SHIMADZU

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Species identification of materials used in cultural heritage objects from Alaska in the British Museum's collection using 'ZooMS' methodology

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Introduction

The British Museum's collection contains a vast number of objects fabricated from animal derived materials where the species of animal used is unidentified. Revealing the animal species of worked materials provides archaeologists and curators with insights into the past including species availability, trading networks and technologies utilised. The information is important to descendants of the makers who wish to understand their history and traditions.

Initial speciation work at the British Museum via MALDI-ToF mass spectrometry is focused on identification of species used in the items collected from Alaska by Captain James Cook and other explorers in the 18th and 19th centuries, including harpoons and cordage used for hunting and fishing.

'ZooMS' (Zooarchaeology by Mass Spectrometry)[1-5] approach and published marker ion libraries have been explored to facilitate species identification.

Figure 1: MALDI-8020 Benchtop linear mass spectrometer

Methods

The methodology developed by Kirby et al[2] was used for extraction and enzymatic digest. In brief, samples were taken by either removing small (<1mm) loose fibres or by gently rubbing 2.5mm diameter discs of 30 µm lapping paper over a small region (1mm^2) of the object. The sample was transferred to a low bind centrifuge tube and 60 µL 50 mM ammonium bicarbonate buffer solution added and heated at 60 °C for 45 minutes, followed by reduction and alkylation of cysteine residues. Digestion with 8 µL trypsin (0.02 µg/µL) was conducted overnight. Samples were stored frozen until required for spotting on MALDI plate. Prior to spotting, the samples were zip-tipped and eluted using the dual elution technique into 10% acetonitrile: 90% 0.1% TFA and 50% acetonitrile: 50% 0.1% TFA. The pH of the samples was adjusted to 2 using 10% TFA and the samples were spotted on a Fleximass-SR48 slide. 5mg/ml CHCA matrix was used for MALDI-TOF analysis. All peaks in the peak list were exported using monoisotopic peak picking.

1

Figure 3: Fishing line (AM.2409) Sample 1.

2

Figure 4: Sinew line (AM.VAN.143) Sample 2.

3

Figure 2: Whale harpoon (AM.NWC.60) Sample 3.

Results

At the beginning of the project, the spectra were manually screened for marker peaks (with a signal to noise above 10). This was extremely time consuming. In addition, the source peak lists were all in unit mass resolution. As the project progressed, we developed an in-house application to scan the data for matches with a species identification list where we could specify the signal to noise cutoff and the mass tolerance. The screening tool made the data scanning fast and removed the potential for interpretation bias although the data still required manual review to remove repeats of modified markers.

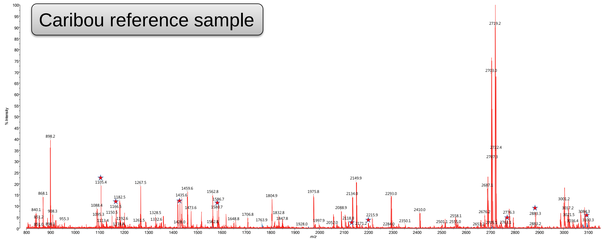

Figure 5: Spectrum obtained from caribou reference sample (relevant marker peaks labelled with a ★).

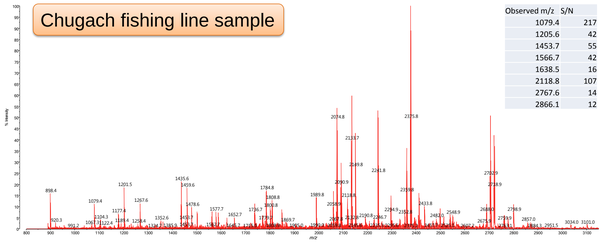

Figure 6: Mass Spectra from fishing line sample (AM.2409) with a table of all potential marker peaks found

Sample and location	Data	Matched peaks
1a Chugach fishing line (2019 04 30 b)	Dolphin:common bottlenose white-beaked euphrosyne	8
1b Chugach fishing line (2019 04 02)	Humpback whale	8
2a Sinew line (2019 01 11 a)	Humpback whale	7
2a Sinew line (2019 01 11 b)	Humpback whale	7
2a Sinew line (2019 04 29)	Humpback whale	7
2a Sinew line (2019 04 30)	Sperm whale	7
2b Sinew line (2019 01 17)	Dolphin:common bottlenose white-beaked euphrosyne	7
2b Sinew line (2019 01 17)	Orca/White-sided dolphin	7
2b Sinew line (2019 01 17)	Porpoise	7
2b Sinew line (2019 01 17)	Humpback whale	7
3a Whale harpoon (2019 04 02)	Right whale	7
3a Whale harpoon (2019 04 02 b)	Orca/White-sided dolphin	8
3a Whale harpoon (2019 04 02 b)	Minke whale	8
3a Whale harpoon (2019 04 02 b)	Fin whale	8
3a Whale harpoon (2019 04 02 b)	Gray whale	8
3a Whale harpoon (2019 04 02 b)	Right whale	8
3b Whale harpoon (2019 01 17)	Ringed seal	7
3c Whale harpoon (2019 04 30)	Dolphin:common bottlenose white-beaked euphrosyne	8

Table 1: Examples of tentative species identification - based on largest number of marker peaks observed in spectrum. Marker peaks reported as proline\hydroxyproline variants counted as a single hit.

Conclusions

Preliminary work has demonstrated the capability of the Shimadzu MALDI-8020 instrumentation for collagen peptide mass fingerprinting and has provided tentative identifications of animal species used in sinews found in museum objects. Further work is required to source materials of known taxonomic provenance to generate an in-house library, enabling complete spectra comparison, provision of validation data and a better understanding of intra-species variability.

Ideally a full set of species-specific marker peaks would be observed for each sample, this has not been achievable, but is not unexpected. Approaches for associating a confidence score to a taxonomic assignment and presenting the data such that close, alternative, matches can be presented alongside the most likely assignment are being investigated.

References

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