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Full Length Research Paper

Proteomic analysis of the endophytic fungus *Undifilum oxytropis*

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The filamentous Ascomycete fungus *Undifilum oxytropis* is an endophyte of locoweed plants of the genera *Oxytropis* that produces a toxic alkaloid swainsonine. Swainsonine, an alpha-mannosidase inhibitor causes a general toxicosis and neurological problems (locoism) when consumed by grazing animals. Swainsonine is also being assessed for its anti-cancer properties. While the ecology of *U. oxytropis* has been studied, little is known about the genetics and proteomics of any swainsonine-producing fungus. To help understand the proteins in *U. oxytropis*, the proteome of *U. oxytropis* was analyzed using 2-dimensional electrophoresis (2-DE). Proteins from *U. oxytropis* mycelia were extracted and separated by in-gel isoelectric focusing (IEF). The entire immobilized pH gradient (IPG) strip was cut into a set of gel sections and each gel section was digested with trypsin and then identified using liquid chromatography tandem mass spectrometry (LC-MS/MS). 2-DE maps were also developed for *U. oxytropis* to define its proteome. In the isoelectric point (pI) range of 3-11 and 10-250 kDa ranges, more than 450 spots were detected in 2-DE silver-stained gels, and 52 proteins were identified by LC-MS/MS. Most of the identified proteins were involved in energy production, oxidoreductase activity, carbohydrate metabolic process, amino acid and cellular ketone metabolic process. A large group of identified proteins were related to stress proteins and heat shock proteins. This work presents the first two-dimensional reference map of this alkaloid-producing fungus. Details of the proteome serve as a baseline for further study of this swainsonine-producing fungus and are essential for a reverse genetic analysis of the fungus.

Key words: *Undifilum oxytropis* fungus, two-dimensional gel electrophoresis, proteome reference map, liquid chromatography tandem mass spectrometry, swainsonine.

INTRODUCTION

Undifilum oxytropis is a recently described endophytic fungus isolated from *Oxytropis* spp. locoweed plants (Pryor et al., 2009; Yu et al., 2010). This fungus produces the alkaloid alpha-mannosidase inhibitor swainsonine, which causes the toxicosis locoism in grazing animals (Braun et al., 2003; James et al., 1981; James and Panter, 1989; Molyneux and James, 1982). *U. oxytropis* synthesizes swainsonine in plants and in culture (Pryor et al., 2009; Braun et al., 2003), and its toxic effects on rats is indistinguishable from toxicosis induced by locoweed consumption (McLain-Romero et al., 2004). Locoweeds are widely distributed poisonous plants in the western U.S. and China, leading to persistent toxicosis problems (Cook

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et al., 2009b; Ralphs and James, 1999). Because the swainsonine content in locoweed plants is highly variable, it is difficult to efficiently manage locoweeds with herbicides to prevent poisoning (Ralphs et al., 1988).

In addition to locoweeds, swainsonine has been isolated from the legume *Swainsona canescens* (Colegate et al., 1979) and from *Ipomoea carnea* Jacq (Molyneux et al., 1995). Swainsonine has also been isolated from the fungi *Rhizoctonia leguminicola* (Harris et al., 1988; Schneider et al., 1983) and *Metarhizium anisopliae* (Hino et al., 1985). Swainsonine is currently being tested as an anti-cancer drug with potential for treating glioma (Sun et al., 2009) and gastric carcinoma (Goss et al., 1994; Sun et al., 2007).

Unfortunately, little is known about the fungi that produce swainsonine. *U. oxytropis* is a slow growing Ascomycete that produces two hyphal types in culture (a compact basal layer and a layer of aerial filamentous hyphae) and septate conidia (Pryor et al., 2009). The fungus can be isolated from all aerial plant parts and appears to be maternally transferred through the seed coat (Oldrup et al., 2010). The fungus is hypothesized to exist in a mutualistic association with its locoweed host plant due to its conservation across *Oxytropis* species and geographical locations (Gardner et al., 2001; Ralphs et al., 2008; Yu et al., 2010). However, specifics about the fungus/plant interaction have proved difficult to determine, in part due to lack of genetic information about either the plant or the fungus (Cook et al., 2009a; Delaney et al., 2011). Knowledge of the genetics of the fungus is limited to a few regions such as ITS (internal transcribed sequence), gpd (glyceraldehyde phosphate dehydrogenase), and mtssu (mitochondrial small subunit) that have been used to taxonomically characterize the fungus (Braun et al., 2003; Pryor et al., 2009). A transformation system has been developed for the fungus and used to knockout a protein in the swainsonine catabolic pathway (Muhkerjee 2010; Muhkerjee et al. 2010). The lack of a genome sequence for any species of *Undifilum* has made genetic analysis of the fungus difficult. A reverse genetic approach was initiated to help address questions in growth, secondary metabolism, and host interaction. A first step toward this approach is identification of the fungal proteins. In our previous work, we developed methods for extracting the fungal proteins and separating them using 2-DE (Li et al., 2012). In this work, we identified and characterized proteins produced by *U. oxytropis*.

