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Full Length Research Paper

Analysis of pig serum proteins based on shotgun liquid chromatography-tandem mass spectrometry

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Recent advances in proteomics technologies have opened up significant opportunities for future applications. We used shotgun liquid chromatography, coupled with tandem mass spectrometry (LC-MS/MS) to determine the proteome profile of healthy pig serum. Samples of venous blood were collected and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis separation and in-gel trypsin digestion. The peptides were then processed using shotgun LC-MS/MS. Serum proteins were subjected to protein identification and bioinformatics analysis. A total of 392 proteins were identified, and 179 were annotated according to their molecular functions and biological processes, excluding 142 hypothetical proteins and 71 immune globulins. To the best of our knowledge, this represents the first porcine serum proteomics analysis based on shotgun LC-MS/MS. This method and the resulting proteomics information may prove valuable for ensuring good animal welfare practice and for monitoring swine health and disease status.

Key words: Analysis, pig serum, shotgun coupled with tandem mass spectrometry (LC-MS/MS).

INTRODUCTION

Serum is a major body fluid. Serum composition thus reflects the overall health status of the individual animal and is often used to monitor health and disease in farm animals (Bendixen et al., 2011; Eckersall et al., 1996). Studies of protein distribution characteristics in serum may provide significant information to help unravel the mechanisms of disease and for the identification of biomarkers associated with new drug targets and early diagnosis (Issaq et al., 2007; Wan et al., 2006). Human serum protein maps have already been established (Millioni et al., 2012). Detailed serum protein two-dimensional gel electrophoresis (2-DE) identification maps have been described for healthy pigs, and 27 high-to-medium-abundance plasma proteins, including some examples of infection/inflammation-regulated proteins in healthy Landrace × Large white pigs (Miller et al., 2009).

Proteome analysis is most commonly accomplished using a combination of 2-DE to separate and visualize proteins and mass spectrometry (MS) for protein identification (Gygi et al., 2000a). However, the disadvantages of this technique include extensive sample handling, a limited dynamic range, and difficulties in resolving low-abundance proteins with extreme isoelectric points (pIs) and molecular weights (MWs), as well as hydrophobic proteins such as membrane proteins (Corthals et al., 2000; Gygi et al., 2000b; Oh-Ishi et al., 2000). Liquid chromatography, coupled with tandem mass spectrometry (LC−MS/MS), represents a powerful technique for the proteomic analysis of complex samples, where peptide masses may still overlap, even with a high-resolution mass spectrometer (Adams and Zubarev, 2005; Wysocki et al., 2005). LC-MS/MS has been increasingly used for the accurate detection of changes in

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Abbreviations: LC-MS/MS, Liquid chromatography-tandem mass spectrometry; **2-D PAGE,** two-dimensional polyacrylamide gel electrophoresis; **MWs,** molecular weights; **pI,** isoelectric point; **PRRSV,** porcine reproductive and respiratory syndrome virus; **PCV-2,** porcine circovirus-2; **PRV,** pseudorabies virus; **HCV,** hog cholera virus; **FMDV,** foot-and-mouth disease virus.

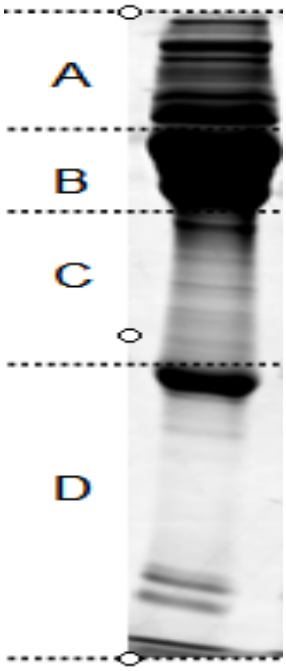

Figure 1. Separation of serum proteins by one-dimensional SDS-PAGE. Samples of 150 μg of proteins were separated on 12% bis-Tris gels and stained to allow protein identification. Four sections were excised and subsequently used for digestion.

protein profiles and to infer biological function (Aebersold and Mann, 2003; Crockett et al., 2005). The shotgun LC-MS/MS proteomics method has been used to identify thousands of proteins in human body fluids, including blood, seminal plasma and tear fluid.

In the present study, gel-LC-MS/MS and bioinformatics analysis methods were used to develop a pig serum protein profile. These results are expected to provide valuable information to assist in the practice of good animal welfare, and for monitoring swine health and disease status.

MATERIALS AND METHODS

Animals

Four Landrace femal pigs, aged about six months, were bought from a local farm and bred in separated rooms. All the pigs were free of the following pathogenic agents: porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus-2 (PCV-2), pseudorabies virus (PRV), hog cholera virus (HCV) and foot-and-mouth disease virus (FMDV), which were detected by polymerase chain reaction or reverse transcription-polymerase chain reaction (datas not shown). The animal experiments were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals, issued by the Council for the International Organizations of Medical Sciences.

