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Analysis of Secreted Proteins from *Undifilum cinereum* by Two Dimensional Gel Electrophoresis and Liquid Chromatography-Mass Spectrometry/Mass Spectrometry

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Abstract: The locoweed plant (Astragalus) is a widely distributed toxic plant in many rangeland regions around the world. It is well known that locoweed plants can produce the alkaloid swainsonine which inhibits α-mannosidases and causing neurological poisonings problems through the consumption of locoweed. Locoweed poisoned grazing animal's exhibit symptoms of locoism. Locoism was caused by locoweed is one of the most destructive disease of rangeland. Recent studies shown that swainsonine was produced by endophytic *Undifilum cinereum* which was isolated from Astragalus locoweed (*Astragalus mollissimus* and *Astragalus lentiginosus* sp.) and responsible for locoism in grazing animals. The toxicosis effect of *U. oxytropis* fungi on rats is indistinguishable from locoweed toxicosis on rats. The mechanisms of swainsonine underlying *U. cinereum* and locoweed are poorly understood. To gain a better understanding of the swainsonine biosynthesis in *U. cinereum* and to facilitate management of locoweed poisoning problems, two-dimensional gel electrophoresis (2-DE) was performed. The 2-DE is a promising tool to study the protein expression profiling and metabolic pathway. To researchers knowledge the present study was the first proteomic reference map using immobilized pH gradients of *U. cinereum.* To identify proteins in *U. cinereum*, proteins extracted from mycelial were separated by 2-DE and IEF, digestion and Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) with an LTQ ion trap mass spectrometer (Thermo Scientific, Waltham, MA). Samples were analyzed by LC-MS/MS and identified using MASCOT MS/MS search in protein databases.

Key words: 2-DE, *Undifilum cinereum* fungus, proteomics, mass spectrometry, astragalus, locoweed, swainsonine

INTRODUCTION

Locoweed is the most common Oxytropis and Astragalus plants that cause locoism in animals (James *et al.*, 1981; Ralphs *et al.*, 1988; Stegelmeier *et al.*, 1995). The alkaloid swainsonine produced by locoweed which inhibits α-mannosidases causing neurological problems that are responsible for locoism (Dorling *et al.*, 1980; Winkler and Segal, 1984). Recently, endophytic fungi were isolated from both Oxytropis and Astragalus, all isolates were found to produce the toxic alkaloid swainsonine. Fungi isolated from Oxytropis were described as *Undifilum oxytropis* based on their growth rate, morphology and molecular analyses. *Undifilun cinereum* endophytes were isolated from locoweed plant species (*Astragalus mollissimus* and *Astragalus lentiginosus* sp.) that described as a new endophytic fungus even though they are similar to *U. oxytropis* but have distinct different features corresponding to new species within the genus. It is well known that fungal endophyte *U. cinereum* is respinsible for the synthesis of swainsonine (Braun *et al.*, 2003; Pryor *et al.*, 2009; Yu *et al.*, 2010). The toxicity symptoms of *U. oxytropis* from locoweed on rats displayed indistinguishable from locoweed toxicosis on rats (McLain-Romero *et al.*, 2004). Locoweed toxicity is the most widespread disease of

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livestock in the Western United States and China and cause significant economic loss to the livestock industry (James, 1972; Zhao *et al*., 2009; Yu *et al*., 2010).

Although, several studies have been described the toxicology and pathology of locoism (Stegelmeier *et al*., 1999; Taylor and Strickland, 2002; Obeidat *et al*., 2005) the problem to protect animals from locoweed is still remain unsolved. Mineral bentonite was tested to bind swainsonine and to alleviate locoweed toxicities, those results indicated that bentonite was ineffective in alleviating animals from swainsonine poisoning (Pulsipher *et al*., 1994). Bacterium have also been used effectively to detoxifying of swainsonine from laboratory studies, many theoretical and practical problems need to be solved prior to employ in field studies (Zhao *et al*., 2009). From that above review, it is difficult to prevent livestock poisoning just focuses on locoweed plants because many factors affect the concentrations of swainsonine in locoweed plants (Ralphs *et al*., 2008; Cook *et al*., 2009; Oldrup *et al*., 2010). Study on fungal endophyte *U. cinereum* will provide a new method for managing locoism instead of locoweed plants.

