J. Microbiol. Biotechnol. (2008), **18**(4), 778–783

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

Bacillus anthracis Spores Influence ATP Synthase Activity in Murine Macrophages

Seo, Gwi-Moon[1], Kyoung Hwa Jung[1], Seong-Joo Kim[1], Ji-Cheon Kim[1], Jang Won Yoon[1], Kwang-Keun Oh[2], Jung-Ho Lee[3], and Young Gyu Chai[1]*

[1]*Division of Molecular and Life Sciences, Hanyang University, Ansan 426-791, Korea*
[2]*Department of Bioprocess Technology, BioPolytechnic College, Nonsan 320-905, Korea*
[3]*Department of Chemical Engineering, Hanyang University, Ansan 426-791, Korea*

Received: June 16, 2007 / Accepted: September 4, 2007

Anthrax is an infectious disease caused by toxigenic strains of the Gram-positive bacterium *Bacillus anthracis*. To identify the mitochondrial proteins that are expressed differently in murine macrophages infected with spores of *B. anthracis* Sterne, proteomic and MALDI-TOF/MS analyses of uninfected and infected macrophages were conducted. As a result, 13 mitochondrial proteins with different expression patterns were discovered in the infected murine macrophages, and some were identified as ATP5b, NIAP-5, ras-related GTP binding protein B isoform CRAa, along with several unnamed proteins. Among these proteins, ATP5b is related to energy production and cytoskeletal rearrangement, whereas NIAP-5 causes apoptosis of host cells due to binding with caspase-9. Therefore, this paper focused on ATP5b, which was found to be downregulated following infection. The downregulated ATP5b also reduced ATP production in the murine macrophages infected with *B. anthracis* spores. Consequently, this study represents the first mitochondrial proteome analysis of infected macrophages.

Keywords: *Bacillus anthracis* spores, mass spectrometry, mitochondrial proteomics, murine macrophages

Bacillus anthracis is a Gram-positive, aerobic, rod-shaped bacterium that forms endospores [8, 13] and interacts with macrophages at various stages of infection. Moreover, anthrax is a zoonosis to which most mammalian cells are susceptible [20]. In the early stages of systemic anthrax, *B. anthracis* spores interact with macrophages at the initial entry site into the host. Once phagocytosed, endospores are simultaneously germinated inside the macrophages and transported to the regional lymph nodes [5, 6]. The germinated spores then transform into vegetative bacilli and are able to grow through replication under the conditions of the host intracellular phagolysosome. Finally, the *B. anthracis* vegetative cells are released from the phagocyte in order to spread [3].

To determine the relationship between the host and *B. anthracis* interactions, a variety of research methods, including proteomics and genomics, have already been carried out. Nonetheless, there is still little evidence regarding the necessary infection conditions for *B. anthracis* spores in macrophages with regard to the interaction factor, cause of host death, and immune response. As the host-pathogen interaction can be better understood through the use of proteomic and genomic techniques, a proteomic analysis between macrophages and *B. anthracis* spores using two-dimensional electrophoresis previously revealed protein changes within macrophages with *B. anthracis* spores. Moreover, various proteins, such as Pak2, related to the infection of *B. anthracis* spores in macrophages, have been revealed in the early stages [18]. A SELDI-TOF analysis was also recently carried out to analyze the macrophage and *B. anthracis* spore interaction and search for differentially expressed proteins in macrophages during infection by *B. anthracis* [17].

Mitochondria are eukaryotic cellular organelles that are important for cell death and known to be targeted for infection by pathogenic bacteria. For example, pathogenic bacteria and toxins are known to localize and induce changes consistent with the permeabilization of mitochondria [4, 22–24].

Accordingly, this study examined the mitochondria of macrophages infected with *B. anthracis* Sterne spores, and the results provide potential avenues for protection against and therapy for the infection of *B. anthracis* spores in macrophages.

*Corresponding author
Phone: 82-31-400-5513; Fax: 82-31-406-6316;
E-mail: ygchai@hanyang.ac.kr

MATERIALS AND METHODS

Materials

The urea, thiourea, 3-[(3-cholamidopropy) dimethyammonio]-1-propanesulfonate (CHAPS), 1% dithiothreitol (DTT), benzamidine, Bradford solution, acrylamide, iodoacetamide, bis-acrylamide, sodium-dodecyl sulfate (SDS), acetonitrile, trifluoroacetic acid, and α-cyano-4-hydroxycinnamic acid were all purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). The pharmalyte (pH 5−8) was obtained from Amersham Biosciences (Piscataway, NJ, U.S.A.), the immobilized IPGStrips (pH 5−8, 7 cm) were from Bio-Rad (Hercules, CA, U.S.A.), and the modified porcine trypsin (sequencing grade) was from Promega (Madison, WI, U.S.A.).

Bacterial Strain and Mammalian Cell Culture

The *B. anthracis* strain used was Sterne (34F2; $pXO1^+$, $pXO2^-$) and grown in a brain-heart infusion medium (BD Science, Ontario, Canada) at 37°C. The total DNA was isolated according to a previously reported method [17]. The Sterne strain was confirmed by a PCR and used in spore formation. The murine macrophages (macrophage-like RAW 264.7 cells) were maintained in Dulbecco's minimal essential medium (DMEM) supplemented with 10% FBS (Gibco, Carlsbad, CA, U.S.A.), and then incubated at 37°C in an atmosphere of 5% CO_2 and saturating humidity.

