

Taxonomic composition of the stool bacteria shows minor changes after standard aerobic FMT preparation

Markus Koglmann¹, Henry Müller¹, Maria Sensen¹, Patrizia Kump², Karl Kashofer³, Gabriele Berg¹ & Christoph Högenauer²

¹Institute of Environmental Biotechnology, Graz University of Technology, Austria

²Department of Internal Medicine, Division of Gastroenterology and Hepatology, Medical University of Graz, Austria

³Institute of Pathology, Medical University of Graz, Austria

Introduction

Fecal microbiota transplantation (FMT) has been applied successfully to treat recurrent *Clostridium difficile* infections. In our preliminary study we have conducted a qualitative analysis of the *live cell fractions* of a donor stool.



The goals of our study:

1. to determine whether standard FMT preparation procedure via blending (e.g. by introduction of oxygen) with saline solution results in changes of the bacterial composition
2. to apply Propidium Monoazide (PMA) in order to distinguish between live and dead cell fractions and follow the bacterial survival rate

Methods



- FMT was prepared with saline solution by using an electric blender under aerobic conditions¹
- live cell fractions were created by using PMA²
- DNA extraction was done by using FastDNA™ Spin Kit for Soil (MP Biomedicals) according to manual

- 16s rDNA amplification and sequencing was performed at the Institute of Pathology using Ion Torrent platform
- bioinformatic analysis was performed with Qiime software package

Results

Beta diversity performed with Qiime using principal coordinate analysis (PCoA) Plot

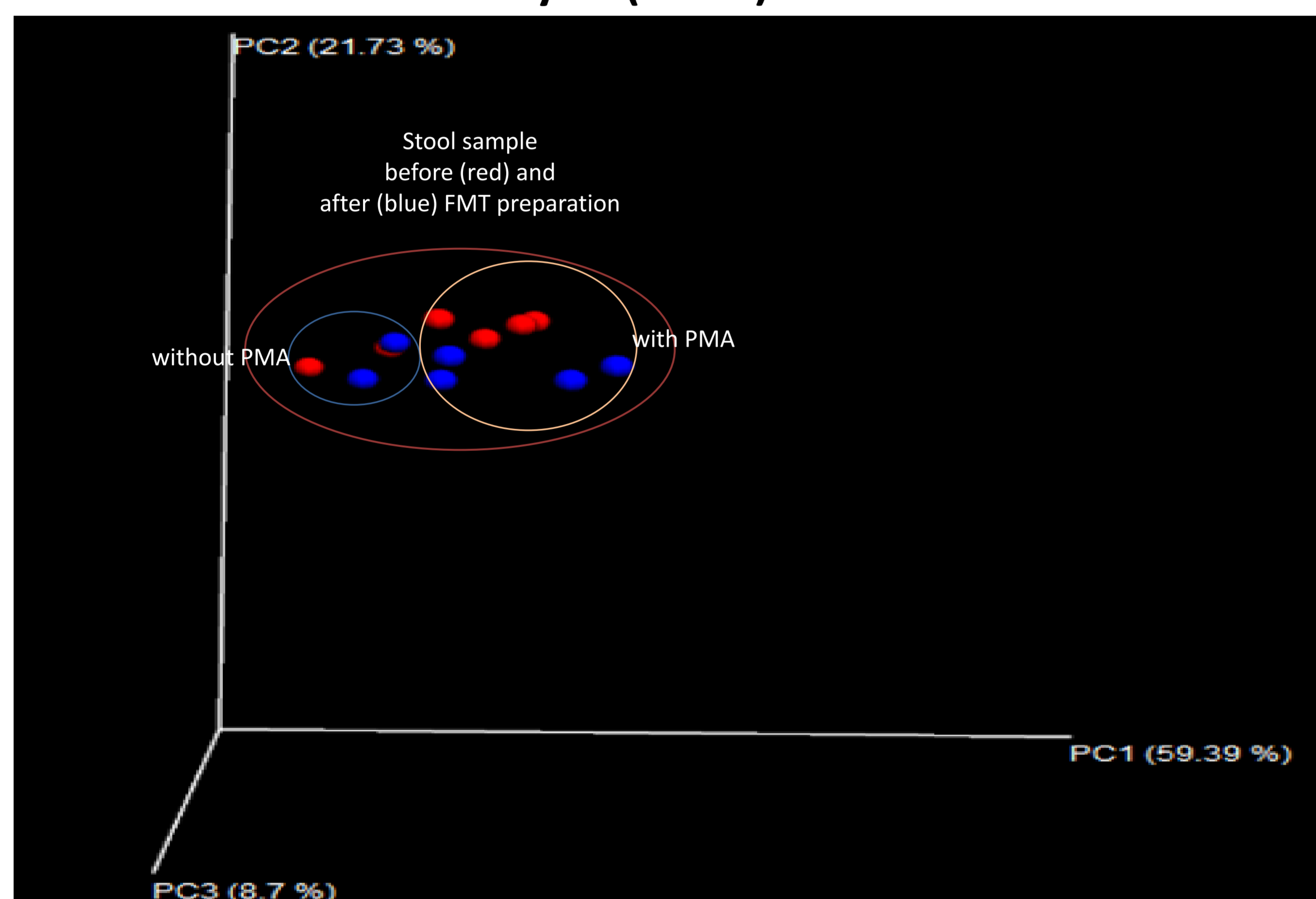


Figure 1: Similarity of the bacterial composition with and without PMA.

