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Analysis of *Escherichia Coli* Inducing Salpingitis-Peritonitis in Layer Geese by Two-Dimensional Electrophoresis and MALDI-TOF/TOF

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Abstract

Geese salpingitis-peritonitis mainly happens in layer geese during egg peak period and leads serious economic losses. This study was conducted in different resource *E. coli* inducing Geese salpingitis-peritonitis and Geese or chicken fibrinous pericarditis-perihepatitis-airsacculitis respectively. The molecular mechanism underlying pathogenicity of Geese salpingitis-peritonitis *E. coli* was investigated through analysis of the special protein of this *E. coli* using proteomics and bioinformatics techniques. Two-dimensional electrophoresis (2-DE) analysis showed a total of 42 protein spots only expressed in Geese salpingitis-peritonitis *E. coli*. A total of 21 proteins were identified by MALDI TOF/TOF mass spectral analysis. According to gene ontology (GO) and Pathway analysis, special expressed proteins in Geese salpingitis-peritonitis *E. coli* were mainly involved in the synthesis of macromolecular material, Cellular metabolism and differentiation, Cytoskeleton composition and other relevant Geese salpingitis-peritonitis *E. coli*, 30S RPS6, KASwere possibly associated with its infection.

Keywords: Geese salpingitis-peritonitis *E. coli*; Mass spectrum; Two-dimensional electrophoresis

Introduction

Escherichia coli are a frequent cause of intestinal and extra intestinal infection in animals. *E. coli* infection in mammals is most a primary enteric disease, whereas in poultry is often extra intestinal infection [1,2]. Salpingitis-peritonitis has been reported in layer hens [3-5]. Since 1960's, this kind of disease was reported happening in layer geese and leading gross economic loss, and the pathogen of it were confirmed as *E. coli* [6]. But little was known about the pathogenesis of salpingitis-peritonitis *E. coli* in layer geese.

The purpose of this study was to explore the specific proteins associated with pathogenesis of salpingitis-peritonitis *E. coli* in layer geese. 2-D gel electrophoresis and MALDI TOF/TOF mass spectral analysis were done to analyze and search the specific proteins.

Materials and Methods

E. coli Strains

The *E. coli* strains G837, G803, G8107, E0056, E0060 used in this research were originally isolated in Ministry of Education Key Lab for Avian Preventive Medicine. *E. coli* strain G837, G803, G8107 were isolated from salpingitis-peritonitis samples in layer geese, E0056, E0060 were isolated from fibrinous pericarditis-perihepatitis-airsacculitis samples in geese. CMCC44251 was isolated from fibrinous pericarditis-perihepatitis-airsacculitis sample in chicken. All these strains were identified and reserved in CMCC.

Preparation of total cellular protein extracts from *E. coli* (Extraction of *E. coli* proteins)

All the *E. coli* strains were plated on Mac-Conkey agar plates and incubated at 37° overnight. The following day, the clone of each strain was inoculated in 3 mL of LB broth. The cultures were grown overnight at 37° with continuous shaking at 200 RPM.

E. coli cells were harvested from cultures by centrifugation at 10,000 g, 4°C for 10 min, and washed three times in sterile phosphate buffer saline (PBS, 0.01 M, pH 7.2). The cells were then lysed in 2-D rehydration solution containing 0.0075 g dithiothreitol (DTT), 5 µl ampholytes. The bacterial cell suspension, in the rehydration solution, was incubated on ice for 30 min and sonicated using three bursts of 75 Watt, 30 s on an Ultrason 250 sonicator (LabPlant, UK). After removal of the insoluble debris at 11,000 g, 4°for 90 min, the protein concentration of supernatant was determined using the Nano Drop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, U.S.A.) and was stored at 70°C or subsequent analysis.

