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Proteome analysis between diverse phenotypes of *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S.* Typhimurium)

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Abstract : Protein expression patterns in *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S.* Typhimurium) strains with diverse phenotypes, such as phage type, antibiotic resistance pattern and plasmid profiles were examined. For detailed analysis of proteins expressed by different *S.* Typhimurium strains, protein fractions were divided into detergent-rich phase (DP) and aqueous phase (AP) using triton X-114 detergent. The two phases were subjected to two-dimensional gel electrophoresis (2-DE), followed by protein identification using peptide mass fingerprinting (PMF). In the results, PMF showed that DP fractions consisted mainly of outer membrane proteins, whereas the AP fractions included cytosolic proteins. Comparison of 2-DE profiles of DP did not show any distinct protein spots which could be correlated with phage type, antibiotic resistance pattern or plasmid profile. However, comparisons of 2-DE profiles of the AP revealed differences in the protein spots, which could be correlated with the plasmid profile and phage types. Among these protein spots, flagellin was specific for strains containing a 90 kb plasmid. Compared to DT193 phage type, three protein spots in the range of pI 5.0-5.5 and MW 8-15 kDa of AP 2-DE profiles were absent in the DT104 phage types. Additionally, a protein spot with PI in the range of 4.5-5.0 and molecular weight (MW) between 51-69 kDa was specific for phage type DT104, while a protein spot with pI in the range of 4.0-4.8 and MW between 18-20 kDa was specific for DT193 phage type. These protein spots may be useful for discriminating phage types of *S.* Typhimurium.

Keywords : OMP, phage type, plasmid, proteome, *Salmonella enterica* serovar Typhimurium, Triton X-114

Introduction

Salmonella enterica subspecies *enterica* serovar Typhimurium (*S.* Typhimurium) is a major food-borne zoonotic pathogen throughout the world [3]. The bacterium has a broad host range including poultry, cattle and pigs, and consumption of contaminated meat, raw milk, or eggs by humans can elicit a variety of illness, ranging from localized gastroenteritis to a life-threatening systemic disease [24, 33, 46]. The *S.* Typhimurium has frequently been isolated from asymptomatic pigs [8]. These carrier animals are the major threats to food safety because of the subclinical nature of the infection and are the main source of infection to other pigs, contamination to the environment, to slaughter plants, and to pork products [4, 42]. Furthermore, the rapid emergence and dissemination of multidrug resistant (MDR) *S.* Typhimurium isolates from infected pigs have become a public health concern worldwide [27].

Phage typing, antimicrobial resistance profiles and plasmid profiles are widely used on epidemiological studies for *S.* Typhimurium. The MDR strains of *S.* Typhimurium are frequently identified into two different definitive phage types, DT104 and DT193 [24, 33, 43]. The phage type DT104 was first isolated in the United Kingdom [43], and later reported from various food animals and pets as well as from human worldwide [26, 37]. On the other hand, 90 kb plasmid of *S.* Typhimurium

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has been known to encode an outer membrane proteins (OMPs) associated with fimbrial biosynthesis, which confers enhanced virulence by promoting bacterial spread after infection [14]. Besides, OMPs of Gram-negative bacteria have been recognized as virulence factors for invasion and adhesion to host, and as transporters for various molecules through the bacterial surface [23]. Recent studies showed that the OMPs significantly change in response to antibiotic stimulation in bacteria [6, 16, 36, 47]. Some OMPs associated with membrane channel proteins, are functional unit for antibiotic efflux systems and contribute to MDR in the bacteria. Resistance to fluoroquinolones in *S.* Typhimurium DT104 and DT204 [1, 2], and multi-drug resistance in *Escherichia* (*E.*) *coli* [34] is reportedly due to overproduction of the AcrAB-TolC efflux system. Since the OMPs play an important function in virulence and MDR in bacteria, they may serve as potential targets for design of antibiotics and vaccines [23].

Proteomic techniques involving, two-dimensional gel electrophoresis (2-DE) followed by peptide mass finger-printing (PMF) have been recognized as an efficient and simple tool in identification of individual proteins, and are widely used for studying the mechanisms involved in pathogenicity, antibiotic resistance and environmental adaptation of bacteria [18]. In these aspects, the protein expression patterns of *S.* Typhimurium have been extensively studied and reference maps of the cytoplasmic and cell envelope proteins have been previously published [11, 12, 31, 32, 35]. However, there is little information about comparisons of protein expression patterns according to phage types, patterns of antibacterial resistance and plasmid profiles of the bacteria. Therefore, we wanted to find differentially expressed proteins using comparative 2-DE analysis among *S.* Typhimurium strains defined using phage type, antibacterial resistance pattern and plasmid profile.

