JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Dietary Fiber from Coffee Beverage: Degradation by Human Fecal Microbiota

DIANA GNIECHWITZ,† NICOLE REICHARDT,‡ MICHAEL BLAUT,‡ HANS STEINHART,† AND MIRKO BUNZEL*,†

Institute of Biochemistry and Food Chemistry, Department of Food Chemistry, University of Hamburg, Grindelallee 117, 20146 Hamburg, Germany, and German Institute of Human Nutrition Potsdam−Rehbruecke, Arthur-Scheunert-Allee 114−116, 14558 Nuthetal, Germany

Arabinogalactans and galactomannans from coffee beverages are part of the dietary fiber complex. Chemical structures and fermentability of soluble dietary fiber obtained from a standard filter coffee beverage (*Coffea arabica*, origin Colombia, medium roasted) by human intestinal bacteria were investigated. One cup (150 mL) of filter coffee contained approximately 0.5 g of soluble dietary fiber (enzymatic−gravimetric methodology), 62% of which were polysaccharides. The remainder was composed of Maillard reaction products and other nonidentified substances. Galactomannans and type II arabinogalactans were present in almost equal proportions. Coffee dietary fiber was readily fermented by human fecal slurries, resulting in the production of short-chain fatty acids (SCFA). After 24 h of fermentation, 85% of total carbohydrates were degraded. In general, arabinosyl units from the polysaccharide fraction were degraded at a slower rate than mannosyl and galactosyl units. In the process of depolymerization arabinogalactans were debranched and the ratio of (1→3)-linked to (1→6)-linked galactosyl residues decreased. Structural units composed of (1→5)-linked arabinosyl residues were least degradable, whereas terminally linked arabinosyl residues were easily utilized. The impact of coffee fiber on numerically dominant population groups of the intestinal microbiota was investigated by fluorescence *in situ* hybridization combined with flow cytometry (FISH-FC). After 24 h of fermentation, an increase of about 60% of species belonging to the *Bacteroides*−*Prevotella* group was observed. The growth of bifidobacteria and lactobacilli was not stimulated.

KEYWORDS: Dietary fiber; coffee; *Coffea arabica*; methylation analysis; fermentation; FISH; SCFA; carbohydrate degradation; bacteroides

INTRODUCTION

Coffee beverages contain considerable amounts of polysaccharides, mainly galactomannans and type II arabinogalactans. Galactomannans from roasted coffee infusions are composed of a backbone of β-(1→4)-linked mannopyranosyl units, which are partially substituted with single galactopyranosyl residues in the O-6-position (*1*, *2*). Compared to commercially available galactomannans derived from carob (*Ceratonia siliqua*), guar (*Cyamopsis tetragonolobus*), or tara (*Caesalpinia spinosa*), mannans from coffee brews are less substituted with galactosyl residues. Only about 4−5% of the mannosyl backbone units are branched (*3*). In contrast, type II arabinogalactans from coffee extracts are described as highly branched polysaccharides with ramified chains of (1→3)- and (1→6)-linked β-D-galactopyranosyl units. The attached side chains are composed of arabinosyl and galactosyl residues (*1*, *2*). Galactomannans and arabinogalactans ingested with coffee beverages belong to the dietary fiber complex, which includes polysaccharides, oligosaccharides, lignin, and associated plant substances that are not degraded by human digestive enzymes (*4*). Thus, they reach the colon and potentially serve as substrates for the colonic microbiota. A high intake of dietary fiber is considered to be positively related to several physiological and metabolic effects, such as the lowering of blood cholesterol (*5*) and the moderation of blood glucose and insulin response (*6*). In addition, fermentable polysaccharides are degraded by the colonic microbiota to short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate, as well as gases such as H_2, CH_4, and CO_2. SCFA production lowers the colonic pH, impeding the growth of certain pathogenic species and supporting the growth of bifidobacteria and other lactic acid bacteria that are considered to be beneficial for human health (*7*). Furthermore, SCFA have a trophic effect on the colonic mucosa and are metabolized by host tissues, with butyrate being the preferred energy substrate of the colonocytes (*8*). Butyrate also plays an important role in the maturation of colonic epithelium and the regeneration of the mucosa in the case of atrophy (*9*). Finally, butyrate is

* Author to whom correspondence should be addressed [telephone +49-(0)40-42838-4379; fax +49-(0)40-42838-4342; e-mail mirko.bunzel@uni-hamburg.de].
† University of Hamburg.
‡ German Institute of Human Nutrition.

10.1021/jf070646b CCC: $37.00
Published on Web 07/21/2007

suggested to induce cell differentiation and to stimulate apoptosis of cancerous cells (*10*).

The objective of the present study was to investigate the fermentability of dietary fiber from coffee brew by the intestinal microbiota to study compositional changes of dietary fiber polysaccharides during fermentation and to analyze the effect of fermentation on the growth and composition of fecal bacteria in batch cultures. For comparison, inulin, which is widely used as a dietary fiber supplement, was fermented under the same conditions.

MATERIALS AND METHODS

General. The reagent kit BIOQUANT Total Dietary Fiber was purchased from Merck KGaA (Darmstadt, Germany). Heat-stable α-amylase Termamyl 120 L (EC 3.2.1.1, from *Bacillus licheniformis*, 120 KNU/g), the protease Alcalase 2.4 L (EC 3.4.21.62, from *B. licheniformis*, 2.4 AU/g), and the amyloglucosidase AMG 300 L (EC 3.2.1.3, from *Aspergillus niger*, 300 AGU/g) were kindly donated by Novo Nordisk (Bagsvaerd, Denmark). Inulin was purchased from Sigma-Aldrich (Schnelldorf, Germany). All other chemicals were of analytical grade or the highest purity available.

Material. Medium-roasted and ground coffee (*Coffea arabica*; Colombia; total weight loss = 12.8%) was kindly provided by Tchibo GmbH, Hamburg, Germany.

Preparation of Coffee Beverage. Medium-roasted and ground Arabica coffee (50 g) was extracted with 1 L of tap water using a standard filter coffee machine. Following volume determination of the extract, the solution was freeze-dried and dry matter was determined gravimetrically.

Determination of Soluble and Insoluble Dietary Fiber Content. The soluble and insoluble dietary fiber contents of the freeze-dried coffee powder were determined using a commercially available reagent kit. The applied enzymatic−gravimetric method using MES/TRIS buffer (pH 8.2) and a sequence of heat-stable α-amylase, protease, and amyloglucosidase is described by Lee et al. (*11*). The dietary fiber content was corrected for residual protein and ash contents. Nitrogen content was determined according to the Kjeldahl method, analyzing Kjeldahl nitrogen colorimetrically as described by Willis et al. (*12*). Protein was calculated as N × 6.25. Ash content was determined gravimetrically by incineration of soluble dietary fiber at 525 °C for 5 h.

