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Lactate- and acetate-based cross-feeding interactions between selected strains of lactobacilli, bifidobacteria and colon bacteria in the presence of inulin-type fructans

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ABSTRACT

Cross-feeding interactions were studied between selected strains of lactobacilli and/or bifidobacteria and butyrate-producing colon bacteria that consume lactate but are not able to degrade inulin-type fructans (ITF) in a medium for colon bacteria (supplemented with ITF as energy source and acetate when necessary). Degradation of oligofructose by *Lactobacillus acidophilus* IBB 801 and inulin by *Lactobacillus paracasei* 8700:2 and *Bifidobacterium longum* LMG 11047 resulted in the release of free fructose into the medium and the production of mainly lactate (lactobacilli) and acetate (*B. longum* LMG 11047). During bicultures of *Lb. acidophilus* IBB 801 and *Anaerostipes caccae* DSM 14662^T on oligofructose, the latter strain converted lactate (produced by the former strain from oligofructose) into butyrate and gases, but only in the presence of acetate. During bicultures of *Lb. paracasei* 8700:2 and *A. caccae* DSM 14662^T or *Eubacterium hallii* DSM 17630 on inulin, the butyrate-producing strains consumed low concentrations of lactate and acetate generated by inulin degradation by the *Lactobacillus* strain. As more acetate was produced during tricultures of *Lb. paracasei* 8700:2 and *B. longum* LMG 11047, which degraded inulin simultaneously, and *A. caccae* DSM 14662^T or *E. hallii* DSM 17630, a complete conversion of lactate into butyrate and gases by these butyrate-producing strains occurred. Therefore, butyrate production by lactate-consuming, butyrate-producing colon bacterial strains incapable of ITF degradation, resulted from cross-feeding of monosaccharides and lactate by an ITF-degrading *Lactobacillus* strain and acetate produced by a *Bifidobacterium* strain.

1. Introduction

The human colon represents one of the most dense microbial ecosystems in terms of bacterial communities. The gut microbiota is highly diverse and contains an estimated 500 to 1000 different species belonging to only a restricted number of dominant phyla, which results in a high level of functional redundancy (Arumugam et al., 2011; Eckburg et al., 2005; Qin et al., 2010; Tap et al., 2009). This microbiota can be represented by about ten bacterial functional groups, which interact with each other and with the host (Kettle et al., 2015). Producers of lactate and acetate in the human colon are essential contributors to the fermentation of non-digestible carbohydrates (De Vuyst and Leroy, 2011; De Vuyst et al., 2014; Kettle et al., 2015). Lactate and acetate produced by members of the bifidobacteria cross-feed butyrate-producing colon bacteria belonging to clostridial clusters IV (*e.g.*, *Faecalibacterium prausnitzii*) and XIVa (*e.g.*, *Anaerostipes caccae*, *Eubacterium hallii*, *Eubacterium rectale*, and *Roseburia* spp.) (Barcenilla et al., 2000; Belenguer et al., 2007, 2011; Duncan et al., 2002a, 2002b, 2002c, 2004, 2006; Duncan and Flint, 2008; Falony et al., 2006; Schwiertz et al., 2002).

Next to bifidobacteria, lactobacilli may make a very important contribution to lactate production in the human colon (Belenguer et al., 2011; Bourriaud et al., 2005; Duncan et al., 2004; Macfarlane and Gibson, 1996). Laboratory studies have shown that lactobacilli are able to degrade non-digestible carbohydrates, such as inulin-type fructans (ITF), in particular *Lactobacillus paracasei* and *Lactobacillus delbrueckii* (Goh et al., 2007; Kaplan and Hutkins, 2003; Makras et al., 2005; Oliveira et al., 2012; Tsujikawa et al., 2013). Also, growth of the dairy isolate *Lactobacillus acidophilus* IBB 801 occurs on oligofructose (Makras et al., 2005). However, a mechanistic study of oligofructose degradation by this strain is still lacking. As for certain bifidobacteria (Ryan et al., 2005), an oligofructose utilization operon has been shown in the case of *Lactobacillus acidophilus* NCFM (Barrangou et al., 2003), *Lactobacillus paracasei* 1195 (Goh et al., 2006, 2007), and *Lactobacillus plantarum* WCFS1 (Saulnier et al., 2007). Possible cross-feeding interactions between lactobacilli and lactate-consuming butyrate-producing colon bacteria have not been investigated up to now.