MATERIALS AND METHODS

Undifilum oxytropis isolate and culture conditions

U. oxytropis was isolated from leaves of *Oxytropis lambertii* plants collected from Winston, NM, USA in June 2000. The isolation was done as previously reported (Braun et al., 2003), and the fungus was grown on potato dextrose agar. The fungus was verified as *U. oxytropis* using morphological characteristics and PCR amplification as described in Oldrup et al. (2010). Cultures of the fungus (and the plants that they were isolated from) were assayed for the presence and concentration of swainsonine and used for toxicity studies with rats (McLain-Romero et al., 2004). Dried host plants and cultures of the fungus were subsequently deposited with the Center for Natural History Collections at New Mexico State University. Dried fungal specimens were stored at -20°C. For these studies, the fungus was added to potato dextrose broth and grown at 25°C with shaking (170 rpm) in 250-ml flasks with 12 h light for 1 month. Under these conditions, *U. oxytropis* grows slowly, producing only mycelium (no sporulation). The mycelium was rinsed with distilled water, lyophilized, and stored at -80°C until further processing.

Protein extraction

Proteins were extracted as described in Li et al. (2012). Briefly, fungal mycelium was ground in liquid nitrogen, extracted with trichloroacetic acid (TCA) in acetone containing 0.07% 2-mercaptoethanol, and precipitated overnight at -20°C. Proteins were concentrated by centrifugation and the pellet washed with cold acetone. The residual acetone was removed by lyophilization. The pellet was resuspended in IEF buffer (Sinha and Chattopadhyay, 2011). Insoluble material was removed by centrifugation. Samples were frozen in liquid nitrogen and stored at -80°C for further processing.

Protein concentration was determined using the Bradford method (Bradford, 1976). Concentration of protein in each sample was calculated against a standard curve of bovine serum albumin.

2-DE and gel analysis

IPG strips (18 cm, 3-11 linear pH gradients, GE Healthcare) were rehydrated for 20 h with 100 µg of protein in 340 µl rehydration solution volume per IPG Strip. IEF was carried out at 20°C with a current of 50 µA/strip in an Ettan IPGphor isoelectric focusing unit (Amersham Biosciences). The following protocol was applied: 500 v for 3 h, 1000 v for 3 h, 8000 v for 4 h and 8000 v for 2 h. After IEF, one IPG strip was used for the second dimension; the other IPG strip was cut into sections for protein digestion. As previously described (Li et al., 2012), IPG strips used for 2-DE were loaded onto 12% polyacrylamide gels in Tris-glycine buffer (Laemmli, 1970). The 2-DE gels were visualized by silver staining as described by Shevchenko et al. (1996) with minor variations. After electrophoresis, the gel was fixed (50% methanol, 5% acetic acid in water) overnight, and incubated for 1 min in 0.02% sodium thiosulfate. The gel was submerged in 0.1% silver nitrate and incubated for 1 h, then developed in 0.04% formaldehyde in 2% sodium carbonate with shaking. Gels were stored in distilled water at 4°C.

In-gel digestion of proteins and sample preparation for mass spectrometry (MS) analysis

The samples were digested into peptides before introduction into the mass spectrometer. In-gel IEF, digestion was performed as described (Giorgianni et al., 2003) with slight modifications. The IPG strip was cut into 8 gel sections (each section about 1 cm in length), and dehydrated with acetonitrile (ACN). Each gel section was reduced using dithiothreitol, and further dehydrated with 200 µl ACN for 20 min. The gel sections were rehydrated in 200 µl of 100 mM ammonium bicarbonate for 30 min. The sections were dehydrated with ACN for 5 min, and then digested with 50 µl of 20 ng/µl sequencing-grade modified trypsin overnight at 37°C. The peptides produced by the digestion were extracted once with 50 mM ammonium bicarbonate and twice with 50% [v/v] ACN in 5% [v/v] formic acid (FA). The volume of the extract was reduced to <20 µl by