Sample preparation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis separation

Samples of intravenous (iv) blood were collected, they were incubated at 37°C for 2 h, then 4°C for 6 h; at last serum were separated at 5000 rpm, for 5 min. The serum protein concentration was determined by quantitative kit (GE) according to the instructions and stored at -80°C until use (Hsieh et al., 2006). Four serum samples (150 μg) were pooled and denatured at 100°C for 5 min in an equal volume of 2× protein loading buffer [0.1 M Tris buffer, pH 6.8, 4% sodium dodecyl sulfate (SDS), 0.2% mercapto-ethanol, 40% glycerol, and 0.002% bromophenol blue], and subjected to SDS-polyacrylamide gel electrophoresis. Samples were separated using 12% homogeneous SDS polyacrylamide slab gels and Tris-glycine-SDS buffer (10 mM Tris, 50 mM glycine, 0.1% SDS, pH 8.0) using a Bio-Rad mini-protean tera system (Bio-Rad). Electrophoresis was carried out at a constant current of 15 mA/gel followed by 30 mA for about 1.5 h until the bromophenol blue reached the bottom of the gel. The gels were then stained with Coomassie brilliant blue G250 (Sigma, USA). Images were acquired using a GS-800 densitometer (Bio-Rad, Hercules, CA).

In-gel digestion

The in-gel trypsin digestion of proteins was conducted according to Wilm et al. (1996). The protein lane of the stained gel was cut into four slices (A, B, C, and D), depending on protein molecular weight (MW) (Figure 1). Each slice was diced into 1 × 1 mm pieces and subjected to in-gel tryptic digestion. The gel pieces were rinsed three times using Milli-Q water and destained with 0.2 ml of 100 mM NH_4HCO_3 in 50% acetonitrile for 45 min at 37°C, until complete depigmentation. The gel pieces were then dried in a vacuum centrifuge. 10 μl of 10 mM dithiothreitol in 100 mM NH_4HCO_3, sufficient to cover the gel pieces, was added to the proteins at 56°C for 1 h. After cooling to room temperature, the dithiothreitol solution was replaced with the same volume of 55 mM iodoacetamide in 100 mM NH_4HCO_3. After 45 min incubation at room temperature in the dark, the gel pieces were washed with 100 μL of 100 mM NH_4HCO_3 for 10 min, dehydrated in 100 μL of acetonitrile, swollen by rehydration in 100 μL of 100 mM NH_4HCO_3, and shrunk again by adding the same volume of acetonitrile. The proteins were subsequently digested with 20 ng/μL porcine trypsin (modified proteomics grade, Sigma) overnight at 37°C. Peptides were extracted by one change of 20 mM NH_4HCO_3 and three changes of 5% formic acid in 50% acetonitrile (20 min for each change) at room temperature (Li et al., 2009; Zhang et al., 2007).

Shotgun LC-MS/MS analysis

The extracted peptides from each gel piece were analyzed using an Ettan MDLC system (GE Healthcare, USA). In this system, samples were desalted on RP trap columns (Zorbax 300 SB C18, Agilent Technologies, USA), and then separated on an RP column (150 μm internal diameter, 100 mm long, Column Technology Inc., Fremont, CA). Mobile phase A (0.1% formic acid in HPLC-grade water) and mobile phase B (0.1% formic acid in acetonitrile) were selected. 20 μg of tryptic peptide mixture was loaded onto the columns and separation was carried out at a flow rate of 2 μL/min using a linear gradient of 4 to 50% B for 120 min. A Finnigan LTQ linear ion trap MS (Thermo Electron, USA), equipped with an electrospray

Table 1. Numbers of peptides and proteins identified in porcine serum.

Parameter	Number of protein	Percentage (%)
Total peptides	5390	100
Total proteins	848	15.7
Protein groups	392	46.2
immune globulin	71	17.1
annotated proteins	179	45.7
Hypothetical proteins	142	36.2

interface, was connected to the LC setup to detect the eluted peptides. Data-dependent MS/MS spectra were obtained simultaneously. Each scan cycle consisted of one full MS scan in profile mode, followed by five MS/MS scans in centroid mode with the following Dynamic exclusion settings: repeat count 2, repeat duration 30 s and exclusion duration 90 s. Each sample was analyzed in triplicate.

Protein identification and bioinformatics analysis

Peptides and proteins were identified using Biowork 3.2 software (Thermo Finnigan, San Jose, CA), which uses the MS and MS/MS spectra of peptide ions to search against the Suina protein database. MASCOT protein scores (based on combined MS and MS/MS spectra) > 72 were considered statistically significant ($p \leq 0.05$). We accepted individual MS/MS spectra with a statistically significant (confidence interval ≥ 95%) ion score (based on MS/MS spectra). The protein identification and annotation criteria were based on Delta CN (≥ 0.1) and Xcorr (one charge ≥ 1.9, two charges ≥ 2.2 and three charges ≥ 3.75). Protein classification was performed using Gene Ontology Annotation (GOA; http://www.ebi.ac.uk/goa/), according to their molecular functions and biological processes. The subcellular locations of different proteins were predicated with PSORT (http://psort.hgc.jp/).

RESULTS

Serum protein SDS-PAGE separation

Serum proteins were separated by one dimensional SDS-PAGE and the gel was cut into four pieces, according to MW, for shotgun LC-MS/MS analysis (Figure 1).

Identification of proteins

A total of 5390 peptides were detected by shotgun LC-MS/MS. A total of 848 proteins were identified, some of which belonged to the same peptides groups. Thus, 392 proteins were finally identified (Table 1).