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The presence of lactic acid bacteria in the human colon has been historically regarded as beneficial for health and well-being due to their therapeutic potential (Kailasapathy and Chin, 2000; Nagpal et al., 2012). Next to the endogenous lactic acid bacterial communities, many of these bacteria enter the colon through the consumption of fermented foods and beverages (Borriello et al., 2003; Fontana et al., 2013; Kailasapathy and Chin, 2000; Nagpal et al., 2012; Oliveira et al., 2012). In the colon, they degrade non-digestible carbohydrates as their energy sources homo- or heterofermentatively, resulting in the production of mainly lactate (Macfarlane and Gibson, 1996). This is confirmed by their stimulation in the human colon of individuals consuming these substrates, besides an increase of the relative proportions of *Bifidobacterium* spp. and *A. caccae* (Dewulf et al., 2013). An increase in the relative proportions of *E. hallii* upon ITF ingestion has also been reported in some individuals (Louis et al., 2010).

In contrast to certain *Roseburia* spp., *E. rectale* and *F. prausnitzii*, butyrate-producing colon bacteria such as *E. hallii* and *A. caccae* are not able to degrade oligofructose and/or inulin (Falony et al., 2009c; Moens et al., 2014b). Instead of consuming oligomers or polymers, their metabolism targets a fast consumption of carbohydrate monomers and lactate (Belenguer et al., 2007, 2011; Duncan et al., 2004; Falony et al., 2009c; Moens et al., 2014b). Hence, the initial degradation of ITF by lactobacilli could result in the production of lactate and the release of fructose, which can serve as ideal substrates for butyrate-producing colon bacteria. A systematic approach to investigate the production of these cross-feeding substrates during ITF degradation by representatives of lactobacilli and bifidobacteria is hence necessary.

The present study aimed at investigating the cross-feeding interactions among two strains of *Lactobacillus* species (one originating from the human colon and one from a dairy product) and two lactate-consuming, acetate-dependent, butyrate-producing colon bacterial strains belonging to the species *A. caccae* and *E. hallii*, in the presence of ITF, both in the absence and presence of a bifidobacterial strain.

2. Materials and methods

2.1. Microorganisms and media

Anaerostipes caccae DSM 14662^T and *E. hallii* DSM 17630 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Göttingen, Germany). *Bifidobacterium longum* subsp. *longum* LMG 11047 was obtained from the Belgian Coordinated Collections of Microorganisms - Laboratory for Microbiology Ghent (BCCM-LMG, Ghent, Belgium). All these strains are human isolates. The lactobacilli used throughout this study were *Lb. acidophilus* IBB 801 and *Lb. paracasei* subsp. *paracasei* 8700:2. *Lactobacillus acidophilus* IBB 801 is a dairy isolate with potential probiotic properties and producing the bacteriocin acidophilin 801 (Zamfir et al., 1999). *Lactobacillus paracasei* subsp. *paracasei* 8700:2 is an isolate from the colon mucosa of healthy individuals (Ahrné et al., 1998). All strains were stored at −80 °C in reinforced clostridial medium (RCM; Oxoid Ltd., Basingstoke, United Kingdom) in the case of *A. caccae* DSM 14662^T, *E. hallii* DSM 17630 and *B. longum* LMG 11047, or in de Man-Rogosa-Sharpe medium (MRS; Oxoid Ltd.) in the case of *Lb. acidophilus* IBB 801 and *Lb. paracasei* subsp. *paracasei* 8700:2. These media were supplemented with 25% (vol/vol) of glycerol as a cryoprotectant. In the case of *E. hallii* DSM 17630, 2 mL L^{-1} of Tween 80 (VWR International, Darmstadt, Germany) was added to RCM.

