J. Vet. Sci. (2003), 4(2), 143-149

JOURNAL OF
Veterinary
Science

Reference Map of Soluble Proteins from *Salmonella enterica* Serovar Enteritidis by Two-Dimensional Electrophoresis

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Received May 17, 2003 / Accepted July 23, 2003

Abstract

Protein identification by peptide mass fingerprinting using matrix-assisted laser desorption ionization time of fight (MALDI-TOF) mass spectrometry (MS) can analyze unambiguously identity of the spots from a 2-dimensional electrophoresis (2-DE) gel.

This study developed a technique for 2-DE of *Salmonella enterica* serovar Enteritidis (*S. enteritidis*) by improving the dissolution conditions by 2-DE using a pH 4 - 7 immobilized pH gradient (IPG) strip.

This report examines the protein components from the patterns of the S. enteritidis protein. The most abundant protein displayed a great number of clusters within the pH 4.5 - 7 range with a molecular mass ranging from 35-80 kDa. Some of these spots were identified as metabolic related enzymes. The protein fraction was also analyzed using an immobilized pH gradient strip. Different proteins were identified on the spot according to the elongation factors. In addition, this study showed that the 2-DE analysis of *S. enteritidis* provides useful information regarding the *S. enteritidis* proteome, and this approach might provide a strategy for identifying bacterial proteins using a proteome technology.

Key words: *salmonella enterica* serovar enteritidis, 2-dimensional electrophoresis (2-DE), matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF), peptide mass fingerprinting (PMF), immoblilized pH gradient (IPG)

Introduction

The genus *Salmonella* comprises gram-negative bacilli, which have many cultural properties and antigens in common with other members of the family Enterobacteriaceae.

Salmonella spp. can cause both avian and human salmonellosis. With the great expansion of the poultry industry, *Salmonella* spp. have acquired greater importance among the avian diseases due to economic losses and public health problems [4, 5, 16].

The *S. enteritidis* pathogen involves many interactions of the pathogen with multiple environments, which include the hamhouse. *S. enteritidis* is currently the only salmonella serotype that causes the frequent human illnesses associated with egg contamination [7]. *S. enteritidis* colonization of poultry remains a major issue of increasing food safety and public health concerns. Its pathogenesis has very different consequences for hatched poultry as compared to mature birds. A *S. enteritidis* infection can sometimes lead to a high frequency of illness and death. The incidence of *S. enteritidis* in poultry flocks is continuously being reported in Korea [8, 13].

The availability of the complete genome sequences necessitates the development of functional assays to analyze huge number of predicted gene products. Such approaches are collectively referred to as functional genomics (differential proteomics). However proteins, not genes, sustain the function. Furthermore, the quantity of mRNA in a cell does not always show correlation with the amount of protein that is produced by the cell [2].

Proteomics aims to make a global analysis of the tissue and cellular proteins. A combination of techniques including two-dimensional gel electrophoresis (2-DE), image analysis, mass spectrometry, and bio-informatics are used to resolve comprehensively, quantify, and characterize the proteins [6].

In particular, 2-DE remains the core technology of choice for separating complex protein mixtures in the majority of proteome projects. This is due to the following reasons: the unparalleled power of 2-DE to separate thousand of proteins simultaneously; the subsequent high-sensitivity visualization of the resulting 2-DE separations, which are amenable to quantitative computer analysis for detecting differentially regulated protein; and the relative ease with which the

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This study was supported by the grant ARPC(No. 20010067)

proteins from a 2-DE gel can be identified and characterized using highly sensitive microchemical methods [19].

Proteomics is a powerful and widely used method to investigate protein expression. As a proteome study develops, the proteome on bacteria becomes increasingly important and popular. By having the protein expression in a database, one can identify any pathogenic bacteria and obtain information on a unique property of the bacteria [9, 11. 14].

A database can be identified by analysis of *S. enterica* serobar Typhimurium with 2-DE, and the patterns of the resolved outer membrane proteins (OMPs) often show remarkable similarities between the enteric species, which offers high hopes for protein identification through cross-species matching. In contrast, the OMPs from C. crescentus do not align well with the enteric OMPs [17]. In addition, Molly *et al* reported some of the benefits and limitations of peptide mass finger printing in cross-species searching [1, 3].