Production of *Bacillus anthracis* Endospores

The *B. anthracis* spores were isolated and purified using a previously reported method [3]. The vegetative cells were then inoculated onto an NBY agar plate, and the cultures incubated at 37°C for seven days until spore formation. The spores were confirmed by spore staining and a PCR. The spores were collected from the plate and isolated using sucrose gradient centrifugation. For nonsucrose solutions, the top 9 ml was transferred to an empty tube. The samples were then centrifuged at 145 ×*g* for 30 min and the supernatant was discarded. The spores in the pellet were removed using sterilized distilled water, and the pellet was resuspended in 5 ml of PBS and stored at -20°C.

Infection of Murine Macrophages by *Bacillus anthracis* Sterne Spores

The murine macrophages were infected with the *B. anthracis* spores according to a previously reported method [3]. The murine macrophages (1.34×10^7 cells) were cultured in a culture plate (Corning, Big Flats, NY, U.S.A.) at 37°C in 5% CO_2 for 1 day. The Sterne spores (1.36×10^8 cells) were then inoculated at 37°C, and transferred 30 min later to DMEM lacking antibiotics.

Protein Sample Preparation

The cultured cell pellets were quickly washed in ice-cold PBS. The mitochondria isolation was performed using a Mitochondria Isolation Kit (Pierce, Rockford, IL, U.S.A.). The cell pellet was collected by centrifugation of the harvested cell suspension in a 2.0-ml microcentrifuge tube at 850 ×*g* for 2 min. The supernatant was then removed and discarded, and Mitochondria Isolation Reagent A (800 μl) added. The cells were vortexed at a medium speed for 5 sec, and the incubated tube was placed on ice for exactly 2 min. Thereafter, 10 μl of Mitochondria Isolation Reagent B was added, and the sample vortexed at the maximum speed for 5 sec. The incubation tube was then placed on ice for 5 min, and vortexed at the maximum speed every min. Next, a total of 800 μl of Mitochondria Isolation Reagent C was added, and then the tube was inverted several times to mix and placed in a centrifuge at 700 ×*g* for 10 min at 4°C. The supernatant was subsequently transferred to a new 2.0-ml tube and placed in a centrifuge at 12,000 ×*g* for 15 min at 4°C. To obtain a more purified fraction of mitochondria, with a >50% reduction of lysosomal and peroxisomal contaminants, the sample was placed in a centrifuge at 3,000 ×*g* for 15 min. The supernatant (cytosol fraction) was transferred to a new tube, while 500 μl of Mitochondria Isolation Reagent C was added to the pellet containing the isolated mitochondria and centrifuged at 12,000 ×*g* for 5 min. This time the supernatant was discarded and the mitochondrial pellets were lysed with 2% CHAPS in Tris-buffered saline (TBS; 25 mM Tris, 0.15 M NaCl; pH 7.2). After centrifugation at 15,000 ×*g* for 1 h at 15°C, the insoluble material was discarded and the soluble fraction used for two-dimensional polyacrylamide gel electrophoresis (2D PAGE). The protein loading was normalized by a Bradford assay [1].

2D PAGE

Immobilized pH gradient dry strips were equilibrated for 16 h with 7 M urea, 2 M thiourea containing 2% CHAPS, 1% DTT, and 1% pharmalyte, and then loaded with 50 μg of the sample. The isoelectric focusing (IEF) was performed at 20°C using a Protean IEF cell electrophoresis unit (Bio-Rad, U.S.A.) following the manufacturer's instructions. For the IEF, the voltage was linearly increased from 150 to 4,000 V for 2 h during the sample entry, followed by a constant 4,000 V, with focusing complete after 20,000 Vh. Prior to the second dimension, strips were incubated for 10 min in an equilibration buffer (50 mM Tris-HCl, pH 6.8, containing 6 M urea, 2% SDS, and 30% glycerol), first with 1% DTT and then with 2.5% iodoacetamide. The equilibrated strips were then placed on SDS-PAGE gels (7−11 cm, 12%). The SDS-PAGE was performed using a Bio-Rad Mini-Protean 3 system (Bio-Rad, Hercules, CA, U.S.A.) following the manufacturer's instructions, and the SDS-PAGE gels were run at 20°C at 15 mA. Thereafter, the 2D PAGE gels were silver stained as described previously [12], with the omission of the fixing and sensitization step with glutaraldehyde.

Image Analysis

A quantitative analysis of the digitized images was carried out using PDQuest (version 7.0, Bio-Rad, Hercules, CA, U.S.A.) software according to the protocols provided by the manufacturer. The quantity of each spot was normalized by the total valid spot intensity. Protein spots with a significant expression level were selected and compared with the *B. anthracis* Sterne spore sample after infection.

Enzymatic Digestion of Protein In-Gel

The protein spots were enzymatically digested in-gel in a manner similar to that previously described [9, 19] using modified porcine trypsin. The gel pieces were washed with 50% acetonitrile to remove the SDS, salt, and stain, dried to remove the solvent, and then rehydrated with trypsin (8−10 ng/μl) and incubated for 8−10 h at 37°C. The proteolytic reaction was terminated by the addition of 5 μl of 0.5% trifluoroacetic acid. The tryptic peptides were recovered by combining the aqueous phase from several gel piece extractions with 50% aqueous acetonitrile. Thereafter, the concentration of the peptide mixture was desalted using C_{18}ZipTips (Millipore, Bedford, MA, U.S.A.), and the peptides were eluted in 1−5 μl of acetonitrile. An aliquot of this solution was mixed with an equal volume of a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% aqueous acetonitrile, and 1 μl of the mixture was spotted onto a target plate.

