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Short communication

Evaluation of the prebiotic properties of wheat arabinoxylan fractions and induction of hydrolase activity in gut microflora

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Abstract

Dietary supplementation with prebiotics may result in the stimulation of the growth of beneficial bacteria such as lactobacilli and bifidobacteria in the human gastrointestinal tract. The effect of water-unextractable arabinoxylans (WU-AX) derived from wheat on the modulation of gut bacterial composition was investigated using a mixed culture fermentation system. A prebiotic index (PI) score of 2.03 was obtained after addition of 1% (w/v) WU-AX to a pH-controlled stirred anaerobic fermentation vessel. Pretreatment of the WU-AX with endo-β-1,4-xylanase resulted in significantly higher PI value (3.48) indicating that pretreatment provided oligomers that were better utilised by the gut bacteria. The extracellular hydrolytic enzymes xylanase and ferulic acid esterase are both required for bacterial metabolism of WU-AX and both activities were present in supernatants derived from the mixed batch cultures. Addition of the WU-AX substrates to the batch cultures produced several fold increases of bacterial synthesis of both enzymes, and these increases were greater when the WU-AX substrate was pretreated with xylanase.

Keywords: Arabinoxylan; Prebiotic; Xylanase; Ferulic acid esterase; Gut bacteria

1. Introduction

Significant quantities of nonstarch polysaccharides (NSP) are consumed by humans on a daily basis. Many of these carbohydrates are not digested in the small intestine but provide fermentable carbon sources for bacteria that inhabit the large bowel (Hopkins et al., 2003). Approximately 50% of this ingested material is derived from cereals in which arabinoxylans (AX) are the predominant hemicelluloses accounting for 30% of the dry weight, compared with 5% of the dry weight of vegetables (Cartano and Juliano, 1970). AX are primary components of the walls that surround plant cells in the starchy endosperm of most cereals, although the major dietary source is likely to be wheat in which approximately 64% of some non-endosperm tissues are composed of AX (Hoffman et al., 1992; McDougall et al., 1996). Wheat AX can be divided into soluble water-extractable (WE-AX) and insoluble water-unextractable (WU-AX) fractions, which have different physicochemical properties (Perlin, 1951).

Arabinoxylans can be degraded by a battery of microbial and plant enzymes such as xylanases, arabinofuranosidases, acetyl esterases, ferulic acid esterases and methyl glucuronidases. Xylanases (EC. 3.2.1.8) belong to two main groups based on their primary sequences and structures (http://afmb.crns-mrs.fr/~cazy/CAZY/). Over the last 16 years ferulic acid esterases, which are responsible for cleaving the ester-bond between plant cell wall polysaccharides and phenolic acids, have been purified and partially characterised (Topakas et al., 2003). These act synergistically with xylanases to release ferulic acid (FA) from arabinoxylan. The factor that determines the rate at which specific esterases release FA from feruloylated insoluble wheat flour arabinoxylan is the ability of different xylanases to produce short chain feruloylated xylooligosaccharides (Vardakou et al.,

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2004). The Advisory Committee on Novel Foods and Processes (Food Standards Agency, UK) sought to increase the base of knowledge regarding the fate of AX in the digestive tract (Charalampopoulos et al., 2002). In recent years a number of oligomers termed prebiotics have been described. These resist digestion in the upper GI tract but are metabolised by certain beneficial bacteria, resulting in a healthier gut flora (Roberfroid, 2001). Most prebiotics, including fructans, galactooligosaccharides, lactulose and raffinose have been shown to have bifidogenic properties (Bouhnik et al., 2004). Previous studies indicated that oat bran, which contains high levels of β-D glucan and arabinoxylans, selectively stimulated the growth of the probiotic strains of *Lactobacillus* and *Bifidobacterium* (Jaskari et al., 1998). An earlier study (Yamada et al., 1993) also indicated that arabinoxylan hydrolysates were utilised by bifidobacterial species.

The aims of this paper were to examine the potential prebiotic effect of wheat arabinoxylan (AX), and to investigate the effect of AX administration on the levels of hydrolytic enzymes produced by a gut microbial community.