Isolation Protocol for Soluble Coffee Fiber. Soluble coffee fiber was prepared by an up-scaled and slightly modified version of the method used for the analytical determination of dietary fiber (*13*). Freeze-dried coffee powder (10 g) was suspended in sodium phosphate buffer (pH 6.0, 80 mM, 300 mL), and α-amylase (750 μL) was added. Beakers were placed in a boiling water bath for 20 min and shaken gently every 5 min. The pH was adjusted to 7.5 (∼60 mL of 0.275 M NaOH), and samples were incubated with protease (300 μL) at 60 °C for 30 min with continuous agitation. After the pH had been adjusted to 4.5 (∼60 mL of 0.325 M HCl), amyloglucosidase (350 μL) was added and the mixture was incubated at 60 °C for 30 min with continuous agitation. The suspension was centrifuged, and the volume of the supernatant was determined. Ethanol [95% (v/v), preheated to 60 °C, 4 volumes] was added, and the precipitate was allowed to form overnight at room temperature. After centrifugation, the residue was washed twice with 78% (v/v) ethanol, 95% (v/v) ethanol, and acetone and dried at 40 °C in a vacuum oven. Soluble dietary fiber was corrected for residual protein and ash contents as described above.

Sample Preparation for Chemical Analysis. Unfermented dietary fiber was analyzed as prepared. An additional cleanup was necessary for the analysis of fermented samples. Cells were removed by centrifugation at 37 °C (6 min, 4000*g*). The supernatant was membrane filtered (0.2 μm pore size) and rotary evaporated at reduced pressure and 40 °C.

Determination of Total Carbohydrates. The total carbohydrate content was determined using the phenol−sulfuric acid method as described by Dubois et al. (*14*). Standard calibration curves were obtained on the basis of mannose (as a representative for hexoses usually read at 490 nm) and arabinose (as a representative of pentoses and deoxysugars usually read at 480 nm). Analyzing of the samples, determination of the absorptions at 480 and 490 nm, and application of a simple mathematical model (elimination method) resulted in hexose contents and pentose/deoxysugar contents. To consider that carbohydrates in coffee dietary fiber are present as polysaccharides, the anhydrosugars were calculated. The total carbohydrate content was estimated as the sum of hexose and pentose/deoxysugar anhydrosugars.

Carbohydrate Analysis. Neutral sugars were released by Saeman hydrolysis, as modified by Englyst et al. (12 M H_2SO_4 for 5 min at room temperature, 2 M H_2SO_4 for 60 min at 100 °C) (*15*), and analyzed as their alditol acetates by GC-FID (*16*) (GC Focus Series, Thermo Electron S.p.A., Milan, Italy) on a DB-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). Chromatographic conditions were as follows: initial column temperature, 150 °C, held for 3 min, ramped at 3 °C/min to 190 °C, ramped at 4 °C/min to 220 min, held for 2 min; split injection (split ratio, 1/6; injector temperature, 250 °C); flame ionization detection (detector temperature, 290 °C). Helium (1.6 mL/min) was used as carrier gas.

Methylation Analysis. Methylation analysis was carried out as described by Nunes and Coimbra (*2*) with minor modifications. In brief, dried samples (1−3 mg) were dissolved in dry dimethyl sulfoxide. NaOH pellets (100 mg) were freshly powdered under nitrogen and added to the solution. Each sample was sonicated for 90 min at room temperature and stood for another 90 min at room temperature. Methyl iodide (1 mL) was added to the ice-cold solution. The mixture was allowed to react for 30 min being sonicated and stood for another 30 min at room temperature without sonication. Sodium thiosulfate (0.1 M, 3 mL) was added, and the methylated carbohydrates were extracted into chloroform (3 mL). The organic layer was washed with water (five times, 5 mL) and rotary evaporated at reduced pressure and 40 °C. The methylation step was repeated, and the remethylated material was hydrolyzed with TFA (2 M, 1 mL) at 121 °C for 1 h. Following rotary evaporation at reduced pressure and 40 °C, a freshly prepared solution of 20 mg of $NaBD_4$ in 0.3 mL of 2 M NH_3 was added to the partially methylated sugars. The mixture was allowed to react for 1 h at room temperature, and the reaction was stopped by adding glacial acetic acid (0.1 mL). Partially methylated alditols were acetylated by adding 1-methylimidazole (0.45 mL) and acetic anhydride (3 mL). Following a reaction time of 30 min, residual acetic anhydride was decomposed by adding water (3 mL). After extraction into dichloromethane (3 mL), the partially methylated alditol acetates were analyzed by GC-MS (HP 5890 series II GC, HP 5972 series mass selective detector, Hewlett-Packard, Waldbronn, Germany) using a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). GC conditions were as follows: initial column temperature, 140 °C, held for 2 min, ramped at 1 °C/min to 180 °C, held for 5 min; splitless injection (splitless time, 0.75 min; injector temperature, 210 °C); mass spectrometric detection (ionization energy, 70 eV; detector temperature, 220 °C; solvent delay, 8 min). He (1.08 mL/min) was used as carrier gas. The quantification of partially methylated alditol acetates was accomplished by GC-FID (GC Focus Series, Thermo Electron S.p.A., Milan, Italy) on a DB-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). Chromatographic conditions were as follows: the temperature program was used as described for GC-MS; split injection (split ratio, 1/6; injector temperature, 210 °C); flame ionization detection (detector temperature, 220 °C). He (1.6 mL/min) was used as carrier gas. Molar response factors according to Sweet et al. (*17*) were used for quantification.

