 1 α 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 p*I* 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 p*I* 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 p*I* 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.[16-23]

3.4.1. Functional Categorization of the Identified Proteins from *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 *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 envelop 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 *S. crispa* 2-D gel. Selenium is an essentially required element for synthesis of selenoproteins including glutathione peroxidase (GPX) that is an important antioxydation enzyme; L-seryl tRNA selenium transferase is involved in the biosynthesis process.[29] Amazingly, it was shown that brown colored *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.[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.[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.[32] 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).[33] 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.[34] 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.[35] Because *N*-terminal amino acid sequences and molecular mass of these three proteins are similar, but their p*I*s 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.[36] 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.[37]

3.4.2. Functional Categorization of the Identified Proteins from *H. erinaceum*. Proteins related to translation, ribosomal structure and biogenesis and energy production and conversion account for large proportion of the identified proteins in *H. erinaceum*. Compared to *S. crispa* protein categories, *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 *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.[38,39] Cyclophilin has been recently shown to be up-regulated in yeast cells under stress condition.[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.[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-β-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.[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, *Neurospora crassa*, and shown to be involved in circadian clock or other light response.[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.[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.[46]

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 p*I* 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.[47] 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".[48] 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.[49]

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).[50] 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 p*I*s (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.[51] 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.[52] 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.[53,54] 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.[55,56] 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.[4] 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, polygaracturonase, 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.

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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 NCBInr 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.

Figure 6. *S. crispa* and *H. erinaceum* proteins belong to 19 functional categories. Out of 115 identified proteins from *S. crispa*, 84 proteins were nonredundant. Among the 172 proteins identified from *H. erinaceum*, 119 proteins were nonredundant. The pie chart shows the distribution of these nonredundant proteins into their biological function classes in percentage. Abbreviations are as follow. EPC : Energy production and conversion. T/RSB : Translation, ribosomal structure and biogenesis. CTM : Carbohydrate transport and metabolism. ATM : Amino acid transport and metabolism. LM : Lipid metabolism. DRRR : DNA replication, recombination, and repair. T : Transcription. PTM/PT/C : Posttranslational modification, protein turnover, chaperones. NTM : Nucleotide transport and metabolism. CEB/OM : Cell envelope biogenesis, outer membrane. ST : Signal transduction. CSD : Chromatin structure and dynamics. CD/CP : Cell division and chromosome partitioning. IITM : Inorganic ion transport and metabolism. IT/S : Intracellular trafficking and secretion. SMBTC : Secondary metabolites biosynthesis, transport and catabolism. RPM : RNA processing and modification. C : Cytoskeleton. S : Sporulation. CM : Coenzyme metabolism.

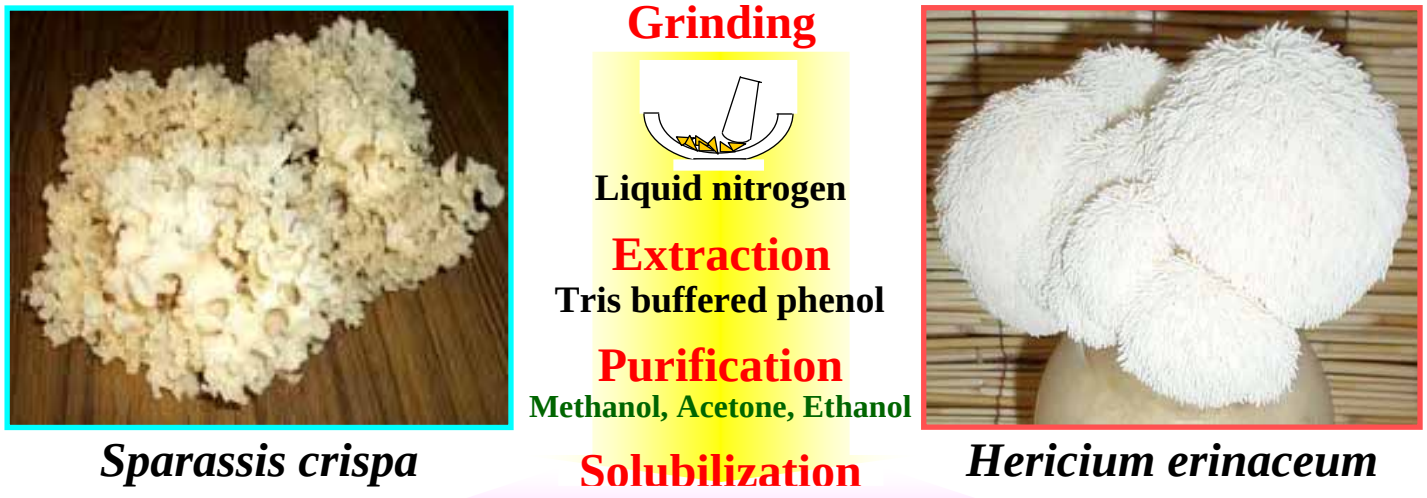

Figure 1
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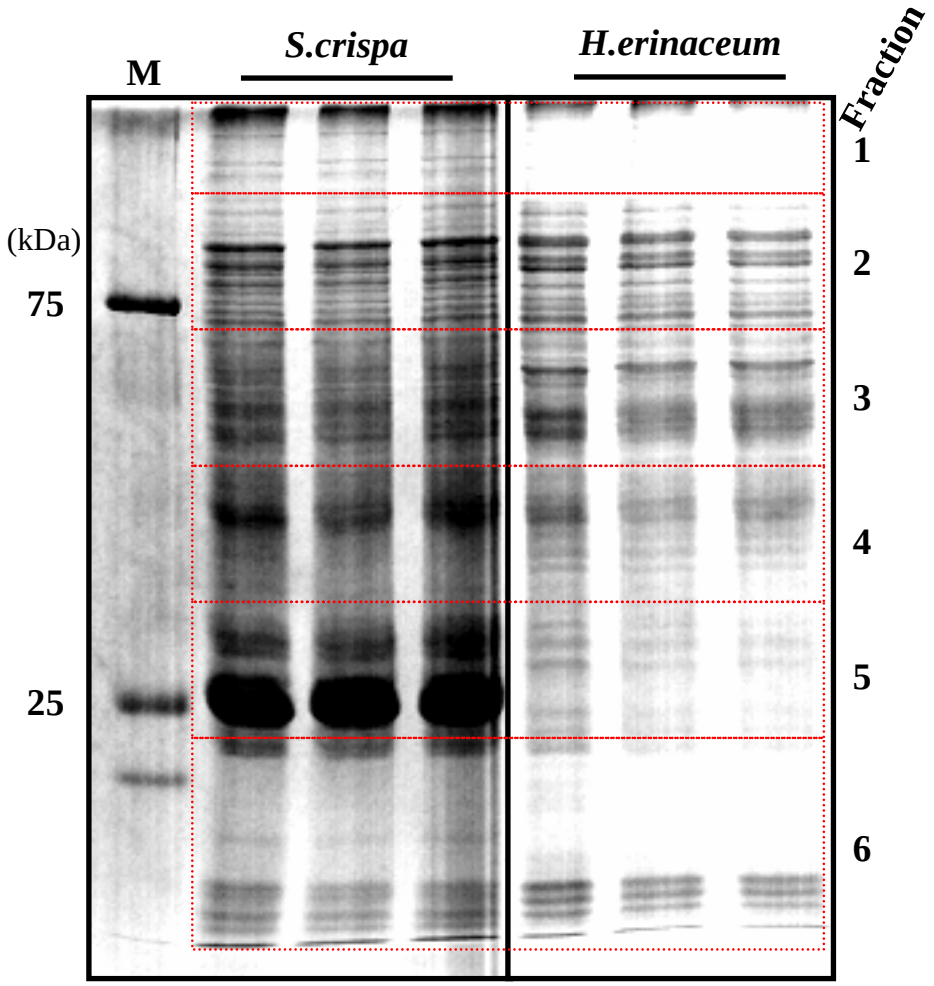

Figure 2
Horie et al.

