Use of a Novel Non-toxic Sample Collection Technology for Accurate Microbial Community Profiling through Long-Term Stabilization of Microbiome Samples at Ambient Temperature

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Summary

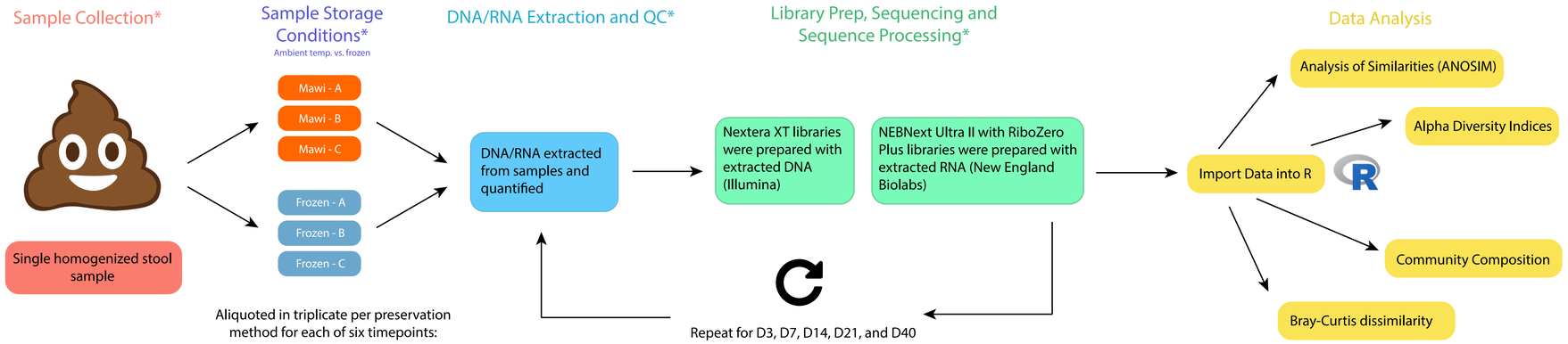
Introduction:

Despite recent and ongoing technological advances that have reduced sequencing costs and enabled high-throughput studies for microbial ecology and systematics, studies are still affected by biases introduced in the process. For instance, frozen samples, the "gold" standard in microbial sample preservation, remain susceptible to microbial community alteration in cold chain transport. In order to address these issues Mawi DNA Technologies has developed iSWAB-Microbiome (MB), a non-toxic stabilizing technology that allows ambient collection and transport of various microbial samples for several weeks, while maintaining the integrity of the sample at the time of collection.

Objective:

To compare the preservation and stabilization of sample microbial communities from samples between iSWAB-Microbiome and frozen samples.

Methods



Results

iSWAB-MB Outperforms the Frozen Sample in Maintaining Sample Alpha Diversity

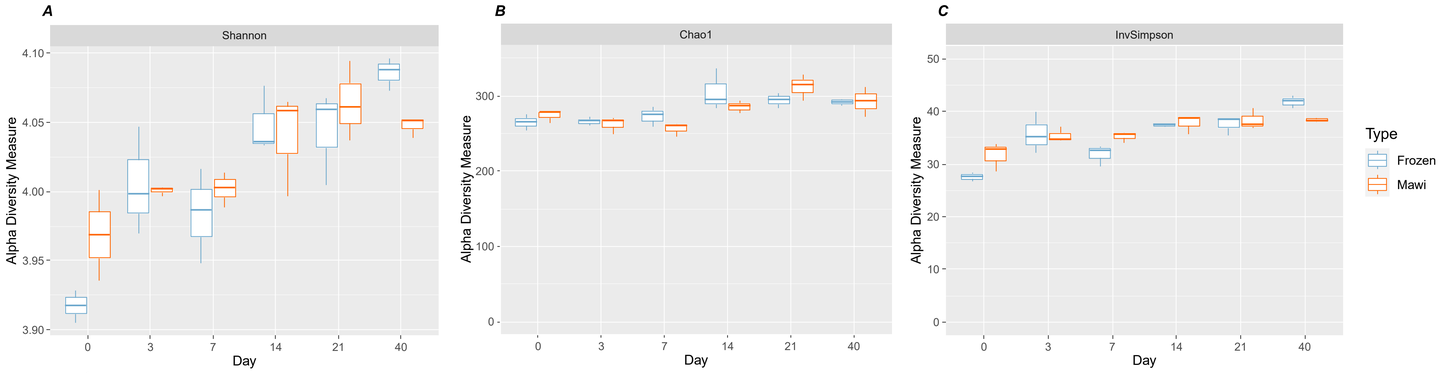


Figure 1:
The Shannon (A), Chao1 (B), and Inverse Simpson (C) indices provided an estimate of overall diversity, richness, and evenness, respectively, within the samples' microbial communities. In general, at $p < 0.05$, richness did not differ significantly between D0 and D40 for samples collected in iSWAB-MB. Evenness and diversity differ (ANOVA $p < 0.05$). However, all indices differed significantly between D0 and D40 for samples that were frozen (Shannon: ANOVA $p < 0.001$, Richness: ANOVA $p < 0.05$, InvSimpson: ANOVA $p < 0.001$).

