Identification of animal furs by MALDI-TOF mass spectrometry

Thomas Elssner[1], Stefan Klepel[1], Guido Mix[1], Klaus Hollemeyer[2], Wolfgang Altmeyer[3], Markus Kostrzewa[1]
[1]Bruker Daltonik GmbH, GERMANY
[2]University of Saarbruecken, GERMANY
[3]Gene-Facts, Saarbruecken, GERMANY

ASMS 2008, Poster WPUUU 580

Introduction

Animal protection organizations have demonstrated that large quantities of furs of domestic dogs and cats derived from breeding farms are being imported mainly from Asian countries into the US and the EU market. To prevent such trade, the US and recently the EU parliament concluded an embargo on imports of furs of domestic dogs and cats. But trading of such furs is difficult to monitor, because different fur types are hard to discriminate. Here, we present a new analytical method for species identification of animals based on MALDI-TOF mass spectrometry. Besides identification of furs from dogs and cats the method may be suitable for monitoring of the Convention on International Trade in Endangered Species of Wild Fauna (CITES).

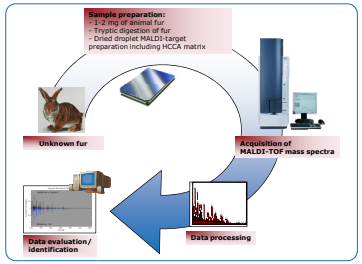

Fig 1: Workflow for animal fur identification by MALDI MS.

Methods

In a first step, small pieces of hairs, furs or feathers were chemically reduced using mercaptoethanol. Subsequently, samples were digested for two hours using trypsin. The extracts were mixed with HCCA matrix solution. From the resulting mixtures, 1 µl was deposited onto a ground steel MALDI target plate. Spectra acquisition was done on an ultraflex III MALDI-TOF mass spectrometer in the reflector mode (mass range 1000-4000 Da). Before analysis spectra processing including smoothing, baseline-correction and mono-isotopic peak-picking was performed to generate peak lists. The peak lists were analyzed using the MALDI Biotyper™ software. Different pattern matching and statistical algorithms were applied to classify and identify the samples based on the mass spectra (Fig 1).

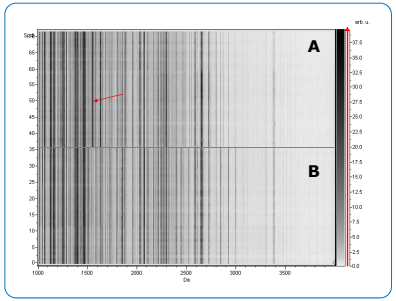

Fig 2: Gelview of 36 MALDI mass spectra from digested raccoon (A) and domestic dog fur (B) in the range from 1kDa to 4 kDa. The peak at *m/z* 1552.7 is suitable to distinguish raccoon dog from domestic dog and other members of the Canidae family.

Results

The primary component of hairs and feathers is keratin. But the amino acid sequence of keratin differs from species to species. Therefore, tryptic digestion of keratins reveals peptide mass fingerprints, which are specific for the mammalian species.
Peptide mass fingerprints were generated from 52 different mammalian species. Reproducibility of digestion followed by acquisition of generated spectra could be demonstrated, successfully (Fig 2). Peak lists of these spectra were merged to one main spectrum and stored as reference in a library. For a library entry, 36 MALDI-TOF mass spectra were acquired for each species. Stored peptide pattern combined with a dedicated pattern matching algorithm allowed the identification of unknown hairs/furs (Fig 3).

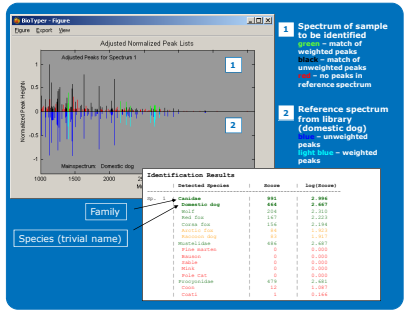

Fig 3: Hierarchical analysis (unweighted pattern matching for family identification followed by weighted pattern matching for species identification) of an unknown animal hair.

Identification is based on a score-calculating algorithm. It is carried out in two steps: 1. Unweighted pattern matching using family-specific superior main spectra. 2. Weighted pattern matching for identification of species belonging to one family.
Furthermore, the phylogenetical memory of the hair enables the allocation of unknown samples to a zoological genus or family, even if there is not an according reference entry in the library is available. Similar species cluster in separated groups within a dendrogram (Fig 4).
The secure identification of closely related raccoon dog and domestic dog (Fig 2) could be successfully demonstrated applying specific differentiating features in a weighted pattern matching algorithm (Fig 3).

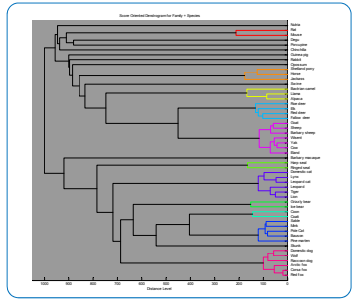

Fig 4: Dendrogram represents relationship between different mammalian species according to their peptide mass fingerprints of digested hairs.

Summary

MALDI-TOF MS peptide mass fingerprinting of tryptic digested mammalian hair samples provided spectra, which were specific for every species.

Reproducibility of peptide mass fingerprints from animal hairs/furs could be demonstrated, successfully.

Usage of these peptide mass fingerprints combined with a standard pattern matching algorithm was found to be a suitable method for identification of hairs and furs on the family and mostly also on the species level.

For identification purposes a score-based algorithm was used.

Separation of closely-related animals (domestic and raccoon dog) was demonstrated.

Conclusions

- ❖ Very simple and rapid method for animal hair and fur identification
- ❖ Minimal costs in sample preparation and analysis
- ❖ MALDI-TOF MS in combination with the MALDI Biotyper™ software has the potential for monitoring fur trade and to control the embargo on imports of furs from secured animals

MALDI-TOF