S. typhimurium or *E. coli* is frequently used as a model organism for studying bacteria proteomics. Subsequent studies identified more of *S. typhimurium* by 2-DE [20, 21].

Many reports on *S. enteritidis* and its induced diseases have been published, but a study of large-scale expressed proteins from the whole genome of *S. enteritidis* has not yet performed.

This study aimed to provide a global analysis of the expressed protein including an antigen of *S. enteritidis* using proteomics. In the first step, we establish a *S. enteritidis* 2-DE protein map using MALDI-TOF MS.

Materials and Methods

Sample preparation for 2-DE

Salmonella enteritidis (ATCC13076) was grown aerobically in a Luria Broth (LB) at 37℃. Growth was stopped in the late exponential phase at an OD of 1.0 at 600 nm. One hundred milliliters of the culture medium was centrifuged at 65,000 rpm for 15 min at 4℃ , and the pellet was washed three times using a solution containing 40 mM Tris-HCl, pH 7.4. The pellet wes dissolved for isoelectricfocusing (IEF) using a solution containing 9 M, urea, 4%, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 40 mM, Tris-base on ice for 1 hr. After centrifugation at 13,000 rpm for 1 h at 4℃, the supernatant was recovered and used in the 2-DE.

2-DE PAGE

Isoelectricforcusing (IEF) was performed using a Phamacia IPGphor forcusing system. The sample was mixed with the appropriate amount of a rehydration buffer containing 9 M urea, 4% CHAPS, 0.5% IPG buffer, 20 mM dithiothreitol (DTT), a few grains of bromophenol blue, and applied to the 13 cm Immobiline Dry Strips (Amercham Biotech, Uppsala, Sweden) by rehydration overnight in a strip holder. The IEF was carried out for 80,000 Volt using the following volt-hours at 20℃: 30 V for 2 hr, 200 V for 1 h, 500 V for 1 h, 1,000 V for 1 h, 2,000 V for 2 h and 8,000 V for 10 h.

The gels were equilibrated for 15 min using an equilibration buffer I , which contained 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, a few grains of bromophenol blue, and 65 mM DTT. The gels were further equilibrate for 15 min by an equilibration buffer II that containing 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, a few grains of bromophenol blue and 260 mM iodoacetamide. The proteins were separated on 12.5 % polyacrylamide gradient gels and stained with silver and Coomassie brilliant blue G-250.

Sample prepariation for MALDI-TOF

The protein spots were excised from the gel, sliced into 1-mm^3 cubes. The gel pieces were washed twice with distilled water for 15 min and twice with 50% (v/v) acetonitrile for an additional 15 min. The gel particles were reduced for 30 min at room temperature in 10 mM DTT/0.1 M NH_4HCO_3. The DTT solution was immediately replaced with 55 mM iodoacetamide/0.1 M NH4HCO3. After 30 min incubation at room temperature in the dark, the gel particles were washed with acetonitrile and dried in a vacuum centrifuge (Heto-Holten, Allerod, Denmark). The dried gel pieces were swollen in a minimum volume of a 2 ㎕ digestion buffer containing 50 mM NH_4HCO_3, and 20 ㎍/㎕ of trypsin in an ice-cold bath. After 15 min, the supernatant was removed and replaced with the 10 ㎕ of the same buffer but without trypsin, in order to keep the gel pieces wet during the enzymatic cleavage (37℃, overnight). The sample was then sonicated for 5 min, and centrifuged for 2 min using a centrifuge [12].

Sample processing on the MALDI-TOF target

The dried extracted peptides were resuspenced in a 2 ㎕ solution containing pure water:actetonitrile:trifluoroacetic acid(TFA) (93:5:2) and sonicated for 5 min in a bath.

Solution-phase nitrocellulose target preparation was used according to the method reported by Landry *et al* [10].

