Pooling of faecal material results in standardized and high taxonomic and functional richness microbiotherapy products

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MaaT013

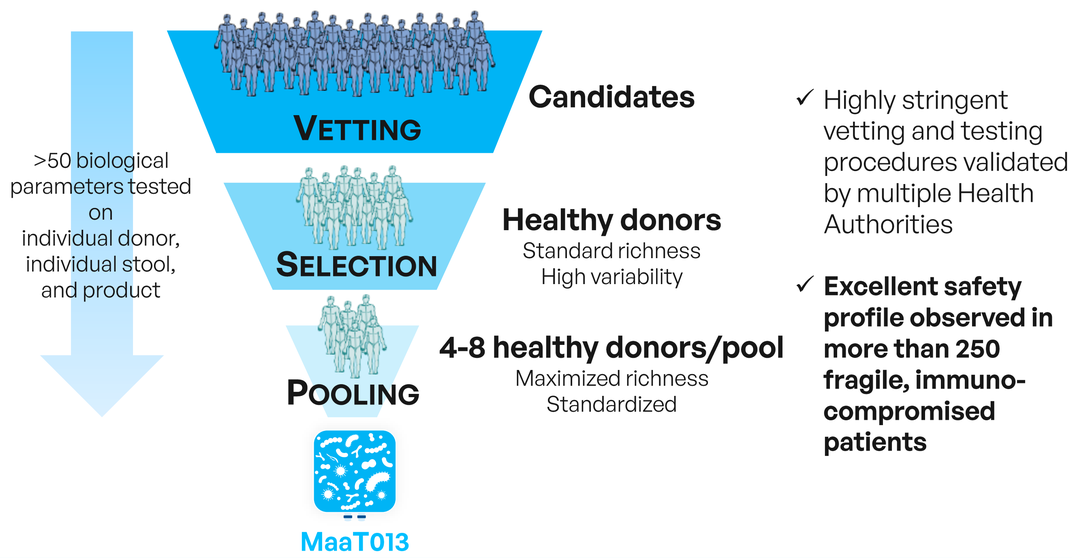
INTRODUCTION

Clinical proof-of-concept studies have evidenced that mixing faecal donations improves FMT efficacy in clinical settings such as ulcerative colitis [1, 2]. Nevertheless, these approaches lack standardization. **MaaT Pharma has developed MaaT013, a standardized, high-richness, pooled allogeneic Microbiome Ecosystem Therapy (MET), derived from strictly vetted healthy donors** dedicated to restore the microbial ecosystem.

METHODS

MaaT013 is a standardized and high-richness microbiotherapy product manufactured in a European cGMP production facility by pooling faecal material from 4 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.**

The microbial composition of 231 MaaT013 products (4 to 8 donors per pool) from 8 distinct productions and 377 samples collected from 120 healthy donors were characterized by 16S-rDNA sequencing using the in-house MgTagRunner v2.0.0 pipeline. In addition, 201 MaaT013 product from 7 distinct productions and 123 samples collected from 111 healthy donors were characterized by shotgun sequencing using the in-house MgRunner V.1.4.0 pipeline.



- ✓ Highly stringent vetting and testing procedures validated by multiple Health Authorities
- ✓ **Excellent safety profile observed in more than 250 fragile, immuno-compromised patients**

RESULTS

Microbial composition (16S-rDNA sequencing)

Taxonomic composition of healthy single-donor fecal samples and MaaT013 product reveals the standardization of product composition and inter-batch consistency with significantly reduced variance for each phylum in MaaT013 batches when compared to single donors

