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1 **Prebiotic role of softwood hemicellulose in healthy mice model**

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27 decision to submit the article for publication.

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1  
2 Abbreviations:  
3 AIEC - Adherent-invasive *Escherichia coli*; AutoH UC - autohydrolysate ultrafiltrated and  
4 treated with activated charcoal; DP - degree of polymerization; FOS - fructo-oligosaccharides;  
5 GGM - galactoglucomannans; MALDI-TOF MS - Matrix Assisted Laser Desorption Ionization  
6 Time of Flight Mass Spectrometry; PP - precipitate; RT- room temperature; SCFA - short  
7 chain fatty acids, TSWD - tryptic soy broth without dextrose.

8

9

1 **Abstract** (150 words)

2

3 We have analyzed the prebiotic effect of softwood hemicelluloses on specific bacterial  
4 species *in vitro* and on microbiota diversity and metabolism in healthy mice *in vivo*. The  
5 ethanol-precipitated glycans from the softwood hemicellulose autohydrolysate were able to  
6 stimulate *in vitro* the growth of *Bifidobacterium adolescentis*, but to a much lesser extent than  
7 that of adherent-invasive *E. coli*. *B. adolescentis* was the best producer of SCFA. When mice  
8 were fed with the ethanol precipitate fraction, the relative abundance of Bacteroidetes  
9 increased while that of Proteobacteria diminished, suggesting change towards a less  
10 obesogenic microbiome. Following treatment, lipid analysis showed a decrease in cholesterol,  
11 bile acids and free fatty acids, indicating a potential cardioprotection role. Furthermore,  
12 analysis of fermentation metabolites revealed an increase in metabolites important for immune  
13 health and well-being. Our results highlight the value of hemicelluloses as a potential source  
14 of prebiotics supporting both a healthy digestive tract and immune system.

15

16

17 **Keywords:** softwood hemicelluloses, prebiotics, healthy mice model, microbiota metabolites.

18

19

## 1 **1. Introduction** 492 words

2 The composition and physiology of the gut microbiota is shaped to a large extent by the  
3 influx of glycans (polysaccharides) from diet and host mucosal secretions into the intestine.  
4 The microbiota confer many metabolic capabilities that the mammalian hosts lack, including  
5 the ability to break down otherwise indigestible dietary glycans. Since microbial species differ  
6 in their glycan preferences, selective consumption of such nutrients can influence both  
7 proliferation and persistence of microorganisms within the gastrointestinal tract.

8 It has been shown thirty years ago that the non-digestible oligosaccharides could be  
9 selectively fermented by Bifidobacteria and at the same time stimulate Bifidobacteria growth  
10 [Mitsuoka et al, 1987]. Gibson and Roberfroid put forward the concept of prebiotics: “Non-  
11 digestible oligosaccharides in general and fructo-oligosaccharides in particular, are prebiotics  
12 [1995]. They have been shown to stimulate the growth of endogenous Bifidobacteria”. In other  
13 words, prebiotic is “a selectively fermented ingredient that results in specific changes in the  
14 composition and/or activity of the gastrointestinal microbial, thus conferring benefit(s) upon  
15 host health” (International Scientific Association for Probiotics and Prebiotics, 2008). First, a  
16 prebiotic oligosaccharide needs to be resistant to gastric acidity, hydrolysis by mammalian  
17 enzymes and direct gastrointestinal absorption. Second, it has to be fermentable by the  
18 intestinal microbiota. Thirdly it should selectively stimulate the growth and/or activity of  
19 intestinal bacteria that are associated with health and well-being. The use of particular dietary  
20 glycans, *i.e.* prebiotics, may provide the basis for a non-invasive strategy by which both the  
21 composition and the physiology of the microbiota could be modulated, in order to promote  
22 host health [Gibson and Roberfroid, 1992; Verbeke et al, 2015].

23 Currently commercialized glycan prebiotics with a degree of polymerization (DP) below 10  
24 are derived mainly from the oligosaccharides and simple polysaccharides found in plants [Al-  
25 Ghazzewi et al, 2012; Yoo et al, 2012]. Thus far, the prebiotic properties of wood  
26 hemicellulose have been described in only two publications. Polari et al. [2012] showed that  
27 the galactoglucomannans, the predominant hemicellulose in the softwood spruce (*Picea*

1 *abies*), might have prebiotic properties, favoring Bifidobacteria growth, while Rivas et al.  
2 [2012] have shown that a fraction obtained from the *Pinus pinaster* (maritime pine) wood  
3 hemicellulose and composed of 14 acetylated hexoses was able to stimulate the human  
4 bifidobacterial population growth. Thus, oligosaccharides derived from wood hemicelluloses  
5 might display prebiotic properties, although their potential impact remains largely unknown.

6 Wood hemicelluloses are an abundant, but still untapped, industrial by-product of the pulp  
7 and paper industry; this industry uses more than 300 million tons of wood per year worldwide,  
8 of which 20 to 30% is hemicellulose. Exploitation of an industrial by-product avoids the use of  
9 arable land and competition with food production resources.

10 Here, we have fractionated softwood hemicelluloses obtained by hot water extraction and  
11 analyzed the impact of these hemicellulose fractions on *in vitro* growth of relevant gut  
12 bacteria. Furthermore, we have confirmed the prebiotic properties of this fraction *in vivo*, by  
13 analyzing its action on both gut microbiota composition and metabolism in healthy mice.

14

## 1 **2. Materials and Methods** 1633 words

2 **2.1 Preparation of hemicellulose fractions.** Hemicellulose was extracted from an  
3 industrial mixture of different softwood species containing Sylvester pine (35%), black pine  
4 (24%), Alep pine (18%), Douglas fir (16%) and spruce (7%) (provided by the Fibre Excellence  
5 Tarascon mill, France) and fractionated. Chemical characterization of fractions was done  
6 according to Deloule et al [2016]. Briefly, hemicelluloses were extracted from industrial  
7 softwoods chips by hot water extraction (170°C for 1 h). The crude autohydrolysate (AutoH)  
8 was fractionated using precipitation with either ethanol: water (PP ethanol) or acetone:  
9 methanol (PP acetone), both at a ratio 9:1. In parallel, the ultrafiltration with a molecular  
10 weight cut-off of 500 Dalton followed by activated charcoal treatment was carried out on  
11 AutoH, yielding AutoH UC.

