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Review

Comparative proteomic analysis of mammalian animal tissues and body fluids: bovine proteome database

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Abstract

Characterizing the complete proteome of multicellular organisms is a challenging task using the currently available technologies. With the increasing degree of genetic complexity, animals acquire a broader repertoire of options to meet environmental challenges. Mammalian cells from different tissues/body fluids express different thousands of proteins with a predicted dynamic range of up to five to six orders of magnitude, thus necessitating the whole arsenal of dedicated analytical strategies for a detailed proteome characterization. Nevertheless, 2D-E analysis of whole cellular lysates still remains the most used initial approach for the proteomic description of specialized cells. It enables to obtain an overview of the main soluble protein components of a specific tissue/body fluid, allowing comparison between different cellular types and molecular description of organ specialization. Massive proteomic investigations have been reported mainly in the case of human, mouse and rat, allowing comparative analysis. For this reason, a research project focused on the 2D-E characterization of tissues and biological fluids from other domestic mammals has been undertaken in our laboratory. A number of high-resolution reference electrophoretic maps have been established for liver, kidney, muscle, plasma and red blood cells samples from Holstein Friesian bovine female individuals. Among the 1863 distinct protein features detected, 534 species were identified and associated to 209 different genes by a combination of MALDI–TOF mass fingerprint, capillary LC–ESI–IT–MS–MS and image gel matching procedures. Identified polypeptide species and differences in expression profiles between various tissues/fluids clearly reflected organ biochemical specialization. This experimental output allowed establishing a 2D-E bovine database accessible at the URL address http://www.iabbam.na.cnr.it/biochem for image comparison.

Keywords: Reviews; Tissues; Fluids; Proteome; Proteomics; Bovine; Proteome database

Contents

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1. Introduction

The function of many of the uncharacterised open reading frames (ORFs) discovered by large-scale genome sequencing projects is now investigated at the level of the expressed gene products. The term "proteome" was initially coined to describe the global set of proteins encoded by the genome [1]. However, the study of the proteome (called proteomics) now evokes not only the description of all protein species in any given cell, but also the set of all protein isoforms and post-translational modifications, the characterization of protein interaction network, and the structural description of proteins and protein complexes. This emerging field of research has been facilitated to a large extent by massive improvements in analytical procedures for the resolution and quantitation of complex protein mixtures [2] as well as in mass spectrometry techniques for identification and structural characterization of each resolved component [3].

Currently, most proteome studies are based on orthogonal electrophoretic separation of complex protein mixtures, followed by peptide fingerprint and/or sequence information-focused mass spectrometric analysis of proteolytic digests from individual components [3]. Recently, new strategies for proteome analysis have been introduced involving global proteolytic digestion of a cell lysate or subfractions, followed by analysis of the resulting complex peptide mixtures by two-dimensional chromatography (2D-LC) coupled to tandem mass spectrometry [4]. Accordingly, various proteome projects have been undertaken to describe protein expression profiles and protein interaction networks in different microrganisms, plants and animals. Detailed proteomic maps have been generated for a large number of organisms; on the contrary, proteomic-based interactome maps are available only for yeast [5] and *C. elegans* [6]. In general, massive results have been obtained preferentially for organisms whose genome has been enterely sequenced. This is the case of microrganisms presenting a reduced genome in size or the poor number of multicellular species that have chosen for their importance or as model systems (*H. sapiens*, *M. musculus*, *P. falciparum*, *C. elegans*, *D. melanogaster* and *O. sativa*) [7–13].

In classical proteomic experiments, samples from cellular crude homogenates are resolved by high-resolution two-dimensional electrophoresis (2D-E) to display the whole body of expressed proteins within the studied system under a given cellular condition [14]. Assuming that the separation system is capable of representing all proteins present in the sample, these maps provide a complete overview of the protein repertoire based on protein spot pattern. Following spots identification, maps obtained under basal cellular conditions may constitute the reference proteome of the organism under investigation. Spot patterns may be compared between two samples obtained from the investigated species under different temporal, environmental or cellular conditions. Original reference maps has been used to establish integrated 2D-E databases accessible through the World Wide Web network,

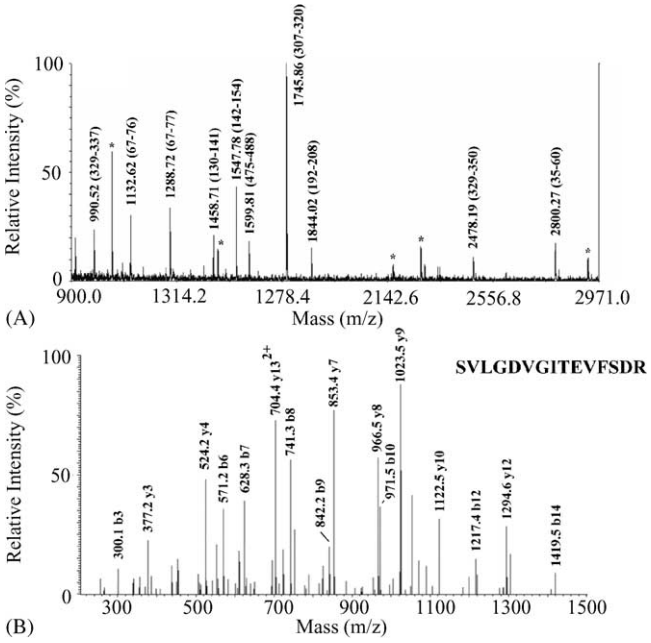

Fig. 1. Mass spectrometric analysis of proteins from bovine liver and plasma. Proteins were excised from the corresponding gels, alkylated and digested with trypsin. Digests were analysed by peptide mass fingerprint MALDI–TOF (panel A) or capillary LC–ESI–IT–MS–MS experiments (panel B). Shown is the CID spectrum generated from the peptide with doubly charged parent ion at *m*/*z* 797.6. Measured mass values and assigned fragment ions are indicated. Masses are reported as monoisotopic values. Asterisks indicate signal generated by trypsin autoproteolysis. Proteins were identified as bovine aldehyde dehydrogenase II (SwissProt entry P20000) and α-1 antitrypsin (SwissProt entry P34955), respectively.

