On the potential of using peculiarities of the protein intrinsic disorder distribution in mitochondrial cytochrome \(b\) to identify the source of animal meats

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ABSTRACT
This study was conducted to identify the source of animal meat based on the peculiarities of protein intrinsic disorder distribution in mitochondrial cytochrome \(b\) (mtCyt-\(b\)). The analysis revealed that animal and avian species can be discriminated based on the proportions of the two groups of residues, Leu\(+\)Ile, and Ser\(+\)Pro\(+\)Ala, in the amino acid sequences of their mtCyt-\(b\). Although levels of the overall intrinsic disorder in mtCyt-\(b\) is not very high, the peculiarities of disorder distribution within the sequences of mtCyt-\(b\) from different species varies in a rather specific way. In fact, positions and intensities of disorder/ flexibility “signals” in the corresponding disorder profiles are relatively unique for avian and animal species. Therefore, it is possible to devise a set of simple rules based on the peculiarities of disorder profiles of their mtCyt-\(b\) proteins to discriminate among species. This intrinsic disorder-based analysis represents a new technique that could be used to provide a promising solution for identification of the source of meats.

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Introduction

Concerns regarding the food authentication and adulteration require development of new means to protect consumers all over the world from products containing inedible meat parts and from products where lower-cost meat species are intentionally mixed into higher-cost meat species for the purpose of economic gain.\(^1\) Mislabeling of the meat products represents a serious concern from the public health viewpoint, since unintended allergens potentially present in such mislabeled products represent significant risks to the allergic consumers.\(^2,3\) Furthermore, there are religious restrictions in the case of pork and its derivatives,\(^4\) whereas horse meat is considered as taboo food in many countries. The need to test meat sources is exemplified by a major 2013 commercial fraudulence scandal in Europe, where horse meat was found in burgers products (as much as 100% of the meat content in some cases), and where many foods advertised as containing beef were shown to include other undeclared meats, such as pork.\(^5\) According to the market studies conducted in Mexico, Turkey, and South Africa, 20–70% of a variety of meat products are mislabeled and contain meat species that are not included to the package labels.\(^6-9\) Meat adulteration is common in USA too. Based on the 1995 analysis of ground meat products in Florida, USA, mislabeling was found in 16.6% of the products tested.\(^10\) Recently, using DNA barcoding of the cytochrome c oxidase I (COI) gene as a means to identify the origin of meats in a variety of ground meat products purchased in USA from online and retail sources, including both supermarkets and specialty meat retailers, revealed high level of mislabeling, with the online specialty meat distributors having the highest rate of mislabeled products.
(35%) followed by local butcher (18%) and local supermarkets (5.8%).

Overall, food authenticity assessment of meat products encompasses several issues, including the detection of fraudulent substitution of higher commercial valued meat species by less expensive ones, the presence of undeclared species, the substitution of animal by vegetable proteins, and fraudulent mislabeling. Various food adulterations cases were detected such as mislabeling, illegal traffic of endangered species, using poor handling, using cheaper materials, and contaminations with unknown meat products. Therefore, reliable methods are needed to protect consumers from inedible parts in meat mixtures, to make consumers aware on the food composition, and to be used to trace the origin and to characterize safety of foods which are available on the market.

Molecular biotechnology provides a set of powerful tools for solving various problems related to the meat adulteration. More and more molecular techniques based on the polymerase chain reaction (PCR) procedure are applied to identification of the animal meat species. Especially, in the field of conservation biology, the molecular genetic diagnosis for the protected species and the interrelated commercial products proved to be very promising.

Mitochondrial DNA (mtDNA) markers have been considered as effective tools for meat species identification and food authenticity assessment. Mitochondria genome is consisted of genes that encode 13 proteins involved in oxidative phosphorylation, 22 tRNAs, and 2 rRNAs. The cytochrome b (cyt-b) gene of the mtDNA was the first tool used for the forensic species identification. The sequence analysis of the hyper variable displacement loop (D-Loop) combined with hylogenetic analysis was used to identify the subspecies of sika deer. Other mtDNA genes have proven useful in wildlife forensics.

