Establishment of a proteomic reference map for the gastrocnemius muscle in the rabbit (Oryctolagus cuniculus)

André M. Almeida a,b,*, Alexandre Campos b, Sofia van Harten a, Luís Alfaro Cardoso a, Ana Varela Coelho b,c

a Instituto de Investigação Científica Tropical, Lisboa, Portugal & Centro Interdisciplinar de Investigação em Sanidade Animal, Lisboa, Portugal
b Instituto de Tecnologia Química e Biológica, Oeiras, Portugal
c Universidade de Évora, Évora, Portugal

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In several laboratory and production species, the establishment of a proteome reference map of a specific tissue has been accomplished. The rabbit is widely used as both a production and experimental animal. A lot of physiology research involving the gastrocnemius muscle of rabbit is described, although no reference proteome map is available. In this work, the first reference map of the rabbit’s gastrocnemius muscle using 2D gel electrophoresis and the identification of proteins through peptide mass fingerprinting (PMF) was established. A total of 45 proteins were localized and identified with three major roles: cell structure and contractile apparatus; metabolic and cell defense proteins. A reference map of major proteins expressed is described enabling possible comparisons with other physiological studies.

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The use of proteomics, the study of protein expression, is considered of major interest in 21st century research in animal science (Fadiel et al., 2005). Aiming to better understand proteome alterations, it is vital to have a reference proteome map for a specific tissue and species. Proteome maps of muscle have been described in laboratory species and are directed towards biomedical. Regarding studies in farm animals, only domestic cattle (Bos taurus) seems to have proteome maps, specific to the Longissimus thoracis (Morzel et al., 2008) or Semitendinosus (Bouley et al., 2004; Chaze et al., 2006). Several other expression studies exist for pig and sheep.

The rabbit (Oryctolagus cuniculus) is an important species worldwide used as a laboratory animal and model for larger species. It is the basis of physiological, anatomical and disease models in humans and plays a major role as a meat-producing animal in Mediterranean Europe and parts of Asia. However, no muscle proteome map seems to be available in the literature. The aim of this study was to construct a reference proteome map for the gastrocnemius muscle of the rabbit, as this muscle is considered to be a model for muscle plasticity (Alford et al., 1987), is readily accessible (Almeida et al., 2006) and frequently used in muscle physiology studies. Proteome mapping will also provide a basis for studies on meat quality.

New Zealand white (NZR, Oryctolagus cuniculus cuniculus, n = 4) and Iberian wild (WR Oryctolagus cuniculus algirus, n = 3) rabbits were used at an age of 10–12 weeks. NZR weighed on average 3495.52 ± 67.67 g and WR 789.63 ± 49.61 g. European Union regulations on laboratory animal experimentation were followed. All animals were fed ad libitum on commercial pellets (Biona 701, Sapprogal, Vila Chã de Ourique, Portugal, composition: 14.9% Crude Protein, 2.7% Crude Fat, 14% Crude Fibre and 13% Ash). Animals kept in individual cages with environmental enrichment, were allowed free access to water. After 30 days, animals were euthanized with 4% isoflurane (IsoFlo®, Veterinaria Esteve, Bologna, Italy). Muscle samples (gastrocnemius) were collected and snap-frozen in liquid nitrogen.

Total protein was extracted (Bouley et al., 2004) and quantified using PlusOne 2D Quant kit (GE Lifesciences, Uppsala, Sweden). Isoelectric focusing (IEF) followed by SDS–PAGE was used. One 2D gel was run per sample. 2D electrophoresis, strip equilibration and gel digitalization was conducted according to Görg et al. (2000) in the IPGphor system, using 24 cm strips with a linear pH gradient 3–10.

Gels were stained using colloidal Coomassie blue (Neuhoff et al., 1988) for 48 h and washed 3 times in double distilled water. Spot matching was conducted according to Soares et al. (2007) using Platinum software. Methodology for protein identification through PMF has been described (Lamy et al., 2008). Three replicates of each spots were excised and used for identification.

We constructed a reference map of the most representative proteins in the gastrocnemius muscle of the rabbit and identified as many proteins as possible. The identification map obtained in this study is presented in Fig. 1a. Most of the proteins present were found in all samples and spots appeared in the similar region.
demonstrating reproducibility of results. To the best of our knowledge, this is the first study attempting to map the proteome of the gastrocnemius muscle in the rabbit. Although a few skeletal muscle proteomes are characterized in cattle (Bouley et al., 2004), information on the mapping of the gastrocnemius muscle proteome in farm animals does not exist, although available for the laboratory rat (Gelfi et al., 2006). A comparison of the identification results obtained in this study and others available in the literature seem to show a consistency of patterns of results, particularly structural proteins.