Materials and Methods

Bacterial strains, growth conditions

Six strains of *S.* Typhimurim used in this study were provided from Busan metropolitan city institute of health and environmental. Phage types, antibiotic resistance patterns and plasmid profiles for strains are summarized in Table 1 [21, 22]. The strains were cultured on Salmonella-Shigella agar (Merck, Germany) at 37°C for 24 h to obtain single colony for 2-DE sample preparation. 2-DE samples were prepared by culturing strains in tryptic soy broth (TSB). The strains were recovered by incubating in TSB at 37°C for 14 h, were pelleted by centrifugation at 14,000 × g for 10 min at 4°C, and washed three times with phosphate buffered saline (PBS, pH 7.2). The final bacterial pellets were stored at −20°C until used for 2-DE sample preparation.

Triton X-114 extraction of proteins from *S.* Typhimurium strains

The Triton X-114 detergent-induced phase partition method was employed for obtaining OMP rich and cytoplasmic protein rich factions as described by Lee *et al.* [20]. The pellets were resuspended in PBS containing 1% Triton X-114, completely vortexed and then centrifuged at 16,600 × g for 30 min at 4°C. The supernatant was transferred into a new tube, incubated for 30 min at 37°C and centrifuged at 100 × g for 1 min. The lower OMP rich detergent phase (DP) and upper aqueous phase (AP) were collected in separate tubes. To improve purity of DP, the extracts were re-extracted with an equal volume of PBS. Finally, the extracted DP and AP were added to ten volumes of cold ethanol and stored at −20°C for over 12 h. The insoluble materials were precipitated by centrifugation at 16,600 × g for 40 min. The pelleted proteins were

Table 1. Phage type, antibioticresistance patterns and plasmid profiles of *S.* Typhimurium isolates

Strains	Host	Phage type	Resistance pattern	Plasmid profile (kb)
SN296	Pig	DT22	P	Nd*
SN75	Pig	RDNC†	DST	40, 6.3
SN164	Pig	DT193	ACCbDNaPSSuT	90, 40, 10.5, 6.3, 3
SNC282	Pig	DT104	ACCbDNaPSSuT	90, 40, 10.5, 6.3, 3
SNC309	Pig	DT104	ACCbDNaPSSuT	40, 10.5, 6.3, 3
SNC310	Pig	DT104	ACCbDNaPSSuT	90, 40, 10.5, 6.3, 3

S. Typhimurium: *Salmonella enterica* subspecies *enterica* serovar Typhimurium. *Nd: not detected, †RDNC: reacts with phages but cannot be confirmed.

air-dried and stored at −20°C until used for 2-DE.

2-DE PAGE

The dried DP and AP samples were dissolved in soluble buffer (7 M urea, 2 M thiourea, 4% CHAPS, 20 mM DTT, 0.5% IPG buffer 3-10), incubated for 10 min on ice and mixed with equal volume of rehydration buffer (9 M urea, 4% CHAPS, 20 mM DTT, 0.5% IPG buffer pH 4-7 and 0.002% bromophenol blue). The protein concentrations were adjusted to 0.3 mg/mL for DP and 0.6 mg/mL for AP. The 2-DE and silver staining were performed according to Shin *et al.* [39]. The stained gels were digitalized using Agfa Arcus 1200 image scanner (Agfa-Gevaert; Mortsel, Belgium) and the digitized images were analyzed using the Phoretix 2D software (Ver. 5.01; NonLinear Dynamics, UK).

PMF

Protein spots were identified by PMF using MALDI-TOF MS as described by Shin *et al.* [39]. In brief, protein spots of interest were picked from silver-stained gels and subjected to in-gel digestion with 12.5 ng/mL porcine trypsin (Promega, USA) at 37°C overnight (approximately 16 h). The supernatant was recovered and extracted twice with equal volumes of 5% formic acid and acetonitrile, pooled, and dried in a vacuum centrifuge. Dried tryptic peptides were re-dissolved in 1 mL sample solution (93 : 5 : 2, v/v ratio of DW, acetonitrile and TFA), and the targeting on MALDI plate was performed using the solution-phase nitrocellulose method [19]. Alpha-cyano-4-hydroxycinnamic acid (40 mg/mL) and nitrocellulose (20 mg/mL) were prepared separately in acetone and mixed with isopropanol at a ratio of 2 : 1 : 1 (v/v). The internal standards, des-Arg-Bradykinin (monoisotopic mass: 904.4681) and angiotensin I (1296.6853) (Sigma-Aldrich, USA) were added to the mixture to generate the matrix solution. The matrix solution (1 μL) was spotted onto target circles on the MALDI plate and dried. The dried samples were analyzed using a Voyager-DE STR MALDI-TOF mass spectrometer (PerSeptive Biosystems, USA). The proteins were identified by comparing the obtained mass spectra to the National Center for Biotechnology Information and SwissProt protein sequence databases using ProteinProspector (University of California, USA).

