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Comparison of proteome and antigenic proteome between two *Neospora caninum* isolates

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Abstract

This study was conducted to explore the relationship between two isolates of *Neospora caninum* (*N. caninum*) (KBA-2 and VMDL-1) using proteomics. To achieve the goal, proteins of *N. caninum* tachyzoite lysates of KBA-2 and VMDL-1 were separated by two-dimensional gel electrophoresis (2-DE), stained with silver-nitrate and analyzed using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) to compare protein profiles. In addition, proteins separated by 2-DE were transferred to membranes, probed with bovine anti-*N. caninum* KBA-2 immunoglobulin G, and reactive proteins were visualized and compared between the two isolates. Most spots on 2-DE profiles and antigenic spots on 2-DE immunoblot profiles were located at similar locations in terms of isoelectric point and molecular weight. Proteins common to both isolates included the following: heat shock protein 70, subtilisin-like serine protease, nucleoside triphosphatase, heat shock protein 60, pyruvate kinase, tubulin α, tubulin β, enolase, putative protein disulfide isomerase, actin, fructase-1,6-bisphosphatase, putative ribosomal protein S2, microneme protein Nc-P38, lactate dihydrogenase, fructose-1,6-bisphosphatase aldolase, serine threonine phosphatase 2C, 14-3-3 protein homologue, *N. caninum* dense granule-1 and NcGRA2. As a consequence, even though *N. caninum* KBA-2 and VMDL-1 isolates were isolated from geographically distinct locations there were significant homology in the proteome and antigenic proteome profiles. In addition, proteomic approach was verified as a useful tool for understanding of host immune response against different isolates of protozoa.

Keywords: *Neospora caninum*; Proteomics; Immunoblot; Proteome comparison

Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; MW, molecular weight; p*I*, isoelectric point

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1. Introduction

Neospora caninum (*N. caninum*) has become an important pathogen of the livestock industry, especially cattle (Dubey, 2003). Pregnant cattle with neosporosis suffer from abortion and stillbirths. On the other hand, no clinical signs have been reported in non-pregnant adult cattle with neosporosis (Anderson et al., 2000; Dubey, 2003). In Korea, bovine neosporosis was first reported in 1997 (Kim et al., 1997). The parasites were isolated in congenitally infected calf and aborted fetus in Korea, and the pathogens were named KBA-1 and KBA-2 isolates, respectively (Kim et al., 1998, 2000).

With the evolution of whole organism genomic sequencing projects, proteomics has become an important area of research (Liebler, 2002). Among its usefulness are characterizing genes and proteins function, understanding functional linkages between protein molecules, thus providing valuable informations regarding the mechanisms of biological processes (Jungblut et al., 1999; Zhu et al., 2003).

Investigation of immunodominant antigens as vaccine candidates may improve efficacy of vaccines. To achieve this goal, understanding host immune response against pathogen through the analysis of pathogen proteins is a critical step (Hanash, 2003). A large number of investigations have used proteomics combined with two-dimensional gel electrophoresis (2-DE) with immunoblot analysis of antigenic proteins, which are useful tools for understanding immunological processes of host against pathogens' proteins (Klade, 2002).

A number of reports have been published about the biological, the morphological, and the molecular features of isolates of *N. caninum* originated from a variety of worldwide-distributed hosts, especially dogs and cattle (Schock et al., 2001; Gondim et al., 2004; Dubey et al., 2004). These reports indicate that *N. caninum* isolates have similarities as well as differences. However, there is limited analysis of the parasite proteome to evaluate the relationships between isolates, but improved methods for protein characterization using 2-DE followed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) provide additional means of characterizing these isolates. In this study, proteomic analysis was carried out to evaluate the relationship between *N. caninum* KBA-2 and VMDL-1 isolates to address the presence of potential differences in protein expression by the different isolates.

2. Materials and methods

If not stated otherwise, all the reagents were purchased from Sigma (St. Louis, MO, USA).

2.1. Parasite

N. caninum isolates KBA-2 (Kim et al., 2000) and VMDL-1 (Hyun et al., 2003) tachyzoites were maintained according to Lee et al. (2003). Tachyzoites of each isolate were purified (Yamane et al., 1998) and stored at −70 °C until use.

2.2. Tachyzoites preparation for 2-DE

Purified tachyzoites (approximately 1×10^8) were dissolved in 40 mM Tris-base, disrupted by freeze-thaw cycles, and then sonicated (XL-2020, Misonix Inc. Farmingdale, NY, USA). The dissolved tachyzoites were lysed in lysis buffer (2.2 M thiourea, 7.7 M urea, 40 mM Tris-base, 4% (w/v) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 1% (w/v) dithiothreitol (DTT), 0.5% (v/v) Triton X-100 and 0.5% (v/v) immobilized pH gradient (IPG)-buffer pH 4–7 (Amersham Bioscience, Uppsala, Sweden)) and the lysates were maintained in ice slurry for 1 h. Protein concentrations of the 2-DE samples were estimated using the Bradford protein assay kit (Biorad, Hercules, CA, USA).