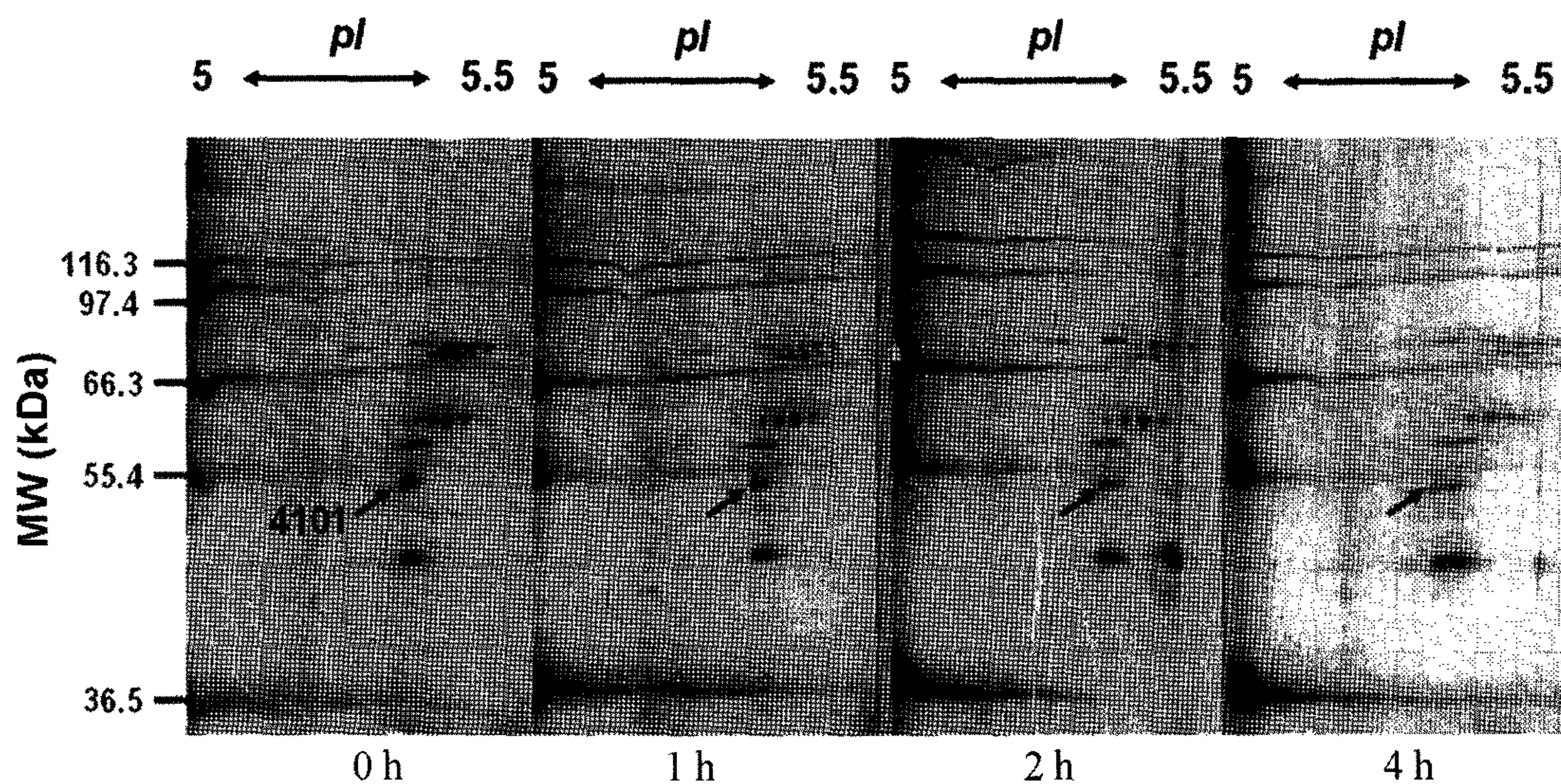


Fig. 1. Comparative proteomic profiles of mitochondrial proteins from macrophage infected with *Bacillus anthracis* Sterne spores. The proteins (50 μg) were separated by 2D PAGE, pH 5–8, and 12% SDS-PAGE gels, and the stains were carried out by alkaline silver staining. The figures display sectors of interest at pH 5–5.5 within the 2D electrophoresis. The position of the 4101 spot is indicated (arrows). The expression of 4101 (arrows) was analyzed after 0, 1, 2, and 4 h, respectively. This spot was identified by MALDI-TOF MS/MS.

MALDI-TOF Analysis and Database Search

A protein analysis was performed using an Ettan MALDI-TOF (Amersham Biosciences, Piscataway, NJ, U.S.A.). The peptides were evaporated with an N_2 laser at 337 nm, using a delayed extraction approach, and then accelerated with a 20-kV injection pulse for a time-of-flight analysis. Each spectrum was the cumulative average of 300 laser shots. The search program Mascot, developed by Matrix Science [http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=PMF], was used for the protein identification based on peptide mass fingerprinting. The spectra were calibrated using trypsin autodigestion ion peaks *m/z* (842.510, 2211.1046) as the internal standards.

Western Blot Analysis

Ten μg of mitochondrial extracts was separated on a 12% SDS-PAGE gel. The gels were then electroblotted onto Western S membranes (Schleicher & Schuell Bioscience, Keene, NH, U.S.A.) and immunodetection was achieved using chemiluminescence (Pierce, Rockford, IL, U.S.A.).