Results

Construction of partial 2-DE maps for DP and AP

2-DE was used for exploring differentially expressed proteins according to phage type, antibiotic resistance patterns and plasmid profiles of *S.* Typhimurium. The results showed that DP and AP extracts were observed to be consist of about 220 (220 ± 10) and 400 (410 ± 30) spots by silver staining after 2-DE, respectively (Fig. 1). Majority of the proteins spots in both 2-DE profiles were distributed at the MW 20 to 70 kDa range. However, there were many differences in spot

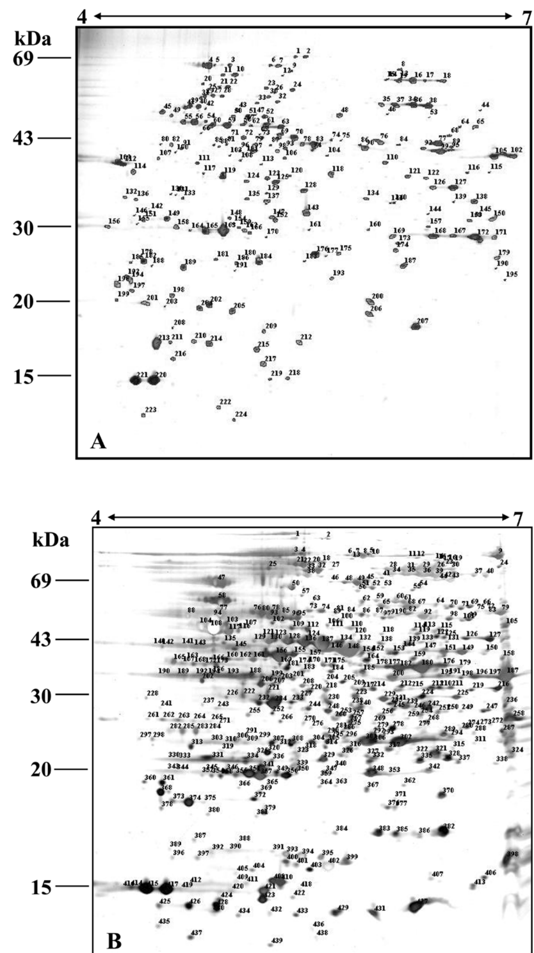

Fig. 1. Partial two-dimensional gel electrophoresis (2-DE) maps of the detergent-rich phase (DP) (A) and aqueous phase (B) fractionated using Triton X-114 from *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S.* Typhimurium) strains.

Table 2. The list of proteins identified in the 2-DE profile of DP of *S.* Typhimurium SN282 using MALDI-TOF MS

Spot No.	Mowse score	Masses matched	Protein kDa/pI	Species	Protein name
4	6.36E + 14	26	89.5/5.0	*S.* Typhimurium LT2	Putative outer membrane protein (antigen) precursor
16	4.97E + 05	9	65.4/6.0	*S.* Typhimurium	Fumarate reductase, flavoprotein subunit
59, 60	1.13E + 09	12	53.6/5.4	*S. typhi* Ty2	Outer membrane protein TolC precursor
77	4.41E + 04	7	45.8/6.8	*S.* Typhimurium	Possible pectinesterase precursor
109	1.38E + 06	9	41.9/4.7	*S.* Typhimurium LT2	Putative serine/threonine protein kinase
163, 165	3.42E + 07	10	29.4/5.4	*S.* Typhimurium	D-methionine-binding lipoprotein MetQ precursor
143	3.89E + 05	9	32.9/5.8	*S.* Typhimurium	Outer membrane phospholipase A
213	4.06E + 04	6	19.6/5.3	*S. paratyphi*	Putative lipoprotein
114	1.34E + 04	6	39.6/4.7	*S.* Typhimurium LT2	Outer membrane porin protein ompD precusor
201, 214	3.16E + 04	7	18.4/5.7	*S. paratyphi*	Outer membrane protein X precursor
83	4.12E + 04	6	43.2/5.3	*S.* Typhimurium	Elongation factor Tu
3	1.70E + 04	6	89.7/5.2	*S.* Typhimurium	Organic solvent tolerance protein precursor
125	5.41E + 04	10	37.4/5.6	*S. typhi*	Outer membrane protein A