2.3. Preparation of anti-N. caninum KBA-2 serum from cow

A cow (Holstein, female) was intra-venously inoculated with 1×10^8 live tachyzoites of the KBA-2 isolate to produce anti-*N. caninum* KBA-2 polyclonal antibodies. Prior to inoculation, the donor was confirmed as *N. caninum* and *Toxoplasma gondii* negative by IFAT (Baszler et al., 2001; Osawa et al., 1998). The cow was provided care according to the guidelines for the care and use of laboratory animals developed by Gyeongsang National University, Republic of Korea. Serum was collected 13 weeks post-inoculation and stored at −20 °C until use. Serum

antibody titers to *N. caninum* and *T. gondii* were evaluated as 1:800 and <1:100 by indirect fluorescent antibody test, respectively.

2.4. 2-DE and its immunoblot

Isoelectric focusing (IEF) was performed using an IPGphor™ system (Amersham Bioscience) according to Görg et al. (2000), with IPG strips (Immobiline DryStrip™, pH 4–7, 0.5 mm × 3 mm × 130 mm; Amersham Bioscience). The prepared tachyzoites 2-DE samples were mixed with a rehydration buffer (2 M thiourea, 7 M urea, 2% (w/v) CHAPS, 0.4% (w/v) DTT, 0.5% IPG buffer, 0.002% (w/v) bromophenol blue) and then focused for a total of 86.1 kVh. After IEF, the IPG strips were subjected to 10% sodium dodecyl sulfate-polyacrylamide gels (160 mm × 160 mm × 1 mm) and run under 10 mA/gel. Immunoblot was performed according to Mansfield (1995). Briefly, each *N. caninum* isolate lysate separated by 2-DE was transferred onto polyvinyldene fluoride (PVDF) membrane (Immobilon-P™, 0.45 mm; Millipore, Billerica, MA, USA) and incubated with anti-KBA-2 serum at 1:1 dilution ratio with 5% (w/v) skim milk in phosphate buffered saline. The dilution ratio between serum and horseradish peroxidase (HRP)-conjugated anti-bovine

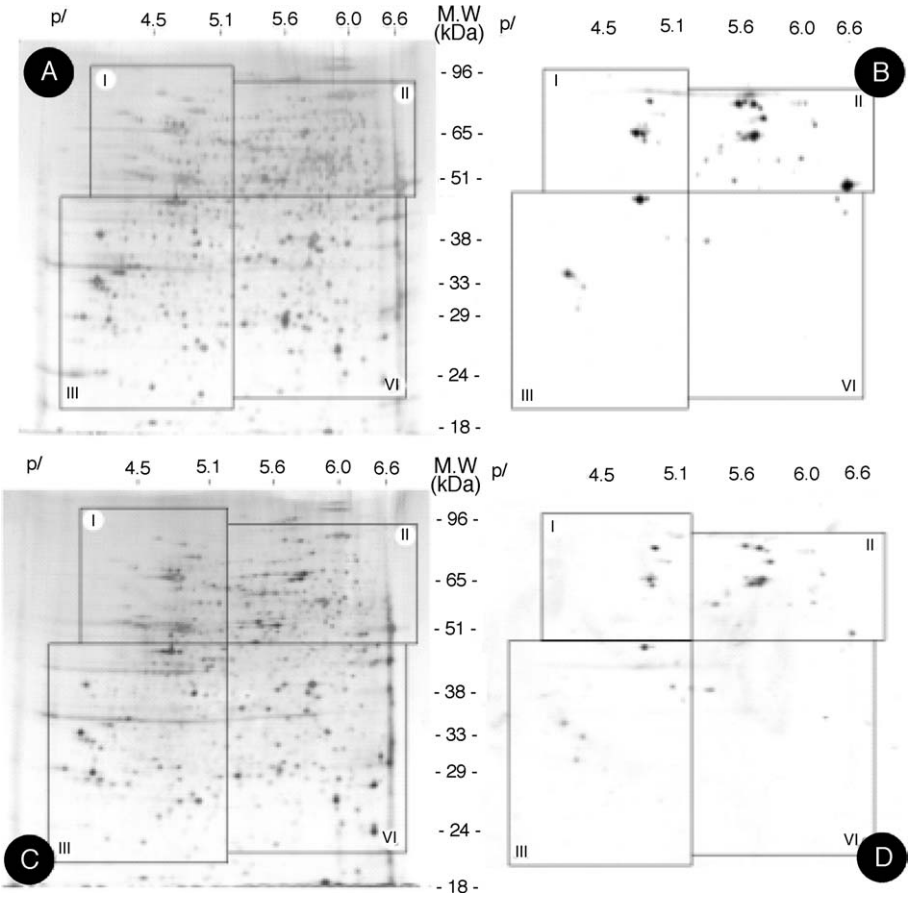

Fig. 1. Two-dimensional gel electrophoresis (2-DE) and its immunoblot profiles of *N. caninum* KBA-2 and VMDL-1. Isoelectric focusing was performed at 86.1 kVh using pH 4–7 immobilized pH gradient strips (13 cm) with 60 μg of lysate from *N. caninum* isolates. SDS-PAGE was performed on a 10% gel then stained with silver-nitrate. Each *N. caninum* isolate lysate separated by 2-DE was transferred onto polyvinyldene fluoride (PVDF) membrane and incubated with anti-KBA-2 serum. Antibody binding was determined using enhanced chemiluminescence solution kit. A, 2-DE profile of *N. caninum* KBA-2; B, 2-DE immunoblot profile of *N. caninum* KBA-2; C, 2-DE profile of *N. caninum* VMDL-1; D, 2-DE immunoblot profile of *N. caninum* VMDL-1; ※, Each profile was divided into four different parts for further analysis (Figs. 2–5).