Characterization of protein profile

The molecular mass and pI value distributions of the 392 identified serum proteins were analyzed. Their molecular masses ranged from 3.2 to 360 kDa, with most being between 10 and 100 kDa (Figure 2A). The pIs of the proteins ranged from 4 to 11.6 (Figure 2B), with most being between 5 and 7. The predicted 2-DE distribution (Figure 3) showed that the pIs of about 95% of the identified proteins were between 4 and 10, representing proteins that are usually difficult to separate by 2-DE. About 40% of the identified proteins fell outside the typical limits of protein resolution obtained by 2-DE. Furthermore, about 14 proteins had higher pIs (> 10), which are also usually difficult to separate by 2-DE, but these proteins were also identified successfully by shotgun LC-MS/MS.

Bioinformatics analysis

A total of 189 peptides were annotated according to the GOA database and were classified on the basis of molecular function or biological process. They could be divided into about five functional molecular groups (Figure 4A): the classical protein group (101, 32.6% of 189 annotated peptides) and the cellular protein group (88, 31.7%) were the most common. The classical serum protein group can be further sub-classified into five subgroups, based on their specific functions (Figure 4B); most proteins were proteases or other enzymes (46, 47.9%), common circulating blood proteins (19, 19.0%), or coagulation and complement factors (18, 18.8%) which are important categories of classical serum proteins. The cellular protein group is also sub-classified into five subgroups according to their function or biological process (Figure 4C): signaling channels, hormone regulation, the cytoskeleton, the nucleus, and cellular metabolic secretions.

DISCUSSION

Serum contains many high-abundance proteins that perform various housekeeping functions, as well as numerous secreted or shed low-abundance proteins that are critical for signal transduction and regulatory events. During necrosis, apoptosis, and hemolysis, cell contents may be released into the serum. In a certain time period, the presence, absence or concentration of a specific protein from serum may be related with the

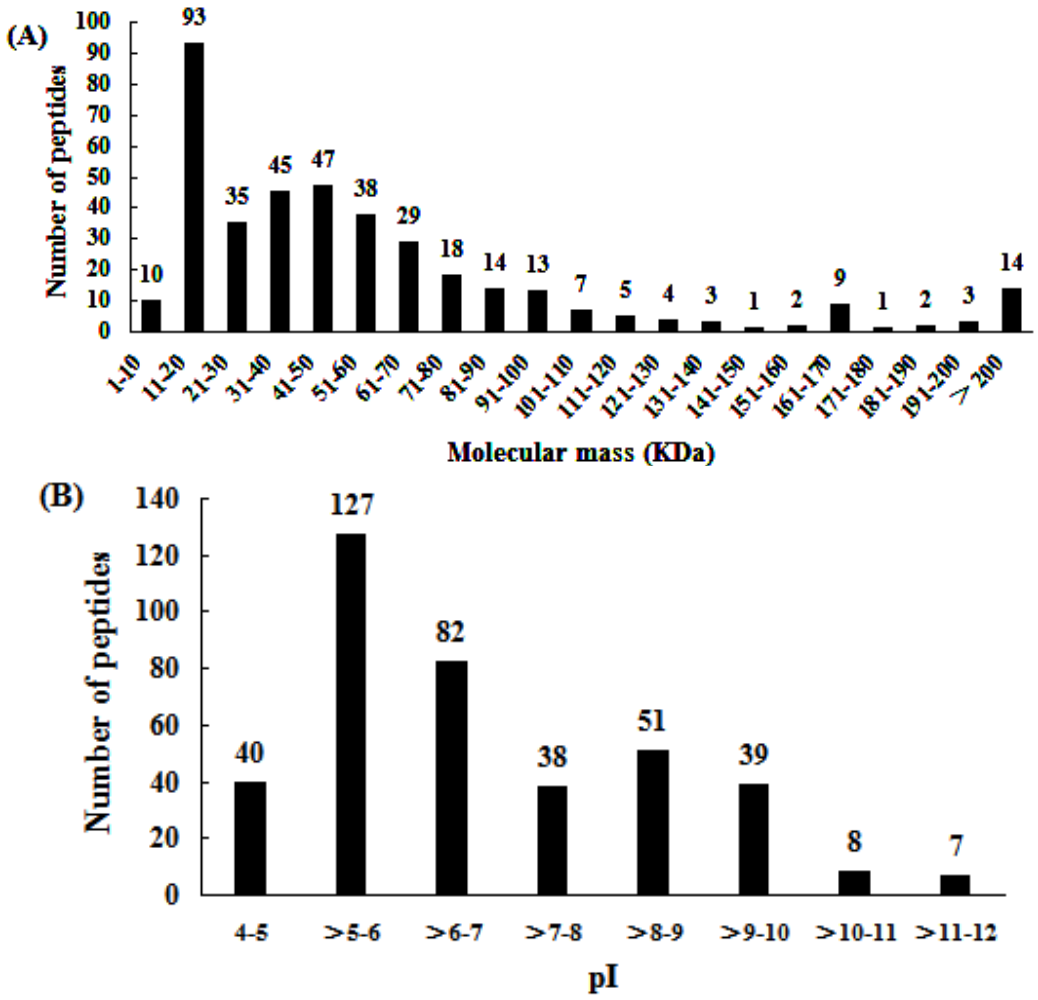

Figure 2. Distributions of molecular weights and pI values for proteins identified by LC-MS/MS, (A) Distribution of molecular weights. (B) Distribution of pI values.

