

Q&A ATCC® *Excellence in Research* Webinar “A Tale of 3 Mummies: A Microbiome Analysis of Life in the Peruvian Andes 1,000 Years Ago”

1. Will we be able to download the presentation?

This presentation will be available to watch on demand on the ATCC website, or [click here](#).

2. How did you ascertain that the sequences you are analyzing originated from the mummy and not the environment?

We used multiple approaches starting with aseptic surgical procedures in a surgical suite to take the biopsy samples. We also used a variety of bioinformatics tools, including mapDamage, Source Tracker, and Operational Taxonomic Unit (OTU) subtraction from control DNA extractions.

3. How did you determine the diet of the Pre-Columbian European mummies? Is it based on the number of hits to a particular database?

We interrogated the NCBI databases with individual reads using the blastx algorithm. After the results were obtained, they were sorted based on e value, % identity, sequence length, and best hit. The number of hits as well as the relative abundance values were used to identify the specific plant and animal genes reported.

4. Did you do the binning analyses with the reads or the assembled reads (contigs)?

We did the binning analysis using the individual reads.

5. In the network analysis of the OTUs, why did you think there are three different clusters for the Pre-Columbian mummies and just one big cluster for the Italian mummies? Does it have to do with diet, culture, and also possibly disease?

This is likely as the Italian nobility mummies had a more homogeneous, carnivorous diet as well as similar cultural habits. In contrast, the Peruvian mummies represented three different groups; one from the pre-Inca period (likely prior to the broad-based use of quinoa as a protein source), and the other two representing a male Inca who probably traveled more extensively than the female Inca, with more restricted travel, which led to different dietary compositions of their corresponding diet.

6. Why did you think the alpha diversity of the Pre-Columbian mummies was lower compared to the Italian mummies?

It is likely that the relatively restricted vegetarian diet of the Pre-Columbian mummies “selected” for a less diverse, more specialized, carbohydrate-digesting microbiota.

7. Does the affordability of a meat-based diet for Italian nobility versus the Inca population have any bearing on the differences you see?

Probably not. Rather, it is likely that cultural habits and agricultural practices played a role in the differences we saw.

8. Would you please explain how the control bacteria (10 to 16 of them as shown in one of your slides) were used to ensure the DNA purification and extraction efficiencies? Were they spiked into the experimental sample? Also, why were these strains chosen?

The mock community consisted of 11 species of bacteria representing the spectrum of cell wall type, G+C ratios, oxygen requirements, and genome size. They were mixed in “approximately” equal densities of 1×10^6 CFUs and ran as an additional sample, in parallel, during DNA extraction, library construction, NGS, and QIIME analysis. They were not spiked as they would have obfuscated the results.

9. Do you have any plans to study mummies of other origins, ages, and cultures? And, are there likely artifacts associated with specific mummification processes?

Yes, we do. We are currently waiting for results from two sets of natural mummies, one set from Libya and another from an abbey in Northern Italy. We are also looking at pre-Colombian samples from the Caribbean.

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