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NDPs from cheaper NMPs.
Results:
By the action of the polyphosphate kinases the cofactors could be formed out of their corresponding NMPs as starting material and were regenerated during 2'-fucosyllactose production from the cheap cosubstrate polyphosphate (GTP ~ 14 times, ATP ~ 28 times). NMPs that are formed as byproducts during HMO synthesis could be recovered by conversion to NDPs. This one-pot reaction system enabled the synthesis of 13 mM of 2'-fucosyllactose (6.5g/L) within 24 hours.
Discussion:
Here we describe for the first time a polyphosphate kinase based nucleotide cofactor regeneration system for in vitro production of fucosylated HMOs. The polyphosphate based system is able to regenerate ATP and GTP from AMP and GMP by the action of two additional enzymes in one pot. The regeneration system worked efficiently as could be seen by accumulation of GDP-L-fucose in the system. Achieved product titers are among the highest reported for in vitro synthesis of fucosylated HMOs and were rather limited by the activity of the fucosyltransferase than by the efficiency of the recycling system.

Keywords: HMO, fucosyllactose, one-pot multienzyme system, cofactor regeneration, polyphosphate kinase

INTERACTION OF FOOD-ASSOCIATED *LACTOBACILLUS PLANTARUM* WITH HUMAN DERIVED INTESTINAL EPITHELIAL CELLS

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Introduction:
It is currently accepted that food-associated microbes can reach the gut as viable cells and interact with the human host providing potential health benefits. *Lactobacillus plantarum* is a flexible species that could be found as a natural inhabitant in several fermented foods and in the human GI tract, from which some strains have already been characterised as probiotics (Corsetti et al. 2015, Reference Module in Food Sciences, Elsevier, pp. 1-8); moreover, it has recently been clarified that food-associated microbes share genetic and physiological traits with probiotic strains. Even though the ability of probiotics to interact by adhering to host cells is commonly considered to be a prerequisite for host-microbe dialogue, the interaction of orally ingested microbes with the intestinal epithelium has just begun to be rigorously studied.
Methods:
The aim of this study was to explore a collection of food-associated *Lactobacillus plantarum* strains, previously characterized for several functional properties (Prete et al. 2017, Frontiers in Microbiology, 8:2349), besides two strains of the same species from human source already patented as probiotics, for their interaction with colonic epithelial cells (NCM460). First, the potential impact on human cell viability of *Lb. plantarum* strains was assessed by the MTT colorimetric assay. Subsequently, all the strains were examined for qualitative adherence by an in vitro Gram-stained adhesion assay, while adhering bacteria were quantified by plating on MRS agar. Furthermore, due to the presence of mucus layer on the GI epithelium, it was also investigated if the mucin can affect the microbial viability and activity by survival assay and adhesion test, respectively. For a better understanding of immunomodulatory properties of food-associated *Lb. plantarum*, all the strains were investigated for their anti-inflammatory potential by evaluating pro- and anti-inflammatory cytokines release in an in vitro inflammation model.
Results:
The cellular metabolic activity of intestinal cells showed that all *Lb. plantarum* strains are likely to leave the cellular metabolic activity of the NCM460 cells unchanged. Microscopical observations showed an overall high adhesion capability of all *Lb. plantarum* strains on intestinal cell lines, with a preference in adhering around cell edges. Data from both quantitative and mucin-related results confirmed the food-borne *Lb. plantarum* adhesion efficiency, showing differences among the strains, with values between 77-98 adhesion percentage. Finally, no *Lb. plantarum* strains, both from human and food source, showed to be affected by growing in presence of porcine mucin. Interaction of *Lb. plantarum* strains with intestinal cells showed a modulation of different pro-and anti-inflammatory cytokines that could lead to a potential reduction of the induced-inflammation status.
Discussion:
Focus of attention on all experiments, the majority of *Lb. plantarum* strains isolated from foods displayed a

similar behaviour of that from human sources, revealing a promising interaction with host cells. All the above, suggest a beneficial cross-talk with the host immune system as an additional functional property of food-associated microbes, that needs to be further investigated in order to select some strains to be used in the development of novel foods and nutraceuticals.

Keywords: *Lactobacillus plantarum*, probiotic characterization, intestinal epithelium, adhesion efficiency, host-microbe interaction

THE CHEESE MATRIX PROTECTS *PROPIONIBACTERIUM FREUDENREICHII*'S IMMUNOMODULATORY SURFACE PROTEIN SLPB FROM PROTEOLYSIS DURING DIGESTION

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Introduction:
Propionibacterium freudenreichii is an emergent probiotic bacterium, belonging to dairy propionibacteria, within the group of Actinomycetales, and possessing the GRAS (Generally Recognized As Safe, USA, FDA) and QPS (Qualified Presumption of Safety, EFSA, European Union) status. It is a traditional Swiss-type cheeses starter and an emergent probiotic, exerting several beneficial effects, including anti-inflammatory modulation of gut inflammation. This feature relies on several metabolites and on surface proteins including in particular the surface protein SlpB.
Methods:
In the present study, we assessed first the impact of proteolytic degradation of SlpB on its immunomodulatory effect on colon epithelial HT-29 cells. Intact *P. freudenreichii* cells, purified surface layer proteins (SLPs), as well as trypsin-proteolyzed SLPs, were used to stimulate HT-29 cells, in the presence or absence of pro-inflamatory LPS. Cytokines were then quantified to evaluate the inflammatory response. We then investigated the potential of cheese matrix as a delivery vehicle of anti-inflammatory *P. freudenreichii* MAMP to the colon, by comparing dairy liquids matrices to a solid Swiss-type cheese matrix, using two in vitro digestion models. Integrity of SlpB was monitored using western blotting.
Results:
In this study, we firstly investigated the relevance to avoid SlpB digestive proteolysis, by comparing the effect of i) *P. freudenreichii* CIRM-BIA 129, ii) its native SLPs, or iii) peptides resulting from SLPs stimulations, with respect to modulation of HT-29 cells response to lipopolysaccharide (LPS) challenge. The anti-inflammatory effect exerted by *P. freudenreichii* CIRM-BIA 129 and by native surface proteins (SLPs) on HT-29 cells was abolished by digestive proteolysis. This result confirmed the importance to protect immunomodulatory surface proteins from digestive proteolysis in order to allow gut immune system modulation. Thus, we examined the effect of dairy matrices on *P. freudenreichii* viability and on SlpB integrity during digestion. In comparison with liquid matrices, the cheese matrix provided an enhanced tolerance to digestive stresses and protection of SlpB towards proteolysis, during two in vitro digestion models: static and dynamic.
Discussion:
These in vitro results provide new insights into the matrix effect on *P. freudenreichii* probiotic functionalities. The cheese matrix offers significant protection against the digestion stresses by enhancing survival, and by protecting surface layer proteins from proteolysis. However, in vivo investigations are necessary to study the matrix effect during digestion, to follow SLPs expression and to evaluate the matrix effect on immunomodulation by *P. freudenreichii* within the gut. Such data accordingly will allow the development of new functional foods for the delivery of *P. freudenreichii* to the gastrointestinal tract of humans in the aim to help prevention or treatment of life-style related diseases with an inflammatory component.

Keywords: probiotic, immunomodulation, surface proteins, digestion, food matrix, stress tolerance