ATP Measurements

The ATP measurements were performed using bioluminescence in a luminometer (Turner Designs, Sunnyvale, CA, U.S.A.) using ATP assay reagents from Sigma-Aldrich.

Results

A proteomic analysis of murine macrophages infected with *B. anthracis* spores was carried out based on 2D PAGE of murine macrophage mitochondrial extracts. In addition, a

Table 1. List of identified proteins.

Number of sample	Name of protein	Sequence coverage of protein (%)	Molecular mass (Da)	Match of peptide	pI	Mascot score
2003	Ras-related GTP binding B, isoform CRAa	7	40,542	3	5.38	28
4101	ATP5b protein	12	56,632	7	5.24	90
4102	NAIP-5 (neuronal apoptosis inhibitory protein 5)	2	161,871	3	5.58	30
5101	Unnamed protein product	4	86,776	3	5.1	31
5102	Unnamed protein product	8	42,039	3	5.29	39
5103	Unnamed protein product	5	29,972	2	5.46	36
5301	4932439K10Rik protein	11	88,220	8	5.2	44
5402	Unnamed protein product	4	68,673	2	5.42	36
6001	Unnamed protein product	20	16,250	4		34
6002	Tropomyosin 2, beta	9	32,933	4	4.66	55
6501	Cdk5rap2 protein	2	157,161	4	5.17	28
6502	Proteasome activator subunit 2 isoform 2	6	26,317	2	5.08	35
7203	Unnamed protein product	5	56,249	3	6.42	28

Western blot was performed on the mitochondrial extracts to confirm the absence of lysosomal and peroxisomal contaminants. No lysosomal or peroxisomal contaminants were detected (data not shown). For the 2D PAGE, 50 μg of the mitochondrial proteins was loaded on pH 5–8 IPG strips. Alkaline silver staining was then used, which detected about 200±20 spots per gel. Partial images are shown for pH 5–5.5 (Fig. 1). The differential spots were analyzed using PDQuest 7.0 image software. Seventeen spots of up-/downregulated proteins were obtained (Table 1), among which 13 proteins were identified and 4 proteins were not. In particular, spot 4101 was monitored as a downregulated protein (Fig. 2A), which was reduced by more than 50% 1 h after infecting the murine macrophages with the *B. anthracis* Sterne spores, and reduced by about 75% after 4 h (Fig. 2B). The ATP5b protein was identified by MALDI-TOF/MS. A chromatogram of the ATP5b protein in the mitochondria is represented in Fig. 3, which also shows the coverage of the ATP5b protein (Fig. 3A). The MALDI-TOF/MS spectrum of the trypsin-digested peptides derived from the spot matched the ATP5b protein (Fig. 3B). The patterns of the measured masses were matched against the theoretical masses of proteins found in the annotated database Mascot (http://www.matrixscience.com/cgi/search_form.pl? FORMVER=2&SEARCH=PMF). The ATP5b protein was identified by the Mascot database with a Mowse Score of 90.

A Western blot analysis confirmed a continued decrease in the ATP5b protein after 2 and 4 h following infection with the *B. anthracis* Sterne spores (Fig. 4A). Meanwhile, the total amount of ATP was analyzed by a bioluminescent assay kit using a luminometer. Although the ATP amount decreased after 1 h following the infection of the murine macrophages with the *B. anthracis* Sterne spores (Fig. 4B),