Intrinsically disordered proteins (IDPs) are biologically active proteins that do not have predetermined tertiary structure while in an isolated state in solution and exist as dynamic conformational ensembles. Structurally, such proteins are highly heterogeneous and range from collapsed (molten globule-like), to partially collapsed (pre-molten globule-like) and to even highly disorganized, coil-like forms. Among major biological functions ascribed to these proteins are recognition, regulation, control, and involvement in various signaling pathways. IDPs are frequently involved in protein-protein, protein-nucleic acid, protein-small molecule, or protein-membrane interactions. The lack of a rigid 3D structure enables disordered proteins to be highly promiscuous binders and take part in a multitude of interactions with multiple often unrelated partners. Furthermore, IDPs and IDP regions (IDPRs) can fold differently at interaction with different binding partners.

These proteins are commonly found in nature and many of them are involved in the pathogenesis of various human diseases. IDPs are more frequently observed in eukaryotes than in prokaryotes. Furthermore, the highest ratio of IDPs (47%) was found in nuclear proteins, with the average disorder level of transcription factors being as high as 63%. In contrast, the lowest ID ratio was obtained for mitochondrial proteins (18% for mitochondrial membrane proteins and 13% for mitochondrial non-membrane proteins).

Although mitochondrial cyt-b gene is frequently used for the forensic species identification, the applicability of the intrinsic disorder-based analysis of the mtCyt-b protein to identify meat species have not been discussed as of yet. To fill this gap, we introduce a novel method to identify the source of animal meats using the analysis of the peculiarities of distribution of the predisposition for protein intrinsic disorder within the amino acid sequences of mtCyt-b from several animal and avian species.

**Material and methods**

**Data collection**

The amino acids sequences of mtCyt-b from various animal and avian species were collected from Uniprot. The mtCyt-b proteins analyzed here were from the most common meat species, such as chicken, turkey, duck, goose, goat, sheep, cattle, buffalo, deer, pig, horse, camel, and donkey.

**Amino acid compositions of the mtCyt-b proteins**

The amino acids compositions of the mtCyt-b proteins was analyzed by Composition Profiler (http://www.cprofiler.org/cgi-bin/profiler.cgi) in order to identify the enrichment or depletion patterns of each amino acid residues in certain protein. The method
utilizes the evaluation of the fractional difference in the amino acids content in a query sample, QS, and a background sample, BS, calculated as \((C_{QS} - C_{BS})/C_{BS}\), where \(C_{QS}\) is the content of a given amino acid in the query set, and \(C_{BS}\) is the corresponding value for the set of background proteins. In our analysis, the amino acid contents of Sus scrofa mtCyt-b (M9TMj) was used as a background protein. In addition, the percentage of each amino acid residues in NK-lysin was used as a background protein. In our analysis, the amino acid contents of multiple proteins were used as the background set of proteins. In our analysis, the amino acid contents of Sus scrofa mtCyt-b (M9TMj) was used as a background protein. In addition, the percentage of each amino acid residues in NK-lysin was used as a background protein.

### Order/disorder propensities of the mtCyt-b proteins

The POND® (Predictor of Natural Disordered Region) VSL2 algorithm \(^80\) was used to evaluate the order/disorder propensities of the mtCyt-b proteins. The POND® VSL2 algorithm is known as one of the more accurate disorder predictor. \(^81,82\) The resulted output of disorder evaluation for a query protein is a disorder plot showing the predicted per-residue disorder probability that ranges from 0 (ideal prediction of disorder) to 1 (ideal prediction of order) to 1 (ideal prediction of disorder). The baseline threshold of \(\geq 0.5\) is utilized to determine ordered residues. Since mtCyt-b is a transmembrane protein and is therefore expected to be highly ordered, we also used the POND® VLYT algorithm, \(^83\) which is known to have high sensitivity to local sequence peculiarities and can be used for identifying disorder-based interaction sites. Finally, the FoldIndex predictor was also used \(^84\) to determine which region of a given protein is expected to be intrinsically disordered according to its mean net charge and mean hydrophobicity. \(^52\)