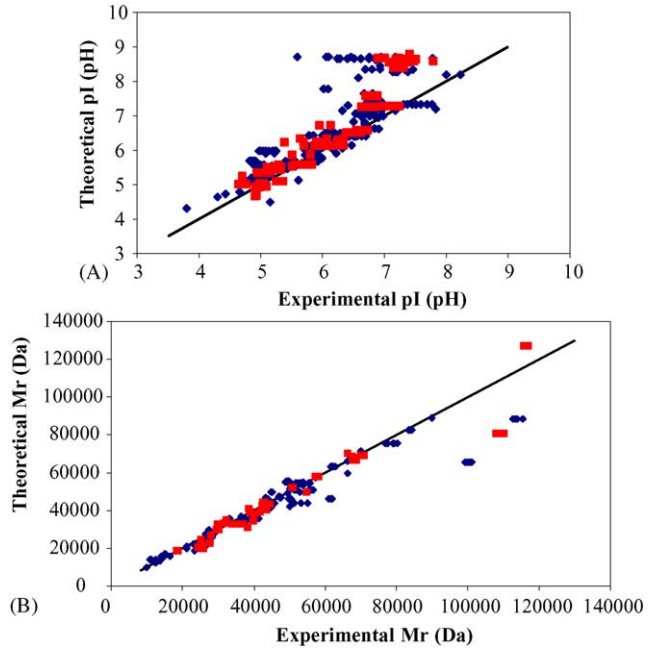

Fig. 2. Comparison of experimentally determined and theoretical p*I* (panel A) and M_r (panel B) values of identified proteins from bovine 2D-E gels. Sequence entries corresponding to bovine or other mammals are reported in black rhombs and gray squares, respectively.

allowing direct use of reported results and comparison between laboratories by optimised image analysis softwares [9,15–18]. In this case, the use of identical experimental conditions for electrophoretic separation is a critical prerequisite. This availability of data is now allowing the diffuse integration of previous and novel results in general studies on the entire protein repertoire of organisms having simple genomes or its variation following environmental challenges. Similarly, this approach has been widely adopted also in differential experiments on more complex organisms focused on highlighting variations in proteomic repertoire as result of cellular response to different physiological conditions or disease [19,20].

Characterizing the complete proteome of multicellular organisms is a challenging and perhaps impossible task using only one of the currently available technologies. In fact, with the increasing degree of genetic complexity, eukaryotic organisms acquire a broader repertoire of options to meet environmental challenges. This increased complexity is realized at three levels: firstly, a larger number of genes are present in their genomes with respect to microrganism counterparts. Secondly, various compartments within a cell contain different and compartment-specific subsets of gene products in order to provide suitable biochemical environments, where they exert particular functions. Thirdly, not all cells of the organism serve the same purpose; the organism contains several different subsets of cells with distinct properties (i.e. epithelial cells, lymphocytes and neurons). Therefore, the proteome of any cell, tissue or biological fluid is a complex mixture of proteins that span a wide range of size, relative abundance, acidity/basicity, and hydrophobicity. Vertebrate cell types may express up to 20,000 proteins with a predicted dynamic range of up to five to six orders of magnitude [21]. These considerations significantly affect the strategies and the procedures to be used in proteomic studies. In fact, proteomic maps of eukaryotic organisms are generally more complex than prokaryotic ones, sometimes necessitating the use of highly resolutive separation procedures or pre-fractionation steps to avoid spot overcrowding [22,23]. In addition, depletion of known proteins or enrichment of particular subcellular structures are eventually necessary in the discovery of gene products that are major components of specific subcellular compartments, but are low-abundance species in whole crude homogenates [24]. Finally, a proteomic description of all cell types, tissues and body fluids is important to describe the distinct cellular functional properties of multicellular species. All these observations resulted from previous work in proteome analysis of human and murine samples, and have to be considered for future investigations on other multicellular organisms.

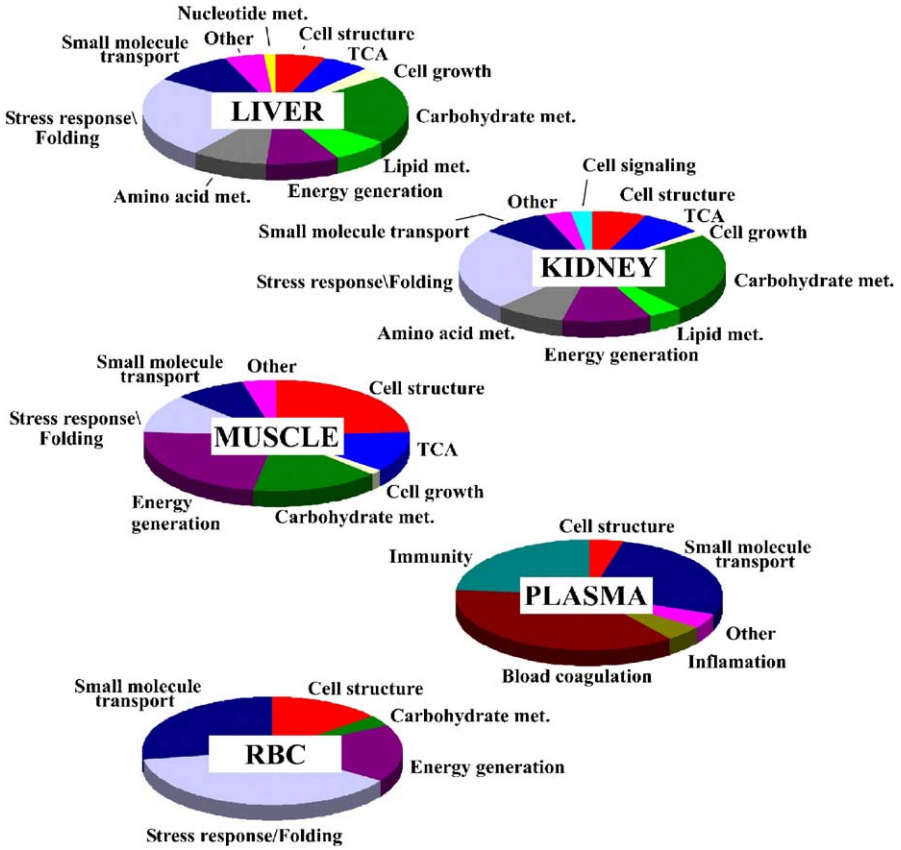

Fig. 3. Distribution of the cellular roles of identified bovine proteins [40]. Functional annotation for each protein was obtained from SwissProt database using SOURCE program [74].

