Detecting bacterial species from ancient human skeletal samples

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Diagnosis of tuberculosis (TB) via morphological analysis is difficult and often inconsistent. With next-generation sequencing (NGS), ancient host microbiomes can be subjected to metagenomic analyses for the detection of TB *in silico*. Suitable bioinformatic workflows are needed for reliable ancient DNA (aDNA) analysis of causative agents. This study aims to enhance available bioinformatic screening methods to create more suitable bioinformatic processes and generate insights in relation to TB.

This research utilizes publicly available NGS data accessed through the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI). Initial quality control steps included adapter trimming with Trim Galore!. Kraken2 was then used for taxonomic classification with a custom-built database comprised of Mycobacterial genomes from the NCBI. Quantitation and visualization were carried out with Bracken and Krona, respectively. Our workflow was first applied to 28 Neolithic skeletons (SRA number PRJNA422903) representing the Middle Neolithic Brześć Kujawski Group of the Lengyel culture (~4400–4000 BC, 26 individuals), and the Late Neolithic Globular Amphora culture (~3100–2900 BC, 2 individuals). Three additional datasets have since been utilized in this research: mummified remains of 265 individuals from Hungary (1731–1838 CE; PRJNA795622), one calcified lung nodule from Lund, Sweden (17th century; PRJNA517266); and dental calculus of four individuals from the Iberian Peninsula (4500-5000 BP; PRJEB46022).

Preliminary results for the 28 Neolithic skeletons revealed an average of 7% of the *Mycobacterium* genus sequencing reads mapping to *Mycobacterium tuberculosis* complex (MTBC) among all individuals. This work also revealed additional species of MTBC and *Mycobacterium avium* complex (MAC) that were previously unreported by the originator of datasets, including the extensively drug-resistant (XDR) Mycobacterium tuberculosis XDR1219 and *Mycobacterium avium hominissuis*. Our bioinformatic workflow has therefore been more effective than previously published approaches and is suitable for future paleopathological studies.