### Results

In this study, the complete amino acid sequences of the mtCyt-b proteins from several animal and avian organisms were obtained from the Universal Protein Resource (UniProt) database to evaluate their propensities for the intrinsic disorder structure. Table 1 lists UniProt IDs of the 349 mtCyt-b proteins from the most common meat species, such as chicken, turkey, quail, duck, goose, goat, sheep, horse, camel, cattle, buffalo, pig, deer, and donkey. The corresponding sets contain 22, 2, 3, 5, 8, 27, 41, 40, 9, 46, 30, 48, 54, and 19 proteins,
respectively. These sets include both annotated (Swiss-Prot) and non-annotated entries (TrEMBL) from UniProtKB. Typically, each set contained at least one Swiss-Prot entry. The exceptions from this rule were duck, goose, and goat sets, which did not have any annotated entries. Furthermore, camel, deer, and pig sets of the mtCyt-b proteins contained two, two, and four annotated Swiss-Prot sequences, respectively.

First, to understand the degree of intra-species variability within these data sets, we conducted multiple sequence alignments of the corresponding sets using the Clustal Omega multiple sequence alignment tool for proteins (version 1.2.3, http://www.ebi.ac.uk/Tools/msa/clustalo/). Results of these alignments together with the corresponding Percent Identity Matrices are provided in Supplementary Materials (see Supplementary Sets). These analyses revealed that proteins within studied meat species were rather well conserved, showing very high levels of sequences identity, that typically exceeded 98%. A representative sequence from each of the 14 meat species was selected for the subsequent inter-species analyses. Selection was based on the quality of sequence annotation. Since UniProtKB/Swiss-Prot is a high quality, manually curated database containing carefully annotated and non-redundant protein sequences, whereas UniProtKB/TrEMBL contains computationally generated unreviewed entries enriched in automatic annotation and classification, for the subsequent studies we selected UniProtKB/Swiss-Prot manually reviewed entries, where it was possible. This selection was attainable for the vast majority of species (except for the duck, goose, and goat sub-sets that do not have any annotated UniProtKB/Swiss-Prot entries). In Table 1, UniProt IDs of all these 14 proteins selected for the subsequent analyses are highlight in bold font, whereas their corresponding amino acid sequences are listed in Supplementary Materials (see Figure S1). Although an alternative approach for selecting proteins for comparative analyses could be based on using multiple sequence alignment of a specific species to generate a consensus sequence representing the clan, we decided to use natural sequences instead. This is because of the relatively low intra-species sequence variability, and also because of the use of the natural sequences better fits the ultimate goal of this study, namely to see how different sources of meet can be differentiated from each other.

Next, these 14 sequences were used for the sequence similarity analysis by the Clustal Omega algorithm (http://www.ebi.ac.uk/Tools/msa/clustalo/). Results of this analysis are shown in Figures S2, S3, and S4 (see Supplementary materials). This multiple sequence alignment revealed that although there is a high conservation degree between the mtCyt-b proteins from the analyzed animal and avian organisms (the degree of similarity between the sequences derived from different species ranges from 70.18% to 99.21% (see Figure S3), these proteins preserve sufficient level of individuality to be reliably grouped into a meaningful phylogenetic tree (see Figure S4). Our phylogenetic analysis was conducted using the Molecular Evolutionary Genetics Analysis (MEGA.6) software (http://megasoftware.net/) that contains a set of tools “for building sequence alignments, inferring phylogenetic histories, and conducting molecular evolutionary analysis.”85 The results of this analysis were used exclusively for the illustration purpose and were not used in the development of the detection method.

