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In silico workflow for determining Metagenome-Assembled Genomes (MAGs) from a pediatric obesity cohort.

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1. Introduction & aim

Exposure to obesogenic xenobiotics, such as Bisphenol A (BPA), disrupts the pediatric gut microbiome. Since reconstructing MAGs is highly sensitive to tool selection, establishing precise bioinformatic strategies is crucial. Therefore, this study aimed to develop and apply an optimized in silico workflow to accurately reconstruct and characterize high-quality MAGs, defining and comparing the gut microbiome structure of children with obesity and normal-weight controls in order to establish a robust baseline to evaluate future metabolic disruptions induced by differential BPA exposure.

2. Material and methods

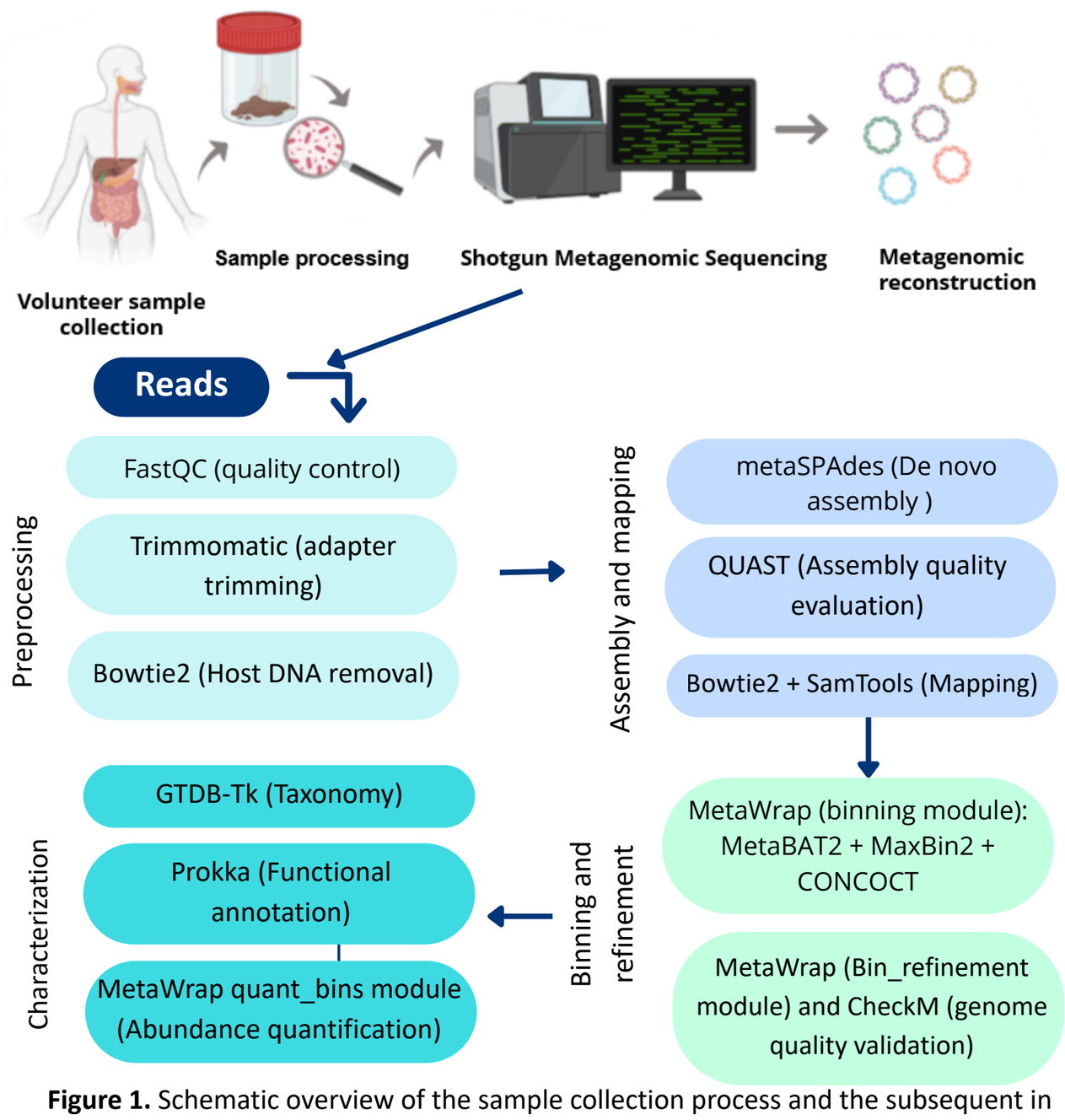

Figure 1. Schematic overview of the sample collection process and the subsequent in silico workflow for MAG reconstruction and characterization.

