Beyond bacteria: Characterization and analysis of the mycobiome and virome in human gut samples

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Abstract

The human gut is inhabited by a tremendous variety of microorganisms that collectively form the gut microbiome. This diverse community is dominated by hundreds of bacterial species, while fungal, archaeal and viral species are also present but in lower relative abundance. The commensal bacterial species found in the human gut have been extensively characterized by 16S and whole genome sequencing (WGS), and bacterial dysbiosis has been linked to a number of human diseases such as IBD, IBS, colorectal cancer and obesity. In contrast, the mycobiome and virome remain largely uncharacterized, primarily because of their much lower abundance but also due to a lack of fully optimized protocols and curated reference databases. In the present study, we tested methodologies to characterize and analyze the mycobiome and virome of human gut samples collected in OMNIgene®•GUT kits (OM-200, DNA Genotek). Preliminary analysis suggests that OMNIgene•GUT gut sample collection devices are able to capture and stabilize the human gut mycobiome and virome as efficiently as the bacteriome. Using internal transcribed spacer (ITS) sequencing for mycobiome taxonomic analysis, we demonstrate that primer selection is paramount to increase identification of fungal species by decreasing biased and non-specific PCR amplification. In virome methodology testing, we were able to enrich endogenous and exogenous bacteriophages by 5 to 10-fold in DNA extracted from OMNIgene•GUT samples. Viral enrichment was achieved using a purification protocol designed to select viral DNA over bacterial DNA. Taken together, our preliminary data indicate that OMNIgene•GUT collected samples could be suitable for mycobiome and virome studies.

OMNIgene•GUT: a validated method for gut microbiome collection and stabilization

- OMNIgene•GUT design enables collection of consistent amounts of fecal material from a wide range of donors: 500 mg ± 160 mg (mean ± SD).
- DNA extraction from a 250 µL OMNIgene•GUT aliquot (~50 mg feces) using established procedures, yields an average of 3.09 ± 1.01 µg to 8.99 ± 4.92 µg (mean ± SD) high molecular weight DNA. This represents enough DNA for multiple downstream sequencing applications.

	16S rRNA sequencing	Metagenomic whole genome sequencing	PCR-free sequencing
Input requirement for application	~5 ng	~ 100 ng	~1-2 µg
Number of assays/ OMNIgene•GUT extraction aliquot†	600-1780	30-90	3-9
Number of assays/ OMNIgene•GUT sample†	>4000	>240	>24

***Table 1**: Number of sequencing reactions that can be performed per OMNIgene•GUT sample.*
† Based on mean DNA yields/extracted aliquot and input requirements from library preps.

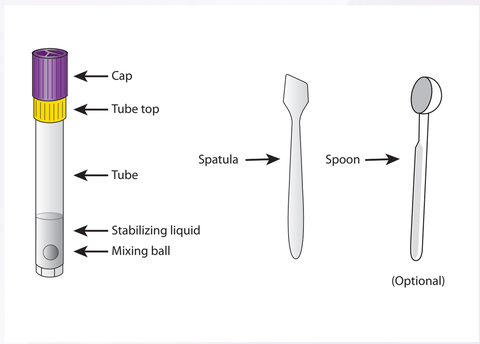


***Figure 1**: Illustration of the OMNIgene•GUT device, an optimized kit for self-collection and stabilization of gut microbiome sample from any Bristol scale stool type*

- OMNIgene•GUT captures an accurate snapshot of the bacterial profile at the time of collection and stabilizes samples during shipping and up to 60 days at 23°C.