12 **2.2 Bacteria and growth media.** The adherent-invasive *Escherichia coli* (AIEC) LF 82 and  
13 *Bifidobacterium adolescentis* CFPL 15196 were isolated from human feces in Lille  
14 Pharmacology Department (France). *Akkermansia muciniphila* (ATCC® BAA-835™) was  
15 purchased from ATCC. *Lactobacillus salivarius* CIP 103140 was purchased from the  
16 Collection of Pasteur Institute (Paris, France). All cultures were carried out in the Don Whitley  
17 Scientific A35 workstations (ShIPLEY, UK) under an anaerobic atmosphere consisting of 10%  
18 H<sub>2</sub>, 5% CO<sub>2</sub> and 85% N<sub>2</sub> (Anaerogaz, Linde). The negative control consisted of tryptic soy  
19 broth without dextrose (TSWD) (Becton-Dickinson, Pont de Claix, France). The positive  
20 controls consisted of 1% glucose and the dietary fiber P95 containing 1% fructo-  
21 oligosaccharides (FOS, BENEEO-Orafti, Oreye, Belgium) with average DP of 4, both in TSWD.  
22 TSWD, supplemented with 1% glucose, FOS or hemicelluloses, was filtered (0.22 µm).

23 **2.3 Growth Analysis.** The intracellular production of ATP was estimated with VIALIGHT  
24 MDA kit (Lonza, Aix-les-Bains, France), using Multimode Reader Tristar LB941 (Berthold  
25 Technologies, Zug, Switzerland), while bacterial growth at 37°C was followed by measuring  
26 optical density at 595 nm, on the iMark Absorbance microplate reader (Bio-Rad, Marnes-la-  
27 Coquette, France).

1       **2.4 SCFA analysis.** Concentrated HCl (10  $\mu$ L) was added to the bacterial supernatants  
2 (100  $\mu$ L), then the mixtures were vortexed for 15 s and extracted for 20 min with 500  $\mu$ L of  
3 diethyl ether. After centrifugation (5 min, 3500 rpm), the upper organic phase was recovered  
4 and 50  $\mu$ L of 1 M NaOH were added, while 500  $\mu$ L of diethyl ether was added to the lower  
5 phase for 20 min, to extract again the SCFA. The SCFA-containing samples were combined,  
6 cleared by centrifugation and adjusted with concentrated HCl to pH 4. Sample aliquots of 20  
7  $\mu$ L were injected into the HPLC-UV apparatus (Ultimate 3000, ThermoFisher Scientific,  
8 Waltham, MA, USA).

9       After mice treatment, the SCFAs were estimated in the fecal water obtained as follows.  
10 Stool samples were thawed and resuspended 1:5 in PBS (0.01 mole L<sup>-1</sup>, pH 7) and  
11 homogenized. The fecal slurry was centrifuged at 35,000 g for 15 min at 4°C, and the  
12 supernatant (“fecal water”) was collected and sterilized by filtration (0.22  $\mu$ m).  
13 Chromatographic separation was performed using a Nucleogel Sugar 810H column (300mm  $\times$   
14 7.8mm) containing 10- $\mu$ m particles (Macherey-Nagel, Düren, Germany). The column  
15 stationary phase was sulfonated polystyrene divinylbenzene spheres in a polymer matrix  
16 (PS/DVB) in H<sup>+</sup> form, kept at 40°C. The mobile phase consisted of 10 mM H<sub>2</sub>SO<sub>4</sub> in water.  
17 The UV detector (Waters 2487) was set at 210 nm wavelength. Data processing was  
18 performed using the Chromeleon version 6.80 software (Thermo Scientific). Good peak  
19 separation was obtained under the following conditions: a flow rate of 0.6 ml/min, room  
20 temperature, 10 mM H<sub>2</sub>SO<sub>4</sub> as eluent, and a detector set for UV and refractometer (RI),  
21 respectively, at 0.0500 and 64 AUFS. A mix of volatile free acids (Sigma) was used as  
22 standard.

23       **2.5 MALDI-TOF-MS.** Mass spectroscopy analyses of oligosaccharides were performed  
24 using Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry, with  
25 apparatus MALDI-TOF-TOF Speed (Bruker Daltonics, Billerica, MA, USA). The matrix used  
26 was 2,5-dihydroxybenzoic acid (DHB); N, N-dimethylaniline (DMA) (Sigma-Aldrich, St. Louis,  
27 Mo, USA).

1       **2.6 Glycans' variations in culture broth.** Dosage of total sugars was done according to  
2 [8]. Briefly, 200  $\mu$ L of 5% phenol and 1 mL of 97% H<sub>2</sub>SO<sub>4</sub> were added to 200 $\mu$ L of growth  
3 medium sample. After 10 min incubation at RT, the solution was vortexed, and after 30  
4 minutes its OD was measured at 490 nm. The assay was calibrated with glucose standards  
5 ranging from 0 to 0.5 mg/mL. The absorbance at 450 nm was obtained with a  
6 spectrophotometer (Kontron, La Garde, France) and plotted against the standards. MALDI-  
7 TOF spectra were obtained for oligosaccharides in media of *B. adolescentis* grown in the  
8 presence of ethanol precipitate fraction. The intensity of carbohydrate-associated peaks was  
9 measured.

10       **2.7 Ethical considerations.** This research project implies the use of mice for *in vivo*  
11 experiments. In line with European laws, this project has been submitted to, and validated by,  
12 Grenoble Ethical Committee for Animal Experimentation (Project N012) as well as French  
13 Ministry of Higher Education, Research and Innovation (agreement number: APAFIS8502-  
14 2016122009136117v1). All along this project mice were carefully handled with respect of  
15 ethical rules on Animal Technology Platform of Grenoble University (the agreement n°  
16 C3851610006 from the French ministry).