Nevertheless, 2D-E analysis of whole cellular lysates has been considered as an important initial step in the proteomic description of specialized cells. It enables to obtain a preliminary view of the main soluble protein components of a specific tissue/body fluid (several hundreds in number), allowing the comparison between different cellular types and the molecular rationalization of organ specialization. Moreover, differential proteomic studies on specific tissues/fluids are characterizing protein expression changes associated to pathological processes, allowing identification of disease biomarkers [25]. For this reason a series of research projects are now in progress to establish 2D-E databases for different mammalian tissues/fluids. Liver, muscle, kidney, heart, white and brown adipose tissues, lens, pancreatic islet cells, mammary epithelial cells, macrophages, lymphocytes, platelet, serum, red blood cells (RBC), cerebrospinal fluid reference maps are progressively annotated [9,15,16,18]. However, most of the data are available on samples from human, mouse and rat individuals.

1.1. Bovine genomic investigation

The importance of Bos taurus on the entire agricultural economy promoted investigation on fundamental mechanisms controlling animal health and productivity, either in terms of genetic analysis, animal physiology and proneness to microbial infections [26]. A combined effort of different genomic sequencing resources now has been coordinated to definitively sequence and annotate the entire bovine genome [27]. An updated bovine gene database is now available on the web at uniform resource locator (URL) address http://www.ncbi.nlm.nih.gov/genome/guide/cow. Bovine genome contains about 35,000 type I coding genes organized in 30 haploid chromosomes [28]. Almost 5000 genes have been sequenced to date, showing a high homology with human and mouse counterparts. The *Bos taurus* genome is the fourth well mapped in Mammalia after human, mice and rat, and almost 4000 loci have been located to specific chromosomes [29]. Preliminary genome projects on this species have already yielded powerful tools for assessing genes that specify hereditary disorders, infectious disease resistance, breed-specific quantitative loci and phenotypes of agricultural relevance [30,31].

On the contrary, a limited number of proteomic studies on bovine tissues and biological fluids have been performed, mainly focused on chondrocytes, mammary glands, cerebrospinal fluid, pulmonary endothelial cells, seminal plasma, milk and corneal lens [32–39]. In these investigations, elec-

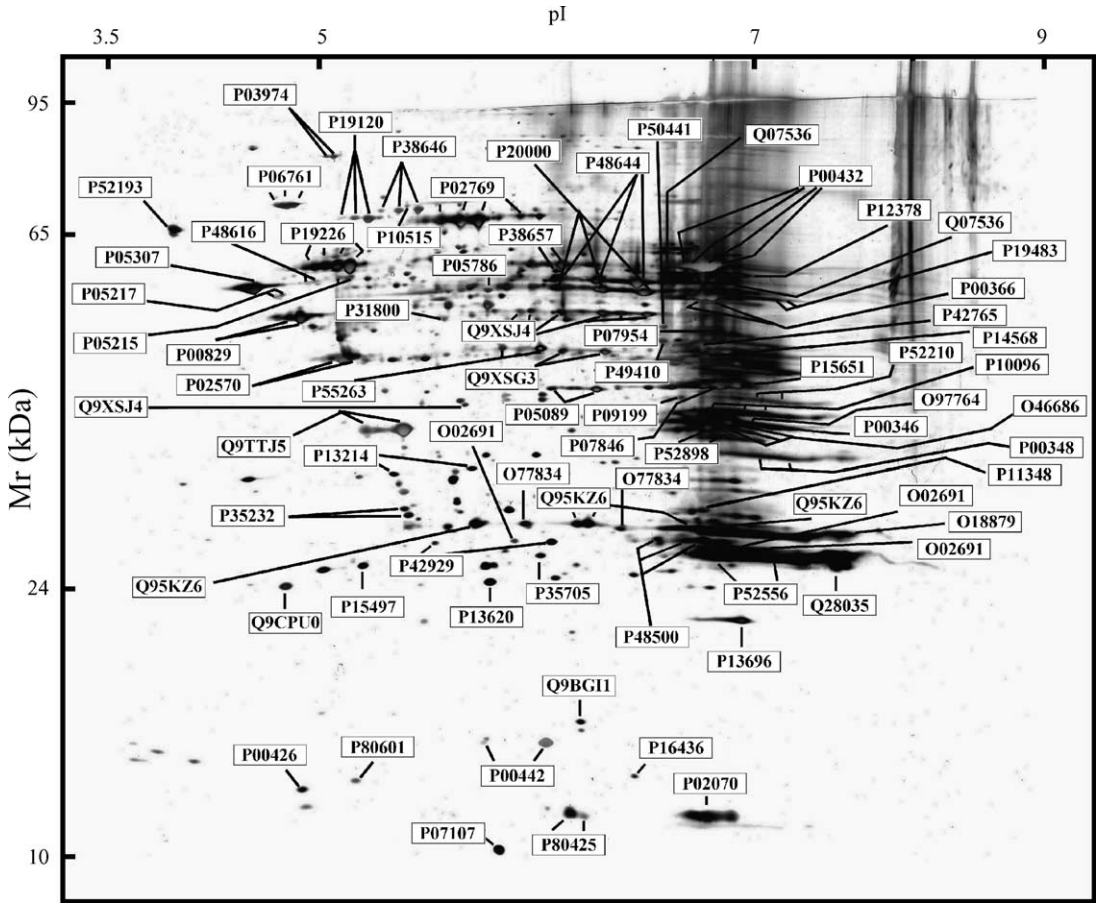

Fig. 4. Identified proteins on the 2D-E map of bovine liver cells. Proteins were resolved and identified as previously reported [40].

trophoretic maps have been obtained under different experimental conditions not allowing tissue/fluid comparison. Moreover, Western blotting and/or automated Edman degradation were the analytical techniques used in the limited identification of specific protein species. No massive proteomic projects have been reported for this animal. On this basis, a research focused on the proteomic analysis of a series of bovine tissues and biological fluids was undertaken in our laboratory [40].

