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Acknowledgments/funding. Projects PID2023-146221OB-I00 and PID2019-104546RB-100 funded by the Spanish MCIN/ AEI. Gutiérrez-Díaz I. has a grant FJC2021-047052-I financed by MCIN/AEI/. Castro AM has a predoctoral contract by ISPA institution (ITM-PRE-DOC-23-11) with support from Fundació Banc Sabadell.

WG3 POSTER PRESENTATIONS

Using a miniature gut model (MiGut) to define microbiota ecologies using different fecal slurry concentrations

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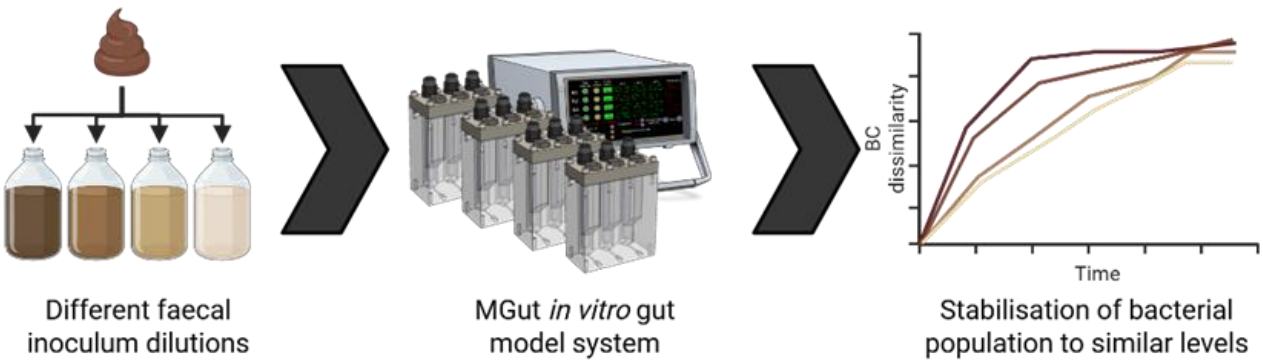

Research group presentation. MiGut was developed by a multidisciplinary team of engineers, microbiologists and food experts of the University of Leeds. WDB is a research fellow in engineering that developed MiGut during his PhD and leads a commercial unit at Leeds looking into technology applications. AB has expertise in nutrition impact on the human gut microbiome and bioreactor experiments. PC brings mechatronics and sensing expertise to MiGut. MW is a clinician expert on gastrointestinal diseases that offers clinical insight. NK has experience in designing controlled fluidic environments for biochemical applications and works closely with industry. IBM has a background studying gastrointestinal diseases and the

impact of antibiotics on the human microbiome, having developed the methods for quantifying samples in MiGut.

Introduction. The human gut microbiome plays a crucial role in food digestion and host immunity. *In vitro* models of the human colon offer a reliable and ethical alternative to *in vivo* testing (1). *In vitro* models can be inoculated with fecal samples to ensure the microbiome is accurately recaptured. However, the large sample requirements of such models can pose practical constraints to complex studies. Here we used a high-throughput, small size model of the human colon, MiGut, to determine whether more dilute fecal inocula can be used, reducing the sample size requirements and facilitating future studies where sample volume is critical.

Materials and Methods. Two independent experiments were performed, each consisting of eight MiGut models (2). Each model consists of three anaerobic chambers maintained at 37°C and continuously fed with a complex nutrient media, replicating the physiological conditions and pH of the proximal (pH 5.5±0.1) medial (6.25±0.1) and distal (6.75±0.1) colon. Each set of models was inoculated in duplicate with a fecal sample from a healthy donor, using four different slurry dilutions (10%, 5%, 2.5%, and 1.25% w/v). All models were run continuously for 20 days with samples collected thrice weekly for analysis via quantitative PCR (qPCR) (3) and 16S rRNA sequencing (2).

Results and Discussion. Bray-Curtis (BC) dissimilarity plots comparing each model to its fecal inoculum (Figure 1, top) throughout the experiment, were used to assess overall population dynamics as previously described for MiGut (2) and other *in vitro* gut model systems (4).