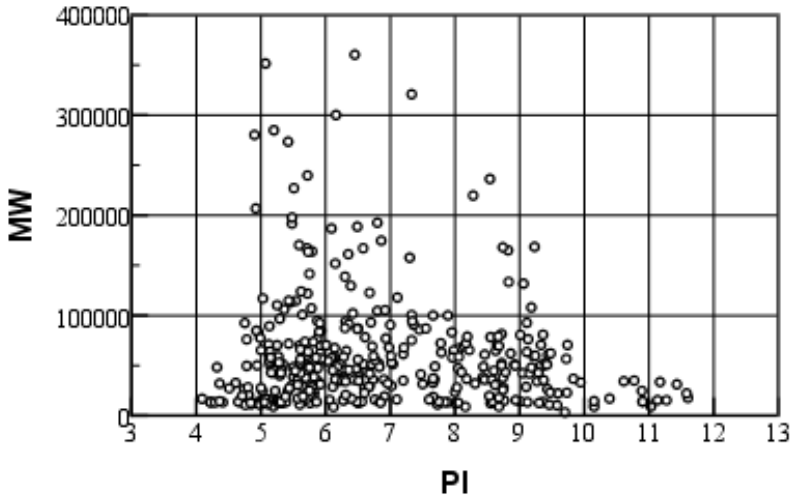

Figure 3. Theoretical 2-DE distribution of proteins from porcine serum. The theoretical pIs and MWs of the proteins were calculated using compute pI/Mw tools according to protein amino acid sequence or ID.

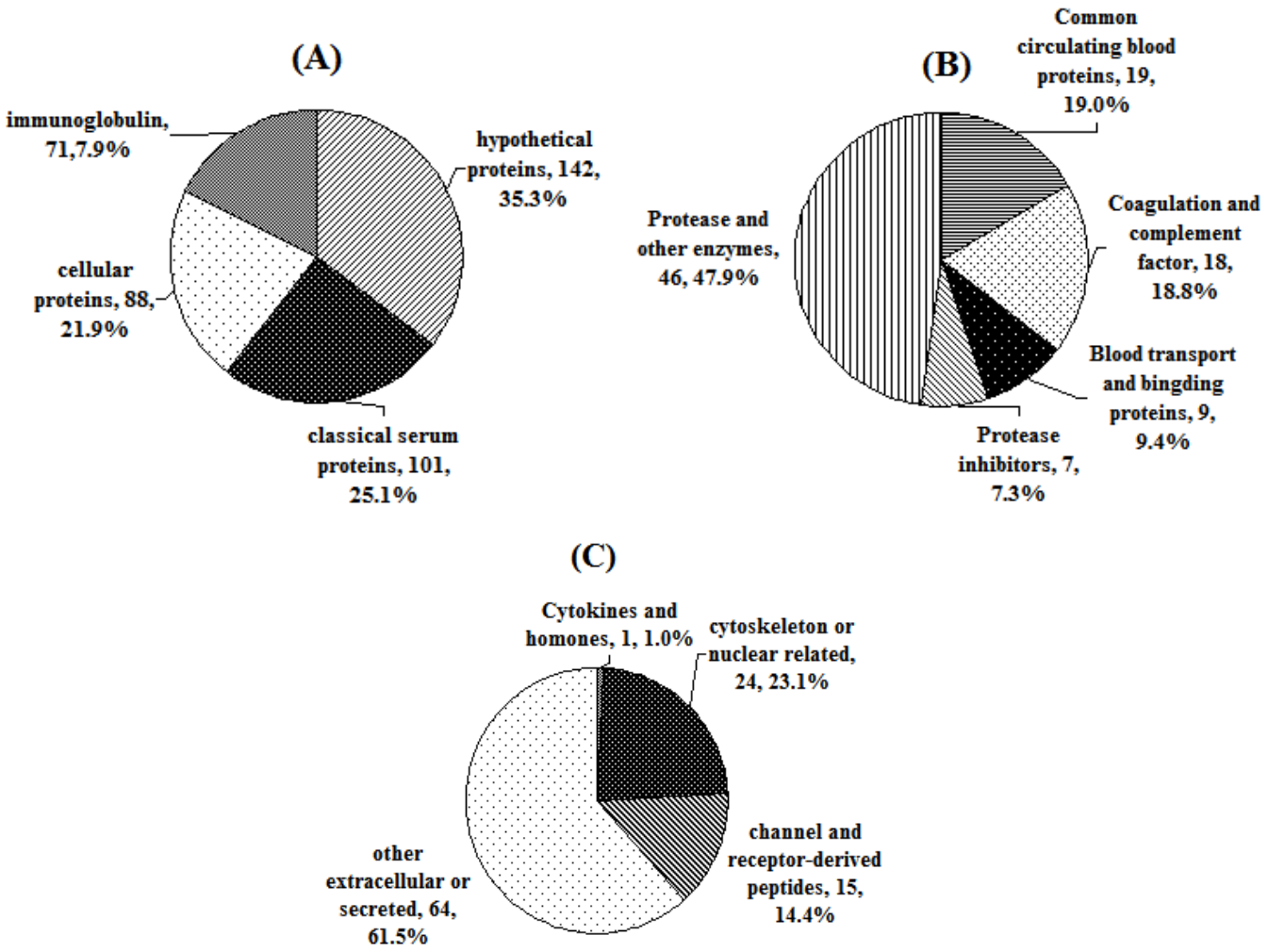

Figure 4. Categorization of 189 serum proteins by molecular functions or biological processes. (A) All identified proteins. (B) Classical serum proteins. (C) cellular proteins.

