18.1

Immunoproteomic Analysis of the Major Allergens from Dust Mites

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House dust mites are ubiquitous and the major source of allergenic components. Very little is known about the mite proteome apart from several allergens characterized previously. Knowledge of the other major proteome components may be useful in enhancing our understanding of host allergic responses, mite control, monitoring and biology. As there are no genome sequences of mites available, proteomic studies on these organisms are a challenging task. In order to understand its underlying components, proteins from *Dermatophagoides farinae* were extracted and separated by 2D electrophoresis. Protein spots were excised and subjected to MALDI-TOF and ESI-MS-MS. The peptide mass fingerprints (PMF) and MS-MS sequences were compared to *in silico* fingerprints generated both from in-house mite expressed sequence tags (EST) and public databases. Various criteria were optimized to identify the proteins against EST database and 100 of the most abundant spots were evaluated. Many proteins appeared as multiple isoforms, matching similar contigs composing of various ESTs. The group 1, 2, 7, 10 and 13 mite allergens were among these major contigs. Careful analysis indicated that about 32 of the top 100 protein spots could be allergenic proteins or their isoforms. The other known allergens however did not appear as abundant proteins. Highly conserved proteins from other mites and different or polymorphic isoforms of the same protein could also be identified via PMF. Full cDNA sequences of these abundant proteins were obtained. Bioinformatic analysis indicated that many of these proteins are novel with unknown functions, while others show homology to putative gene products from the *Drosophila*, common structural, biosynthetic or metabolic proteins such as actins, cuticle-like proteins, esterases, kinases, and ferritin. Further, the pan-allergen arginine kinase was first found in the dust mite as major proteins and the isoforms of arginine kanse could be differentiated by de novo sequencing. We have demonstrated in this study that even without the genome sequence, protein identification can still be performed to a certain degree using PMF and ESI- MS-MS with the help of expressed sequence tags, in particular the major proteins and high copy transcripts. With the combined EST and proteomic strategy, we have successfully identified, cloned and evaluated more than 40 groups of allergens, believed to be the full spectrum of the allergenic components from major dust mite species.

18.2

Deciphering Embryo-Maternal Cross-Talk by Holistic Proteome Approaches

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In mammals, early development of embryos, their implantation and the maintenance of pregnancy are critically dependent on an efficient exchange of molecular signals between the embryo and the maternal environment. Dysfunctions during these embryo-maternal communication processes lead to increased rates of embryonic mortality. Only few molecular signals involved in this process have been identified so far. We investigate this cross-talk by differential proteome approaches using *in vivo* models. Endometrial tissue samples from synchronized (estrous cycle) non pregnant and pregnant cows were collected. To avoid variations caused by different genetic backgrounds, we use monozygotic twins, generated by embryo splitting at the morula or blastocyst stage, giving rise to animals with identical genomes. Sample collection as well as sample preparation were optimized by extensive test series, with a major focus on minimizing serum proteins. Two strategies are applied to obtain quantitative proteome data of endometrium of pregnant versus non pregnant cows. LC-MS/MS is used in combination with cleavable ICAT reagent for protein quantification, and multidimensional LC is applied for online peptide separation. The identification and quantification of tagged peptides is performed using an ion trap mass-spectrometer. In a second approach, 2D-DIGE fluorescent labelling, including internal standards, is used for quantification of differentially expressed proteins. Overlapping pH-gradients are applied to enhance resolution in the first dimension. Biological samples of at least three twin pairs are analyzed, and two replicas are performed per twin pair. Significant differences of protein expression, found by means of these complementary strategies, are described.

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18.3

Proteomics Analysis of Differential Protein Expression in Rat Brain Regions

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CNS is a challenging area for proteomics for a couple reasons; first, much of the response in the brain is thought to be “electrical” rather than changes in protein expression and second, the brain is the primary CNS tissue, but this tissue has many sub-regions known to respond differently to different conditions and stimuli. In the current study, we tested the feasibility of the second issue. A study was designed to look at five brain regions from six rats. The objectives of the study were to look at the combined technical and biological variation seen across the six animals in each brain region, and two, to assess the degree of difference in protein composition across the five brain regions.

Five brain regions—hypothalamus, hippocampus, MPFC, striatum and mesolimbic—were dissected out of each of the six rats studied. Each brain tissue was separated by 2D PAGE (PI range 4–7). Gel images were analyzed using software Z4000 (Compugen). No protein identifications were done in this study. Statistical analysis provided an overall assessment, but in addition, particular proteins with high variance were studied in more detail.

Animal to Animal Variation: When comparisons were made across all six animals for each brain region, we observed an average value of 0.23 $\pm$ 0.13 for the analysis of correlation of variation where hippocampus remained as one region that has the lowest variation in protein expression across animals.