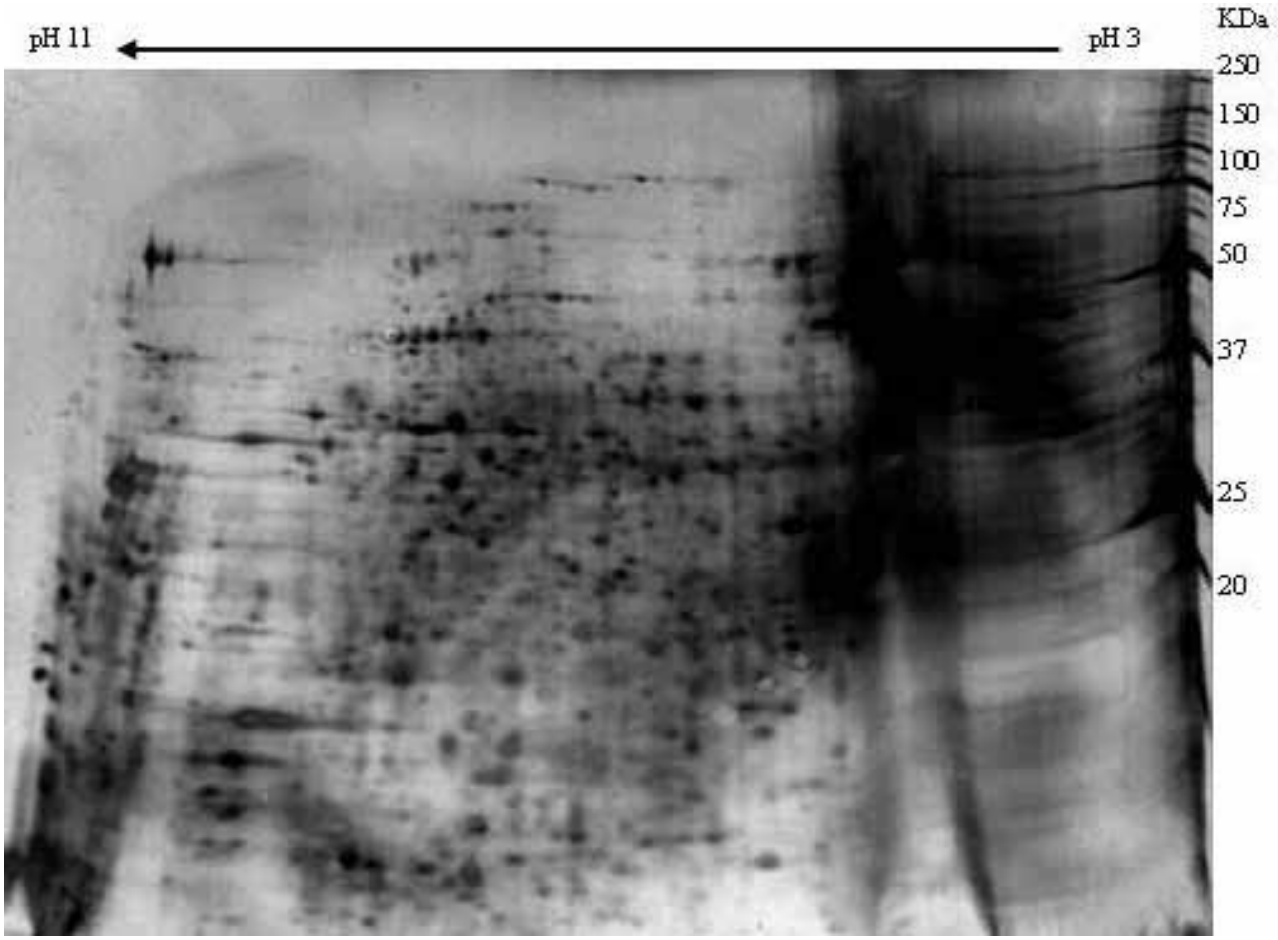

Figure 1. 2-DE gel map of *U. oxytropis* proteins. The pH of the isoelectric focusing gel ranging from 3 to 11 is shown on the top of each gel. Molecular weight standards used to estimate the M_r of induced proteins are shown on the right of the gel.

evaporation and stored at -20°C until later analysis by LC-MS/MS.

Tandem mass spectrometry and database searching

Samples were digested into peptides and proteins were identified directly from the complex mixtures. The peptides were sequenced using tandem mass spectrometry, and the computer used the patterns of sample peptides to determine the sequence of the peptide. This sequence information was used to search against commercially available databases. Tryptic peptides were analyzed via liquid chromatography (Agilent 1100) coupled to a Thermo Linear ion trap Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer. The peptides were infused into a hybrid linear ion trap FT-ICR mass spectrometer (LTQ-FT, Thermo, San Jose, CA) through a micro electrospray ion source from an ultra-performance liquid chromatograph (UPLC, Acquity, Waters Corp., Milford, MA). Samples were separated using a 40 min linear gradient from 95% Solvent (0.1% formic acid in water)/Solvent Ⅱ (0.1% formic acid in acetonitrile) to 70% Solvent / Ⅱ with a 1.0 × 150 mm 5 µm reversed phase trapping column (Zorbax, 300SB-C18). Throughout the gradient, FT-ICR mass spectra were collected at a mass resolving power of 100,000 (m/z = 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 (Hwang et al., 2006). Mass spectral data files were searched against the Swiss Prot fungi database for protein identification with the Bioworks browser. Protein identifications with an xcorr value > 2 were selected.

