Development of a culture manufacturing process preserving the profile of gut microbiota for therapeutic purposes

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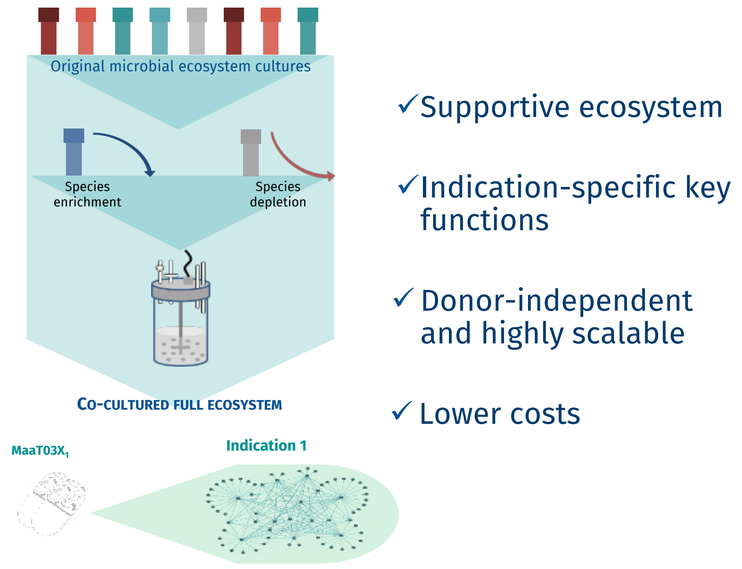
INTRODUCTION

Recent clinical studies have shown that faecal microbiota transfer (FMT) can be successfully used to modulate the gut microbiome for therapeutic purposes. However, single-donor FMT does not allow to purposefully design a therapeutic product, while therapeutic approaches based on single-strain or small consortia of strains may be too reductionist to fully replicate the results obtained with an ecosystem such as FMT. Hence, there is a need for the development and manufacturing of complex, defined ecosystems for therapeutic use. Building upon its expertise in healthy donor-derived, standardized Microbiome Ecosystem Therapies™ (MET-N), MaaT Pharma has recently developed an **innovative co-culture technology** (*in vitro* colic fermentation), enabling the production of **large-scale biomass of defined, complex microbial communities for its next generation of product candidates (MET-C)**. *In silico* modelling tools were also developed for prediction and in-depth analysis of co-culture products.

METHODS

MET-C product candidates are produced using a proprietary *in vitro* colic fermentation technology. Here, we assessed several aspects of our technology including preservation of key ecosystem functionalities, robustness of the process and accuracy of an *in-silico* modeling tool. All results were analyzed by 16S-rDNA sequencing using the in-house MgTagRunner V2.0.0 pipeline.

- Three single-donor materials selected for their different metagenomic profiles were used to assess key ecosystems functionalities. Each was independently co-cultured over 15 days.
- The robustness of the process to produce replicable outcomes was verified with multiple co-cultures from the same starting material. Results were compared to intra-donor variability observed in our healthy donor's database.
- MaaT Pharma's predictive tool, based on Artificial Intelligence, was used as a proof-of-concept to design combinations of starting materials used as seedings in two independent experiments and then co-culture outputs were evaluated to determine if similar products profiles were obtained.