Amino acids composition of cytochrome b protein from different species

The amino acid compositions of the mtCyt-b proteins were determined using the Composition Profiler software that provides information on the enriched and depleted amino acid residues in a certain protein or a set of proteins.79 The relative enrichment/depletion of the avian and animal mtCyt-b proteins (or ordered proteins from PDB) in various residues was analyzed using the \((C_x - C_{UniProt})/C_{UniProt}\) equation, where \(C_x\) corresponds to the content of a given residue in a query data set (ordered proteins, avian mtCyt-b proteins or animal mtCyt-b proteins) and \(C_{UniProt}\) represents the content of this residue in the background set (proteins from UniProt).79 This analysis revealed that in comparison with the typical UniProt proteins, the mtCyt-b proteins were enriched in Trp, Phe, Tyr, Ile, Leu, His, Met, and Pro residues, and depleted in Val, Ala, Arg, Gly, Asp, Gln, Glu, and Lys residues, as noted in Table 2 and shown in Fig. 1. It is also seen that the compositions of the mtCyt-b proteins are rather different from the amino acid composition of typical ordered proteins from PDB, which were also analyzed by the Composition Profiler for comparison (see Fig. 1A). Fig. 1B shows that there are some differences between the relative amino acid compositions of
the avian and animal mtCyt-b proteins, with avian proteins being noticeably enriched in Ala, Gln, Asn, Glu, and Leu residues, and being noticeably depleted in Met, Asp, Ile, and Cys residues.

Figure 2 represents the amino acid compositions of the individual mtCyt-b proteins from different species evaluated by the ProtParam tool of the ExPasy Bioinformatics Resource Portal. Here proteins are grouped by their origin, with individual avian and animal species varied as follows: chicken (16.0% and 8.7%), quail (16.8% and 8.9%), turkey (17.6% and 8.7%), duck (16.6% and 7.4%), goose (17.1% and 8.4%), horse (16.4% and 15.8%), cattle (11.3% and 9.8%), sheep (14.0% and 11.30%), goat (14.5% and 11.1%), camel (14.5% and 14.8%), pig (10.3% and 11.3%), deer (15.0% and 10.0%), buffalo (15.6% and 10.5%), and donkey (16.4% and 11.30%). Fig. 3A clearly shows that by their Leu/Ile content, the mtCyt-b proteins of avian and animal origin are segregated in two distinct groups.

Analysis of the second group of residues, Ser, Pro, and Ala, showed that proteins differ in their Ser/Pro/Ala content as follows: chicken (7.1%, 6.6%, and 6.6%), quail (7.1%, 6.8%, and 6.6%), turkey (6.6%, 6.6%, and 7.1%), duck (6.3%, 6.6%, and 8.2%), goose (6.6%, 6.6%, and 8.9%), horse (7.1%, 6.1%, and 5.3%), cattle (5.8%, 5.8%, and 7.1%), sheep (8.3%, 6.3%, and 6.3%), goat (5.5%, 6.1%, and 5.8%), camel (6.6%, 6.1%, and 6.9%), pig (6.3%, 5.8%, and 6.1%), deer (5.8%, 6.1%, and 6.1%), buffalo (6.3%, 5.8%, and 7.1%), and donkey (7.1%, 6.1%, and 5.0%). Fig. 3B confirms findings of Fig. 3A and shows the mtCyt-b proteins of avian and animal origin can also be segregated in two distinct groups by their Ser/Pro/Ala content.

### Intrinsic disordered and flexible regions in the mtCyt-b proteins

The PONDR® VSL2 and PONDR® VLXT algorithms were used in order to evaluate and visualize the order/disorder propensities of the mtCyt-b proteins. Results of this analysis are shown in Fig. 4, which indicates that although the mtCyt-b proteins are expected to be rather ordered, they are characterized by specific disorder profiles, peculiarities of which can be potentially used for identification of the species of the protein’s origin. This is especially the case for the general differentiation of avian and animal proteins, which clearly possess rather different disorder profiles obtained by PONDR® VLXT (cf. Figs 4A and 4C) and PONDR® VSL2 (compare Figs 4B and 4D). In fact, data shown in Fig. 4 and Table 3 indicate that the PONDR® VLXT-based level of intrinsic disorder in these proteins ranges from 2.6% (sheep) to 4.2% (chicken, quail, horse, and donkey), or from 7.6% (goose) to 13.7% (horse and donkey), when evaluated by PONDR® VSL2.