17       **2.8 Mice gavage with ethanol precipitate fraction.** C57BL/6N female mice (5 weeks of  
18 age) were purchased from Janvier SA (Le Genest-Saint-Isle, France) and were housed (4  
19 mice per cage) with free access to wood and water. Eight control mice were fed for three  
20 weeks with control A04 diet (SAFE, Villemoisson-sur-Orge, France), while other 8 mice were  
21 fed with A04 supplemented with the ethanol precipitate fraction at 0.3 g/day per mouse  
22 (unfortunately, one mouse in the treatment group died from an unknown cause). The freeze-  
23 dried ethanol precipitate fraction was dissolved in water to reach 86g/L and filtered through  
24 0.22 $\mu$ m membrane. Feeding bottles (100 mL) were changed every 2 - 3 days and replaced  
25 with freshly prepared solution. At the end of experimental period the mice were euthanized  
26 with isoflurane, and serum was withdrawn by cardiac puncture. A ventral midline incision was  
27 made to excise cecum and colon. Cecal contents were collected and immediately frozen in

1 liquid nitrogen. Samples of cecal content were prepared through homogenization with PBS  
2 (cecal water), similarly as for fecal water, and frozen.

3 **2.9 Cecal microbial diversity.** The 16S rDNA sequencing (including DNA extraction and  
4 PCR) was subcontracted to Vaiomer (Vaiomer SA, Labège, France). PCR amplification was  
5 performed using 16S universal primers targeting the V3-V4 region of the bacterial 16S  
6 ribosomal gene (Vaiomer universal 16S primers). The joint pair length was set to encompass  
7 467 base pairs amplicon thanks to 2 x 300 paired-end MiSeq kit V3. The detection of the  
8 sequencing fragments was performed using MiSeq Illumina® technology. Sequences were  
9 analyzed and normalized with the pipeline FROGS (Find Rapidly OTU with Galaxy Solution)  
10 [Escudié et al, 2018]. Reads were clustered into Operational Taxonomic Units (OTUs) using  
11 the Swarm clustering method. Phylogenetic affiliations were manually checked (using RDP  
12 seqmatch ([https://rdp.cme.msu.edu/seqmatch/seqmatch\\_intro.jsp](https://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp)) and/or BLAST  
13 ([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=tblastn&PAGE\\_TYPE=BlastSearch&LIN  
15 K\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=tblastn&PAGE_TYPE=BlastSearch&LIN<br/>14 K_LOC=blasthome)). The alpha diversity, which represents the richness and diversity within a  
16 group, was evaluated using the number of Observed OTU and the Chao1 index (community  
17 richness), using the Shannon index (community diversity) and the Simpson and InvSimpson  
18 indexes. Beta diversity, which reports diversity between groups was assessed by computing  
19 weighted UniFrac distances between each sample pairs.

20 To normalize data, 18763 reads were randomly selected for each sample. 289 OTUs were  
21 assigned at different taxonomic levels (from phylum to species). *P* values of  $\leq 0.05$  from  
22 statistical analyses (t-tests) were considered significant.

23 **2.10 Tryptophan catabolites.** Kynurenine and tryptophan concentrations were determined  
24 in 40µL of mice serum by HPLC coupled to mass spectrometry according to [Boulet et al,  
25 2017]. Briefly, 10 µL of internal standard mixture (13C-labeled tryptophan and kynurenine)  
26 was added to samples. Protein precipitation was made by addition of 700 µL of MeOH, 79 g/L  
27 zinc sulfate in water (80/20, v/v) containing 0.05% trifluoroacetic acid. After mixing, samples  
were left at 4°C during 15 min and centrifuged at 10,000 g for 15 min at 4°C. 700 µL of clear

1 supernatant were transferred into a new tube and dried under a stream of nitrogen. The dried  
2 residues were reconstituted with 35  $\mu$ L of water containing 0.08% ascorbic acid. LC-MS/MS  
3 was performed using a Kinetex C18 column (100 mm x 2.1 mm; 5  $\mu$ M particle size) with a 2.1  
4 mm C18 guard column, both obtained from Phenomenex (Torrance, USA), piloted by a  
5 Dionex Ultimate 3000 liquid chromatography instrument (ThermoFisher, Waltham, USA)  
6 connected to a Sciex API 4000 tandem mass spectrometer (SCIEX, Ontario, Canada). Mann-  
7 Whitney *U* test gave values  $p < 0.05$  (\*),  $p < 0.01$  (\*\*).

8 **2.11 Lipid analysis.** Total lipids were extracted from 10  $\mu$ L serum in the presence of 1 nmol  
9 tridecanoic acid and 1 nmol dihenecosanoyl phosphatidylcholine (extraction control), with 600  
10  $\mu$ L chloroform/methanol, 2:1 (v/v), followed by biphasic separation through the addition of 150  
11  $\mu$ L 0.2% KCl (w/v). Organic phase was dried with 1 nmol pentadecanoic acid and derivatized  
12 online to fatty acid methyl ester (FAME) with trimethylsulfonium hydroxide (TMSH, Macherey-  
13 Nagel). For cholesterol, the organic phase was dried with 1 nmol pentadecanoic acid and  
14 derivatized to cholesterol-TMS with N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) +  
15 TMCS, 99:1 (Sigma). Resulting FAME and cholesterol-TMS was analyzed by GC-MS (5977A-  
16 7890B, Agilent). FAME was then quantified using Mass Hunter Quantification software  
17 (Agilent). All statistical analyses were conducted using GraphPad Prism software. *P* values of  
18  $\leq 0.05$  from statistical analyses (Ttests) were considered significant.

19 **2.12 Other metabolites.** Lipopolysaccharide-Binding Protein was estimated in mice sera,  
20 using Mouse LBP PicoKine ELISA kit (Boster Biological Technology, Pleasanton, CA, USA).  
21 Secretory IgA, myeloperoxidase, folic acid and bile acids were estimated in cecal water (cecal  
22 samples diluted in PBS at 1:5 ratio w/v) by using Mouse sIgA ELISA Kit (ElabSciences,  
23 Houston, TX, USA), Mouse MPO ELISA Kit (HycultBiotech, Uden, Netherlands), Mouse Folic  
24 Acid ELISA Kit (Cusabio Technology, Houston TX, USA) and Mouse total Bile Acids assay Kit  
25 (Crystal Chem, Zaandam, Netherlands), respectively, according to manufacturer's  
26 instructions. Statistical differences were considered significant when \* $p < 0,005$  or \*\* $p < 0,001$   
27 using unpaired t-test, when comparing control versus PP ethanol fed mice (n= 7-8 / group).