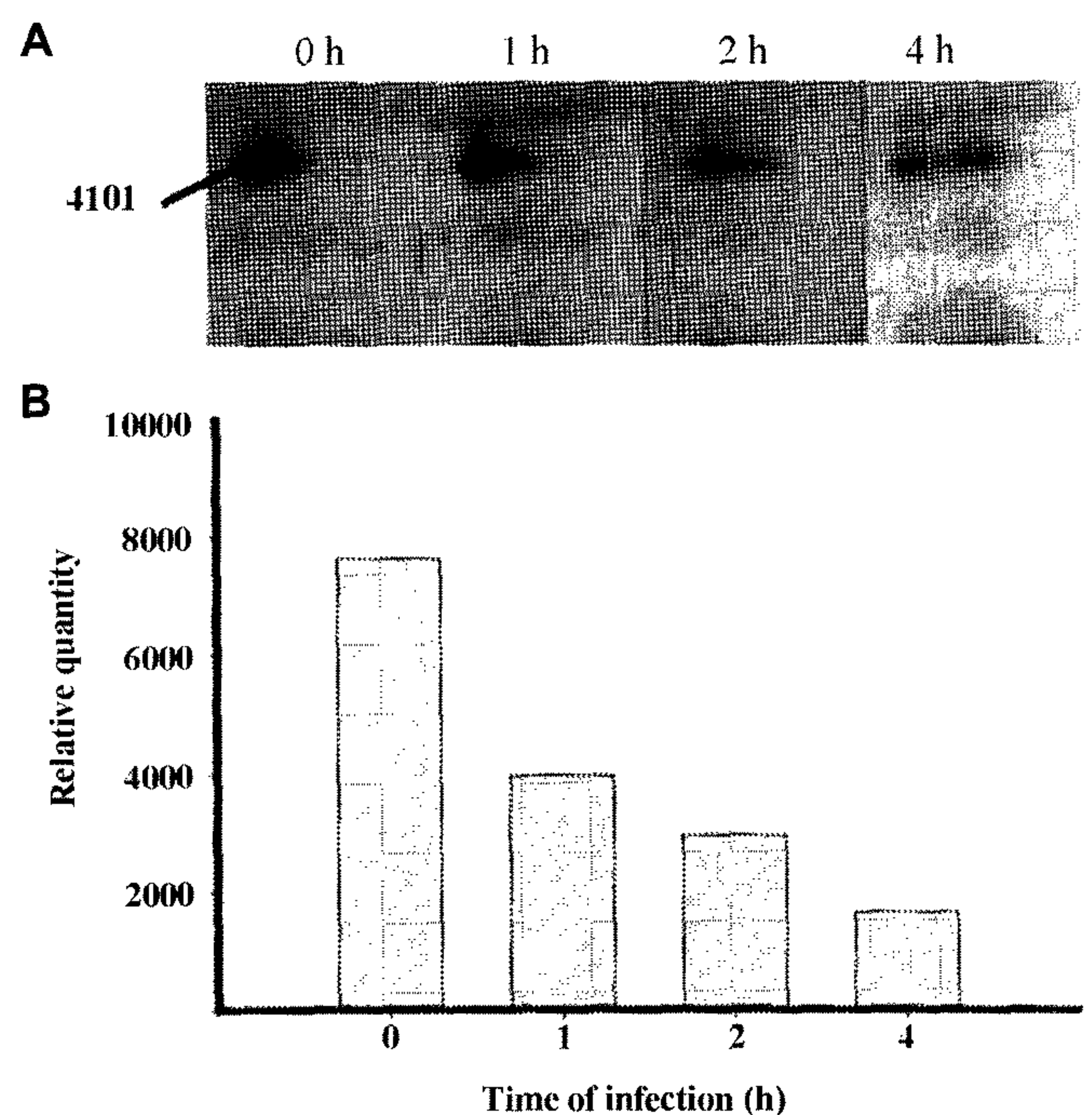
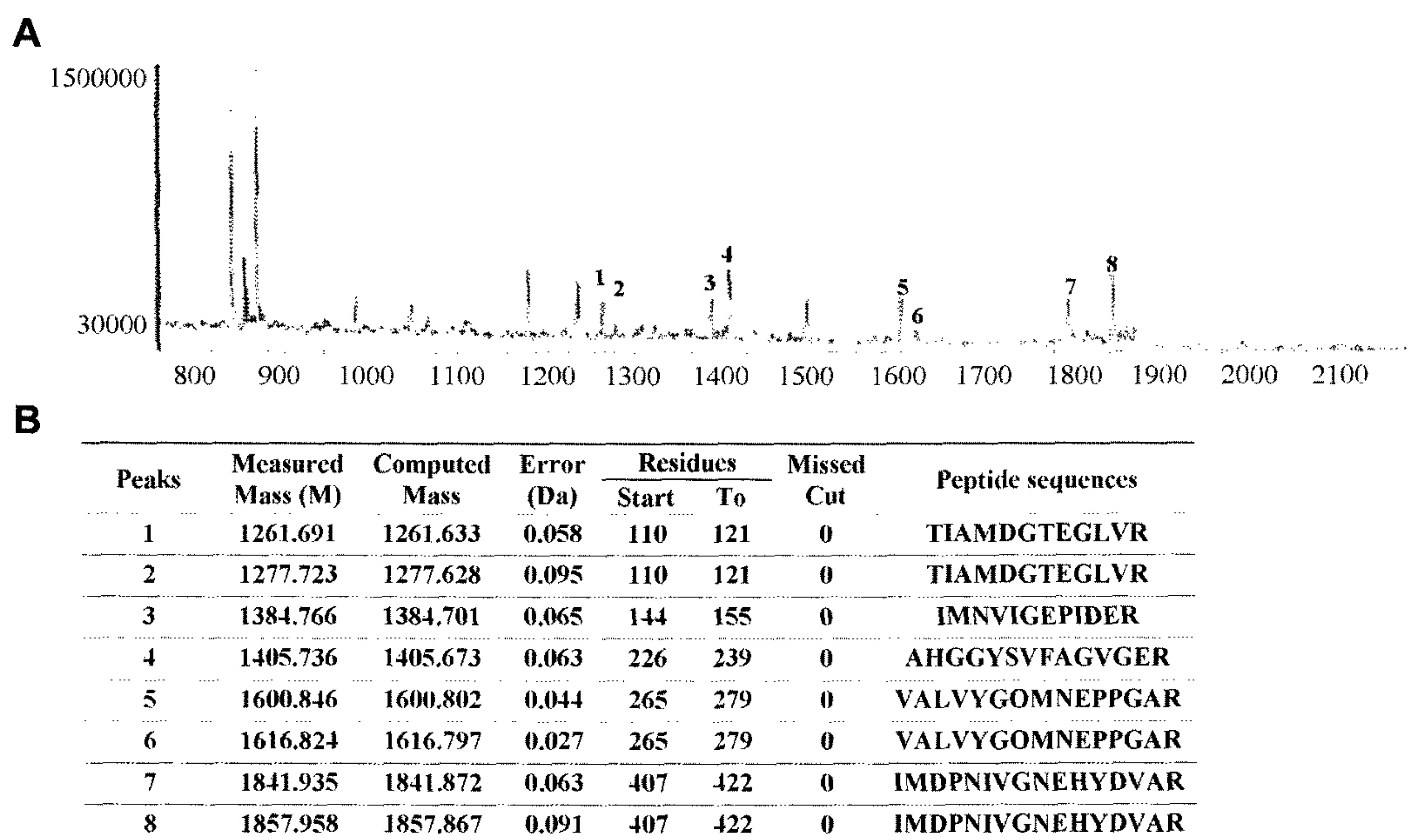


Fig. 2. Comparison of mitochondrial protein expression patterns of murine macrophage cells infected with *Bacillus anthracis* Sterne spores from 0 to 4 h following infection.
A. Expression of protein indicated at spot 4101. **B.** Quantities of spot 4101 during *B. anthracis* spore infection of murine macrophage cells. Spot 4101 that appeared in the 2D PAGE gels was quantitatively analyzed using the PDQuest analysis program.