In addition, each of the analyzed mtCyt-b proteins was shown to contain several intrinsically disordered protein regions (IDPRs), the number of which ranged from 2 (5) to 4 (6) when the proteins
were analyzed by PONDR® VLXT (PONDR® VSL2) (see Table 3 and Fig. 4). Furthermore, Fig. 4 clearly indicates that in addition to the IDPRs, there proteins contains flexible regions (i.e., regions with the disorder score noticeably deviating from 0). Although disorder profiles were rather similar within the groups of avian and animal proteins, these profiles noticeably diverged between the groups. In fact, Fig.s 4 A and 4B show that from the viewpoint of PONDR® VLXT, the mtCyt-b proteins might have from 7 to 9 disordered/flexible regions. Positions of some of these regions are similar for avian and animal proteins, whereas sequence distributions of others are specific for avian and animal proteins. For example, all animal proteins are characterized by the presence of 9 disordered/flexible regions centered at residues 5 (peak 1), 65 (peak 3), 150 (peak 4), 209 (peak 5), 258

Figure 1. Peculiarities of the amino acid compositions of avian and animal mtCyt-b proteins evaluated by Composition Profiler. (A) Relative amino acid compositions of typical ordered proteins (black bars), avian (red bars) and animal mtCyt-b proteins calculated as $(C_x - C_{UniProt})/C_{UniProt}$, where $C_x$ corresponds to the content of a given residue in a query data set (ordered proteins, avian mtCyt-b proteins or animal mtCyt-b proteins) and $C_{UniProt}$ represents the content of this residue in the background set. (B) Relative amino acid compositions of avian and animal mtCyt-b proteins evaluated based on the following equation: $(C_{avian} - C_{animal})/C_{animal}$, where $C_{avian}$ and $C_{animal}$ correspond to the content of a given residue in avian and animal mtCyt-b proteins.
(peak 6), 291 (peak 7), 346 (peak 8), and 375 (peak 9), whereas for all the avian proteins, there are 7 such disordered/flexible regions that are centered at residues 3 (peak 1), 16 (peak 2), 208 (peak 5), 259 (peak 6), 292 (peak 7), and 375 (peak 9). In other words, an avian-specific N-terminally located disorder doublet (peaks 1 and 3 at residues 3 and 16) is substituted in the animal species by a single IDPR (peak 1 at residue 5). On the other hand, animal proteins contain three new flexible regions (peaks 3, 4 and 8). Relative intensities of several strong disorder/flexibility signals (peaks 5, 6, and 7) are also group-specific, which avian proteins being characterized by a well-resolved triplet with comparable intensities, and with animal proteins being characterized by more intensive peaks 5 and 6 and by the less pronounced and more diffused peak 7 (compare Fig. 4A and 4C). Importantly, Fig. 4 also

![Amino acid compositions of individual avian (A) and animal mtCyt-b proteins (B) evaluated by the ProtParam tool of the ExPasy Bioinformatics Resource Portal.](image-url)
shows that the most variability in the propensity for intrinsic disorder between different mtCyt-b proteins is observed for their most disordered regions.

Figure 3. Peculiarities of the Leu/Ile (A) and Ser/Pro/Ala contents (B) in avian (blue) and animal mtCyt-b proteins.

Careful analysis of data shown in Fig. 4 and especially of the PONDR® VLXT-based disorder profiles of individual proteins (see Figure S5) suggested that the positions and intensities of peaks corresponding to
the disordered (i.e., with this disorder scores ≥ 0.5) or flexible regions (i.e., regions with the disorder scores noticeably deviating from 0) in the disorder profiles of individual proteins are rather unique and can be considered as specific disorder/flexibility “signals” suitable for the identification of individual proteins. For example, all the avian species shared two IDPRs in their mtCyt-b proteins (residues 1–8 and 258–260), however, positions of other disordered regions are unique for each species; i.e., chicken and quail both have two additional IDPRs (residues 16–17 and 209–211, and 16–20 and 209–211, respectively). The mtCyt-b of turkey has only two avian-specific IDPRs (residues 1–8 and 258–260), whereas mtCyt-b proteins of duck and goose have a new IDPR at residues 16–17, which is predicted as a flexible region in other avian species.

