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Proteomics: an efficient tool to analyze nematode proteins

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Soybean cyst nematode (*Heterodera glycines*, SCN) is the most destructive pathogen of soybean (*Glycine max* (L.) Merr.), and is responsible for estimated annual yield losses of $457 to $819 million (2003-2005) in the United States (5). Although plant parasitic nematodes can sometimes be controlled using chemicals, the vast acreage dedicated to soybean in the U.S. eliminates these methods as environmentally and economically unfeasible for the control of SCN. Crop rotation and planting of soybean cultivars resistant to some SCN populations are two approaches commonly used to reduce SCN damage. However, resistant soybean varieties are not always the highest yielding varieties. Furthermore, soybean varieties may be resistant to some SCN populations, but not to others. There are fourteen different SCN races in the U.S. that have been identified. The other popular means of SCN control is crop rotation. However, when soybean is not grown in a field, another crop of equal value must be found and substituted, which is not always practical. Therefore, new methods of SCN control need to be investigated including genetically engineered soybean with resistance to all SCN populations. To achieve these goals, understanding of SCN proteins is important. There are six stages of the nematode's life cycle, namely egg, four juvenile (J1 - J4), and adult. Among them, the second-stage juvenile (J2) is important since it hatches from the egg, moves through the soil, and infects by penetrating host plant roots to become parasitic J2. Therefore, we used J2 stage of nematode for our proteomic study. Proteomic technologies are powerful tools for characterizing proteins from complex biological mixtures that are part of biological research on varied fronts, from evolutionary investigations to cancer research. Recent advances in mass spectrometry, the establishment of various protein databases, and availability of high-throughput methods increased the adoption of these technologies by the scientific community. Sample extraction and preparation are always critical for the analysis of proteins, and these are highly dependent upon the source of the sample. In this investigation, we used protein expression profiling using two-dimentional polyacrylamide gel electrophoresis (2D-PAGE). This technique separates proteins according to two independent properties in two discrete steps. The first step is called isoelectric focusing that separates proteins according to their isoelectric points; the second dimension, SDS-PAGE, separates proteins according to their molecular weights. The separated protein spots were digested using trypsin and purified before analysis of mass spectrometry. The resulting data were analyzed using bioinformatic tools to extract information from different databases. Based on probability scores, the proteins were identified by comparing them with known proteins.

Soybean cyst nematode was grown at the United States Department of Agriculture, Beltsville, MD according to Klink *et al*. (4). The second stage juvenile nematodes were used for protein analysis. Proteins were extracted with a modified phenol extraction procedure (3). The nematode protein samples (100-600µg) were separated in the first dimension using 13cm (pH 3-10) Immobiline IPG Drystrips (GE Healthcare). We used 12.5% SDS-PAGE gels

for 2nd dimensional gel electrophoresis. After staining the gels, we randomly selected some protein spots for MS.

In our laboratory, we evaluated three different methods, phenol/ammonium acetate (phenol method), thiourea/urea solubilization (lysis method) and trichloroacetic acid/acetone (TCA method), and selected the phenol extraction procedure as an efficient method based on the higher protein resolution and spot intensity to extract soybean J2 nematodes compared to other methods (1). In this study, we compared four different protein amounts, 100 µg, 200 µg, 400 µg, and 600 µg of SCN proteins to separate and analyze both abundant and low abundant proteins by 2D-PAGE. Among these four amounts (Figure 1-A, 1-B, 1-C, and 1-D), we observed that 400 µg (Figure 1-C) of protein was a suitable amount for separating a large number of both abundant and low abundant SCN proteins. The first dimension was run using a pH gradient from 3.0-10.0. The second dimension was a 12% SDS-PAGE. To identify proteins following 2D-PAGE, spots were manually picked from Coomassie-stained gels, digested with trypsin and analyzed by MS. We analyzed ten gel regions to determine the quality and concentration effect on mass spectrometry identification. These regions are boxed in Figure 1 and labeled 1 to 10. The identified proteins were actin (1), Unc-87(2), As-37 (3), galactoside-binding lectin family protein(4), probable glutathione S-transferase gst-36 (5), glutathione S-transferase-1(6), glutathione peroxidase (7), myosin regulatory light chain (8), *Caenorhabditis briggsae* CBR-UNC-60 protein and superoxide dismutase (9), and immunoglobulin I-set domain containing protein (10). Important protein information, such as assigned group ID number, theoretical isoelectric point (pI), and molecular weight (Mr), protein identity, number of peptides matched, percentage sequence coverage, MOWSE score, and accession number of the best match for the identified protein spots are listed in Table 1. This investigation demonstrates that 400 to 600 µg protein is better for the separation of both low and high abundant proteins in terms of spot intensity as well as total number of spots, whereas 100 and 200 µg protein concentration were suitable for the analysis of high abundant proteins. Therefore, we used 400 µg protein concentrations for our further investigations. We have isolated 803 SCN protein spots, digested with trypsin and further identified 426 proteins using mass spectrometry (2). Using the Gene Ontology, the identified proteins were further annotated different functional categories. The results of our investigation showed that proteins involved in metabolic, developmental and biological regulation process were the most dominant (2). Currently, we are investigating the soybean interaction with the SCN, and compared protein expression profiles of soybean roots infected by the SCN. The results will be useful to scientists who wish to develop nematode resistant soybeans.