The objectives of this study were to develop a protein extraction method for *U. cinereum* and obtain an overall view of total proteins isolated from filamentous fungus *U. cinereum*. Protein separation was analyzed by two-dimensional polyacrylamide gel electrophoresis and LC-MS/MS to analyze followed by BLAST to identify the proteins. To the knowledge this is the first application of 2-DE on *U. cinereum*, 2-DE reference map has been optimized using immobilized pH gradients of *U. cinereum* in the hope of identifying these polypeptides which could play a regulatory role in swainsonine synthesis may be extremely useful in further to develop novel strategies to control this important animal poisoning problem.

MATERIALS AND METHODS

***U. cinereum* isolate and growth condition:** *U. cinereum* that was used in this study was isolated from leaves and small stems of *Astragalus mollissimus* as before described (Braun *et al*., 2003). The stem and leaf were placed onto petri dishes containing water agar at 30°C for 1 week to attain conidia production. Conidia were collected and transferred to PDA. The conidia preparation was made from PDA and then grown in potato dextrose broth in 250 mL flasks and cultures were growing in an orbital shaker at 170 rpm, 28°C and 12 h light for 4 weeks. Mycelia were harvested by filtration, washed with sterile water and immediately frozen and stored at -80°C until protein extraction.

Protein extraction: Lyophilized mycelia (100 mg) were ground to a fine powder in liquid nitrogen using a cooled mortar. The powder was homogenized in five volumes NP-40 buffer (0.5 M Tris-HCl, pH 7.0, 1% NP-40, 30 mM magnesium chloride, 1% β-mercaptoethanol, 10 mM phenylmethanesulfonyl fluoride and 2% polyvinylpolypyrrolidone) with vortex (10 times, 1 min). The insoluble materials were eliminated by centrifuge at 13200 rpm for 30 min at 4°C. The supernatant was homogenized in five volumes 10% w/v TCA in acetone containing 0.07% 2-mercaptoethanol. The suspension incubated at -20°C for 4 h with intermittent stirring, centrifuged as described before and the pellet was washed three times with acetone containing 0.07% v/v 2-mercaptoethanol and centrifuged as described before. The final pellet was dried by vacuum centrifuge and resuspended in lysis buffer (7 M urea, 4% CHAPS, 60 mM Dithiothreitol (DTT), 2% ampholytes, 0.002% bromophenol blue). Insoluble material was removed by centrifugation. Samples were stored at -80°C until two-dimensional gel electrophoresis. The protein concentration was determined by the Bradford (1976) Method. Protein extracts from mycelia were prepared from at least three independent experiments.

2-DE and gel analysis: Isoelectric Focusing (IEF) was carried out in Immobilized pH Gradient (IPG) strips (pH 3-11; 18 cm, GE Healthcare) with a current 50 μA/strip in an Ettan IPGphor isoelectric focusing (Amersham Biosciences). After IPG strips were rehydrated for 20 h with 100 μg of protein in 340 μL rehydration solution volume. IEF was carried out at 20°C by using the following voltage program: 500 V for 4 h, 1000 V for 4 h, 8000 V for 4 h and 8000 V for 2 h. After IEF, one IPG strip was used for the second dimensional to develop a proteome map, the other IPG strip was cut into gel sections for protein digestion. The IPG gel needs to equilibration before the second dimensional. The IPG-strip was incubated two times each for 15 min with 10 mL equilibration buffer under gentle shaking. The focused gel was equilibrated in equilibration buffer I (50 mM Tris-HCl, (pH 8.8), 6 M urea, 30% glycerol, 2% SDS, 0.002% Bromophenol blue, 1% DTT). After 15 min, the procedure was repeated with another 10 mL equilibration Buffer II 50 mM Tris-HCl, [pH 8.8], 6 M urea, 30% glycerol, 2% SDS, 0.002% Bromophenol blue, 2.5% iodoacrtamide). The second-dimension electrophoresis was performed by Laemmli (Gorg *et al*., 1988). IPG strips were then placed onto 12% SDS-polyacrylamide gels and overlaid with agarose solution (25 mM Tris base, 192 mM glycine, 0.1% SDS, 0.5% agarose, 0.001 bromophenol blue). The SDS-PAGE was run at a current setting of 200 V at 4°C until the