Similar situation was also observed for the mtCyt-b proteins from animal species as depicted in Table 3, Figure 4, and Figure S5. For example, based on the results of the PONDR® VLXT analysis, four IDPRs were found in the goat and deer mtCyt-b proteins (residues 2–2, 4–4, 209–210, 255–261/262), whereas the sheep protein had only two IDPRs (residues 209–210 and 254–262), and the cattle and buffalo mtCyt-b proteins too had two IDPRs (residues 2–6/7 and 255–262). The mtCyt-b proteins from horse and donkey had the same set of three IDPRs (residues 2–4, 208–211 and 254–262), whereas the camel protein was characterized by an exclusive set of three IDPRs located at residues 2–6, 254–262, and 378–378. On the other hand, one should keep in mind that the results of our analysis can be used as indication or tendency only and not for making solid conclusions, since a careful analysis of the values shown in Table 3 indicates an overlap in the disorderedness of the avian and animal species.

Figure 5 further addresses an issue of intra-species variability of proteins in various data sets by representing PONDR® VLXT-based disorder profiles of all 22 chicken proteins (Fig. 5A) and all 46 cattle proteins listed in Table 1 and shown in Supplementary Materials. Data in Supplementary Set show that chicken proteins are characterized by the sequence identities ranging from 98.16% to 100%, whereas cattle proteins are characterized by the largest intra-species variability, with the sequences identity being varying from 94.46% to 99.47%. Fig. 5 clearly provides an important illustration of the fact that the intra-species variability of disorder predispositions is noticeably less pronounced than the inter-species differences, even if only avian or cattle species would be taken into account. In fact, the vast majority of the mtCyt-b proteins have almost identical disorder profiles, with the noticeable exceptions being Q9G296, Q9B623,
Table 3. Intrinsic protein disordered regions (IDRs) in cytochrome b gene of mtDNA in different meat species. The disorder level was predicted by using PONDR VSL2 algorithm.

| Organism (UniProt ID) | Total number of amino acids | Number of disordered residues: VLXT/VSL2 | Percent of predicted disorder: VLXT/VSL2 | Position of predicted IDPRs: VLXT | Position of predicted IDPRs: VSL2 |
|-----------------------|-----------------------------|------------------------------------------|----------------------------------------|----------------------------------|----------------------------------|
| Chicken (P18946)      | 380                         | 16/40                                    | 4.2/10.5                               | 1–8 16–17 209–211 258–260        | 1–9 204–219 253–256 312–316 375–380 |
| Turkey (P50663)       | 380                         | 11/33                                    | 2.9/8.7                                | 1–8 258–260                      | 1–9 204–216 253–256 314–315 376–380 |
| Quail (P35075)        | 380                         | 16/40                                    | 4.2/10.5                               | 1–8 16–20 209–211                | 1–9 204–209 253–255 312–316 374–380 |
| Duck (Q34160)         | 380                         | 13/30                                    | 3.4/7.9                                | 1–8 16–17 258–260                | 1–9 203–209 253–256 312–315 375–380 |
| Goose (Q6JK7)         | 380                         | 13/29                                    | 3.4/7.6                                | 1–8 16–17 258–260                | 1–9 203–209 253–256 314–315 374–380 |
| Goat (O78789)         | 379                         | 11/39                                    | 2.9/10.3                               | 2–2 4–4 209–210 255–261          | 1–7 59–61 204–217 260–264 311–314 374–379 |
| Sheep (P24959)        | 379                         | 10/46                                    | 2.6/12.1                               | 2–2 208–211 254–262              | 1–7 59–61 204–217 252–265 310–314 376–379 |
| Horse (P48665)        | 379                         | 16/52                                    | 4.2/13.7                               | 2–4 208–211 254–262              | 1–7 59–61 203–217 252–265 310–315 375–379 |
| Camel (P24952)        | 379                         | 15/47                                    | 4.0/12.4                               | 2–6 254–262 378–378             | 1–7 56–61 204–217 252–265 310–315 378–379 |
| Cattle (QSEG37)       | 379                         | 13/48                                    | 3.4/12.7                               | 2–6 255–262                      | 1–7 56–61 204–215 253–265 311–315 374–379 |
| Buffalo (P92571)      | 379                         | 14/50                                    | 3.7/13.2                               | 2–7 255–262                      | 1–7 58–61 204–217 252–265 310–315 375–379 |

Table 3. (Continued).