The identification of proteins was accomplished through the analysis of spectra (Fig. 1b). Identified proteins were classified according to their role (Table 1, Fig. 1c). Results demonstrate that the majority of metabolic proteins are found in the alkaline region (pI 7–10), in contrast to cell structure and contractile apparatus proteins that were found in the pI 4–7 region.

We identified one protein involved in cell defense, α-crystallin B chain. Although abundantly expressed (Kamradt et al., 2002), this protein is located to have low levels of expression under control situations; hence difficult to detect during two-dimensional electrophoresis separation at pI ranges where the depletion of most abundant proteins is not conducted. The proportion of cell defense proteins identified by Bouley et al. (2004) is higher (16%) than the 2% obtained in our study. As those authors loaded 800 µg versus 600 µg in our study, results indicate that if higher amounts of protein are loaded, we are likely to obtain higher identification success.

Several metabolic proteins were identified, with special reference to glycolysis enzymes. Contractile apparatus and cell structure proteins identified included actin, myosin light, regulatory light and heavy chains and its fragments, tropomyosin α and β, and troponin I. Other proteins identified were parvalbumin, hemoglobin and myoglobin.

Despite the fact that identification was inconclusive for 20 spots, a total of 45 relevant spots were positively identified using the PMF methodology, a success rate of 70%. Protein identification through PMF was successful for abundant proteins and with relative success for low abundant proteins. However, identification was not fully achieved for proteins with lower molecular weights or located in the vicinity of abundant proteins due to a lower amount of peptides resulting from tryptic digests or possible spot overlapping. A strategy to overcome such difficulties would include 2D electrophoresis using different pH ranges, increasing the amount of loaded protein or conduct studies using immuno-

**Fig. 1.** Two dimensional reference map, mass spectra and distribution of proteins according role. a – Two dimensional reference map of wild rabbit gastrocnemius muscle. First dimension run was carried out in 24 cm pH 3–10 IPI strip and second dimension in a 12% SDS–PAGE gel, respectively, separating proteins according to their isoelectric point (pI) and the molecular mass (MW). Gels were stained with Coomassie Colloidal G250. Identified proteins are indicated with the most commonly used name. Indications in parenthesis concern reference numbers that can be found in Table 1. Molecular weight markers (MW) are presented on the left hand side. b – Mass Spectra obtained by Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technique for spots N. The monoisotopic mass of each individual peak considered is marked and used for protein identification by peptide mass fingerprinting using the Mascot program. Peaks submitted are marked with an arrow (Peptides Searched), black for unmatched and grey for matched peptides. As shown in Table 1, protein N was identified as Tropomysin α from rabbit. c – Distribution of proteins identified for the gastrocnemius muscle in the rabbit, classified according to general biochemical and physiological roles: metabolism; contractile apparatus, cell structure; cell defense and other.
depletion. Such strategies, if using PMF, would still depend on the characterization of rabbit muscle proteins or their homology to other species’ proteins. If the similarity would prove to be low, it would be advisable to use MS/MS or de novo sequencing approaches.