### 1 **3. Results** 2779 words

#### 2 **3.1 Wood hemicellulose-derived fractions extraction and characterization**

3 Hemicellulose (20-30% of wood) is usually a rejected by-product of paper production from  
4 wood. It consists of polymers of arabinose, glucose, xylose, galactose and mannose with DP  
5 = 50-150, that undergo hydrolysis during hot water extraction. The extraction of crude  
6 autohydrolysate yielded three different fractions containing the same major component,  
7 galactoglucomannan (GGM, the main hemicellulose glycan of softwoods), but differed in purity  
8 and molecular weight (Fig. 1, and [8]). The three fractions derived from crude autohydrolysate  
9 were acetone precipitate, ethanol precipitate and autohydrolysate-treated by ultrafiltration (cut-  
10 off 500) and activated carbon. The PP ethanol contained glycans with the highest DP (Fig.  
11 1B). Naturally occurring acetylation of GGM is also visible, as each peak on Fig. 1B exhibits a  
12 second and a third, slightly delayed, replicates. Acetylation has been shown to impact the  
13 solubility of polysaccharides in water through structural reorganization, and chemical  
14 acetylation of wood has been used to improve its durability and resistance to fungi, bacteria,  
15 and termites [Kishani et al, 2018; Pawar et al, 2013 and references therein]. In our hands, the  
16 acetyl groups represented 7.9% of the mass in crude autohydrolysate, 8.9% in ethanol  
17 precipitate and 8.3% in PP acetone (measured through hydrolysis to acetic acid and  
18 estimated by HPLC). The ratio of one O-acetyl per three monomers was persistent over a  
19 wide range of DP. The acetylated GGMs exhibited peaks of higher intensity than the non-  
20 acetylated ones for ethanol precipitate, with DP 12 to 22, and with DP 6 to 16 for acetone  
21 precipitate.

#### 22 **3.2 Effect of hemicelluloses on bacterial growth *in vitro***

23 In order to determine the effect of the hemicellulose fractions and glucose or FOS (as  
24 control) on different gut bacteria, we first used a growth control experiment. To follow bacterial  
25 growth and metabolism, we followed intracellular ATP production measurements rather than  
26 rely on OD<sub>595</sub> measurements. Indeed, the ATP assay detects bacteria that are dying and is  
27 not influenced by size, shape and sedimentation ability of involved bacteria. In our choice of

1 bacterial species, we were guided by their gut predominance. The AIEC LF82 (Gram  
2 negative) is abnormally predominant in the ileac mucosa of Crohn's disease [Carvalho et al,  
3 2012]. It has been recently shown recently that this AIEC is able to adhere, invade and survive  
4 within prostate epithelial cells, inducing strong inflammatory response, and could conceivably  
5 play a role in prostate infection/inflammation [14]. This bacterium was used to pinpoint the  
6 hemicellulose fractions to be eliminated, in case they would boost AIEC growth.  
7 *Bifidobacterium adolescentis*, together with the *B. catenulatum* group, dominate in healthy gut  
8 microbiota [Conte et al, 2016]. This Gram-positive bacterium is present in 79% of the adults  
9 [Kim et al, 2008] and was found to inhibit harmful fecal enzymes, hamper proliferation of  
10 human colon cancer cells *in vitro* and exhibit an antiviral activity [Lee et al, 2013; Derrien et al,  
11 2017]. *Akkermansia muciniphila* is a Gram-negative mucin-degrading bacterium that resides  
12 in the gastrointestinal tracts of humans and animals. Reduced levels of *A. muciniphila* have  
13 been observed in patients with inflammatory bowel diseases and metabolic disorders, which  
14 suggests it may have anti-inflammatory properties [Neville and O'Toole, 2010]. *Lactobacillus*  
15 *salivarius* is a Gram-positive bacterium, that is able to influence composition of gut microbiota  
16 through production of bacteriocins, some with an anti-Listeria activity [Messaoudi et al, 2013].  
17 *L. salivarius* is also able to stimulate the human immune system, mainly through increased  
18 production of interleukins [Barker, 1981].

19 Medium containing glucose induced a rapid ATP production by all examined bacteria (Fig.  
20 2), but it was also the fastest to become depleted medium, with low or no ATP produced on  
21 glucose after about 100 h of growth. In contrast, oligomers in all other fractions continued to  
22 sustain low ATP production for quite a long time. Importantly, hemicelluloses were rather  
23 poorly fermented by *E. coli* LF82. These media gave growth values close to that of FOS or  
24 even to that of TSWD. *A. muciniphila* was not able to achieve high ATP production on  
25 glucose, in contrast to the other three bacteria. When the effect of the autoH UC, fraction  
26 obtained from autoH but devoid > 500 kDa compounds and soluble lignins, was compared  
27 with that exerted by autoH, some increase of ATP production (meaning bacterial growth) was  
28 observed for all bacteria, probably due to the removal of deleterious impurities. Interestingly,

1 growth of *B. adolescentis* was significantly prompted by 1% and even more by 3% PP ethanol  
2 fraction, this fraction inducing a second ATP production peak at between 50 and 170 hours of  
3 growth (Fig. 2). It is conceivable that these bacteria produce, after induction, glycan-  
4 hydrolyzing enzymes, thus providing the low-molecular weight glycans to sustain bacterial  
5 growth. For the remaining three bacteria, a second ATP production peak (albeit significantly  
6 smaller) was also observed around 150 h of growth. When 2% of AutoH, FOS and glucose  
7 were used, no major changes were observed (not shown).

8 As bacterial growth was also monitored with OD<sub>590</sub> readings, we could extract the  
9 maximum OD<sub>590</sub> values just before bacteria reached the stationary phase was reached.  
10 Maximum OD<sub>590</sub> values not only show the effect of hemicellulose fractions on both the level  
11 and rate of bacterial growth (Fig. 3A). These measurements showed again that the highest  
12 growth was obtained on glucose. However, while the *E. coli* grew exponentially on glucose for  
13 only 9 h, *A. muciniphila* grew efficiently on glucose for 64 hours. The latter also grew longer on  
14 FOS and TSWD, without, however, reaching a high OD. Surprisingly, all bacteria could weakly  
15 propagate on TSWD (no sugar). Of note, amino acids can serve as energy sources for some  
16 anaerobic bacteria [19]. *A. muciniphila* had the longest growth on these different fractions,  
17 followed by *B. adolescentis*, *L. salivarius* and *E. coli* LF 82, with the highest OD obtained by *B.*  
18 *adolescentis*. These observations suggest that AIEC LF 82 grew well on the simple glycans  
19 present in control media but was not very efficient at hydrolyzing the longer oligomers that  
20 could sustain lengthier growth periods. On the other hand, the longer growth periods  
21 demonstrated by the three other bacteria suggest that they might extend their growth by  
22 hydrolyzing higher-DPs glycans and utilizing the resulting shorter sugars.

