MULTICENTER EVALUATION STUDY OF GUT MICROBIOME PROFILING BY NEXT-GENERATION SEQUENCING

Hugo Roume [1,3], Stanislas Mondot [2], Adrien Saliou[4], Sophie Le Fresne-Languille[5], Joël Doré [1,2]

[1]Université Paris-Saclay, INRAE, MGP, 78350, Jouy-en-Josas, France

[2]Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350, Jouy-en-Josas, France

[3]Discovery & Front End Innovation, Lesaffre Institute of Science & Technology, Lesaffre Marcq-en-Barœul, France

[4]OMICS Hub, BIOASTER, Microbiology Research Institute, 40 Avenue Tony Garnier, 69007 Lyon, France

[5]Biofortis, Mérieux NutriSciences Compagny, 3 Route de la Chatterie, Saint-Herblain, 44800 Nantes, France

Keywords: human, gut, microbiota, metabarcoding, metagenomic

Objectives: Next-generation sequencing workflows, using either metabarcoding or metagenomic approaches, have massively contributed to expanding knowledge of the human gut microbiota, but methodological bias compromises reproducibility across studies. Where these biases have been quantified within several comparative analyses on their own, none have measured inter-laboratory reproducibility using similar DNA material.

Methods: Here, we designed a multicenter study involving seven participating laboratories dedicated to partial- (P1 to P5), full-length (P6) metabarcoding, or metagenomic profiling (MGP) using DNA from a mock microbial community or extracted from 10 fecal samples collected at two time points from five donors. Fecal material was collected, and the DNA was extracted according to the IHMS protocols. The mock and isolated DNA were then provided to the participating laboratories for sequencing. Following sequencing analysis according to the laboratories' routine pipelines, relative taxonomic-count tables defined at the genus level were provided and analyzed.

Results: Large variations in alpha-diversity between laboratories, uncorrelated with sequencing depth, were detected among the profiles. Half of the genera identified by P1 were unique to this partner and two-thirds of the genera identified by MGP were not detected by P3. Analysis of beta-diversity revealed lower inter-individual variance than inter-laboratory variances. The taxonomic profiles of P5 and P6 were more similar to those of MGP than those obtained by P1, P2, P3, and P4. Reanalysis of the raw sequences obtained by partial-length metabarcoding profiling, using a single bioinformatic pipeline, harmonized the description of the bacterial profiles, which were more similar to each other, except for P3, and closer to the profiles obtained by MGP.

Conclusion: This highlights the major impact of the bioinformatics pipeline, and primarily the database used for taxonomic annotation. Laboratories need to benchmark and optimize their bioinformatic pipelines using standards to monitor their effectiveness in accurately detecting taxa present in gut microbiota.