HCCA (40 ㎎/㎖) and nitrocellulose(20 ㎎/㎖) were prepared separately in acetone and mixed with 2-propanol at a ratio of 2:1:1. An internal calibrant (des-Arg-Bradykinin and angiotensin I) was added to the mixture to make the matrix solution. The matrix solution was mixed with the sample at a ratio 1:1. And 1 ㎕ was spotted onto the target and dried. The immobilized samples were washed with 5% formic acid, which was followed by washing in pure water. The samples were then dried for a second time prior to the MALDI-TOF mass spectrometry analysis

MALDI-TOF mass spectrometry

The sample was analyzed using Voyager DE STR MALDI-TOF MS (Perseptive Biosystems, Framingham, MA, USA). All the spectra were acquired in positive-ion reflector /delayed extraction mode with an acceleration voltage of 20

kV, a grid voltage of 76.000%, guide wire woltage of 0.010% and a delay time of 150 nanosec. The pressure in the TOF analyzer was approximately 2×10^{-7} ton. The mass spectra were acquired using the sum of the ion signals which were generated by irradiating the target with 128 laser pulses.

Protein mass fingerprinting

The mass spectra were acquired using the sum of the ion signals that were generated by irradiating of the target with 128 laser pulses, and subjecting it to a two-point internal calibration using the des-Arg-Bradykinin peak (m/z 904. 4681) and the angiotensin I peak (m/z 1296.6853) to give a typical mass accuracy of ±50 ppm. The monoisotopic peptide masses were assigned and used in the database search. The proteins were identified by comparing the observed mass fingerprints to the NCBIs and the SWISS-PROT databases using the protein Prospector website (http://prospector.ucsf. edu).

Results

Primary experiment

This study used the soluble protein fraction of *S. enteritidis*. Fig. 1 shows a representative 2-DE analysis result of the soluble fraction on the 7 cm, pH 3-10 IPG strip. Approximately 400 spots could be detected by silver staining. The greatest population of the protein components need within pI 4.5-7 range.

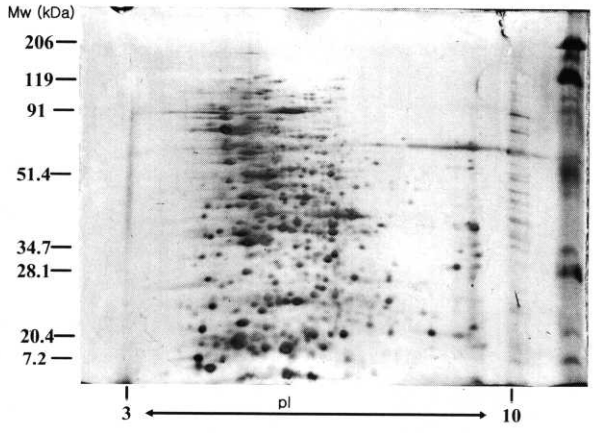

Fig. 1. Separation of the soluble fraction of the *Salmonella* entertidis extracts over the pIs range on the 3-10 IPG strip. The pIs range and location of the molecular weight mark is indicated.

Analysis of the 2-DE protein profiles of *S. enteritidis*

Fig. 2 shows a silver stained 2-DE pattern of the soluble fraction analysis of *S. enteritidis* on the pH 4-7 IPG strip. The resulting 2-DE pattern showed highly resolved protein spots even in the crowded area between pH 4 and 7.

Using silver staining we observed that several abundant protein groups dominated the gel, although many other minor components suitable for MALDI-TOF analysis were also detected.

The first step in obtaining a peptide mass map is the in-gel digestion of a protein spot by trypsin. Fig. 3 shows the performance of spot No. 265 in the peptide mass fingerprinting. However, the identification was constructed based on the *S. typhimurium*, *S. typhi*, and *E. coli*, as there was no information on the identified spots and links to *S. enteirdidis* (Table 1). Spot No. 265 peaks were a database search of this study based on *S. typhi*, *S. typhimurium* and *E. coli*, as there was no gene sequence database on *S. enteritidis* (Fig. 3, Table1).