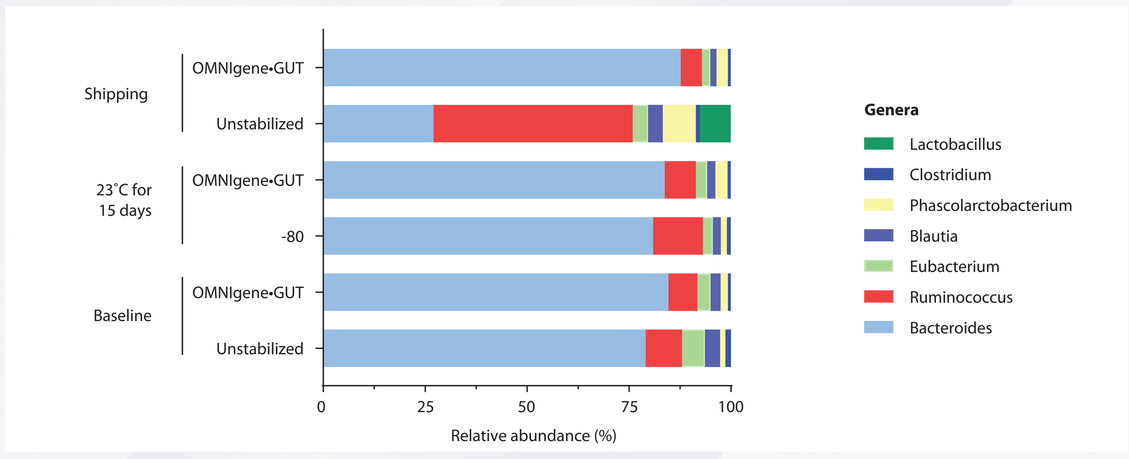


***Figure 2**: Relative abundance of bacterial genera in an unstabilized and OMNIgene•GUT stabilized gut sample subjected to storage at room temperature or -80°C for 15 days and subjected to simulated shipping. Representative donor from a cohort of 13 healthy adults.*

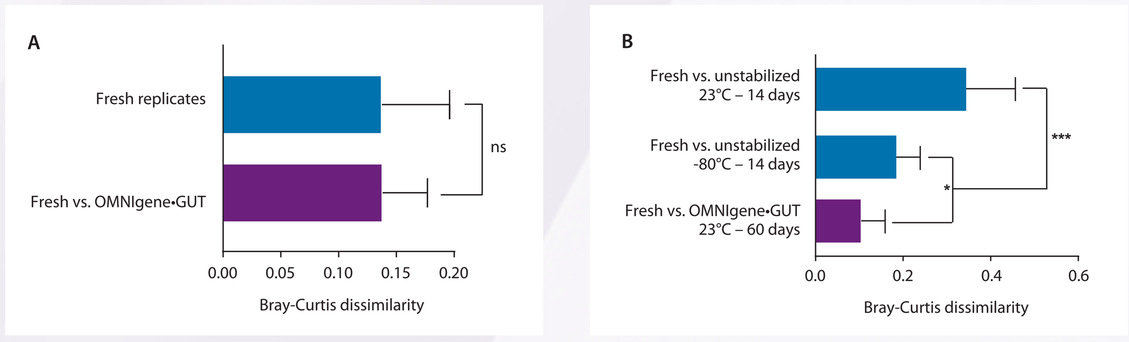
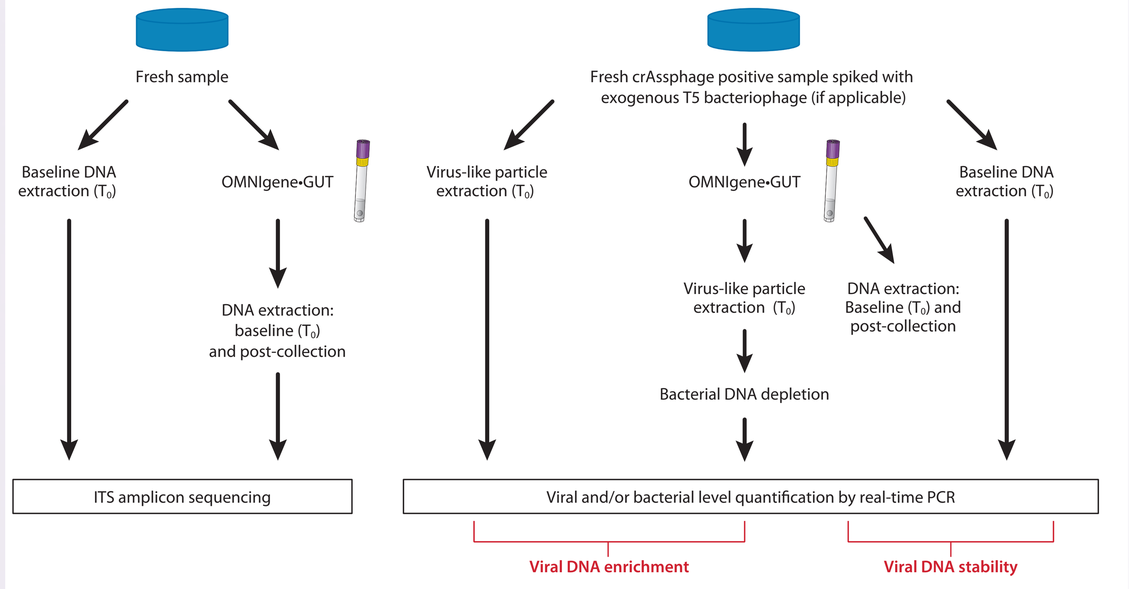