immunoglobulin G (IgG) as reagents were basically titrated by the Checker-Board method and established the proper ratio (Crowther, 1995). HRP-conjugated anti-bovine IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) at 1:2000 was used as the secondary antibody. Antibody binding was determined using enhanced chemiluminescence solution (ECL™: Amersham Bioscience) for 1 min, exposed to X-ray film (Fuji, Tokyo, Japan), and developed.

2.5. Protein visualization and identification

Silver-nitrate staining of gels, image analysis, in-gel digestion of protein spots on gels, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis, and database searches (Ms-Fit; The Mass Spectrometry Facility, the University of California San Francisco) were performed according to Lee et al. (2003). Gel staining, image analysis, and protein identification were each performed a minimum of three times, and several minor spots were not analyzed. Spots with p*I* and MW values within 5–10% of each other were regarded as having the same p*I* and MW (Jungblut and Thiede, 1997). In addition, only sequences that contained at least five matching peptides that comprised at least 15% of the protein sequence were selected (Mann et al., 2001).

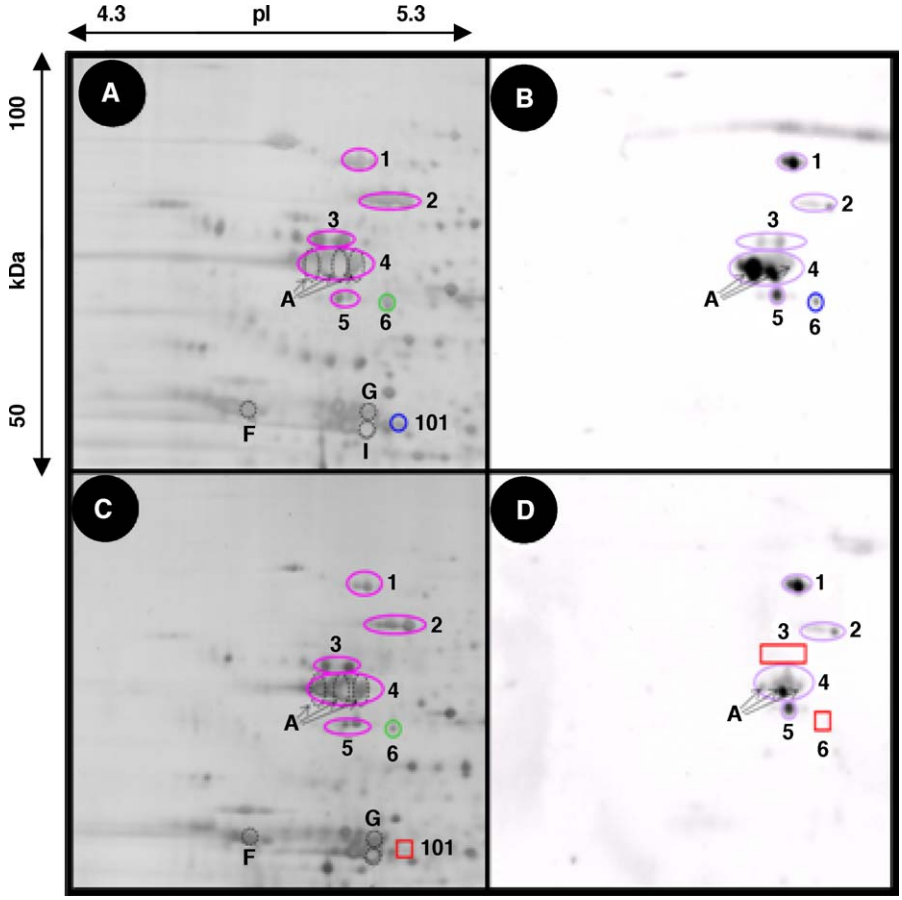

Fig. 2. Proteome and antigenic proteome profiles of part I on Fig. 1 ranged p*I*s between 4.3 and 5.2 and MWs from 48 to 100 kDa. Most spots stained with silver-nitrate were found to locate in similar places between the isolates. In the immunoblot profiles, spots recognized as immunoreactive also showed high similar locations between isolates. A, proteome profile of *N. caninum* KBA-2; B, antigenic proteome profile of *N. caninum* KBA-2; C, proteome profile of *N. caninum* VMDL-1; D, antigenic proteome profile of *N. caninum* VMDL-1.

