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From the Ocean to the Table – An Integrated Mass Spectrometry Approach to Identify the Fish on Your Plate

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ABSTRACT

The fishery market has growth in sales for the last 15 years. As a result, fish demand is producing a worldwide overexploitation of resources and fraudulent practices in the industry that account for 30% of the sales. In most cases, high priced fish species are substituted for lower value species. Here we described an integrated proteomic approach to authenticate fish species from muscle tissue.

INTRODUCTION

The identification of commercial fish species is a relevant issue to ensure correct labeling, maintain consumer confidence and enhance the knowledge of the captured species, benefiting both, fisheries and manufacturers. Here we propose a proteomic approach, based on top down proteomic analyses using ESI-MS/MS in a high resolution orbitrap mass spectrometer for the identification of fish species with commercial interest. ESI-Orbitrap protein mass fingerprint from thermo-stable proteins purified from fish tissue were used for the identification of a commercial hake filet with no label regarding the fish other than Product from South Africa. Further identification and characterization of this sample was performed using standard shotgun proteomics and PRM targeted analysis. We believe that fisheries and manufacturers may take advantage of this methodology as a tool for a rapid and effective seafood product identification and authentication, providing and guaranteeing the quality and safety of the foodstuffs to consumers.

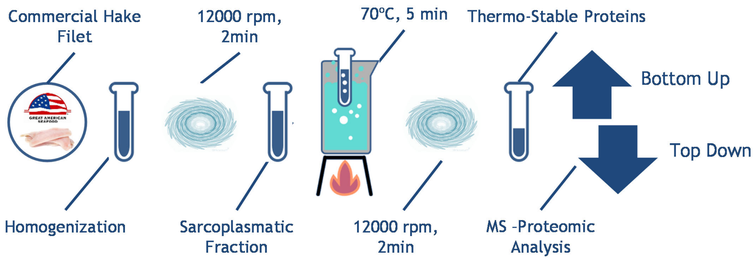
MATERIALS AND METHODS

1 gr of fish muscle tissue was homogenized in water. The sample was then centrifuged to remove the insoluble material. Water soluble proteins were then heated at 70ºC for 5 min. After the heat treatment the sample was again centrifuged and the supernatant was aliquoted. One of the aliquots was submitted for bottom up and PRM proteomics analysis. A second aliquot was submitted for top down analysis. For bottom-up proteomics, the pH of the sample was adjusted to 8, trypsin was added and digestion was performed for 3 minutes using high intensity ultrasound. After digestion the sample was desalted using Pierce™ Micro-Spin Columns following the instructions of the manufacturer. After desalting the samples were resuspended in 0.1% formic acid and summited for LC-MS analysis using a nLC 1200 hyphenated to a Q Exactive Classic. Peptides were separated using a 15 cm Easy NanoSpray column. After LC-MS analysis, raw files were submitted for database search using Protein Discoverer 2.1 and a composite protein database of all fish species from Uniprot.

For top down analysis, water soluble proteins after the heat treatment were diluted 10X and directly infused into a Q Exactive mass spectrometer. Mass spectra were acquired from 800 to 1200 m/z range at 140K@m/z 200. MS/MS acquisition was performed using HCD fragmentation at 15% NCE. Data analysis was performed using Thermo Scientific Deconvolution 4.0 and Prosight PD node in Proteome Discoverer 2.0 software.

WORKFLOW

FIGURE 1. General overview of the analytical workflow. Commercial hake filet samples were processed as described in the workflow. First, 1 gr of tissue was physically disrupted with a mortar and later with ultrasound in water. Muscle debris was removed by centrifugation and the supernatant was submitted to a heat treatment for five minutes. After the heat treatment, the sample was centrifuged to removed denaturalized proteins and submitted to either bottom-up or top-down proteomic analysis.



RESULTS

TABLE 1. List of the top protein group out of over ~200 proteins identified from the bottom-up proteomic analysis using Protein Discoverer 2.1. As can be noticed the very high protein sequence homology among three very different species of hake does not allow to identify what is the specie that the test sample belongs.

Accession	Description	Coverage %	#AAs	MW [kDa]	# Unique Peptides
P86765	Parvalbumin beta 2 OS=Merluccius merluccius	65.74	108	11.27	8
P86764	Parvalbumin beta 1 OS=Merluccius hubbsi	65.74	108	11.29	8
P86768	Parvalbumin beta 1 OS=Merluccius paradoxus	65.74	108	11.39	8

FIGURE 2. Blast alignment of the three proteins shows the highly conserved sequence for this calcium binding proteins among the three species. Blue stars indicate were the amino acid sequence varies among the three proteins. Only three different peptides could allow for the specific identification of fish under study AEGTFK, SPADIK and SPAADIK. However the short sequence of these peptides does not allow a straight identification of the species because their mass to charge ratio are below the typical scanning range in DDA experiments in case of +2 charge state peptide, or if they are in their +1 charge state usually +1 charge ions are not targeted for fragmentation.