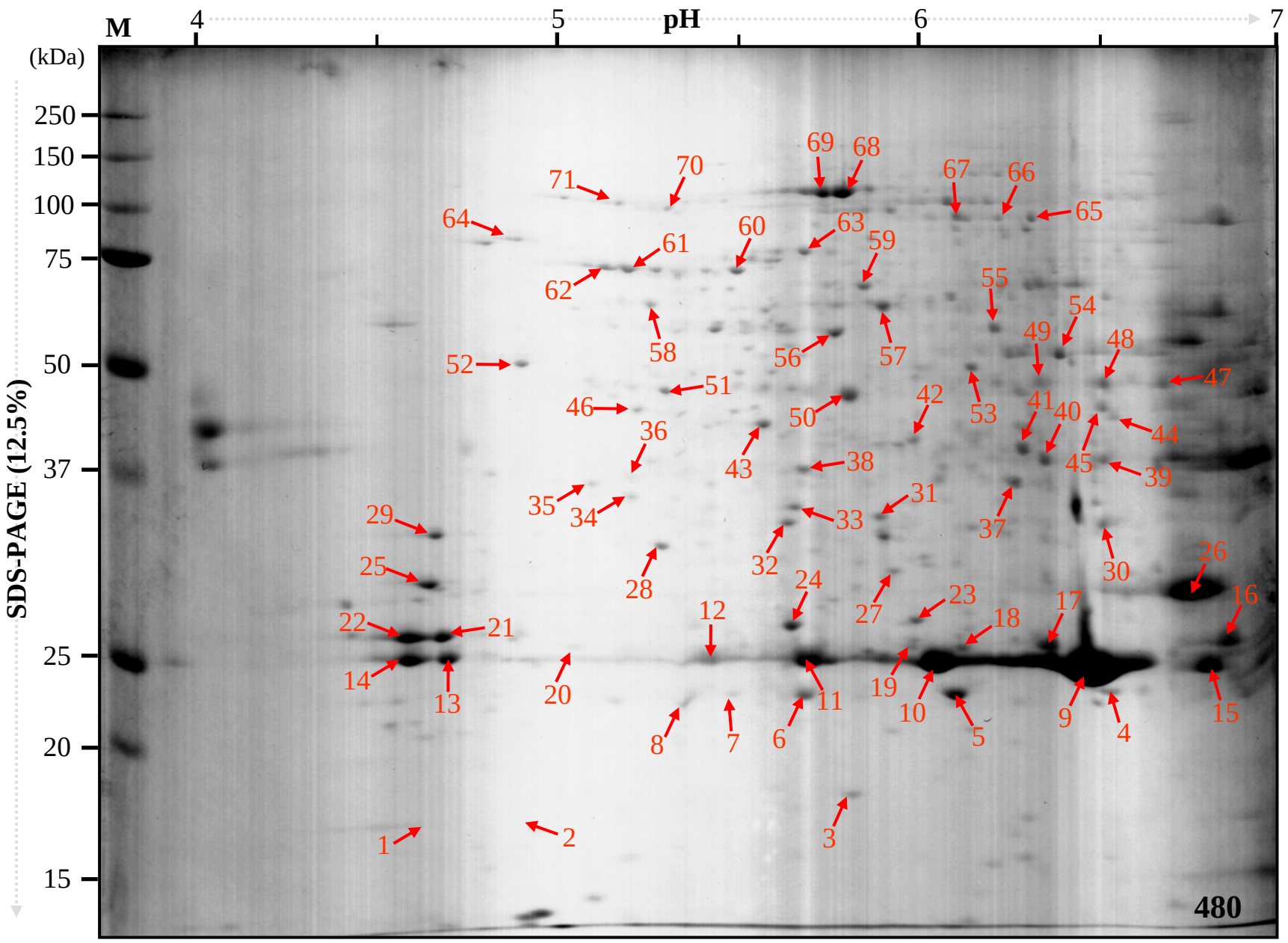

Figure 3
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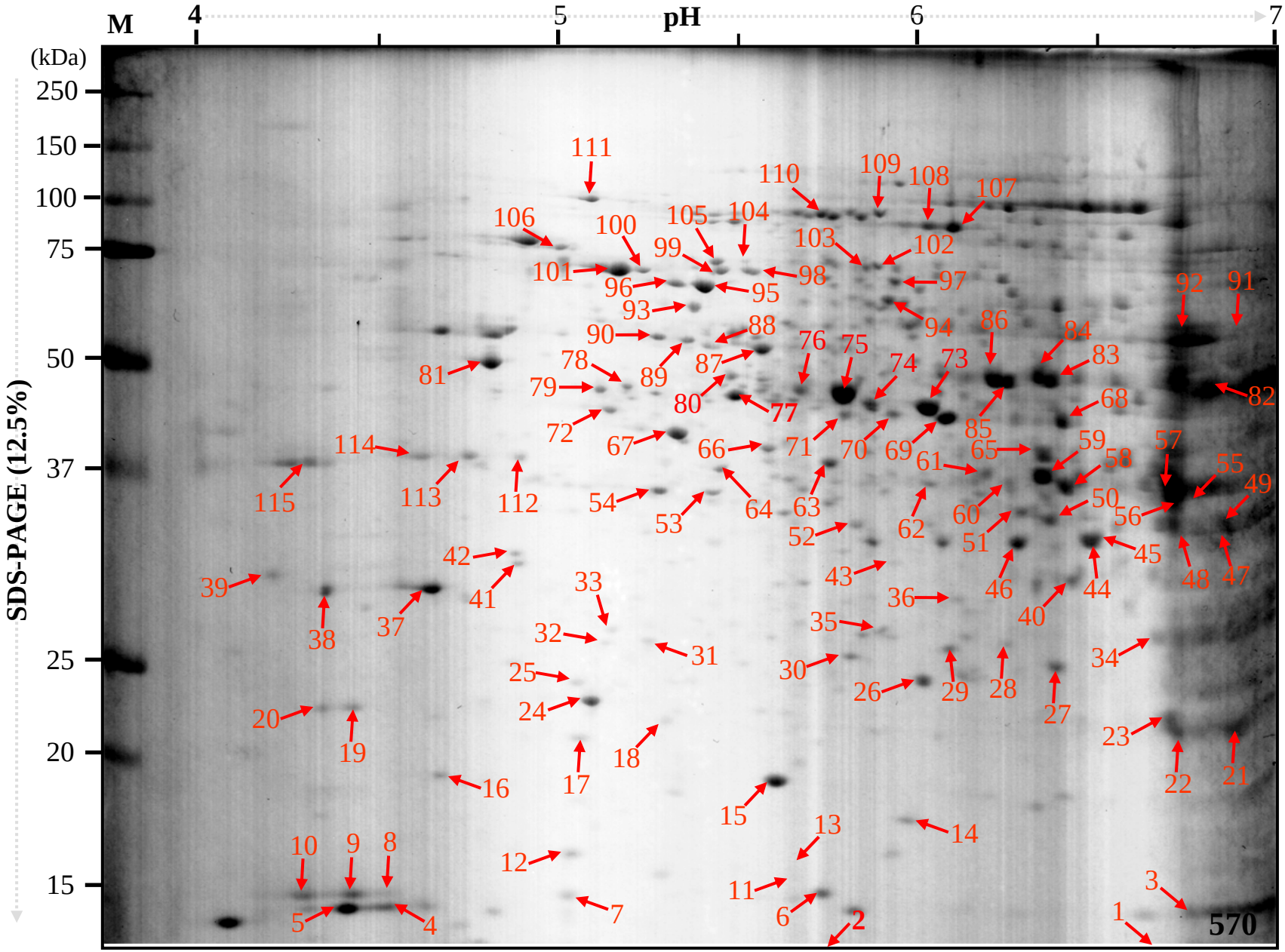

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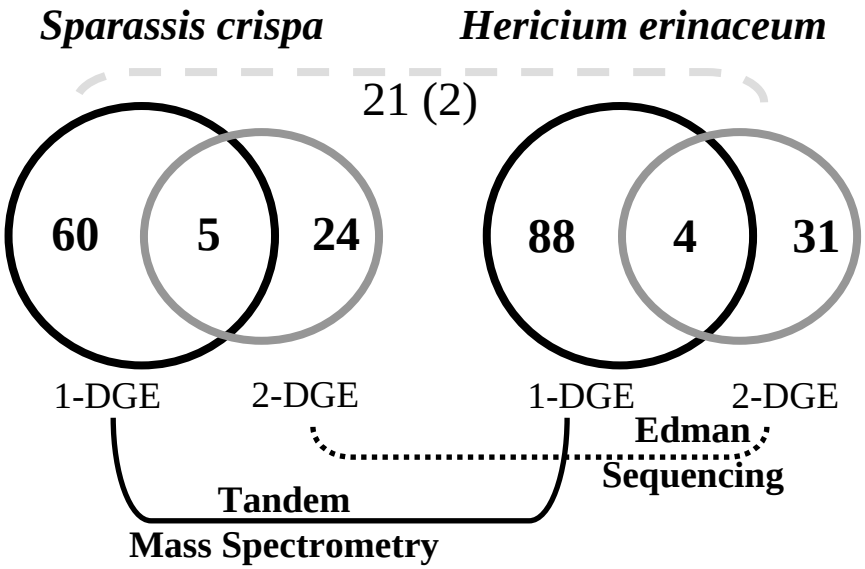