Pairwise Comparisons to Initial Time Point and ANOSIM Show Improved Sample Stability for iSWAB-MB Over Frozen Sample

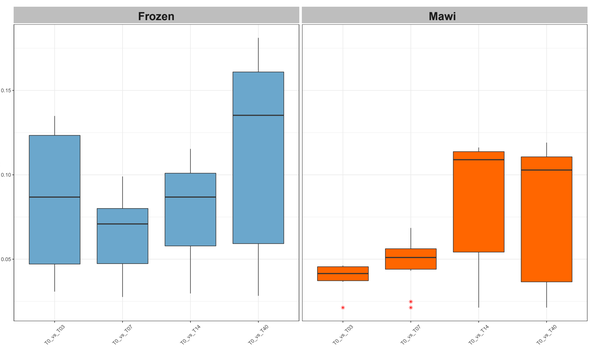


Figure 2:
Pairwise comparisons of the microbial communities between each post-collection time point against the time of collection (T0) based on the calculated Bray-Curtis distance index. Time point 21 was omitted due to great replicate variation that were skewing the dissimilarities for both collection methods. iSWAB-MB shows overall better sample stability, especially over the course of the initial seven days post-collection.

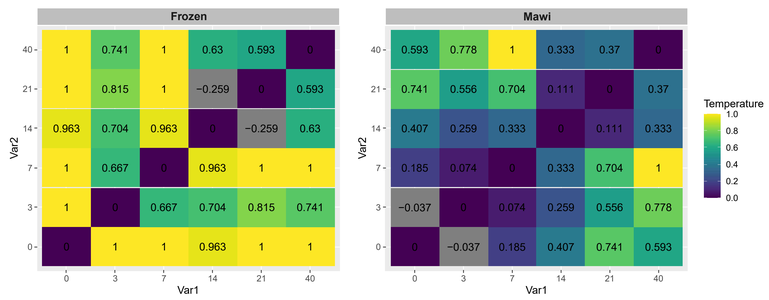


Figure 3:
ANOSIM (Analysis of Similarities) was performed between each replicate sample and time point separately for frozen and iSWAB-MB-preserved samples to test for significant differences in community composition. The value of the ANOSIM R statistic ranges from 0 (no separation) to 1 (complete separation) of the groups tested. All frozen samples analyzed post collection were completely separated from time of collection T0, suggesting loss of diversity immediately after freezing the stool sample. In contrast, samples suspended in Mawi buffer and analyzed post collection showed high similarity to time of collection T0 at least until T7, indicating efficient preservation of diversity for at least seven days.

Comparable Stability in Microbial Community Composition to Frozen Samples

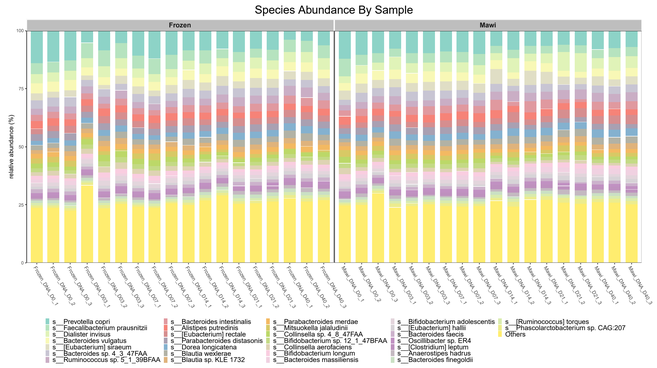


Figure 4:
Compositional differences of bacteria recovered from each replicate per time point for samples preserved in iSWAB-MB or frozen immediately after collection. The top 30 most abundant species across all samples are shown, comprising approx. 75% of total sequences retrieved. The different colors indicate each of these species. Overall species abundance remained similar for both solutions across all time points.

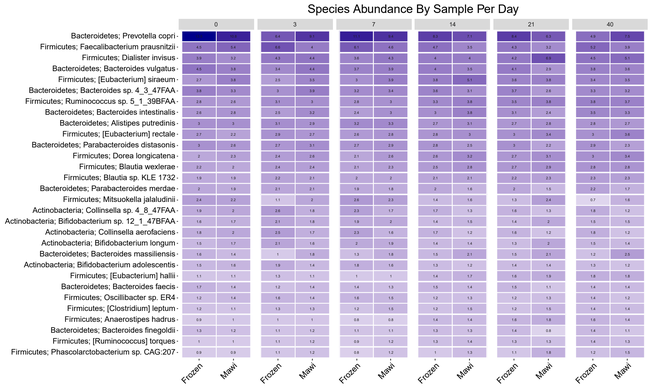


Figure 5:
Heatmap of the compositional differences of bacteria collected in iSWAB-MB side-by-side with frozen samples. The 30 most abundant species present in the dataset are shown. Color scale reflects abundance with white corresponding to lower and dark purple to higher abundances (color scale 1-10%).

Conclusions

- iSWAB-Microbiome stabilizes sample microbial community better over 40 days than frozen samples under multiple quantitative metrics.
- iSWAB-Microbiome at ambient room temperature performs to the same standard as frozen samples, eliminating the need for cold-chain transport and storage.