Region-region variation: When protein spots were compared across all five regions for each animal, we saw a matching in the higher end of 41% for one animal and in the lower end of 32% for another. Average correlation of coefficient for expression intensity of the protein spots matched between two regions for each animal was around 0.80.

18.4

Study of Apoptotic Changes in In Vitro Condition

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Apoptosis or cell suicide is important mechanism in both development and homeostasis in adult tissues for removal of either superfluous, infected, transformed or damaged cell by activation of an in trinsic suicide program.

One of the signals that induced apoptosis is Glucocorticosteroides which can promote apoptosis in rat thymocyte.

Dexamethasone as a prototype of corticosteroids may induct indigenous endonuclease-mediated cell apoptosis. Studies or involvement of the SM signaling system showed that several cytokines including TNFα, CD95/ FAS/ APO-1 and environmental stresses induce rapid ceramid generation while affecting an apoptotic response. In Particular, and early generation of ceramide, through the sequential activation phosphatidylinositol-specific phospholipase c (PI-PLC) and acid SMase, is required for.

Dexmethazon induced Caspase activation and apoptosis.

Dexamethasone using objects.

1. Evaluation the effects of Dexamethasone on rat cells.
2. Study of light and electorn microscopic changes.
3. Demonstrating relevance between doses of drag and extent of apoptosis.

However, we organized treatment group consisting of four sub-group (every one having 5 rats), naming, T-a, T-b, T-c, and T-d Sub groups which every subgroup received Dexamethasone intraperitoneally in 0.5, 1.5, 2.5 and 3.5 mg/kg body weight, respectively. We organized a four-sub-grouped group as Control and six hours after administration the thymus gland of all treatment and control group rats were ectomized and were provided for preparing light microscope (LM) and electronic microscopic (EM) section. The results of this investigation suggest that apoptotic bodies appear as round or oval cytoplasmic masses with or without contained basophilic clumps of chromatin and EM studies demonstrate the peripheral nuclear chromatin form aggregates of osmiuphilic granules, (X6200) which separate from the fibrillar core and irregular cell outline nuclear fragments and whirling endoplasmic reticulum (X8200). In this therapeutic and over does excite and stimulate DNA fragmentation in thymocytes. Corticosteroides death of thymocyte is recognized as a calcium dependent process.

18.5

Proteomics and Its Appplication in Plant Nutrition Research

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As one of the most active and important research fields in the post genomic era, proteomics-based approaches, which examine the expressed proteins of a tissue or cell type, complement the genome initiatives and are increasingly being used to address biological questions. Though the field of plant proteomics is just at the very beginning, it has achieved great progresses. In this paper, the outline of proteomics and the various experimental approaches used in proteomics were introduced, such as two-dimensional polyacrylamide gel electrophoresis, mass spectrometry, and protein database. Recent advances in understanding proteomic changes of the plants under nutrient and environmental stresses were reviewed, and The application of proteomic approaches to the studies on plant hormone function and regulation was also addressed in this paper. The perspectives for proteomic application to plant nutrition research were discussed.

At present, many plant nutrition scientists have made great efforts to find plant mutants under nutrient stresses, and then the physiological mechanisms of nutrient uptake or transport by the mutants have obtained great achievements. Meanwhile, they have started to study the proteomic changes of the mutants by proteomics approaches, expecting to reveal the cell regulation mechanisms of the plants. Take these for examples. Kazuya Suzuki *et al.* has found that formate dehydrogenase is induced by iron deficiency in Barley roots. Herbik A. *et al.* has studied Iron and Copper-nutrition dependent changes in protein expression in a tomato wild type and the nicotanamine-free mutant chloronerva. Gh. Hosseini Salekdeh *et al.*, has carried out proteomic analysis of rice leaves during drought stress and recovery.

However, application of proteomics approaches in plant nutrition science is just at the beginning. As far as the applications, there are two outstanding fields, one is to draw the protein expressing maps of the plants, which may reveal nutrition uptake and transport mechanisms; Another is to indicate proteomic changes of the plants under nutrient and environmental stresses so as to make clear the physiological or cell regulation mechanisms of the plants under adversities.

18.6

Proteomic Analysis of Cultured First Branchial Arch Cells from Avian Embryos of Early Facial Patterning Stage

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Growth of the maxilla and mandible plays a critical role in facial developmental patterning and morphology. We wanted to the *in vitro* system. Therefore we developed dissect out the factors that control the growth of the facial development a functional assay of first branchial arch cells with micromass culture. After the culture of first arch cells from chicken embryos at the stages immediately after the end of neural crest cell migration and also at the stages of early facial patterning, we could observe two different types of cultured cells; one with the embryonic stem cell colony and the other with dispersed fibroblastic cell layer. In order to characterize the nature of cultured cells as well as to identify the differences of *in vivo* and *in vitro* cells for the first branchial arch, we performed the proteomic analysis. The cultured embryonic stem cells displayed the similar proteomic profiles to the early cultured, single cell-isolated first arch. In addition, some differentially expressed proteins were identified at each group of cells. From above results, we could figure out the characteristics of early first branchial arch cell, which is similar to embryonic stem cell in terms of proteomic profiles.