Analysis of bacterial proteins by 2-D gel electrophoresis

The bacterial proteins were analysed by 2-DE as described previously and modified [7] For each sample, 350 µg of solubilized proteins was dissolved in 300 µl of rehydration solution, 8 M urea, 4% 3-(3-cholamidopropyl dimethylammonio)-1-propanesulfonic acid (CHAPS), 1% dithiothreitol (DTT), 0.5% ampholytes, trace bromophenol blue, and loaded onto a 17 cm immobilized pH gradient (IPG) linear pH 4-7 strip (Bio-Rad Laboratories, USA). The IPG strips were rehydrated at 20°C for 14 h at 50 V. The rehydrated strips were automatically focused using the following parameters: 250 V, linear, 30 min; 500V, 30 min; 1000 V, linear, 2 h; 8000 V, linear, 5 h; 8000 V, rapid, 65,000 Vh. The current for each strip was limited to 50 A. Isoelectric Focusing (IEF) strips (Protean IEF Cells, Bio-Rad) were incubated for 15 min with gentle shaking in equilibration buffer (6 M

urea, 20% glycerol, 2% SDS and 0.375 mM Tris-HC1, pH 8.8) containing 2% (w/v) DTT, followed by an equilibration for 15 min in equilibration buffer containing 2.5% (w/v) iodoacetamide instead of DTT. Equilibrated IPG strips were further resolved with 10% SDS-PAGE gels (1 mm gel thickness) at 50 V for 30 min, then 200 V until the bromophenol blue dye marker reached the bottom of the gel.

Protein visualization and image analysis

Gels were stained by blue silver staining and scanned at a resolution of 600 d.p.i. using a Powerlook 2100XL-USB scanner (UMAX, Taiwan) [8]. Spot detection, matching and quantitative intensity analysis were performed using PDQuest 2-D software, version 8.0.1 (Bio-Rad Laboratories, USA). The 2-DE was performed in salpingitis-peritonitis strains with fibrinous pericarditis-perihepatitis-airsacculitis strains. Each strain was repeated three times using total proteins. Finally, we selected representative gels from each strain, which reproducibility in the same stain was no less than 85%, which reflected the small variances between strains or running. Relative comparison between the two different types of strains. Only the protein spots which were showed expression only in salpingitis-peritonitis strains not in fibrinous pericarditis-perihepatitis-airsacculitis strains were selected and subjected to identification by MS.

Mass spectrometry (MS)

Spots of special proteins were manually cut from gels and sent to Southern Medical University (Guangzhou, China) to be identified by MALDI-TOF/TOF (ABI 4700 TOF-TOF Proteomics Analyzer, Applied Biosystems, USA).

Bioinformatics analysis of special expressed proteins

The data of those special expressed proteins showed in Goose salpingitis-peritonitis *E. coli* strains were searched and analysed by GPS Explorer using MASCOT (Matrix Science, London), Gene Ontology (GO) Analysis (http://www.geneontology.org/) and Pathway Analysis (http://www.genome.jp/kegg/).

Results

Electrophoretic analysis of special proteins in salpingitis-peritonitis strains and fibrinous pericarditis- perihepatitis-airsacculitis strains from Geese

Total bacterial protein extracts of the *E. coli* strains were analysed by 2-DE as described in Section 2. Approximately 600-700 protein spots can be detected on each 17 cm 2-D gels (pI 4-7) loaded with 350 μg of total protein (Figure 1).

Comparing of multiple 2-DE gel images with PDQuest 2-D software (version 8.0.1), 42 protein spots were found that they were only showed in salpingitis-peritonitis strains not in fibrinous pericarditis- perihepatitis-airsacculitis strains (Figure 2). Twenty-nine proteins spots were selected for identification by MALDI-TOF/TOF (Figure 3). Twenty-one of them were successfully identified (Table 1).