***Figure 3**: **(A)** Bray-Curtis dissimilarity between Fresh sample extraction replicates and Fresh vs OMNIgene•GUT collected samples shows no significant difference. **(B)** Bray-Curtis dissimilarity comparison between fresh sample vs unstabilized samples kept 14 days at -80°C or 23°C and between fresh samples vs OMNIgene•GUT stabilized samples kept at 23°C for 60 days showing that lack of stabilization increases dissimilarity over time. *p≤0.05, ***p<0.001 Mann-Whitney U test.*

Methodology for non-bacterial species analysis



Preliminary analysis of the gut mycobiome composition and stability in OMNIgene•GUT by ITS sequencing

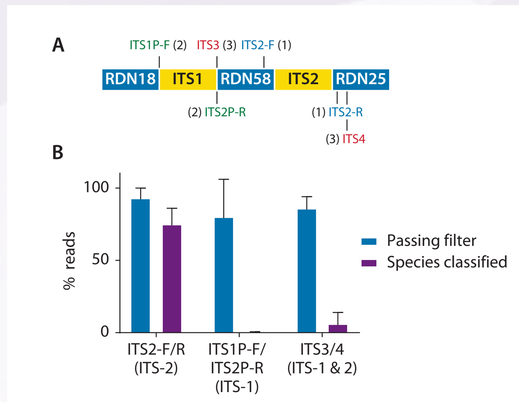


***Figure 4**: **(A)** Schematic map of the fungal rDNA region with the 28S, 18S, 5.8S ribosomal RNA and ITS-1/ITS-2. The various primer binding sites are indicated. **(B)** Comparison of MiSeq read quality and species identification for the 3 primer sets tested: ITS2-F/R (280 bp), ITS1P-F/ ITS2P-R (480 bp) and ITS3/4 (420 bp). Recommended amplification strategies, master mix and cycling conditions, were followed for each primer set. ITS-2 primer set was chosen for further studies based on species classification performance.*

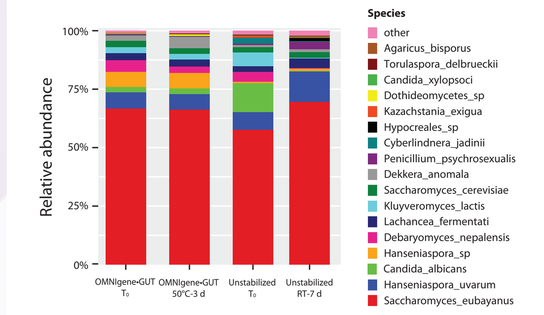
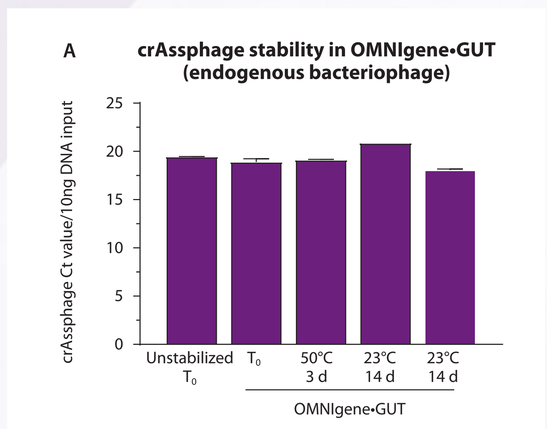


***Figure 5**: Comparison of the mean relative abundance of the fungal species detected by ITS-2 sequencing in unstabilized and OMNIgene•GUT collected samples at various time points post-collection. The OMNIgene•GUT device was incubated at 50°C for 3 days (simulating extreme transport) while the unstabilized sample was kept at 23°C (ambient) for 7 days.*

Characterisation of the gut virome in OMNIgene•GUT samples: capture, stability and strategies for enrichment