18.7

Analysis of Proteins Expressed in Striploin at Muscle Development Stage and Fat Development Stage of Hanwoo Steers: Using Two-dimension Gel Electrophoresis (2-DE)

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Adult bovine skeletal muscle (striploin) contains a lot of intramuscular fat (marbling) that plays a major role in the combined contribution of flavor and juiciness toward overall consumer acceptance in Korea. The adipogenesis within muscle begins between 11 and 13 month and continues to develop with aging in Hanwoo steers. However, there is almost nothing known about the physiological mechanism and stimulus for marbling, but once the marbling begins, the muscle may undergo characteristic changes as expression of specific proteins. Therefore, our study was conducted to determine a more effective method for the proteome analysis of striploin using 2-DE, and compare to the difference of striploin proteins between muscle development stage (11-month) and fat development stage (17 or 24 month) in Hanwoo steers. We modified the solution for rehydration in IEF separation on IPG strips: Urea (7M), Thiourea (2M), DTT (65mM), Ampholytes (Pharmalyte (3–10 NL, 0.5%)). Using this rehydration solution, more than 700 protein spots on each gel were detected by silver staining. Different protein spots between 11 and 17-month in modified rehydration solution were increased more than 80 spots compared with normal solution (Urea (8M), DTT (65mM), IPG-buffer (3–10 NL, 0.5%)). Gels of pooled 11, 17 and 24-month samples exhibited a total 1002, 782 and 798 protein spots respectively, using image master platium 5.0. The spots matched among the 11, 17 and 24 month were 513 spots. The 19 specific spots were detected between 11 and 17-month and 25 spots were detected between 11 and 24-month, but between 17 and 24-month were only 2 spots. Our results suggest that the increased or decreased specific proteins may be related to inducing of adipogenesis (determination of multipotent stem cell) within striploin muscle.

18.8

Identification of Phosphorylation Sites of a Rice ABF Family of bZIP Transcription Factor by Mass Spectrometry and Phosphopeptide Mapping

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Abscisic acid plays key role in responses of plants to stress, especially water deprivation. ABA response is controlled by trancriptional regulation of genes. It is believed that interactions of more than five kinds of transcription factors are involved in tissue specific responses of ABA in *Arabidops*is. Though there are many reports on ABA mediated signal transduction in *Arabidopsis*, few works were achieved in rice. Recently we ascertain that phosphorylation of rice bZIP1 protein, a class of ABI5 gene, is regulated by specific rice Ser/Thr protein kinase. As an effort to understand the regulatory mechanism of ABA mediated signal transduction pathway and function of the OsbZIP1 gene, we studied characteristics of phosphorylation of this protein. In vitro kinase assay showed that OsbZIP1 is phosphorylated at multiple sites. Phosphoryalted proteins were digested with trypsin or endoproteinase Glu-C. We identified phophorylation site using immobilized metal affinity chromatography, matrix associated laser-desorption post-source decay (MALDI-PSD) and de novo sequencing using MALDI TOF TOF mass spectrometry. Phosphorylation site identified in this study will give a clue for understanding ABA mediated responses.

18.9

Proteomic Analysis of Plasma Membrane Proteins from Undifferentiated and Differentiated Mesenchymal Stem Cells

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Human bone marrow-derived mesenchymal stem cells (hMSCs) differentiate into osteocyte, adipocyte, chondrocyte, and myocyte. As expected, many important receptors and adhesion molecules known to be involved in osteogenesis and adipogenesis were activated during differentiation. We examined the expressed plasma membrane (PM) protein profiles of undifferentiated hMSCs and hMSCs induced to osteogenesis and adipogenesis by 2-dimentianal electrophoresis and MS/MS analysis and confirmed that proteins by RT-PCR, western blotting, immunocytochemistry. We identified that more PM proteins were differentially expressed than previously identified PM proteins of hMSCs and compared both of that profiles. These studies indicate if undifferentiated hMSCs is capable of differentiating to ways different from formal studies. In addition, in vitro-differentiated cultured hMSCs should be applicable to the validation of cells for the cell therapy.

