



UNTARGETED MASS SPECTROMETRY-BASED PROTEOMICS FOR SPECIES AND TISSUE IDENTIFICATION IN MIXED FEED AND FOOD SAMPLES

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INTRODUCTION

Due to globally rising demands for food and feed, novel protein ingredients are being introduced into our food systems on an increasing scale. This gives rise to novel challenges in relation to the detection of feed and food fraud and the determination of feed and food authenticity, respectively. In this context, the development and increased implementation of rapid, sensitive and robust molecular methods are key. However, progress in the application of such tools has been hampered by a general lack of well-annotated reference genomes of target species commonly used or newly introduced in feed or food preparations. Our group has been working on the development and implementation of untargeted mass spectrometry-based approaches in regulatory settings to identify, differentiate, and quantify proteic ingredients of animal and plant origin in various food and feed mixes without using any genomic information.

METHODOLOGY

The workflow presented here comprises the enzymatic digestion of samples using trypsin, analysis of extracts using high-performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS), data processing using direct spectra comparison (compareMS2) and proteomics spectra library (SL)-based analyses using tools of the Trans-Proteomics Pipeline (TPP). All data generated and published by our group are being made available on public repositories for MS data such as the Mass Spectrometry interactive Virtual Environment (MassIVE) hosted at the University of California San Diego (UCSD), and also are added to an in-house SL database specifically developed for the species and tissue-specific screening of food and feed samples of marine and terrestrial origin.

RESULTS

The untargeted proteomics workflow described above has already been used successfully to differentiate processed animal proteins (PAP). It has also been used to identify and authenticate food and feed-grade insect species and to detect whether black soldier fly (BSF) larvae fed on prohibited substrates could be differentiated from conventionally fed

insect species. We also found that SL-based untargeted proteomics can be used for the identification of common allergens in food-grade insect samples. Currently, we are investigating whether untargeted MS-based methods can also be used to successfully differentiate and authenticate 30 marine fish species both in pure fillet and mixed samples containing two or more fish species.

DISCUSSION

The untargeted proteomics SL-based approach implemented in our laboratory was shown to be capable of both species and tissue-specific identification of proteinaceous food and feed ingredients, including processed animal proteins (PAP), insect proteins, and plant, mammalian and fish proteins. Future work is focusing on the differentiation and detection of seafood fraud, which has recently been highlighted as an emerging issue in the global food market. We are also in the process of making all species and tissue-specific MS data collected in the above-listed (and in parts already published) studies available on request to researchers in the field using a dedicated web-based service. The latter is currently being developed in-house and, following proper quality testing, is envisaged to be released publicly to provide research and regulatory laboratories with an easily accessible platform for authenticating and identifying proteinaceous ingredients in feed and food in both pure and mixed samples.