2. Proteome analysis of bovine tissues and body fluids

2.1. 2D-gel electrophoresis

2D-E maps were obtained in multiple copies from freshly prepared samples of 1-year-old healthy female Holstein Friesian bovine individuals. Particular care was taken to preserve tissue cellular homogeneity avoiding stroma, blood and serum contamination. In particular, all tissues were washed in a cold rinse buffer containing different protease inhibitors and antibiotics to remove cell debris and blood [40]. Fresh blood samples were separated into different components by centrifugation. Sample preparation and solubilisation was performed slightly modifying the SWISS-2DPAGE sample preparation procedures [7,9] according to Rabilloud's laboratory recipes [41], verifying the increased protein solubilisation by preliminary sodium dodecyl-sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. Samples were separated on non-linear 3.5–10 immobilized pH gradient (IPG) strips (0.5 mm × 180 mm) (Amersham Bioscience). First and second dimension separations were performed as previously reported [40]. Protein detection was achieved by ammoniacal silver staining that allowed visualization of proteins in the range of 10,000–150,000 relative molecular mass units [42]. Optimal resolution was obtained by loading samples containing 50–150 μg of proteins. Attempts to increase the amount of loaded material were problematic and resulted in gels poorly resolved. These findings were evident for RBC and plasma for which a high proportion of the total protein is accounted for by abundant species such as globins and albumin, in agreement with previous observations [43]. Isoelectric points were estimated by mixing samples with p*I* protein standards (BioRad, Richmond, CA).

Image analysis of multiple electrophoresis 2D-E copies allowed defining a master gel for bovine liver, kidney, skeletal muscle, plasma and red blood cells, comprising 484, 677, 218, 303 and 181 reproducible protein spots, respectively [44]. Previous systematic investigations on similar tissues/fluids have been performed on human, mouse and rat,

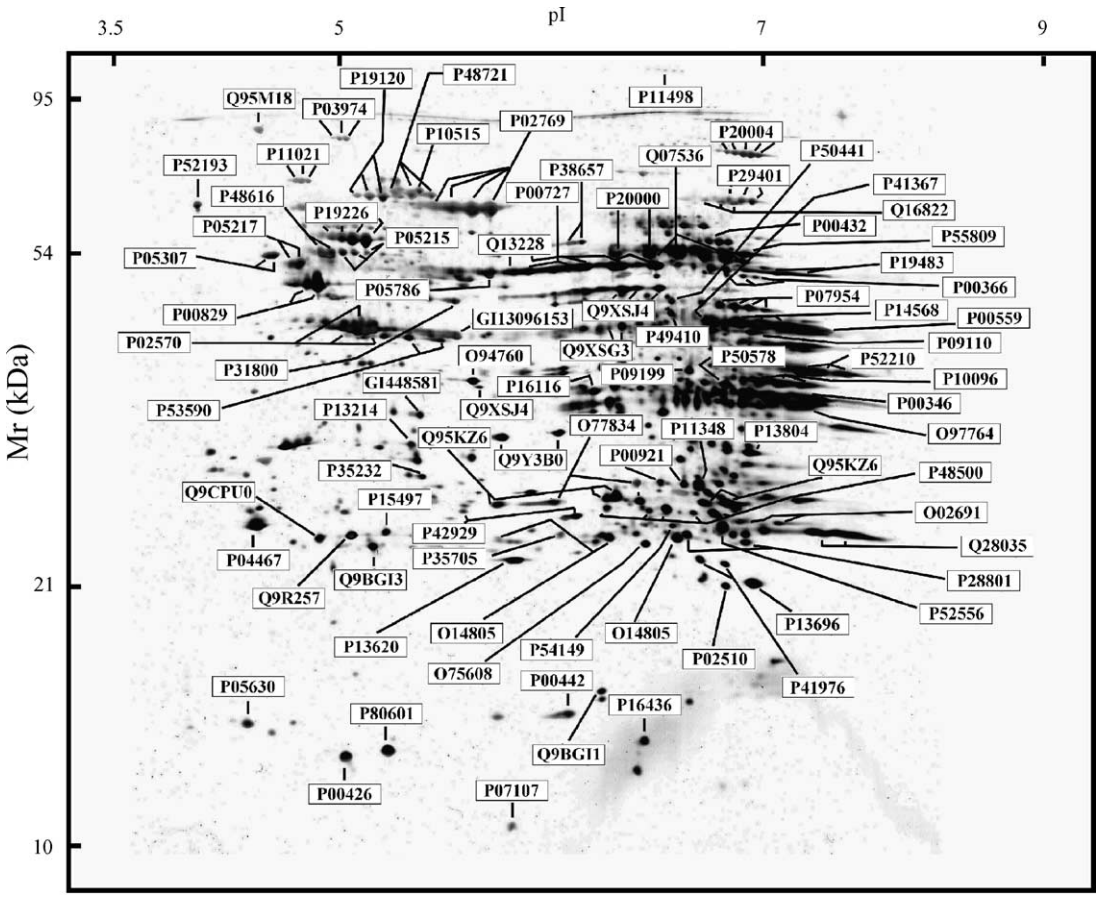

Fig. 5. Identified proteins on the 2D-E map of bovine kidney cells. Proteins were resolved and identified as previously reported [40].

determining massive protein identification [6,7]. However, although a general similarity was observed for plasma, liver and kidney samples from different mammals experimentally processed in parallel (data not shown), differences in protein relative abundance, p*I* and molecular mass were obvious for major as well as for minor components. Furthermore, initial attempts to identify bovine species by gel-matching procedures, on the basis of the known nature of proteins already identified in human and mouse 2D-E databases, were unsuccessful. Significant problems were evident in regions with high spot overcrowding. Additional attempts to match gels following preliminary identification of a dozen of bovine protein markers already present in human and mouse 2D-E database were still unsuccessful. On this basis, we concluded that gel matching procedures with maps already published for other mammals were ineffective for identification of bovine proteins.

2.2. Mass spectrometric identification of bovine proteins

To identify bovine proteins, spots from 2D-E gels were manually picked, submitted to trypsin digestion and extracted as previously reported [40]. Samples were analysed in parallel by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI–TOF–MS) and capillary liquid chromatography coupled to electrospray ion trap tandem mass spectrometry (cLC–ESI–IT–MS–MS). Peptide mass fingerprint experiments were performed using a Voyager-DE PRO spectrometer (Applera, USA) as previously reported [40]. ProFound software package was used to identify spots from independent non-redundant sequence databases [45]. Collision-induced dissociation (CID)-derived information were obtained using a LCQ Deca XpPlus mass spectrometer (ThermoFinnigan, USA) equipped with an electrospray source connected to a Phenomenex C18 column (250 mm × 0.30 mm) eluted as previously reported [40]. Data were elaborated using the BioWorks software provided by the manufacturer [46]. Candidates with ProFound's Est'd *Z* scores >2 from peptide matching analysis or with more than five identified CID spectra of peptides belonging to the same protein and TurboSequest Xcorr values >2.5 were further evaluated by the comparison with their calculated molecular mass and p*I* using the experimental values obtained from 2D-E. Fig. 1 shows the spectrum of aldehyde dehydrogenase class 2 and α-1 antitrypsin as examples of proteins identified by peptide mass fingerprint or tandem mass spectrometry experiments, respectively. Samples for whom a positive identification by MALDI–TOF–MS was associated to proteins of other mammalian species (whose bovine counterpart was absent in sequence databases) were confirmed by peptide tandem mass spectrometry experiments.