3. Results

For ease and detailed analysis, the overall profiles of *N. caninum* KBA-2 and VMDL-1 (Fig. 1) were divided into four different parts (Figs. 2–4), and potentially important proteins were indicated using circular or cubic marks. Specifically, circles and cubics indicated the existence and the non-existence of spots between the two isolate profiles, respectively. Alphabet letters on the profiles indicate spots that were identified by MALDI-TOF MS that correspond to a known protein.

3.1. Proteome profiles of N. caninum isolate KBA-2 and VMDL-1

Approximately 540 and 520 spots were observed on the silver-nitrate stained gel using pH 4–7, 13 cm IPG strip of *N. caninum* isolate KBA-2 and VMDL-1, respectively. To identify their respective protein names, some spots indicated on the profiles were examined by MALDI-TOF MS, and 28 protein spots corresponding to 19 different proteins were successfully identified by peptide mass fingerprinting (PMF) analysis. The identified protein spots (alphabet letters A–S) were marked onto the gel image as shown in Figs. 2–5 and listed in Table 1. Table 2 lists spots' identification numbers exclusively observed for one isolate but not both via 2-DE analysis.

3.2. Antigenic proteome profiles of N. caninum isolate KBA-2 and VMDL-1

Approximately 53 and 46 spots were detected as antigenic spots on 2-DE immunoblot profiles of KBA-2 and VMDL-1, respectively. Among these, 13 spots corresponding to 7 different proteins, such as heat shock protein 70 (HSP70), subtilisin-like serine protease, nucleoside triphosphatase (NTPase), HSP60, enolase, actin and *N. caninum* dense granule-1 (NCDG-1), were successfully identified and corresponded with proteins in the MALDI-TOF MS analysis. The antigenic spots were marked onto the profiles as shown in Figs. 2–5 and listed in Table 3.

3.3. Comparison of N. caninum isolate KBA-2 and VMDL-1 profiles

Fig. 2 shows proteome and antigenic proteome profiles of part I setting in the range of p*I*s 4.3 and 5.2, with MWs ranging from 48 to 100 kDa. In the 2-DE

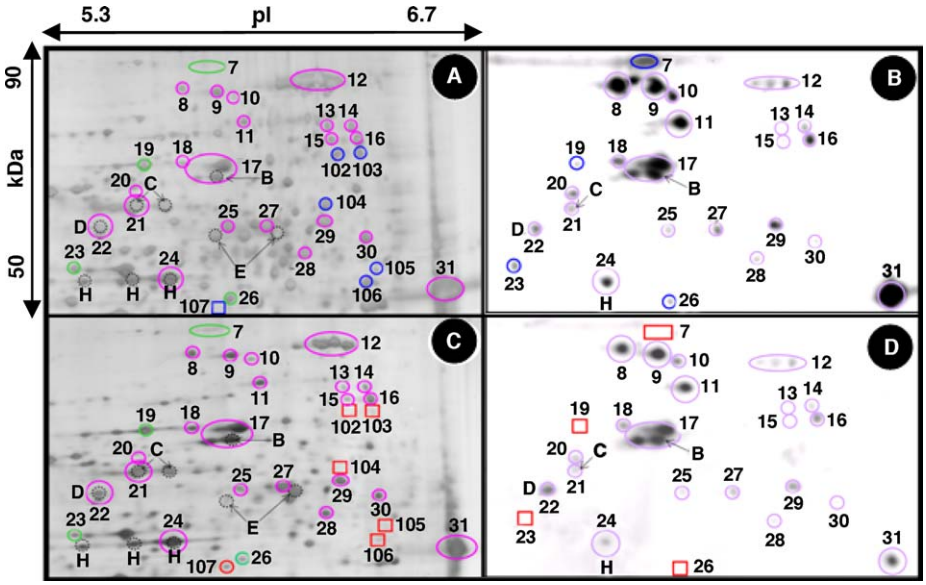

Fig. 3. Proteome and antigenic proteome profiles of part II on Fig. 1. The range of the map was setting between p*I*s 5.3 and 6.7 with the MWs ranging from 50 to 90 kDa. Spots were observed at the same locations in both isolates of silver-nitrate staining profiles and the same patterns were also demonstrated in the immunoblot profiles. A, proteome profile of *N. caninum* KBA-2; B, antigenic proteome profile of *N. caninum* KBA-2; C, proteome profile of *N. caninum* VMDL-1; D, antigenic proteome profile of *N. caninum* VMDL-1.