23 The degradation of carbohydrate oligomers was measured in *B. adolescentis* culture broth  
24 using the MALDI-TOF MS and total sugar dosage (Fig. 3B). *B. adolescentis* easily fermented  
25 the low-DP glycans and acetylated glycans, which were consumed quite rapidly but also  
26 reappeared at longer culture times – see results for 4HOAc, 5H and 5HOAc. Furthermore, the  
27 longer oligomers (DP 9 and above) diminished clearly at 144h. Taken together these results  
28 indicate quite probable the induction of *B. adolescentis* glycolases, able to digest high-DP

1 glycans in the PP ethanol fraction. Note that the MALDI-TOF MS technique was applied to  
2 ascertain the degree of acetylation on spruce-derived AcGGM, and to characterize the  
3 acetylated hemicelluloses in hardwoods (Jacobs et al, 2002; Teleman et al, 2003; Lundquist  
4 et al, 2003).

5 In conclusion, *B. adolescentis* achieved the highest growth on all media, with an ability to  
6 efficiently ferment hemicelluloses, in particular during later growth periods. The health-  
7 detrimental *E. coli* LF82 was unable to reach significantly better growth on hemicelluloses  
8 than on low-molecular-weight oligosaccharides present in control media, displaying a rather  
9 low ability to ferment softwood hemicelluloses.

10

### 11 **3.3 Effect of hemicellulose fractions on SCFA production *in vitro***

12 The important end products of fermentation of dietary fibers by the anaerobic intestinal  
13 microbiota are short-chain fatty acids (SCFAs). The combined concentrations of SCFAs  
14 produced in the colon reach ~150 mM, making SCFAs the most abundant anions in the colon.  
15 SCFAs are absorbed in the colon and either utilized in colonocytes or transported via the  
16 portal vein to reach the blood circulation and other organs [Kim et al, 2014]. We determined  
17 the amount of SCFA induced during growth of *E. coli*, *B. adolescentis* and *A. muciniphila* on  
18 different hemicellulose-derived fractions (Fig. 2S). It appears that the acetate was the main  
19 SCFA produced by all bacteria, with *B. adolescentis* producing more SCFA than *E. coli* LF82  
20 or *A. muciniphila*. In addition, *B. adolescentis* produced significant amount of propionic acid,  
21 which was formed at a negligible level by the two other bacteria (Figs. 2S). Interestingly, at the  
22 beginning of LF82 growth, formic acid was produced, with, however, a steady decline in all  
23 media. The analyzed bacteria did not seem to produce butyric acid, known for its anti-cancer  
24 effects and immunity modulation [Fung et al, 2012; Meijer et al, 2010]. It is relevant that  
25 among 313 bacterial isolates from human fecal samples, only 74 were observed to produce  
26 butyrate [Barcenilla et al, 2000]. As acetic and propionic acids are fuel for colonocytes [Floch,  
27 2010], we wanted to better characterize the SCFA-generating effect of hemicellulose fractions,  
28 by following the changes in the ratio of propionic/acetic acid (Fig. 4). This comparison

1 confirmed quite low production of SCFAs by *E. coli* LF82 and *A. muciniphila*. The ratio  
2 observed with *B. adolescentis*, using the hemicellulose fractions underlines the positive effect  
3 of autohydrolysates on propionic acid production, with a peak appearing at about 30 h of  
4 growth. It seems that autohydrolysates containing glycans with lower DP are able to sustain  
5 propionate production by *B. adolescentis* at earlier stages (up to ~47 h, see the first peak of  
6 bacteria growth on Fig. 2S). In contrast, the PP ethanol fraction with higher DP glycans rather  
7 efficiently maintained the longer growth phase of *B. adolescentis* (after 60 h, see Fig. 2S),  
8 which correlated with an efficient propionate production.

### 10 **3.4 Effect of PP ethanol fraction on mice microbiota**

11 Mice were fed with PP ethanol fraction and the different phyla from their caecum were  
12 identified through analysis of 16S rRNA genes as previously [Mangin et al, 2018]. Microbiota  
13 of 15 mice were analyzed: 8 for the control group and 7 for the PP ethanol fraction gavage  
14 group. 18763 sequences were analyzed per sample. The 289 OTUs belonged to six  
15 phylogenetic phyla: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria,  
16 Verrucomicrobia and Candidatus Saccharibacteria. The phyla Bacteroidetes, Firmicutes and  
17 Proteobacteria accounted for more than 99.5 % of the diversity. Only 32 OTUs were known  
18 (less than 2% divergence with a sequence of a cultivated species in databases), for example  
19 *Acetatifactor muris*, *Akkermansia muciniphila*, *Bacteroides acidifaciens*, *Bifidobacterium*  
20 *animalis*, *Clostridium disporicum*, *Escherichia coli*, *Lactobacillus gasseri*, *Pseudomonas*  
21 *aeruginosa* and *Streptococcus salivarius*. The phylum Bacteroidetes was dominant (based on  
22 the number of sequences), but the phylum Firmicutes was the most diversified (based on the  
23 number of OTUs) (Figs. 5 and 3SA). Analysis of the beta diversity enlightened the microbiota  
24 modification due to the prebiotic fraction: weighted UniFrac distances allowed to separate  
25 samples from treated and untreated mice (Figs. 3SB). There were two main consequences on  
26 the microbiota when feeding mice with the PP ethanol fraction. Firstly, the number of OTUs  
27 was less important in the caecum of treated mice, 246.4 for the PP ethanol fraction  $\pm$  8.5