pathophysiological performance of body, and the presence of these components in blood reinforces the significance of a proteomic approach to identifying biomarkers for disease status. Previous proteomic characterizations of pig serum have used two-dimensional PAGE (Miller et al., 2009). Result shows (Figure 3) 40% proteins that are usually difficult to separate by 2-DE. This study used the shotgun LC-MS/MS proteomics technique combined with informatics analysis to determine the proteome profile of pig serum. This technique represents an efficient strategy for swine serum proteomics research, and overcame the disadvantage of 2DE that cannot separate polarity protein. To reduce the individual differences, serum from four pigs were merged together and used for gel electrophoresis separation. In order to identify proteins according to molecular weight, brand A and B mixed, and brand C and D mixed, respectively (Figure 1).

In this study, given profile of serum protein from porcine, we identified a total of 392 proteins (Table 1), of which 189 (Table 2) were annotated and classified based on their molecular function or biological process (Figure 4). As we expected, besides immune globulin, albumin, apolipoprotein, hemoglobin and actin, which are both ubiquitous in the red blood cells were successfully identified. These represented the main serum proteins, and are involved in the combination and transportation of small molecules (Alaupovic, 1996; Bondarenko et al., 2002). Few coagulation or complement factors associated with whole process of blood coagulation were identified successfully as well. Sodium channel protein, transmembrane channel-like protein related to signaling pathways and receptors were identified, with important functions in signal transduction (Naren et al., 1997). The identified proteins also included 46 kinds of proteases or enzymes related to many important biological processes, such as biosynthesis, metabolic regulation, nucleotide replication, damage repair, transcription and post-translational modification. Fibronectins involved in cell adhesion, cell motility, opsonization, wound healing, and

Table 2. List of 189 annotated serum proteins.

Protein name	PepCount	Unique PepCount	Cover percent	MW kD	p*I*	Accession number
Common circulating blood protein 15						
Albumin	787	52	74.1	69.4	5.92	gi|833798
Alpha-2-macroglobulin	91	37	32.2	167	5.58	gi|311256211
Ceruloplasmin	71	26	33.7	121.8	5.72	gi|311269519
Apolipoprotein B	31	22	12.3	300	6.19	gi|951375
Apolipoprotein A-I	63	15	52.1	30.3	5.48	gi|461519
Apolipoprotein A-II	8	3	26	11.1	7.73	gi|297747304
Apolipoprotein C-III	4	2	28.1	107	4.76	gi|416627
Hemoglobin subunit beta	33	9	72.1	16.2	7.1	gi|3041678
Porcine hemoglobin	30	9	67.8	16	6.76	gi|5542425
Haptoglobin	22	9	30.8	38.5	6.51	gi|41019122
Hemopexin precursor	37	12	45.3	51.3	6.59	gi|47522736
Apolipoprotein E	12	7	24.6	36.6	5.62	gi|461527
Apolipoprotein D	3	2	13.7	21.5	4.76	gi|311269822
Angiotensinogen-like	3	2	6.3	37.8	8.74	gi|311271188
Spectrin alpha chain	2	2	1.7	284.9	5.2	gi|311246557
Coagulation and complement factor 19						
Complement C3	272	55	46.8	186.8	6.09	gi|47522844
Coagulation factor X protein	1	1	2.3	53.1	5.28	gi|113205818
Coagulation factor IX	1	1	2.7	45.5	5.19	gi|60392241
Complement component C3	70	12	60.5	33.4	5.69	gi|295656640
Complement factor B	36	11	22.6	85.9	7.45	gi|162138242
Complement component 4	65	20	19.5	192.5	6.8	gi|158537756
Complement C2	1	1	3	83.3	7.95	gi|156120138
Complement component C5	11	6	5.9	188.6	6.49	gi|37677940
Complement component C6	5	3	5.1	105.3	6.92	gi|148226535
Complement component C7 precursor	1	1	1.5	93.1	6.7	gi|47523630
Complement component C8A	6	4	13.1	66	5.61	gi|147905213
Complement component C8B	7	3	7.4	69.2	8.14	gi|148235410
Component C8G	2	2	10.9	22.3	5.59	gi|148223227
Complement component C9	5	1	3.3	62.3	5.92	gi|148233690
Complement factor I	11	4	11.8	67.1	8.06	gi|311262683
Complement C1	5	2	15.8	26.5	9.43	gi|51491906
Blood coagulation factor XIV	9	4	13.9	51.8	6.23	gi|571399
Coagulation factor XII	1	1	3.6	68	6.98	gi|35039077
Galectin-8	1	1	6.3	36.3	7.86	gi|218664463
Protease inhibitors 7						
Serpin A3-1	89	17	36.9	60.9	8.46	gi|311261515
Inter-alpha-trypsin inhibitor	40	15	22.8	102.1	6.42	gi|48374067
Inhibitor of carbonic anhydrase	34	13	26.9	77.6	5.88	gi|47523160
Alpha-1protease inhibitor	16	6	26.8	47.2	5.54	gi|1703026
Clusterin precursor	12	6	20.4	51.7	5.62	gi|47522770
Plasma protease C1 inhibitor	7	2	6.5	54.6	6.77	gi|178056710
Plasminogen activator inhibitor	2	1	4.7	44.8	8.6	gi|311259199

Table 2. Count'd.

