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Recovering bone and tooth proteins in arid environments using palaeoproteomics

A case study in the Late Stone Age site of Toteng, Botswana

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The advent of herding practices remains a key event in human societies in terms of social, cultural, economical changes and regarding their interactions with animals.
In Africa, no remain of wild ancestors species of sheep *Ovis aries* nor goat *Capra hircus* has never been reported. This suggests that those domestic species have been introduced from the epicentre of their domestication : the Near East[1].
The first occurences of those animals remains in the archaeological record are attested since the 7th millenium BCE in Egypt and Lybia[2].

But their appearance in the Southern part of the continent is only dated to the 2nd century BCE [3].
The modalities of their diffusion accross the continent in such large amount of time is still debated : cultural diffusion (percolation) or population migrations; Eastern or Western route?
Regarding the strong evidences of extant exchanges between Eastern and Southern Africa, we propose to document the hypothesis of the Eastern route using biomolecular approaches through a larger project (Fig. 1). Here, we will focus on methodological developments of palaeoproteomics in the context of African remains.



Fig. 1: Archaeological sites included to the PhD project

Because of the strong morphological similarities between the caprines species and with other small wild bovids, emphasized by the African burying conditions, the distinction of archaeological remains is not always possible.
We aimed to use a novel biomolecular method for species determination of archaeological material: palaeoproteomics (Fig. 2).
Based on the amino acid variations on preserved proteins, this method allow species identification of remains where DNA might not be conserved, due to increased diagenetic processes amplified by arid environmental conditions.

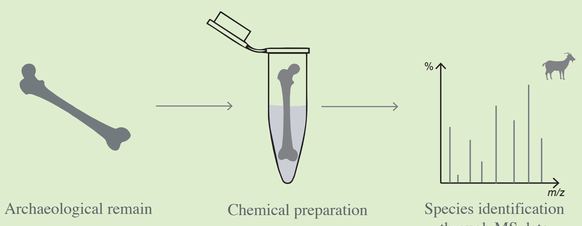
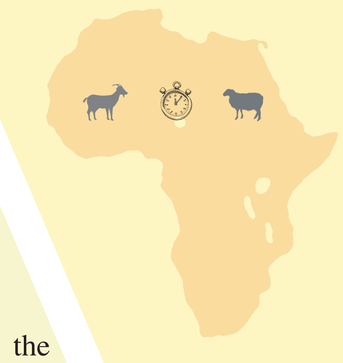


Fig. 2: General principle of palaeproteomics analyses on archaeological material



In Africa, the absence of wild ancestors' remains of domestic caprines indicates an exogenous origin of domestic sheep and goat. Archaeological evidences suggest that they were introduced in the Northern part of the continent by the 7th millenium BCE, but only arrived in the Southern part by the 2nd century BCE. Moreover, the morphological similarities existing between the two species among them, and with other small wild bovids like antelopes combined with the large amount of highly fragmented remains due to arid environnemental conditions have substantially limited the use of classical comparative anatomy analyses.

We report here the use of a promising method, palaeoproteomics, on degraded bone and tooth splinters from the Late Stone Age site of Toteng, Botswana. This study is among the first to focuse on African archaeological remains, strongly degraded by arid burying conditions and diagenesis. The purpose of our work is to both document the presence of domestic caprines at this site, but also to develop a new methodology adapted to archaeological remains from arid environments.

Abstract

Context of the PhD

Aim

Material & Methods

Results

Conclusion

A collagen content threshold of ~ 3 wt% under which it would be risky to perform palaeoproteomics analyses is proposed.

Tris-EDTA buffer is identified as the best demineralizing agent when dealing with remains from arid environment.

The presence of domestic sheep *Ovis aries* at the Late Stone Age site of Toteng, Botswana, is confirmed based on preserved proteins from one of the degraded splinters.

Material

18 unidentified bones and teeth splinters from the Late Stone Age site of Toteng, Botswana, were selected for this study. Toteng is a complex of open-air sites discovered in 1991 by A.C. Campbell where a rich sequence of typical Late Stone Age lithic industry, a large faunal spectrum and Bambata sherds[4,5,6] were excavated. New excavations in 2003 and zooarchaeological analyses unraveled the presence of domestic sheep (*Ovis aries*) directly dated to 2020 ± 40 BP (2σ, [7]). Remains included to this study were exported from the National Museum of Botswana under authorization and examined by one of the authors (JL). Most of them were too fragmented and altered to propose morphological identifications beyond family[8].

Methods

Estimation of remains organic phase preservation

In order to estimate the organic content of Toteng remains, 0.5 mg of powdered sample were applied on the diamond of a Attenuated Total Reflectance Fourier transform infra-red spectrometer. Anvil pressure was adjusted to normalise ν_3PO_4 band to an absorbance of 0.5 (Fig. 3).
The ratio between amide I and PO_4 bands area is then used to estimate nitrogen and collagen weight percent content (%wt) according to a method described by Lebon and collaborators[9].