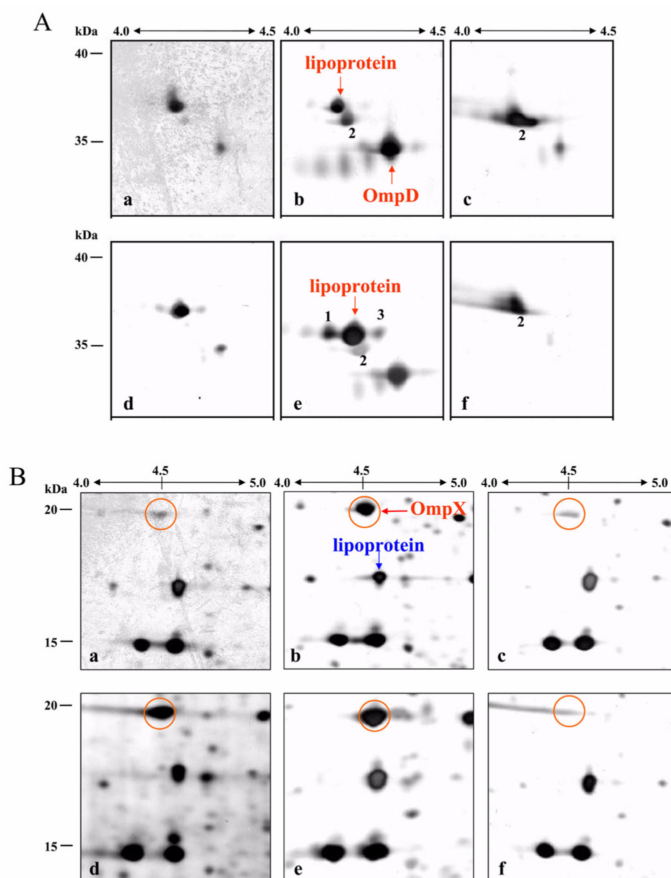

Fig. 2. Comparison of DP 2-DE profiles from 6 different isolates of *S.* Typhimurium, SN72 (a), SN164 (b), SN282 (c), SN296 (d), SN309 (e) and SN310 (f). (A) The range of pI 4.0-4.5 and MW 35-40 kDa of DP 2-DE profiles. (B) The range of pI 4.0-5.0 and MW 15-20 kDa of DP 2-DE profiles.

distribution of 2-DE profile for individual strain in terms of pI. Spots for DP proteins were gathered in two ranges of pI 4.5-5.5 and pI 6.0-7.0 (Fig. 1A). In the case of 2-DE profile for AP, most of the spots were

Table 3. Proteins identified in the 2-DE profile of AP of *S.* Typhimurium SN282 using MALDI-TOF MS