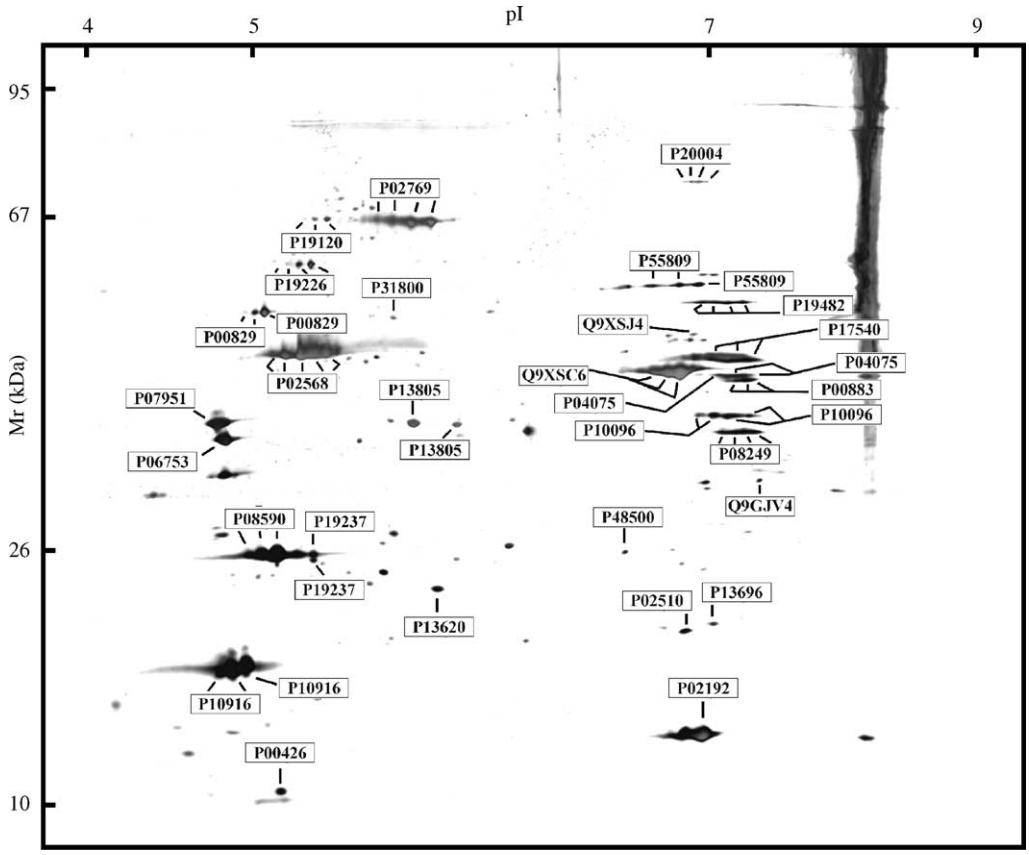

Fig. 6. Identified proteins on the 2D-E map of bovine skeletal muscle cells. Proteins were resolved and identified as previously reported [40].

In general, this combined mass fingerprint/tandem mass spectrometry approach allowed the identification of 509 protein species, corresponding to 209 different genes. Identification succeeded in almost 61% of the analysed samples; this result was probably a consequence of the limited number of bovine sequences present in databases. Among the identified proteins, 141 were associated to bovine genes already sequenced and 68 to mammalian proteins whose bovine counterparts are missing in the databases. In the latter case, initial identifications obtained by mass fingerprint analysis always found a positive verification by tandem mass spectrometry experiments. These results confirmed the possibility to identify proteins from mammals whose genomes are still poorly characterised by simple mass fingerprint experiments and searching against non-redundant databases. These findings were in agreement with previous investigations where, similarly to our approach, confirmatory experiments were performed nevertheless [47,48].

2.3. Comparative analysis of bovine proteomes

A comparative analysis of the 2D-E bovine master gels revealed that identical proteins, directly identified by mass spectrometry procedures, presented very similar experimental p*I* and molecular mass values among the different tissues and fluids analysed. These findings were verified for marker proteins common to some analysed samples. No problems of reproducibility or calibration of the gels were observed during image processing, thus confirming the suitability of these 2D-E gels to be used as reference standards. Comparative analysis of different bovine tissues and fluids by gel matching procedures allowed a positive identification of 25 additional protein spots, thus determining a final number of 534 identified bovine protein species.

A cumulative analysis of all identified proteins from bovine tissues/fluids demonstrated that it ranged from theoretical p*I* 4.31–8.19 and from theoretical M_r 9000–127,000 units. We avoided any experimental conditions favouring undesidered modifications known to be the cause of some electrophoresis artefactual phenomenona. Molecular weight and isoelectric point values of protein spots were experimentally determined and compared with gene-deduced M_r/p*I* coordinates (Fig. 2). In the case of bovine proteins associated to mammalian genes whose bovine counterparts are missing in the databases, we used the theoretical M_r/p*I* values of the identified species. The theoretical M_r/p*I* distributions were predicted by the use of the ExPASy server TagIdent tool [49] on mammalian proteins present in the Swiss-Prot/TrEMBL databases. The majority of gel-estimated and theoretical M_r/p*I* values matched quite well