Table 1
Summary of protein spots identified by 2D gel and peptide mass fingerprinting technologies in rabbit gastrocnemius muscle. Each spot includes number of peptide matched per peptide search, estimated and theoretical molecular weight, coverage percentage, protein scores, MSDB database accession number and the protein names including the species on which it is identified.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Peptide search/match</th>
<th>Estimated MW (kDa)</th>
<th>Theoretical MW/peptide (Da/peptide)</th>
<th>% coverage</th>
<th>Score</th>
<th>MSDB database accession number; protein name and species</th>
</tr>
</thead>
<tbody>
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<td><strong>Metabolism proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>R8</td>
<td>32/12</td>
<td>125</td>
<td>11657/6.2</td>
<td>18</td>
<td>89</td>
<td>S0196S GTase-activating protein – bovine</td>
</tr>
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<td>55</td>
<td>57305/7.7</td>
<td>16</td>
<td>70</td>
<td>I4AA9 pyruvate kinase (EC 2.7.1.40), chain A – rabbit</td>
</tr>
<tr>
<td>#826</td>
<td>65/17</td>
<td>200</td>
<td>92995/6.5</td>
<td>22</td>
<td>82</td>
<td>2GPN glycogen phosphorylase b (EC 2.4.1.11) – (fragment)</td>
</tr>
<tr>
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<td>18/10</td>
<td>90</td>
<td>96311/6.6</td>
<td>12</td>
<td>84</td>
<td>1NQJ glycogen phosphorylase (EC 2.4.1.1) – rabbit</td>
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<tr>
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<td>55</td>
<td>47428/6.2</td>
<td>26</td>
<td>79</td>
<td>A23126 phosphorphyruvate hydratase (EC 4.2.1.11) or alpha- enolase (EC 4.2.1.11)</td>
</tr>
<tr>
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<td>40</td>
<td>43313/6.6</td>
<td>35</td>
<td>90</td>
<td>KIRBCM creatine kinase (EC 2.7.3.2) chain M – rabbit</td>
</tr>
<tr>
<td>R10</td>
<td>34/11</td>
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<td>47174/8.1</td>
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<td>47174/8.1</td>
<td>30</td>
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<td>A37210 phosphorphyruvate hydratase (EC 4.2.1.11) beta – or beta- enolase</td>
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<td>39774/8.3</td>
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<td>DEPGC3 glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) – pig</td>
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<td>44/9</td>
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<td>26894/7.1</td>
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<td>ISRBT triose-phosphate isomerase (EC 5.3.1.1) – rabbit</td>
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<td>26894/7.1</td>
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<td>ISRBT triose-phosphate isomerase (EC 5.3.1.1) – rabbit</td>
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<td>57/15</td>
<td>45</td>
<td>32718/4.7</td>
<td>35</td>
<td>112</td>
<td>TMRBA tropomyosin alpha chain, cardiac and skeletal muscle –</td>
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<td>21719/8.9</td>
<td>40</td>
<td>94</td>
<td>Q53EY8_HUMAN Adenylate kinase 1 variant (fragment) human</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>N</td>
<td>29/9</td>
<td>70</td>
<td>32718/4.7</td>
<td>20</td>
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<td>TMRBA tropomyosin alpha chain, cardiac and skeletal muscle – rabbit</td>
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<tr>
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<td>57/15</td>
<td>45</td>
<td>32718/4.7</td>
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<td>TMRBA tropomyosin alpha chain, cardiac and skeletal muscle – rabbit</td>
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<td>29/7</td>
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<td>MLE1_RABIT Myosin light chain 1, skeletal muscle isoform – rabbit</td>
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<td>25</td>
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<td>MLE1_RABIT Myosin light chain 1, skeletal muscle isoform – rabbit</td>
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<td>20</td>
<td>18997/4.8</td>
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<td>31/6</td>
<td>17</td>
<td>16573/4.6</td>
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<td>64</td>
<td>MLE3_RABIT Myosin light chain 3, skeletal muscle – rabbit</td>
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<td>125</td>
<td>78732/5.5</td>
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<td>SO0084 myosin heavy chain, fast skeletal muscle – rabbit</td>
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<td>58/16</td>
<td>115</td>
<td>103804/5.3</td>
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<td>77</td>
<td>Q63939_9MUR Myosin heavy chain (fragment) – Rattus sp.</td>
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<td>55/33</td>
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<td>223841/5.6</td>
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<td>A59293 skeletal myosin heavy chain – rabbit</td>
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<td>40/10</td>
<td>29</td>
<td>21241/8.9</td>
<td>46</td>
<td>79</td>
<td>TNN2_RABIT Troponin I, fast skeletal muscle – rabbit</td>
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<tr>
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<td>27/10</td>
<td>60</td>
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<td>38/12</td>
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<td>42090/5.2</td>
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<td>ATBOSM actin, aortic smooth muscle – bovine</td>
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<td>49</td>
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<td>22</td>
<td>76</td>
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<td>36</td>
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<td>42/9</td>
<td>29</td>
<td>42052/5.3</td>
<td>28</td>
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<td><strong>Cell defense proteins</strong></td>
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<td>37</td>
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<td>11/6</td>
<td>15</td>
<td>11926/5.1</td>
<td>40</td>
<td>77</td>
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<td>22980/10.6</td>
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<td>Q63H1P_HUMAN Hypothetical protein DKFzP686M0519. – Homo sapiens (human)</td>
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<td>16048/8.1</td>
<td>75</td>
<td>100</td>
<td>HBB_RABIT Hemoglobin subunit beta-1/2 rabbit</td>
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</table>

MW – molecular weight; pl – isoelectric point; score is -10 + Log(P) and P is the probability that the observed match is a random event and protein scores greater than 59 are significant when p < 0.05.
Acknowledgements

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References