Figure 5
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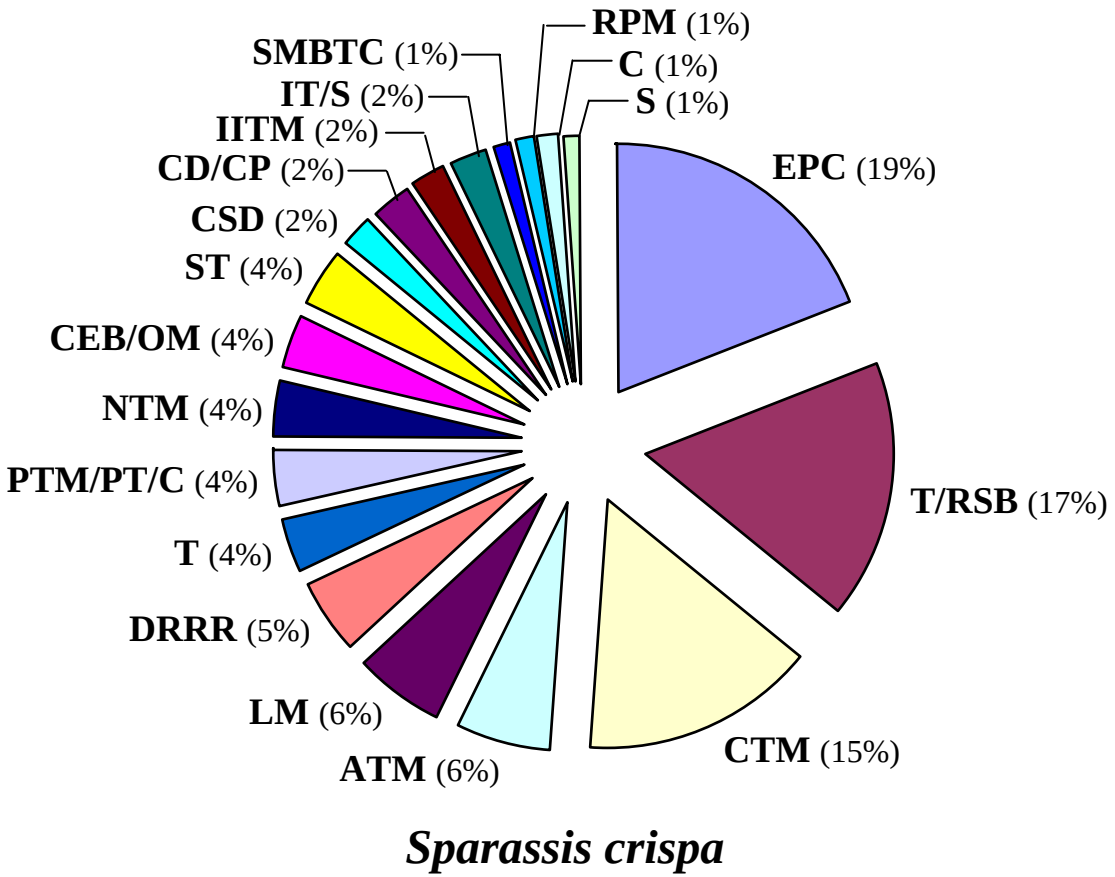

Figure 6
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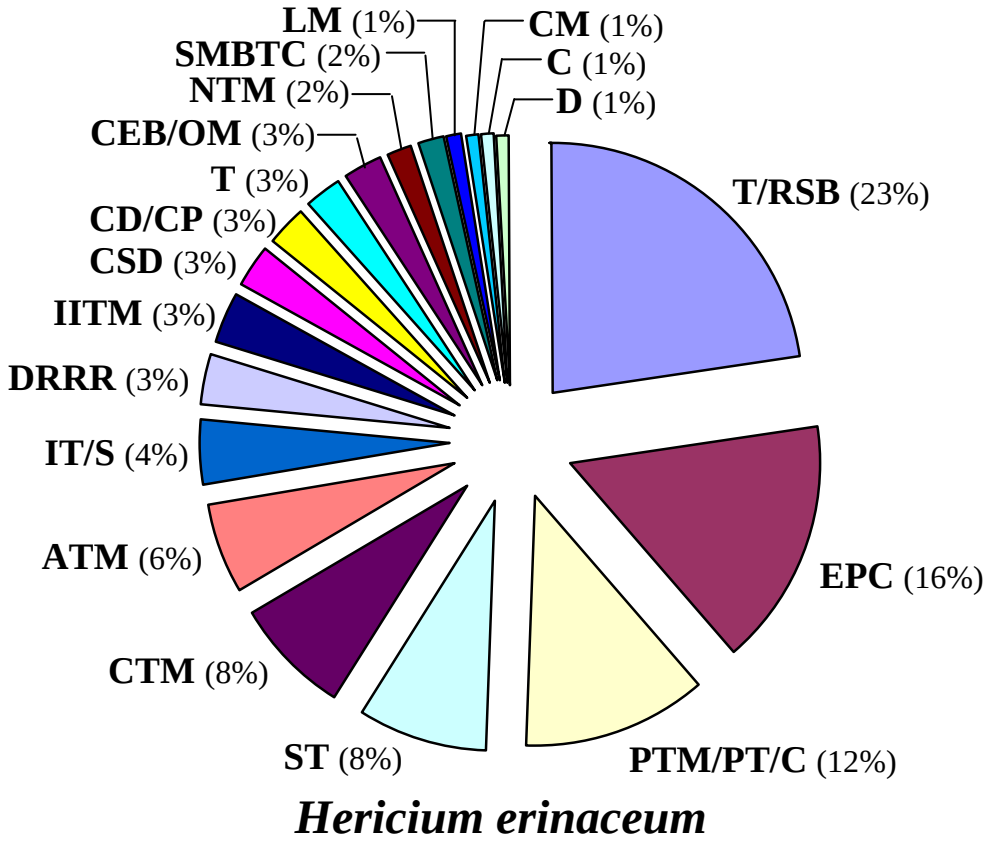

Figure 6 contd…
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Table 1.

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, accesion numbers, protein names, theoretical mass (kDa), theoretical pH left to right.

Fraction Number	Matching Peptide	score	accession number	protein name	analytical MW	theoretical p*I*	peptide
Chromatin structure and dynamics							
6	1	92	gi|1742929	Histone H2B *	15165.40	10.16	AMAILNSFVNDIFER
6	1	67	gi|3142	Histone H4*	11366.34	11.36	TVTALDVVYALK
DNA replication, recombination, and repair							
2	1	57	gi|14245691	Reverse transcriptase *	40781.01	9.06	QILADLK
3	1	69	gi|908896	DNA repair protein rad32*	73688.98	5.48	QINLLVK
5	1	47	gi|47132515	recQ family Helicase*	169673.76	8.04	LEAGTAHDSSDGVGGGSAPAAPGK
Cell division and chromosome partitioning							
4	1	102	gi|10241488	Cell division control protein 10 *	38657.99	7.20	STLINTIFASHLIDSK
4	1	51	gi|6977953	Septin*	53737.59	5.41	VNIIPIIAK
Transcription							
4	1	49	gi|57228114	Transcriptional activator*	133338.53	8.14	EEGEGLGEEGK
4	1	47	gi|19068759	Heat shock transcription factor *	33685.36	6.62	EDLLGFDDSLR
Translation, ribosomal structure and biogenesis							
1	3	208	gi|58758727	Translation elongation factor EF1-α *	44184.90	8.55	EHALLAFTLGVR, TLLDAIDAIEPPVRPSDKPLRLPLQDVYK, YAWVLDKLK, YAWVLDK, AGMIVTFAPTNVTTEVK
1	3	193	gi|11078214	Translation elongation factor EF1-α	46634.83	8.79	IGGIGTVPVGR, AGMIVTFAPTNVTTEVK
3	5	263	gi|2897607	Translation elongation factor EF1-α	50133.84	9.21	EHALLAFTLGVR, TLLDAIDAIEPPVRPSDKPLRLPLQDVYK, QTVAVGIIK, YAWVLDKLK, YAWVLDK
3	5	259	gi|58758727	Translation elongation factor EF1-α	44184.90	8.55	EHALLAFTLGVR, TLLDAIDAIEPPVRPSDKPLRLPLQDVYK, YAWVLDKLK, YAWVLDK, AGMIVTFAPTNVTTEVK
3	4	237	gi|2313	Translation elongation factor EF1-α	49828.51	9.12	QLIVAINK, EHALLAFTLGVR, YAWVLDKLK, YAWVLDK

3	2	149	gi|32567511	Translation elongation factor EF1-α	14692.27	6.95	TLLDAIDANEPPVRPSDKPLR, EHALLAFTLGVR
3	1	71	gi|8927046	Translation elongation factor EF2*	89733.93	6.75	GTVAFGSGLHGWAFTVR
5	1	59	gi|46431916	Ribosomal protein L3*	43948.70	10.26	HGSLGFLPR
5	1	50	gi|3766376	Ribosomal protein L10 *	33565.30	4.71	TSFFQALGIPTK
6	2	109	gi|16943769	Ribosomal protein S19*	14522.61	9.48	LEVPTWVDLVK, ELAPYDPDWYYVR
6	1	81	gi|3806	Translation initiation factor 5A-2*	17114.28	4.81	VHLVAIDIFTGK
6	1	56	gi|3560198	Ribosomal protein L18E *	21189.79	11.66	AGGEVLTLDQLALR
6	1	56	gi|11595555	Ribosomal protein S28*	15909.58	10.60	VSGVGLLALWK
6	1	53	gi|1164943	Ribosomal protein S7e*	21987.65	9.73	ILEDLVFPTEIVGK
6	1	42	gi|1850540	Ribosomal P2 phosphoprotein*	11145.20	4.17	DINELIASGPEK
Posttranslational modification, protein turnover, chaperones							
2	4	271	gi|7521943	Heat shock protein 70 *	71055.79	5.02	IINEPTAAAIAYGLDKK TQDLLLLDVAPLSLGIETAGGVMTALIK, SINPDEAVAYGAAVQAAILSGDTSEK, IINEPTAAAIAYGLDK
2	1	66	gi|19069227	26S Proteasome regulatory subunit 4*	47377.75	6.39	TMLELLNQLDGFDTR
2	1	65	gi|56199674	60 kDa Chaperonin*	19860.09	5.99	GFISPYFITDVK
3	2	160	gi|7521943	Heat shock protein 70	71055.79	5.02	IINEPTAAAIAYGLDKK TQDLLLLDVAPLSLGIETAGGVMTALIK, SINPDEAVAYGAAVQAAILSGDTSEK, IINEPTAAAIAYGLDK
4	1	56	gi|7521943	Heat shock protein 70	71055.79	5.02	IINEPTAAAIAYGLDKK TQDLLLLDVAPLSLGIETAGGVMTALIK, SINPDEAVAYGAAVQAAILSGDTSEK, IINEPTAAAIAYGLDK
5	1	60	gi|172714	Heat shock protein 70	16141.27	5.42	FELSGIPPAPR
Energy production and conversion							
2	1	50	gi|4376093	Pyruvate kinase *	58248.74	5.88	GDLGIEIPASQVFLAQK
3	1	45	gi|4376093	Pyruvate kinase	58248.74	5.88	GDLGIEIPASQVFLAQK
3	2	140	gi|584806	ATP synthase alpha chain*	59522.39	9.07	STVAQLVKTLEENDAMK, TGEIVDVPVGPELLGR
3	1	79	gi|44985523	F0F1-type ATP synthase*	58941.62	9.29	TAVALDTILNQK
3	1	76	gi|5190	Phosphoglycerate kinase *	44406.05	6.15	DGAITNNNR
3	1	53	gi|2654	Vacuolar ATPase subunit b *	57199.76	4.96	TIFESLDLAWSLLR
3	1	52	gi|469103	Fumarase *	53156.73	6.84	SLQNFDIGGPTER