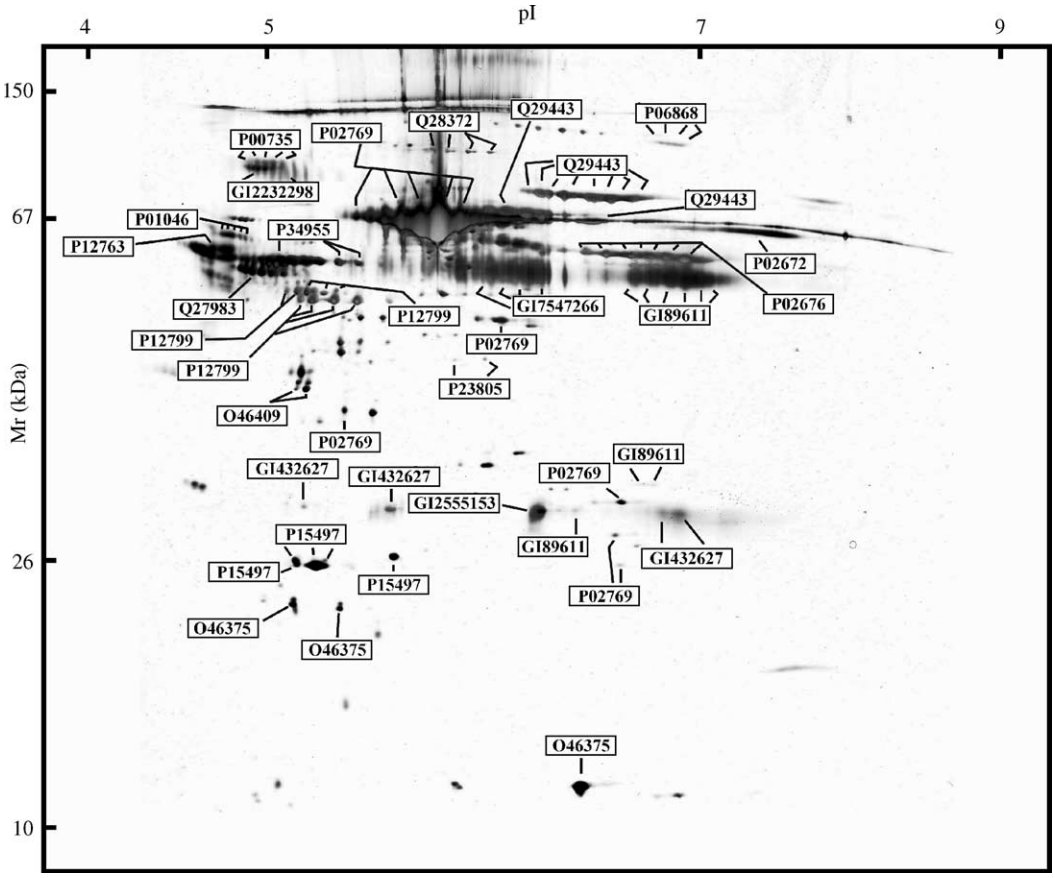

Fig. 7. Identified proteins on the 2D-E map of bovine plasma. Proteins were resolved and identified as previously reported [40].

(Fig. 2). Discrepancies between theoretical and experimental values were more frequent for p*I* that in general was lower than expected. These findings were maybe more reliable to predict on the basis of the known post-translational modifications affecting mammalian proteins. In fact, it has been estimated that one-third of mammalian gene products can be modified by phosphorylation [50]. When M_r differences were considered, both decreased or increased experimental values respecting to theoretical ones were found. The first case should be due to proteolytic processing or alternative splicing events; the second case should reflect the occurrence of post-translational modifications causing covalent addition of large chemical moieties to the polypeptide backbone. In this case, glycosylation should be the most probable modification causing the M_r changes observed.

Overall, similarly to other mammalian species, a large number of bovine proteins were found to be present as multiple isoforms. Heterogeneity was mainly due to their variable p*I* values. Many of these proteins have a catalytic activity; isozymes provide a better efficiency in biocatalysis. Others are polypeptide species secreted from particular cells in body fluids (plasma); glycoproteins are progressively processed through endoplasmic reticulum and finally sorted for extracellular delivery to their final destination.

The nature of the identified polypeptide species and the differences in expression profiles between various tissues/body fluids significantly reflected organ/fluid specialization (Fig. 3). In fact, liver and kidney presented the largest number of protein species, in agreement with its numerous specialized biochemical and physiological functions. They presented enzymes involved in energy generation, carbohydrate, lipid, amino acid and xenobiotic metabolism, as well as proteins involved in polypeptide synthesis, folding and cell structure. On the contrary, skeletal muscle showed a relative abundance of myofibrillar polypeptide components, and proteins involved in glycolysis and ATP metabolism. Plasma was enriched in proteins associated to immunity response, blood clotting, inflamation and transport of small molecules. Our results were in perfect agreement with those already published on bovine serum [43]. Finally, RBC presented high levels of proteins involved in oxygen transport and response to oxidative stress. All these data well matched with previous findings on similar tissues/fluids from other mammals. In fact, in addition to a series of proteins already reported in the corresponding 2D-E maps [9,15,16,18], we were able to identify in bovine a series of polypeptide species revealed in each mammalian tissue only by nucleotide hybridization techniques [40].