Fecal Samples and Culture Conditions. Fecal samples were collected from four healthy volunteers (aged 25−45 years) who had no previous history of gastrointestinal disorders and had not undergone antibiotic therapy within at least 6 months prior to the study. Fresh fecal samples were diluted 10-fold (w/v) in prereduced phosphate-buffered saline (PBS) (containing per liter 8.5 g of NaCl, 0.3 g of KH_2PO_4, 0.6 g of Na_2HPO_4, 0.1 g of peptone, and 0.25 g of cysteine) and centrifuged (1 min, 300*g*) to remove large particles. Before inoculation, the fecal bacteria were washed three times with prereduced PBS (3 min, 6000*g*), optical density (OD) was adjusted (final value in the batch culture was ∼0.4), and the pellet was transferred to the fermentation

Table 1. 16S rRNA Targeting Oligonucleotide Probes Used for FISH-FC

probe	sequence (5′–3′)	OPD code	target organism	ref
EUB338	GCTGCCTCCCGTAGGAGT	S-D-Bact-0338-a-A-18	all bacteria	*41*
Clep866	GGTGGATWACTTATTGTG	S-*-Clep-0866-a-A-18	*Clostridium leptum* group	*42*
Fprau645	CCTCTGCACTACTCAAGAAAAAC	S-*-Fprau-0645-A-A-23	*Faecalibacterium prausnitzii* group	*43*
Erec482	GCTTCTTAGTCARGTACCG	S-*-Erec-0482-a-A-19	*Clostridium coccoides–Eubacterium rectale* group	*44*
Bac303	CCAATGTGGGGGACCTT	S-*-Bacto-0303-a-A-17	*Bacteroides–Prevotella* cluster	*45*
Bfra998	GTTTCCACATCATTCCACTG	S-S-Bfrag-0998-a-A-20	*Bacteroides fragilis*	*46*
Bvulg1017	AGATGCCTTGCGGCTTACGGC	S-S-Bvulg-1017-a-A-21	*Bacteroides vulgatus*	*46*
Bif164	CATCCGGCATTACCACCC	S-G-Bif-0164-a-A-18	bifidobacteria	*47*
Lab158	GGTATTAGCAYCTGTTTCCA	S-G-Lab-0158-a-A-20	lactobacilli and enterococci	*48*
NON EUB338	ACATCCTACGGGAGGC	NA[a]	negative control	*49*

[a] NA, not applicable.

vessels. Fermentations were carried out in 250 mL glass vessels containing basal liquid medium [100 mL, composition per liter: 0.35 g of K_2HPO_4, 0.23 g of KH_2PO_4, 0.5 g of NH_4Cl, 2.25 g of NaCl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.07 g of $CaCl_2 \cdot 2H_2O$, 0.005 g of $FeSO_4 \cdot 7H_2O$, 0.00015 g of $NaSeO_3$, 3.5 mg of tryptically digested peptone from casein, 3.5 mg of yeast extract, 0.5 g of cysteine·HCl, 4.0 g of $NaHCO_3$, and 3 mL of trace element solution SL10 (*18*)]. The pH was adjusted to 7.5, and the medium was gassed with N_2/CO_2 (80:20, v/v) and autoclaved at 121 °C for 15 min. Following autoclaving 5-*x*-vitamin solution (4 mL) (*19*) and coffee fiber or inulin, respectively, were added. The final dietary fiber concentration was 1.5 mg/mL. Aliquots (20 mL) were taken after 0, 3, 6, 12, and 24 h of incubation and analyzed as described.

Determination of OD, pH, and SCFA. Batch culture (0.5 mL) was centrifuged (5 min, 14000*g*), the pellet was resuspended in PBS (1 mL), and the resulting OD was determined at 600 nm (Beckman DU-640 spectrophotometer, Beckman Instruments Inc., Fullerton, CA). The pH was determined by using a MultiCal pH-meter (WTW, Weilheim, Germany).

Gas chromatographic SCFA determination was performed by adding isobutyric acid (12 mM, 23,6 μL) as internal standard, $HClO_4$ (0.36 M, 280 μL), and NaOH (1 M, 270 μL) to the batch culture supernatant (200 μL). The mixture was lyophilized and redissolved in a mixture of 400 μL of acetone and 100 μL of 5 M formic acid. After centrifugation (5 min, 14000*g*) at room temperature, the supernatant (1 μL) was analyzed using an HP GC 5890 series II (Hewlett-Packard, Waldbronn, Germany) equipped with a HP-FFAP capillary column (30 m × 0.53 mm i.d., 1 μm film thickness). Helium (1 mL/min) was used as carrier gas. Chromatographic conditions were as follows: initial column temperature, 85 °C, ramped at 7 °C/min to 140 °C, ramped at 70 °C/min to 190 °C, held for 5 min; split injection (split ratio, 1/10; injector temperature, 200 °C); flame ionization detection (detector temperature, 260 °C).

Cell Fixation and Hybridization of Fixed Cells with Fluorescent Oligonucleotide Probes and Flow Cytometric Analysis. Batch culture aliquots (6 mL) were washed with PBS and centrifuged (5 min, 10000*g*), and the pellet was resuspended in PBS (1.5 mL). The resulting suspension was mixed with 4% paraformaldehyde in PBS (1/3, v/v). Samples were fixed for 4 h at 4 °C and stored at −80 °C until hybridization. Hybridization was done as described by Rigottier-Gois et al. (*20*). Briefly, after one wash in TE buffer (10 mM Tris-HCl, 1 mM EDTA) and centrifugation (3 min, 8000*g*), pellets were resuspended in TE buffer containing 1 mg/mL lysozyme and incubated for 10 min at room temperature. Cells were washed in PBS (1 mL), centrifuged (3 min, 8000*g*, 4 °C), and equilibrated in hybridization buffer [1 mL, 900 mM NaCl, 20 mM Tris-HCl, 0.01% sodium dodecyl sulfate (SDS), 30% formamide, pH 8.0]. An aliquot of this suspension (50 μL) was used for FISH. Hybridization was performed overnight at 35 °C in 96-well microtiter plates containing 0.7 mM of the appropriate labeled oligonucleotide probe in the hybridization solution. All probes used are listed in **Table 1**. The NON EUB 338 probe was used as negative control probe, whereas the EUB 338 probe was used as positive control probe. The labeling quality of the probes was controlled as described previously (*21*). After hybridization, hybridization buffer (150 μL) was added to each well and the cells were pelleted (4500*g*, 20 min). Cells were then incubated for 20 min at 37 °C in a washing solution (65 mM NaCl, 20 mM Tris-HCl, 0.01% SDS, pH 8.0), pelleted (4500*g*, 20 min), and resuspended in PBS (200 μL). Aliquots (100 μL) were added to 250 μL of Facs Flow (Beckton Dickinson, Franklin Lakes, NJ) for flow cytometry. Flow cytometry was performed as described previously (*22*) using a Facs Calibur flow cytometer (Becton Dickinson) equipped with an air-cooled argon ion laser providing 15 mW at 488 nm combined with a 635 nm red diode laser. Data acquisition and subsequent analyses using CellQuest software (Beckton Dickinson) was performed as described previously (*22*).

