

Modulation of an *in vitro* reconstructed human gut microbiota in presence of probiotic strains and a prebiotic Maitake extract

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Abstract: This study aims to evaluate the modulation of an *in vitro* reconstructed human gut microbiota in the presence of probiotics and prebiotics and its consequent potential beneficial effects on human intestinal and immune system cell lines. The *in vitro* reconstructed gut microbiota was designed to include bacteria representative of the major phyla of the intestinal bacterial community (Firmicutes, Bacteroides and Proteobacteria). Its modulation was evaluated in the presence of probiotic strains (*Lactobacillus* spp. and *Bifidobacterium* sp.) and a prebiotic Maitake extract.

Initially, the model was validated employing a well-known prebiotic source such as short-chain fructooligosaccharides (FOS DP 3-5) monitoring both the growth of the single strains composing the model (through qRT-PCR analysis) and the production of bacterial metabolites (through GC-MS analysis).

Then the modulation of the *in vitro* microbiota was investigated utilising as a prebiotic source the mushroom Maitake extract (previously characterised by De Giani et al., 2021) analysing the single strain growth and the production of bacterial metabolites. The results showed that the mushroom extract primarily supported the growth of the probiotic strains, particularly *L. plantarum* and *B. animalis* subsp. *lactis*. Consistently the analysis of the metabolites revealed the production of short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs), beneficial bacterial metabolites known in the literature for their anti-inflammatory, antioxidant and antitumoral effects. Consequently, cell lines representative of the two most important compartments at the intestinal level, the epithelium (HT-29) and the immune system (PBMCs), have been selected to test the effects of the produced bacterial metabolites. The results revealed positive effects on the antioxidant response (increasing SOD1 and NQO1 expression levels in the epithelium) and an increase in the production of the immuno-regulatory IL-10. Therefore, altogether the findings indicate that the probiotic selected strains and the Maitake extract were able to positively modulate the *in vitro* reconstructed human gut microbiota leading to an enhancement of the tolerogenic intestinal microenvironment simulating what happens naturally in the host.