Rapid and efficient microbial community viability assessment using marker gene targeting

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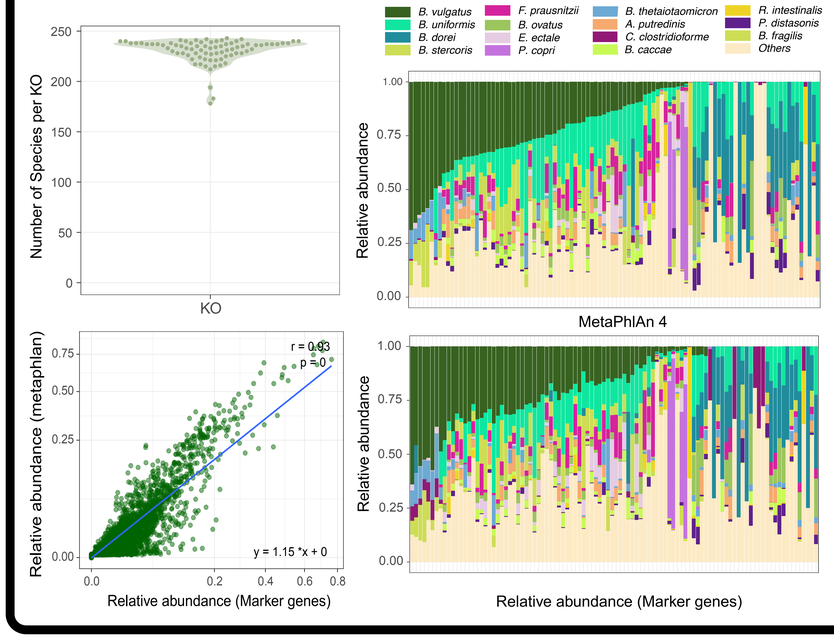
Distinguishing viable from non-viable microbes remains a major challenge in microbiome research, limiting our ability to interpret community structure, function, and transmission. Since only living microbes actively contribute to processes like metabolism and host interaction, assessing viability is critical. Existing methods, including chemical assays and 16S rRNA transcript detection, often fall short in complex communities, while shotgun metagenomics and metatranscriptomics are costly and computationally intensive. To address this, we are developing a high-throughput, cost-effective approach based on sequencing transcripts from optimized marker genes.

Preliminary results show that rationally selected marker genes accurately identified viable microbes and capture taxonomic profiles in human and environmental samples. We have built a marker gene database, designed primers, and optimized amplification protocols using paired metagenomic and metatranscriptomic data. Current work focuses on refining marker selection, improving amplification efficiency, and validating the method in synthetic and complex communities with known viability controls. This marker gene-based amplicon sequencing approach provides a scalable, accessible tool for profiling active microbial members, with broad applications in microbiome diagnostics, therapeutic development, and environmental monitoring.

The microbial marker genes are reliable for microbial community profiling

We first evaluated the feasibility of using a set of core marker genes for assessing microbial community composition in human stool samples.

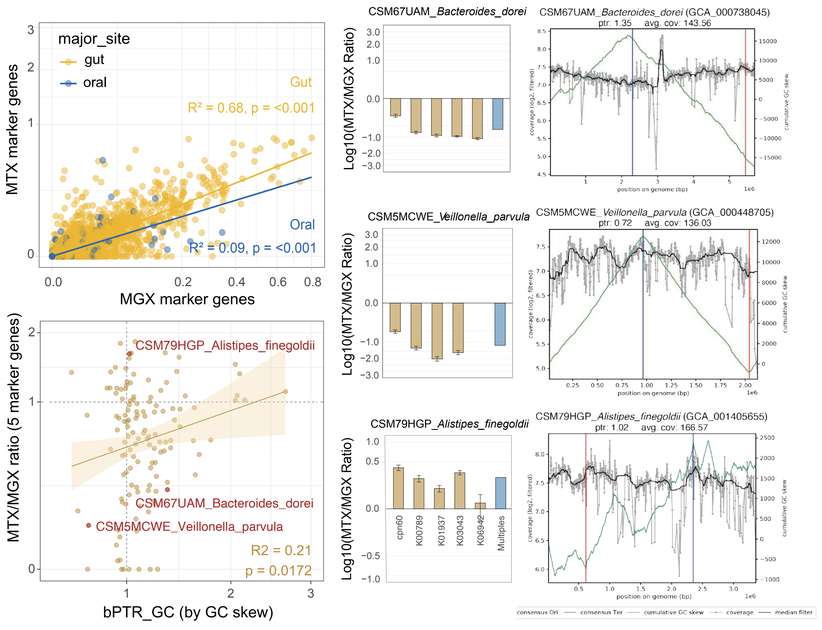
- The marker genes were universally present in 245 bacterial species identified in HMP2 stool samples, supporting their applicability across diverse human-associated microbes
- Taxonomy composiiton profiled using marker genes closely matched those generated using MetaPhlAn4.