Statistical Analyses. Standard deviations indicated for the characterization of unfermented soluble coffee dietary fiber are valid for the whole procedure (preparation of coffee beverage; determination of dry matter; preparation of soluble coffee fiber; determination of total sugar, sugars analysis, and methylation analysis, respectively) and do not just reflect reproducibility of the final analytical methodology. The whole procedure was carried out in triplicate. For fermentation experiments the soluble coffee fiber preparations were pooled and the fermentations were carried out in triplicate. Standard deviations indicated for the results of the analysis of fermented samples are valid for the whole procedure (fermentation; processing of fermented samples; determination of total sugar, sugars analysis, methylation analysis, respectively) and the four different fecal samples. Determination of OD, pH, and SCFA was carried out in duplicate. *p* values were obtained by two-tailed homoscedastic Student tests.

RESULTS AND DISCUSSION

Determination and Chemical Characterization of Coffee Dietary Fiber. Using a customary filter coffee machine, a coffee beverage with an average dry matter content of 1.6 g/100 mL was prepared from a medium-roasted Arabica coffee (Colombia). The dietary fiber content was determined by applying the widely accepted AOAC method 985.29 (*23*), as modified by Lee et al. (*11*) to allow the determination of insoluble and soluble dietary fiber. One cup (150 mL) of coffee beverage contained 0.5 g of soluble dietary fiber but no insoluble dietary fiber. However, using this enzymatic−gravimetric methodology, nondigestible oligosaccharides and other nondigestible substances that are soluble in 76% ethanol but are part of the dietary fiber complex are not determined. As expected, carbohydrates were the major constituents of the fiber fraction. The total carbohydrate content as determined using the phenol−sulfuric acid method was 62.1 ± 1.1%. Acid hydrolysis, derivatization, and subsequent GC-FID determination of the resulting alditol acetates indicated a deviating carbohydrate content of 54.4 ± 0.9%. Differences between the results using these two methodologies might have their origin in a low specificity of the phenol−sulfuric acid assay or incomplete hydrolysis and/or partial destruction of liberated monosaccharides using the GC-FID determination of alditol acetates. However, using varying hydrolytic conditions did not significantly enhance the total carbohydrate content. The polysaccharide fraction was composed

Table 2. Glycosidic Linkage Composition of Soluble Coffee Fiber Polysaccharides

glycosidic linkage	mol % (±SD)[a]
T-Rha*p*	2.0 (±0.4)
T-Ara*f*	8.6 (±0.8)
5-Ara*f*	2.9 (±0.4)
T-Man*p*	5.8 (±0.1)
4-Man*p*	45.2 (±2.0)
4,6-Man*p*	2.2 (±0.1)
4-Glc*p*	0.5 (±0.0)
T-Gal*p*	8.5 (±0.5)
3-Gal*p*	12.4 (±0.3)
6-Gal*p*	4.2 (±0.2)
3,6 Gal*p*	7.8 (±0.3)

[a] Standard deviations are valid for soluble dietary fibers isolated from three identically prepared filter coffee infusions.

of mannose (50.59 ± 1.60 mol %), galactose (38.91 ± 1.04 mol %), arabinose (8.82 ± 0.45 mol %), rhamnose (0.97 ± 0.13 mol %), and glucose (0.71 ± 0.01 mol %). Considering that galactomannans from roasted coffee are described as poorly substituted with galactose side chains (*24*), the neutral sugar composition reveals that the soluble coffee fiber contained galactomannans and arabinogalactans in almost equal proportions as confirmed by methylation analysis (**Table 2**). Galactomannans—identified by the presence of 4-Man*p*, T-Man*p*, and 4,6-Man*p*—accounted for approximately 55% of the polysaccharide fraction. Their average substitution degree was 4.0%. Nunes and Coimbra (*25*) demonstrated that galactomannans from coffee infusions also contain low amounts of β-(1→4)-linked glucopyranosyl residues (1 mol %). Consequently, 4-Glc*p* residues (0.5 mol % of total carbohydrates) detected in the polysaccharide fraction of soluble coffee fiber may have their origin from galactomannans.

The detected 3-Gal*p*, 6-Gal*p*, 3,6-Gal*p*, 5-Ara*f*, and T-Ara*f* units are typical structural elements of type II arabinogalactans from roasted coffee (*1*, *3*). The highly branched nature of arabinogalactans in coffee dietary fiber is indicated by a 3-Gal*p*/3,6-Gal*p* ratio of 1.6. Similar results have been published by Redgwell et al. (2.0−2.1) and Nunes and Coimbra (1.2−1.7) for arabinogalactans from Arabica coffees with different roasting degrees (*3*, *26*). Because 3,6-Gal*p* residues may also be part of (1→6)-linked galactan side chains, the (1→3)-linked galactan backbone is possibly less branched than suggested by the 3-Gal*p*/3,6-Gal*p* ratio. When side-chain galactosyl units were incorporated in the ratio, a (3-Gal*p* + 6-Gal*p*)/3,6-Gal*p* ratio of 2.1 was found. Branched galactosyl residues may bear single T-Ara*f* and T-Gal*p* residues as well as galactan or arabinan side chains, terminated with arabinosyl or galactosyl residues. Assuming that arabinosyl units exclusively stem from type II arabinogalactans, it is theoretically possible that each side chain contains one T-Ara*f* residue, as 3,6-Gal*p* and T-Ara*f* residues are present in almost equal amounts (T-Ara*f*/3,6-Gal*p* ratio of 1.1).

A slight brown color and UV absorbance of the soluble coffee fiber indicated the presence of Maillard reaction products. To date, little is known about the structural features of coffee melanoidins. It was proposed that low molecular weight chromophores are covalently linked to polysaccharides in roasted coffee infusions (*24*). However, high molecular weight Maillard reaction products may also contribute to the soluble fiber fraction of coffee infusions.

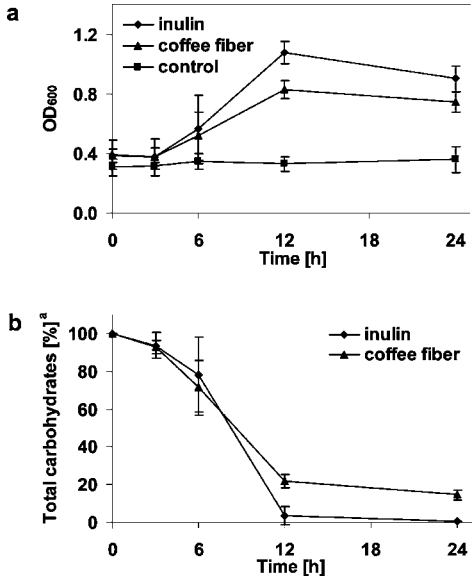

Figure 1. Changes of optical density (**a**) and total carbohydrate content (**b**) during fermentation of inulin and soluble coffee fiber with four different fecal samples. [a]Results refer to the total carbohydrate content determined at 0 h, which was set to 100%.

