

UNVEILING THE MICROBIOME AND RESISTOME IN PAST HUMAN POPULATIONS - PRELIMINARY RESULTS

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Introduction

The combination of bioarchaeology and palaeomicrobiology data provides a deeper understanding of microbial communities and pathogen evolution.

Bioarchaeology, involves the study of human skeletal remains in archaeological contexts, with a focus on health, diet and disease in past populations.

Palaeomicrobiology, meanwhile, identifies pathogenic microorganisms and antimicrobial resistance (AMR) genes in ancient human remains, shedding light on the origins of AMR.

This study focuses on the skeletal remains of nuns from the Convent of Jesus in Setúbal, Portugal, from the 15th to the 19th centuries. Historical records document deaths due to respiratory infections (e.g. tuberculosis and pleurisy), fever and stroke.

Aims

The present study investigates the oral and systemic microbiome in a sample of skeletonized remains of nuns lived in Setúbal, Portugal. Furthermore, it explores the presence of antimicrobial resistance genes in ancient DNA. The objective of this study is to integrate osteological, dental and molecular data in order to reconstruct health and disease profiles within this historical religious community.

Material and methods

- Samples:
- 15 adult female subjects were observed, 10 of whom exhibited visible signs of dental calculus.
- Dental selection is defined as the process of removing excess tartar from teeth.
- The bone samples obtained for analysis included a femur and rib fragments.



Figure 1 – Tartar in a female adult of burial (2015.5)

- The process of DNA extraction is outlined as follows:
- The NZY Soil DNA Isolation Kit (Nzytech[®]) was adapted from Weyrich et al (2015).
- The environment is maintained under stringent conditions to ensure the absence of contamination.
- Molecular analyses:
- Polymerase chain reaction (PCR) was performed using universal primers for the 16S rRNA gene.

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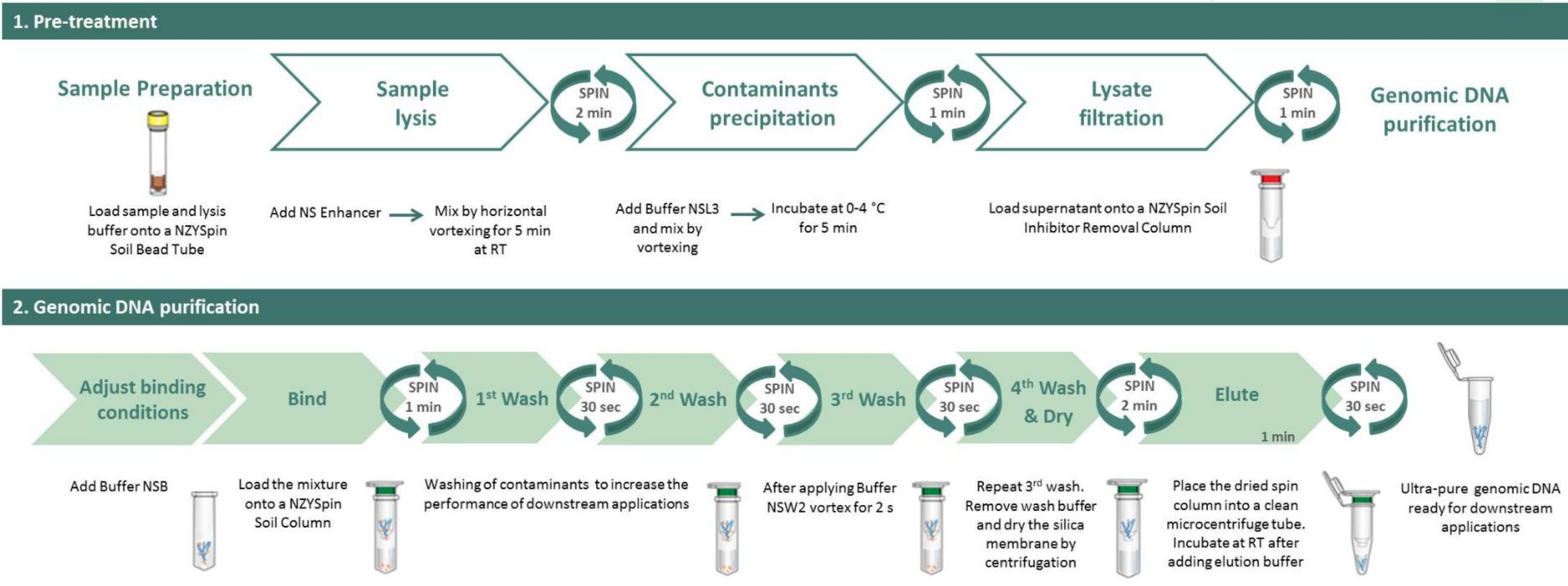


Figure 2 - Schematic diagram of the sample preparation according to the manufacturer.

