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Comparing the Microbiomes of Dental Plaques in 19th Century Ancestral Remains

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VCU Microbiomes of Dental Plaques in 19th Century Ancestral Remains

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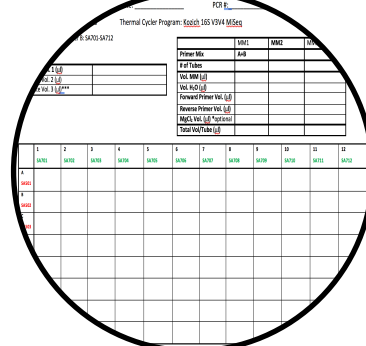
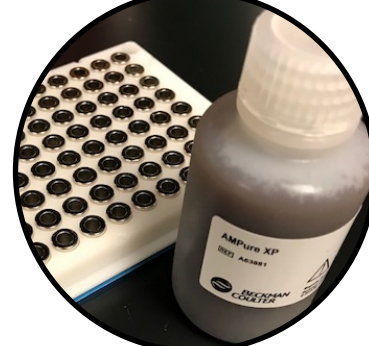


INTRODUCTION

During the construction of VCU's Hermes A. Kontos Medical Sciences Building in April 1994, nineteenth century ancestral remains were found in an abandoned well on the Medical College of Virginia (MCV) campus (Figure 1). This well, now known as the East Marshall Street Well (EMSW), is thought to have been used as a disposal location for cadaver dissection for surgical and other medical waste in the past. VCU is now seeking to use DNA sequencing technology to match and assemble bones from each individual for a proper burial, uncover the cultural and historical context in which these people lived, and bring a sense of closure to our community and to these ancestors. The development of oral diseases can result from disruptions to the ecological balance of the oral microbiome, especially by pathogenic bacteria, so studying the dental plaque microbiome and recognizing the different species present can provide insight into the health and disease status of these individuals. The composition of the ancient oral microbiome, which preserves dietary and environmental debris, might also provide us with insight into the dietary patterns and environmental conditions of these individuals. This research includes a preliminary study (n=8) on the calcified dental plaques (dental calculus) found on the teeth of the skulls using 16S rDNA high throughput sequencing to uncover information about microbes associated with dental calculus.



Figure 1. The excavation site at East Marshall Street (<https://emsw.vcu.edu/about/>).

METHODS

 <p>Sample Collection</p> <ul style="list-style-type: none"> Dental plaques scrapped from several skeletal remains (N=18) Stored at -20°C 	 <p>DNA Extraction</p> <ul style="list-style-type: none"> CTAB Organic extraction Sample weight/extraction: 7-14mg Agarose Gel Electrophoresis (0.8%) N=8 	 <p>DNA Quantification</p> <ul style="list-style-type: none"> Qubit® Fluorometer (ThermoFisher Scientific) Qubit dsDNA HS Assay Kit 	 <p>Library Preparation</p> <ul style="list-style-type: none"> 16S rDNA V4 dual-index amplification using Kozich et al. 2013. 	 <p>Post-PCR Clean Up & Quantification</p> <ul style="list-style-type: none"> Agencourt AMPure XP (Beckman Coulter) Qubit® Fluorometer (ThermoFisher Scientific) 	 <p>DNA Sequencing</p> <ul style="list-style-type: none"> Illumina's MiSeq FGx™ MiSeq Reagent Kit v.2 	 <p>Data Analysis</p> <ul style="list-style-type: none"> Sequence analysis: mothur MiSeq SOP
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RESULTS

Table 1. Total DNA recovered from dental plaque samples associated with the East Marshall Street Well (EMSW) skeletal remains.

Sr. #	Sample Name	DNA Concentration (ng/μL)	16S rDNA PCR Success	Sequencing Success
1	AU_M01C	0.144	No	No
2	AU_M02C	0.233	No	No
3	AU_M03C	0.042	Yes	Yes
4	AU_M09C	0.127	Yes	Yes
5	AU_M10C	0.0825	Yes	Yes
6	AU_V01C-N	0.359	No	No
7	AU_V01C-X	0.145	No	No
8	AU_V02C	0.348	Yes	No