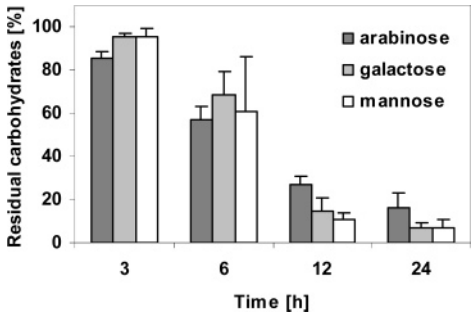

Figure 2. Degradation of arabinosyl, galactosyl, and mannosyl residues of coffee fiber polysaccharides during fermentation with four different fecal samples. Results refer to the total amounts of arabinose, galactose, or mannose determined at 0 h, which were set to 100%.

Fermentability of Soluble Coffee Fiber as the Sole Carbon Source by Human Fecal Microbiota. Soluble coffee fiber or inulin was added to batch cultures of diluted fecal bacteria and incubated anaerobically at 37 °C. The OD at 600 nm, which reflects bacterial cell density, was maximal after 12 h of incubation for both substrates. Inulin resulted in a 24% higher OD as compared to coffee fiber (**Figure 1a**). However, it must be considered that inulin is a pure polysaccharide, whereas coffee fiber additionally contains unknown components, which might be less easily utilized by human intestinal bacteria. Whereas inulin was fermented completely within 24 h, the coffee fiber polysaccharides were not fully degraded (**Figure 1b**). After 24 h of fermentation, 14.7% (phenol−sulfuric acid method) or 8.7% (determination as alditolacetates), respectively, of the fiber carbohydrates were not degraded, possibly due to more complex polysaccharide structures. Larch arabinogalactans were similarly reported to be fermented more slowly than other polysaccharides such as starch and pectins (*27*). However, it cannot be completely ruled out that Maillard reaction products negatively affect the degradability of coffee fiber polysaccharides.

The rate of carbohydrate degradation during the first hours of fermentation considerably differed depending on the human donor of the feces sample as indicated by larger standard

Table 3. Concentration (Millimolar) of SCFA after 24 h of Fermentation[a]

substrate	acetate	propionate	butyrate	total amount
inulin	8.04 ± 0.94	1.24 ± 0.74	1.57 ± 0.41***	10.73 ± 0.52***
coffee fiber	7.44 ± 0.07	1.34 ± 0.03	0.41 ± 0.03	9.17 ± 0.10

[a] Values given as mean ± SD; ***, $p < 0.001$

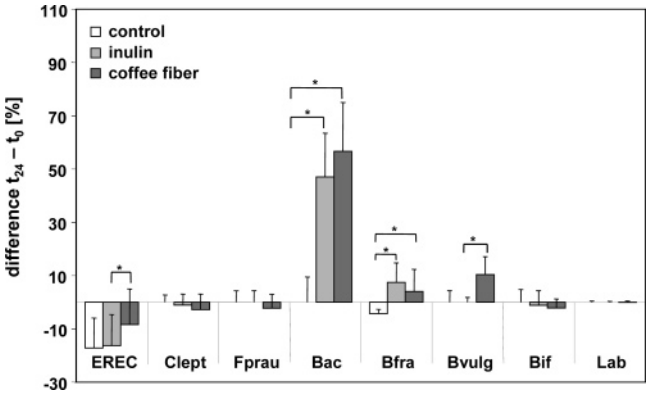

Figure 3. Changes of microbial composition in batch cultures after 24 h of fermentation of inulin or soluble coffee fiber with four different human fecal samples as determined by FISH-FC. EREC, *Eubacterium rectale–Clostridium coccoides* group; Clep, *Clostridium leptum* subgroup; Fprau, *Faecalibacterium prausnitzii*; Bac, *Bacteroides–Prevotella* group; Bfra, *Bacteroides fragilis*; Bvulg, *Bacteroides vulgatus*; Bif, bifidobacteria; Lab, lactobacilli. Values are given as mean ± SD; *, $p < 0.05$.

deviations, but after 12 h, similar levels of residual carbohydrates were observed (**Figure 1b**). This shows that the colonic microbiota from different human subjects degraded coffee fiber polysaccharides at different rates but to a similar extent.

Acetate, propionate, and butyrate (**Table 3**) were the fermentation products formed from coffee fiber and inulin. SCFA concentrations after 24 h of incubation were 9.17 ± 0.10 mM (coffee fiber) and 10.73 ± 0.52 mM (inulin). This difference was mainly based on a higher butyrate production from inulin, whereas no significant differences were found for acetate and propionate. In agreement with the formation of higher SCFA concentrations from inulin, the pH after 12 h was slightly lower during the fermentation of inulin (pH 7.17 ± 0.22) than during the fermentation of coffee fiber (pH 7.31 ± 0.19) (data not shown). The relatively high concentrations of acetic acid and propionic acid indicate that members of the genus *Bacteroides* played a major role in the degradation of coffee fiber and long-chain inulin. This is in agreement with the bacterial cell counts (**Figure 3**).