| Organism (UniProt ID) | Total number of amino acids | Number of disordered residues: VLXT/VSL2 | Percent of predicted disorder: VLXT/VSL2 | Position of predicted IDPRs: VLXT | Position of predicted IDPRs: VSL2 |
|-----------------------|-----------------------------|------------------------------------------|----------------------------------------|----------------------------------|----------------------------------|
| Pig (P24964)          | 379                         | 14/40                                    | 3.7/12.9                               | 2–6 254–262                      | 1–7 58–61 204–217 252–265 310–314 376–379 |
| Deer (P24960)         | 379                         | 12/47                                    | 3.2/12.4                               | 2–2 4–4 209–210 255–262          | 1–7 59–61 204–216 253–265 310–315 375–379 |
| Donkey (P92487)       | 379                         | 16/52                                    | 4.2/13.7                               | 2–4 208–211 254–262              | 1–7 58–61 202–217 252–265 310–315 375–379 |

E5DEQ7, and A0A0B4ZVA3 in the set of chicken proteins and Q85UJ1, Q45LM9, Q3S224, and Q3S243 among the cattle mtCyt-b proteins (see Fig. 5).

Discussion

In this study, we considered a possibility of using of the peculiarities of distribution of intrinsic disorder propensity within the amino acid sequences of the mtCyt-b proteins as a novel method to identify the source of animal species in commercial meats. Recently, the DNA-based methods have been considered as essential tools for species identification in animal products and meat foodstuffs, and this use of such techniques is becoming widespread. Compared to the protein-based techniques, the DNA-based approaches possess several advantages, such as the ubiquity of nucleic acids in every type of cells and the higher stability of DNA when compared with proteins. Among DNA-based methods, polymerase chain reaction (PCR) is the most well-developed molecular technique because of its simplicity, high speed, specificity and sensitivity, that determine the ability of PCR to identify species of origin even in the complex and processed foods.

Esposti et al. (1993) reported that among proteins encoded by the mitochondrial DNA genes the cytochrome b is one of the best candidates for the phylogenies analysis with respect to its structure and function. This protein possesses some clear advantages such as slow and rapid amino acid evolving coding position...
and presence of conserved and variable domains. Therefore, it was used in diversity phylogenetic studies.13,90-96

The effects of cooking methods on the reliability of the identification of the meat source using the cyt b gene from the mitochondrial DNA were studied.97 It was also shown that the meat species can be successfully identified in cooked chicken sausage based on the cyt b information.98 Similar results were obtained when cytochrome b gene was applied to identify species in heated sausages.17 The awareness on the food composition and authentication is required for many reasons, such as food born disease, illegal traffic of animal species and biodiversity studies. Therefore, new and reliable techniques are needed to provide a useful promising solution.

Our analysis revealed that it is possible to differentiate between avian and animal meat species based on the content of amino acid residues in the corresponding mtCyt-b proteins. In fact, different Leu/Ile and Ser/Pro/Ala contents in analyzed avian and animal species can be used to reliably identify the avian or animal origin of the protein. Furthermore, the contents of these two groups of residues in the mtCyt-b protein can be potentially used to discriminate among avian and animal meat species (see Fig. 2 and 3).

The intrinsic disorder predisposition analysis revealed that although the mtCyt-b proteins from all avian and animal species analyzed in this study are predicted to be rather ordered, these proteins have specifically located disordered and flexible regions (see Fig. 4). Furthermore, our analysis suggested that not
only avian and animal proteins can be distinguished from each other, but proteins within each of these groups can also be identified based on the specific features of their disorder profiles. These observations suggest that it is possible to devise a simple rule based on the specific amino acid compositions and peculiarities of disorder profiles of the mtCyt-b proteins to discriminate among species. In our view, this intrinsic disorder-based analysis represents a new technique that could be used to provide a promising solution for identification of the source of meats.

Conclusions

We introduced a novel method to identify the source of animal species in the commercial meats using intrinsic disorder-based analysis of the mitochondrial cytochrome b. Our analysis revealed that it is possible to differentiate between and within the avian and animal meat species based on the content of amino acid residues in their mtCyt-b proteins. Another level of differentiation can be added based on the analysis of the peculiarities of the intrinsic disorder profiles of these proteins.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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