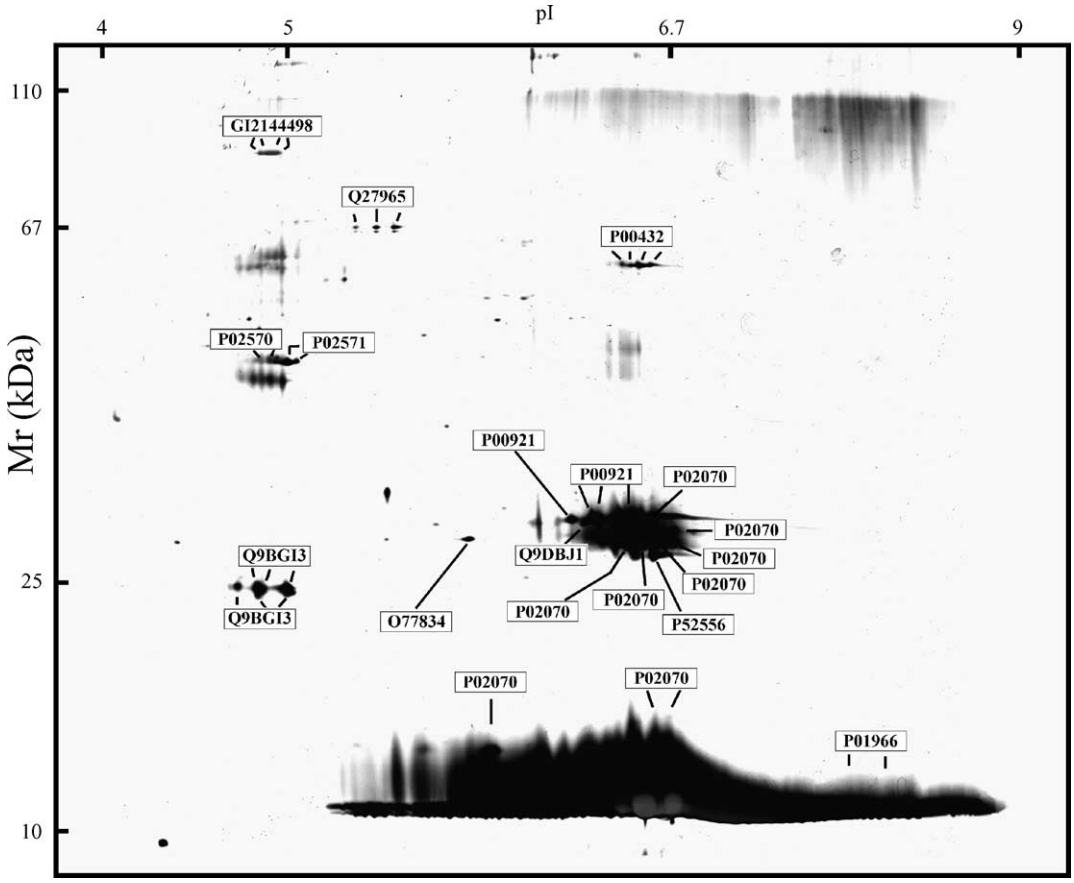

Fig. 8. Identified proteins on the 2D-E map of bovine red blood cells. Proteins were resolved and identified as previously reported [40].

2.4. Bovine tissues/body fluids 2D-E database

The recent developments in computer-based image analysis systems have allowed the efficient process of detection, matching, quantitation and statistical analysis of the thousands of spots on 2D-E maps [51,52]. These dedicated softwares also allow the user to match his local gel with any gel image from any of the 2D-E databases available on public domains. These programs make inter-laboratory gel comparisons possible and enhance protein spot identification. Despite technological improvements in gel repeatability, ensured by highly reproducible IPG gradients and standardized electrophoretic conditions, Web-based exchange of data for inter-laboratory gel comparisons have to be treated with extreme caution. Particular care has to be spent in limiting minor variations in tissue/fluid handling by standardized sample procedures, thus avoiding separation artifacts, loss or gain of specific protein components. Positive and negative examples of web-based gel matching applications are available in the literature [53,54].

With these considerations in mind, our experimental output was used to establish a bovine 2D-E database, worldwide available for search and image comparison, where each bovine entry contains data on protein species occurring in the gels [40]. This database currently presents the following five maps containing altogether 1863 spots: liver (Fig. 4), kidney (Fig. 5), skeletal muscle (Fig. 6), plasma (Fig. 7) and red blood cells (Fig. 8). Master images were stored in joint photographic experts group (JPEG) format on our local server computer and are available at the URL address http://www.iabbam.na.cnr.it/Biochem. In particular, 134 spots have been identified in liver (Fig. 4), corresponding to 71 protein entries. Similarly, 170 spots from kidney (Fig. 5) were related to 85 bovine genes in the database and 71 spots from skeletal muscle (Fig. 6) corresponded to 29 protein entries. Finally, 122 spots have been identified in bovine plasma

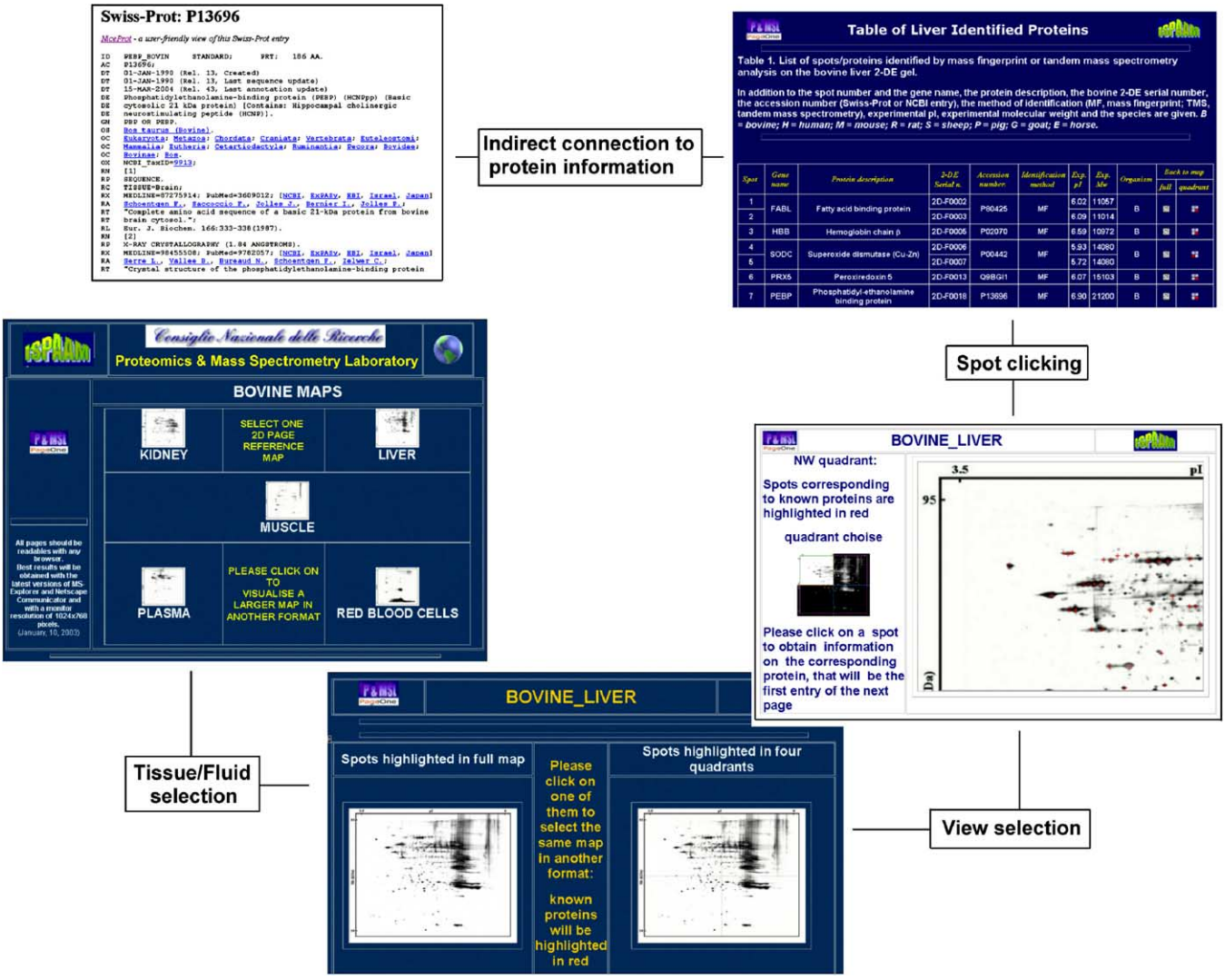

Fig. 9. Bovine 2D-E web-browser interface. Reported is the way to access any given information on entries present in the database.