18.10

Proteomic Analysis of Rice Seed During Grain Filling and Seed Maturation Highlights That Most of Enzymes Involve in Carbohydrate Metabolic Pathway Are Products of Multigene Family and/or Posttranslational Modification

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We adopted 2-DE to analyze protein profiles expressed during different developmental stages of rice seed maturation. Rice seeds from 1 week and 3 weeks after fertilization (WAF) and matured stage were used for the analysis. More than 1,000 peptide spots could be detectable on a 2-DE. 199 proteins out of about 240 peptide spots on 2-D gels were identified by MALDI-TOF mass spectrometer (MS) and nano LC ESI Q-TOF MS (identification rate = 82.9%). Sequence coverage of the 199 proteins by the peptides identified by MS was 21.3%. The high rate of sequence coverage was due to continuous development of proteome analysis methods while conducting this project. The 199 proteins identified by MS could be categorized into 6 different groups according to their expression patterns during seed maturation.

Out of the 199 peptides, 99 peptides were identified different, i.e. 39 protein spots were identified as 2–10 times. By careful analyses of the multiple spot proteins, it was interestingly found that most proteins which show more than three spots are belonged to the enzymes involved in carbohydrate metabolic pathway. Moreover it was surprisingly found that the enzymes in carbohydrate metabolic pathway are products of multigene family and/or posttranslational modification. Here in this study, we demonstrate by high throughput proteome analysis that the regulatory mechanisms for starch biosynthesis in the developing rice seed are far complex than those published so far.

18.11

The Use of Biotechnology in Iran

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Biotechnology is the commercial application of biological processes to provide more efficient routes to such important end products and in recently research in Iran for breeding most of plant it is used. One of the important oil plant in Iran is rapeseed. Rapeseed breeding for oil quality is very important and necessary. For the first step we must optimizing gene transformation by the Reporter Gene. The aim of this project was optimizing transformation and regeneration systems in *Brassica napus* using GUS reporter gene too. Four strains of *Agrobacterium* and 3 explants (root, stem and leaf) were tested. The infected explants were transferred to shoot inducing media and after 3 weeks shoots were transfered to solid dark and light media (without any hormon). After assay the regenration transformed plants the results showed that there were a great difference between the various Strains and shoots in the dark media have had a highly regeneration rate.

18.12

Comparison of Soluble Proteins Profiling of Gastrula to That of Blastula

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Soluble proteins profiling of early stage embryos for the pattern organism supplied abundant information to explain embryonic developmental course and discover molecular mechanism of development. The soluble protein expression profile of gastrula and blastula of *Bufo bufo gargarizens* was developed using 2DE strategy. More than 1000 protein spots were visualized and about 200 proteins were analyzed by PDQest. Soluble proteins from gastrula and blastula of *Bufo bufo gargarizens* were separated by 4% IEF and 10% SDS-PAGE. After scanned by GS-800 protein densitometer of Bio-Rad, the total soluble protein spots' distribution were examined. The results show that the signal ratio r which stands for the similarity of protein spots' distribution in gastrula and blastula is 0.807175 and there are 217 soluble protein spots in blastula while 145 soluble protein spots in gastrula. In contrast to blastula, 114 protein spots disappeared and 42 new protein spots added in gastrula, in which most proteins were in the range of high molecular weight and high basic pI on the gel and the change occurred mainly above 5.00 of pI and over 20.1kD of molecular weight. Also comparing to blastula, there are 43 protein spots two fold upper and 5 protein spots two fold lower which distribute in different pI and different molecular weight in gastrula. Thus, proteins' expression changed greatly when blastula developed to gastrula while detail explanation to its mechanism needs further research.

18.13

Liver, Spleen, and Heart Tissue Preparation for Proteomic Analysis in Transplantation Immunology Study with Rodent Models

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Liver allografts are spontaneously accepted in some rat strain combinations without immunosuppressive therapy while heart and other organ and tissue allografts are rejected. In order to better understand the mechanism of allograft rejection and tolerance, a proteomic approach has been proposed. Liver tolerance and heart rejection have been chosen as model systems. The aim of this study was to establish sample preparation methodology as the first step for proteomic analysis. Liver, spleen and heart tissues were taken from adult recipient or normal rats. The tissues were flash frozen in liquid nitrogen and transferred into vapour phase of liquid nitrogen tank at below -130°C for storage. Before two-dimensional electrophoresis, the tissue was homogenized by mechanical disruption. Proteins were separated using pre-cast immobilised pH gradients (IPGs, Bio-Rad) with pH ranges of initially 3–10 and 4–7. The IPG strips were reduced, alkylated and detergent exchanged, and second dimension SDS-PAGE performed. Gels were fixed in 40% (v/v) methanol, 10% (v/v) acetic acid for 1 h, and then stained with Sypro Ruby (Molecular Probes, Eugene, OR) overnight. Gels are de-stained for 2 h in 10% (v/v) methanol and 7% (v/v) acetic acid and scanned using a Bio-Rad Molecular Imager Fx. The results showed that satisfactory protein separation was achieved and conditions required for each tissue varied. Optimization of conditions for individual tissues is being carried out.