Peaks	Measured Mass (M)	Computed Mass	Error (Da)	Residues Start	Residues To	Missed Cut	Peptide sequences
1	1261.691	1261.633	0.058	110	121	0	TIAMDGTEGLVR
2	1277.723	1277.628	0.095	110	121	0	TIAMDGTEGLVR
3	1384.766	1384.701	0.065	144	155	0	IMNVIGEPIDER
4	1405.736	1405.673	0.063	226	239	0	AHGGYSVFAGVGER
5	1600.846	1600.802	0.044	265	279	0	VALVYGOMNEPPGAR
6	1616.824	1616.797	0.027	265	279	0	VALVYGOMNEPPGAR
7	1841.935	1841.872	0.063	407	422	0	IMDPNIVGNEHYDVAR
8	1857.958	1857.867	0.091	407	422	0	IMDPNIVGNEHYDVAR

Fig. 3. Peptide mass fingerprinting (**A**) and protein coverage map of ATP5b protein (**B**).
This protein was predicted by peptide fragments corresponding to the observed *m/z* values for ATP5b.

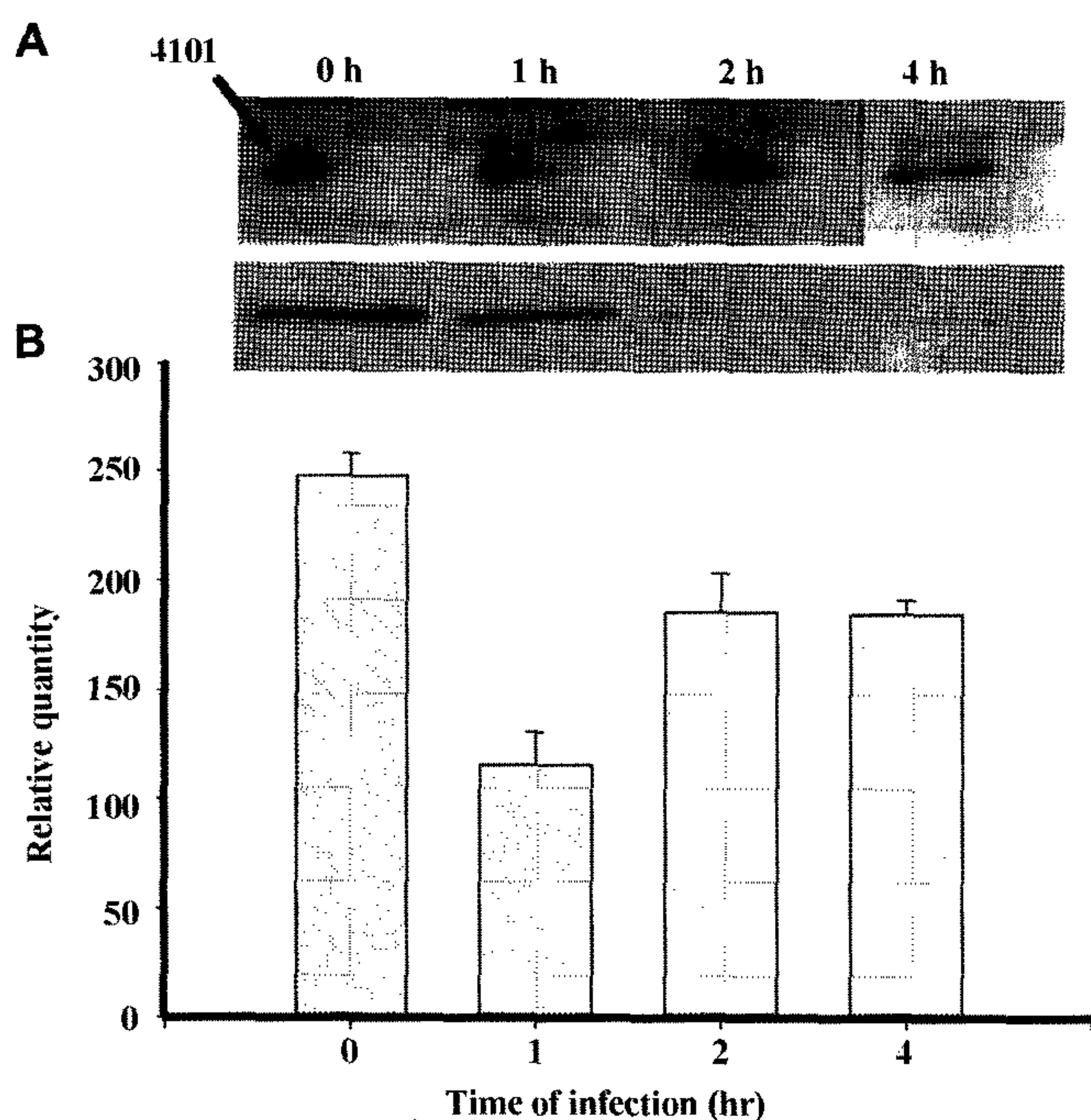


Fig. 4. Comparison of protein expression patterns of ATP5b protein. **A.** Expression patterns of ATP5b protein using 2D-PAGE and Western blot analysis. **B.** ATP assays of murine macrophage cells infected with *Bacillus anthracis* Sterne spores. The ATP5b protein was confirmed by a Western blot analysis. The mitochondrial proteins were loaded with 10 μg of proteins. The ATP assay was carried out in three independent experiments with the standard deviation (SD) value depicted as an error bar.

the ATP levels after 2 and 4 h were above the levels seen at 1 h after infection.