Spot No.	Mowse score	Masses matched	Protein kDa/pI	Species	Protein name
9	5.34E + 15	30	96.2/6.2	*S.* Typhimurium LT2	Iron dependent alcohol dehydrogenase
18	6.72E + 09	14	95.4/5.3	*S.* Typhimurium	Chaperone ClpB
32	8.35E + 04	10	77.0/5.3	*E. coli*	Phosphate acetyltransferase (phosphotransacetylase)
50	4.34E + 08	14	63.5/5.2	*S. typhi*	Prolyl tRNA synthetase
40	8.98E + 03	8	81.2/6.0	*S. typhi*	Lysine decarboxylase
43	8.42E + 04	9	65.4/6.0	*S. paratyphi*	Fumarate reductase, flavoprotein subunit
47	5.94E + 06	16	69.2/4.8	*S.* Typhimurium	Chaperone protein DnaK (heat shock protein 70; HSP70)
58	1.27E + 05	8	57.2/4.8	*S.* Typhimurium	60 kDa chaperonin (protein Cpn60; GroEL protein)
77	8.29E + 10	20	48.0/4.8	*S.* Typhimurium	Trigger factor
79	1.01E + 10	17	51.9/6.2	*S.* Typhimurium	Inosine 5′-monophosphate dehydrogenase
80	2.53E + 06	10	56.2/5.1	*S.* Typhimurium	2,3 bisphosphoglycerate independent phospho-glyceromutase
85	2.00E + 07	13	52.2/5.1	*S.* Typhimurium	Aspartate ammonia-lyase
92	8.21E + 11	22	55.1/5.7	*S.* Typhimurium	ATP synthase alpha subunit
95	9677	7	52.4/5.2	*S.* Typhimurium LT2	Aminoacyl-histidine dipeptidase
104, 108	8.88E + 06	13	51.6/4.9	*S.* Typhimurium	Phage 1 flagellin
128, 136, 137	1.21E + 07	10	45.6/5.3	*S.* Typhimurium	Enolase (2-phosphoglycerate dehydratase) (2-phospho-D-glycerate dehydratase)
130	5.74E + 04	8	42.3/5.1	*S. typhi*	3-oxoacyl-[acyl carrier protein] synthase I
148	3.67E + 10	15	43.2/5.3	*S.* Typhimurium	Elongation factor Tu
149	1.91E + 06	9	43.2/5.8	*S.* Typhimurium	Acetate kinase
156, 169	9.23E + 05	10	41.1/5.1	*S. typhi*	Phosphoglycerate kinase
165	4.56E + 10	17	40.3/4.7	*S. paratyphi*	Cell division protein FtsZ
180	3.54E + 04	8	39.1/5.7	*S. paratyphi*	Fructose 1,6-bisphosphate aldolase
187	3.90E + 06	14	35.5/6.3	*S.* Typhimurium	Glyceraldehyde-3-phosphate dehydrogenase
188	3.96E + 04	13	36512/5.0	*Shigella flexneri*	RNA polymerase, alpha subunit
192	1.12E + 05	8	23.4/5.5	*S.* Typhimurium	Adenylate kinase (ATP-AMP transphosphorylase)
202	1.18E + 05	11	26.9/5.7	*S.* Typhimurium	Triosephosphate isomerase
216	8.48E + 04	8	32.4/6.0	*S.* Typhimurium	Malate dehydrogenase
225, 229, 259	1.189E + 06	9	35/5.8	*S.* Typhimurium	PTS system, mannose-specific IIAB component
234	1.38E + 07	12	30.3/5.1	*S.* Typhimurium	Elongation factor Ts
244	2.23E + 05	9	30.9/5.3	*S.* Typhimurium LT2	Putative hydrolase
301	4.22E + 04	9	23.9/5.4	*S.* Typhimurium	Oxygen insensitive NAD(P)H nitroreductase
304	4.33E + 06	11	24.2/5.2	*S.* Typhimurium LT2	Stringent starvation protein A
329	1.68E + 05	9	22.5/5.3	*S.* Typhimurium	Uracil phosphoribosyltransferase
339	5.92E + 04	9	22.3/5.2	*S. typhi*	Probable peroxidase
355	9.37E + 04	8	19.6/5.0	*S.* Typhimurium	Inorganic pyrophosphatase
356, 357	5.00E + 05	8	20.7/5.0	*S.* Typhimurium	Alkyl hydroperoxide reductase protein C22
402	1.31E + 04	8	15.5/5.3	*S.* Typhimurium	DNA-binding protein H-NS
408	8.98E + 03	5	16.0/5.1	*S.* Typhimurium	6,7-dimethyl-8-ribityllumazine synthetase
410	6.07E + 04	6	16/5.4	*S.* Typhimurium	Universal stress protein A
421	1.05E + 05	9	14.2/5.1	*E. coli* K12	Putative formate acetyltransferase

widely spread at range of over pI 5.0 (Fig. 1B). Individual spots of the 2-DE were randomly selected and subjected to MALDI-TOF MS for identification and annotation using PMF. Among spots detected in the silver staining, 16 spots for DP could be identified as 13 different proteins, whereas 46 spots from AP 2-DE profile were annotated with 40 different proteins. Of these proteins, fumarate reductase and elongation factor-Tu were detected on both 2-DE profiles for DP and AP (Tables 2 and 3, Fig. 1). Except for two proteins, 11 protein spots for DP were identified as outer membrane proteins of genus *Salmonella*. The isoforms of TolC precursors (Spot No. 59 and 60) and lipoprotein MetQ precursors (Spot No. 163 and 165 for MetQ) identified in the DP, had the same MW but different pI (Fig. 1A and Table 2). However, the isoforms of ompX (Spot No. 201 and 214) of the DP showed differences in their MW as well as pI. In the case of 2-DE profiles for AP, the identified proteins were mainly cytosolic proteins (Table 3). Of the identified proteins of AP, two isoforms of phage 1 flagellin (No. 104 and 108), phosphoglycerate kinase (Spot No. 156 and 169) and alkyl hydroperoxide reductase protein C22 (Spot No. 356 and 367) were identical in a molecular mass but a slightly different in pI. In addition, enolase was detected on three spots (Spot No. 128, 136 and