18.14

Proteomic Analysis of Nasopharyngeal Tumorigenesis Tissues of Rat in the Different Stages

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Proteomic techniques have recently become available for large-scale protein analysis. We constructed a proteome map of nasopharyngeal tumorigenesis tissues in the rats by using two differential fractionated techniques to isolate the proteins. The rat nasopharyngeal carcinomas were induced with N,N-Dinitrosopiperazine (DNP).The simple hyperplasia, atypical hyperplasia, and nasopharyngeal carcinoma tissue were got, from which proteins were isolated, separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and identified by matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry followed by peptide mass fingerprinting. To contrast with simple hyperplasia, atypical hyperplasia, and nasopharyngeal carcinoma tissue, a total of 67 unique proteins were identified from carcinoma tissue, including ILGF, IL-14, onco-protein WNT-2, MAPK-8 et al. Bioinformatic analysis predicted glycosylation to be the most common explanation for multiple forms of the same protein. These imply that, proteomic analysis of nasopharyngeal carcinoma is a promising tool to study nasopharyngeal carcinogensis and to determine biomarkers of nasopharyngeal carcinoma. ILGF, IL-14, onco-protein WNT-2, MAPK-8 may be key factors in the development of NPC.

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18.15

Proteomic Study on Regulatory Mechanism of Oxalate Metabolism in Rice

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Oxalate is widely distributed in plant kingdom, which may account for 6–10% of tissue dry weight in many plants. Accumulated data have been showing that oxalate may play an important role not only in physiological processes but also in stress resistance of plants. In order to understand the regulatory mechanism of oxalate metabolism in plants, two-dimensional electrophoresis (2-DE) was employed to compare the global protein patterns between the rice leaves with contrasting oxalate content regulated by nitrogen forms. More than 800 protein spots were clearly visualized with 62 protein spots expressed differentially. There were 3 proteins that were specifically expressed and 41 proteins were increasingly expressed in high oxalate level leaves as compared with the leaves with low oxalate content. In contrast, 2 specific proteins were observed and 16 proteins were expressed at a higher level in the low oxalate level leaves. Thirteen interested proteins were analyzed by MALDI-TOF-MS, and the data from peptide mass fingerprinting were used in protein database search. The results indicated that an aminotransferase was likely up-regulated in the leaves with low oxalate content. The active amino acid syntheses need consuming more organic acids as the carbon skeleton so as to decrease the organic acids. Another protein spot up-expressed in high oxalate content leaves was a photosynthesis related protein. Photosynthesis serves as the carbon source for organic acid synthesis and its increase may lead to more oxalate yield. In addition, putative 33 kDa oxygen evolving protein translation initiation factor, eIF-5A antibody variable domain putative SCARECROW gene regulator actin cytokeratin were up-expressed in the leaves with higher oxalate content. The findings provided cues for the further understanding of the regulatory mechanism of oxalate metabolism in plants.

18.16

Proteomic Analysis of the Mitochondrial Membrane Proteins of HL Type Cytoplasmic Male Sterile Rice

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Rice is one of the most important food crops in the world and is the main nutritional staple for approximately 40% of the world's population. Annual rice production on a worldwide basis must increase to support the rapid increase in the human population. Since rice hybrids show heterosis, which subsequently result in them having yields 15–30% higher than inbred varieties, these hybrids may offer a solution to this problem. The combination of cytoplasmic male sterility (CMS) and a nuclear gene for restoration of fertility (Rf) are essential for breeding hybrid varieties and for hybrid seed production. CMS, which eliminates the possibility of self-pollination, is commercially used in the production of hybrid seeds for economically important plants. Mitochondrial defects account for all instances in which the nature of the lesion responsible for CMS has been identified. However, the specific mechanisms of Mitochondrial defects causing CMS are only poorly understood. In order to understand the unique functional characteristics and probe the regulation mechanisms from genome to transcriptome and proteome, it is necessary to compare the comprehensive protein expression profiling of mitochondrion membrane of YTA (CMS), YTB (maintenance line) and HL-6 (F1) with each other. The proteins of mitochondrial membrane were separated by two-dimensional electrophoresis with immobilized pH (3–10) no-line gradients as the first dimension and the SDS-PAGE as the second. And the expression profile of the three kinds of mitochondrion membrane was developed. With 2DE strategy, total 750 protein spots were visualized and there are more than 100 proteins were different between them, and 20 proteins were identified by MALDI-TOF/MS (this work is going on). Functional categories of the identified proteins showed that some of these proteins were involved in the signal transduction.