(Fig. 7) and 39 spots in red blood cells (Fig. 8), corresponding to 26 and 13 protein entries, respectively.

Fig. 9 shows the bovine 2D-E database Web pages available at our Web address. It reports the way to access any given entry present in the database. A main menu page allows selecting the tissue/body fluid of interest. Graphical interfaces have been developed to query 2D-E maps by direct clicking on a red-highlighted spot from the selected reference map. Information on the nature of the selected spot are provided, including spot number, gene name, protein description, bovine 2D-E serial number, SwissProt accession number of the bovine protein or the mammalian homologue (human, mouse, rat, dog, pig, sheep, goat, horse and rabbit), method of identification, experimental p*I*, experimental molecular weight and mammalian species whose protein has been identified. Different formats of the same map are available for selection. No query is now possible by means of different keywords such as protein name, SwissProt accession number or author reference name. At the moment our database is not a Federated 2-DE database; in fact, it does not conform to all the guidelines for standardization of 2D-E data exchange over the Net [55]. Efforts are now spent to overcome this condition.

3. Conclusions and future perspectives

Current proteomic methodologies applied to mammalian cells analysis still suffer from several technical limitations not allowing a systematic comprehensive description of the entire protein expression profile and missing detection of many important low soluble or less abundant components. However, despite this, even preliminary tissues/fluids analysis by conventional 2-DE procedures are still able to yield suitable data on poorly characterized animals, contributing to a more detailed understanding of important biological processes. In fact, measuring gene expression by 2D-E platforms can still provide information on specific polypeptide species, highlighting the occurrence of eventual post-translational modifications, or easily detect global dynamic changes in the protein repertoire associated to different physiological and pathological conditions, using user-friendly comparative experiments with multiple samples taken under different experimental conditions. On this basis, several electrophoresis-based investigations have positively described the molecular determinants of a series of human pathologies [25,56], or have been used in developmental and infectivological studies on animal models [57,58].

In contrast, poor examples of systematic studies have been performed to evaluate the entire gene expression repertoire in tissues and fluids of domestic mammals, or to detect novel markers specific for bovine pathologies. In fact, although metabolic profiles are routinely performed to evaluate animal conditions [59], in classical veterinary studies single enzymatic activities or genetic anomalies are conventionally associated to specific physiological and pathological conditions. Examples are the case of myostatin gene in muscular hypertrophy (double muscle) trait [60], melanocyte stimulating hormone receptor and tyrosinase genes in coat colour disorders [61] and altered expression of major histocompatibility complex genes in animal propensity to leucosis by retrovirus or to mastitis [62]. Only very recently, cDNA microarray approaches have been used to characterize pregnancy-associated changes in genome-wide gene expression profiles in the liver and blood neutrophils of cow throughout pregnancy [63,64], lymphocyte subpopulations and major histocompatibility complex haplotypes of mastitis-resistant and susceptible cows [65], mammary gene expression profiles for mammary-specific transcripts [66] or gene expression in ileal tissues of cattle infected with Mycobacterium [67]. Similarly, only recently proteomic methodologies have been used to investigate global protein repertoire in bovine tissues and fluids. In fact, the recent works on proteins involved in bovine dilated cardiomyopathy, an endemic disease in Swiss animals [68], on polypeptides associated to high lean muscle mass character in Belgium Blue bovines [69], on serum proteins in major acute-phase [43] and on the comparative analysis of liver, kidney, skeletal muscle, plasma and RBC [40] are the only examples reported in the literature to date. The bovine 2D-E database here reported will provide the users a tool to identify protein pattern changes in bovine samples as result of inflammation, exposure to infectious agents, genetic diseases, or breed-specific quantitative phenotypes of agricultural relevance. Comparison of 2D-E pictures of pathological or agriculturally significant samples with reference maps will allow the detection of various modifications such as presence or absence of particular spots as well as their eventual micro-heterogeneity.

A preliminary analysis of the 500 protein species identified in bovine samples indicated a general similarity with those already reported in the same murine and human tissues/fluids [7,9], thus suggesting a common pattern of biological activities among organs from different mammals. Similar results were reported in previous comparative 2-DE studies on serum [57], skeletal muscle [70], fibroblasts [71], or atrial and ventricle tissues from various vertebrates [72]. These reports, dealing on systematic 2D-E-based detection of evolutionary changes, were performed before the dramatic advances reached in proteomic analysis during recent past years. Future comparative investigations based on integrated proteomic platforms will allow a reclassification of the traditional three domains (Bacteria, Archaea and Eukarya) not only according to DNA sequence information but also on the basis protein expression profiles. These studies will be possible only following a detailed characterization of each organ's protein repertoire by an in-depth proteomic assault. Significant efforts have been coordinated in the case of human proteomic initiative by Human Proteome Organization, trying to benefit from lessons learned by genome-sequencing consortia. In this case, concomitant projects on human plasma, liver and brain are now in progress. Following their completion, the final comparison of the relative protein expression

profiles among different tissues/body fluids will provide important information on the differences and the specialization of various cell types. We firmly hope that similar initiatives will be coordinated, in a next future, to study the proteome of different tissues from other vertebrates. Futuristic comparative investigations on the proteome repertoire of same cellular types from different organisms, will finally allow studying phylogenetics on the basis of proteomic approaches.

In conclusion, there is a huge amount of work currently taking place on the human proteome but most other animals of importance to humans are poorly studied. Proteomic investigations on mammalian tissues and fluids used as raw materials in human diet also will allow evaluating the extent of protein modifications resulting from technological processes adopted in food industry [73]. In a next future, applied genomics and proteomic technologies will significantly contribute to nutritional sciences and food technologies, allowing quality and authenticity verification of foods, identification of allergic proteins, control of fermentation processes and detection of food spoilage and contamination by pathogenic microrganisms.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found at doi:10.1016/j.jchromb.2004.07.017.

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