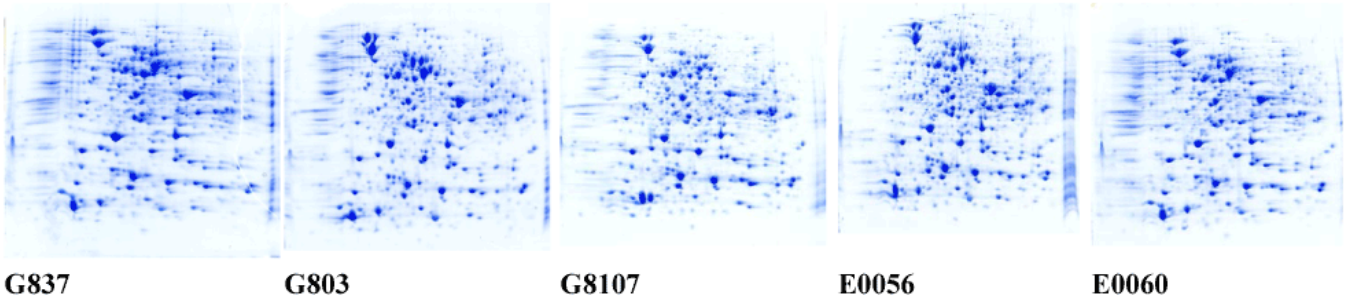

Figure 1: Representative 2-D protein profile of the total cellular proteins of *E. coli* isolate. A total protein extract from the salpingitis-peritonitis E. coli strains and fibrinous pericarditis-perihepatitis -airsacculitis *E. coli* strains was prepared and analysed as described in Section 2.

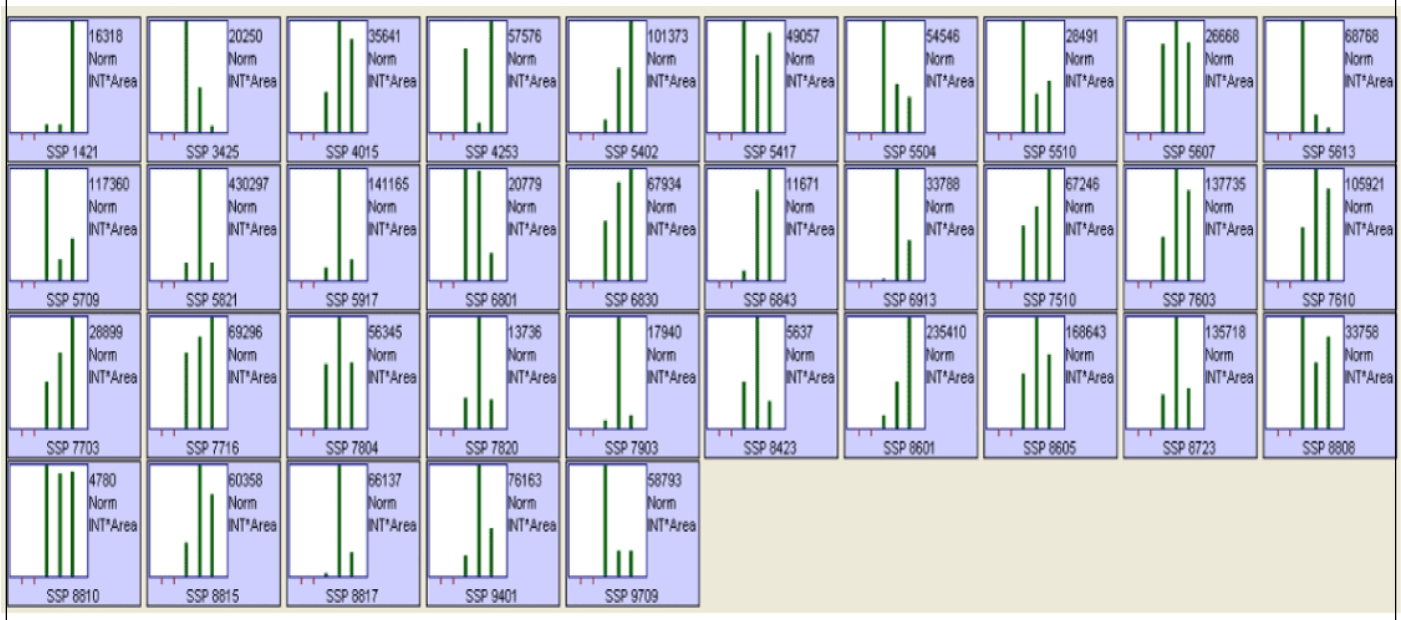

Figure 2: PDQuest 2-D software (version 8.0.1) analysis indicated that 42 protein spots were only showed in salpingitis-peritonitis strains not in fibrinous pericarditis- perihepatitis-airsacculitis strains.