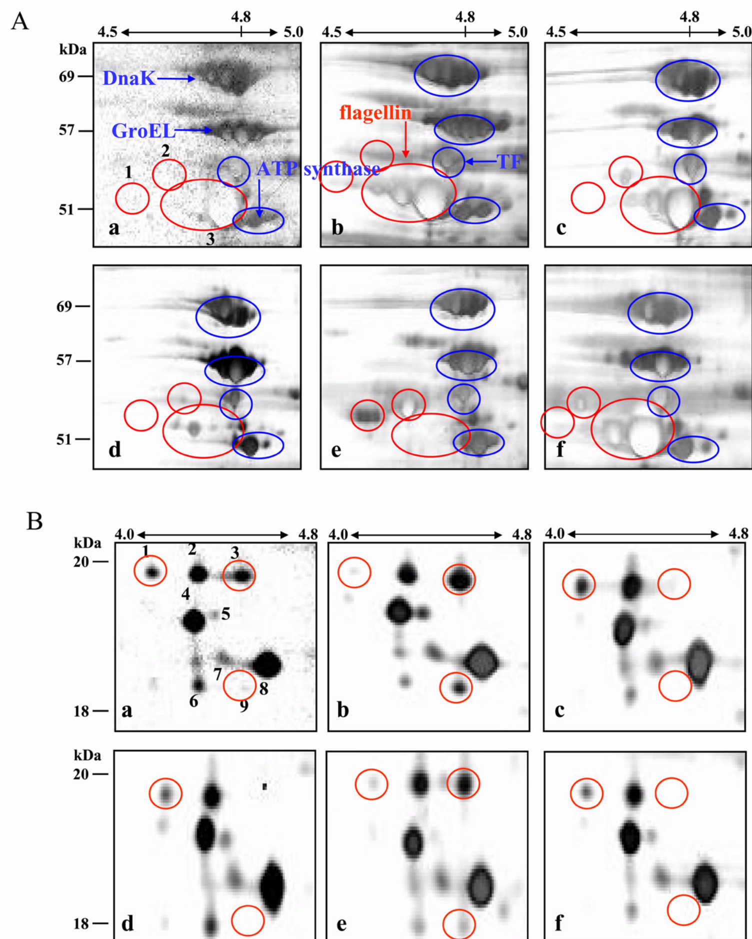

Fig. 3. Comparison of DP 2-DE profiles from 6 different isolates of *S.* Typhimurium SN72 (a), SN164 (b), SN282 (c), SN296 (d), SN309 (e) and SN310 (f). (A) the range of pI 4.5-5.0 and MW 51-69 kDa of DP 2-DE profiles. (B) the range of pI 4.0-4.8 and MW 18-20 kDa of DP 2-DE profiles. (C) the range of pI 4.5-5.5 and MW 20-24 kDa of DP 2-DE profiles. (D) The range of pI 5.0-5.5 and MW 8-15 kDa of DP 2-DE profiles.

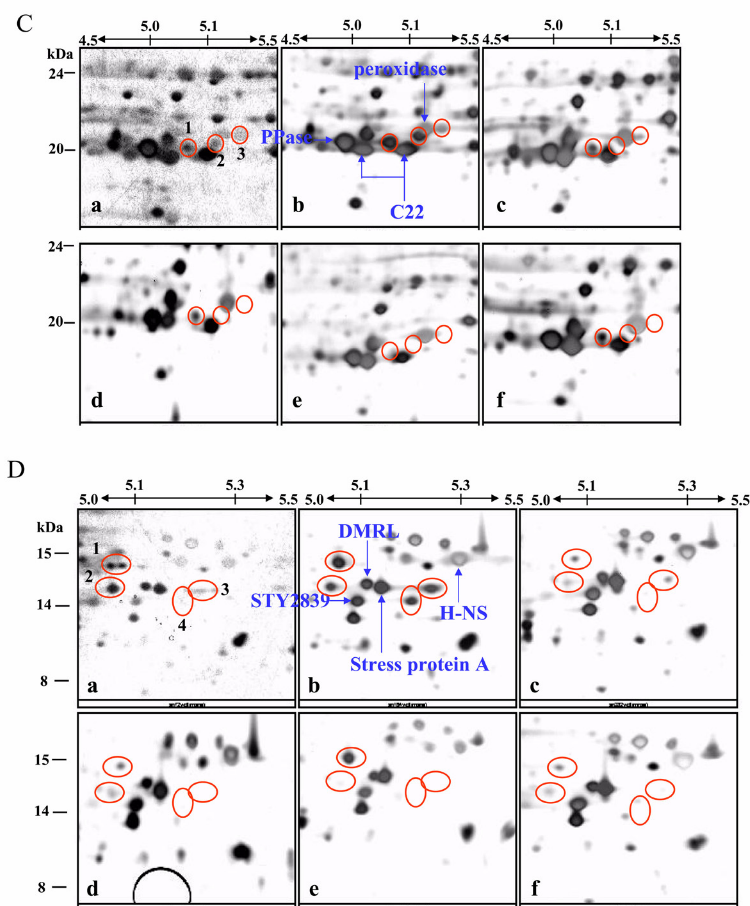

Fig. 3. continued

137) of 43 kDa in 2-DE profile for AP. Mannose-specific IIAB (Spot No. 225, 229 and 259) was detected on three different spots in 2-DE profiles for AP (Fig. 1B and Table 3). Of the proteins spots, spots No. 225 and 229 had an identical molecular mass but a slightly different pI. However, Spot No. 259 had lower molecular mass compared with two spots for mannose-specific IIAB (Fig. 1B and Table 3).