DISCUSSION

This study successfully used 2D PAGE to assess the infection of murine macrophages with spores of *B. anthracis*. Although the effects of *B. anthracis* spores on macrophages are not yet clear, mitochondria are known to be the main target of various intracellular pathogens. Thus, to examine the protein changes within infected macrophages, proteomic techniques were used to identify up- and downregulated proteins within the mitochondria (Fig. 1). A novel approach was also adopted to analyze the effects of infection with *B. anthracis* Sterne spores using organelle proteomics.

Previous studies of mitochondria revealed several pathogenic bacteria as cellular targets for mitochondria, including *Helicobacter pylori*, *Neisseria gonorrhoeae*, and *Staphylococcus aureus* [4, 22–24].

The present study used 2D PAGE to examine the mitochondrial protein expression in macrophages infected with *B. anthracis*. Thirteen among about 200 mitochondrial proteins were observed to have a significant differential expression after infection. These proteins were identified by MALDI-TOF/MS and the Mascot database. Thus, based on a good spectrum and high score obtained from the Mascot database, this study focused on the ATP5b protein that is related to mitochondrial ATP synthesis. Previous studies of the ATP5b protein have reported that macrophages infected with *Francisella tularensis* upregulated the ATP5b protein [10], whereas macrophages exposed to the lethal toxins of *B. anthracis* showed a reduction of the ATP5b protein [2]. Recently, Sapra *et al.* [11, 16] reported that the ATP5b protein was upregulated when a low concentration of a lethal toxin was used to treat murine macrophages. Additionally, Sapra *et al.* [16] reported an elevation of cytoskeletal proteins and chaperones, such as Hsp70 and Hsp60, in J774.1 and RAW264.7 macrophages exposed to a lethal toxin. These chaperones are known to be involved in protein folding and unfolding, and targeting irreversibly denatured proteins for clearance. In addition, Hsp70 and Hsp60 are both needed for binding ATP to protect against apoptosis [14, 15, 21]. Cytoskeletal rearrangement also needs ATP. Thus, the upregulation of ATP synthase in lethal-toxin-treated macrophages may be generally correlated to the production of ATP. However, Chandra *et al.* [2] reported the downregulation of ATP and reduction of chaperones, such as Hsp70, inducing cell death for macrophages. Yet, this discrepancy may have resulted from the use of different protocols for the experiments performed by the different groups. Meanwhile, the present results indicated that the ATP5b protein was downregulated in the macrophages infected with *B. anthracis* (Fig. 2). This reduction of ATP5b was also confirmed using a Western blot (Fig. 4A), and the reduction of ATP confirmed by an ATP assay (Fig. 4B). However, after 3 h, the amount of ATP was found to have increased, while the amount of ATPase was decreased. In mycobacteria, the acidification of the host cells is protected by blocking the accumulation of the vacuolar proton ATPase [8], which may explain the decrease in ATPase during the early stage of *Bacillus anthracis* infection. Nonetheless, *Bacillus anthracis* is dependent on the ATP from the host cell for survival and proliferation. Similarly, *Chlamydia* are entirely dependent on the ATP from the host cells, so even if the expression of ATP5b decreases, the amount of ATP increases [8]. Another possible reason was that the macrophages infected with *B. anthracis* were unable to generate cytoskeletal rearrangement and chaperones, and thus the functions of the chaperones in the macrophages were eliminated because of ATP depletion, thereby inducing cell death. However, the cytoskeleton rearrangement and expression of chaperone proteins were not confirmed in this study. Therefore, it was assumed that the ATP5b protein expression change was related to the use of the host cell system and death of the host after infection with *B. anthracis*. Although several previous reports have demonstrated the differential mitochondrial protein expression of macrophages infected with *B. anthracis* spores using

proteomic techniques, this study used 2D PAGE to analyze the mitochondrial proteins related to infection with *B. anthracis* spores, and it is expected that this approach may help reveal the organelle targets of *B. anthracis* in host cells, such as macrophages.

Acknowledgments

The authors would like to thank Mr. S. Shahid for his fruitful discussions. This work was supported by a grant (034475) from the BioGreen 21 Program, a grant (KOSEF-2007-04918) from the Basic Research Program of the Korea Science & Engineering Foundation, and by the Agency of Defense Development under contract UD060021AD.

