Pooling of faecal material results in standardized and high-richness microbiotherapy products MaaT013 and MaaT033

CYRIELLE GASC, ENORA GASCHET, EMILIE PLANTAMURA, MARIANNE ROBIN, CAROLE SCHWINTNER
Affiliation: MaaT Pharma, 70 avenue Tony Garnier, 69007 Lyon, France

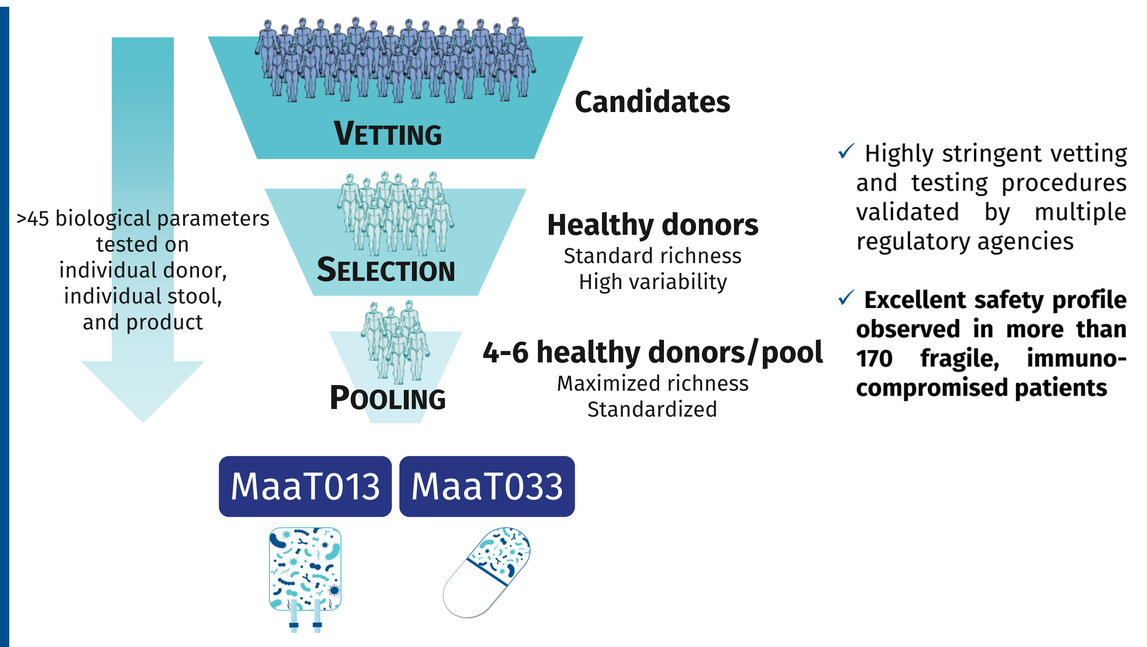
INTRODUCTION

Clinical proof-of-concept studies have evidenced that pooling faecal donations leads to improved FMT efficacy in clinical settings such as ulcerative colitis [1, 2]. Nevertheless, these approaches lack standardisation. **MaaT Pharma has developed two standardized, high-richness, pooled allogeneic Microbiome Ecosystem Therapies (METs), derived from strictly vetted healthy donors** dedicated to restoring the microbial ecosystem and normalize the immune response of patients who receive HCT and those who develop aGvHD.

METHODS

MaaT013 and MaaT033 are standardized and high-richness microbiotherapy products manufactured in a European cGMP production facility by pooling faecal material from 3 to 8 strictly vetted, healthy donors, using a proprietary cryoprotectant preserving a high concentration of viable cells in the drug substance. **MaaT013 is a rectal formulation and MaaT033 is an oral lyophilized encapsulated formulation designed to target the ileo-colic region.**

The microbial composition of 109 MaaT013 products (3 to 8 donors per pool) from 5 distinct production campaigns, 10 MaaT033 products and 180 samples collected from 63 healthy donors were characterized by 16S-rDNA sequencing using the in-house MgTagRunner v2.0.0 pipeline. The metabolite composition of 1 MaaT013 product and the 8 stools of the 7 donors used for its manufacturing was measured using CE-TOFMS and LC-TOFMS.