1 versus  $285.5 \pm 18.6$  for the control microbiota (mean  $\pm$  SEM),  $p < 0.01$ . This decrease was  
2 statistically significant for the phyla Firmicutes, Proteobacteria, Actinobacteria and  
3 Verrucomicrobia (Fig. 5A). The alpha diversity measure was in agreement with this finding,  
4 with all indexes smallest for the PP ethanol group (Observed, Chao1, Shannon, Simpson and  
5 InvSimpson). Secondly, the amount of Bacteroidetes sequences increased from 58 to 65 % ( $p$   
6  $< 0.05$ ) and the Proteobacteria sequences decreased from 11 to 7 % ( $p < 0.01$ ) (Fig. 5B). The  
7 observed decrease for the Firmicutes phylum was not statistically significant. Of note, *B.*  
8 *adolescentis* belongs to phylum Actinobacteria, while *E. coli* LF82 belongs to Proteobacteria,  
9 confirming our *in vitro* results. Glycans from the PP ethanol fraction impacted the cecal  
10 microbiota of mice: the proportion of the Bacteroidetes phylum was increased, the proportion  
11 of Proteobacteria decreased and the number of species diminished except for the  
12 Bacteroidetes phylum. It has been observed that gut dysbiosis leading to several human  
13 diseases is characterized by an increased Firmicutes/Bacteroidetes (F/B) ratio. Importantly,  
14 the F/B ratio is a marker of an obesogenic microbiome, a high F/B ratio is more likely to  
15 support obesity, while a low F/B ratio is associated with weight loss [Ussar et al, 2016]. Our *in*  
16 *vivo* experiments led to a decrease in F/B ratio from 0.527 in control mice to 0.433 in mice fed  
17 with PP ethanol glycans, indicating a potential beneficial effect of this fraction on mice  
18 microbiome, reshaping it toward less obesogenic. This could be tested in a further study more  
19 focused on the physiological impacts of these fibers.

20

### 21 **3.5 Effect of hemicellulose fractions on SCFA production *in vivo***

22 SCFAs, that play a key role in the maintenance of colonic homeostasis and seem to be the  
23 most important microbial end products of the colon fermentation involved in the regulation of  
24 the immune cell function [Flint et al, 2015; Corrêa-Oliveira et al, 2016]. Administration of a  
25 hemicellulose-derived fraction had a significant impact by increasing the production of acetate  
26 ( $50$  vs  $39 \mu\text{mol. g}^{-1}$ ) and propionate ( $11$  vs  $7 \mu\text{mol. g}^{-1}$ ), with little effect on the levels of formate  
27 or butyrate in the cecum (Fig. 6A). The bacterial species belonging to the *Bacteroidetes*  
28 phylum produced predominantly acetate and propionate, whereas butyrate is primarily

1 produced by the *Firmicutes* phylum [Vital et al, 2014] SCFA production (Fig. 6A) correlates  
2 well with changes in bacterial populations (Fig. 5).

3

4 **3.6 Tryptophan metabolites.** Since gut microbial dysbioses are linked to aberrant  
5 immune responses, we subsequently carried out a detailed analysis of some immune system-  
6 relevant products and fermentation metabolites. A major group of immunomodulatory gut  
7 bacteria metabolites derives from catabolism of the aromatic amino acids. A principal route for  
8 tryptophan catabolism is the kynurenine pathway. It plays an important role in several  
9 fundamental biological processes, because tryptophan metabolites are ligands of aryl  
10 hydrocarbon receptor (AhR), which regulates immune responses and inflammation through its  
11 effects on cytokine production [Haase et al, 2018]. It might also directly influence gut health,  
12 as 5-hydroxytryptamine (5-HT, serotonin) produced by the gut epithelial cells contributes to  
13 the development of microvilli in the intestine [Nakamura et al, 2008]. The hemicellulose diet  
14 induced in mice an increase in tryptophan level and a decrease in kynurenine level (Fig. 6B).  
15 As a result, the ratio of Trp to Kyn increased significantly, from 100 to 170. In addition, the  
16 level of 5-HT rose, suggesting that the neurotransmitter serotonin pathway might be  
17 privileged. Indeed, it has been recently shown, that gut microbes promote colonic serotonin  
18 production through action of SCFA [Reigstad et al, 2015].

19

20 **3.7 Lipid analysis.** Lipid analysis included an estimation of the changes in the level of  
21 serum free fatty acids (FFA) and cholesterol in treated mice. Upon feeding mice with the PP  
22 ethanol fraction, the total level of FFA significantly diminished with reduction noted for palmitic  
23 acid (C16:0), stearic acid (C18:0) and linolenic acid (C18:2) (Fig. 7A). In parallel, a reduction  
24 of cholesterol was also observed (Fig. 7B). Free fatty acids, in addition to be utilized as an  
25 important source of energy, are involved in a variety of both beneficial and detrimental effects.  
26 Under conditions of overload, FFAs become toxic, inducing reactive oxygen species  
27 production, endoplasmic reticulum stress, apoptosis and inflammation [Legrand-Poels et al,  
28 2014]. Elevated cholesterol is known to be one of the main risk factors for cardiovascular

1 diseases, with reduction of cholesterol being cardioprotective [London et al, 2014]. Thus,  
2 reduction of FFA and cholesterol is obviously health-beneficial.

3 Furthermore, the analysis of the toxic effect on Microbiota bile acids was carried out (Fig.  
4 7C). Bile acids are synthesized by the liver from cholesterol, transformed into secondary bile  
5 acids by the intestinal microbiota and reabsorbed back into the blood. Nearly 100% of the bile  
6 acid pool in the large intestine consists of bacterially modified bile acids, implicated in a variety  
7 of signaling pathways relevant to metabolic and hepatic diseases [Klaassen and Cui, 2015].  
8 Almost two-fold reduction observed in bile acid of treated mice is significantly health-  
9 beneficial, as bile acids convert the intestinal microbiota to higher Firmicutes/Bacteroidetes  
10 ratios that are observed after a high-fat diet [Klaassen and Cui, 2015], during obesity and gut  
11 dysbiosis [Mangin et al, 2018].