4	2	130	gi|46444820	Malate dehydrogenase *	34728.38	5.72	DDLFNTNASIVR, VAVLGAGGGIGQPLSLLL
4	1	77	gi|4029338	Malate dehydrogenase *	33029.29	6.13	VAVLGAAGGIGQPLSLLLK
4	1	81	gi|46433360	NAD-formate dehydrogenase *	41801.55	5.51	LLGTVENELGIR
4	1	74	gi|13785197	Inorganic pyrophosphatase *	32390.49	5.24	VLGIMALLDEGETDWK
4	1	45	gi|218041	Phosphoglycerate kinase *	44656.34	8.14	ALESPQRPFLAILGGAK
Carbohydrate transport and metabolism							
1	1	44	gi|929983	Glycerol-3-aldehyde dehydrogenase *	30768.23	6.13	VPTNDVSVVDLVVR
1	1	42	gi|5441844	Polygalacturonase 1*	38063.31	6.53	FFYAHSLDDSTITGLNVK
2	1	56	gi|15808971	Glycogen synthase *	80847.52	6.03	GVDMFIESLAR
2	1	88	gi|6651233	Trehalose phosphorylase *	83653.35	6.38	IINEPTAAAIAYGLDK
2	1	51	gi|171569	1,4-glucan-6-(1,4-glucano)-transferase*	81129.57	5.76	LPAWITR
3	1	95	gi|169852	Glyceraldehyde-3-phosphate dehydrogenase*	36055.25	7.01	VPTLDVSVVDLVVR
3	1	74	gi|3367647	Enolase *	46877.80	5.15	LGANAILGVSLAVAKAGAAEK
3	1	47	gi|40713647	Glucose-6-phosphate isomerase*	61445.79	6.38	ILLSNFFAQPEALAFGK
4	2	175	gi|30038515	Glyceraldehyde-3-phosphate dehydrogenase	36607.76	7.67	VPTIDVSVVDLVVR, AVGNNIIPSSTGAAK
4	2	152	gi|929979	Glycerol-3-phosphate dehydrogenase *	30895.28	5.71	VPTLDVSVVDLVVR, KVIISAPSSDAPMFVCGVNLDAYDPK
4	1	59	gi|3289019	Xylose reductase *	36021.33	5.77	WAAQRGIAVIPK
4	1	44	gi|19068698	Alpha alpha trehalase *	76692.14	5.92	LFEVVEGKRSK
5	1	65	gi|68429	Triose-phosphate isomerase *	27234.99	6.13	IVIAYEPVWAIGTGK
5	1	42	gi|19068698	Alpha alpha trehalase	76692.14	5.92	LFEVVEGKRSK
6	1	74	gi|169852	Glyceraldehyde-3-phosphate dehydrogenase	36055.25	7.01	VPTLDVSVVDLVVR
Amino acid transport and metabolism							
3	1	97	gi|16415894	Aspartyl-proteinase*	18517.90	5.77	VILDTGSSNLWVPSTK
3	1	51	gi|44980853	Aminopeptidase I *	53590.96	6.08	FFAGFLTDWR
Nucleotide transport and metabolism							
1	1	49	gi|44981739	Carbamoylphosphate synthase *	248131.78	5.48	AASTVDEALEAVK
1	1	45	gi|22095310	C1-tetrahydrofolate synthase *	67745.49	6.28	AGCVNLKKHIQNAK
Lipid metabolism							

2	1	43	gi|4959943	Esterase *	170506.56	4.67	DLHLADWDGDGACDIIWTDPDNLNR
3	1	43	gi|12718322	C-8, 7 sterol isomerase*	30464.65	6.51	AGDVGGGLLEKVVR
4	1	47	gi|44986286	Diacylglycerol kinase *	63153.68	5.37	SSIISVDGENFPFEPIQVEVLPR
5	1	42	gi|5725410	Phosphocholine cytidylyltransferase *	41556.61	5.39	FFYAHSLDDSTITGLNVK
Inorganic ion transport and metabolism							
3	1	44	gi|2776	Catalase *	57848.89	6.42	LFSYPDTHR
Cytoskeleton							
3	2	137	gi|508701	Actin *	41736.68	5.17	SYELPDGQVITIGNER, TTGIVLDSGDGVTHTVPIYEGFSLPHAILI
Cell envelope biogenesis, outer membrane							
6	1	60	gi|46442658	GPI-protein transamidase *	55466.46	5.40	EQTNVKDLIVK
6	1	44	gi|51215619	Chitin biosynthesis protein *	27327.64	5.31	K.NGLRLGKIPK.A
Intracellular trafficking and secretion							
6	1	48	gi|2072023	GABA-receptor-associated protein*	22479.01	6.66	YLVPSDLTVGQFVYYVIR
Signal transduction							
4	5	314	gi|11262436	14-3-3 protein homolog *	28913.33	4.67	QAFDDAIAELDTLSEESYKDSTLIMQLLI , AASDVAVTELPPTHPIR, QAFDDAIAELDTLSEESYK, DSTLIMQLLR, YLAEFATGDK
5	1	53	gi|3218407	Adenylate cyclase*	190334.33	5.86	ELNIANNK
Unclassified							
1	1	53	gi|6321879	Protein involved in RNA metabolism	12009.47	5.05	IEEVIDLILR
2	1	52	gi|6321879	Protein involved in RNA metabolism	12009.47	5.05	IEEVIDLILR
4	3	179	gi|30024660	guanine nucleotide binding protein	34556.86	5.98	DGITMLWDLNEGK, GWVTAIATSSENPDMILTASR, LWDLNTGLTTR
4	1	44	gi|28564107	SOL1	16776.24	5.58	FAGRDSVK
5	1	67	gi|46438514	ATP/ADPtranslocator	32754.91	9.62	EFNGLVDVYKK

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 2.

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

The table lists indicated spot numbers, amino acid sequences, scores, accesion numbers, protein names, identity/similality, theoretical mass (kDa), theoretical pH, organism and function left to right.

spot number	amino acid sequence	score	accession number	protein name	identity /similarlity	analytical MW	theoretical *pI*	organism	functional category
1	gefsddsdqagaydq	74	A1CE10	Phosphoglycerate mutase*	73 / 87	12190.12	4.81	Fungi	Carbohydrate transport and metabolism
2	sntgvvaatv			No significant hits found					
3	sptvxvndvvpsgtftyvpy	74	Q2PCV2	Laccase*	69 / 81	5404.94	6.26	Fungi	Secondary metabolites biosynthesis, transport, and catabolism
4	sxraaxgsgavnags			No significant hits found					
5	raakgsgavnagstqsfvgnlseln			No significant hits found					
6	rgkggsgvnnggtqqffv	65	Q6ZXX3	Histone H4	53 / 100	11382.34	11.36	Fungi	Chromatin structure and dynamics
7	aplgievadapstaafgv	74	Q1IL41	Hypothetical protein	53 / 88	32116.70	5.64	Bacteria	Unclassified
8	aplsievadapstiafgv	61	Q9FAX2	DNA gyrase subunit B*	56 / 78	53556.69	6.33	Bacteria	DNA replication, recombination, and repair
9	sdsiearlhfienln	56	P46963	CTD kinase subunit gamma*	69 / 92	34809.18	5.88	Fungi	Transcription
10	sdsiearlhfienln	56	P46963	CTD kinase subunit gamma	69 / 92	34809.18	5.88	Fungi	Transcription
11	sdsiearlhfienln	56	P46963	CTD kinase subunit gamma	69 / 92	34809.18	5.88	Fungi	Transcription
12	sdsieaqlhfienln	54	Q753W3	Vacuolar sorting protein SNF7*	64 / 79	26685.19	4.84	Fungi	Intracellular trafficking and secretion
13	speqelaavngrllqssvgdldpvv	79	A0V740	Phosphatidylserine decarboxylase*	54 / 75	30888.40	9.09	Bacteria	Lipid metabolism
14	speqelaavngrllqssvgd			No significant hits found					
15	sldvearalyiesldifg	59	Q3IYU1	tRNA pseudouridine synthase B	56 / 88	32194.57	5.20	Bacteria	Translation, ribosomal structure and biogenesis
16	rvpketlidqlakay			No significant hits found					
17	gldklvnvnglqdqmgvgdl	68	Q8ZVM9	Probable exosome complex exonuclease 1*	56 / 94	27148.26	6.86	Bacteria	Amino acid transport and metabolism
18	rlgkvgnvrgagg			No significant hits found					

