

Modulation of a reconstructed *in vitro* gut microbiota by prebiotic Maitake extract and pre-probiotic mediated protective effects on colorectal cell lines

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Abstract: In this work, a *Grifola frondosa* (Maitake) ethanol/water extract derived from the sporophora of Chinese wild mushrooms was characterized through enzymatic and chemical assays for the presence of different sugars, proteins, and polyphenols. Due to the detection of glucans, the prebiotic potential of the Maitake extract was tested by monitoring the growth of 8 probiotic strains (Lactobacilli and Bifidobacteria), and then mixed as a probiotic consortium. The results revealed Maitake extract's prebiotic properties due to the high stimulation of the growth of the probiotic strains, combined also as a consortium. Considering the growth also of potential strains composing the human gut microbiota, the registered prebiotic index was 2.31. Short-chain (SCFAs) and branched-chain (BCFAs) fatty acids were extracted and characterized through GC-MSD analyses. After the probiotic consortium fermentation of the Maitake extract, lactic, succinic, and valeric acids were detected. These collected secondary metabolites were then administered to healthy (CCD841) or tumoral colorectal cell lines (CACO-2 and HT-29), highlighting positive effects in terms of protection from reactive oxygen species (De Giani et al. 2021).

Subsequently, the possible beneficial effects of the Maitake extract included the modulation of the levels of the strains mimicking a defined human gut microbiota in a batch reactor. The basal community was composed of *Bacteroides*, *Clostridium* and *Escherichia* representatives, while the probiotic intervention was simulated using *L. plantarum*, *L. acidophilus*, and *B. animalis* subsp. *lactis*, which effectiveness was tested in an *in vivo* trial (De Giani et al. 2022). The synbiotic favoured *L. plantarum* growth and the production of beneficial SCFAs, such as lactic, propionic, and succinic acid. Quinoline (a possible component of Maitake) was also detected.

These results support a beneficial modulation of the *in vitro* reconstructed gut microbiota due to the administration of the synbiotic formulation, suggesting future employment *in vivo*.