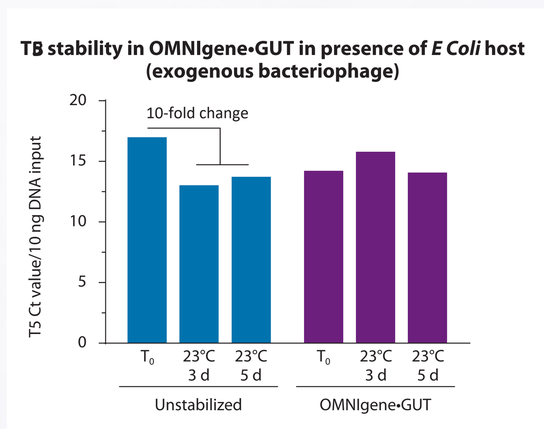


***Figure 6**: (A) Endogenous viral DNA crAssphage bacteriophage stability over time in OMNIgene•GUT devices incubated at various temperatures following collection. Ct values were measured by real-time PCR using crAssphage specific primers and 10ng total DNA as input. (B) T5 bacteriophage stability over time at room temperature following spiking in PBS (unstabilized) or OMNIgene•GUT chemistry with its bacterial host E. Coli. Ct values were measured by real-time PCR using T5-specific primers and 1ng total DNA as input.*

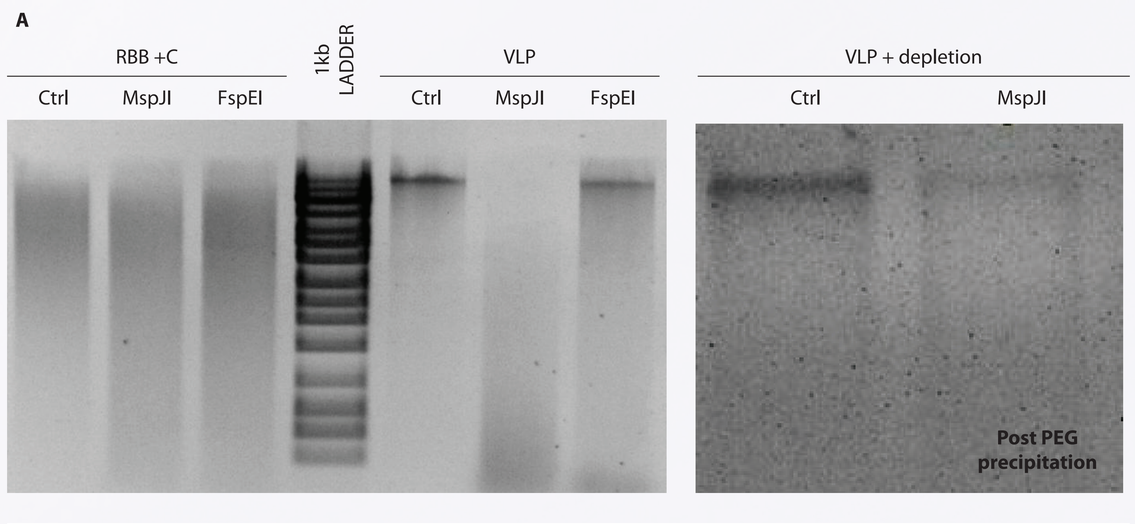


***Figure 7**: **(A)** DNA gel following total DNA extraction (RBB+C) or Virus-like particle enrichment (VLP) and bacterial DNA depletion using methylation-dependent restriction endonucleases (MspJI or FspEI) and polyethylene glycol precipitation (for size selection). **(B)** Real-time PCR quantification of bacteriophages and bacterial levels following RBB+C or VLP extraction with or without bacterial depletion. Ct values were measured using primers specific for crAssphage (endogenous), T5 (exogenous), bacterial 16S (universal), Faecalibacterium prausnitzii and Bifidobacterium longum.*

Conclusions

- OMNIgene•GUT devices are not restricted to bacteriome analysis but are also suitable for the study of the less abundant human gut mycobiome and virome.
- Preliminary data demonstrate that OMNIgene•GUT can accurately capture and stabilize both fungal and viral species.
- Analysis of fungal species in OMNIgene•GUT samples by ITS sequencing revealed that primer selection is paramount for accurate taxonomic identification, with ITS-2 giving optimal species identification.
- A 10-fold enrichment of viral DNA from OMNIgene•GUT collected samples is possible using an adapted VLP protocol. However, presence of bacterial DNA from cells that were lysed *in vivo*, as well as, any lytic potential of chemistry may hinder further enrichment of viruses.

References:

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