The microbial marker genes are feasible for community viability assessment

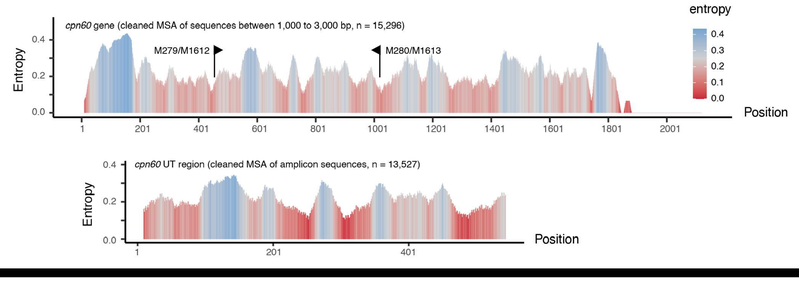
We evaluated the feasibility of using marker genes for viability assessment in human stool samples.

- The marker gene method successfully differentiated activity levels of gut- and oral-associated microbes in stool samples.
- Transcript-based activity estimates showed a positive correlation with PTR values calculated using the iRep tool.
- When the two methods produced discordant results, marker gene transcript-based method provided complementary information.



Developing a marker gene-based amplicon-sequencing protocol

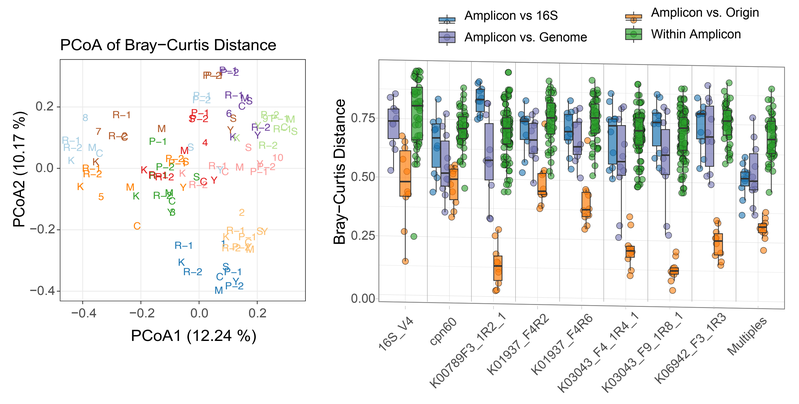
To develop a cost-effective and accessible method for microbial community viability assessment, we constructed nucleotide databases for each gene using UniRef90 and ChocoPhlAn database, and performed multiple sequence alignments to identify conserved and variable regions for primer design. Seven amplicons derived from five core marker genes (*cpn60* chaperonin protein, *pyrG*, *rpoB*, *ychF*, *metK*).



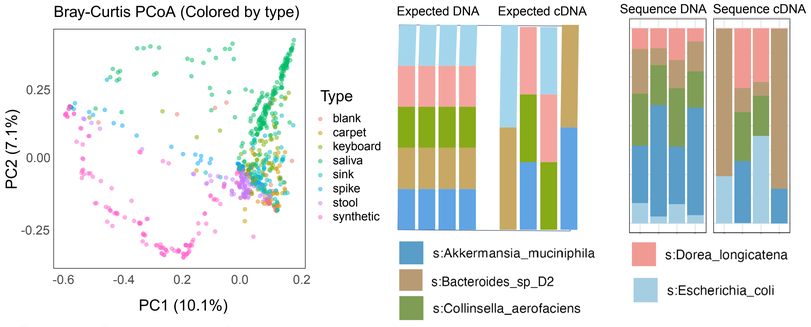
Marker gene-based amplicon-sequencing is able to differentiate active microbes

We evaluated the marker gene-based amplicon-sequencing protocol *in silico* in over 1,000 known bacterial genomes across various phyla. Taxonomic profiles derived from individual marker gene amplicons closely matched original genome compositions.

Combined marker gene amplicons produced profiles that more closely resembled the original genomes and 16S profiles.



We further tested this approach in synthetic microbial communities as well as realistic human-associated and built environment samples. In synthetic communities, the marker gene-based method accurately profiled community composition in DNA samples and successfully identified active microbial members in RNA-derived cDNA samples, supporting its potential for viability-aware microbiome profiling.



Ongoing works

Ongoing and future work involves evaluating the marker gene–based viability assessment in both synthetic and complex real-world communities, with applications in human health (e.g., IBD and colorectal cancer) and diverse environments—including built and natural settings—to better understand microbiome functions defined by the viable fraction of the community.

Acknowledgments

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