RESULTS

Proteome map of *U. oxytropis*

A reference proteome map was constructed for analyzing the *U. oxytropis* proteomic profile (Figure 1). Several hundred spots where detected where most protein spots were concentrated in the pI range of 3 to 11 and 20 to 100 kDa ranges.

Protein identification and classification

Through 2-DE and LC-MS/MS, 52 proteins were clearly identified (Table 1). While many peptides were obtained from each gel slice, only a few statistically significant proteins were identified. Data listed in Table 2 include IEF pH range, total number of peptide, number of peptide

Table 1. List of the proteins identified in *U. oxytropis* by LC-MS/MS.

Range	Protein name	Organism	Accession number	Protein MW	Protein pI	P Value	Peptide sequence
pI 3-4	Calmodulin	*Ajellomyces capsulata*	P60206	17001.9	4.0	7.59E-99	KVFDRDNNGFISAAELRH
	U6 snRNA-associated Sm-like protein LSm6	*Phaeosphaeria nodorum*	Q0UWI9	9414.6	4.4	6.61E-88	KGDLQSVDGYMNIALERC
	Pre-mRNA-splicing factor cwc2	*Schizosaccharomyces pombe*	P87126	44253.8	5.4	2.09E-40	MSENGLEQEVTVEEKNNDVTEKILVEGEK
	Transposon Ty4-H Gag-Pol polyprotein	*Saccharomyces cerevisiae*	P0C2J7	207839.0	8.4	2.14E-33	G.KGNIADLLTKP
	Heat shock 70 kDa protein	*Emericella nidulans*	Q5B2V1	69873.8	4.9	1.86E-31	RLSKEDIERM
pI 4-5	Protein dopey	*Schizosaccharomyces pombe*	Q9C0Z4	192530.0	5.6	6.03E-31	YPDPVKDYACMDVFIAKL
	Heat shock protein 60, mitochondrial	*Ajellomyces capsulata*	P50142	61850.7	5.6	1.20E-106	RTALVDASGVASLLGTTEVAIVEAPEEKG
	Enolase	*Alternaria alternata*	Q9HDT3	47176.2	5.0	2.75E-99	KVNQIGTITEAIQAAKD
	Minor allergen Alt a 7	*Alternaria alternata*	P42058	22042.0	5.1	1.41E-68	KLFQVAETLPQEVLDKM
	Protein disulfide-isomerase	*Alternaria alternata*	Q00002	46246.4	5.1	1.66E-68	KLVTIAKV
	ATP synthase subunit beta, mitochondrial	*Kluyveromyces lactis*	P49376	54035.5	5.0	1.51E-52	KVALVFGQMNEPPGARA
	Histone H2B	*Agaricus bisporus*	P78567	15156.2	10.6	8.51E-52	HPDTGISNKAMAILNSFVNDIFERI
	Alcohol dehydrogenase 3, mitochondrial	*Saccharomyces cerevisiae*	P07246	40344.1	8.5	7.59E-48	KLPLVGGHEGAGVVVKLG
	Heat shock protein 60, mitochondrial	*Paracoccidioides brasiliensis*	O60008	62094.8	5.4	3.55E-46	KATPDMLGSTGSITITKE
	Alcohol dehydrogenase 2	*Candida albicans*	O94038	36784.1	6.3	5.75E-44	KLPLVGGHEGAGVVVALG.E
	Woronin body major protein	*Neurospora crassa*	P87252	19114.6	6.5	6.17E-43	RLGDILILQGRP
pI 5-6	Probable NADP-dependent mannitol dehydrogenase	*Alternaria alternata*	P0C0Y4	28716.3	5.8	5.13E-109	KVVIVTGASGPTGIGTEAARG
	Enolase	*Alternaria alternata*	Q9HDT3	47176.2	5.0	2.00E-89	KVNQIGTITEAIQAAKD
	Minor allergen Alt a 7	*Alternaria alternata*	P42058	22042.0	5.1	1.00E-65	KLFQVAETLPQEVLDKM
	Heat shock 70 kDa protein	*Ajellomyces capsulata*	Q00043	77510.1	5.4	2.45E-61	KFADPEVQADMKH
	Tubulin alpha chain	*Encephalitozoon hellem*	P92120	42453.2	7.1	8.32E-60	RIIAQVVSSITASLRF
	Tubulin alpha-1 chain	*Emericella nidulans*	P24633	50327.4	4.8	8.32E-60	RLIAQVVSSITASLRF
	Actin-1	*Pneumocystis carinii*	P43239	41876.9	5.3	2.14E-51	KEITALAPSSMKI
	Actin	*Pichia pastoris*	Q9P4D1	41664.8	5.2	2.14E-51	KELTALAPSSMKV
	Probable formate dehydrogenase	*Emericella nidulans*	Q03134	41549.4	6.3	1.51E-50	G.LLGTTENELGIRK
pI 6-7	Minor allergen Alt a 7	*Alternaria alternata*	P42058	22042.0	5.1	3.02E-63	KLFQVAETLPQEVLDKM