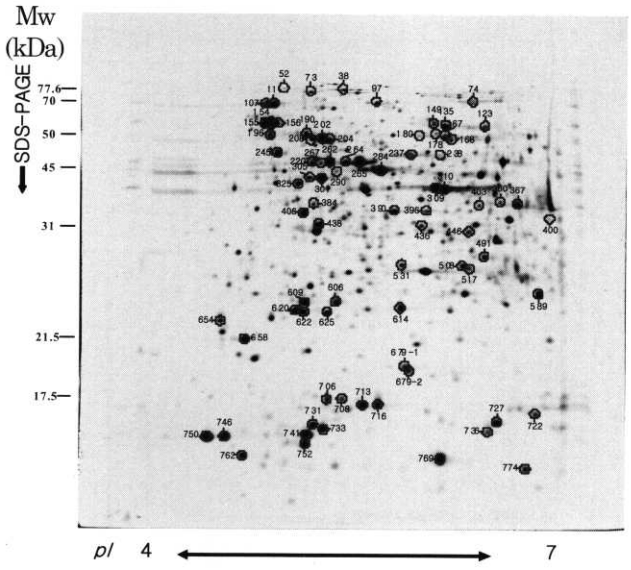

Fig. 2. Silver-stained 2-DE patterns of the *Salmonella enteritidis* soluble proteins. The approximate molecular weights are shown on the left. The approximate pIs are shown along the bottom.

Table 2 shows the identified proteins from *S. enteritidis*. The data is accessible by a protein description. The number of peptides detected by MALDI-TOF MS was 38, 284 and 438. Particularly, Table 1 shows that the number of peptide peaks from No 38, 284 and 438, were the elongation factors, Tu, G and Ts, respectively. The NCBI and SWISS-PROT gene database was analyzed using the MS-FIT program. Different spectra identified the spot as pyruvate kinase I, II (spot No. 132, 267), fumarate hydratase (spot No. 136), enolase (spot No. 264, 265) phosphoglycerate kinase (spot No.301), and fructose 1,6-bisphosphate aldolase, GroEL (spot No. 154.155.156) and DnaK (spot No. 107). All were sufficient for making an unambiguously identification, and the optimized peptide mass fingerprinting could, unambiguously identity the spots found on the sliver stained gel (Table 2).

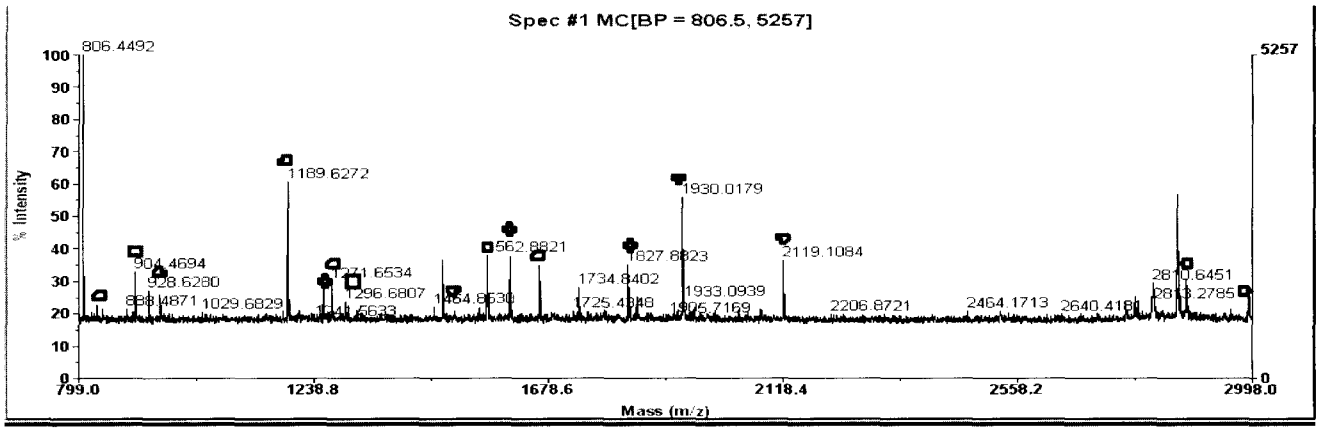

Fig. 3. MALDI-TOF mass spectrometry analysis of spot 265. MS detection was achieved using the Voyager DE-RP mass spectrometer reflectron/delayed extraction mode. The marked peaks (□) correspond to the calibrants, and the other marked peaks(○) are the tryptic peptides. A database search with the mass data identified the protein as an enolase.

