Journal of Functional Foods 40 (2018) 9–17

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Effect of butyrate and fermentation products on epithelial integrity in a mucus-secreting human colon cell line

Ditte Søvsø Gundelund Nielsen*, Bent Borg Jensen, Peter Kappel Theil, Tina Skau Nielsen, Knud Erik Bach Knudsen, Stig Purup

Department of Animal Science, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark

ARTICLE INFO

Keywords:
Fermentation
Arabinoxylan
Butyrivibrio fibrisolvens
Short-chain fatty acids
Butyrate
Intestinal epithelial barrier function

ABSTRACT

Butyrate produced by microbial fermentation of dietary fibres beneficially affect the colonic epithelium. Here we enhanced butyrate production through *in vitro* incubations of different sources of arabinoxylan (AX) with butyrogenic bacteria (*Butyrivibrio fibrisolvens, Eubacterium rectale*) to explore their potential synbiotic effects on the intestinal epithelial barrier function. *B. fibrisolvens* incubated with AX produced the highest butyrate levels (15 mM). Sodium-butyrate (1–10 mM) and fermentation products (5% v/v) from *B. fibrisolvens* increased the barrier function in a human colonic epithelial cell line. This was associated with regulation of different genes involved in epithelial integrity, mucus secretion, apoptosis, oxidative stress, and butyrate transport. In conclusion, butyrate in concentrations that can be achieved by dietary intervention *in vivo* enhanced the epithelial barrier function *in vitro*. *B. fibrisolvens* might be a potential new probiotic for increasing colonic butyrate production in humans, specifically in synbiotic combination with AX, a common DF component of food cereals.

1. Introduction

The highest prevalence of colonic diseases such as inflammatory bowel diseases and colorectal cancer is found in western countries. However, incidences in African and South-Central Asian populations are increasing as nations become more industrialized and adapt a westernized lifestyle (Jemal et al., 2011; Molodecky et al., 2012). These colonic diseases are often associated with alterations in the colonic microbiota, which is largely affected by diet. Specifically, intake of carbohydrates not digested in the small intestine (dietary fibre, DF) contributes to maintain a diverse and active microbiota and to the health of the colon (Wong, de Souza, Kendall, Emam, & Jenkins, 2006). The average daily intake of DF for adults in Denmark is 18 g in adults (Pedersen et al., 2010) compared to 12 and 18 g in countries like the UK and Spain (PHE., 2014; Serra-Majem et al., 2007); for all European countries the DF intake is below the recommended intake of 25–35 g per day (Jonsdottir et al., 2013).

The human colon harbours a diverse microbiota with approximately 10^{11} colony forming units (cfu)/g content, consisting of 500–1000 different bacterial species (Sekirov, Russell, Antunes, & Finlay, 2010). These microbes ferment DF and protein and produce short-chain fatty acids (SCFA) and essential micronutrients (Hill, 1997; Windey, De Preter, & Verbeke, 2012). The SCFAs formed during the colonic fermentation process include acetate, propionate and butyrate, which provide energy for the colonic cells (Scheppach, 1994) and up to 10% of the daily energy demand for maintenance in humans (McNeil, 1984). Butyrate induces several positive effects on the colonic epithelium (Leonel & Alvarez-Leite, 2012) through regulation of gene expression by inhibition of histone deacetylases (Candido, Reeves, & Davie, 1978). Butyrate has been shown to increase the epithelial barrier function in Caco-2 cells (Peng, He, Chen, Holzman, & Lin, 2007; Peng, Li, Green, Holzman, & Lin, 2009), cdx2-IEC monolayers (Wang, Wang, Wang, Wan, & Liu, 2012) and IPEC-J2 cells possibly by increasing the tight junction proteins (Ma et al., 2012). Also, butyrate induces the formation of mucins produced by goblet cells lining the colon (Finnie, Dwarakanath, Taylor, & Rhodes, 1995; Gaudier et al., 2004). Such modulations enhance the colonic epithelial barrier function and indeed, a link between low colonic levels of butyrate and IBD has been suggested (Fava & Danese, 2011).