Table 1. Contd

	Enolase	*Cryphonectria parasitica*	Q6RG04	47330.6	5.1	2.88E-59	RSGETEDVTIADIVVGIRA
	Enolase	*Alternaria alternata*	Q9HDT3	47176.2	5.0	2.88E-59	RSGETEDVTIADIVVGLRS
	Pyruvate decarboxylase	*Aspergillus fumigatus*	Q4WXX9	62959.2	6.1	4.27E-38	RKPVDVAEYLFRR
	Heat shock protein 60, mitochondrial	*Ajellomyces capsulata*	P50142	61850.7	5.6	5.62E-36	RTALVDASGVASLLGTTEVAIVEAPEEKG
pI 7-8	ATP synthase subunit beta, mitochondrial	*Kluyveromyces lactis*	P49376	54035.5	5.0	4.47E-60	KTVFIQELINNIAKA
	Minor allergen Alt a 7	*Alternaria alternata*	P42058	22042.0	5.1	1.58E-58	KLFQVAETLPQEVLDKM
	Pyruvate decarboxylase	*Aspergillus fumigatus*	Q4WXX9	62959.2	6.1	4.47E-48	RKPVDVAEYLFRR
	ATP-dependent RNA helicase dbp8	*Aspergillus fumigatus*	Q4WXW1	57034.3	9.8	8.51E-33	RVGRQMEEWSEEGVSIEGRLVR
pI 8-9	Probable NADP-dependent mannitol dehydrogenase	*Alternaria alternata*	P0C0Y4	28716.3	5.8	1.00E+00	KVVIVTGASGPTGIGTEAARG
	Enolase	*Cryphonectria parasitica*	Q6RG04	47330.6	5.1	1.70E-109	NPTVEVDLVTETGLHRA
	Enolase	*Alternaria alternata*	Q9HDT3	47176.2	5.0	4.90E-86	NPTVEVDIVTETGLHRA
	Serine/threonine-protein kinase plo1	*Schizosaccharomyces pombe*	P50528	77253.1	9.3	4.90E-86	KIGPETKPVPS*KLSTALHAA
	Enolase	*Tuber borchii*	Q6W3C0	47901.9	5.3	1.86E-39	VLKAVENVNTIIAPALIKE
	Uncharacterized protein YGR117C	*Saccharomyces cerevisiae*	P53270	53404.3	6.9	8.32E-38	RFKTLSINDDLAPIDNAKYG
pI 9-10	ADP, ATP carrier protein	*Neurospora crassa*	P02723	33866.7	10.2	4.68E-82	RYFPTQALNFAFRD
	Probable NADP-dependent mannitol dehydrogenase	*Alternaria alternata*	P0C0Y4	28716.3	5.8	5.75E-81	KVVIVTGASGPTGIGTEAARG
	Histone H2A.1	*Ashbya gossypii*	Q74ZL4	13856.6	11.0	2.24E-79	AVLEYLAAEILELAGNAARD
	Woronin body major protein	*Neurospora crassa*	P87252	19114.6	6.5	6.92E-52	RLGDILILQGRPCQVIRI
	Histone H4.1	*Ashbya gossypii*	Q757K0	11331.4	11.8	1.82E-51	KTVTSLDVVYALKR
	Histone H2B.1	*Ashbya gossypii*	Q74ZL5	14275.6	10.6	5.13E-46	RLILPGELAKH
	ADP,ATP carrier protein	*Kluyveromyces lactis*	P49382	33073.3	10.1	5.25E-44	KLLIQNQDEMIKQ
	ADP,ATP carrier protein 3	*Saccharomyces cerevisiae*	P18238	33291.4	10.2	5.25E-44	KILIQNQDEMIKQ
	Uncharacterized protein YOL036W	*Saccharomyces cerevisiae*	Q08206	84300.7	9.6	3.31E-42	PTLSTSSTFESDLNARQPMLIRR
pI 10-11	Histone H2B.1	*Ashbya gossypii*	Q74ZL5	14275.6	10.6	9.12E-48	LNSFVNDIFERI
	60S ribosomal protein L11	*Neurospora crassa*	Q7RVN0	20071.6	10.6	2.88E-47	KVLEQLSGQTPVYSKA
	Histone H2A.1	*Ashbya gossypii*	Q74ZL4	13856.6	11.0	3.80E-40	AVLEYLAAEILELAGNAARD

Table 2. Peptide database coverage of current fungi proteome database.