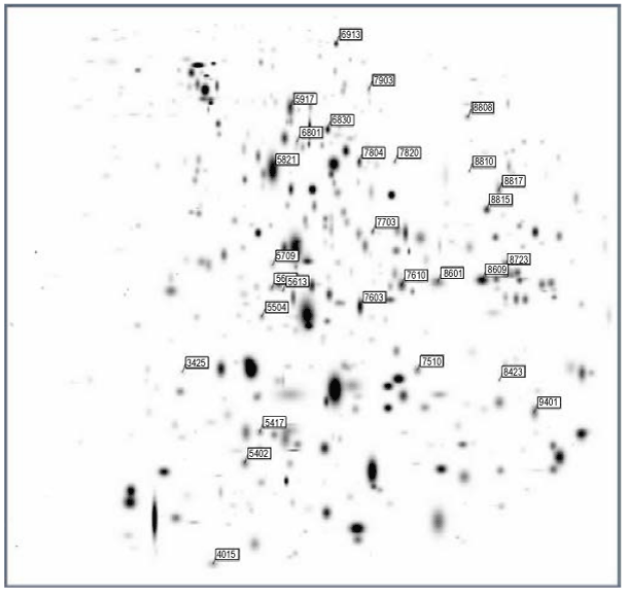

Figure 3: Twenty-nine protein spots were selected for identification by MALDI-TOF/TOF Protein spots identified as part of the current study are numbered automatically by the software. These proteins identified in salpingitis-peritonitis strains differentiation from fibrinous pericarditis-perihepatitis- airsacculitis *E. coli* strains and the locations have been mapped to the above profile using the PDQuest 2-D software package.

Analysis of all these special expressed proteins

According to annotations from the Uniprot Knowledgebase (Swiss-Prot/TrEMBL) and Gene Ontology Database, the identified proteins were classified based on the cellular component, molecular function and biological process (Figure 4 and Figure 6). By the bio-information analysis, the identified proteins were related with the synthesis of macromolecular material, Cellular metabolism, Cell proliferation and differentiation, Cytoskeleton composition and so on.

Discussion

Geese salpingitis-peritonitis was also named geese egg plague and goose *Escherichia coli* sex reproductive organs disease in China [6]. This kind of disease mainly happens in layer geese during egg peak period and leads serious economic losses. At present, few researches associated with geese salpingitis-peritonitis was reported. In this study, 2-D electrophoresis method was applied in the research of the proteomic heterogeneity of geese salpingitis-peritonitis *E. coli* strains to investigate the pathogenesis of geese salpingitis-peritonitis.

The data presented in this report showed the proteomic variation between geese salpingitis-peritonitis *E. coli* and pericarditis-perihepatitis-airsacculitis *E. coli*. Throughout this work, standard protein extraction and analytical protocols were used to compare the *E. coli* cellular proteomes. Bacterial cell proteins were prepared by sonication and lysis of the cells in a buffer compatible with 2-DE and then analysed using 2-D gel system.

In this work, 21 special expressed proteins in geese salpingitis-peritonitis *E. coli* were identified, and associated with the synthesis of macromolecular material, Cellular metabolism, Cell proliferation and differentiation, Cytoskeleton composition and so on.

In recent years, the research of related with the protein of *E. coli,* has become a hotspot research, especially in application of developing

a new drug [9-13]. The protein found in this study can be used for resistance research and super bacteria with conditions research.

