IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN DIFFERENT GROWING STAGES IN CHICKEN LIVER BY PROTEOMICS APPROACH

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ABSTRACT

닭의 간은 해독작용, 당의 저장, 혈장 단백질의 합성 등 주요 기능을 하는 것으로 알려져 있다. 본 연구는 간에서 성장 단계별로 발현량에 차이를 보이는 단백질들을 비교해 보았다. 2차원적 전기 영동에 의해 분리된 300개 이상의 단백질들이 확인되었으며 이 중 성장 단계별 특이적인 13개의 단백질은 MALDI-TOF MS에 의하여 분석이 되어졌다. 본 연구를 통하여 밝혀진 단백질들은 생화학적인 연구에 중요한 자료를 제공할 것으로 사료된다.

▶**Key words** : chicken liver, different growing stages, MALDI-TOF MS, two-dimensional electrophoresis

INTRODUCTION

Proteomics is a recently developed technique and can see all protein profiles expressed from in a given tissue or cell. Even though all the cells have the same set of genes, proteome expressions from different tissues or cells are different. Therefore, proteomic study can be very useful for identifying protein-protein interaction in a given environment and the identification of differentially expressed proteins. In chicken, skeletal muscle proteome was previously investigated for identification of growing stage specific proteins in a layer strain (Doherty et al., 2004). In this study, chicken livers in different growing stages were investigated in order to identify the biological roles for the differentially expressed proteins.

MATERIALS AND METHODS

Liver samples from White Leghorn breed were obtained at 0, 10, 21 and 32 weeks of age. Obtained liver samples were immediately dissected and stored at -70℃ until use. Frozen liver samples (200 mg) were used for protein extraction. Protein content of each sample was determined according to Bradford (Bradford, 1976) using BSA as a standard. For first dimensional separation, 1 mg protein of each sample was loaded onto immobilized pH-gradient (IPG) strips (pH 3-10 NL, 18 cm : Amersham Biosciences, Sweden) and rehydrated for 12 hr. Focusing was performed

in 4 steps containing 200 V 1 hr, 500 V for 1 hr, 500 V for 1 hr, 1000 V for 1 hr and final focusing step of 8000 V for 8 hr at 20℃. After equilibration, The second dimension was run on a 12% polyacrylamide SDS gel using a PROTEAN II xi electrophoresis kit (Bio-Rad, USA). Staining was carried out using a Coomassie Brilliant Blue G-250 (Fluka, Germany). Gels were scanned using a Powerlook III image scanner (UMAX data system, Taiwan) and gel images were saved as TIFF file formats. The gels were compared using the 2D Image Master (Amersham Biosciences, Sweden).

RESULTS and DISCUSSION

In this study, chicken liver proteome was investigated for the possible solution of detoxification, glycogen storage, plasma protein synthesis and fat metabolism in different growing stages. The identified proteins are currently under investigation for their biological roles specifically in different growing stages in chicken liver. Upon finishing, this information will give valuable informative for improvement of chicken productivities.

REFERENCE

1. Doherty M.K., McLean L. et al. 2004. The proteomes of chicken skeletal muscle : Changes in soluble protein expression during growth in a layer strain. Proteomics. 4:2082-2093.

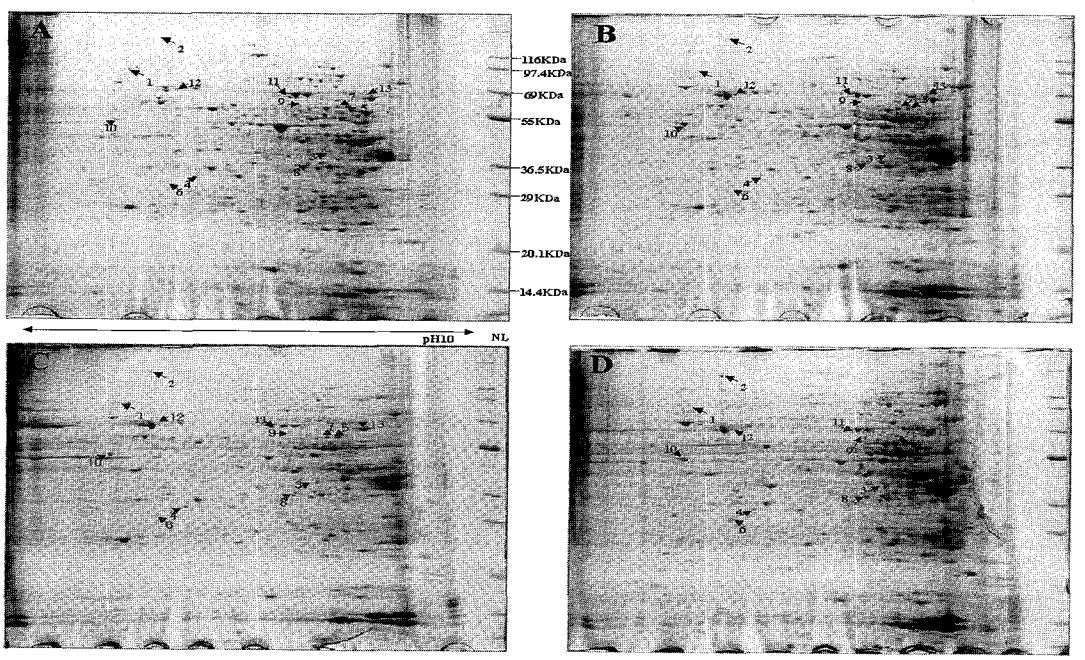

Figure1. 2-DE Gel images of chicken liver. Chicken livers were obtained at 0 (A), 10 (B), 21 (C) and 32 (D) weeks of age. Total 13 differentially expressed protiens spots were selected.

Table1. The identification results for the differentially expressed proteins in four growing stages in chicken liver. Note that three proteins (spot No. 6, 8, 9) were identified as lipid related proteins.

Spot No.	Est'd Z	Accession No	Protein Information	Coverage (%)	pI	kDa
1	-	-	albumin	30	69.872	5.51
2	-	-	albumin	39	69.872	5.51
3	1.89	XP_413719.1	PREDICTED: similar to Sorbitol dehydrogenase (*L*-iditol 2-dehydrogenase) [*Gallus gallus*]	29	7.1	38.91
4	2.17	1ALA	Annexin V	44	5.6	36.34
5	2.34	XP_421486.1	PREDICTED: similar to catalase [Gallus gallus]	31	7.3	55.77
6	2.21	NP_990486.1	fatty acid synthase; thioesterase [Gallus gallus]	5	5.9	271.27
7	2.18	XP_421486.1	PREDICTED: similar to catalase [Gallus gallus]	30	7.3	55.77
8	-	XP_419350.1	PREDICTED: similar to Malate dehydrogenase, cytoplasmic [Gallus gallus]	18	10.3	68.15
9	2.34	AAK97531.1	malic enzyme [Gallus gallus]	17	6.4	62.69
10	1.77	XP_414512.1	PREDICTED: similar to Stress-70 protein, mitochondrial precursor (75 kDa glucose regulated protein) (GRP 75) (Peptide-binding protein 74) (PBP74) (Mortalin) (MOT) [Gallus gallus]	21	6.1	73.5
11	2.4	XP_421486.1	PREDICTED: similar to catalase [Gallus gallus]	26	7.3	55.77
12	2.4	CAE45562.1	triosephosphate isomerase [Anser anser]	51	6.2	22.84
13	1.73	NP_990451.1	enolase [Gallus gallus]	29	6.2	47.71