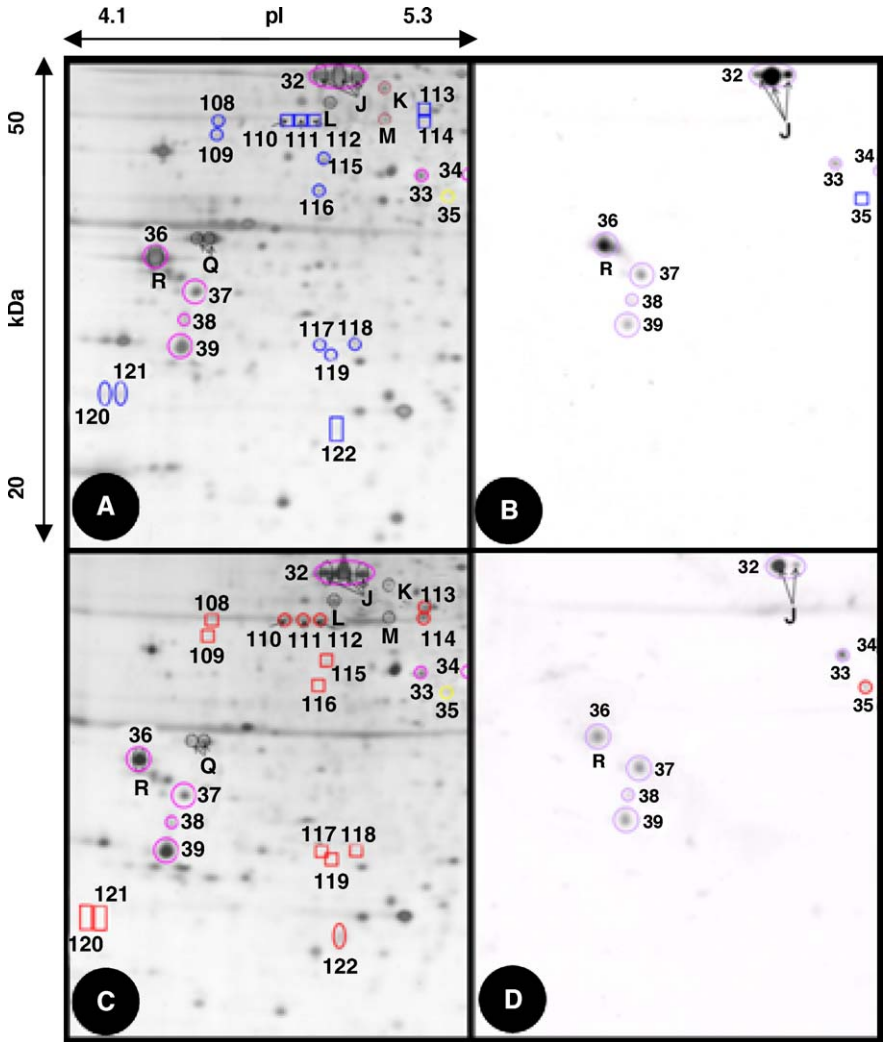

Fig. 4. Proteome and antigenic proteome profiles of part III on Fig. 1. The map was located on the range of p*I*s between 4.1 and 5.2 with the MWs ranging from 20 to 48 kDa. Most spots on 2-DE and the corresponding immunoblot profiles were exhibited to have the same location (p*I* and MW) between isolates. A, proteome profile of *N. caninum* KBA-2; B, antigenic proteome profile of *N. caninum* KBA-2; C, proteome profile of *N. caninum* VMDL-1; D, antigenic proteome profile of *N. caninum* VMDL-1.

profiles (Fig. 2A and C), most spots including HSP 70 (spot A), tubulin α-chain (spot G), tubulin β-chain (spot F) and putative protein disulfide isomerase (spot I) were found to locate in similar places between the isolates. However, only spot no. 101 was present on 2-DE profile of KBA-2. In the immunoblot profiles (Fig. 2B and D), spots recognized as immunoreactive (spot nos. 1–5) also showed high similar locations between isolates but differences were noticed, such as spot no. 6 was only exhibited on immunoblot profile of KBA-2. Spot nos. 4 and 5 on KBA-2 were more strongly reacted as antigenic spots probed with bovine anti-*N. caninum* KBA-2 IgG.

Proteins in between p*I*s 5.3 and 6.7 with MWs in the range 50–90 kDa (Fig. 3) were identified as follows: subtilisin-like serine protease (spot B), NTPase (spot C), HSP60 (spot D), pyruvate kinase (spot E) and enolase (spot H). Other unidentified spots