Preliminary Results

The extraction of bacterial DNA from dental calculus and bone was successful.

The presence of viable cells was verified by PCR analysis, which is a widely accepted method for genetic analysis.

- Macroscopic dental analysis revealed:
- Presence of occlusal wear;
 - Presence of caries;
 - Antemortem tooth loss;
 - Signs of periodontitis.

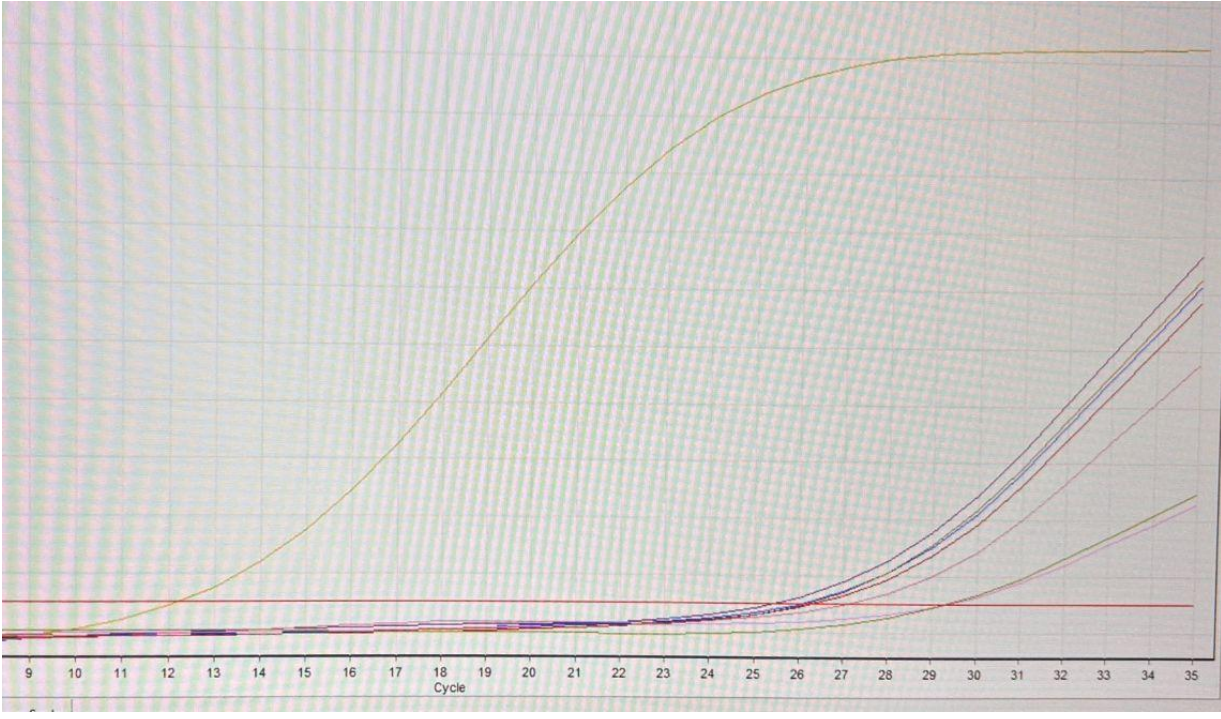


Figure 3- Results of the RT-PCR curve that validates the presence of bacterial DNA.

The presence of calculus is indicative of a high-carbohydrate diet and poor oral hygiene.

Discussion

Dental calculus has been demonstrated to function as a reservoir of ancient DNA and information pertaining to oral health. The preliminary results provide validation for the laboratory methods employed. Moreover, the successful extraction of DNA from bone facilitates metagenomic analyses of the ancient microbiome and resistome.

Conclusions

The findings of this study demonstrate the feasibility of working with ancient bacterial DNA retrieved from past human remains. Dental calculus provides valuable insight into the aetiology of oral diseases and dietary habits, while DNA extracted from bones contributes to understanding the evolution of antimicrobial resistance over time. This research underscores the significance of interdisciplinary collaboration in the reconstruction of health patterns in past populations.

Next Steps

- Implementation of complete metagenomic sequencing;
- Identification of the microorganisms that constituted the oral microbiome of this past religious community;
- Detection of the antimicrobial resistance genes in past human remains samples;

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