A modified version of a medium for colon bacteria (mMCB) to allow growth of *F. prausnitzii* was used for the cultivation experiments (Moens et al., 2016). This medium was composed as follows (g L^{-1}): bacteriological peptone (Oxoid), 6.5; soy peptone (Oxoid), 5.0; yeast extract (VWR International), 3.0; tryptone (Oxoid), 2.5; NaCl (VWR International), 4.5; K_2HPO_4 (Merck, Darmstadt, Germany), 0.45; KH_2PO_4 (Merck), 0.45; $MgSO_4 \cdot 7H_2O$ (Merck), 0.09; $CaCl_2 \cdot 2H_2O$ (Merck), 0.09; cysteine-HCl (Merck), 0.4; $NaHCO_3$ (VWR International), 0.2; $MnSO_4 \cdot H_2O$ (VWR International), 0.05; $FeSO_4 \cdot 7H_2O$ (Merck), 0.005; $ZnSO_4 \cdot 7H_2O$ (VWR International), 0.005; hemin (Sigma-Aldrich, Steinheim, Germany), 0.005; menadione (Sigma-Aldrich), 0.005; and resazurin (Sigma-Aldrich), 0.001. To this medium, 2 mL L^{-1} of Tween 80 (VWR International) was added, except for the monoculture fermentation with *B. longum* LMG 11047 on inulin. In some cases (see below), acetate (50 mM or 6.8 g L^{-1} of $CH_3COO^- Na^+.3H_2O$; Merck) was added to mMCB. The pH of the medium was adjusted to 6.3, corresponding with the average pH of the colon, and the media were autoclaved at 210 kPa and 121 °C for 20 min. After sterilization, fructose (VWR International), oligofructose (Raftilose P95; Beneo-Orafti NV, Tienen, Belgium; Falony et al., 2009a), or inulin (OraftiHP; Beneo-Orafti; Falony et al., 2009a) were added as the sole energy sources aseptically, always at a final concentration of 50 mM fructose equivalents (FE), using sterile stock solutions. Stock solutions of oligofructose and inulin were made sterile by membrane filtration using Minisart filters (pore size, 0.2 μm; Sartorius, Göttingen, Germany); for sterilization of fructose, see below.

For the cultivation experiments in bottles, stock solutions of fructose were initially made anaerobic through autoclaving at 210 kPa and 121 °C for 20 min. The solutions were subsequently filter-sterilized and transferred into glass bottles, which were sealed with butyl rubber septa that were pierced with a Sterican needle (VWR International) connected with a Millex-GP filter (Merck) to assure sterile conditions. For the cultivation experiments in fermentors, fructose stock solutions were autoclaved under the same conditions as the media.

2.2. Comparative genomics in view of the presence of β-fructofuranosidase genes in Lactobacillus acidophilus IBB 801

The genome of *Lb. acidophilus* IBB 801 was sequenced recently (V. Pothakos, M. Verce, and L. De Vuyst, unpublished results). Gene prediction and annotation of the draft genome was carried out using the bacterial genome annotation system GenDB version 2.2 (Meyer et al., 2003). Based on this genome sequence and the publicly available complete genomes of *Lb. acidophilus* NCFM (Altermann et al., 2005), 30SC (Oh et al., 2011), and La-14 (Stahl and Barrangou, 2013), a comparative genomic analysis was carried out, using Mauve Aligner (Darling et al., 2010; Rissman et al., 2009), as to assess the presence of β-fructofuranosidase genes.

2.3. Cultivation experiments in stationary bottles

Monoculture cultivation experiments with *A. caccae* DSM 14662^T and *E. hallii* DSM 17630 in mMCB without controlling the pH were performed in stationary glass bottles to investigate their consumption of fructose and lactate, whether or not with the addition of acetate. The bottles contained 100 mL of mMCB, supplemented with either fructose, lactate, or fructose and lactate as the sole energy sources. The inocula were prepared as follows. Cells of the strains under study were transferred from −80 °C to test tubes containing 10 mL of RCM (supplemented with 2 mL L^{-1} of Tween 80 for *E. hallii* DSM 17630) that were incubated anaerobically at 37 °C for 16 h (*E. hallii* DSM 17630) or 24 h (*A. caccae* DSM 14662^T). Subsequently, the strains were propagated twice for 24 h in glass bottles containing 100 mL of mMCB (with or without acetate depending on the strain), supplemented with the energy source under study. These precultures were finally added to the glass bottles aseptically. During the inoculum build-up, the transferred volume was always 5% (vol/vol). All bottles were equipped as described above and incubated anaerobically in a modular atmosphere-controlled system (MG anaerobic work station; Don Withley Scientific Ltd., West Yorkshire, United Kingdom) that was continuously sparged with a mixture of 80% N_2, 10% CO_2, and 10% H_2 (Air Liquide, Paris, France). Samples for further analyses were withdrawn after 0, 6, 12, 24, and 48 h. All experiments were performed in duplicate. The results presented onward are representative for both experiments.