19	spdkltnvnl			No significant hits found					
20	aapapkapkgvekrd			No significant hits found					
21	slnmvyrlmf			No significant hits found					
22	slimqlrltf			No significant hits found					
23	kldldllvrlrldah			No significant hits found					
24	kadmvtrvrl	47	Q9MUU6	Chloroplast 30S ribosomal protein S8*	75 / 100	14764.25	9.73	Plant	Translation, ribosomal structure and biogenesis
25	kqsredlvylaklgr	64	Q562H7	14-3-3 protein	92 / 92	28815.19	4.76	Fungi	Signal transduction
26	snpnrkpgqhdlall			No significant hits found					
27	kpgagengdg			No significant hits found					
28	kpgvhfmpfddfgld			No significant hits found					
29	kapegnilggggdrg			No significant hits found					
30	lkdlqedhagvrlqv	80	O14460	Elongation factor 2	73 / 93	93230.90	6.02	Fungi	Translation, ribosomal structure and biogenesis
31	kpqytpanigapgrg	69	O57683	Splicing factor 3B subunit 1*	57 / 100	146214.52	6.48	Insect	RNA processing and modification
32				N-terminal amino acid blocked					
33				N-terminal amino acid blocked					
34	asatehtltvrdgln	53	Q9B6D8	Cytochrome c oxidase subunit 3*	73 / 91	30467.04	6.32	Fungi	Energy production and conversion
35	psakifydsdadlsl	73	Q46JF6	Ketol-acid reductoisomerase*	85 / 100	36309.51	5.21	Bacteria	Amino acid transport and metabolism
36	slpegytvlsnrdsi			No significant hits found					
37	slgrygiggfl			No significant hits found					
38	pnvqrvtexvfqsps	50	Q9FNE0	Putative cytochrome c oxidase polypeptide Vc-4*	43 / 86	7421.61	8.03	Plant	Energy production and conversion
39				N-terminal amino acid blocked					
40	qpgdelrdef	59	Q8DJ62	Urease accessory protein ureE*	89 / 100	16285.70	6.49	Bacteria	Amino acid transport and metabolism
41	qpgydgvlaryrlgp	64	Q73V62	UPF0061 protein MAP 3154	67 / 87	53324.47	5.07	Bacteria	Unclassified
42	spgnalevgy	54	P22553	S-locus-specific glycoprotein BS29-2 precursor*	70 / 100	49543.04	8.48	Plant	Sporulation
43	qleydynghq			No significant hits found					
44	lqtaadstqleda	52	P48465	Actin	82 / 100	41736.68	5.17	Bacteria	Cytoskeleton
45	rigyngytnrfggrf			No significant hits found					
46				N-terminal amino acid blocked					

47	rvtglklgavailrv	57	Q8NSS8	Hypothetical transport protein Cgl0590/cg0683	67 / 87	55665.64	5.78	Bacteria	Unclassified
48	kgngglgqnadlgr	64	P43852	Bifunctional purine biosynthesis protein purH*	83 / 92	58349.59	5.75	Bacteria	Nucleotide transport and metabolism
49	qiggasgggr			No significant hits found					
50	mvlveelnvlva	45	Q83PY1	Protein slyX	64 / 82	8184.30	4.89	Bacteria	Unclassified
51	kgyatpiftmd			No significant hits found					
52	ateatgtigqvktvigavvd	86	Q98EV8	ATP synthase subunit beta*	65 / 95	50775.77	4.97	Bacteria	Energy production and conversion
53	slgahgakry	59	Q00094	Gene 73 protein kinase*	80 / 100	106567.73	8.80	Viruses	Signal transduction
54	pltfsqqfntsv	52	Q8CWA2	Cation efflux system protein cusB precursor*	67 / 92	44409.95	5.70	Bacteria	Cell envelope biogenesis, outer membrane
55	rtagepydtv			No significant hits found					
56	paiaeikvpathkti	53	Q4WGM4	Ubiquinol-cytochrome c reductase complex*	47 / 80	14662.88	5.55	Fungi	Energy production and conversion
57	apqtkllqatgdaay	51	P94186	Mercuric transport protein periplasmic component precursor*	73 / 91	9505.12	9.26	Bacteria	Inorganic ion transport and metabolism
58	ahkdikfsnegrasi	70	Q0H0L2	Heat shock protein 60	71 / 83	63039.38	5.91	Fungi	Posttranslational modification, protein turnover, chaperones
59	slqvaliayqkndyh	55	Q7VI68	L-seryl-tRNA(Sec) selenium transferase*	53 / 93	50580.15	8.50	Bacteria	Translation, ribosomal structure and biogenesis
60	klgdklgrkg	67	Q0K852	NADH dehydrogenase type2 *	100 / 100	47467.77	8.64	Bacteria	Energy production and conversion
61	glearvptdsqp	67	Q2H0Z4	Hypothetical protein	75 / 100	51089.66	4.91	Fungi	Unclassified
62	qteeyfvitlqflhv	52	P37485	Hypothetical protein yybS	64 / 91	34534.02	9.65	Bacteria	Unclassified
63	spgtlprgkhrgmgs	77	Q2LEM4	Hypothetical protein	71 / 86	25414.61	5.89	Bacteria	Unclassified
64	sqgeglgtnier			No significant hits found					
65	atvsegnigaty			No significant hits found					
66	alvgeggrgt			No significant hits found					
67				N-terminal amino acid blocked					
68	slgrvdllrt			No significant hits found					
69	sllvvgygth			No significant hits found					
70	klraelnen			No significant hits found					

71	klrayrnvlk	49	Q6EW16	Chloroplast 30S ribosomal protein S8*	88 / 100	15447.03	11.30	Plant	Translation, ribosomal structure and biogenesis

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 (*Hericium erinaceum*) identified by 1-DGE and nESI-LC-MS/MS

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

fraction number	matching peptide	score	accesion number	protein name	analytical MW	theoretical p*I*	peptide
Chromatin structure and dynamics							
2	1	44	gi|44982537	Chromatin remodeling complex subunit RSC8*	64437.55	5.46	DLAQLNISKK
6	1	91	gi|1742929	Histone H2B *	15165.40	10.16	AMAILNSFVNDIFER
6	1	77	gi|3142	Histone H4.2 *	11366.34	11.36	TVTALDVVYALKR
DNA replication, recombination, and repair							
1	1	42	gi|57898980	DNA topoisomerase II *	176899.32	7.14	SIGMGRRGK
2	1	47	gi|550429	Helicases*	124537.62	6.82	EINSNTKTLENIR
4	1	49	gi|2213553	DNA repair protein RAD4 *	79023.81	8.76	EEALLPNAKPVK
6	1	42	gi|2340169	Telomerase reverse transcriptase 1 *	116457.25	9.75	KFLNLSLR
Cell division and chromosome partitioning							
3	1	73	gi|5725417	Septin *	40119.94	5.01	VNVIPVIGK
3	1	56	gi|38704270	Beta-tubulin	25238.83	5.07	AVLNDLEPGTMDAVR
4	1	100	gi|10241488	Cell division control protein 10 *	38657.99	7.20	STLINTIFASHLIDSK
5	1	44	gi|984572	Beta-tubulin*	48984.54	4.92	AVLIDLEPGTMDAVR
Transcription							
3	1	45	gi|21745321	Transcription factor *	56479.77	9.38	LPPITNHSEK
4	1	45	gi|5055	RNA polymerase II large subunit *	194162.51	5.62	QILSLIIPK
Translation, ribosomal structure and biogenesis							
1	2	151	gi|11078222	Translation elongation factor EF1-α	40282.27	8.71	TLLEAIDAIEPPSRPSDKPLR LPLQDVYK, TLLEAIDAIEPPSRPSDKPLRLPLQD VYK
1	2	119	gi|27960789	Translation elongation factor EF1-α	36015.52	8.81	SVEMHHEQLQEGLPGDNVGFNVK , QLIVAINK
1	1	45	gi|42547595	60S ribosomal protein L15 *	24017.93	11.27	YYEVILVDPQHK
2	2	113	gi|10637881	Translation elongation factor EF1-α *	50051.61	9.15	GDVASDSKNDPAK ,YMVTVIDAP(
2	1	70	gi|1364060	Translation elongation factor EF1-α	49663.33	9.22	DDLFNTNASIVR, IQFGGDEVVK