marker reached the end of the gel. Broad molecular weight protein standards (Bio-Rad) were used for molecular weight determination. The gels were subsequently visualized by silver staining with a modification of the method described by Damerval *et al.* (1986) and Shevchenko *et al.* (1996). The gel was fixed in 50% methanol containing 5% acetic acid for 12 h. Then, wash the gel for 20 min with 50% methanol followed by treated with 0.02% sodium thiosulfate 1 min and rinsed with water for 10 min. Incubated the gel for 1 h in 4°C cold 0.1% silver nitrate solution and rinsed with water for another 10 min and then developed the gel in 0.04% formaldehyde and 2% sodium carbonate with intensive shaking. The development was terminated by 5% acetic acid when the gel turns yellow and the staining is sufficient. Finally, wash the gel by water and leave the gel at 4°C in 1% acetic acid for storage and analyzed.

Peptide sample preparation for LC-MS/MS analyses: The gel cut sections from the IPG strip were cut into 1-2 mm^3 sections. The digestion protocol was performed according to the method of Jeno *et al.* (1995), Shevchenko *et al.* (1996), Wilm *et al.* (1996) and Lahm and Langen (2000). The excised gels were dehydrated with acetonitrile, vacuum dried, rinsed with 10 mM DTT in 100 mM ammonium bicarbonate for 1 h at room temperature. Followed by shrunk with acetonitrile at room temperature for 0.5 h and dehydrated with acetonitrile for 20 min at room temperature. Gel pieces were subsequently rehydrated with 100 mM ammonium bicarbonate for 30 min at room temperature and dehydrated with acetonitrile for 5 min at room temperature, vacuum dried and incubated with 20 ng μL^{-1} sequencing-grade modified trypsin in 50 mM ammonium bicarbonate at 37°C overnight. Thereafter, the supernatant was removed and stored. The peptides produced were recovered from the gel by sequential extraction three times with 50% (v/v) acetonitrile in 5% (v/v) formic acid. Finally, all extracts were reduced to <20 μL by evaporation in a vacuum centrifuge and kept frozen for later MS analysis.

Tandem mass spectrometry and database searching: The samples were digested with trypsin and peptides were subjected to LC-MS/MS by an ion trap mass spectrometer (Thermo Scientific, Waltham, MA) with a Waters NanoACQUITY UPLE. Protein identifies were determined by the SyQuest algorithm as implemented by the BioWorks Browser V 3.2 (Thermo Scientific) and searching against the database. Chromatography consists of isocratic elution of 5% solvent (0.1% formic acid in water) for 5 and 40 min linear gradient to 70% Solvent II (0.1% formic acid in acetonitrile) with a 1.0×150 mm 5 μm reversed phase trapping column (Zorbax, 300SB-C18). FT-ICR mass spectra were collected at a mass resolving power of 100,000 (m z^{-1} = 400) and data dependent linear ion trap tandem mass spectra were collected for the five most abundant ions observed in each FT-ICR parent ion mass spectrum. Mass spectral data was searched against the Swiss Prot fungi database for protein identification with Bioworks browser. Protein identifications with an xcorr value >2 were selected. About 49 proteins were identified by LC-MS/MS using an ion trap LTQ mass spectrometer. Identified proteins were shown in Table 1.