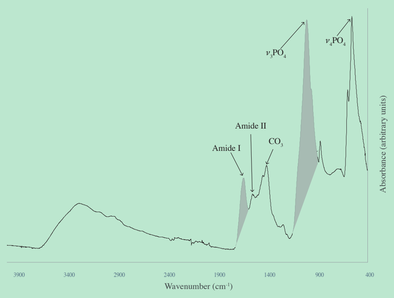


Fig. 3: ATR FT-IR spectrum of a modern bone reference (*B. taurus*). Areas used for organic content preservation estimation appear in grey.

Paleoproteomics

Because no chemical preparation protocol was never adapted to remains from arid environments, we aimed at identifying one that allow the best protein sequence coverage and permits species identifications (Fig. 4).
Once proteins were properly extracted and enzymatic hydrolysis succeeded, we performed high performance liquid chromatography coupled to tandem mass spectrometry analyses for characterization[9].

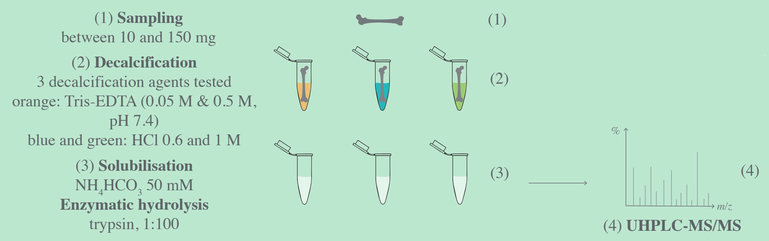


Fig. 4: Flow chart of palaeoproteomics protocol conducted in this study

Best suited protocol for remains from arid environments

All 18 Toteng remains presented contrasted organic phase preservation. Nine out of the 18 were selected for palaeoproteomics analyses regarding a range of preservation distributed between 1.91% and 5.38%[8] of collagen content.

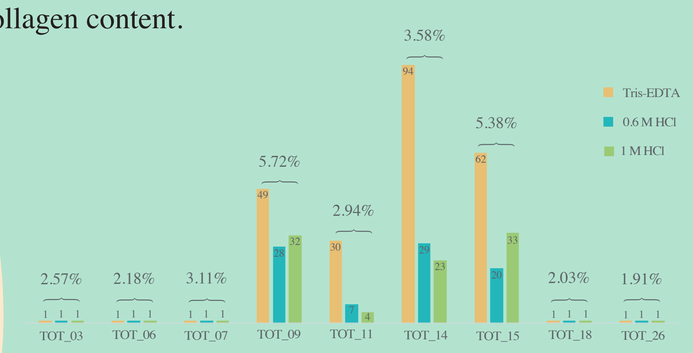
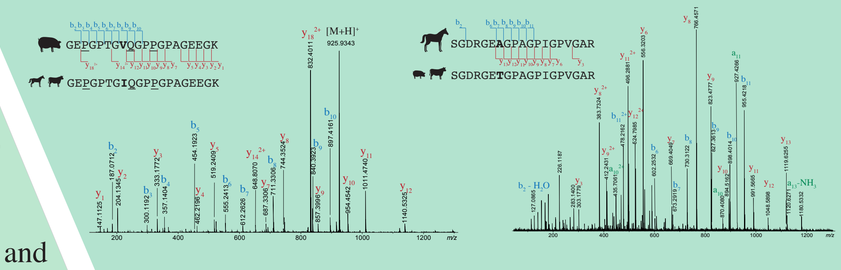


Fig. 5: Number of assigned peptides using available databases per sample and per decalcification protocol used. Preserved collagen content (coll. wt%) are presented above each sample.

Within the nine tested remains for palaeoproteomics analyses, four yielded preserved peptides that were assigned to proteins using available databases (Fig. 5).
Identified remaining proteins were α1 and α2 chains of collagen type I . Tris-EDTA buffer allowed the best protein sequence coverage.

Species identification based on protein characterisation



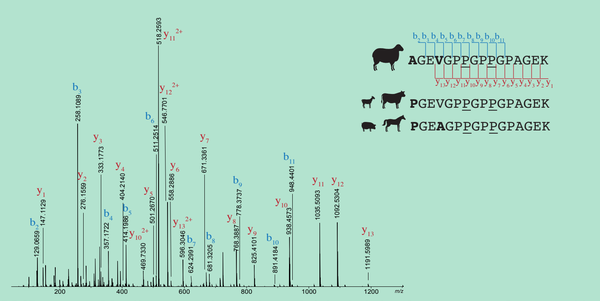


Fig. 6: MS/MS spectra of family and species markers of alpha 1 chain of collagen type I preserved in Toteng samples and identified in this study.

A1 chain of collagen type I allowed the refining of species identification. One remain presented the suidae marker (Fig. 6, top left), two other the equidae marker (top right) and one remain was attributed to the domestic caprine species, *Ovis aries* (bottom center)[8].
All the α1 chain of collagen type I markers presented here were never reported in the litterature and will allow species identification based on the least changing chain of this type of collagen.

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Acknowledgements

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