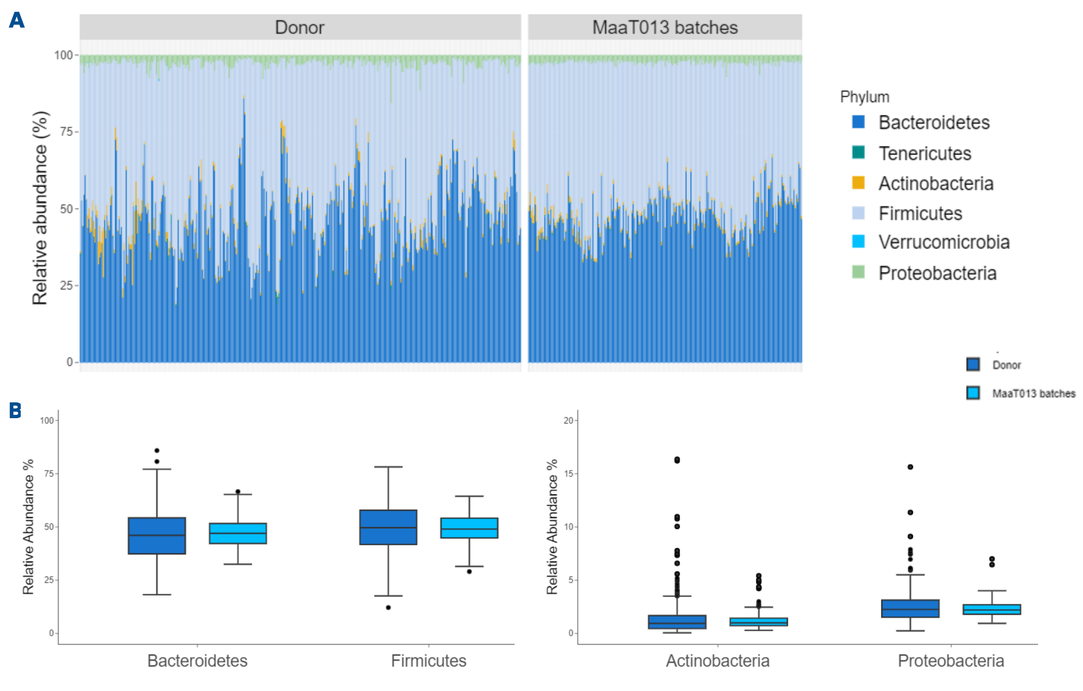


Figure 1 - Metagenomic composition of microbiome of healthy donors and MaaT013. **A.** Stacked barplot of phyla relative abundances. **B.** Relative abundance of main phyla (Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria). The test for equality of variance for all phyla showed that variances were significantly different between donors and MaaT013 batches (p<0.0001)

Batches consistency is also observed for specific genera associated with clinical benefits like the Butycore®, a group of 15 short-chain-fatty-acids-producing bacterial genera and the core microbiota, a set of 11 genera found in most healthy individuals

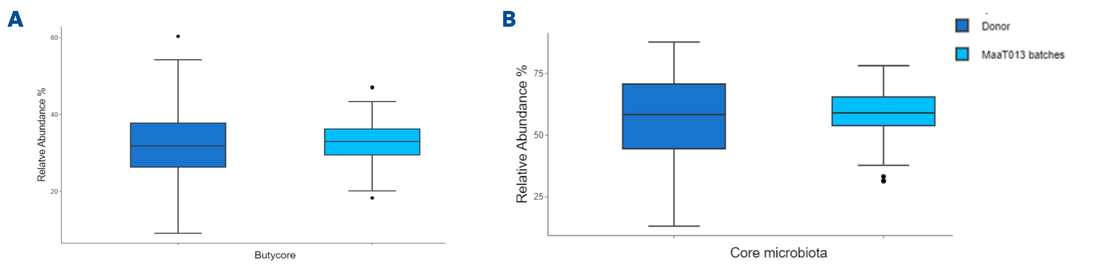


Figure 2 – MaaT Pharma indexes. **A.** Butycore®. **B.** Core microbiota. The test for equality of variance for both MaaT Pharma indexes showed that variances were significantly different between donors and MaaT013 batches (p<0.0001)

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. MaaT013 is also characterized by a high OTU (Operational Taxonomic Unit) richness while that of donors is significantly lower (p<0.0001)