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          1         11        22        31        41        51        61
P86765 AFAGILADAD ITAA LAACK AEGSFKHGE FFTKIGLKGK S_AADIKKVF GIIDQDKSDF VEEDELKLFL
P86764 AFAGILADAD ITAA LAACK AEGTFKHGE FFTKIGLKGK S_AADIKKVF GIIDQDKSDF VEEDELKLFL
P86768 AFAGILAEAD ITAA LAACK AEGTFKHGE FFTKIGLKGK SPA_DIKKVF GIIDQDKSDF VEEDELKLFL

          71        81        91       101       111
P86765 QNFSAGARAL TDAETATFLK AGDSDGDGKI GVDEFAAMVK G
P86765 QNFSAGARAL TDAETATFLK AGDSDGDGKI GVDEFAAMVK G
P86768 QNFSAGARAL TDAETATFLK AGDSDGDGKI GVDEFAAMVK G
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FIGURE 3. Intact mass analysis: Left panel shows the mass spectrum obtained after direct infusion of the undigested sample. Showing ~11 kDa group of proteins. After protein deconvolution (right panel) using the Extract algorithm the most abundant mass corresponds to Parvalbumin beta 2 from Merluccius *paradoxus.*

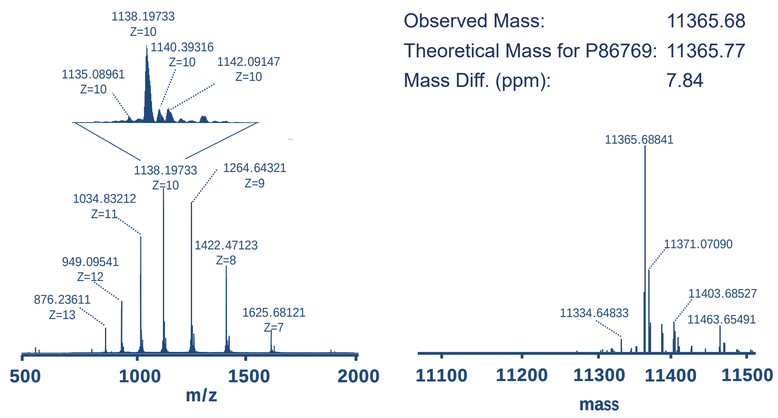


FIGURE 4. The 11365.68 mass was further selected for top-down analysis to verify that the protein sequence belongs to Parvalbumin beta 2 from Merluccius paradoxus. Left panel show the MSMS spectra for the 1138.19733 mass. The right panel show the sequence coverage obtained that allows for the explanation of 45% of the residues cleavages.

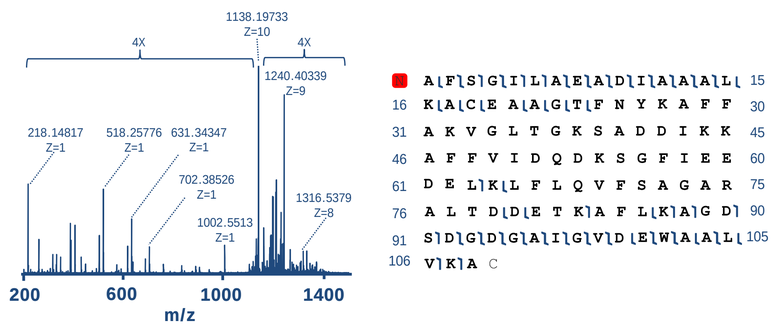
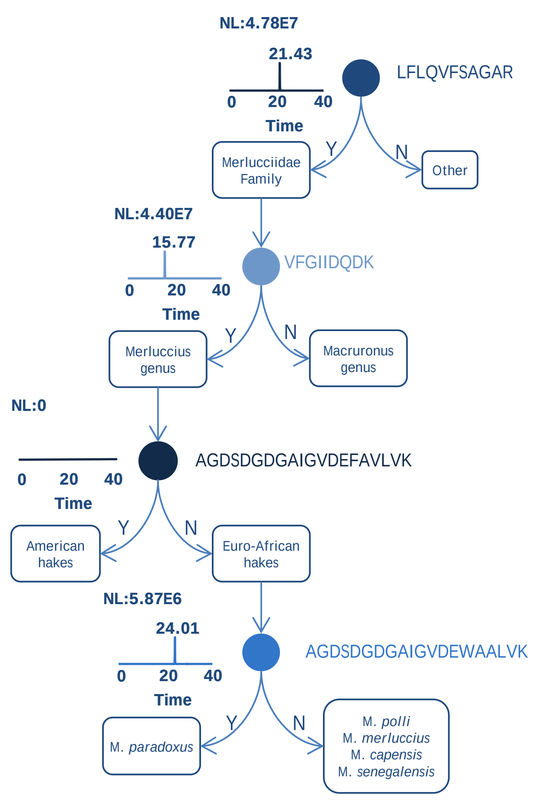


FIGURE 5. A PRM decision tree for a systematic discrimination of Merlucciidae species using specific tryptic peptides from parvalbumins based on previously published peptide biomarkers[1].



CONCLUSIONS

- We successfully identify the fish specie from an unlabeled commercial hake filet.
- Intact MS analysis of thermostable proteins represents a promising technique for fish identification.
- The workflow developed here allows for fish authentication in less than 30 minutes.

REFERENCES

1. Monica Carrera, Benito Canas, Daniel Lopez-Ferrer et al. Anal. Chem. 2011, 83, 5688–5695

TRADEMARKS/LICENSING

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