REFERENCES

1. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
2. Chandra, H., P. K. Gupta, K. Sharma, A. R. Mattoo, S. K. Garg, W. N. Gade, R. Sirdeshmukh, K. Maithal, and Y. Singh. 2005. Proteome analysis of mouse macrophages treated with anthrax lethal toxin. *Biochim. Biophys. Acta* **1747:** 151–159.
3. Dixon, T. C., A. A. Fadl, T. M. Koehler, J. A. Swanson, and P. C. Hanna. 2000. Early *Bacillus anthracis*-macrophage interactions: Intracellular survival and escape. *Cell. Microbiol.* **2:** 453–463.
4. Galmiche, A., J. Rassow, A. Doye, S. Cagnol, J. C. Chambard, S. Contamin, V. de Thillot, I. Just, V. Ricci, E. Solcia, E. Van Obberqhen, and P. Boquet. 2000. The N-terminal 34 kDa fragment of *Helicobacter pylori* vacuolating cytotoxin targets mitochondria and induces cytochrome *c* release. *EMBO J.* **19:** 6361–6370.
5. Guidi-Rontani, C. 2002. The alveolar macrophage: The Trojan horse of *Bacillus anthracis*. *Trends Microbiol.* **10:** 405–409.
6. Guidi-Rontani, C., M. Weber-Levy, E. Labruyere, and M. Mock. 1999. Germination of *Bacillus anthracis* spores within alveolar macrophages. *Mol. Microbiol.* **31:** 9–17.
7. Joiner, K. A. 1997. Membrane-protein traffic in pathogen-infected cells. *J. Clin. Invest.* **99:** 1814–1817.
8. Kim, H. T., G. W. Seo, K. Y. Jung, S. J. Kim, J. C. Kim, K. G. Oh, B. S. Koo, and Y. G. Chai. 2007. Generation of a specific marker to discriminate *Bacillus anthracis* from other bacteria of the *Bacillus cereus* group. *J. Microbiol. Biotechnol.* **17:** 806–811.
9. Kim, I. S., H. S. Yun, and I. N. Jin. 2007. Comparative proteomic analyses of the yeast *Saccharomyces cerevisiae* KNU5377 strain again menadion-induced oxidative stress. *J. Microbiol. Biotechnol.* **17:** 207–217.
10. Kovarova, H., P. Halada, P. Man, I. Golovliov, Z. Krocova, J. Spacek, S. Porkertova, and R. Necasova. 2002. Proteome study of *Francisella tularensis* live vaccine strain-containing phagosome in Bcg/Nramp1 congenic macrophages: Resistant allele contributes to permissive environment and susceptibility to infection. *Proteomics* **2:** 85–93.
11. Kuhn, J. F., P. Hoerth, S. T. Hoehn, T. Preckel, and K. B. Tomer. 2006. Proteomics study of anthrax lethal toxin-treated murine macrophages. *Electrophoresis* **27:** 1584–1597.
12. Oakley, B. R., D. R. Kirsch, and N. R. Morris. 1980. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* **105:** 361–363.
13. Park, T. J., J. P. Park, G. M. Seo, Y. G. Chai, and S. Y. Lee. 2006. Rapid and accurate detection of *Bacillus anthracis* spores using peptide-quantum dot conjugates. *J. Microbiol. Biotechnol.* **16:** 1713–1719.
14. Polla, B. S., D. Francois, S. Salvioli, G. Franceschi, C. Marsac, and A. Cossarizza. 1996. Mitochondria are selective targets for the protective effects of heat shock against oxidative injury. *Proc. Natl. Acad. Sci. USA* **93:** 6458–6463.
15. Samali, A., J. Cai, B. Zhivotovsky, D. P. Jones, and S. Orrenius. 1999. Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of Jurkat cells. *EMBO J.* **18:** 2040–2048.
16. Sapra, R., S. P. Gaucher, J. S. Lachmann, G. M. Buffleben, G. S. Chirica, J. E. Comer, J. W. Peterson, A. K. Chopra, and A. K. Singh. 2006. Proteomic analyses of murine macrophages treated with *Bacillus anthracis* lethal toxin. *Microb. Pathogen.* **41:** 157–167.
17. Seo, G. M., S. J. Kim, and Y. G. Chai. 2004. Rapid profiling of the infection of *Bacillus anthracis* on human macrophages using SELDI-TOF mass spectroscopy. *Biochem. Biophys. Res. Commun.* **325:** 1236–1239.
18. Seo, G. M., S. J. Kim, J. C. Kim, D. H. Nam, M. Y. Yoon, B. S. Koo, and Y. G. Chai. 2004. Targeting of *Bacillus anthracis* interaction factors for human macrophages using two-dimensional gel electrophoresis. *Biochem. Biophys. Res. Commun.* **322:** 854–859.
19. Shevchenko, A., M. Wilm, O. Vorm, and M. Mann. 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal. Chem.* **68:** 850–858.
20. Stephen, J. 1981. Anthrax toxin. *Pharmacol. Therap.* **12:** 501–513.
21. Tonge, R., J. Shaw, B. Middleton, R. Rowlinson, S. Rayner, and J. Young. 2001. Validation and development of fluorescence two-dimensional differential gel electrophoresis proteomics technology. *Proteomics* **1:** 377–396.
22. Willhite, D. C. and S. R. Blanke. 2004. *Helicobacter pylori* vacuolating cytotoxin enters cells, localizes to the mitochondria, and induces mitochondrial membrane permeability changes correlated to toxin channel activity. *Cell. Microbiol.* **6:** 143–154.
23. Willhite, D. C., T. L. Cover, and S. R. Blanke. 2003. Cellular vacuolation and mitochondrial cytochrome *c* release are independent outcomes of *Helicobacter pylori* vacuolating cytotoxin activity that are each dependent on membrane channel formation. *J. Biol. Chem.* **278:** 48204–48209.
24. Yamasaki, E., A. Wada, A. Kumatori, I. Nakagawa, J. Funao, M. Nakayama, J. Hisatsune, M. Kimura, J. Moss, and T. Hirayama. 2006. *Helicobacter pylori* vacuolating cytotoxin induces activation of the proapoptotic proteins Bax and Bak, leading to cytochrome *c* release and cell death, independent of vacuolation. *J. Biol. Chem.* **281:** 11250–11259.