Structural Changes of Polysaccharides in the Course of Fermentation. More detailed information about the fermentability of mannans and arabinogalactans was obtained by investigating the degradation process of the main monosaccharide units (**Figure 2**). Whereas 93% of mannosyl and galactosyl residues were degraded within 24 h, only 84% of arabinosyl residues were utilized, indicating that galactomannans were more extensively degraded than arabinogalactans. Arabinose-containing sections of the arabinogalactans might hinder the polysaccharide degradation. Similar effects have been observed for larch arabinogalactans in fermentation experiments with *Bifidobacterium adolescentis* and *Bacteroides thetaiotaomicron* (*28*), showing that arabinosyl side chains were degraded to a lesser degree than the galactose backbone. However, arabinogalactan structures from larch and coffee brews are not fully comparable. Larch arabinogalactans contain β-(1→3)-linked arabinofuranosyl residues in their sidechains (*29*), whereas arabinosyl residues in coffee arabinogalactans are α-(1→5)-linked. Structural changes in the coffee fiber polysaccharides were investigated in more detail by methylation analysis of fermented material after 0, 6, and 12 h (**Table 4**). Time-dependent structural changes were expressed by calculating characteristic molar ratios of glycosidic linkages. The degradation process was quite similar and widely independent from the fecal samples investigated. Galactomannans were extensively utilized. A slight decrease in the total Man*p*/4,6-Man*p* ratio, which expresses the galactose substitution degree of galactomannans, was shown between 0 and 6 h, indicating that nonbranched galactomannan sections were degraded more quickly than branched sections. However, after 12 h, possible hindrances of side chains in galactomannan degradation were overcome as indicated by increasing total Man*p*/4,6-Man*p* ratios. The (3-Gal*p* + 6-Gal*p*)/3,6-Gal*p* ratio describes the overall degree of branching of galactan chains. An increase in the ratio (3-Gal*p* + 6-Gal*p*)/3,6-Gal*p* reveals that side chains were easily removed from galactans. Depending on the glycosidic linkage of the galactan [(1→3)-linked in the backbone, (1→6)-linked in the side chains], the resulting galactosyl residue following side-chain removal could be either (1→3)-linked or (1→6)-linked. Hence, the observed decrease in the 3-Gal*p*/6-Gal*p* ratio does not prove that (1→3)-linked galactosyl residues were degraded more quickly than (1→6)-linked galactosyl residues. Debranching of 3,6-Gal*p* residues from (1→6)-linked galactans would produce relevant amounts of 6-Gal*p* residues, thus decreasing the 3-Gal*p*/6-Gal*p* ratio. In the first 6 h of fermentation, T-Ara*f* units were degraded at comparable rates as galactosyl residues became debranched, whereas after 12 h, the T-Ara*f*/3,6-Gal*p* ratio increased. A possible explanation is that T-Ara*f* which are part of more complex side chains are not as easily accessible as single T-Ara*f* side chains of the arabinogalactan backbone. The decreasing T-Ara*f*/5-Ara*f* ratio indicates that single arabinofuranosyl residues linked to the galactan backbone are degraded more quickly than arabinan side chains composed of 5-Ara*f* residues. In our study 5-Ara*f* units were the least fermentable structural units in coffee fiber polysaccharides. However, pectins were shown to be degraded completely within 6 h by human fecal microbiota, although they contain α-(1→5)-linked arabinofuranosyl residues (*30*). In coffee polysaccharides 5-Ara*f* units appear to be integrated into complex arabinogalactan side chains, which are hard to degrade.

Effects of Coffee Fiber Fermentation on the Composition of the Microbiota. Changes in the microbial composition during fermentation of soluble coffee fiber or inulin, as carbon and energy source, with human fecal samples were analyzed using FISH-FC. Significant changes in the relative proportions of dominant microbial population groups were determined for both substrates (**Figure 3**). Fermentation of coffee fiber and inulin, respectively, led to an increase of cell counts of bacteria belonging to the *Bacteroides*−*Prevotella g*roup by ca. 60% (compared to the control), which thereby became the dominant bacterial group. Within the *Bacteroides*−*Prevotella g*roup a slight increase of *B. fragilis* was observed for both coffee fiber (4.0%) and inulin (7.4%), whereas an increase of *B. vulgatus* was observed for only coffee fiber (10.4%) (**Figure 3**). This indicates that mainly members of the *Bacteroides*−*Prevotella* group other than *B. fragilis* or *B. vulgatus* were responsible for the observed increase. The increase of the *Bacteroides*−*Prevotella* group was not completely surprising as members of the genus *Bacteroides* are well-known for their ability to degrade a large variety of complex carbohydrates (*31*), which is also

Table 4. Changes of Selected Molar Ratios of Glycosidic Linkages Depending on Fermentation Time

	fecal sample A			fecal sample B			fecal sample C			fecal sample D		
glycosidic linkage ratio	0 h	6 h	12 h	0 h	6 h	12 h	0 h	6 h	12 h	0 h	6 h	12 h
3-Gal*p*/6-Gal*p*	3.6	3.6	2.9	3.8	2.7	2.0	3.0	2.9	1.6	3.0	2.5	1.9
(3-Gal*p* + 6-Gal*p*)/3,6-Gal*p*	1.8	2.5	4.0	1.8	2.3	3.7	1.9	2.3	3.4	1.8	2.1	2.9
T-Ara*f*/3,6-Gal*p*	0.9	1.1	1.9	0.8	0.6	2.7	1.0	1.0	4.3	0.9	0.9	3.0
T-Ara*f*/5-Ara*f*	2.4	2.0	1.0	2.2	1.4	0.7	2.4	1.7	0.8	2.4	1.7	1.0
total Man*p*/4,6-Man*p*	24.7	23.2	26.7	25.2	23.6	26.8	19.8	13.7	23.1	20.7	17.9	20.8

evident by information from recent meta genome analysis (*32*). However, many authors reported a decrease or no change in the number of bacteroides in response to the administration of inulin or inulin-type fructans but an increase in the number of bifidobacteria and lactobacilli (*33*−*35*). No bifidogenic effect was observed in our study for either of the two substrates, and the proportion of lactobacilli did not change either. A possible explanation is that we used long-chain inulin, whereas the bifidogenic effects mostly referred to inulin or oligofructose with a DP of less than 13. Because in our study coffee fiber or inulin was the only energy substrate available for the fecal bacteria, our results indicate that members of the genus *Bacteroides* were particularly able to utilize these substrates and thereby outcompete other bacterial groups. Although many saccharolytic bacterial species may play a role in polysaccharide degradation, it appears that especially bacteroides are very effective because the enzymes involved in carbohydrate depolymerization are constitutively expressed and excreted or attached to the cell surface (*36*). In bifidobacteria, polysaccharide-degrading enzymes are inducible and located in the cytoplasm (*37*, *38*). This may be a disadvantage in the competition for nutrients. The proportions of the *Clostridium coccoides*−*Eubacterium rectale* group decreased in the control slurry as well as in the slurries supplemented with coffee fiber and inulin, but the decrease was smaller for coffee fiber-containing samples. Within the *C. coccoides*−*E. rectale* group, which includes many butyrate-producing species such as clostridia, fusobacteria, and eubacteria (*39*), no changes were observed for the *Clostridium leptum* group and the *Faecalibacterium prausnitzii* group.