12 In addition, we monitored the changes in the level of the lipopolysaccharide-binding protein  
13 (LBP) that is associated with obesity and which through lipopolysaccharide binding may  
14 promote immune responses [Moreno-Navarrete et al, 2012; Kheirandish-Gozal et al, 2014], of  
15 the secretory IgA (sIgA) that promote intestinal homeostasis [Rogier et al, 2014], of the  
16 myeloperoxidase (MPO) that has been detected in a wide range of inflammatory conditions  
17 [Nauseef, 2014]. We also measured changes in the levels of folic acid, an essential vitamin,  
18 exclusively supplied by commensal microbes in the gut (by Bifidobacteria among others  
19 [Sugahara et al, 2015]) or by dietary supplementation. The changes observed for these  
20 metabolites were not significant upon feeding mice with the PP ethanol fraction (Fig. 4S).  
21 Thus, the levels of some metabolites were not changed, which is understandable, as only  
22 healthy mice were treated with the PP ethanol fraction.

23

#### 1 4. Discussion

2 Hemicellulose (20-30% of wood) is a rejected by-product of paper production. Hardwoods  
3 hemicelluloses (from dicot angiosperm trees that reproduce by flowering, such as teak,  
4 mahogany, maple, walnut, oak, cherry, apple) contain 15-30% of xylan but only 2-5% of  
5 glucomannan. In contrast, the softwoods hemicelluloses (from gymnosperm trees such as  
6 evergreen conifers, like pine or spruce) contain only 5-10% xylan but 14-20% glucomannan  
7 [Timmel, 1964; Álvarez et al, 2016]. Not much is known about the eventual biological role of  
8 glucomannans, apart from the fact that galactomannan does not support growth of some  
9 human *Lactobacilli* [McLaughlin et al, 2015]. When the fermentability of GGM was studied in  
10 broiler chickens, it was observed to protect against *S. typhimurium* colonization [Rajani et al,  
11 2016]. Very recently, when the effect of acetylated galactoglucomannan (AcGGM) from  
12 Norwegian lignocellulosic feedstock has been investigated, it appeared to promote beneficial  
13 bacteria, suggesting a prebiotic activity *vis-a-vis* human gut health [La Rosa et al, 2019].

14 In our hands, the majority of hemicellulose-derived fractions contained glycans with a  
15 degree of polymerization comprised between 3 and 8, while the ethanol-precipitated fraction  
16 contained GGM with DP comprised between 4 and 13. In order to measure growth of chosen  
17 gut bacteria, we monitored ATP production, which follows the growth and metabolism of living  
18 bacteria better than OD measurements. We observed that the ethanol precipitate was able to  
19 modify growth behavior of analyzed bacteria, inducing robust growth of *B. adolescentis*.  
20 Moreover, this hemicellulose fraction induced quite high second peak of bacteria growth at  
21 later culture time, suggesting the transformation of initial GGMs to lower-DP glycans, readily  
22 used by bacteria. Analysis of glycan kinetics upon feeding *B. adolescentis* with the ethanol  
23 precipitate fraction showed that at lengthier culture times the concentration of longer glycans  
24 decreased, with a concomitant appearance of shorter glycans. It is relevant that *B.*  
25 *adolescentis* produces several secreted glycan-degrading enzymes [van den Broek et al,  
26 2008]. *E. coli* LF82 was able to quickly and efficiently use furnished glycans, which confirms  
27 high fitness of adherent-invasive *E. coli* LF82 [Martinez-Medina and Garcia, 2014]. *E. coli*

1 LF82 is a pathogenic strain, and our hemicelluloses fractions did not induce a strong growth of  
2 this bacterium. The highest growth on both controls and hemicellulose-derived fractions was  
3 achieved by *B. adolescentis*, but the longest periods of growth on these fractions were  
4 observed for *A. muciniphila*, showing a slow increase of viable bacteria (and suggesting rather  
5 long doubling time).

6 Commensal microbes attune the innate and adaptive immune responses [Donia and  
7 Fischbach, 2015; Lee and Hase, 2014, among others], through the production of short-chain  
8 fatty acids, the important end products of fermentation of dietary fibers by the anaerobic  
9 intestinal microbiota. SCFAs act as a link between the microbiota and the immune system, by  
10 modulating different aspects of intestinal epithelial cells and leukocytes development, survival  
11 and function through activation of G protein coupled receptors and by modulation of the  
12 activity of enzymes and transcription factors [Corrêa-Oliveira et al, 2016]. In addition, the  
13 SCFAs exert multiple beneficial effects on mammalian homeostasis of healthy mucosa. The  
14 most abundant SCFAs produced by colonic bacteria are acetic acid (C2), propionic acid (C3),  
15 and butyric acid (C4). Butyric acid is an energy source for the colonic epithelial cells  
16 [Tsuji-kawa et al, 2003], the other SCFA are absorbed in the host circulation, joining various  
17 metabolic pathways [Cummings JH and Macfarlane, 1991; Wolever et al, 1993; Henningsson  
18 et al, 2002]. The low level of SCFAs in the human gut has been associated with the  
19 development of different diseases ranging from metabolic and inflammatory diseases to  
20 cancers [den Besten et al, 2013; Bultman, 2016; Stilling et al, 2016]. Furthermore, the  
21 presence of SCFA is proposed to confer colonization resistance against Enterobacteriaceae  
22 (containing disease-causing bacteria) through “metabolic exclusion” [Lawley and Walker,  
23 2013].

24 *B. adolescentis* is known as the best producer of acetate among different Bifidobacteria  
25 [Marx et al, 2013], and the production of acetate by *B. adolescentis* may result in cross-  
26 feeding to *Faecalibacterium prausnitzii*, leading to production of health-promoting butyrate by  
27 the latter [Moens et al, 2016]. Upon supplementing medium with hemicellulose-derived

1 fractions, *B. adolescentis* was able to produce significant amounts of acetate but also of  
2 propionic acid, in particular induced by autohydrolysate and PP ethanol fraction. Propionic  
3 acid is generally associated with hypocholesterolemic effects and might be an anti-obesity,  
4 satiety-enhancing and anti-cancer factor [Illman et al, 1988; Arora et al, 2011; Havenaar,  
5 2011]. Furthermore, it is associated with reduced stress behavior and protection of blood-brain  
6 barrier (BBB) from oxidative stress, which suggests that gut-derived microbial metabolites  
7 might interact with the BBB [Burokas et al, 2011; Hoyles et al, 2018]. Interestingly, mice fed  
8 with Western diet (high sugar, high fat) showed a decrease in propionate production and were  
9 more susceptible to colonization with AIEC strains [Agus et al, 2016]. We did not detect *in*  
10 *vitro* production of butyrate. It is known that Bifidobacteria do not produce butyrate, as no  
11 butyrate production pathway is present in their genomes [Klijn et al, 2005]. It has also been  
12 noted that the mucin-degradation activity of *A. muciniphila* led to the production of propionate  
13 and acetate only [Derrien et al, 2004].