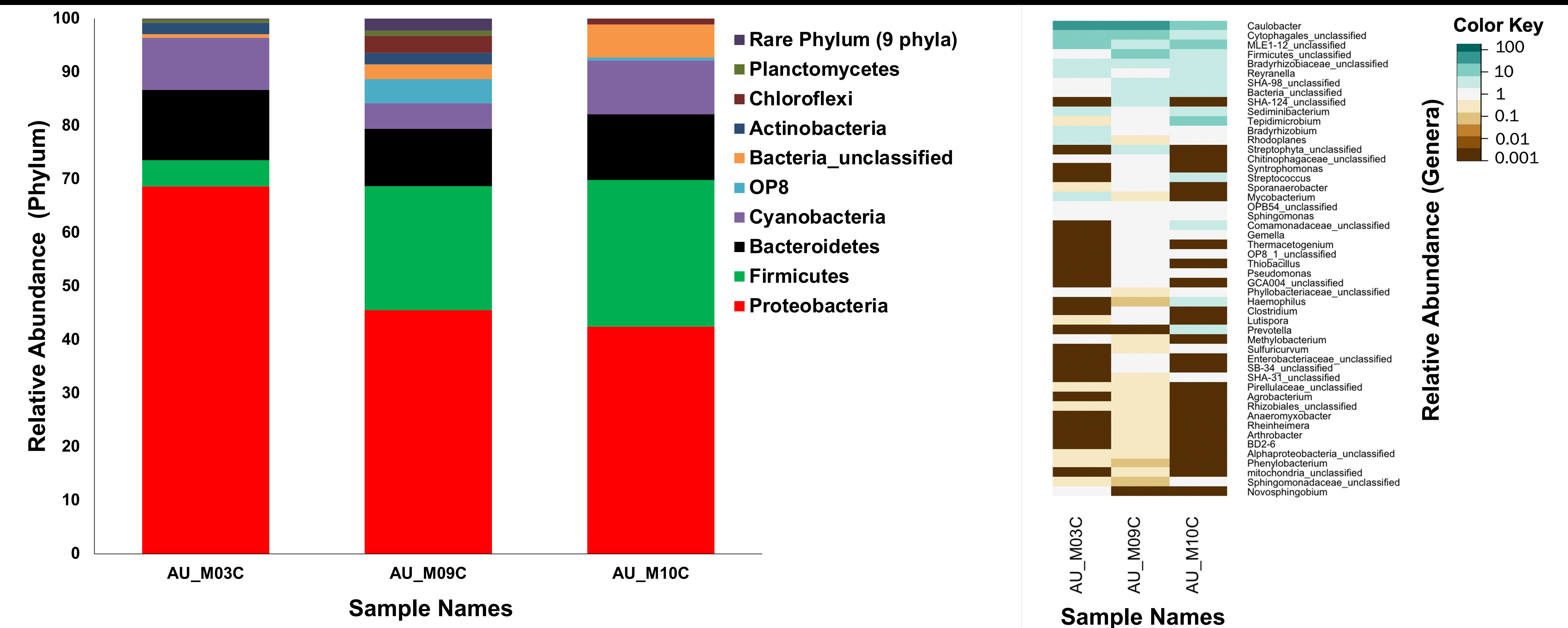


Figure 2. Average relative abundance of the top nine bacterial phyla associated with three dental plaque samples collected from East Marshall Street Well (EMSW) skeletal remains. "Rare Phylum" includes combined relative abundances of all classified phyla that did not rank in the top nine.

Figure 3. Heatmap of the top 50 bacterial genera associated with three dental plaque samples collected from East Marshall Street Well (EMSW) skeletal remains.

CONCLUSIONS

- All samples resulted in DNA yield, but 16S rDNA of only four samples were successfully amplified and sequenced. One sample had very low sequence reads and hence was removed from further analysis.
- Proteobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria were the top four bacterial phyla (in decreasing order) associated with the EMSW dental plaques, which is consistent with major phyla reported in expanded Human Oral Microbiome Database (eHOMD), and other ancient dental calculus microbiome studies.
- Caulobacter*, Unclassified Cytophagales, Unclassified MLE1-12, Unclassified Firmicutes, and Unclassified Bradyrhizobiaceae were the top five bacterial genera (in decreasing order) associated with the EMSW dental plaques. *Caulobacter* and Unclassified Bradyrhizobiaceae have potential application in bioremediation, which is possibly a result of the environment these remains were found in. Members of the order Cytophagales (including genus *Cytophaga*) have been associated with human infections and bacteremia. Order MLE1-12 is a non-photosynthetic cyanobacteria, whose abundance tends to increase in patients with selected neurodegenerative, gastrointestinal, hepatic, metabolic, and respiratory diseases.
- Dental plaque of individual AU_M03C had a high abundance of genus *Mycobacterium*. *M. tuberculosis* is a causative agent of TB and hence the cause of death of this person may be TB. On the other hand, individual AU_M10C had high abundance of genus *Streptococcus*, a common oral bacteria. One species of *Streptococcus*, *S. mutans* is the main pathogen of dental caries disease. Pathogenic *Pseudomonas* was also present in very low abundances in two (AU_M09C and AU_M10C) individuals.

ACKNOWLEDGEMENTS AND REFERENCES

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