In conclusion, we have shown that coffee beverages contribute to the dietary fiber intake. An enhanced dietary fiber intake is desired because most people in industrial countries fail to meet the recommended intake of 30 g of dietary fiber per day. In a Western diet, cereals and vegetables are usually the main sources of dietary fiber. However, 600 mL of filter coffee, equivalent to four cups, provides ca. 2 g of soluble dietary fiber. This is a significant contribution, compared to the actual dietary fiber intakes (about 20 g/day in Germany and 14 g/day in the United States). Coffee fiber polysaccharides undergo rapid fermentation in the human colon and thereby contribute to the physiological effects generally associated with fiber fermentation. Epidemiological studies indicate an inverse effect of coffee consumption and type II diabetes. Several putative mechanisms have been proposed (*40*), but the role of polysaccharides present in coffee beverages has not been discussed. Further studies are required to increase the knowledge about the influence of coffee fiber polysaccharides on human health.

ABBREVIATIONS USED

5-Ara*f*, (1→5)-linked arabinofuranosyl residues; T-Ara*f*, terminally linked arabinofuranosyl residues; DP, degree of polymerization; EtOH, ethanol; FISH-FC, fluorescence in situ hybridization combined with flow cytometry; 3-Gal*p*, (1→3)-linked galactopyranosyl residues; 6-Gal*p*, (1→6)-linked galactopyranosyl residues; 3,6-Gal*p*, (1→3,6)-linked galactopyranosyl residues; T-Gal*p*, terminally linked galactopyranosyl residues; 4-Glc*p*, (1→4)-linked glucopyranosyl residues; T-Glc*p*, terminally linked glucopyranosyl residues; 4-Man*p*, (1→4)-linked mannopyranosyl residues; 4,6-Man*p*, (1→4,6)-linked mannopyranosyl residues; T-Man*p*, terminally linked mannopyranosyl residues; OD, optical density; PBS, phosphate-buffered saline; T-Rha*p*, terminally linked rhamnopyranosyl residues; SCFA, short-chain fatty acids; SDS, sodium dodecyl sulfate.

LITERATURE CITED

(1) Navarini, L.; Gilli, R.; Gombac, V.; Abatangelo, A.; Bosco, M.; Toffanin, R. Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: isolation and characterization. *Carbohydr. Polym.* **1999**, *40*, 71−81.

(2) Nunes, F. M.; Coimbra, M. A. Chemical characterization of the high molecular weight material extracted with hot water from green and roasted arabica coffee. *J. Agric. Food Chem.* **2001**, *49*, 1773−1782.

(3) Nunes, F. M.; Coimbra, M. A. Chemical characterization of galactomannans and arabinogalactans from two arabica coffee infusions as affected by the degree of roast. *J. Agric. Food Chem.* **2002**, *50*, 1429−1434.

(4) AACC report. The definition of dietary fiber. *Cereal Foods World* **2001**, *46*, 112−126.

(5) Delaney, B.; Nicolosi, R. J.; Wilson, T. A.; Carlson, T.; Frazer, S.; Zheng, G.-H.; Hess, R.; Ostergren, K.; Haworth, J.; Knutson, N. Beta-glucan fractions from barley and oats are similarly antiatherogenic in hypercholesterolemic Syrian golden hamsters. *J. Nutr.* **2003**, *133*, 468−475.

(6) Jenkins, D. J.; Goff, D. V.; Leeds, A. R.; Alberti, K. G.; Wolever, T. M.; Gassull, M. A.; Hockaday, T. D. Unabsorbable carbohydrates and diabetes: decreased post-prandial hyperglycaemia. *Lancet* **1976**, *2*, 172−174.

(7) Gibson, G. R.; Roberfroid, M. B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* **1995**, *125*, 1401−1412.

(8) Prasad, K. N. Butyric acid: a small fatty acid with diverse biological functions. *Life Sci.* **1980**, *27*, 1351−1358.

(9) Sakata, T. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *Br. J. Nutr.* **1987**, *58*, 95−103.

(10) Hague, A.; Butt, A. J.; Paraskeva, C. The role of butyrate in human colonic epithelial cells: an energy source or inducer of differentiation and apoptosis? *Proc. Nutr. Soc.* **1996**, *55*, 937−943.

(11) Lee, S. C.; Prosky, L.; deVries, J. W. Determination of total, soluble, and insoluble dietary fibre in foods—enzymatic-gravimetric method, MES-TRIS buffer: collaborative study. *J. AOAC Int.* **1992**, *75*, 395−416.

(12) Willis, R. B.; Montgomery, M. E.; Allen, P. R. Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate. *J. Agric. Food Chem.* **1996**, *44*, 1804−1807.

(13) Bunzel, M.; Ralph, J.; Marita, J. M.; Hatfield, R. D.; Steinhart, H. Diferulates as structural components in soluble and insoluble cereal dietary fibre. *J. Sci. Food Agric.* **2001**, *81*, 653−660.