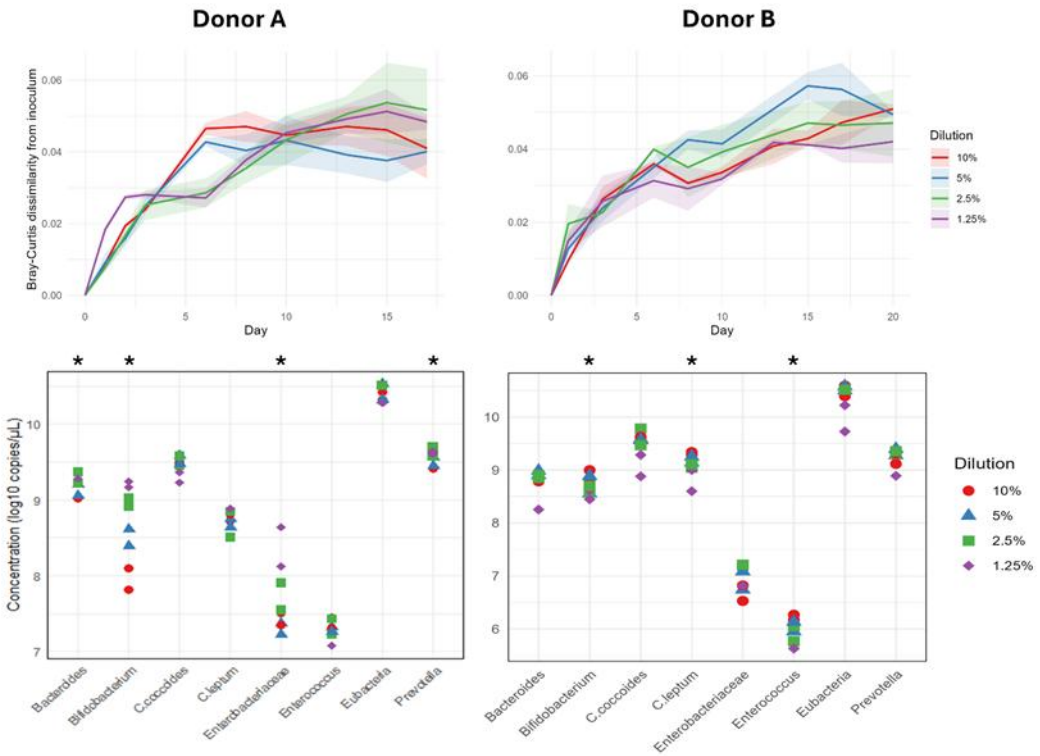

Figure 1. Top: Bray-Curtis dissimilarity from the original fecal inoculum over time for Donor A (left) and Donor B (right) across different fecal dilutions. Lines represent the mean of biological replicates, with shaded areas indicating standard error. Bottom: Linear regression analysis of bacterial populations

quantified by qPCR (log_{10} copies/µL) at experimental day 3. Asterisks (*) indicate bacterial populations showing significant correlations between concentration and dilution level ($p < 0.05$, linear regression analysis).

Although some differences were evident in the early days of the study (e.g. Donor A, day 6), the populations in all reactors stabilized by the end of the experimental period, shown by the plateauing of the plots. Linear regression analysis of qPCR data at day 3 (Figure 1, bottom) revealed that some populations were significantly correlated with dilution: more dilute slurries favored the growth of *Bifidobacterium* spp. and Enterobacteriaceae in donor A, while *Enterococcus* spp. and *C. leptum* were more prevalent in concentrated slurries for donor B. These initial differences are likely due to different growth rates between populations, whereby some groups had an initial competitive advantage before a stable microbial community is established (5). These differences appear to be donor-specific and transient, having resolved as populations stabilised, with no populations being significantly correlated with dilution by the end of the study. 16S rRNA sequencing analysis confirmed convergence to similar communities as both prevalent (e.g. Bifidobacteriaceae and Lachnospiraceae) and low abundant (e.g. Acidaminococcaceae and Coriobacteriaceae) bacterial families were recaptured at similar abundance levels, resulting in consistent compositions and Shannon diversity indices.

Conclusion. MiGut models successfully recaptured the gut microbial composition of the healthy microbiota using reduced fecal slurry concentrations. While initial population dynamics varied between dilutions, all models converged to a similar profile from day 10 onwards, recapturing the donor microbial communities. Unlike batch culture systems which exhibit blooms of some populations (6), the extended run time of MiGut and strict environment control allow for a stable equilibrium to be established *in vitro* even when using low inoculum concentrations. Reducing the donor material allows repeat testing with donors of interest and the ability to use clinical samples, such as those from clinical trials or patients, where sample quantity is often a limiting factor. Future work should also evaluate the metabolic profile of the microbiota to ensure that functional output is retained at reduced concentrations.

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Acknowledgments/funding. Funding for this work has been provided by the UKRI IAA (project IAA4179) at the University of Leeds (UK).