2. Materials and methods

2.1. Arabinoxylan substrates

Insoluble feruloylated arabinoxylan (WU-AX), the water insoluble pentosan fraction from wheat flour, was purchased from Megazyme (Wicklow, Ireland). The fraction was free from starch, β-glucan and protein. Its purity was 95% with an arabinose to xylose ratio (A/X) of 0.69. The alkali-extractable ferulic acid (FA) content of WU-AX was 0.43% of dry weight. It was measured by incubation of 10 mg of WU-AX with 40 ml of 0.1 M NaOH, for 22 h, at room temperature, in the dark. After centrifugation of the preparation at 10,000 ×g for 10 min, the supernatant was neutralised with 1 M HCl and the FA content was assessed by HPLC using a Nucleosil column (250 mm × 4.6 mm, Agilent technologies, Santa Clara, USA) with acetonitrile:water:formic acid (1.3:7:1) as the mobile phase at a flow rate of 1.0 ml min^{-1} at ambient temperature. The ferulic acid elution was detected by a UV detector (Jasco, Great Dunmow, UK) set at 300 nm (Vardakou et al., 2003).

Endo-β-1,4-xylanase from *Thermoascus aurantiacus* was produced and purified as described previously (Kalogeris et al., 1998). The WU-AX was hydrolysed by adding 1 g of WU-AX to 100 ml of a solution containing 1 U ml^{-1} of xylanase in 50 mM sodium phosphate–citric acid buffer, pH 5, and incubating the mixture for 4 h at 50 °C. Xylanase activity was determined using the soluble substrate arabinoxylan (WE-AX) as described previously (Katapodis et al., 1999). The hydrolysed mixture was freeze dried.

2.2. Preparation and operation of batch fermenters

Fresh faecal samples from a healthy human volunteer who had no history of antibiotics treatment in the previous 6 months were first diluted (1:10) in anaerobic buffer containing 0.1 M phosphate-buffered saline (PBS) solution, pH 7.4, and then were homogenised using a Stomacher 400 (Seward, Worthing, UK) at 200 rpm for 2 min and filtered via stomacher bags (Seward). A 30 ml portion of the filtered faecal slurry was added to 270 ml growth medium in 300 ml batch fermentation vessels. Before inoculation with the faecal material 3 g of the arabinoxylan fractions was placed in two of the sample vessels. The third vessel, without arabinoxylan, was used as the control. Batch fermentation was performed as described by Vulevic et al. (2004). The vessels were magnetically stirred and maintained under anaerobic conditions by continuous sparging with oxygen-free nitrogen. Temperature and pH were automatically controlled at 37 °C and pH 6.8, respectively. Three separate fermentation experiments were carried out. At each experiment a 3 ml sample was taken from each vessel at 0, 8, 24, and 48 h.

2.3. Bacterial enumeration

The samples from each vessel were immediately transferred to an anaerobic cabinet (Don Whitley Scientific, Shipley, UK) containing an atmosphere of 10% H_2, 10% CO_2, and 80% N_2, and were serially diluted with pre-reduced half strength peptone water, pH 7, supplemented with 0.5 g cysteine–HCl l^{-1}. Portions of 20 μl from each dilution were plated, in triplicate, onto agar plates. The selective growth media used were: Wilkins Challegren agar, for total anaerobes; brucella blood-based agar, supplemented per litre with 75 mg kanamycin, 5 mg haemin, 75 mg vancomycin and 50 ml laked horse blood, for *Bacteroides* spp.; reinforced clostridial agar, supplemented per litre with 8 mg novobiocin and 8 mg colistin, for *Clostridium* spp.; rogosa agar, supplemented with 1.32 ml glacial acetic acid l^{-1}, for *Lactobacillus* spp.; Columbia agar containing per litre 5 g glucose, 0.5 g cysteine HCl, and 0.5 ml propionic acid, pH 5.0, for *Bifidobacterium* spp.; nutrient agar, for total aerobes; MacConkey No. 3 agar, for enterobacteria; and azide agar, for gram positive cocci. All agars were purchased from Oxoid (Basingstoke, Hants, UK) and prepared according to the supplier's instructions. All the antibiotics used in the preparation of media were purchased from Sigma (UK). For further confirmation of culture identity, ten randomly selected isolates from each agar were characterised by identification of their 16S rDNA sequence. PCR amplifications were performed using primers AmpF (5′-GAGAGTTTGATY[1]CTGGCTCAG-3′) and AmpR (5′-AGGAGGTGATCCAR[2]CCGCA-3′) to amplify the V6 region of the 16S rDNA gene. DNA sequencing of the amplified fragment was carried out using a DNA sequencer (model 373A; Applied Biosystems, Warrington, UK) following the manufacturer's instructions.

2.4. Enzymes assays

Two synthetic chromophoric substrates based on 4-methylumbelliferone (MU), MU-xylobioside (MU-X_2) and MU-dihydroferulate (MU-F), both kindly provided by Prof M. Claeyssens, University of Ghent, were used to detect xylanase and ferulic acid esterase activities, respectively, in the samples.