(14) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.
(15) Englyst, H. N.; Quigley, M. E.; Geoffrey, J. H. Determination of dietary fibre as non-starch polysaccharides with gas−liquid chromatographic, high performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* **1994**, *119*, 1497−1509.
(16) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291−299.
(17) Sweet, D. P.; Shapiro, R. H.; Albersheim, P. Quantitative analysis by various g.l.c. response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydr. Res.* **1975**, *40*, 217−225.
(18) Widdel, F.; Kohring, G. W.; Mayer, F. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magnum* sp. nov. *Arch. Microbiol.* **1983**, *134*, 286−294.
(19) Wolin, E. A.; Wolfe, R. S.; Wolin, M. J. Viologen dye inhibition of methane formation by *Methanobacillus omelianskii*. *J. Bacteriol.* **1964**, *87*, 993−998.
(20) Rigottier-Gois, L.; Le Bourhis, A.-G.; Gramet, G.; Rochet, V.; Dore, J. Fluorescent hybridisation combined with flow cytometry and hybridisation of total RNA to analyse the composition of microbial communities in human faeces using 16S rRNA probes. *FEMS Microbiol. Ecol.* **2003**, *43*, 237−245.
(21) Fuchs, B. M.; Wallner, G.; Beisker, W.; Schwippl, I.; Ludwig, W.; Amann, R. Flow cytometric analysis of the in situ accessibility of Escherichia coli 16S rRNA for fluorescently labeled oligonucleotide probes. *Appl. Environ. Microbiol.* **1998**, *64*, 4973−4982.
(22) Clavel, T.; Henderson, G.; Alpert, C.-A.; Philippe, C.; Riottier-Gois, L.; Dore, J.; Blaut, M. Intestinal bacterial communities that produce active estrogen-like compounds enterodiol and enterolactone in humans. *Appl. Environ. Microbiol.* **2005**, *71*, 6077−6085.
(23) Prosky, L.; Asp, N. G.; Furda, I.; deVries, J. W.; Schweizer, T. F.; Harland, B. F. Determination of total dietary fiber in foods and food products: collaborative study. *J. AOAC Int.* **1985**, *68*, 677−679.
(24) Nunes, F. M.; Reis, A.; Domingues, M. R. M.; Coimbra, M. A. Characterization of galactomannan derivatives in roasted coffee beverages. *J. Agric. Food Chem.* **2006**, *54*, 3428−3439.
(25) Nunes, F. M.; Domingues, M. R.; Coimbra, M. A. Arabinosyl and glucosyl residues as structural features of acetylated galactomannans from green and roasted coffee infusions. *Carbohydr. Res.* **2005**, *340*, 1689−1698.
(26) Redgwell, R. J.; Trovato, V.; Curti, D.; Fischer, M. Effect of roasting on degradation and structural features of polysaccharides in Arabica coffee beans. *Carbohydr. Res.* **2002**, *337*, 421−431.
(27) Englyst, H. N.; Hay, S.; Macfarlane, G. T. Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiol. Ecol.* **1987**, *95*, 163−171.
(28) Macfarlane, G. T.; Macfarlane, S.; Gibson, G. R. Co-culture of *Bifidobacterium adolescentis* and *Bacteroides thetaiotaomicron* in arabinogalactan-limited chemostats: effects of dilution rate and pH. *Anaerobe* **1995**, *1*, 275−281.
(29) Ponder, G. R.; Richards, G. N. Arabinogalactan from Western larch, part III: alkaline degradation revisited, with novel conclusions on molecular structure. *Carbohydr. Polym.* **1997**, *34*, 251−261.
(30) Gulfi, M.; Arrigoni, E.; Amado, R. In vitro fermentability of a pectin fraction rich in hairy regions. *Carbohydr. Polym.* **2007**, *67*, 410−416.
(31) Salyers, A. A.; Vercellotti, J. R.; West, S. E. H.; Wilkins, T. D. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. *Appl. Environ. Microbiol.* **1977**, *33*, 319−322.
(32) Xu, J.; Bjursell, M. K.; Himrod, J.; Deng, S.; Carmichael, L. K.; Chiang, H. C.; Hooper, L. V.; Gordon, J. I. A genomic view of the human−*Bacteroides thetaiotaomicron* symbiosis. *Science* **2003**, *299*, 2074−2076.
(33) Wang, X.; Gibson, G. R. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *J. Appl. Bacteriol.* **1993**, *75*, 373−380.
(34) Menne, E.; Guggenbuhl, N.; Roberfroid, M. Fn-type chicory inulin hydrolysate has a prebiotic effect in humans. *J. Nutr.* **2000**, *130*, 1197−1199.
(35) Langlands, S. J.; Hopkins, M. J.; Coleman, N.; Cummings, J. H. Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. *Gut* **2004**, *53*, 1610−1616.
(36) Blatch, G. L.; Woods, D. R. Molecular characterization of a fructanase produced by *Bacteroides fragilis* BF-1. *J. Bacteriol.* **1993**, *175*, 3058−3066.
(37) Imamura, L.; Hisamitsu, K.; Kobashi, K. Purification and characterization of β-fructofuranosidase from *Bifidobacterium infantis*. *Biol. Pharm. Bull.* **1994**, *17*, 596−602.
(38) Ehrmann, M. A.; Korakli, M.; Vogel, R. F. Identification of the gene for β-fructofuranosidase of *Bifidobacterium lactis* DSM10140(T) and characterization of the enzyme expressed in *Escherichia coli*. *Curr. Microbiol.* **2003**, *46*, 391−397.
(39) Barcenilla, A.; Pryde, S. E.; Martin, J. C.; Duncan, S. H.; Stewart, C. S.; Henderson, C.; Flint, H. J. Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl. Environ. Microbiol.* **2000**, *66*, 1654−1661.
(40) Ranheim, T.; Halvorsen, B. Coffee consumption and human health—benificial or detrimental? Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 diabetes mellitus. *Mol. Nutr. Food Res.* **2005**, *49*, 274−284.
(41) Amann, R. I.; Binder, B. J.; Olson, R. J.; Chisholm, S. W.; Devereux, R.; Stahl, D. A. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl. Environ. Microbiol.* **1990**, *56*, 1919−1925.
(42) Lay, C.; Sutren, M.; Rochet, V.; Saunier, K.; Dore, J.; Rigottier-Gois, L. Design and validation of 16S rRNA probes to enumerate members of the *Clostridium leptum* subgroup in human fecal microbiota. *Environ. Microbiol.* **2005**, *7*, 933−946.
(43) Suau, A.; Rochet, V.; Sghir, A.; Gramet, G.; Brewaeys, S.; Sutren, M.; Rigottier-Gois, L.; Dore, J. *Fusobacterium prausnitzii* and related species represent a dominant group within the human fecal flora. *Syst. Appl. Microbiol.* **2001**, *24*, 139−145.
(44) Franks, A. H.; Harmsen, H. J.; Raangs, G. C.; Jansen, G. J.; Schut, F.; Welling, G. W. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.* **1998**, *64*, 3336−3345.
(45) Manz, W.; Amann, R.; Ludwig, W.; Vancanneyt, M.; Schleifer, K. H. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology (Reading, U.K.)* **1996**, *142*, 1097−1106.
(46) Rigottier-Gois, L.; Rochet, V.; Garrec, N.; Suau, A.; Dore, J. Enumeration of *Bacteroides* species in human faeces by fluorescent in situ hybridisation combined with flow cytometry using 16S rRNA probes. *Syst. Appl. Microbiol.* **2003**, *26*, 110−118.
(47) Langendijk, P. S.; Schut, F.; Jansen, G. J.; Raangs, G. C.; Kamphuis, G. R.; Wilkinson, M. H. F.; Welling, G. W. Quantitative fluorescence in situ hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. *Appl. Environ. Microbiol.* **1995**, *61*, 3069−3075.

(48) Harmsen, H. J. M.; Elfferich, P.; Schut, F.; Welling, G. W. A 16S rRNA-targeted probe for detection of lactobacilli and enterococci in faecal samples by fluorescent in situ hybridization. *Microb. Ecol. Health Dis.* **1999**, *11*, 3−12.

(49) Wallner, G.; Amann, R.; Beisker, W. Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry* **1993**, *14*, 136−143.

Received for review March 6, 2007. Revised manuscript received June 6, 2007. Accepted June 11, 2007. This research project was supported by the German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn). Project AiF 14085 BG.

JF070646B