Aug 9th, 10:15 AM - 12:00 PM

Investigating the origin of coprolites from three great basin caves

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Investigating the origin of coprolites from three great basin caves

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Introduction

The study of coprolites (mummified feces) is a relatively new endeavor, which enables investigations of the health and diet of ancient people and provides some of the oldest evidence to date for the human habitation in North America (2). In this project, 18 coprolites were examined from archeological digs at three Great Basin caves: the Bonneville Estates Rockshelter (UT), Hidden Cave (NV), and Top of the Terrace Rockshelter (UT). The main objectives were: 1) to verify human origin through the presence of mitochondrial DNA (mtDNA) and 2) assuming human origin, characterize intestinal microflora of Native Americans prior to European contact. Primer sets specific for human mtDNA were employed to obtain products and establish human origin in general and Native American origin specifically (through SNP analysis). Initial microbiological efforts targeted the bacterial genus, *Bacteroides*, which tend to dominate gut flora in modern humans and thus is considered an ideal indicator for human fecal contamination (1,6). Primers targeting human-associated *Bacteroides* spp. strains were used in conjunction with human mtDNA results to further verify human origin. A major obstacle in this project, as might be expected, was damage to ancient DNA (aDNA). aDNA from coprolite samples is usually degraded into short fragments due to hydrolytic or oxidative damage, greatly reducing the possibility of long polymerase chain reaction (PCR) amplifications (4). The suggestion is that if large fragments are obtained from PCR, that the sample is most likely contaminated (3). To repair the fragmented aDNA, a technique termed reconstructive polymerization (RP) developed by Golenberg et al. (3) was applied. If these samples are found to be of human origin, it could provide an interesting lens into not only humans, but also the colonization of Western North America and beyond.

Archeological dig at Bonneville Estates Rockshelter

Archeological dig at Hidden Cave

Materials and Methods

Dr. Dave Rhode (DRI) provided 18 samples, 13 suspected to be of human origin and 5 known to be wood rat coprolites. Through carbon dating performed by Dr. Rhode, the samples are predicted to be between 1,500-6,000 years old. DNA from coprolite samples, modern human and non-human (horse) fecal matter and human mtDNA were extracted using MoBio Ultraclean Soil DNA isolation kit. The presence of bacterial 16S RNA was found using universal primers: 27F YM+3, 926R and 1496R. Prior to PCR, 1:40 dilutions of some of the templates were needed to negate the activity of inhibitors which co-purified with the DNA. Damaged aDNA was repaired using the method of reconstructive polymerization adapted from Golenberg et al. (3). *Bacteroides-Prevotella* primers Bac32F/Bac708R (Field 2000) amplified specific bacterial DNA. Primers L00654 and H00686 (Gilbert et al. 2008) were used to amplify human mtDNA.

Results

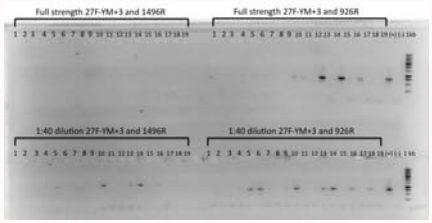

Figure 1. PCR amplification using universal 16S RNA bacterial primers 27F-YM+3 and 926R/1496R. Dilutions performed in second row to determine presence of inhibitors. Refer to figure 5 for specific sample information.

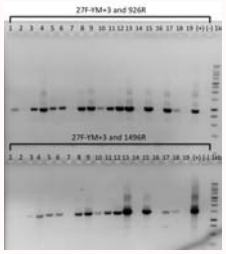

Figure 2. PCR amplification using universal 16S RNA bacterial gene primers 27F-YM+3 and 926R/1496R after reconstructive polymerization. Refer to figure 5 for specific sample information.

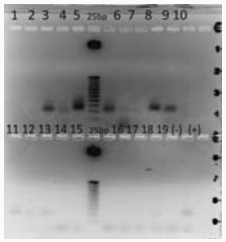

Figure 3. PCR amplification using human mtDNA primers L00654 and H00686. Refer to figure 5 for specific sample information.

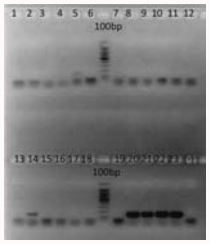

Figure 4. PCR amplification using *Bacteroides* spp. primers specific to *Bacteroides* human fecal matter. Refer to figure 5 for specific sample information.

#	Sample	12	HC 449
1	BER N7W23 Acc 24433 FS 14C0	13	HC 914
2	BER N7W23 Acc 24433 FS 14C1	14	TOT 2.8 Rat Dung not processed for DNA
3	BER N6W16 Acc 19665 FA 389	15	TOT 4 Rat Dung not processed for DNA
4	BER Acc 4482 FS 98	16	TOT 4 Rat Dung not processed for DNA
5	BER Acc 5725 FS 26	17	TOT 5 Rat Dung not processed for DNA
6	BER Acc 3425 FS 20	18	TOT 5 Rat Dung not processed for DNA
7	BER Acc 6698 FS 15	19	Blank
8	BER Acc 9588 FS 104	20	Human fecal sample
9	HC 65	21	Human Fecal sample
10	HC 342	22	Horse fecal sample
11	HC 363	23	Bacteroides DNA

Figure 5. Description of coprolite samples. BER represents samples collected from Bonneville Estates Rockshelter, HC represents samples collected from Hidden Cave and TOT represents samples collected from Top of the Terrace.

Conclusions

Original amplification using 16S universal bacterial primers showed 6 positive results at full strength and 9 positive results at a dilution of 1:40 (figure 1). This is probably due to the dilution of inhibitors that are common with aDNA (5). Coprolite DNA amplification with 16S rRNA gene universal bacterial primers after reconstructive polymerization yielded 15 positive amplifications (figure 2). Repairing the aDNA allowed for a more effective amplification for future reactions and reconstructive polymerization product was successfully used as a template for said reactions. The amplification of mtDNA as seen is promising but does not guarantee human origin (figure 3). It will be necessary to properly sequence these products to verify Native American origin and to show lack of contamination with scientists who have handled the coprolites. Samples 5 and 14 were positive for mtDNA and *Bacteroides* spp., making these samples more likely to be of human origin (figure 4).

Future Directions

- Clone mtDNA fragments into a vector to be sequenced and compared to humans of Native American origin, compare to scientist's mtDNA who have come in contact with samples
- Create 16S rRNA gene libraries of bacteria in samples
- Cultivate spore forming bacteria from samples

Acknowledgments

This work was funded by NSF Research Experience for Undergraduates (REU) program A Broad View of Environmental Microbiology at the University of Nevada, Las Vegas (DBI 1005223) and by the General Frederick Lander Endowment. A special thanks my mentor, Duane Moser, and fellow lab members.

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