Table 1. MS-Fit result of spot No. 265

	MOWSE Score	#/34(%) Masses Matched	% Cov	% TIC	Mean Err ppm	Data Tol ppm	MS-Digest Index #	Protein M/W (Da)/pI	Accessi on #	Species[a)]	Protein Name
1	1.09E+11	15(44)	44	44.1	27.4	33.4	106019	45599/ 5.3	Q8XGP6	SALTY	Enolase (2-phoshoglycerate dehydratase) (2-phosho-D-glycerate hydrolyase)
2	3.56E+09	13(38)	37	38.2	28.8	32.7	96030	45655/ 5.3	P08324	ECOLI	Enolase (2-phoshoglycerate dehydratase) (2-phosho-D-glycerate hydrolyase)
3	1.69E+06	8(23)	30	23.5	28	32.8	24273	45807/ 5.0	Q0KPC5	VIBCH	Enolase (2-phoshoglycerate dehydratase) (2-phosho-D-glycerate hydrolyase)
4	4.20E+05	8(23)	25	23.5	27.1	37.4	73647	45469/ 5.2	Q8ZBN2	YERPE	Enolase (2-phoshoglycerate dehydratase) (2-phosho-D-glycerate hydrolyase)
5	1.29E+04	6(17)	18	17.6	27	32.8	97877	46117/ 5.0	P43806	HAEIN	Enolase (2-phoshoglycerate dehydratase) (2-phosho-D-glycerate hydrolyase)

a) SALTY, *Salmonella typhi* and *typimurium*, ECOLI, *Escherichia coli*; VIBCH, *Vibrio cholerae*; YERPE, *Yersinia pestis*; HAEIN, *Haemophilus influenzae*. Swiss prot database was used.

Discussion

Even though *S. enteritidis* is the only human pathogen that contaminates eggs, the on-farm environment of the chicken is still a rich source of a number of Salmonella serotypes [15].

Salmonella had complete sequence genomes database. However, characterization of most predicted open reading frames is still lacking. To afford such functional genomics , proteome analysis is a powerful global approach as it allows us to functionally characterize many proteins in parallel.

The detection of peptides and proteins using MALDI-TOF mass spectrometry largely depends on the preparation of the target, which promotes the efficient laser desorption of the sample. MALDI-TOF mass spectrometry is often considered to be a more attractive method for protein analysis because it allows a rapid analysis and has high sensitivity.

This study first concentrated on the 2-DE patterns for *S. enteritidis* after evaluating and optimizing the optimal condition of these steps.

The evaluation and processing of the 2-DE *S. enteritidis* samples were conducted by comparing the observed mass fingerprints to the NCBIs and SWISS-Prot database using a protein prosector (http//prosector.ucsf.edu).

Approximately 400 spots were detected on the silver stained 2-DE gel on the 7cm pH3-10 IPG strip (Fig. 1). The results of the 2-DE pattern from the soluble fraction analysis of *S. enteritidis* on pH 4-7 IPG strips showed highly resolved protein spots even in the crowded area between pH 4-7 with molecular masses ranging from 35-80 kDa (Fig. 2).

The reproducibility of the separation was high, although some variations were observed in acidic, high molecular mass region, particularly after applying relatively large quantities of protein in the first-dimensional separation. This is due to the linear gradient.