Blood transport and binding proteins 9						
Serotransferrin	258	46	74.7	77	6.93	gi|136192
Vitamin D-binding protein	12	6	24.3	24.5	5.02	gi|5186337
Hemoglobin subunit alpha	10	6	57.4	150.3	8.76	gi|122465
Transthyretin	7	5	48	16.1	6.29	gi|1717817
C4b-binding protein alpha chain-like	4	3	8.3	67.4	6.14	gi|311265150
Transgelin-2-like	3	2	12.6	54.7	6.04	gi|311254018
Polyadenylate-binding protein 4-like	2	1	3.8	70.5	9.33	gi|311258948
Polypyrimidine tract-binding protein 1	1	1	5	59.9	9.24	gi|47523538
Telethonin binding protein	1	1	9.6	18.9	5.38	gi|224809550
Channel and receptor derived proteins 15						
Voltage-dependent anion-selective						
Channel protein 1	3	1	6.7	30.7	8.62	gi|7505046
Signal sequence receptor, alpha	2	1	5.2	32	4.36	gi|297632426
Calreticulin	2	1	7	48.3	4.32	gi|290750002
Signal recognition particle 68 kda protein	1	1	2.4	70.4	8.65	gi|311266756
Lycine receptor subunit alpha-1	1	1	1.4	50.2	8.93	gi|311274089
Sodium channel protein	1	1	0.8	206.8	4.92	gi|311266955
Transmembrane channel-like protein	1	1	1.8	92.5	5.91	gi|311245910
Phosphoinositide 3-kinase adapter protein 1	1	1	1.6	100.7	5.64	gi|194041783
Insulin receptor substrate 4	1	1	2.3	53.1	8.83	gi|258590765
Transient receptor potential cation channel	1	1	0.9	236.2	8.54	gi|311245919
Sodium channel and clathrin linker 1	1	1	6.4	11	4.98	gi|311262562
Mitochondrial import receptor subunit TOM34	1	1	3.1	50.9	9.42	gi|311274903
Calmodulin-like	1	1	14.8	16.8	4.09	gi|311252670
Syntaxin-3-like	1	1	3.9	48.9	8.44	gi|311247613
Protease or other enzymes 44						
Alpha-1-antichymotrypsin 2	32	9	40.2	46.7	6.28	gi|47523270
Plasminogen	18	9	15.8	90.6	7	gi|146345485
Fumarate hydratase	20	8	11.8	13.9	6.3	gi|47523636
Antithrombin-III	28	7	24.8	52.4	5.84	gi|194018664
Prothrombin precursor	19	7	18.8	70.1	5.62	gi|172072659
Membrane primary amine oxidase-like	12	6	15.7	78.3	6.61	gi|311267153
Glyceraldehyde 3-phosphate dehydrogenase	18	4	29.4	35.8	8.57	gi|2407184
Serum paraoxonase/arylesterase 1	8	4	18.8	39.9	5.29	gi|167621416
Kininogen-1 isoform 2	5	4	11	43.8	6.64	gi|311269761
Plasma kallikrein	8	3	7.9	72.3	7.78	gi|47522962
L-lactate dehydrogenase B chain	5	3	17.7	36.6	5.57	gi|1107387
Beta-enolase	19	2	9.22	47.1	8.05	gi|113205948
Pyruvate kinase isozymes	7	2	5.4	64.9	7.98	gi|311260850
ATP synthase subunit alpha, mitochondrial	6	2	7.8	59.7	9.21	gi|297591975
ATP-dependent RNA helicase A	1	1	4.5	44.6	5.53	gi|311264941
Alpha-1-antichymotrypsin 1	5	2	13.8	24.7	5.22	gi|9968809
Transketolase	4	2	9.3	67.8	7.21	gi|162952052
Carbonic anhydrase 1	3	2	12.7	29	6.67	gi|194037099
Pig muscle 3-phosphoglycerate kinase	3	2	8.5	43.4	8.78	gi|13399644
ADP/ATP translocase 1-like isoform 1	3	1	6.7	24.7	10.89	gi|311254417
Phosphoglycerate mutase 1-like isoform 2	3	1	10.5	28.9	6.51	gi|194041795
Ribose-phosphate pyrophosphokinase	3	1	4.7	34.8	8	gi311276762|
Carboxypeptidase B2	2	1	4.3	48.6	6.83	gi|194040626
Transmembrane protease serine 4-like	2	1	2.5	64.5	8.22	gi|311264000

Table 2. Count'd.