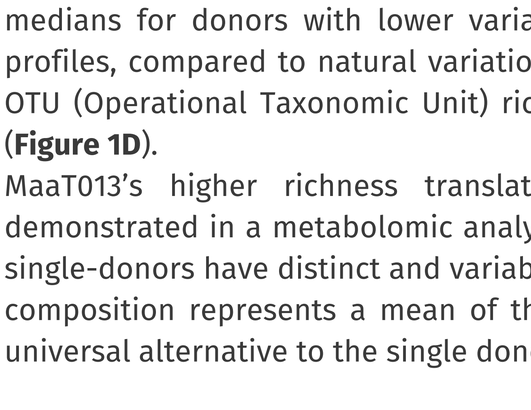
RESULTS

MaaT013

Comparison of the taxonomic composition of donor stools with that of MaaT013 products show that phyla relative abundances are very different from one single donor to another, while MaaT013 batches present consistent profiles (**Figure 1A**). Relative abundance analysis of the main phyla shows that pooling allows the standardization of the product composition and inter-batch consistency, with significantly reduced variance for each phylum in MaaT013 batches when compared to single donors (**Figure 1B**). Similar results are observed at other taxonomic levels (data not shown). Batches consistency and significant reduction of variance are also observed for specific genera associated with clinical benefits, such as *Blautia* and the Butycore™, a group of 15 short-chain-fatty-acids-producing bacterial genera (**Figure 1B**). Donors' consistency was also evaluated using the Bray-Curtis similarity index (**Figure 1C**). Bray-Curtis inter-donor similarity varies from 4.5% to 84.8% (median = 32,8%), whereas the inter-batch product similarity levels vary from 37.4% to 86.7% (median = 64,5%). The Bray-Curtis medians for MaaT013 batches are significantly higher than medians for donors with lower variation, indicating that pooling allows standardization of the profiles, compared to natural variations between donors. MaaT013 is also characterized by a high OTU (Operational Taxonomic Unit) richness while that of donors is significantly lower ($p<0.0001$) (**Figure 1D**).

MaaT013's higher richness translates into a rich and consistent metabolite content as demonstrated in a metabolomic analysis (**Figure 2**). These data illustrate that faecal microbiota of single-donors have distinct and variable metabolite profiles. On the contrary, MaaT013's metabolite composition represents a mean of the donor metabolite composition, constituting a consistent, universal alternative to the single donor products.

Figure 1 (right) - Metagenomic composition of microbiome of healthy donors and MaaT013. **A**. Stacked barplot of phyla relative abundances. **B.** Relative abundance of main phyla (Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria), and specific genera associated with clinical benefits, *Blautia* and Butycore. Test for equality of variance showed that variances were significantly different between donors and MaaT013 batches ($p<0.0001$). **C.** Intra-group Bray-Curtis similarities at OTU level calculated for donors (all campaigns) and MaaT013 batches per production campaign. Statistical significance for median comparison was evaluated using unpaired t-test with Welch's correction. Test for equality of variance showed significantly differences between donors and MaaT013 batches except for one campaign with fewer batches produced: p=0.0017 (n=24); p=0.0008 (n=16); p=0.65 (n=7); p<0.0001 (n=24); p<0.0001 (n=38). **D**. OTU richness. Statistical significance was evaluated using unpaired t-test with Welch's correction.