Arabinoxylan (AX) is an important DF constituent in food cereals (Izydorczyk & Biliaderis, 1995; Saulnier, Sado, Branlard, Charmet, & Guillon, 2007). AX has a complex structure consisting of a backbone of xylose units substituted with arabinose monomers and with a structure that varies between cereals and between the different tissue layers ((Izydorczyk & Biliaderis, 1995; Saulnier et al., 2007). AX has shown prebiotic potential, beneficially affecting the colonic

* Corresponding author.
E-mail addresses: DitteG.Nielsen@anis.au.dk (D.S.G. Nielsen), BentBorg.Jensen@anis.au.dk (B.B. Jensen), Peter.Theil@anis.au.dk (P.K. Theil), TinaS.Nielsen@anis.au.dk (T.S. Nielsen), Knuderik.Backknudsen@anis.au.dk (K.E.B. Knudsen), Stig.Purup@anis.au.dk (S. Purup).

http://dx.doi.org/10.1016/j.jff.2017.10.023
Received 9 January 2017; Received in revised form 19 September 2017; Accepted 12 October 2017

epithelium (Cloetens et al., 2010). The prebiotic potential have further been found to vary depending on whether AX is provided as a polymer or as AX oligosaccharides (AXOS) (Cloetens et al., 2010). In the current study we investigated butyrate production following incubations between the butyrate-producing bacteria *B. fibrisolvens* and *E. rectale* with different AX substrates. The effects were tested on the epithelial barrier function by measuring transepithelial electrical resistance (TEER) and the paracellular permeability. Finally, treatment effects on expression of genes related to epithelial integrity, mucus production, cell turnover, oxidative stress and butyrate transport were evaluated. It was hypothesized that AX and butyrate producing bacteria in synergy might enhance butyrate production with positive effects on the intestinal epithelial barrier function.

2. Experimental section

2.1. Substrates

The substrates used were commercial and unhydrolysed wheat bran (UWB, kindly provided by Lantmännen Cereala, Vejle, Denmark), enzymatically treated wheat bran (ETWB, kindly provided by DuPont Industrial Biosciences, Brabrand, Denmark), wheat flour AX (Megazyme, Batch MWP 90801) and wheat starch (Sigma-Aldrich, Brøndby, Denmark). The ETWB was prepared as described by (Nielsen et al., 2014).

2.2. HT29-MTX-E12 cell culture

The human mucus-secreting colorectal adenocarcinoma cell line HT29-MTX-E12 (E12) was kindly donated by Dr. David Brayden and Dr. Sam Mayer (UCD Conway Institute, Dublin, Ireland). The cells were maintained in 75 cm^2 cell culture flasks in Dulbecco's Modified Eagle medium (DMEM, Life Technologies, Naerum, Denmark) supplemented with 4 mM GlutaMax (Life Technologies, Naerum, Denmark), 10 mM HEPES buffer solution (Life Technologies, Naerum, Denmark), 1% (v/v) penicillin-streptomycin (Sigma-Aldrich, Brøndby, Denmark) and 10% fetal calf serum (FCS, Bio-Whittaker, Vallensbæk Strand, Denmark). Cells were passaged using 0.05% Trypsin-EDTA (Life Technologies, Naerum, Denmark) when reaching 80–90% confluence. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Cell culture assays

E12 cells were seeded at a density of 2×10^5 cells/cm^2 on polyethylene terephthalate cell culture inserts with a pore size of 0.4 μm in 6-well cell culture plates (BD FALCON, Albertslund, Denmark). Cells were cultured in DMEM containing 10% FCS and the media were changed every 48 to 72 h. All assays were carried out within cell passage 46–51 to ensure reproducibility. Cells were cultured for 43 days until reaching full differentiation as determined by transepithelial electrical resistance (TEER) and paracellular flux measurements. Cell layers were treated with 50 μM deoxynivalenol (DON, Sigma-Aldrich, Brøndby, Denmark) for 72 h followed by sodium-butyrate or selected fermentation products at 5% (v/v) for 5 days in cell culture medium without FCS in duplicate wells (Fig. 1). Deoxynivalenol was dissolved in 96% ethanol and butyric acid (Sigma-Aldrich, Brøndby, Denmark) was dissolved in serum-free media at final concentrations of 0.1, 1, 5, 10, 50 and 100 mM. All solutions were sterile-filtered.