Bifunctional aminoacyl-trna synthetase	2	1	1	161.1	1.35	gi|311265228
Glucosamine--fructose-6-phosphate	2	1	2.7	59.5	7.01	gi|311249541
Aminotransferase	2	1	2.7	59.5	7.01	gi|311249541
Polypeptide N-acetylgalactosaminyltransferase	2	1	4.8	64.2	8.63	gi|194042623
Threonyl-trna synthetase	1	1	4	37.7	6.28	gi|311273548
Nicotinamide N-methyltransferase	1	1	9	29.5	5.63	gi|118573081
Phosphoinositide 3-kinase adapter protein	1	1	1.5	107.1	5.78	gi|28860138
Mismatch repair endonuclease PMS2	1	1	1.9	94	6.31	gi|311250873
Alanyl-trna synthetase, cytoplasmic-like	1	1	23.5	8.6	6.12	gi|311257020
Adenylosuccinate synthetase	1	1	2	50.1	8.72	gi|189031714
Inorganic pyrophosphatase	1	1	6.6	27.5	5.44	gi|311271315
Ubiquitin carboxyl-terminal hydrolase 22-A	1	1	2.2	94.4	7.34	gi|311276293
Tyrosine-protein kinase	1	1	1	122.6	6.68	gi|311249266
Ubiquitin-conjugating enzyme E2 L3	1	1	16.2	17.9	8.68	gi|297591969
Serum paraoxonase/arylesterase 1	7	4	14.3	45.6	6.36	gi|118403912
Cholinephosphotransferase	1	1	2.2	42.3	9.05	gi|311262709
Serine/threonine-protein kinase 25-like	1	1	3.5	48.7	6.18	gi|311273415
Serine/threonine-protein kinase Nek5	1	1	0.1	79	8.67	gi|311266294
Rho gtpase-activating protein 23-like	1	1	1	131.8	9.06	gi|311268532
Carbonyl reductase [NADPH] 3-like	1	1	6.1	30.7	5.57	gi|311270205
Cytokines or homones 1						
Interleukin enhancer-binding factor 2	3	2	13.1	43.1	5.19	gi|311254260
Other extracellular or secreted 55						
Spreading factor	6	4	10.7	52.6	5.6	gi|1351418
Heparin cofactor 2	6	4	11.2	55.8	6.5	gi|194043402
Matrin-3-like isoform 1	2	2	5.1	94.7	5.87	gi|311250254
Elongation factor 1-alpha 2	6	1	6.3	50.2	9.33	gi|311263706
60S ribosomal protein L15	2	1	7.8	17.7	11.6	gi|6174950
40S ribosomal protein S5-like isoform 1	2	1	9.8	22.9	9.73	gi|311259613
Vitamin K-dependent protein S	2	1	5.9	27.6	5	gi|311270126
Leucine-rich alpha-2-glycoprotein	2	1	3.8	29.7	7.02	gi|311248408
Macrosialin-like isoform 1	1	1	4.3	42.8	9.29	gi|311261974
Fibronectin isoform 3	27	18	13.9	239.7	5.72	gi|311273025
Histidine-rich glycoprotein	56	12	25.3	61.5	7.2	gi|311269757
Gelsolin	30	11	25.7	84.8	5.93	gi|121118
Alpha-1B-glycoprotein	42	10	30.8	54.4	5.99	gi|311259609
Alpha-2-HS-glycoprotein	59	8	36.3	38.8	5.5	gi|311269753
60 kda heat shock protein	21	8	25.1	60.9	5.7	gi|194044029
Actin	50	6	31.2	41.7	5.29	gi|311250866
Heat shock cognate 71kda protein	26	5	25.9	50.4	5.41	gi|311264120
Heat shock 70 kda protein 1B	18	5	17.5	70.1	5.6	gi|56748897
Heat shock cognate protein HSP 90-beta	13	5	12.3	83.2	4.96	gi|31160516
Heat shock protein HSP 90-alpha	8	3	7.4	84.7	4.93	gi|47522774
T-complex protein 1 subunit alpha-like	13	4	15.4	60.3	5.71	gi|194033404
Vimentin-like	13	4	11.5	70.3	6.01	gi|257096532
Complex of Bdellastasin With Porcine Trypsin	27	3	22.4	23.4	8	gi|257472074
Clathrin heavy chain	7	3	3.8	191.6	5.48	gi|224492556
Fetuin-B-like	4	3	15.4	41.2	7.4	gi|31126975
Zinc-alpha-2-glycoprotein-like	3	3	17.1	34.4	5.88	gi|311250971
Eukaryotic translation initiation factor 3	1	1	16.8	12.6	4.84	gi|311253491
Lumican-like	7	2	11.4	38.8	5.82	gi|194037683

Table 2. Count'd.