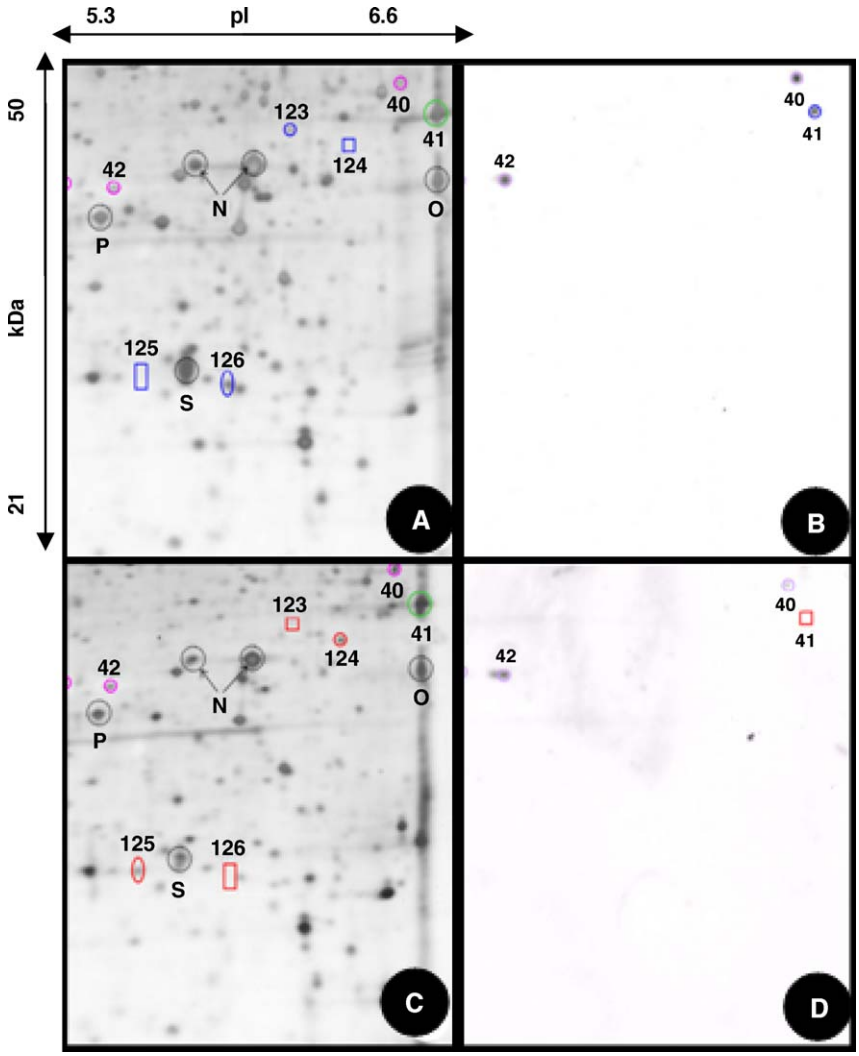

Fig. 5. Proteome and antigenic proteome profiles of part IV on Fig. 1. At the range of p*I*s between 5.3 and 6.6 with MWs between 21 and 50 kDa were focused. The locations of the spots presented also showed high similarities between KBA-2 and VMDL-1. A, proteome profile of *N. caninum* KBA-2; B, antigenic proteome profile of *N. caninum* KBA-2; C, proteome profile of *N. caninum* VMDL-1; D, antigenic proteome profile of *N. caninum* VMDL-1.

were observed at the same locations in both isolates (Fig. 3A and C), and the same patterns were also demonstrated in the immunoblot profiles (Fig. 3B and D). Of the spots on 2-DE profiles, spot nos. 102–106 were only detectable with KBA-2 2-DE profile, whereas spot no. 107 was present in VMDL-1 isolate but was not present in KBA-2. In the immunoblot profiles, there were similarities and differences between isolates. Spot nos. 7, 19, 23, 24 and 26 were only detected in KBA-2, whereas spot no. 22 was only detected in VMDL-1. Spot nos. 8–11, 16–18, 29 and 31 were present in both isolates.

As seen in Fig. 4, in the range between p*I*s 4.1 and 5.2 with the MWs ranging from 20 to 48 kDa (part III), most spots on 2-DE and the corresponding immunoblot profiles were exhibited to have the same location (p*I* and MW) between isolates. Of the spots on 2-DE profiles, spot nos. 108, 109 and 115–121 were only detected in KBA-2, and spot nos. 110–114 and 122 were only detected in VMDL-1. Of the spots on the immunoblot

Table 1
Protein spots identified on the two-dimensional gel electrophoresis (2-DE) profiles of *Neopora caninum* (*N. caninum*) KBA-2 and VMDL-1 after 2-DE followed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis

Spot no.	Protein name	No. of matched peptides	Sequence coverage (%)	Theoretical[a]		Measured[a]		Accession no.[b]	Species
				MW (Da)	p*I*	MW (Da)	p*I*		
A	Heat shock protein 70 (HSP70)	13	22	70,627	5.2	69,998	4.81	11277111	*T. gondii*
A	HSP70	12	23	70,627	5.2	69,716	4.88	11277111	*T. gondii*
A	HSP70	11	23	70,627	5.2	69,364	4.91	3850197	*T. gondii*
B	Subtilisin like serine protease	9	13	93,650	5.4	67,713	5.76	6119851	*N. caninum*
C	Nucleoside triphosphatase (NTPase)	13	20	68,688	5.4	64,123	5.52	3298332	*N. caninum*
C	NTPase	13	23	68,688	5.4	64,076	5.61	3298332	*N. caninum*
D	HSP60	15	30	61,014	5.8	61,336	5.42	5052052	*T. gondii*
E	Pyruvate kinase	9	22	57,530	6.0	61,100	5.94	13928580	*T. gondii*
E	Pyruvate kinase	9	19	57,530	6.0	60,036	5.76	13928580	*T. gondii*
F	Tubulin β	20	32	50,060	4.7	54,290	4.69	135499	*T. gondii*
G	Tubulin α	10	27	50,114	5.0	53,301	4.99	135439	*T. gondii*
H	Enolase	7	19	48,291	5.7	53,081	5.63	12619316	*T. gondii*
H	Enolase	7	24	48,291	5.7	53,027	5.52	12619316	*T. gondii*
H	Enolase	7	20	48,291	5.7	52,697	5.37	12619316	*T. gondii*
I	Putative protein disulfide isomerase	9	27	52,802	5.1	52,807	4.99	14494995	*T. gondii*
J	Actin	18	51	41,908	5.0	46,324	4.88	1703160	*T. gondii*
J	Actin	19	47	41,908	5.0	45,940	4.83	1703160	*T. gondii*
J	Actin	14	40	41,908	5.0	45,830	4.94	1703160	*T. gondii*
K	Fructase-1,6-bisphosphatase	10	34	42,398	6.1	43,753	5.02	21715907	*T. gondii*
L	Putative ribosomal protein S2	9	39	31,512	5.2	41,165	4.85	22035888	*T. gondii*
M	microneme protein Nc-P38	17	44	38,084	5.4	39,700	5.02	6606507	*N. caninum*
N	Lactate dihydrogenase	4	24	35,549	6.0	38,331	5.67	1695772	*T. gondii*
N	Lactate dihydrogenase	13	51	35,549	6.0	38,331	5.85	1695772	*T. gondii*
O	Fructose-1,6-bisphosphatase aldolase	15	36	39,097	7.6	37,946	6.42	25989716	*T. gondii*
P	Serine threonine phosphatase 2C	9	37	36,791	5.4	35,891	5.38	27817640	*T. gondii*
Q	14-3-3 Protein homologue	11	32	30,650	4.8	34,167	4.46	3023191	*N. caninum*
Q	14-3-4 Protein homologue	9	34	30,650	4.8	34,147	4.50	3023191	*N. caninum*
R	*N. caninum* dense granule-1 (NCDG-1)	7	33	22,495	4.6	33,182	4.33	3023896	*N. caninum*
S	NcGRA2	8	33	22,418	8.6	28,600	5.64	11066176	*N. caninum*

[a] Molecular weights (MW) and iso-electric points (p*I*) were calculated as an average value ($n = 3$).
[b] No. of protein sequence database hosted by the national center for biotechnology information (NCBI), USA.

profiles, spot nos. 32, 36–39 were present in both, whereas spot no. 35 was only present in VMDL-1 (Fig. 4B and D).

In the range of p*I*s between 5.3 and 6.6 with MWs ranging from 21 to 50 kDa as shown in part IV (Fig. 5), the locations of the spots presented also showed high similarities between KBA-2 and VMDL-1. In this figure, spot nos. 123 and 126 were only shown with KBA-2, and spot nos. 124 and 125 were only found with VMDL-1. Antigenic spot no. 41 was expressed on KBA-2 immunoblot profile. There were fewer protein spots recognized by bovine antiserum in this section of the immunoblot (Fig. 5B and D) as compared with other immunoblot profiles.

4. Discussion

Identifying molecular, structural and antigenic proteins between pathogens is very important in epidemiological studies as well as developing diagnostic methods and vaccines. Proteomic analysis

Table 2
Spots only observed two-dimensional gel electrophoresis (2-DE) profiles either *Neopora caninum* (*N. caninum*) KBA-2 or VMDL-1

Spot no.	MW (Da)[a]	p*I*[a]	Spot no.	MW (Da)	p*I*	Spot no.	MW (Da)	p*I*
101	52,642	5.06	110	39,554	4.72	119	28,292	4.86
102	71,478	6.12	111	39,554	4.77	120	26,736	4.17
103	71,760	6.19	112	39,554	4.81	121	26,720	4.22
104	64,360	6.09	113	40,l417	5.14	122	25,272	4.87
105	54,588	6.23	114	39,235	5.14	123	39,501	5.96
106	52,697	6.21	115	38,225	4.84	124	38,890	4.09
107	49,785	5.78	116	36,794	4.82	125	28,261	5.49
108	39,620	4.52	117	28,804	4.83	126	27,999	5.77
109	39,076	4.51	118	28,896	4.92			

[a] Molecular weights (MW) and iso-electric points (p*I*) were calculated as an average value ($n = 3$).

using 2-DE, 2-DE with immunoblot analysis combined with MALDI-TOF MS analysis provides powerful, highly specific and sensitive method for proteins and antigenic proteins analysis (Klade, 2002; Seliger and Kellner, 2002). In this study, these techniques were used to evaluate differences between *N. caninum* isolates, KBA-2 and VMDL-1 isolated from geographically distinct regions.

According to several reports, there have been little biological or genetic differences detected among isolates of *N. caninum* (Atkinson et al., 1999; Schock et al., 2001). This conservation between *N. caninum* isolates suggests this parasite is not as diverse as other related parasites such as *T. gondii* (Howe and Sibley, 1999; Innes et al., 2000). However, recently published result found small differences among *N. caninum* isolates specifically within the internal transcribed spacer 1 (ITS1) region sequence among isolates derived from several regions: Brazil, North America and Europe (Gondim et al., 2004). This report indicate the need for comparisons of additional *N. caninum* isolates in terms of biological, genetic and antigenic studies using methods with extremely fine resolution capabilities.