IEF pH range	Total number of peptide MS2	Number of peptides with a database ID	% Coverage of current database
pH 3-4	126	30	23.8
pH 4-5	109	50	45.9
pH 5-6	77	27	35.1
pH 6-7	70	24	34.3
pH 7-8	52	16	30.8
pH 8-9	51	29	56.9
pH 9-10	73	35	47.9
pH 10-11	57	14	24.6

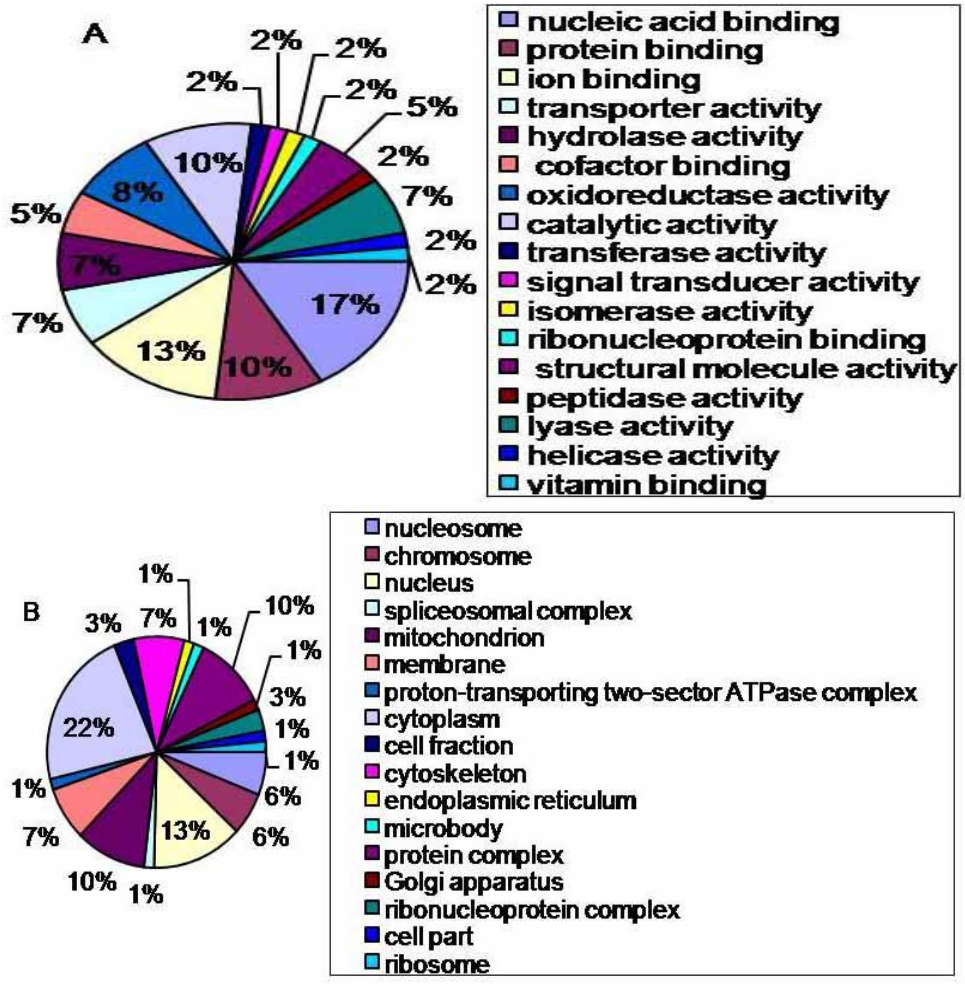

Figure 2. Classification of identified proteins from *U. oxytropis*: (A) functional category; (B) cellular component; (C) biological process.

matched, and percentage sequence coverage.When the protein information resource (PIR) ontology was used to classify proteins according to their molecular function category, cellular component and biological process, 17 functional groups were identified. Of these proteins, 17% were related to nucleic acid binding, 13% to ion binding, 8% with oxidoreductase activity, 7% to hydrolase activity, and 5% to lyase activity (Figure 2A).