No	Accession No	Protein name	MW	pI
1	gi|38704234	aspartate ammonia-lyase	52950	5.19
2	gi|20149796	Chain A, E. coli Cofactor-Dependent Phosphoglycerate Mutase Complexed With Vanadate	28408	5.86
3	gi|3318853	Chain A, The Structure Of Clpp At 2.3 Angstrom Resolution Suggests A Model For Atp-Dependent Proteolysis	21663	5.55
4	gi|15804334	F0F1 ATP synthase subunit alpha	55416	5.80
5	gi|16130258	3-oxoacyl-[acyl-carrier-protein] synthase I	42928	5.35
6	gi|213581781	acetate kinase	14835	5.72
7	gi|110807394	6-phosphofructokinase	37503	5.81
8	gi|170769713	triosephosphate isomerase	27094	5.64
9	gi|290586581	30S ribosomal subunit protein S6	15694	4.93
10	gi|89107229	regulator protein that represses from RAB operon	10425	5.84
11	gi|15799843	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase	24624	5.09
12	gi|110591231	Chain A, Crystal Structure Of An N-Terminal Deletion Mutant	51547	5.27
13	gi|77416725	3-oxoacyl-[acyl-carrier-protein] synthase 2	43247	5.71
14	gi|238690047	Enolase	45627	5.25
15	gi|84028790	Single-stranded DNA-binding protein	18963	5.44
16	gi|167008648	ATP-dependent Clp protease proteolytic subunit	23286	5.52
17	gi|67473134	Transaldolase A	35865	5.89
18	gi|166990504	Serine hydroxymethyltransferase	45459	6.03
19	gi|84027800	Succinyl-CoA ligase	30044	6.32
20	gi|84028666	Glutamine-binding periplasmic protein	27173	8.44
21	gi|81645945	Uridylate kinase	26129	5.96

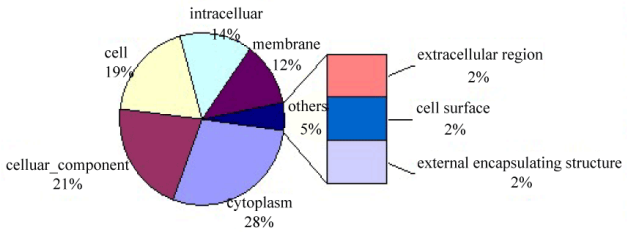

Figure 4: Proteins classification based on the cellular component

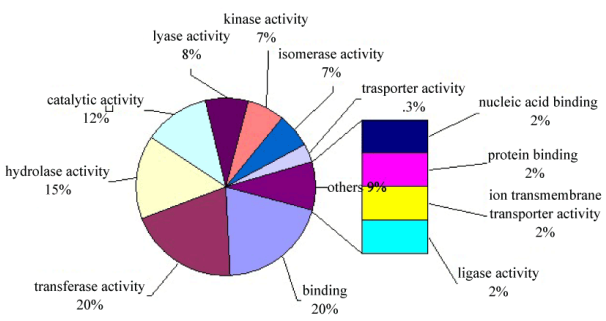

Figure 5: proteins classification based on the molecular function

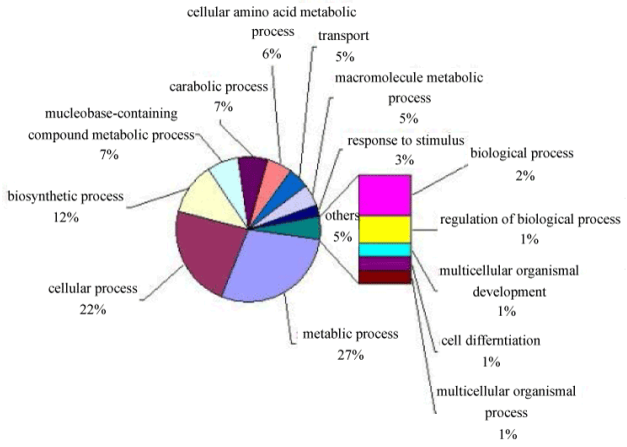

Figure 6: proteins classification based on the biological process

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