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# Identification of novel synbiotics by *in vitro* colonic fermentation and a human clinical trial.

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Prebiotics and probiotics used in combinations as synbiotics, may change composition and activity of the human gut microbiota and thereby potentially affect host health beneficially. However selection of synbiotic combinations are rarely based upon prebiotics screened for growth-stimulation of well-characterised probiotic strains.

In the present study, a library of 37 potential prebiotics, comprising carbohydrates with a large range of degrees of polymerization (DP), monomeric units and glycosidic linkage, was investigated with regard to growth stimulation of the well studied probiotic bacteria *Lactobacillus acidophilus* NCFM (NCFM) and *Bifidobacterium animalis* subsp. *lactis* Bl-04 (Bl-04). For each probiotic strain, the most effective carbohydrates were selected for further investigation, using a four-stage semi-continuous model system of the human colon. Selected combinations were NCFM combined with isomaltulose, cellobiose, raffinose and oat β-glucan hydrolyzed with endo-1,3-β-glucanase, respectively and Bl-04 combined with melibiose, xylobiose, raffinose and maltotriose, respectively. Growth support of the probiotic bacteria and influence on the microbiota was investigated using qPCR while the formation of short-chain fatty acids (SCFA) was investigated by gas chromatography. Based on the *in vitro* studies, NCFM in combination with cellobiose was selected for a randomized, double-blinded, placebo-controlled crossover human trial and collected fecal samples were analyzed by qPCR, next generation sequencing and gas chromatography.

Carbohydrates selected for fermentation in the colonic model in combination with NCFM or Bl-04, were all able to support growth of the probiotic bacteria, increasing quantities of NCFM and Bl-04 by $10^3$–$10^4$ fold and 10–$10^2$ fold, respectively, compared to control. Interestingly all combinations decreased the ratio of *Bacteroidetes/Firmicutes,* and levels of SCFA, especially acetic and butyric acid were increased by three to eight fold, as compared to the control. In the human trial, large variation was observed between individuals, and no differences were found for the ratio of *Bacteroidetes/Firmicutes* or SCFA, as compared to placebo. Increases in lactobacilli and bifidobacteria, observed in the colonic model, were confirmed for the human trial ($p=0.04$). In conclusion, using monoculture screening and a colonic model, several potential synbiotics were identified and for NCFM and cellobiose the potential was confirmed by a human clinical trial. However the synbiotic potential of the investigated combinations should be further validated by larger human clinical trials.

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