18.17

Identification and Characterization of Meat-based Allergens Via Immunoproteomic Approach

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Many allergenic components from food have been identified, but little is known about vertebrate meat allergy or the allergens as it is thought to be rare. Study on cross-reactivity among different meat-based allergens is also limited due to insufficient meat allergens being identified, lack of B-cell and T-cell epitope sequences and structural information in major public databases. Our study based on a novel immunoarray system showed that the frequency of IgE binding to 4 commonly consumed meat is especially high in 1096 allergic patients [pork 46% (504/1096), beef 39% (428/1096), lamb 37% (403/1096), chicken 33% (366/1096)]. Cross-inhibition ELISA showed that pork, beef and lamb are cross-reactive. In order to identify and understand various allergenic components, proteins from *Sus scrofa* were extracted and separated by both 1D and 2D electrophoresis. Subsequently, 1D and 2D IgE immunoblotting were performed using sera from meat allergic patients. IgE binding protein spots were excised, subjected to in-gel trypsin digestion and analyzed by MALDI-TOF-TOF mass spectrometry. The peptide mass fingerprints (PMF) and MS-MS sequences were analyzed with Mascot for identification and characterization of proteins. More than 20 reactive spots were analyzed, some of which were characterized as heat shock protein, serum albumin precursor, IgG heavy chain precursor, troponin, and LIM protein. Most of the characterized proteins were known allergens homologues previously not recognized as allergens in *Sus scrofa*, while others may be putative allergens. Functional groups of these sequences with potential antigenic properties will be constructed according to their putative biological functions and analyzed for IgE-binding. Our current work has extended to include cross reactive allergens from *Bos taurus* (beef), *Ovis aries* (lamb), *Gallus gallus* (chicken) and together with those from major staple plant foods such as *Oryza sativa* (rice), *Triticum aestivum* (wheat) and *Glycine max* (soybean). We have demonstrated that immunoproteomic approach is able to administer comprehensive identification and characterization of novel allergens from various allergy sources.

18.18

A Proteomic Study of Salt Stress Response in Rice

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Salt stress is one of the major abiotic stresses in agriculture worldwide. It was estimated that about 20% of the world land and nearly half of all irrigated land were affected by salinity. Identifying novel genes, determining their expression patterns in response to salt stress, and understanding of their functions in stress adaptation will provide us with the basis for the effective engineering strategies to improve crop stress tolerance. Rice (*Oryza sativa* L.) is not only an important crop, but also a model plant for monocots because of its relatively small genome size. In order to investigate the cellular responses upon salt stress in rice at the protein level, we used a proteomics approach. Three-week-old rice seedlings (cv. Nipponbare) were treated with 150 mM NaCl for 24, 48 and 72 h. Total root protein were extracted and separated by two-dimensional electrophoresis. More than 1100 protein spots were reproducibly detected, including 34 up-regulated and 20 down-regulated ones. Mass spectrometry analysis and database searching helped us to identify 12 spots representing 10 different proteins. Three spots were identified as the same protein enolase. While four of them were previously confirmed as salt stress-responsive proteins, 6 are novel ones, i.e. UDP-glucose pyrophosphorylase, cytochrome c oxidase subunit 6b-1, glutamine synthetase root isozyme, putative nascent polypeptide associated complex alpha chain, putative splicing factor-like protein and putative actin-binding protein. These proteins are involved in carbohydrate, nitrogen and energy metabolism, reactive oxygen species scavenging, mRNA and protein processing, and cytoskeleton stability. This study gave new insights into salt stress response in rice roots and demonstrated the power of proteomics approach in plant biology study.

18.19

Towards Comprehensive Proteomic Analysis of Complexes, Organelles and Cells

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A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies have benefited from large scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and straightforward analysis of protein data from experiments. Proteins separated by gel electrophoresis and 2-dimensional gel electrophoresis can now be rapidly identified enabling more comprehensive analyses of biochemical and molecular biology experiments. New approaches have also emerged for protein analysis such as direct identification of proteins in mixtures without gel separation. By digesting protein mixtures and separating peptides with liquid chromatography directly into tandem mass spectrometers, sufficient information can be obtained to identify the peptides and subsequently the proteins present in the mixture. As peptide mixtures become more complex better separation techniques such as 2-dimensional liquid chromatography are required to resolve the peptide components for analysis. Technologies and methods of proteomics will be described and applications illustrated by discussing experiments to identify proteins, modifications membrane proteins and membrane protein topology.