Table 1: MS identification of proteins from *U. cinereum*

Proteins	Organism	Protein name	Accession number	Molecular weight	pI	Peptide sequence
1	*Neurospora crassa*	Formate dehydrogenase	Q07103	40931.1	5.93	K.LAVTAGIGSDHVDLNAANK.T
2	*Alternaria alternata*	Enolase	Q9HDT3	47176.2	5.19	R.AIVPSGASTGSHEACELR.D
3	*Ashbya gossypii*	Glucose-6-phosphate isomerase	Q758L0	61219.1	5.96	K.MLASNFFAQAEALMVGK.D
4	*Alternaria alternata*	Probable NADP-dependent mannitol dehydrogenase	P0C0Y4	28716.3	5.86	K.VVIVTGASGPTGIGTEAAR.G
5	*Phaeosphaeria nodorum*	Catalase-peroxidase	Q0U324	83446.6	5.84	R.FAPLNAWPDNVSLDK.A
6	*Ajellomyces capsulata*	Heat shock 70 kDa protein	Q00043	77510.1	5.53	K.STAGDTHLGGEDFDNR.L
7	*Schizosaccharomyces pombe*	Peptidy-prolyl cis-trans isomerase	P18253	17390.7	8.81	R.VIPQFMLQGGDFTR.G
8	*Trichoderma koningii*	Glyceraldehyde-3-phosphate dehydrogenase 2	P17730	36083.5	6.25	R.VPTANVSVVDLTVR.I
9	*Neosartorya fischeri*	Probable beta-qlucosidase F	A1DMR8	93047.0	5.83	R.TLHELYLWPFAEGIR.A
10	*Kluyveromyces lactis*	ATP synthase subunit beta, mitochondrial	P49376	54035.5	5.16	R.GISELGIYPAVDPLDSK.S
11	*Neurospora crassa*	ADP, ATP carrier protein	P02723	33866.7	9.84	R.YFPTQALNFAFR.D
12	*Alternaria alternata*	Minor allergen Alt a 7	P42058	22042.0	5.19	K.LFQVAETLPQEVLDK.M
13	*Aspergillus flavus*	Probable beta-glucosidase A	B8NRX2	93356.8	4.82	K.LFQVAETLPQEVLDK.M
14	*Emericella nidulans*	Probable formate dehydrogenase	Q03134	41549.4	6.24	K.LAVTAGIGSDHVDLDAANK.T
15	*Neurospora crassa*	V-type proton ATPase catalytic subunit A	P11592	67079.2	5.32	R.TTLIANTSNMPVAAR.E
16	*Humicola lutea*	Superoxide dismutase [Cu-Zn]	P83684	15853.9	6.36	R.TLVVHAGTDDLGR.G
17	*Candida albicans*	Aconitate hydratase, mitochondrial	P82611	84168.8	5.96	K.TTTDHISMAGPWLK.Y
18	*Neurospora crassa*	Woronin body major protein	P87252	19114.6	6.43	R.LGDILILQGRPCQVIR.I