2	1	63	gi|4056551	Translation initiation factor 3 subunit*	62636.95	6.59	EQRGRQGAFAGR
3	5	333	gi|58618695	Translation elongation factor EF1-α	46048.02	8.79	EHALLAFTLGVR, YMVTVIDAPGHR, LPLQDVYK, LVPSKPMCVESYNEYPPLGR, YMVTVIDAPGHRDFIK
3	4	278	gi|58758729	Translation elongation factor EF1-α	34283.63	8.28	EHALLAFTLGVR, SVEMHHEQLEQGLPGDNVGFNVK , LPLQDVYK, LVPSKPMCVESYNEYPPLGR
3	3	213	gi|11078222	Translation elongation factor EF1-α	40282.27	8.71	TLLEAIDAIEPPSRPSDKPLR LPLQDVYK, TLLEAIDAIEPPSRPSDKPLRLPLQD VYK
3	1	70	gi|542225	Ribosomal protein L4.e *	39005.13	10.64	NIPGVEIVNVR
4	1	80	gi|11078222	Translation elongation factor EF1-α	40282.27	8.71	TLLEAIDAIEPPSRPSDKPLR LPLQDVYK, TLLEAIDAIEPPSRPSDKPLRLPLQD VYK
4	1	79	gi|32810507	Translation elongation factor EF1-α	20360.35	8.66	AGMIVTFAPTNVTTEVK
4	1	78	gi|3766376	Ribosomal protein L10*	33565.30	4.71	TSFFQALGIPTK
4	1	77	gi|1039443	Ribosome-associated protein*	31684.85	4.91	FTPGSFTNYITR
4	1	63	gi|57225965	60s Ribosomal protein l5-b*	34650.37	8.79	ELDAEVLQK
4	1	42	gi|2104451	Threonyl-tRNA synthetase *	80138.01	6.84	WELNAGDGAFYGPK
5	2	135	gi|1364060	Translation elongation factor EF1-α	49663.33	9.22	DDLFNTNASIVR, IQFGGDEVVK
5	2	118	gi|1164943	Ribosomal protein S7e*	21987.65	9.73	ILEDLVFPTEIVGK, ILEDLVFPTEIVGKR
5	2	106	gi|46098158	40S Ribosomal protein S9 *	22237.86	10.68	QIVNVPSFVVR, LFEGNAIIR
5	2	94	gi|32563304	Translation elongation factor EF1-α	30650.33	6.61	YAWVLDKLK, IGGNGTVPVGR
5	1	72	gi|1039443	Ribosome-associated protein	31684.85	4.91	FTPGSFTNYITR
5	1	62	gi|12329975	Ribosomal protein S2 *	28310.92	10.16	SMEEIYLFSLPVK
5	1	53	gi|3859691	Ribosomal protein L13e *	23053.64	10.61	GFTLAELK
5	1	49	gi|44885669	S-phase specific ribosomal protein	29423.16	9.87	LFAIGFTK
5	1	48	gi|16944409	related to GTPase MSS1*	56936.39	4.98	SQDPILISCR
5	1	48	gi|2414648	60S ribosomal protein L7*	28449.26	10.06	QAANFLWPFK
5	1	46	gi|3766376	Ribosomal protein L10	33565.30	4.71	TSFFQALGIPTK
5	1	44	gi|14994314	40S Ribosomal protein S8*	23557.83	10.93	LIGVVYNASNNELVR
6	2	150	gi|19571756	Ribosomal protein L18E *	11145.20	4.17	APTGSNTVLLR
6	3	148	gi|1742935	40S Ribosomal protein S13 *	17080.03	10.78	GISSSALPYR, KGLTPSQIGVTLR, LILIESR
6	2	137	gi|46098158	40S Ribosomal protein S9	22237.86	10.68	QIVNVPSFVVR, LFEGNAIIR

6	1	86	gi|11595555	Ribosomal protein S28 *	15909.58	10.60	VSGVGLLALWK
6	1	74	gi|1039443	Ribosome-associated protein	31684.85	4.91	FTPGSFTNYITR
6	1	56	gi|38524278	40S Ribosomal protein S5*	23680.17	9.30	DISLTDYIQIR
6	1	55	gi|3620	Ribosomal protein L29 *	16721.55	10.62	INMDKYHPGYFGK
6	1	54	gi|3859690	40S Ribosomal protein rps16 *	15739.42	10.29	INMDKYHPGYFGK
6	1	53	gi|3806	Hypusine containing protein HP2 *	17114.28	4.81	VHLVAIDIFTGK
6	1	49	gi|6323236	Ribosomal protein L6*	95480.78	6.15	AFKQGNIDAGVVAGDIYFQMQNY
Posttranslational modification, protein turnover, chaperones							
1	1	45	gi|2551	Ubiquitin *	8540.78	6.56	IQDKEGIPPDQQR
1	1	44	gi|21388600	Heat shock protein 70	25092.47	5.52	DAGVIAGLNVLR
2	5	385	gi|7521943	Heat shock protein 70	71055.79	5.02	IINEPTAAAIAYGLDKK IINEPTAAAIAYGLDK, TQDLLLLDVAPLSLGIETAGGVMT ALIK, NGLESYAYNLR, FELSGIPPAPR
2	3	222	gi|44983832	Heat shock protein 70 *	70303.76	5.12	IINEPTAAAIAYGLDKK, IINEPTAAAIAYGLDK,FELTGIPPA PR
2	2	135	gi|25990446	Heat-shock protein 90 *	79295.85	4.92	HSEFISYPIQLVVTK, DSPFLEVLK
2	2	100	gi|44981480	Heat shock protein 70 *	73898.47	4.91	FELSGIPPAPR, EDIDDIVLVGGSTR
3	1	81	gi|19069227	26S Proteasome regulatory subunit 4 *	47377.75	6.39	TMLELLNQLDGFDTR
3	1	60	gi|3687465	26S Proteasome regulatory subunit*	43552.98	5.28	ENAPAIIFIDEIDAIATK
5	1	65	gi|984687	Proteasome alpha type 1*	25604.02	6.90	LFQVEYALEAIK
6	2	109	gi|16943775	Cyclophilin *	17614.90	9.36	FADENFQLK, VIPQFMLQGGDFTK
6	1	77	gi|849081	Cyclophilin B *	21969.06	8.59	VIKDFMIQGGDFTR
6	1	49	gi|6323236	Ubiquitin *	95480.78	6.15	AFKQGNIDAGVVAGDIYFQMQNY
Energy production and conversion							
1	5	347	gi|57227717	Pyruvate carboxylase*	121206.58	6.27	NIIVEQGPEAFAK, TWTTFIDDTPELFK, SAFGDGTVFIER, LVPNIPLQALVR, HYFIEINPR
1	2	108	gi|4255	Pyruvate carboxylase *	130907.76	6.25	QFNGTLLMDTTWR, HYFIEINPR
1	1	51	gi|46433360	NAD-formate dehydrogenase *	41801.55	5.51	LLGTVENELGIR
1	1	49	gi|7320601	Pyruvate carboxylase	43215.42	5.57	ASDSSVFDHEMPGGQYTNLMFQA SQLGLGTQWTEIK
2	1	81	gi|1749734	Succinate dehydrogenase*	53767.12	7.71	LGANSLLDIVVFGR
3	2	160	gi|584806	ATP synthase alpha chain*	59522.39	9.07	STVAQLVKTLEENDAMK,TGEIVD VPVGPELLGR

3	1	69	gi|46433360	NAD-formate dehydrogenase *	41801.55	5.51	LLGTVENELGIR
3	1	59	gi|4376093	Pyruvate kinase *	58248.74	5.88	GDLGIEIPASQVFLAQK
3	1	51	gi|1420756	PYK2 *	55195.22	6.43	IENQQGLDNFDEILEVTDGVMIAR
4	2	137	gi|59803007	Malate dehydrogenase*	35191.31	8.26	DDLFNTNASIVR, LFGVTTLDVVR
4	2	110	gi|4029338	Malate dehydrogenase *	33029.29	6.13	IQFGGDEVVK, VAVLGAAGGIGQPLSLLLK
4	2	96	gi|462075	Formate dehydrogenase *	39910.56	6.84	GLVNAELLK, GAWLVNTAR
4	1	81	gi|46433360	NAD-formate dehydrogenase	41801.55	5.51	LLGTVENELGIR
4	1	66	gi|13785197	Inorganic pyrophosphatase *	32390.49	5.24	VLGIMALLDEGETDWK
4	1	42	gi|18149179	Arylalcohol dehydrogenase *	44382.60	6.88	NITAVAIAYVMQK
5	1	72	gi|34765759	NADH-ubiquinone oxidoreductase*	30373.33	8.83	VVYEPLQLTQAFR
Carbohydrate transport and metabolism							
1	1	89	gi|929983	Glycerol-3-aldehyde dehydrogenase *	30768.23	6.13	VPTNDVSVVDLVVR
2	1	53	gi|29409959	Transketolase *	74759.56	5.98	ANSGHPGAPMGMAPVSHVLFNK
2	1	43	gi|21264637	Endo beta 1,4 glucanase *	36395.44	4.40	VASATAWLK
3	2	123	gi|870831	Glucose-6-phosphate 1-dehydrogenase	58950.31	6.17	GGYFDEFGIIR,TFPALFGLYR
3	1	87	gi|929983	Glycerol-3-aldehyde dehydrogenase	30768.23	6.13	VPTNDVSVVDLVVR
3	1	70	gi|40713647	Glucose-6-phosphate isomerase *	61445.79	6.38	ILLSNFFAQPEALAFGK
4	3	237	gi|57232460	Glyceraldehyde 3-phosphate dehydrogenase *	25354.62	8.81	VPTNDVSVVDLVVR, VVNDKFGIVEGLMSTIHATTATQK, FGIVEGLMSTIHATTATQK
4	1	80	gi|30525994	Glyceraldehyde 3-phosphate dehydrogenase	11364.02	9.10	GVNGNIIPSSTGAAK
4	1	63	gi|3289019	Xylose reductase *	36021.33	5.77	TPAEVLLR
5	1	81	gi|929983	Glycerol-3-aldehyde dehydrogenase	30768.23	6.13	VPTNDVSVVDLVVR
5	1	74	gi|40739109	Triosephosphate isomerase *	27156.97	5.88	VVIAYEPVWAIGTGK
6	1	58	gi|929983	Glycerol-3-aldehyde dehydrogenase	30768.23	6.13	VPTNDVSVVDLVVR
Amino acid transport and metabolism							
4	2	151	gi|6746633	Glutamine synthetase*	38306.81	5.88	IWDFDGSSTNQAPGNDSDVYLRF AAIFKDPFR, IWDFDGSSTNQAPGNDSDVYLRP AAIFK
4	1	55	gi|57223024	Endopeptidase*	47547.37	4.77	EPGLAFAFGK
Nucleotide transport and metabolism							
6	1	47	gi|44981739	Carbamoyl-phosphate synthase *	248131.78	5.48	AASTVDEALEAVK
Lipid metabolism							