18.20

The Extraction of Keratin from Egg-shell Membrane by Biological Enzyme

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With improving of the living standards of people and development of the food industry, the consumption of eggs are increasing by large degree, at the same time a large number of egg-shell are abandoned. According to the experimental results, the egg-shells that our country throws away every year have been reached 4 million tons. If they are not be used, they must cause to pollute the environment seriously. Eggshell is mainly made up of real shell and eggshell membrane, and the real shell is made up of calcium carbonate. It is a natural green source of calcium, and may be used for preparation industrial calcium gluconate, calcium citrate, calcium acetate, etc. medical additional calcium agents and food additives. Some researches on recycling egg-shell have been done and reported. But the reports on recycling membranes of egg-shell have not been seen. If recycle the membrane of egg-shell could not only avoid the environmental pollutions, but also fully utilize resources, realize that "change wastes into wealth, utilize synthetically".

Egg membrane is one kind of complicated protein which is mainly made up of keratin and combined with mucoitin together, and is one of the main natural keratin sources. After hydrolyzing, acetyl amino glucose aldehydic acid, hyaluronic acid, chondroitin sulfate, amino acid etc. soluble polymeric compounds can be gained. And these compositions are making important physiological actions in every organ of the human body. So it can be used in pharmacy and cosmetics industry extensively.

It is reported that the nature keratin is close to that of the skin of human, and is easy to be absorbed by the skin, and has good protective action for the skin. The skin protecting agent that the keratin is added, such as the cold cream of egg membrane and the liquid shampoo etc., it can avoid and lighten the coarse skin, accelerate productive new epidermal area, prevent and relieve wrinkle, freckle, acne etc., and the cost of material is reduced constantly by 85% than that of pearl powder. In addition, the keratin also can moisten the lung, relieve a cough, stop and breathe heavily, turn on sound, dispel eye efficiency, and can use as treating chronic bronchitis, swallow ache, aphonic, alimentary couldal.

The extraction methods of keratin to be material that come from the egg-shell membrane are mainly acid process and alkaline process. This paper describes one of enzyme processes of extraction technology and finds that the hydrolysis speed is fast, the extraction time is short, there are no environmental pollution. The conditions of enzyme process are mild, amino acids are not destroyed, the type can't be damaged. The purity of keratin drawn is high, it is soluble easily to water, the property of physics and chemistry is stabled, and is more advanced abstraction method at present.

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Biologically Active Peptides Derived from Fresh Pigskin by Enzymatic Hydrolysis

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Pigskin, the major wastes from poultry and tannery industries posing wide disposal problems, were used in this study for the preparation of biologically active peptides. Disposed pigskin were degreased by 0.3% NaOH and then trypsin was used to enzymatic hydrolysis. After deactivating, filtering, decolourizing, concentrating and drying, the biologically active peptides were obtained. Through experiments, the optimum condition was obtained as follows: pH = 8, 50 min, 1‰ enzyme dosage and 50°C.Under this condition, yield of 50 g pigskin was 10.6 g and content of nitrogen was 95.55%.

So-called biologically active peptides (BAP) are polypeptides which may have substantial benefits to biological activity of organisms or have certain physiological function .As the swiftly developing of biotechnology, researchers become more and more interested in BAP and now the preparation of BAP has actually been an study focus all across the world. Modern biological metabolism studies suggest that proteins absorbed by a man are not always in the form of amino acids as we conventionally supposed, but mostly in the form of peptides. Some low peptide or oligopeptides among them can offer indispensable nutrition during the growing process of the human, and at the same time show some important physiological function. Pigskin is a kind of byproduct in the course of meat processing. Its main component is the moisture, protein and fat and a small amount of cellulose and minerals are also contained. Through biological enzymatic hydrolysis, protein in the pigskin can be hydrolyzed into low peptides of which chain is about 10, namely the biologically active peptide. Its characteristic and use as the following: (1) Have good viscoplasticity and water resistance, can increase the viscoplasticity of the meat products to use among the meat products, reduce losing of moisture in the course of braizing and cooking. (2) They consists of oligopeptide, easy digestion and absorption by the human body, may increase the content of protein of the meat products at the same time. (3) Can stand by high temperature, and not take place degeneration of the protein. (4) Improve flavor of meat products.

This paper focuses on the preparation condition of BAP derived from fresh pigskin by enzymatic hydrolysis. Through experiment, discuss some influenceable factor including enzymatic hydrolytic temperature, pH value, enzyme dosage and enzymatic hydrolytic time and ascertained optimum reaction conditions. The new technology was put forward to extract BAP from fresh pigskin. Enzymiatic hydrolysis is potentially an effective method for the recovery of BAP. This process is simple, efficient and lead to a higher yield. BAP has potential to be used in a wide range of applications due to its excellent characteristic: diet foods, high protein sports drinks, hypoallergenic baby food, etc.