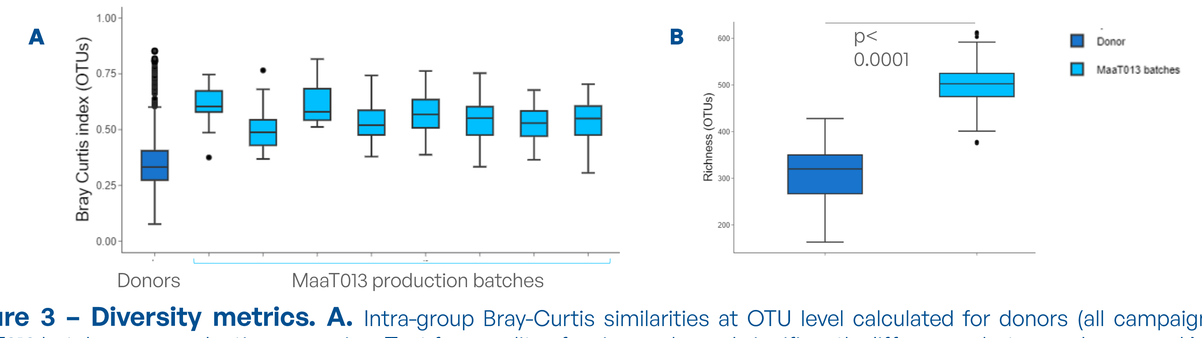


Figure 3 – Diversity metrics. A. Intra-group Bray-Curtis similarities at OTU level calculated for donors (all campaigns) and MaaT013 batches per production campaign. Test for equality of variance showed significantly differences between donors and MaaT013 batches except for one production with fewer batches: p=0.00012 (n=21), p=0.47 (n=7), p<0.0001 (n=16), p<0.0001 (n=24), p<0.0001 (n=38), p<0.0001 (n=47), p<0.0001 (n=34), p<0.0001 (n=40). **B.** OTU richness. Statistical significances were evaluated using Wilcoxon test

Functional features (Shotgun sequencing)

MaaT013 is also characterized by a high gene richness while donor richness is lower (p<0.0001)

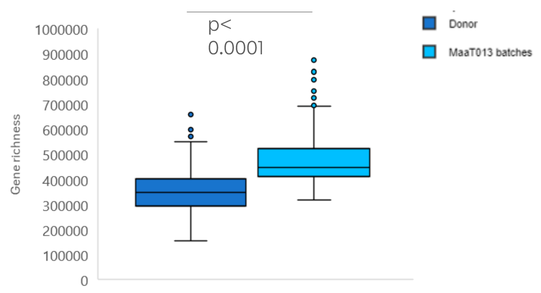


Figure 4 – Gene richness. Statistical significance was evaluated using Wilcoxon test

Short-chain fatty acids (SCFA) production genes including butyrate, propionate and acetate are standardized by pooling (p<0.0001)

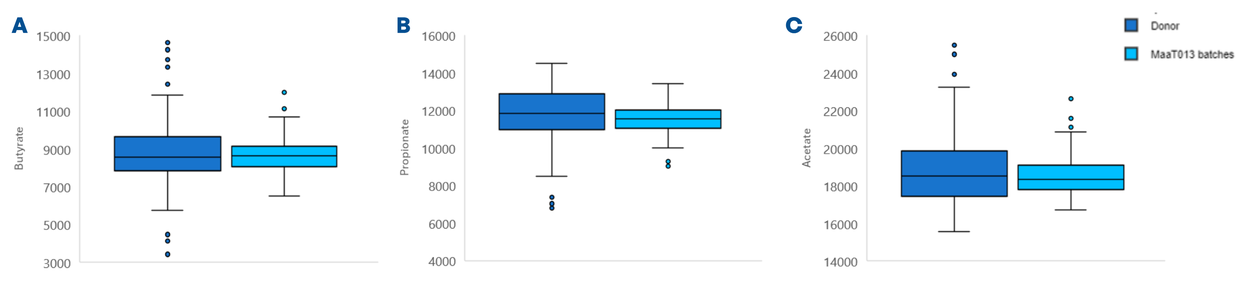


Figure 5 – SCFA production genes. **A**. Butyrate. **B.** Propionate. **C.** Acetate. The test for equality of variance for all the three SCFA showed that variances were significantly different between donors and MaaT013 batches (p<0.0001)

CONCLUSION

Pooling stool donations results in a broadly applicable medicinal product profile that **standardizes microbiome composition and increases taxonomic diversity and functional richness.** MaaT013 has 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** [3]. 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

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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.
3. Malard et al, eclinical medicine 2023

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