Table 2. Identified proteins of *Salmonella enteritidis*

spot No.	Accession #	MOWSE score	Protein Name	Protein MW (Da)/pI	No. of peptide matched	% Coverage
38	16762837 M	6.424E+03	elongation factor G	77600/5.2	7 (14)	15
52	[illegible] M	1.213E+04	outer membrane protein precursor	89467/4.9	7 (13)	12
73	[illegible] M	6.339E+05	polynucleotide phosphorylase, member of mRNA degradosome	77039/5.1	11 (24)	17
74	[illegible] M	2.345E+07	fumarate reductase, flavoprotein subunit	65493/6.0	12 (42)	25
107	[illegible] M	2.330E+04	DnaK protein (heat shock protein 70)	69232/4.8	11 (28)	23
111	[illegible] M	3.720E+04	30S ribosomal protein S1	61174/4.9	17 (43)	32
132	[illegible] M	5.093E+04	pyruvate kinase I (formerly F), fructose stimulated	50658/5.7	12 (30)	31
135	[illegible] M	5.856E+06	ABC superfamily (periplasm), oligopeptide transport protein with chaperone properties	65568/6.3	14 (40)	35
136	[illegible] M	4.818E+05	fumarate hydratase class I	60096/5.9	9 (19)	25
154	[illegible] M	2.826E+05	GroEL protein	57286/4.8	13 (17)	28
155	[illegible] M	6.963E+03	GroEL protein	57286/4.8	7 (11)	21
156	[illegible] M	3.405E+04	GroEL protein	57286/4.8	11 (26)	21
167	[illegible] M	2.381E+08	ATP synthase alpha subunit	55113/5.7	15 (26)	39
168	16766917 M	3.370E+06	ABC superfamily (peri_perm), dipeptide transport protein	60220/6.1	13 (33)	28
190	[illegible] M	5.910E+06	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	55555/5.0	10 (14)	27
196	[illegible] M	2.304E+03	phase-1 flagellin	51794/5.0	6 (20)	14
202	[illegible] M	1.559E+05	aspartate ammonia-lyase	52287/5.1	9 (17)	19
220	[illegible] M	4.316E+05	ADP-heptose synthase	51125/5.1	12 (35)	30
237	[illegible] M	1.882E+05	putative arginine deiminase	45562/5.5	10 (38)	26
238	[illegible] M	5.946E+04	serine protease	47311/6.8	10 (38)	29
245	[illegible] M	6.787E+09	ATP synthase beta subunit	50284/4.9	15 (36)	43
248	477806 M	1.159E+10	glycine hydroxymethyltransferase (EC 2.1.2.1)	45415/6.0	16 (47)	39
249	[illegible] M	1.956E+08	survival protein SurA precursor	47251/6.7	17 (27)	33
249	[illegible] M	2.590E+03	nitrite reductase, large subunit	93063/6.0	8 (27)	14
262	[illegible] M	5.972E+04	3-oxoacyl-[acyl-carrier-protein] synthase I	42373/5.1	7 (27)	26
264	[illegible] M	2.651E+03	enolase	45599/5.3	7 (19)	21
265	[illegible] M	1.499E+06	enolase	45599/5.3	12 (54)	40
267	[illegible] M	2.369E+03	pyruvate kinase II, glucose stimulated	51388/6.1	8 (18)	19
280	[illegible] M	1.852E+05	mannitol-1-phosphate dehydrogenase	40900/5.6	13 (44)	34
281	[illegible] M	2.900E+02	putative peptidase T(aminotripeptidase)	44850/5.5	4 (17)	12
284	[illegible]	1.882E+11	translation elongation factor EF-Tu,A	43252/5.3	19 (31)	58
290	[illegible] M	1.248E+05	unknown function in glycerol metabolism	35656/5.3	10 (31)	41
301	[illegible] M	9.390E+02	phosphoglycerate kinase	41133/5.1	6 (13)	20
309	[illegible] M	2.643E+06	periplasmic maltose-binding protein	43153/6.3	14 (54)	40
310	[illegible] M	1.534E+05	fructose 1,6-bisphosphate aldolase	39157/5.7	11 (46)	32
325	[illegible] M	2.387E+07	RNA polymerase, alpha subunit	36512/5.0	16 (80)	45
348	[illegible] M	6.291E+04	glutathione synthetase	35380/5.0	7 (17)	26
361	[illegible] M	5.618E+06	6-phosphofructokinase	34915/5.6	15 (38)	51
361	[illegible] M	1.250E+02	putative carbamate kinase	33351/5.4	5 (17)	18
380	[illegible] M	6.430E+02	glyceraldehyde 3-phosphate dehydrogenase A	35587/6.3	5 (16)	15
390	[illegible] M	5.878E+04	putative carbamate kinase	33351/5.4	10 (12)	26
396	[illegible] M	4.350E+01	threonine dehydratase (EC 4.2.1.16), biosynthetic	56277/5.7	4 (20)	9
400	[illegible] M	1.075E+03	malate dehydrogenase	32476/6.0	5 (12)	20
406	[illegible] M	1.341E+06	transaldolase B	35171/5.1	12 (20)	52
417	[illegible] M	2.362E+09	PTS system, mannose-specific IIAB component	35018/5.8	16 (27)	44
422	[illegible] M	1.479E+08	PTS system, mannose-specific IIAB component	35018/5.8	15 (51)	33
430	[illegible] M	4.235E+04	putative enzymes related to aldose 1-epimerase	32560/5.7	8 (18)	24
431	[illegible] M	3.470E+07	acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	35344/5.4	16 (41)	54
436	[illegible] M	1.072E+04	enoyl-[acyl-carrier-protein] reductase (NADH)	27775/5.6	7 (23)	27