The proteins identified were categorized into eighteen groups based on predicted cellular location. Most of the proteins were localized in the cytoplasm (22%), nucleus (13%), mitochondria (10%), and protein complex (10%) (Figure 2B). Identified proteins were also classified into 38 categories on the basis of the biological process in which

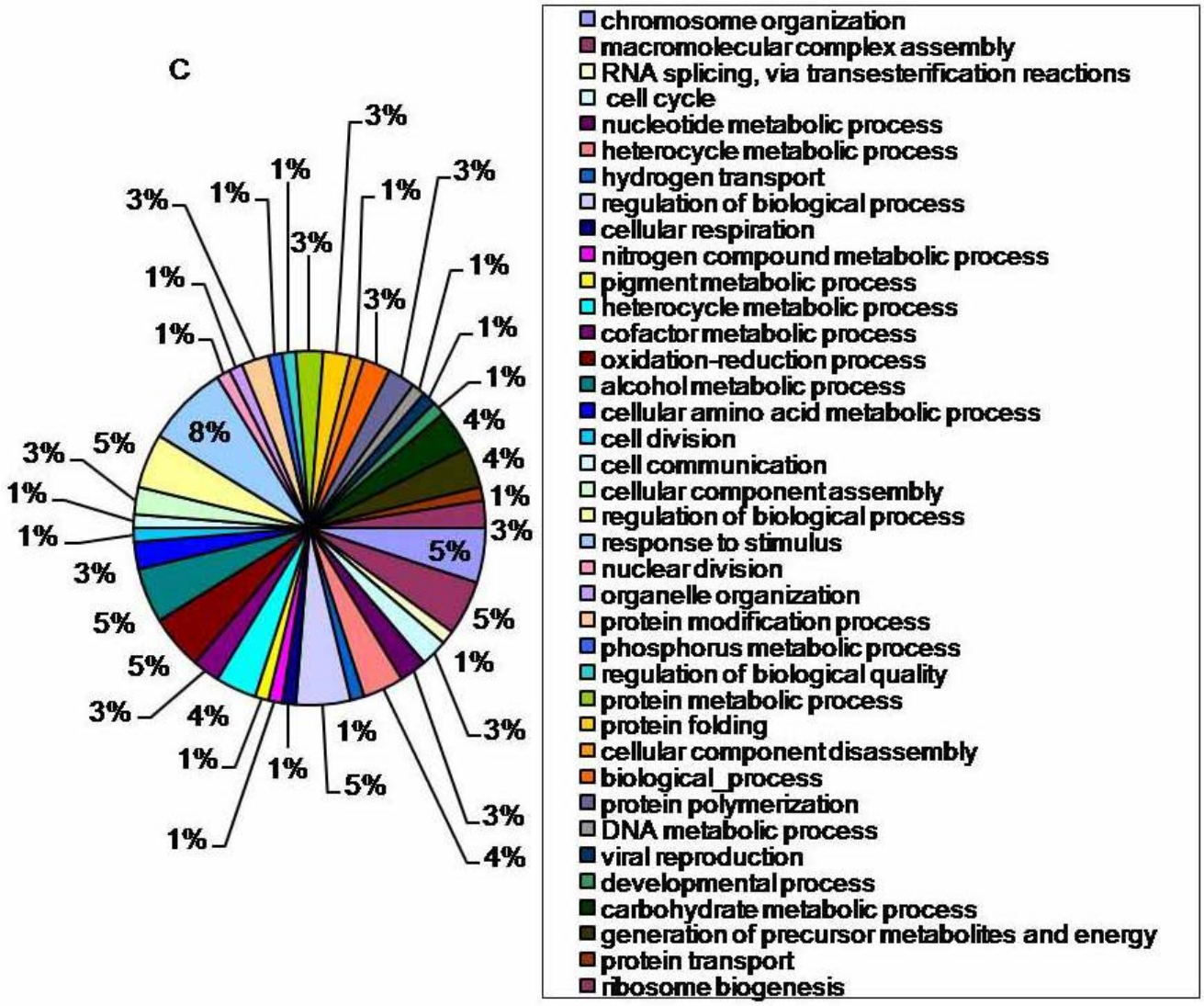

Figure 2. Contd.

they are involved (Figure 2C). Of these 8% was related to response to stimuli, 5% to oxidation-reduction processes, 5% to regulation of biological processes, 5% to organelle organization, and 5% to alcohol metabolic processes.

DISCUSSION

The analysis of *U. oxytropis* proteins from digested IPG strips was successful in identifying many diverse proteins. Compared to the approximately 450 protein spots that were visible when the IPG strips were used for two-dimensional (2-D) gel electrophoresis, only a small proportion, 52 proteins were clearly identified (Table 1). The lack of genomic data and protein sequences for *U. oxytropis* caused difficulty in matching the MS^2 spectra to known proteins. However, compared to the very few proteins that were previously identified from 2-DE gels when each spot had to be individually excised from the gel prior to trypsin digestion (Li et al., 2012), the method outlined here was much more efficient for identification of large numbers of proteins.