Table 1: Continue

Proteins	Organism	Protein name	Accession number	Molecular weight	pI	Peptide sequence
19	*Pichia guilliermondii*	Elongation factor 1-alpha	A5DPE3	50016.10	9.14	K.SVEMHHEQLVEGV PGDNVGFNVK.N
20	*Candida albicans*	6-phosphogluconate dehydrogenase, decarboxylating	O13287	56888.20	6.14	K.GVLDSFLIEITR.D
21	*Aspergillus fumigatus*	Catalase B	Q92405	79861.20	5.50	R.FGFDLLDPTK.I
22	*Arxula adeninivorans*	Elongation factor 1-alpha	P41745	50084.20	9.06	K.YYVTVIDAPGHR.D
23	*Leptosphaeria maculans*	Isocitrate lyase	Q86ZF1	60087.90	6.30	R.AYGEIIQEPEAEN KVDVLTHQK.W
24	*Alternaria alternata*	Protein disulfide-isomerase	Q00002	46246.40	5.10	KLVTIAKV
25	*Pichia pastoris*	S-(hydroxymethyl) glutathione dehydrogenase	O74685	40533.30	6.08	K.VDEFITHR.H
26	*Aspergillus fumigatus*	Vacuolar protease A	O42630	43327.70	4.81	K.YDSSASSTYK.A
27	*Saccharomyces cerevisiae*	Down-regulator of invasive growth 1	Q03063	49325.90	8.62	K.EADHEDSETATAKKR.K
28	*Saccharomyces cerevisiae*	ATP-dependent RNA helicase DBP7	A6ZZY8	83270.10	9.33	R.DPDVVIYK.L
29	*Saccharomyces cerevisiae*	Protein transport protein SEC61	P32915	52902.50	9.39	K.RETSIYRELK.K
30	*Bordetella avium*	Homoserine O-acetyltransferase	Q2KU63	45061.50	5.62	RNTTSPDTTSHRP
31	*Yersinia pseudotuberculosis* serotype	p-hydroxybenzoic acid efflux pump subunit AaeB	A7FDT6	72390.78	9.47	R.VITVNNDQKRR.Y
32	*Buchnera aphidicola* sp.	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Q89AE9	44861.80	9.90	R.FQEKLQSIGAK.I
33	*Homo sapiens*	Beclin 1-associated autophagy-related key regulator	Q6ZNE5	55274.63	6.59	R.IEQLKQTICK.G
34	*Prochlorococcus marinus*	Aspartate-semialdehyde dehydrogenase	P49420	37533.97	6.38	R.KILNQSELAITATCVR.V
35	*Roseobacter denitrificans*	DNA-directed RNA polymerase subunit beta'	Q160X8	156625.20	5.68	K.DHAIIAEIDGYVRYGK.D
36	*Mycoplasma pneumoniae*	Uncharacterized protein MG061 homolog 1	P75041	61591.68	9.43	K.EQMQLGNQPSAGDILK.R
37	*Schizosaccharomyces japonicus*	40S ribosomal protein S0-A	B6K180	31386.76	4.94	R.FTPGNFTNYITR.T
38	*Nitrosococcus oceani*	Chaperone protein dnaK	Q3J7D8	69910.19	4.90	K.HLNVRITR.A
39	*Caenorhabditis elegans*	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	Q09508	70354.37	6.37	R.LGANSLLDLVIFGR.A
40	*Homo sapiens*	Isovaleryl-CoA dehydrogenase, mitochondrial	P26440	46289.67	8.45	R.NGNEAQKEK.Y
41	*Homo sapiens*	Glutamate-rich protein 1	Q86X53	48954.66	4.68	K.NPNNVLIEQAELEK.Q
42	*Rhamdia sapo*	Fatty acid-binding protein	P80856	13979.33	8.63	KDVKPVTEIQQTGNDFVITSKT
43	*Dictyostelium discoideum*	Putative actin-25	Q54HF0	43187.57	5.29	R.TAFPSIVGRPR.C
44	*Phaeosphaeria nodorum*	Nascent polypeptide-associated complex subunit alpha	Q0UKB5	23310.47	4.63	K.NILFVINQPDVYK.S
45	*Trypanosoma cruzi*	Heat shock-like 85 kDa protein	P06660	80706.63	5.07	K.AELVNNLGTIAR.S
46	*Neurospora crassa*	Guanine nucleotide-binding protein subunit beta-like protein	Q01369	35108.59	6.79	K.DGTTMLWDLNESK.H
47	*Plasmodium falciparum* (isolate 3D7)	Uncharacterized protein PFC0810c	O77374	132899.30	6.21	K.NVIELKEYLEDLK.K
48	*Pichia pastoris* (strain GS115)	Carboxypeptidase Y	P52710	59409.91	4.76	R.TGKNVYDIR.K
49	*Arabidopsis thaliana*	Malate dehydrogenase 1, mitochondrial	Q9ZP06	35781.88	8.54	K.LFGVTTLDVVR.A

RESULTS AND DISCUSSION

Construction of the Astragalus cinereum proteomic reference map: A method described protein extraction protocol (NP-40 buffer plus TCA-acetone precipitation and solubilization), optimized and employed for the preparation of protein extracts from *U. cinereum*. After 2-DE electrophoresis and silver nitrate staining of the gels, 49 protein were identified from *U. cinereum*. A reference proteomic map was constructed for analyzing the *U. cinereum* (Fig. 1). The molecular mass and the isoelectric points (pI) in the gel separated proteins varied between 20 and 250 kDa and pH 3 and 11, respectively. The 2-DE gel clearly illustrates the proteins that involved in biological processes such as carbohydrate metabolic process, oxidation-reduction process and nitrogen compound metabolic process.