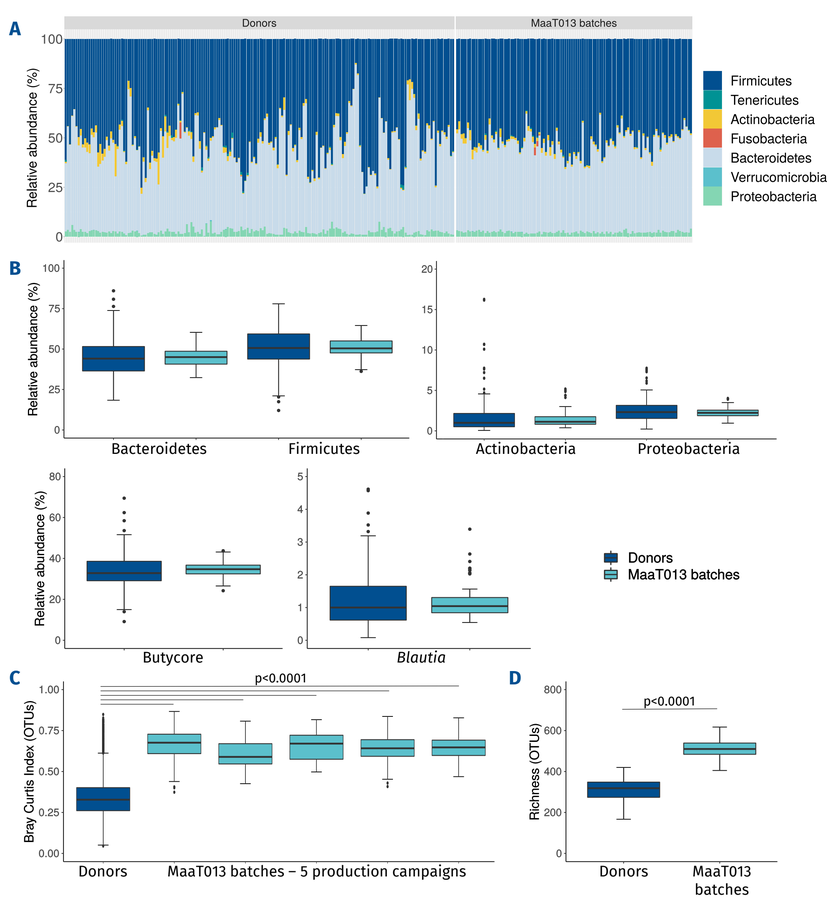


Figure 2 (left) - Metabolite composition of one MaaT013 batch and its individual donors. Color represents the level of detection of each metabolite (one line is one metabolite). Donor 1 provided two different stools used for batch production.

MaaT033

Similar to MaaT013, the oral formulation MaaT033 also allows to standardize the microbial profile of products with a similar richness (Wilcoxon paired test: p=0.7213) and diversity (**Figure 3A**), and a similar microbial community structure in terms of present taxa and relative abundances, as measured by the Bray-Curtis index (Bray-Curtis similarity at OTU level: from 75,4% to 92,9%, median = 85,1%) (**Figure 3B**). Administered in *in vitro* models, these two products lead to the development of similar microbial communities (data not shown). MaaT033 is intended to address different medical needs than MaaT013, and supports easier-to-use, potentially chronic medication.

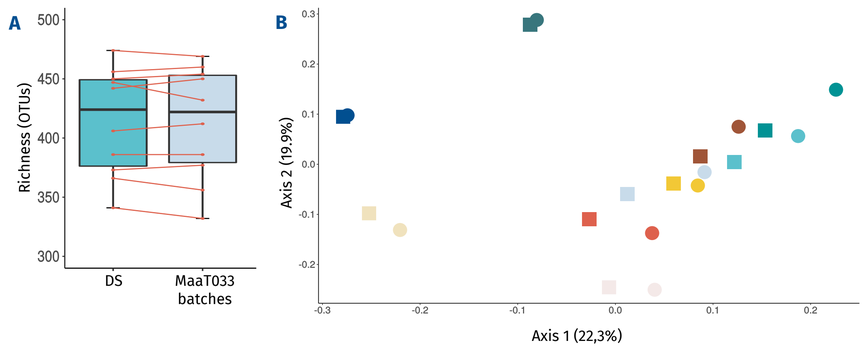


Figure 3 - Similarity in microbiota composition between MaaT033 products and corresponding drug substances. The microbial composition of drug substances can be assimilated to that of corresponding MaaT013 products. **A.** OTU richness. DS = Drug substance. The red lines indicate the correspondence between the DS and the MaaT033 products. **B.** Principal coordinate analysis of Bray-Curtis similarity at OTU level. Circle = MaaT033 batch; Square = Drug substance. MaaT033 products and the corresponding drug substance are represented in the same color.

CONCLUSION

Pooling stool donations results in a broadly applicable medicinal product profile that standardizes microbiome composition, increases microbial richness and optimizes the diversity of metabolites provided to the patients, covering the pleiotropic functions of the gut microbiome.

With two different formulations, MaaT013 and MaaT033 have similar properties that benefit from the pooling of stool from multiple strictly vetted donors. These products have been tested in different clinical studies, especially in GvHD patients, and have shown a beneficial effect on health and on the restoration of a healthy microbiota.

This supports the potential benefit of pooled products to restore a balanced and diverse microbiome that can in turn help to restore the immune homeostasis in patients.

REFERENCES:
1. Paramsothy S, *et al.*, Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. Lancet 389:1218-1228, 2017.
2. Costello SP *et al.*, Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. Jama 321:156-164, 2019.