3. Results and discussion

The designed in silico script successfully standardized MAG reconstruction (Figure 2). Average MAG yields did not differ significantly between Normoweight and Obese groups (69.2 vs. 62.4; p = 0.2851). This statistical uniformity confirms that the sequencing and assembly processes were homogeneous and free of bioinformatic bias. However, host body fat percentage showed a significant negative correlation with total recovered MAGs (R = -0.48; p = 0.0173). This lower recovery of genomes indicates a reduction in taxonomic richness, which current literature identifies as a primary marker of intestinal dysbiosis associated with metabolic alterations (1).

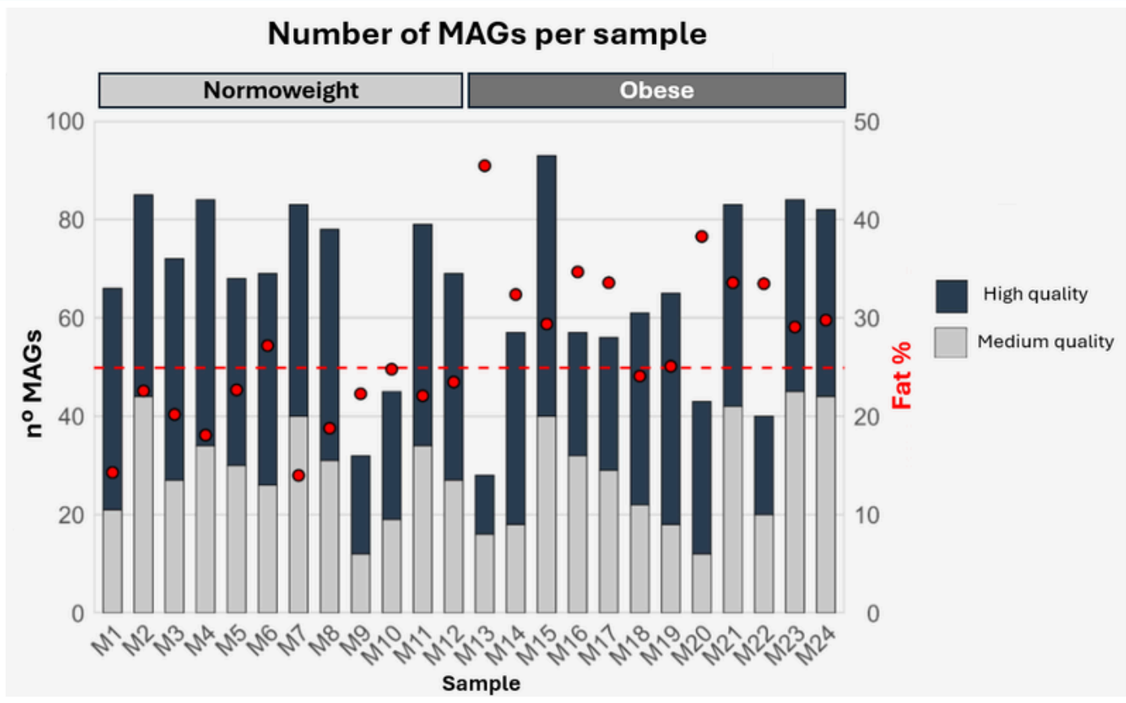

Figure 2. Number of MAGs recovered per sample. Stacked bars indicate the total number of MAGs obtained per individual, stratified by CheckM quality thresholds into High quality (>90% completeness, <5% contamination) and Medium quality (50% completeness, <10% contamination). Red dots represent the corresponding body fat percentage for each subject.

At the Phylum level, Bacillota and Bacteroidota dominated the cohort (Figure 3). At Genus level, Obese group displayed an expansion of *Enterococcus* and species like *Bifidobacterium adolescentis*, which directly displaced core *Bacteroides*. Conversely, *Escherichia coli* predominated in Normoweight subjects, showcasing a high inter-individual variability that matches baseline pediatric benchmarks (2) (Figure 4).