Table 3
Antigenic spots found on the two-dimensional gel electrophoresis (2-DE) immunoblot profiles of *Neopora caninum* (*N. caninum*) either KBA-2 or VMDL-1, and both

Spot no.	MW (Da)[a]	p*I*[a]	Spot no.	MW (Da)	p*I*	Spot no.	MW (Da)	p*I*
1	83,034	4.99	10	82,259	5.82	26	50,225	5.80
1	83,105	4.97	11	77,679	5.84	27	61,761	5.91
1	83,175	4.94	12	85,360	6.03	28	57,218	6.03
2	78,242	4.99	12	85,289	6.00	29	62,328	6.07
2	78,172	5.01	13	76,763	6.09	30	59,785	6.20
2	78,172	5.04	14	76,692	6.16	31	51,378	6.42
2	78,102	5.07	15	74,296	6.10	32	46,324	4.88
3	72,605	4.87	16	74,155	6.18	32	45,940	4.83
3	72,253	4.93	17	69,364	5.79	32	45,830	4.94
4	69,998	4.81	17	67,713	5.76	33	37,636	5.13
4	69,716	4.88	17	67,572	5.71	34	37,718	5.31
4	69,646	4.85	18	69,787	5.66	35	36,692	5.21
4	69,364	4.91	19	69,646	5.55	36	33,182	4.33
4	69,364	4.96	20	66,533	5.52	37	31,452	4.45
5	66,013	4.92	21	64,123	5.52	38	30,119	4.42
6	65,635	5.03	22	61,336	5.42	39	28,730	4.41
7	84,655	5.70	23	54,713	5.35	40	40,110	6.32
8	83,739	5.66	24	53,081	5.63	41	40,656	6.41
9	83,246	8.76	25	61,667	5.79	42	37,390	5.42

[a] Molecular weights (MW) and iso-electric points (p*I*) were calculated as an average value ($n = 3$).

In the comparison of the two isolates, most spots on 2-DE profiles and antigenic spots on 2-DE immunoblot profiles were found at similar p*I* and MW between the two isolates. In contrast, there were limited numbers of proteins observed solely in either KBA-2 or VMDL-1 by 2-DE and the companion immunoblot. These protein spots were regarded as isolate-specific spots. Approximately, 17 spots and 8 immunoreactive spots were found in KBA-2, and 6 spots and 1 immunoreactive isolate-specific spots were found in VMDL-1, respectively, but these spots were not identified to proteins within the existing database. Unfortunately, present study used only anti-KBA-2 serum as a probe against isolates, since we could not obtain anti-VMDL-1 serum. Previously, Lee et al. (2005) reported that *N. caninum* isolate KBA-2 and JPA-1 (Yamane et al., 1997) showed high protein similarity between isolates, but the proteome and antigenic proteome analysis showed clear differences when *T. gondii* was included in the comparison. The *N. caninum* isolates, KBA-2 and VMDL-1, were isolated from geographically distinct regions, Republic of Korea and the USA, respectively. Nonetheless, this study was able to suggest that there were similarity between *N. caninum* isolates at the proteome and antigenic proteome level but potentially some individual differences.

A number of examinations have been conducted to explore immunodominant proteins in neosporosis. These reports mentioned that the immunodominant proteins could be utilized for efficient diagnostic markers (Lally et al., 1996; Louie et al., 1997; Nishikawa et al., 2001a; Howe et al., 2002; Ahn et al., 2003) and vaccine candidates (Nishikawa et al., 2001b; Cannas et al., 2003a,b; Liddell et al., 2003). In this study, spot nos. 8–11, 16–18, 29, and 32 of the spots unidentified were commonly recognized as immunodominant antigens on the immunoblot profiles in both isolates. In the case of the spots identified, such as HSP70 (spot A), subtilisin like serine protease (spot B), and actin (spot J), they were exhibited as immunodominat antigens on the immunoblot profiles of both isolates. Except for the unidentified spots, spots recognized as immunodominant antigens from both isolates were associated with a process of invasion, proliferation and egression of apicomplexian parasites (Dobbin et al., 2002; Blackman et al., 1998; Morrissette and Sibley, 2002). Thus, it might be supposed that the host immune response was activated vigorously by these tachyzoite proteins related to the survival strategy of apicomplexans, and they could be potential candidates for vaccine and diagnostic markers.

It would be desirable to target antigens that show a high similarity between *N. caninum* KBA-2 and VMDL-1 isolates. The tools of studying proteome and antigenic proteome profiling were proven to be highly useful for exploring the relationship between isolates. Moreover, the study methodology offers a better resolution to specifically identify common and distinct antigens between isolates as compared to conventional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein separation or whole parasite staining techniques. These results contribute to additional information needed to pursue a better understanding of proteins present in *N. caninum* and vaccine development.

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