Functional classification of the identified proteins: The functional of the protein is one of the central topics in cell biology. The proteins identified in the study were sorted into functional categories based on their annotations in

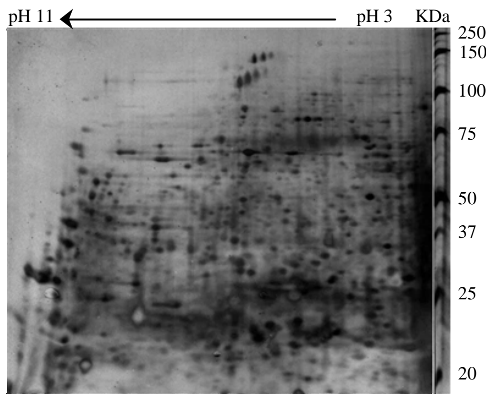

Fig. 1: 2-DE gel map of *U. cinereum* proteins. Proteins were separated in the first dimension on an 11 cm, 3-11 pI range IPG strip followed by SDS-PAGE separation on a 12% gel. For numbers of identified proteins (Table 1)

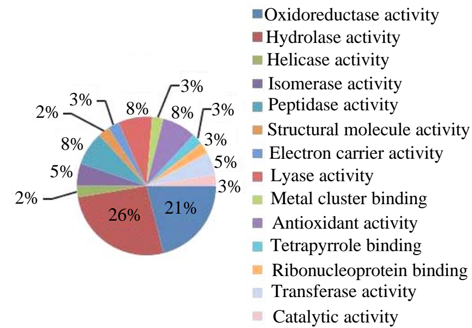

Fig. 2: Functional category of identify proteins. Sorting of the function of proteins was based on their annotations in the database

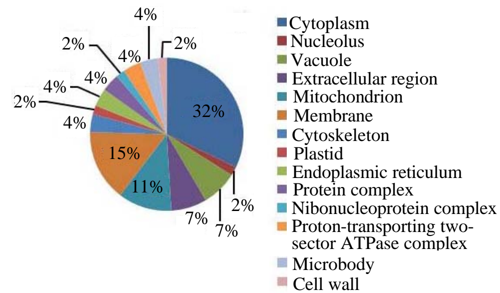

Fig. 3: Cellular component grouped of the identified proteins from *U. cinereum*

the database as shown in Fig. 2. Many of which were discovered based on hydrolase proteins and the oxidoreductase proteins. A total of 14 functional groups were categorized. This classification provides a general overview of the different types of protein detected in *U. cinereum*. Not surprisingly, Fig. 2 revealed that the majority of identified proteins and the large group (26%) have hydrolase activity. A portion of the enzymes (21%) had functions in oxidoreductase activity, 8% peptidase activity, 8% lyase activity and 8% antioxidant activity. Others 5% have the peptidase activity, 5% have the transferase activity and other kind of proteins (Fig. 2).

To determine the sub cellular localization of a protein, the identified proteins were categorized according to their cellular localization. Protein localization data are a valuable information resource helpful in elucidating the molecular mechanisms, cell biology and function. The results indicate that the 49 identified proteins were localized in *U. cinereum* to a wide variety organelles and intracellular structures including the cytoplasm, endoplasmic reticulum, vacuole, nucleolus, membrane, mitochondrion (Fig. 3). The identified proteins are categorized into fourteen types of cellular component. Among the identified proteins, 32% of *U. cinereum* proteins are cytoplasm, 15% membrane and 11% mitochondrion.

Organization of the identified proteins based on metabolic pathways revealed that the majority of the identified proteins were involved in oxidation-reduction process and response to stimulus. Approximately 11% of the identified proteins were involved in regulation of biological process. A portion of the identified proteins (11%) were involved in the carbohydrate metabolic process and 8% were involved in the alcohol metabolic process and involved in other process (Fig. 4).