1. There is no significant change in the total composition of live and dead cells (without PMA) in stool before and after FMT preparation (similarity 99.2%).
2. The composition of the live cell fraction (with PMA) is slightly less similar (similarity 88.5%) comparing to the total composition.

Species richness displayed using rarefaction

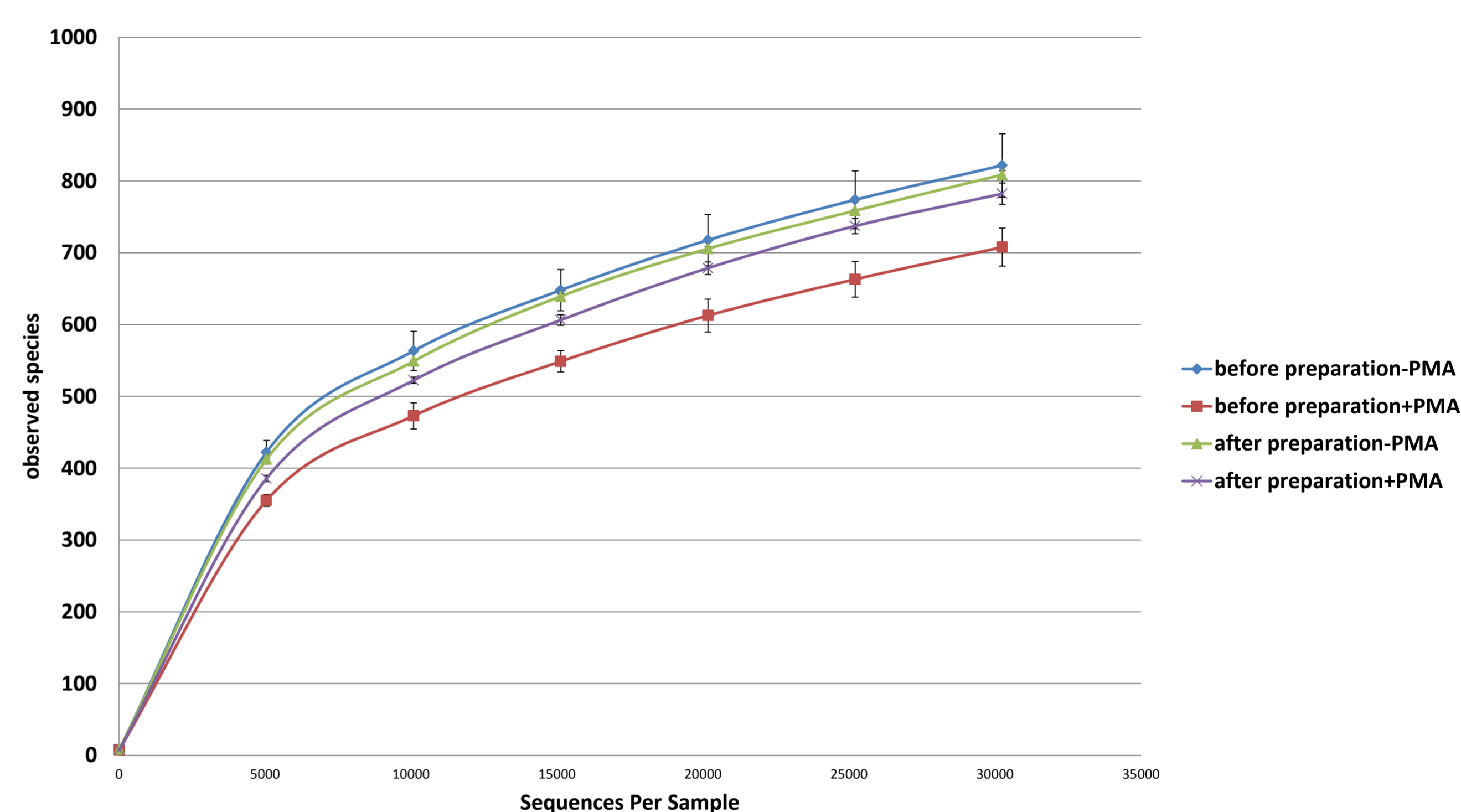


Figure 2: Alpha diversity before and after FMT preparation, with (+) and without (-) PMA.

1. Before preparation 87.6% of the total species can be detected in the live cell fraction.
2. After preparation there is an even higher survival rate of 96,3%.

