Fructooligosaccharide-driven metabolism of a probiotic consortium modulates hepatocyte lipid accumulation in an *in vitro* microbiota-liver interaction model

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Metabolic-dysfunction-associated fatty liver disease (MAFLD) is a metabolic disorder in which the gut-liver axis plays a central role. Microbial-derived metabolites generated from dietary substrates are key mediators linking gut microbiota activity to host metabolic regulation. Specifically, fructooligosaccharides (FOS) can modulate microbial metabolism and functional outputs.

We investigated the impact of FOS-driven microbial metabolism on hepatic lipid accumulation using a defined *in vitro* human gut microbiota model. A probiotic consortium composed of *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, and *Limosilactobacillus reuteri* was evaluated alone and in combination with a reconstructed minimal human gut microbiota core (*Clostridum symbiosum*, *Flavonifractor plautii*, *Bacteroides cellulosilyticus*, and *Escherichia coli*). Bacterial growth, strain-level dynamics, and expression of genes involved in FOS utilization were assessed. Microbial metabolites were characterized by GC-MS and tested on HepG2 cells in a palmitic acid-induced steatosis model.

All strains grew on FOS, with strain-dependent efficiency. The probiotic consortium and the overall microbial community were shaped by substrate utilization, with *L. plantarum* and *L. reuteri* dominating in both contexts. Genes involved in FOS metabolism were identified in all probiotic strains, showing differences in gene organization, and expression across different experimental conditions.

FOS fermentation resulted in the production of short-chain fatty acids and organic acids. HepG2 cells pre-treated with metabolites derived from the probiotic consortium and the reconstructed community showed reduced intracellular lipid accumulation under steatotic conditions, associated with decreased *cd36* lipid transporter gene expression.

These findings highlight the role of microbial context in shaping metabolic outputs and support the contribution of microbial interactions to the regulation of host lipid metabolism in a liver cell model.