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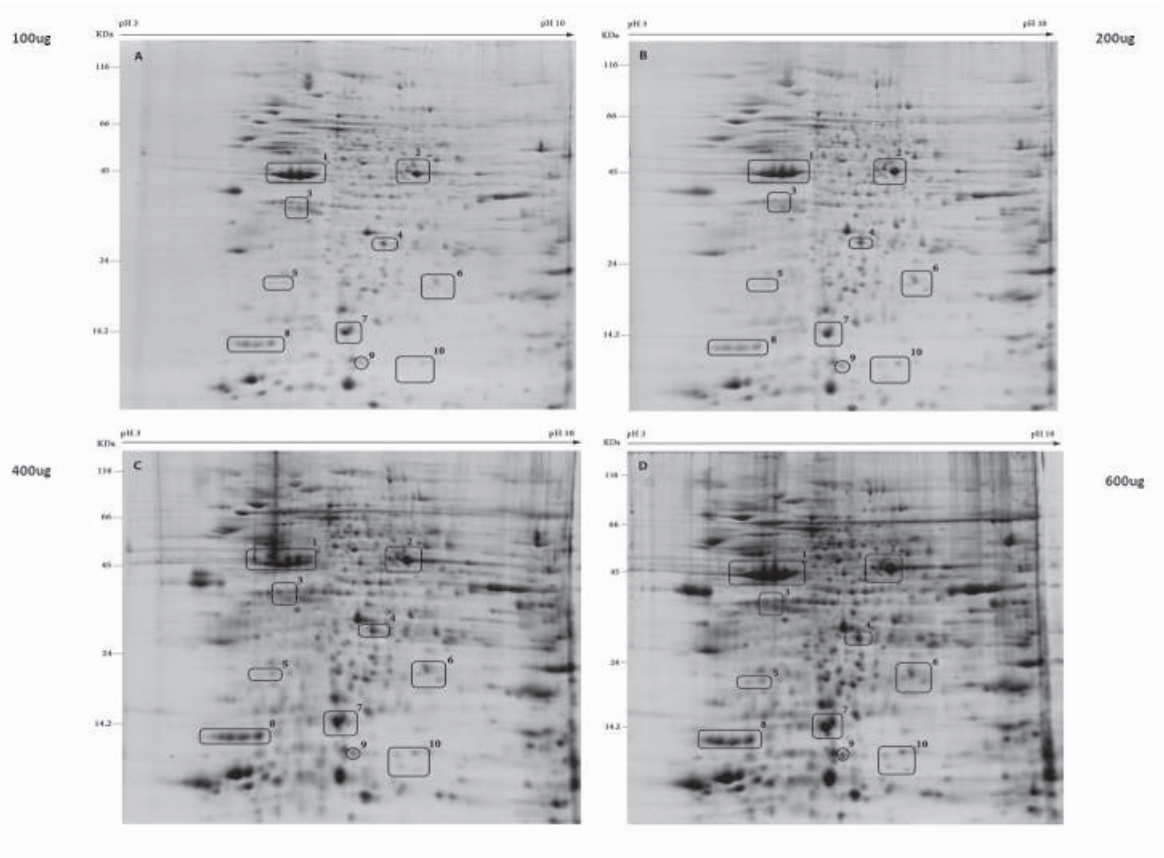

Fig. 1 (A-D) Two-*dimensional electrophoretograms of SCN proteins*

Literature Cited

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Table 1.
Proteins identified from soybean cyst nematode

Group ID	Protein Name	Calc pI/Mr	MOWSE score	# PM	% SC	gi #
1	Actin	5.30/42150	1408	20	64%	18314323
2	Unc-87	7.04/41827	453	7	20%	50086703
3	As-37	4.98/35750	492	7	23%	22036079
4	Galactoside-binding lectin family protein	8.83/35803	514	10	38%	170581205
5	Probable Glutathione S-transferase gst-36	5.88/23876	377	7	31%	21542413
6	Glutathione S-transferase-1	6.76/23716	609	12	54%	126009882
7	Glutathione peroxidase	5.53/19788	356	7	31%	21739127
8	Myosin regulatory light chain	5.36/18989	497	9	43%	17530145
9	- *C. briggsae* CBR-UNC-60 protein	6.84/32562	179	4	16%	268566209
	- Superoxide dismutase	6.30/12917	129	2	15%	256857922
10	Immunoglobulin I-set domain containing protein	6.11/22472	472	9	47%	170587487

Group ID numbers correspond to the proteins labeled in Figure 1; Calc pI/Mr, theoretical isoelectric point (pI) and molecular weight, (Mr); MOWSE score, represents results from searches performed by Mascot; #PM, number of peptide matched; % SC, percent sequence coverage; gi #, database NCBI accession number of the best match.