Comparisons of 2-DE profiles of *S.* Typhimurium strains

In 2-DE profiles for DP from *S.* Typhimurium, lipoprotein was detected in all strains, except for SN282 and SN310 strains (Fig. 2A). On the other hand, it's neighboring spots (Fig. 2A), Nos. 1 and 3, were evident only in SN296 and SN309 strains. In the case of spot No. 2, it was absent only in 2-DE profile for DP of an SN296 strain resistant only to penicillin. OmpD spot disappeared only in 2-DE profile for DP of SN310 strain (Fig. 2A). The OmpX spot identified in the DP of SN164, SN296 and SN309 strains were not detected in the rest of the strains (Fig. 2B). When comparing the AP 2-DE profiles from all strains, major differences were evident at 10 different spots (Fig. 3). In Fig. 3A, the phage 1 flagellin spots were absent in AP 2-DE profile of SN72 and SN309 strains. However, the spots of SN296 strain showed lower intensities compared to other strains (Fig. 3A). Spot No. 1 in the AP 2-DE profiles was shown only in SN309 strain. In the case of spot No. 2, it was observed with higher intensity in DT104 compared with that of other phage types (Fig. 3A). When comparing the range of pI 4.0-4.8 and MW 18-20 kDa on AP 2-DE profiles (Fig. 3B) of all strains, spot No. 1 showed highest intensity in

SN72 and SN282 strains, intermediate intensity in SN296 and SN310 strains, and lowest intensity in SN164 and SN309 strains. Spot No. 3 was detected in the AP of SN72, SN164 and SN296 strains but were absent in other strains. Spot No. 9 was unique to the SN164 strain, which was DT193. In the range of pI 4.5-5.5 and MW 20 kDa (Fig. 3C), spot No. 1 was not detected in the AP only from SN309 strain. Spot No. 2 was not detected in the 2-DE profiles for AP from SN282, SN296, SN309 and SN310 strains (Fig. 3C). In the case of spot No. 3, it disappeared on AP 2-DE profiles for DT22 (SN296) and DT104 (SN309 and SN310 strains). In addition, spot No. 3 of SN282 strain showed lower intensity compared to those of SN72 (RDNC) and SN164 (DT193). The 2-DE profile of AP in the range of pI 5.0-5.5 and MW 14-15 kDa showed differences at four different spots (Fig. 3D). Spot No. 1 consisted of two spots on AP 2-DE profiles only in the SN72 strain. However, the spot for SN164 and SN309 strains showed higher intensity than those of the other strains. Finally, spot No. 2 disappeared on 2-DE profiles for DT104 and DT22 strains. Spot No. 3 and 4 showed higher intensity only in DT193 and SN164 strain (Fig. 3D).

Discussion

In this study, comparative 2-DE analysis was employed for investigating protein expression patterns of *S.* Typhimurium strains according to phage type, antibiotic resistance pattern and plasmid profile. The traditional 2-DE analysis has some disadvantages, such as low load ability, poor separation of highly hydrophobic proteins and low resolution due to complexity of sample proteins. In this aspect, we used the phase partitioned with Triton X-114 to reduce complex proteins from *S.* Typhimurium strains and comparative 2-DE analysis for exploring differentially expressed proteins from the bacterial strains.

Our previous study reported a 2-DE reference map for *S.* Enteritidis ATCC13076 using traditional extraction buffer (Mixture of urea and CHAPS). In the map, only one OMP was identified by PMF using MADLI-TOF MS [29]. The study might suggest that OMPs were insufficiently dissolved in the buffer. Therefore, the present study needed alternative methods to obtain both OMP-rich fraction and cytoplasmic proteins-rich fractions from six strains of *S.* Typhimurium. There are a number of extraction methods for OMPs, such as using strong surfactants or high pH buffers combined with ultracentrifugation and temperature-dependent phase partitioning by Triton X-114 [5]. Of these methods, we selected phase partitioning using Triton X-114, because of technical simplicity. Whole cell lysates from six strains were divided into two phases using Triton X-114, DP and AP. PMF results showed that most of the OMPs were present in DP 2-DE profiles, whereas most spots for cytoplasmic proteins were detected in AP 2-DE profiles. Based on the results, partition using Triton X-114 was thought to be a useful method for extraction of OMPs and cytoplasmic proteins of *S.* Typhimurium.