Taxonomic summary of the live cell fractions (with PMA)

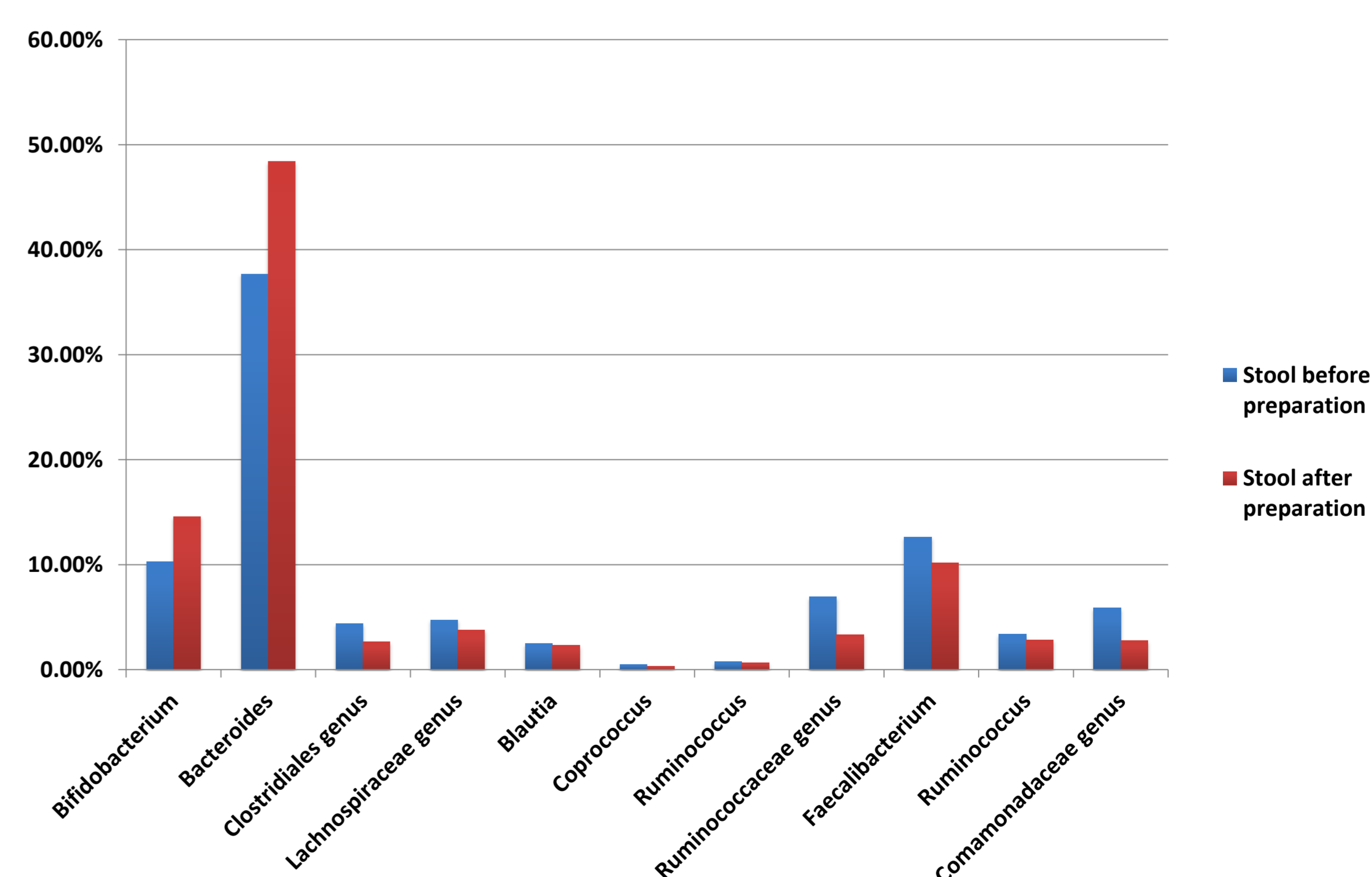


Figure 3: Comparison of the bacterial composition in the stool sample before and after FMT preparation. Bacteria with a total occurrence of >1% are displayed.

1. The FMT preparation seems to be tolerated by *Bifidobacterium sp.* (+4.3%) and *Bacteroides sp.* (+10.7%) well.
2. However, *Clostridiales sp.* (-1.75%), including *Faecalibacterium sp.* (-2.45%), have a slight decrease in ratio.

Conclusion

1. The results indicate that standard aerobic FMT preparation procedure of donor stool only minimally affects the bacterial composition.
2. We could successfully apply PMA in order to follow the survival rate of bacteria during FMT standard preparation.

References

1. Kump K. P., Gröchenig Hans-Peter, Lackner S., Trajanoski S., Reicht G., Hoffmann K. M., Deutschmann A., Wenzl H. H., Petritsch W., Krejs J. G., Gorkiewicz G., and Högenauer C. Alteration of Intestinal Dysbiosis by Fecal Microbiota Transplantation Does not Induce Remission in Patients with Chronic Active Ulcerative Colitis (Inflamm Bowel Dis 2013;19:2155–2165)
2. Nocker A., Cheung C.-Y., Camper A.K. (2006) Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells; Journal of Microbiological Methods 67 (2006) 310–320.