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Impact of storage conditions and DNA extraction protocols on the assessment of bacterial and fungal fecal microbiota

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Background: The human intestinal microbiota contains a vast community of microorganisms that is increasingly studied using high-throughput DNA sequencing of fecal samples. Standardized protocols for storage of fecal samples and DNA extraction have been established for bacterial microbiota analysis only, rather than concomitant bacterial and fungal microbiota analyses. Here, we investigated the impact of two storage conditions and two DNA extraction protocols on bacterial and fungal community structures detected in human stools.

Material/methods: Three stool samples from healthy adults were stored, as such or diluted in RNALater® (ThermoFisher Scientific, USA), at -80°C for one month. They were then subjected to two different genomic DNA extraction protocols, including a step of mechanic lysis, using either the Powersoil® MoBio kit (MoBio, USA) or the standardized Protocole Q recommanded by International Human Microbiome Standards (IHMS) based on the QIAamp DNA stool kit (Qiagen, France). Amplicon libraries, targeting the V3-V4 16S region for bacteria and the ITS1 region for fungi, were prepared using the Metabiote® protocole (Genoscreen, Lille, France) and sequenced on GSFLX 454, Roche. Sequencing data were analysed using the SHAMAN pipeline (http://shaman.c3bi.pasteur.fr/). Composition of the bacterial and fungal microbiota for each storage condition and extraction protocol were compared in terms of diversity and relative abundance.

Results: Overall, 172 000 and 156 000 reads were obtained for 16S and ITS sequencing, respectively. All bacterial sequences were assigned to 42 taxa (38 at genera level), while only 34% of fungal sequences were assigned to 34 taxa (28 at genera level) even though 3 different fungal databases (UNITE, Findley, Silva) were used. The rarefaction curves were satisfactory for every

condition for 3/3 and 2/3 individuals in 16S and ITS analyses, respectively. The ITS data of this third individual were excluded. PCoA analyses showed that >80% of between-sample differences in bacterial or fungal diversity were related to inter-individual variability, and <10% to storage conditions or the extraction methods. For 16S, extraction methods had a significant influence on the relative abundance of a large number of taxa (19/42) while only a low number (4/42) were impacted by storage conditions. For ITS, both extraction methods and storage conditions had a low impact on relative abundance of taxa, with only 3/34 and 4/34 taxa impacted, respectively.

Conclusions: Our data confirm that methods of DNA extraction highly influence the structure of bacterial communities in terms of relative abundance of major and minor taxa, while it has a lower impact on fungal communities. By contrast, the use of RNALater® for stool storage does not impact significantly the structure of bacterial and fungal communities. Therefore, IHMS standard protocols should be recommended to investigate concomitantly bacterial and fungal microbiota.