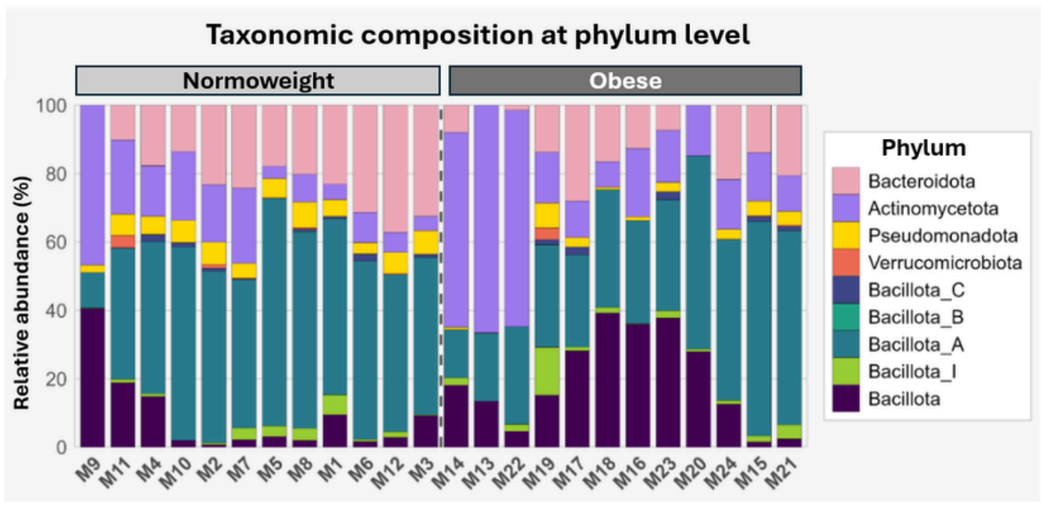

Figure 3. Relative abundance of gut microbiota at the Phylum level.

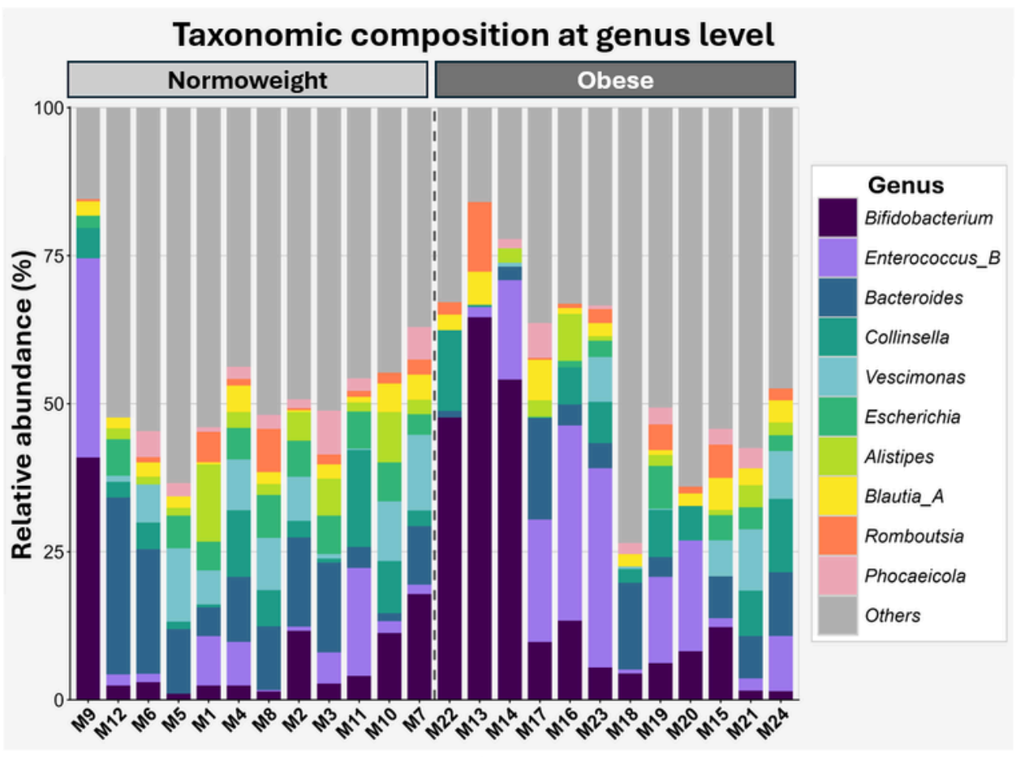

Figure 4. Relative abundance of gut microbiota at the Genus level.

4. Conclusions

1. The *in silico* workflow ensures homogeneous genome reconstructions across samples, confirming that MAG recovery yields are statistically uniform and free of inter-group bioinformatic bias.
2. The findings reveal that rather than altering global diversity, both the obesity phenotype and body fat percentage drive a qualitative reconfiguration of the gut architecture.
3. This obesity-associated restructuring characterizes a specific dysbiotic profile, marked by the expansion of the opportunistic *Enterococcus* complex and a concomitant displacement of core *Bacteroides*

REFERENCES

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2. Ignacio A, Fernandes MR, Rodrigues V a. A, Groppo FC, Cardoso AL, Avila-Campos MJ, et al. Correlation between body mass index and faecal microbiota from children. Clin Microbiol Infect. 1 de marzo de 2016;22(3):258.e1-258.e8. doi:10.1016/j.cmi.2015.10.031