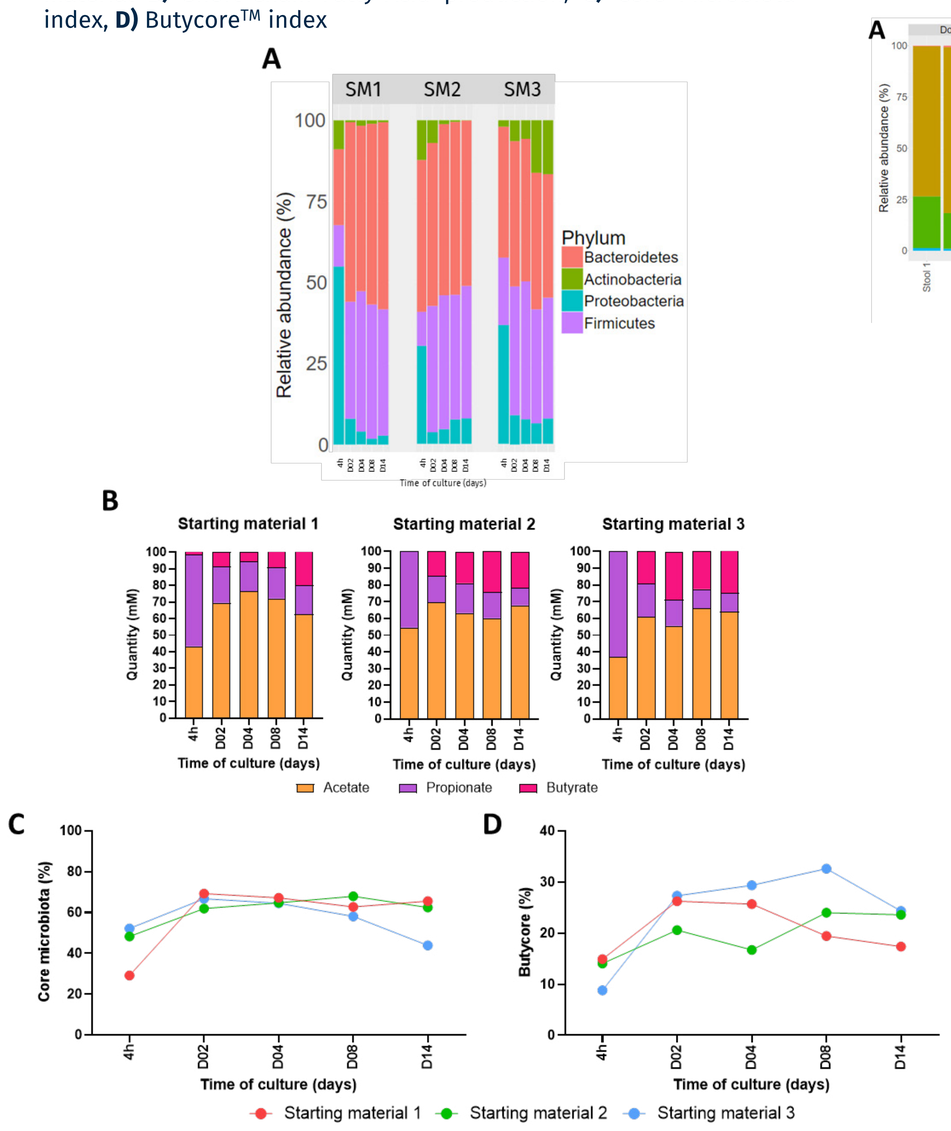


RESULTS

Preservation of key ecosystem functionalities

The *in vitro* co-culture over 15 days demonstrated that fast-growing bacteria such as Proteobacteria, was observed at the early stages of the co-culture (**Figure 1A**). Nevertheless, our process allowed to foster growth of all key phyla including Bacteroidetes, Firmicutes and Actinobacteria, with a stabilization of profiles, correlated with Proteobacteria decrease as well as metabolite production stabilization, for all three single-donor starting materials used (**Figure 1A and 1B**). The preservation of initial ecosystem functionalities after the co-culture process was confirmed by the preservation of the Core Microbiota index (set of eleven bacterial genera found in most healthy individuals), (**Figure 1C**) as well as Butycore™ index (a group of 15 Short-Chain-Fatty-Acids-producing bacterial genera), (**Figure 1D**).

Figure 1: Metagenomic composition of three co-cultured ecosystems. A) Stacked barplots of phyla relative abundances, SM= Starting material **B)** Short-Chain-Fatty-Acid production, **C)** Core Microbiota index, **D)** Butycore™ index

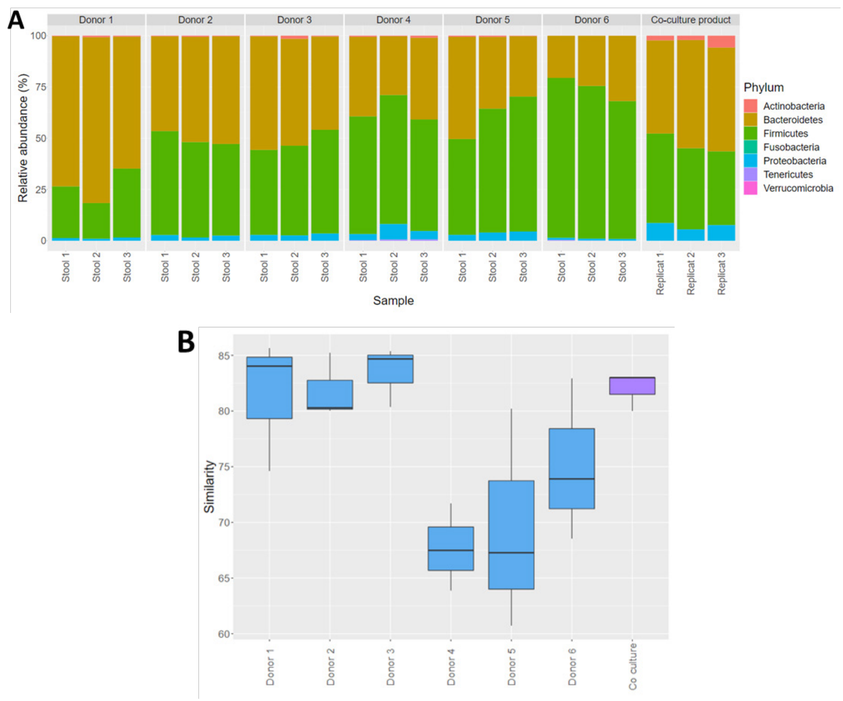


Process robustness

Based on previously collected samples from 63 single healthy donors, intra-donor variability in terms of OTU's relative abundances, as assessed by the Bray-Curtis index, ranged from 57.8% to 93.5% (median = 75.6%) of similarity. As an example, 6 donors were used to generate the Figure 2.