As expected, proteins encoded by genes that have been previously characterized for *U. oxytropis* were identified including the Alt a 1 allergen (Hong et al., 2005), beta-tubulin (Braun et al., 2003), and glyceraldehyde 3-dehydrogenase (Pryor et al., 2009). Saccharopine dehydrogenase, known to be involved in the swainsonine metabolic pathway in *U. oxytropis* (Mukherjee, 2010), was not detected in this analysis, however, it is known to be a minor protein involved primarily in the secondary metabolic pathway.

Some identified proteins were detected repeatedly in each section of the IPG strip. Heat shock proteins were

the most abundant proteins found in pI range of 3-4, 4-5, 5-6 and 6-7. Enolase was also abundant, found in pI range of 4-5, 5-6, 6-7 and 8-9. The 52 identified proteins were categorized according to different ontology (Figure 2).

Heat shock proteins (HSPS) encompasses a large number of proteins that can be grouped into several families based on their molecular weight of ~19 to ~110 kDa, many of which are involved in environmental stress response, such as heat, including HSPS 90, HSPS 70 pI 3-4, 5-6, and HSPS 60 pI 4-5, 6-7. HSPS have different molecule functions and act in many aspects of cell biology. Major HSPS are involved in cell growth and differentiation (Mehlen et al., 1997), physiological and cytoprotective functions (Bukau and Horwich, 1998) and play an important role in the survival of extreme environmental conditions (Ulmasov et al., 1992). Functions of HSPS in *U. oxytropis* remain to be elucidated, but current research suggests that the fungus may help moderate plant response to drought, heat, and nutrient deficiencies (Delaney et al., 2011; Oldrup et al., 2010). Enolase is a major glucan-associated cell wall protein (Angiolella et al., 1996; Pitarch et al., 2002). Actin, which was identified in pI 5-6 proteins, are sorted into the cytoskeleton grouping (Qinghua et al., 2006; Shevchenko et al., 2005), and is known to contribute to cell polarity (Ahren et al., 2005).

Swainsonine is synthesized in *Metarhizium* and *Rhizoctonia* via pipecolic acid, which is produced by the catabolism of L-lysine (Sim and Perry, 1997; Wickwire et al., 1990a, b). Although all the intermediates in the swainsonine biosynthetic pathway are not known, suspected enzymes include oxido-reductases, polyketide synthase, and cytochrome P450. The proteome analysis identified multiple proteins of all of these types, although many are not well characterized. Two polyketide synthases (PKS7 and PKS2) and seven cytochrome P450 enzymes were identified as minor components. Protein disulfide-isomerase (PDI) found in pI range of 4-5 is found within the endoplasmic reticulum (ER) protein family (Zhang and Putti, 2010). PDI enzymes act as molecular chaperones for protein folding, cell adhesion and DNA binding (Ferraro et al., 1999). Other reports found that PDIs act as oxido-reductases, 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).

We compared the identified proteins with other similar fungi such as *Metarhizium anisopliae*, which is a insect pathogen, of which some isolates can produce swainsonine (Tamerler et al., 1998) and *Alternaria* sp. which are closely related plant pathogens that produce toxins (Lawrence et al., 2008). The whole genome of *M. anisopliae* has been analyzed and its number of encoded secreted proteins is remarkably large (Gao et al., 2011). For *U. oxytropis*, the most identified proteins (that is, Enolase, Minor allergen Alt a 7, Histone H4.1, U6 snRNA-associated Sm-like protein LSm6, Pre-mRNA-splicing factor cwc2, Heat shock protein 60, ATP synthase subunit beta, Histone H2B, Alcohol dehydrogenase 2, Tubulin alpha chain, Actin, Pyruvate decarboxylase, 60S ribosomal protein L11, etc.) showed significant similarity to both *M. anisopliae* and *Alternaria brassicicola* proteins in the database.

This study will serve as a base line study for this agriculturally and medically important fungus. The proteome data and the approach described here will be used to study the differential expression of proteins between swainsonine producing and nonproducing species of *Undifilum* to identify enzymes active in the swainsonine biosynthesis pathway. In addition, this protein data will be used in a reverse genetics approach to develop primers to search for the genes in the pathway. The differential expression of proteins between plant pathogenic and mutualistic *Undifilum* species may provide insight into the nature of plant pathogenesis as it has for pathogenic and saprophytic *Alternaria* species (Lawrence et al., 2008). Because of the large losses caused by locoism, the poorly understood interaction between these swainsonine-producing fungi and locoweed plants, and the lack of a genome sequence for the fungus, any insight into the proteins produced by these fungi are particularly valuable.

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Abbrebviation

2-DE, 2-Dimensional electrophoresis; **IEF,** in-gel isoelectric focusing; **LC-MS/MS,** liquid chromatography tandem mass spectrometry; **IPG,** immobilized pH gradient; **pI,** isoelectric point.

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