To the knowledge, this is the first proteomic study of *U. cinereum* which resulted in the identification of 49 proteins. Since, no DNA or amino acid sequence information for *U. cinereum* in public databases, these identified protein data provide valuable information in *U. cinereum*. In the following paragraphs, researchers present the analysis of some of these identified proteins.

Formate Dehydrogenase (FDH), a soluble mitochondrial enzyme found in both prokaryotes and eukaryotes that catalyze the oxidation of formate to CO_2 (Colas *et al*., 1993; Andreadeli *et al*., 2009). FDH has already been reported from green leaf mitochondria (Halliwell, 1974). This enzyme from plants was first described in 1951 by Davison (1951). The immune detection and immune gold labeling experiment showed that the arabidopsis FDH was present in the chloroplast and target both the chloroplasts and mitochondria of leaf cells (Peeters and Small, 2001). FDH has been shown to be

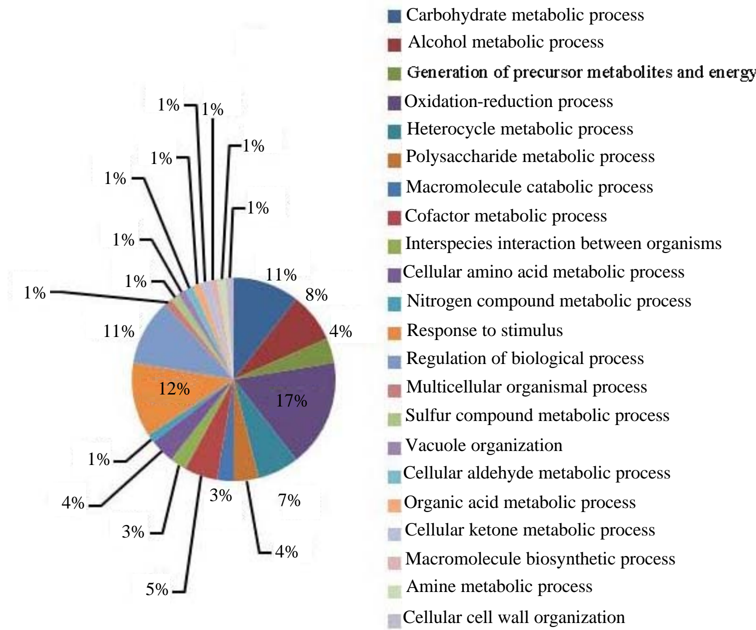

Fig. 4: Pie chart grouping the identified proteins from the 2-DE gels according to metabolic pathways they can be associated with

a shock protein and its transcript is strongly induced by a number of environmental stresses such as drought, hypoxia, low temperature, wounding, chilling and heat (Hourton-Cabassa *et al*., 1998; Suzuki *et al*., 1998; Thompson *et al*., 1998). The enzyme has also been found previously in animals (Mathews and Vennesland, 1950), *Candido boidinii* (Schutte *et al*., 1976) and from pea (Uotila and Koivusalo, 1979).

One protein isolated from the 2-DE gels was identified as glucose-6-phosphate isomerase. Glucose-6-Phosphate Isomerase (GPI) is a key enzyme in glycerol synthesis from glucose hydrolyzed to fructose-1, 6-diphosphate (Chen and Jiang, 2009). It has been shown to salt stress not only at the gene level but also at the protein level.

Fatty Acid Binding Proteins (FABPs) are a family of small and highly conserved cytoplasmic proteins which function as an energy transporter from mitochondria to high energy and involved in amino acid and fatty acid metabolism (Kucharski and Maleszka, 1998; Storch and Thumser, 2000).

Homoserine O-acetyltransferase is the first enzyme in the methionine biosynthetic pathway in several organisms such as in gram-positive bacteria of the genus *Bacillus*, fungi, yeast (Langin *et al*., 1986; Saint-Girons *et al*., 1988; Andersen *et al*., 1998). This enzyme catalyzes the transfer of acetyl group from acetyl-CoA to homoserine (Wang *et al*., 2007).

