

Mucin- and carbohydrate-stimulated adhesion and subproteome changes of the probiotic bacterium *Lactobacillus acidophilus* NCFM - DTU Orbit (09/11/2017)

Mucin- and carbohydrate-stimulated adhesion and subproteome changes of the probiotic bacterium *Lactobacillus acidophilus* NCFM

Adhesion to intestinal mucosa is a crucial property for probiotic bacteria. Adhesion is thought to increase host-bacterial interactions, thus potentially enabling health benefits to the host. Molecular events connected with adhesion and surface proteome changes were investigated for the probiotic *Lactobacillus acidophilus* NCFM cultured with established or emerging prebiotic carbohydrates as carbon source and in the presence of mucin, the glycoprotein of the epithelial mucus layer. Variation in adhesion to HT29-cells and mucin was associated with carbon source and mucin-induced subproteome abundance differences. Specifically, while growth on fructooligosaccharides (FOS) only stimulated adhesion to intestinal HT-29 cells, cellobiose and polydextrose in addition increased adhesion to mucin. Adhesion to HT-29 cells increased by about 2-fold for bacteria grown on mucin-supplemented glucose. Comparative 2DE-MS surface proteome analysis showed different proteins in energy metabolism appearing on the surface, suggesting they exert moonlighting functions. Mucin-supplemented bacteria had relative abundance of pyruvate kinase and fructose-bisphosphate aldolase increased by about 2-fold while six spots with 3.2-2.1 fold reduced relative abundance comprised elongation factor G, phosphoglycerate kinase, BipAEFTU family GTP-binding protein, ribonucleoside triphosphate reductase, adenylosuccinate synthetase, 30S ribosomal protein S1, and manganese-dependent inorganic pyrophosphatase. Surface proteome of cellobiose- compared to glucose-grown *L. acidophilus* NCFM had phosphate starvation inducible protein stress-related, thermostable pullulanase, and elongation factor G increasing 4.4-2.4 fold, while GAPDH, elongation factor Ts, and pyruvate kinase were reduced by 2.0-1.5 fold in relative abundance. Addition of recombinant *L. acidophilus* NCFM elongation factor G and pyruvate kinase to a coated mucin layer significantly suppressed subsequent adhesion of the bacterium. Biological significance: Human diet is important for intestinal health and food components, especially non-digestible carbohydrates can beneficially modify the microbiota. In the present study, effects of emerging and established prebiotic carbohydrates on the probiotic potential of *Lactobacillus acidophilus* NCFM were investigated by testing adhesion to a mucin layer and intestinal cells, and comparing this with changes in abundance of surface proteins thought to be important for host interactions. Increased adhesion was observed following culturing of the bacterium with fructooligosaccharides, cellobiose or polydextrose, as well as mucin-supplemented glucose as carbon source. Enhanced adhesion ability can prolong bacterial residence in GIT yielding positive health effects. Higher relative abundance of certain surface proteins under various conditions (i.e. grown on cellobiose or mucin-supplemented glucose) suggested involvement of these proteins in adhesion, as confirmed by competition in case of two recombinantly produced moonlighting proteins. Combination of *Lactobacillus acidophilus* NCFM with different carbohydrates revealed potential bacterial determinants of synbiotic interactions, including stimulation of adhesion.

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