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The Proteomic Changes During Maturation of Rice Ear

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The maturation of rice ear is an important process that is tightly correlated with the grain yield. Although the physiological changes of rice ear have been carefully detailed, the biochemical development is remained to be explored. Proteomic analysis paves an accessible approach to monitor the molecular events related to functions during the development of rice ear. A rice variety, 9311 (Oryza sativa L. ssp. Indica), was selected and the rice ears were collected from four different growth periods, booting, heading, milk and mature phases, for studying the proteomic changes during rice maturation. The rice ear proteins were extracted by TCA-Acetone precipitation, separated by 2DE followed by Commassie staining, and identified by MALDI-TOF/MS and LC-MS/MS. On the 2DE images, the stained spots were found decreasing with rice development, the total of detected spots, 769 at booting, 604 at heading, 524 at milk and 329 at matured seed, respectively. On average, 85% spots were verified to be rice proteins based on the PFM and MS/MS data. Carefully analyzing the growth-dependent proteins in rice ear according to their functions, some cellular skeleton and transcript regulation proteins, such as sigma-54 and ribosome proteins, were attenuated in expression even disappeared in the late phase, however, the glutelin, a major storage protein, became the highest abundant protein (>50%) in the maturated seed. Interestingly, several proteins participating in glycolysis and amino acid metabolism, such as pyruvate kinase and alanine aminotransferease, raised their expression during rice ear maturation.

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The Proteomic Dynamics of Rice Leaf in Six Different Growth Periods

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An important issue for high yield of rice is how solar radiation is transformed into biomass through rice leaves. We have analyzed the proteomic changes in the rice leaves collected from six differently developing stages, from vegetative to ripening phase. The profiles of protein expression were studied by 2DE, image analysis and MALDI-TOF/MS. A total of 49 gel spots throughout all of development stages were identified with significant changes in staining intensity. Of these spots, 89.8% of them were confirmed to be rice proteins. The protein expression is attenuated during growth, especially some chloroplast proteins. However, the change is slow and most proteins maintain relatively stable expression during development. Regardless of at each growth stage, rubisco was found to be a major protein in rice leaf, as other reported. Interestingly, a high ratio of degradation of rubisco large subunit was found in all of samples, detected either by mass spectrometry or by immunoblot. These fragments are similar to the degraded products of rubisco mediated by free radials. Although some rubiscos are degraded, the photosynthesis capacity of rice is reasoned to be insignificantly weakened until ripening phase, because rubisco small subunit and rubisco activase remain a constant expression at that period. In contrast to some observations, antioxidant proteins such as SOD and peroxidase were found in the declined level of expression at the early ripening stage. This study is the first document describing the dynamic proteomes of rice leaves, which will benefit to explore the relationship between photosynthesis and grain yield.

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Analysis of Tibia-fibulae Musculature Proteome of Rat Fetus by Two-dimensional Gel Electrophoresis

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Wistar rat fetus has been commonly used in embryological and toxicological research. Analysis of its proteomes may provide important information for the understanding of molecular basis for the development of various parts of the body. Immobilized pH gradient isoelectric focusing (IPG IEF)–2D PAGE has become a primary tool for the analysis of protein complex in proteomic research. In this study, proteins were extracted from tibia-fibulae musculature isolated from 21-day rat fetus with lysis solution (5 M urea, 2 M thiourea, 2% CHAPS, 2% SB3-10, 40 mM Tris, 0.2% Bio-Lyte 3–10) (1 ml/50 mg tissue). For 2D PAGE, IPG gel (pH 3–10 and pH 5–8) IEF was set as the first dimension, with 12% SDS polyacrylamide gel electrophoresis as the second dimension. Loaded sample sizes were: 0.5 mg (350 μl) for pH 3–10 IPG and 1.0 mg (350 μl) for pH 5–8 IPG. Following Coomassie Brilliant Blue or silver staining, the results were analyzed with a PDQuest 7.0 software package, where more than 600 and 1200 protein spots were detected, respectively. The total muscular proteome was separated with good reproducibility. The proteins had mainly distributed within the range of pH 5~7 and molecular weight of 20~100 kD, and were more clearly separated on the narrow pH gel (pH 5–8 IPG). The reproducibility of 2D gel electrophoresis was verified through comparing of peptide mass fingerprints from MALDI-TOF mass spectrometry analysis of one matching protein spots (apparent molecular weight 22 kD, pI = 5.3) from three separate gels. Searching protein databases (SwissProt and NCBInr) using three software including PeptIdent, Mascot and MS-Fit had consistently suggested a same protein (light chain 1 of skeletal muscle myotonin). We thereby conclude that utilization of 2D electrophoresis can satisfactorily separate muscular proteins from rat embryos and achieve a high resolution and good reproducibility for protein verification.

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