NADP-dependent mannitol dehydrogenase, a protein with a molecular mass of 28.7 kDa and pI of 5.86 is an important fungal allergen and similar to the apparent molecular mass of *C. herbarum* (Schneider *et al*., 2006). This enzyme catalyzes the NADPH-dependent conversion of both D-fructose reduction and D-mannitol oxidation. Two-dimensional protein analysis indicated that NADP-dependent mannitol dehydrogenase is present as a single isoform that might be suitable for improving diagnostic and therapeutic procedures (Simon-Nobbe *et al*., 2006). The characterization of this new protein has been suggested that has a role in fungal-plant and host-pathogen interactions (Stoop and Mooibroek, 1998).

Aspartate semialdehyde dehydrogenase has been expressed heterologously in *E. coli* and with a subunit molecular mass of 39 kDa (Hadfield *et al*., 2001). This enzyme involved in lysine biosynthetic which catalyzing the formation of L-2, 3-dihydrodipicolinate (Cahyanto *et al*., 2006) and the aspartate semialdehyde can be further reduced to homoserine which finally leads to methionine, threonine, isoleucine or with pyruvate and cylized into dihydrodipicoline to produce lysine (Hadfield *et al*., 2001). Early studies on the enzyme (Holland and Westhead, 1973; Biellmann *et al*., 1980) indicated that RNA binding by glyceraldehyde-3-phosphate dehydrogenase which catalyzes a similar reaction to Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (Faehnle *et al*., 2005). Aspartate semialdehyde

dehydrogenase is an essential enzyme found in many organisms such as bacteria, fungi and higher plants but absent in mammals. The aspartate pathway is responsible for the biosynthesis of L-lysine, aspartate semialdehyde dehydrogenase catalyze the initial reactions in the pathway and may be therefore serve as useful antibacterial or fungicidal agents.

Protein Disulfide-Isomerase (PDI) found in pI 4-5 are from the Endoplasmic Reticulum (ER) protein family (Zhang and Putti, 2010). PDI enzymes are found in the endoplasmic reticulum and other subcelluar compartments and act as molecular chaperones for protein folding, cell adhesion and DNA binding (Ferraro *et al*., 1999). Other reports found that the function of PDI are oxido-reductase, isomerases and chaperones (Ellgaard and Ruddock, 2005). Histone represents (pI 9-10, 10-11) a highly complex of mixture of proteins such as (H4, H2B, H2A and H3). Acetylation, phosphorylation, methylation, sumoylation and ubiquitination are commonly known modifications of lysine in histone (Jenuwein and Allis, 2001; Kouzarides, 2007). The synthesis of swainsonine in Metarhizium and Rhizoctonia was via pipecolic acid which was thought to be formed by the catabolism of L-lysine (Wickwire *et al*., 1990a, b; Sim and Perry, 1997). Histone has been applied in drug discovery and it has been shown that many cancers are accompanied by histone hypoacetylation (Mai *et al*., 2005). Histone Deacetylases inhibitors (HDACi) can alter the process of regulating gene expression and effect on tumors (Bolden *et al*., 2006). Researchers also identified many other proteins, the description and classifications for these are shown in Table 1 and Fig 2-4.

CONCLUSION

The main aim of this study was to develop a protein extraction method and obtain an overview of proteins from *U. cinereum*. To analyze protein expression in *U. cinereum*, researchers used 2-DE to detect, silver nitrate staining, LC-MS/MS to analyze and peptide sequencing followed by searching in database to identify the proteins. From the 2-DE profile, about 600 proteins spots were found. This is the first application of 2-DE on *U. cinereum* and such resolution of 2-DE protein profile has never been achieved in *U. cinereum* and this is found to be a useful approach for proteomic analysis of the *U. cinereum* and for both basic and applied research. The results of this study will enable further studies of gene expression and protein function analysis. It will also be a valuable tool to give an invaluable molecular basis for metabonomics approaches of this complex super molecule.

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