In this study, isoforms of various proteins including, phage 1 flagellin, enolase, phosphoglycerate kinase, mannose-specific IIAB, alkyl hydroperoxide reductase, protein C22, OmpX, TolC precursor and MetQ precursor were observed in the detergent and aqueous phase extracts of *S.* Typhimurium. Most of these isoforms showed differences in the range of their PI values. The presences of several isoforms of the same protein have been reported in the 2-DE maps of various bacteria including *E. coli* [28], *Chlamydia pneumoniae* [44] and *Staphylococcus aureus* [7]. Multiple isoforms of several proteins are also reported for *S.* Typhimurium, Enteritidis, Choleraesuis, Pullorum and Dublin [11]. The occurrence of protein isoforms may have been due to post translation modifications such as phosphorylation and glycosylation, or amino acid substitution, which contributed to the overall protein diversity observed amongst the six isolates of *S.* Typhimurium.

In this study we could identify the proteins associated with virulence and antibiotic resistance mechanisms in the *S.* Typhimurium strains. The overproduction of AcrAB-TolC efflux system was reported to be associated with multi-drug resistant mechanisms in *S.* Typhimurium [1] and *E. coli* [34]. It was reported that the OmpD porin acts as an outer membrane channel protein and was associated with the efflux of methyl viologen [38] and resistance to ceftriaxone [16] in *S.* Typhimurium. The MetQ is the substrate-binding domain of the MetD methionine transport system, which is associated with multidrug resistant pumps [25]. The OmpX, a small outer membrane protein common to gram-negative bacteria, is involved in virulence and resistance to β-lactams and fluoroquinolones in various enterobacterial species [10, 41, 45]. The OmpA, a major OMP of *S.* Typhimurium that is 94% identical to *E. coli* OmpA

[13], is involved in stabilization of the envelope structure and porin activity [29]. The OMP precursors, YaeT/Omp85, are required for OMP assembly of gram-negative bacteria [9, 45], and are also essential components for secretion of bacterial autotransporters, such as YaeT [17]. Their deficiency had been reported to induce impairment in membrane insertion, folding, and oligomerization of many OMPs, such as OmpA, TolC, and phospholipase A [9]. The bacterial phospholipase (outer membrane phospholipase A or OMPLA) is an integral membrane protein located in the outer membrane of many Gram-negative bacteria and has been implicated as a virulence factor [40]. In this study, the histone-like structuring nucleotide protein was also identified in the AP extracts of the *S.* Typhimurium isolates. This protein is reported to be associated with condensation of bacterial chromosome and regulation of many genes associated with adaptation to stressful environmental conditions [15] and multidrug resistance in *E. coli* [30]. These proteins identified in the present study might be useful information in studies on pathogenesis and mechanisms of resistance against antibiotics of *S.* Typhimurium. However, no relationship could be established between the 2-DE protein profiles for the DP extracts of the *S.* Typhimurium strains used in the present study. The strains might have acquired resistance to antibiotics within the host body or in slaughter house environment before infecting the host. Previous studies on the antibiotic resistance mechanisms had reported differences in the expression of proteins, such as TolC and OmpD, in antibiotics treated resistant mutants and their parent bacterium, using proteomic techniques [6, 16, 36, 47].

In this study, we could identify spots specific for phage type, antibiotics resistance and plasmid profile of *S.* Typhimurium. Spots for phage 1 flagellin disappeared in 2-DE profiles from strains, SN296, SN75 and SN309, without 90 kb plasmid. Previous studies showed that a 90 kb plasmid encoded an outer membrane protein associated with fimbrial biosynthesis in enteric bacteria including *Salmonella sp*. Based on the previous and present studies, the 90 kb plasmid is thought to play an important role in synthesis of flagellin in *S.* Typhimurium. On the other hand, we could find one spot specific for DT104 at the range of pI 4.5-5.0 and MW 51-69 kDa on AP 2-DE profiles. In the case of spot No. 9, it was specific for the DT193 strain. In addition, spot Nos. 2, 3 and 4 were absent at the range of pI 5.0-5.5 and MW 8-15 kDa on 2-DE profiles for AP from DT104 strains. Although these spots could not be identified, the spots might be useful for discriminating phage types of *S.* Typhimurium.

Conclusion

Partial 2-DE maps were constructed from DP and AP of *S.* Typhimurium extracted using Triton X-114. PMF from 2-DE gel showed that DP consisted mainly of OMPs, whereas most of the AP included cytosolic proteins. Comparative proteomic analysis showed that synthesis of flagellin was associated with a 90 kb plasmid. In addition, we also found several non-identified spots specific for DT104 and DT193.

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