spot No.	Accession #	MOWSE score	Protein Name	Protein MW (Da)/pI	No. of peptide matched	% Coverage
438	16759207 M	8.009E+05	elongation factor Ts	30358/5.1	11 (31)	34
456	16760665 M	2.391E+03	2-dehydro-3-deoxyphosphooctonate aldolase	30796/5.9	7 (45)	32
462	16765898 M	6.256E+04	carries out condensation and ring closure step after PdxA in pyridoxine biosynthesis	26343/5.7	9 (29)	30
470	16761363 M	8.156E+03	putative oxidoreductase	27870/5.0	8 (15)	37
491	16767908 M	2.091E+05	2-deoxyribose-5-phosphate aldolase	28369/5.7	9 (19)	49
508	16759697 M	9.420E+02	phosphoglycerate mutase 1	28494/5.8	5 (13)	20
517	16760352 M	1.854E+05	putative oxidoreductase	27043/5.8	8 (16)	35
520	16760106 M	4.190E+02	transcriptional regulatory protein PhoP, regulator of virulence determinants	25624/5.3	4 (26)	21
531	16765850 M	6.440E+01	putative anaerobic dimethylsulfoxide reductase	88758/6.9	4 (10)	4
534	16767347 M	7.695E+03	triosephosphate isomerase	26917/5.7	8 (20)	26
543	16763955 M	1.288E+03	dihydropteridine reductase/oxygen-insensitive NAD(P)H nitroreductase	23955/5.4	9 (41)	31
606	16759380 M	1.252E+04	probable peroxidase	22318/5.2	8 (13)	54
620	16763234 M	1.817E+03	inorganic pyrophosphatase	19677/5.0	6 (28)	33
622	16759567 M	3.392E+04	alkyl hydroperoxide reductase c22 protein	20748/5.0	7 (16)	52
658	16761352 M	2.247E+03	pts system, glucose-specific IIA component	18247/4.7	5 (33)	38
664	16765025 M	9.900E+02	thiol peroxidase	18026/4.9	4 (16)	32
713	16760132 M	9.390E+02	DNA-binding protein (histone-like protein Hlp-II)	15543/5.3	7 (19)	41
716	16763151 M	3.650E+02	GroES protein	10318/5.4	4 (22)	53
731	16759394 M	5.201E+03	6,7-dimethyl-8-ribityllumazine synthase (riboflavin synthase beta chain)	16008/5.1	6 (8)	40
746	15803726 M	1.106E+03	50S ribosomal subunit protein L21	11564/9.8	5 (35)	35
769	20521562	2.800E+02	100 pct identical to gp:AB021078_3(YcgC of plasmid ColIb-P9)~93 pct identical to gp:AP000342_35(YcjA of plasmid R100)	15767/4.5	5 (33)	18

a) Protein spots analyzed but not identified: 97, 204, 208, 180, 178, 403, 360, 367, 446, 589, 609, 614, 625, 654, 658, 706, 708, 722, 727, 733, 736, 741, 750, 752, 762, and 774.

The protein mass fingerprint was widely successful when searching against the fully sequenced genomes. However, it was unclear whether this technique would be useful when searching across species boundaries. Such a rapid, high-throughput approach would certainly be attractive for identifying the protein in the organisms whose genomes remain coded [18].

Therefore, a useful database was obtained using the analysis criteria for protein identification by peptides mass mapping. For example, an analysis of the peaks from spot No.265 shows the great potential for many uses such as a library in *S. enteiritidis* proteome study. However a database search was not conducted on *S. typhi*, *S. typhimurium* and *E. coli*, because there was no gene sequence database on *S. enteiritidis* (Fig. 3, Table 1). In addition, this study identified the proteins from *S. enteiritidis*, and showed that the major components were the elongation factors Tu, G and Ts. High expression levels could be demonstrated for glycolytic-related enzymes such as pyruvate kinase I, II, famarate hydratase, phosphor glycerate kinase, fructose1,6-biphosphoate aldolase in addition to a number of hypothetical proteins.

In summary, a 2-DE database of the proteome of *S. enteiritidis* was prepared. The soluble *S. enteritidis* protein fractions were analyzed and identified. The most abundant proteins were the elongation factors, the heat shock protein and metabolicrelated enzymes. This identification will make a basic database for the further proteomic analysis using *S. enteiridis*.

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