Comparatively, three independent replications of the co-culture process from the same starting material demonstrated that phyla's relative abundances were at least as similar between replicates as within a single healthy donor (**Figure 2A**). Indeed, co-culture replicates presented Bray-Curtis similarities between 80-83% at the OTU level, supporting the process robustness (**Figure 2B**).

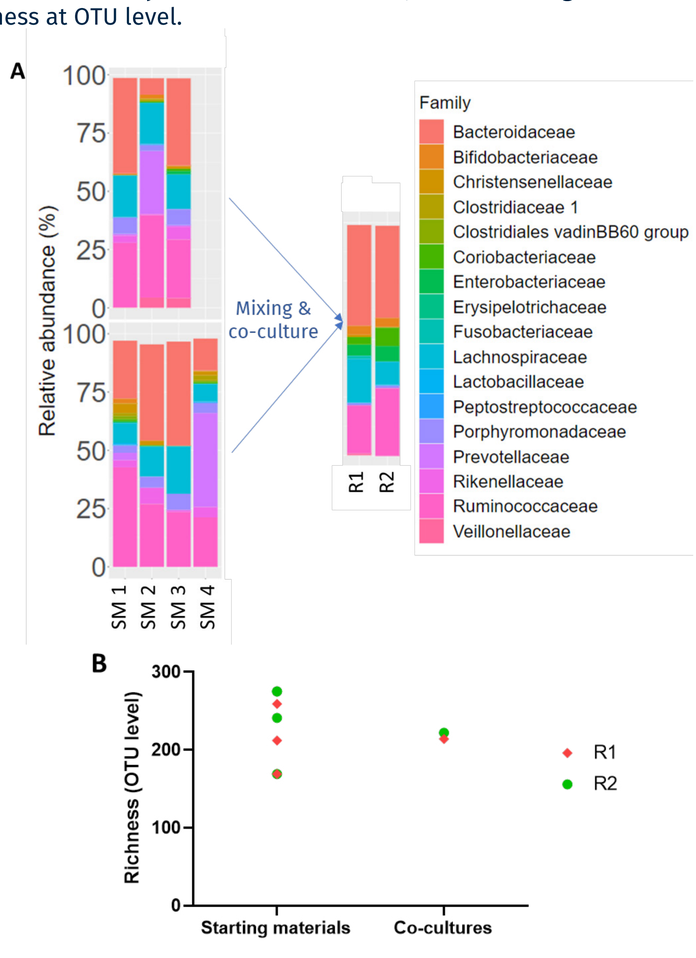
Figure 2: Metagenomic composition (A) and similarity assessment (B) of stools of healthy donors (3/donor) and replicates of co-cultured ecosystems derived from the same starting material. A) Stacked barplots of phyla relative abundances, **B)** Bray-Curtis similarity at OTU level between stools of donor (3/donor) or co-culture replicates.



Proof of concept of a modelling tool

To demonstrate the ability of reliably obtaining a pre-defined profile, we used our predictive tool to target the same profile after co-culture using different starting materials. We selected 7 samples which were mixed in two culture seeds of 3 or 4 starting materials. The resulting co-culture of both mixes harbored a highly similar profile (**Figure 3A**) as confirmed by a Bray-Curtis index at genus level of 81% and a richness level comparable to that of the starting materials (**Figure 3B**).

Figure 3: Metagenomic composition of co-cultured ecosystems from two culture seeds made from different starting materials. A) Stacked barplots of family relative abundances, SM= Starting material. **B)** Richness at OTU level.



CONCLUSION

MaaT Pharma developed a **platform enabling the design and manufacture of a new generation of Microbiome Ecosystem Therapies™ (MET-C)** by combining modelling and predictive tools and an innovative *in vitro* co-culture process. This controlled process allows to purposefully select starting materials and orient the co-culture to generate defined, stabilized bacterial communities in a reproducible way.

This supports the possibility to design and develop drug candidates dedicated to modulating or restoring the gut microbiome and composed of relevant ecosystems with preserved functionalities, as compared to reductionist approaches.