2.4. Transepithelial electrical resistance (TEER)

TEER was measured in triplicates/well using a Millicell-ERS volt-ohm meter combined with a MERSSTX03 electrode (Millipore, Hellerup, Denmark) according to the manufacturer's guidelines.

2.5. Paracellular tracer flux assay

Fluorescein-isothiocyanate (FITC)-dextran (Sigma-Aldrich, Brøndby, Denmark) was added apically at a concentration of 2.2 mg/ml in cell culture medium (0% FCS) and incubated at 37 °C for 1 h as described previously (Pinton et al., 2009). The flux of FITC-dextran across the E12 monolayer was measured in duplicates from each basolateral compartment. Measurements were performed on an EnVision 2103 Multilabel Reader (Envision, PerkinElmer). Excitation wavelengths were set at 485 nm and emission was recorded at 535 nm.

2.6. Microorganisms and media

Butyrivibrio fibrisolvens (DSM3071, Braunschweig, Germany) and *Eubacterium rectale* (Bent Borg Jensen, Aarhus University) were grown at room-temperature in sterile and anaerobic CGCM broth as described previously (Holdeman, Cato, & Moore, 1977) except the rumen extract was replaced by pig colon extract.

2.7. In vitro fermentations and butyrate analysis

One hundred mg of UWB, ETWB, AX and wheat starch were added to separate Hungate tubes containing 10 ml 90% (v/v) CGCM broth (without sugars) and inoculated with 100 μl of a 48 h pre-culture of bacteria (1% v/v). The bacteria-substrate mixtures were incubated anaerobically at 37 °C for 48 h. The supernatant was collected by centrifugation at 15.000*g* for 10 min at 4 °C. The concentration of butyrate from *in vitro* fermentations were quantified by gas chromatography as described previously (Canibe, Hojberg, Badsberg, & Jensen, 2007), except that 1 ml of fermentation solution was diluted 10-fold with a 0.028 M sodium hydroxide solution containing 100 mM of ethylbutyric acid (Sigma-Aldrich, Brøndby, Denmark) as the internal standard. The standard mixture was as described previously (Jensen, Cox, & Jensen, 1995). The mixtures were analysed on a Hewlett Packard 6890 series gas chromatograph (Agilent Technologies, Naerum, Denmark) equipped with a flame-ionization detector. The samples were injected with an AutoSampler (HP6890 injector) and data were analysed with GC ChemStation (Agilent Technologies, Glostrup, Denmark). A 30 m BP1 column (Scientific glass engineering) with an internal diameter of 0.25 mm coated with 100% dimethyl polysiloxane with a film thickness of 0.25 μm were used for the separation. Helium was used as the carrier gas with a pressure of 17 psi. Inlet and detector temperature was set at 250 °C and 300 °C, respectively. Two microliter sample was injected with a split-mode of 25:1 and the compounds were eluted with the following temperature gradient: 70 °C for 2 min, 4 °C/min to 110 °C, 15 °C/min to 180 °C, 25 °C/min to 300 °C and finally held at 300 °C for 3.03 min.

2.8. Real time reverse-transcription PCR

E12 cells were collected in RNAlater and stored at -80 °C. Total RNA was isolated and purified according to the NucleoSpin RNA II manual (Machery-Nagel, Düren, Germany). Total RNA concentration and purity was assessed by measuring the absorbance at 260 and 280 nm. The RNA was reverse transcribed using oligo(dT) primers, random primers and SuperScript III Reverse Transcriptase (Invitrogen, Naerum, Denmark). The cDNA samples were diluted 1:10 and cDNA synthesis was performed on an Esco Swift MaxPro Thermal Cycler (Holm & Halby, Brøndby, Denmark). Real time PCR quantitation was performed by SYBR GREEN PCR master mix (Applied Biosystems, Stockholm, Sweden) and appropriate primers (Table 1, Life Technologies, Naerum, Denmark). For probe-based detection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) TaqMan Universal PCR master mix (Applied Biosystems, Stockholm, Sweden) was used with the probe 5′ CGCCTGGTCACCAGGGCTGCT 3′. PCR was performed using a ViiaA7 Real-Time PCR System (Applied Biosystems, Stockholm, Sweden) with 40