5	1	55	gi|1907190	Acyl-CoA sterol acyltransferase *	71613.08	8.53	ESLSPETREILQK
Inorganic ion transport and metabolism							
2	1	46	gi|30580366	Catalase 3*	79227.71	5.75	FEASHVTNEQVKK
4	1	66	gi|57226036	Voltage-dependent ion-selective channel*	30636.63	9.13	INNAGVLSLGYTQALRPGVK
6	1	51	gi|38566870	Glutathione peroxidase *	18888.76	6.43	FLIGKDGKVK
Secondary metabolites biosynthesis, transport, and catabolism							
1	1	44	gi|44982853	Cytochrome P450 *	60505.74	6.77	VMTVYLGTK
Cytoskeleton							
3	3	164	gi|5053107	Actin 1 *	41617.51	5.30	TTGIVLDSGDGVTHTVPIYEGFALP HAILR, LDLAGRDLTDFLIK, DLTDFLIK
4	1	62	gi|31581446	Actin 1	33445.88	5.46	SYENPDGQVITIGNER
5	1	91	gi|31581482	Actin 1	33559.39	5.46	SYENPDGQVITIGNER
Cell envelope biogenesis, outer membrane							
3	1	87	gi|15321714	UDP-glucose dehydrogenase *	51311.93	5.53	ILTVGLWSSELSK
Intracellular trafficking and secretion							
5	1	84	gi|46099486	GTP-binding protein ypt1*	22931.88	5.89	SATNVEQAFLTMAK
5	1	64	gi|46444053	Clathrin-associated protein AP-1 complex *	50205.14	7.08	LKTFPGGK
5	1	61	gi|30024664	Ras-related protein Rab7 *	22969.85	4.75	EAINVEQAFQTVAK
5	1	48	gi|311752	Ran*	24810.36	6.12	SNYNFEKPFLWLAR
6	2	108	gi|461532	ADP-ribosylation factor*	20912.06	5.79	ILMVGLDAAGK, DALLLVFANK
6	1	82	gi|46099486	GTP-binding protein ypt1	22931.88	5.89	SATNVEQAFLTMAK
Signal transduction							
1	1	54	gi|3560251	Serine/Threonine protein kinases*	55222.27	8.00	EVSILER
2	1	44	gi|3560	Calmodulin-binding protein 2*	68499.64	5.92	SSDAIRNTEQINAAIKIIENK
2	1	42	gi|44985926	Serine/Threonine protein kinases*	51673.68	5.61	NLKPDAVDLLEK
4	1	50	gi|474400	Serine/Threonine protein kinases*	42450.42	9.35	TVLLLADQLISR

5	6	414	gi|11262436	14-3-3 protein homolog *	28913.33	4.67	LAEQAERYEEMVENMKR, QAFDDAIAELDTLSEESYKDSTLI MQLLR, AASDVAVTELPPTHPIR, DSTLIMQLLR, YLAEFATGDKR, NLLSVAYK
Unclassified							
2	1	53	gi|28950128	Related to sporulation protein SPO72 *	219672.55	4.84	HILLDNLR
2	1	48	gi|44981703	ACR227Wp	87703.32	5.52	MISECLGDDSESILVPRLK
3	1	43	gi|57222792	protein-nucleus import-related protein	78291.35	5.11	LIDLGRISGNLK
4	1	45	gi|44983595	AER010Cp	84506.58	8.70	SDEVALLKHRR
6	1	71	gi|1052793	SPAC2F7.10	72521.60	8.74	QALISNGLK
6	1	53	gi|531492	RRP3p	122491.61	4.52	ENNGNEEDK
6	1	45	gi|27948809	SNT1	93751.27	7.77	ISNFMGGLR

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 4.

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

The table lists indicated spot numbers, amino acid sequences, scores, accesion numbers, protein names, identity/similality, theoretical mass (kDa), t pH, organism and function left to right.

Spot Number	amino acid sequence	score	accession number	protein name	identity /similarlity	analytical MW	theoretical *pI*	organism	function
1	qlttkvrvlrdkrin	51	Q6FJ50	Guanine nucleotide-binding protein subunit gamma*	53 / 73	10251.63	6.05	Fungi	Signal transduction
2	gwfhddsdeaqaysq vndaphkael	118	Q4IID0	Hypothetical protein	67 / 83	11878.88	4.88	Fungi	Unclassified
3	anpggdyamn			No significant hit found					
4	qftvvlkaan	44	P58724	Blue-light photoreceptor*	70 / 80	28816.77	4.73	Bacteria	Signal transduction
5	qgatsikalgfnipl	59	Q9LA06	Serine protease do-like htrA*	62 / 92	41647.97	5.17	Bacteria	Amino acid transport and metal
6	hgeaeefilg	49	Q31G56	Ribonuclease HII*	70 / 90	22808.57	6.30	Bacteria	Nucleotide transport and metab
7	rakvyvmnls	40	P61174	50S ribosomal protein L36*	75 / 100	4694.62	12.14	Bacteria	Translation, ribosomal structure biogenesis
8	txgdpsaavgflray			No significant hit found					
9	txgdpsaavgflraylef	57	Q9YAD8	Hypothetical protein APE2001	57 / 86	15980.57	5.40	Bacteria	Unclassified
10	txgdpsaavgflraylef	57	Q9YAD8	Hypothetical protein APE2001	57 / 86	15980.57	5.40	Bacteria	Unclassified
11	flekaqikvgekvslset vk	57	Q88QM6	30S ribosomal protein S17*	56 / 75	10057.76	9.81	Bacteria	Translation, ribosomal structure biogenesis
12				N-terminal amino acid blocked					
13	axpasflekaqikvgek vsl	67	Q6H806	Putative ubiquitin fusion degradation protein*	50 / 94	35143.74	5.77	Plant	Posttranslational modification, turnover, chaperones
14	vektahaaarsflekaqi kv	68	Q8U778	Transcriptional regulator, ROK family*	58 / 89	42235.10	5.97	Bacteria	Transcription
15	qpilfiaddgyafty	62	Q9AJ64	Antigenic heat-stable 120 kDa protein	64 / 79	108518.46	5.08	Bacteria	Unclassified
16				N-terminal amino acid blocked					
17				N-terminal amino acid blocked					
18				N-terminal amino acid blocked					
19	mllysdiitddemfsda fpv	101	Q5K7S2	Translationally-controlled tumor protein homolog *	65 / 95	18763.25	4.55	Fungi	Signal transduction