Ubiquitin-like modifier-activating enzyme 1	5	2	3.4	114.6	5.54	gi|311276235
Elongation factor 1-gamma	5	2	11.4	50	6.15	gi|311247489
Fascin	3	2	8.5	54.7	6.07	gi|226372953
40S ribosomal protein S15	3	2	28.3	17	10.39	gi|51338618
Zinc finger protein 7	1	1	1.9	76.3	9.13	gi|311253237
Zinc finger protein AEBP2-like	1	1.4	1.4	54.2	5.13	gi|311250679
Zinc finger protein 425-like	1	1	2.6	70.6	9.74	gi|311264767
Polyubiquitin-C-like	1	1	1.2	80.8	9.36	gi|31127000
Adiponectin	1	1	10.3	15.5	8.94	gi|33694199
Troponin T	1	1	6.11	31.2	5.92	gi|66773803
Transcription activator BRG1	1	1	1.4	165	8.82	gi|311248656
Mitogen-activated protein kinase 9	1	1	4	48.4	5.5	gi|311249537
Cytotoxic T-lymphocyte protein 4	1	1	4.5	24.4	5.42	gi|12644505
Centromere protein F-like	1	1	0.5	351.5	5.07	gi|311265008
Transcription factor AP-2 gamma	1	1	4.9	49.1	7.69	gi|178056536
Cell division control protein 42 homolog	1	1	6.8	21.3	5.76	gi122063302
94 kda glucose-regulated protein	1	1	2.1	92.5	4.75	gi|17865698
Ubiquilin-4-like	1	1	3.3	63.9	5.14	gi|311254132
Annexin A5-like	1	1	3.9	42.9	5.16	gi|311262609
Apoptosis regulator protein 1-like	1	1	2.2	71.8	5.43	gi|311271288
C-reactive protein	1	1	11.3	24.9	5.75	gi|628999899
Myosin-9	1	1	1	227	5.51	gi|311255169
ADP-ribosylation factor 1-like	1	1	15	20.7	6.31	gi|311249487
Golgi membrane protein 1-like	1	1	5.9	27.2	4.51	gi|311265509
40S ribosomal protein S28	1	1	17.5	9.1	11.03	gi|45268967
78 kda glucose-regulated protein	4	4	9.5	73.8	5.68	gi|194033595
Leucine-rich PPR motif-containing protein	1	1	2.1	87	7.55	gi311252711
Sytokeleton or nuclear related 24						
Heterogeneous nuclear ribonucleoprotein A1	10	4	20.9	34.2	9.27	gi|116175259
Eukaryotic initiation factor 4A-I	11	3	14.5	46.1	5.32	gi|154147660
Tubulin beta chain isoform 1	6	3	14	49.7	4.78	gi|194040122
Histone H2A type 2-C-like	7	2	37.2	14	10.9	gi|311254405
Plastin-2 isoform 1	3	2	6.4	70.2	5.25	gi|194040624
Collagen alpha-3(VI)	1	1	0.8	32.1	7.33	gi|194043712
Microtubule-associated protein 4-like	3	1	1.87	116.9	5.03	gi|311268808
Heterogeneous nuclear ribonucleoprotein A/B	3	1	6.3	32	8.31	gi|162951821
Histone H3.1-like	3	1	23.5	15.4	11.13	gi|311259879
RNA-binding protein FUS-like	3	1	6.2	52.5	9.4	gi|311251250
Plastin-3 isoform 1	2	1	2.4	63.9	5.73	gi|311276826
Heterogeneous nuclear ribonucleoprotein F	2	1	4.1	45.7	5.32	gi|311271228
Vinculin	2	1	1.7	123.9	5.62	gi|50403675
Heterogeneous nuclear ribonucleoprotein Q	1	1	2.9	69.6	8.68	gi|194035295
Actin related protein	1	1	8.3	19.7	8.53	gi|19556223
Actin	50	6	31.2	41.7	5.29	gi|311250866
Nucleolysin TIAR isoform 2	1	1	4.6	43.4	8.1	gi|311271911
Heterogeneous nuclear ribonucleoprotein D0	1	1	9.5	22.9	9.47	gi|311262905
Small nuclear ribonucleoprotein E	1	1	27.2	10.8	9.46	gi|147903209
Histone H3.2	1	1	23.5	15.4	11.27	gi|311254411
Histone h1t-like	1	1	5.2	22.2	11.58	gi194039830
Nucleophosmin-like isoform 1	1	1	4.8	32.6	4.61	gi|311273930
Nuclear envelope pore membrane protein POM	1	1	7.3	31.2	11.43	gi|311265618
Sister chromatid cohesion protein PDS5	1	1	3.6	37.1	7.66	gi|311262027

maintenance of cell shape were identified (Hakkinen et al., 2010). Some organellar proteins were found (including 40S ribosomal protein S15 and 40S ribosomal protein S28), as well as a few eukaryotic translation initiation factors. Heat-shock proteins (HSPs) are specific proteins that can protect cells and play an important role in growth, development, differentiation and other physiological activities (Arrigo and Simon, 2010; Burel et al., 1992). In a word, this overview map of pig serum protein provided a large number of reference information.

According to function information of proteins, we can make a particular study of partially serum protein in some aspect of disease. Additionally, as can be seen from the sub-cellular localization of identified proteins, these proteins distributed mainly in nucleus, cytoplasm, extracellular matrix, mitochondrion cytoskeleton, and perform their functions in these areas. Therefore, sub-cellular localization of protein from serum has potential values in research on diseases.

Due to the limited number of pig proteins available in the public databases, protein annotation for some of the proteins was impossible and a number of peptide mass fingerprinting was unmatched effectively. The identification of total proteins in pig serum will be achievable as soon as the complete and accurately annotated genome and protein sequence databases for pig become available.

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