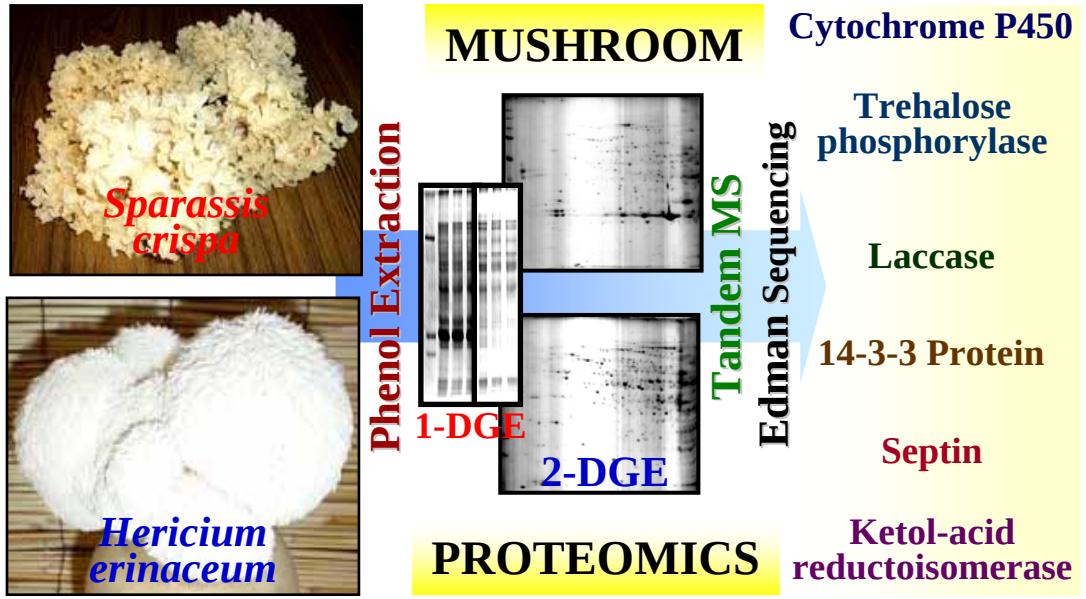
20	mllysdiitddemfsda	79	Q5K7S2	Translationally-controlled tumor protein homolog	65 / 94	18763.25	4.55	Fungi	Signal transduction
21	vhtlpdlpyaydalepyfsr	123	Q9P4T6	Superoxide dismutase [Mn]*	90 / 100	22194.25	6.03	Fungi	Inorganic ion transport and met
22	vhtlpdlpyayd	77	Q9P4T6	Superoxide dismutase [Mn]	80 / 90	22194.25	6.03	Fungi	Inorganic ion transport and met
23	vlklhgsphstgttrvvvvl	84	Q1EBD9	Glutathione S-transferase*	75 / 85	23889.39	6.60	Fungi	Secondary metabolites biosynth transport, and catabolism
24	snavkeglffta			No significant hit found					
25				N-terminal amino acid blocked					
26	tltggrgafgir	56	Q1CX38	ATP synthase epsilon chain*	78 / 89	14595.68	5.54	Bacteria	Energy production and convers
27	aprkffvggnfkmnpstqae	97	P04828	Triosephosphate isomerase	71 / 94	27156.97	5.88	Fungi	Carbohydrate transport and me
28				N-terminal amino acid blocked					
29	rplaglyrga			No signifcant hit found					
30	kplfgv			No signifcant hit found					
31	tqgpivtgtsilalk	71	A1CE34	Proteasome component Pre4*	67 / 100	29023.61	5.67	Fungi	Posttranslational modification, turnover, chaperones
32	mlgng			No significant hit found					
33	qglpganyit			No significant hit found					
34	kvgges			No significant hit found					
35				N-terminal amino acid blocked					
36				N-terminal amino acid blocked					
37	pesredsvylaklaeqaery	125	Q4G2I8	14-3-3 protein	100 / 100	28721.07	4.71	Fungi	Signal transduction
38				N-terminal amino acid blocked					
39	sihpevllgq			No significant hit found					
40	mrliirddktavgdy	73	Q4PDU5	hypothetical protein	73 / 86	32424.87	6.35	Fungi	Unclassified
41				N-terminal amino acid blocked					
42				N-terminal amino acid blocked					
43	nkynagkygl	58	A1FRZ0	Lytic transglycosylase*	80 / 90	35129.87	8.28	Bacteria	Cell envelope biogenesis, outer
44				N-terminal amino acid blocked					
45	tkvavlgagggigqpls	113	P17505	Malate dehydrogenase*	95 / 100	35649.96	8.46	Fungi	Energy production and convers
46	spryilatdq			No significant hit found					
47	pkavvlgaaggigqp	75	P17505	Malate dehydrogenase	79 / 100	35649.96	8.46	Fungi	Energy production and convers
48	skavvlgaaggigqp	75	P17505	Malate dehydrogenase	79 / 100	35649.96	8.46	Fungi	Energy production and convers

49				N-terminal amino acid blocked					
50				N-terminal amino acid blocked					
51	dyqtakpaievgklk	62	P36498	Transport protein comB*	83 / 92	49601.49	5.64	Bacteria	Cell envelope biogenesis, outer
52	paltvtklnt	45	P92133	Cathepsin B-like CP3 precursor*	83 / 100	32673.83	5.19	Insect	Amino acid transport and metal
53				N-terminal amino acid blocked					
54	dillfkvlr	49	Q5VV43	Uncharacterized protein KIAA0319 precursor	89 / 100	117762.74	5.34	Human	Unclassified
55				N-terminal amino acid blocked					
56				N-terminal amino acid blocked					
57	vvkvgingfgrigri	97	Q8J1H3	Glyceraldehyde-3-phosphate dehydrogenase	100 / 100	36400.27	6.46	Fungi	Carbohydrate transport and me
58				N-terminal amino acid blocked					
59				N-terminal amino acid blocked					
60				N-terminal amino acid blocked					
61				N-terminal amino acid blocked					
62	ivgrpghqlvmv	61	P10982	Actin-1	75 / 83	15693.88	5.30	Fungi	Cytoskeleton
63	mltelekalnsidsv	68	P05109	Protein S100-A8*	87 / 93	10834.51	6.51	Human	Signal transduction
64	mltelekalnsidsv	68	P05109	Protein S100-A8	87 / 93	10834.51	6.51	Human	Signal transduction
65				N-terminal amino acid blocked					
66	lpiheyqsvkllnsy	82	Q5KN95	Succinate-CoA ligase*	87 / 93	44998.54	5.62	Fungi	Energy production and convers
67				N-terminal amino acid blocked					
68	vykvadislaafgrkeiega	104	**P39954**	Adenosylhomocysteinase*	90 / 95	49125.51	5.83	Fungi	Amino acid transport and metal
69	vaywepgtqynygsiveyeg	81	Q8U1H4	Putative chitinase*	65 / 88	39701.99	5.18	Bacteria	Carbohydrate transport and me
70	vaydepgtqynhgsi	54	P47009	Hypothetical 11.7 kDa protein in IDS2-MPI2 intergenic region	64 / 91	11659.54	6.40	Fungi	Unclassified
71				N-terminal amino acid blocked					
72				N-terminal amino acid blocked					
73	ginkgklg	53	Q5NF19	Probable inorganic polyphosphate /ATP-NAD kinase*	100 / 100	32505.52	8.63	Bacteria	Coenzyme metabolism
74				N-terminal amino acid blocked					
75				N-terminal amino acid blocked					

76				N-terminal amino acid blocked					
77				N-terminal amino acid blocked					
78				N-terminal amino acid blocked					
79				N-terminal amino acid blocked					
80	atavtqgpftevttl	61	Q9Y8B5	Mitochondrial-processing peptidase subunit beta*	60 / 93	51162.66	5.69	Fungi	Amino acid transport and metal
81	ateakgaigavktvigavxd	81	Q6FYM3	ATP synthase subunit beta*	60 / 83	57135.98	5.61	Bacteria	Energy production and convers
82	apegvgdigliglah			No significant hit found					
83				N-terminal amino acid blocked					
84				N-terminal amino acid blocked					
85				N-terminal amino acid blocked					
86				N-terminal amino acid blocked					
87				N-terminal amino acid blocked					
88				N-terminal amino acid blocked					
89				N-terminal amino acid blocked					
90	papadflkgvdsneeafiqr	66	Q98N59	Elongation factor G*	50 / 77	77208.28	5.28	Bacteria	Translation, ribosomal structure biogenesis
91	hlqqvf			No signifcant hit found					
92	pnqtvfdirdgavyt	62	Q93LL2	Acetyl-coenzyme A synthetase*	62 / 85	37143.22	6.14	Bacteria	Energy production and convers
93	ahkeikfsnegraai	73	P50142	Heat shock protein 60*	73 / 93	61888.74	5.69	Fungi	Posttranslational modification, turnover, chaperones
94	qlgseadftqisfdh			No signifcant hit found					
95	lggggvlslg			No signifcant hit found					
96				N-terminal amino acid blocked					
97				N-terminal amino acid blocked					
98	qglpnprqgaed	60	P36627	Cellular nucleic acid-binding protein homolog*	100 / 100	19342.51	8.34	Fungi	Signal transduction
99	rlkvggpggg			No signifcant hit found					
100				N-terminal amino acid blocked					
101	qtdpaeitidgvfgi	55	P01007	Plasminostreptin*	54 / 85	11398.86	6.07	Bacteria	Defense
102				N-terminal amino acid blocked					
103				N-terminal amino acid blocked					
104	qglnlpg	50	Q927D8	UPF0133 protein	100 / 100	11438.28	4.59	Bacteria	Unclassified
105				N-terminal amino acid blocked					

106	dasnkseygtvigig	60	Q24895	78 kDa glucose-regulated protein precursor*	83 / 92	71675.38	5.09	Insect	Posttranslational modification, turnover, chaperones
107	pgqllaqanphgdsg			No signifcant hit found					
108	qgqllaqanphg			No signifcant hit found					
109				N-terminal amino acid blocked					
110				N-terminal amino acid blocked					
111	spgnllgdgarg	61	P04922	Circumsporozoite protein precursor	67 / 83	34782.33	5.38	Insect	Unclassified
112	fpapgateqvkgvis			No signifcant hit found					
113	aapvstndrfvpsaa	57	P78972	WD repeat containing protein	64 / 91	53417.86	9.30	Fungi	Unclassified
114	aapvslndqfgpsaa	60	Q5A387	Hypothetical Protein MSS1	73 / 91	42895.97	5.98	Fungi	Unclassified
115	gghsvpltnfm	65	P07267	Saccharopepsin precursor*	73 / 100	44499.00	4.70	Fungi	Amino acid transport and metal

Asterisks indicate unique proteins identified from *S. crispa* fruiting body using LC-MS/MS and Edman sequencing. Commonly identified proteins i
and *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.

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JPR for presentation