14 During *in vivo* experiments, no significant effect on body mass of mice was observed with  
15 the hemicellulose diet. The analysis of 16S rRNA genes in cecal samples of control and  
16 ethanol PP-fed mice showed a significant increase in the proportion of Bacteroidetes and a  
17 diminished proportion of Proteobacteria. Some Proteobacteria, in particular AIEC strains such  
18 as LF 82, have been associated with the pathogenesis of inflammatory bowel conditions  
19 [Mazzarella et al, 2017]. Bacteroidetes and Firmicutes are prevalent phyla (more than 90%) in  
20 normal human gut microbiota and also in the cecum of our mice. The Bacteroidetes phylum  
21 generally makes up half or more of the gut microbiome, with other phyla, such as  
22 Actinobacteria and Verrucomicrobia, comprising 10% or less of the microbiome [Jandhyala et  
23 al, 2015; Lloyd-Price et al, 2017]. Similar proportions were found in our study (see control in  
24 Fig. 5). Bacteroidetes genomes encode a high number of inducible carbohydrate-active  
25 enzymes that enable the use of dietary and host mucosal glycans [El Kaoutari et al, 2013],  
26 explaining the rise of Bacteroidetes upon feeding mice with hemicelluloses. Similarly, in the  
27 study on the effect of fiber diet in humans, levels of Bacteroidetes correlated positively with  
28 fiber intake (Simões et al, 2013). A high Firmicutes/Bacteroidetes ratio has been implicated in

1 predisposition to obesity [Ley et al, 2006; Turnbaugh et al, 2009; Compare et al, 2016].  
2 Therefore, an increase in Bacteroidetes observed upon feeding with glycans contained in PP  
3 ethanol fraction suggests potentially an anti-obesity effect of this fraction.

4 Our *in vivo* data showed the potential of hemicellulose-derived fractions to modulate the  
5 intestinal microbiota. By feeding healthy mice with particular glycans, we observed the diverse  
6 and varied effects resulting in gut microbiota modification, boosting of the immune system,  
7 lipids alterations and bile sequestration. Data obtained on murine models have shown that  
8 tryptophan catabolites are able to enhance regulatory T cells and protect mice from chronic  
9 hyperinflammatory responses [Mezrich et al, 2010]. The increase of the Trp/Kyn ratio is a sign  
10 of good health [Keszthelyi et al, 2009]. Moreover, the increase in serotonin (5-HT) might be a  
11 sign a well-being since serotonin is well known for mood control in the brain (Liu and Deneris,  
12 2011). Accordingly, the administration of prebiotics (FOS and GOS) reduced depression and  
13 anxiety in mice [80]. Finally, the increased 5-HT might also be a sign of good bowel health,  
14 serotonin being involved in the peristaltic reflex pathway [Burokas et al, 2017] and the  
15 development of microvilli in the intestine [Nakamura et al, 2008].

16 The total level of fatty acids was significantly diminished in mice fed with PP ethanol  
17 fraction, with a reduction noted for palmitic, stearic and linolenic acids. It has been described  
18 that the Palmitic acid has been shown to induce anxiety-like behavior in mice [Moon et al,  
19 2014] and it has been associated with an increased risk of coronary heart disease and tumor  
20 development [Fattore and Fanelli, 2013], while stearic acid apparently induces  
21 proinflammatory cytokine production [Miao et al, 2015]. Linoleic acid (C18:2) is an essential  
22 fatty acid that te human body cannot synthesized. When released from membrane  
23 phospholipids, it can be enzymatically oxidized to a variety of derivatives involved in cell  
24 signaling. It is a precursor of arachidonic acid that can be converted to prostaglandins and  
25 leukotrienes, which, when produced in excess, are known to contribute to a number of chronic  
26 diseases, such as inflammation and cancer [Fritsche, 2014]. These data should, however, be  
27 treated with caution as it seems that the response of fatty acid metabolism to the  
28 administration of Bifidobacteria is strain-dependent [Wall et al, 2012], and we do not know

1 whether these changes in lipidome result from an increase in *B. adolescentis* due to the mice  
2 being fed with the PP ethanol fraction. In parallel, a reduction in cholesterol level was  
3 observed. Elevated blood cholesterol is a major risk factor for coronary heart disease, a risk  
4 factor for breast cancer and a significant public health problem. Despite use of cholesterol-  
5 lowering statins, quite often a residual vascular risk remains [Sampson et al, 2012]. Also, the  
6 reduction of bile acids was observed for treated mice. It is known that dietary or genetic  
7 obesity inducing alteration of gut microbiota increases the level of deoxycholic acid (DCA), a  
8 gut bacterial metabolite known to cause DNA damage, which may lead to liver cancer  
9 [Yoshimoto et al, 2013]. Decreasing bile acid concentration in the colon in cirrhosis leads to  
10 reduction in bile acid 7 $\alpha$ -dehydroxylating gut bacteria, which produce DCA [Ridlon et al,  
11 2013]. Until now, the manipulation of the bile acid pool was done with drugs able to sequester  
12 bile acids. The bile sequestrants have found clinical application for dyslipidemia leading to  
13 regression of atherosclerotic plaque [Insull, 2006; The Lipid Research Clinics, 1984] and,  
14 more recently, application to type 2 diabetes [Ooi and Loke, 2014]. Remarkably, the action of  
15 some anti-diabetic drugs is accompanied by increase in Lactobacilli and Bifidobacteria [Gu et  
16 al, 2017]. In our hands, upon feeding healthy mice with glycans in PP ethanol fraction, the  
17 amount of bile acids diminished significantly, suggesting a decreased toxicity to microbiota. In  
18 conclusion, the observed modulation of healthy mice metabolites might conceivably lead to a  
19 better health through prevention of some diseases. These effects can be obtained through  
20 utilization of the usually rejected softwood fraction and achieved without deleterious side  
21 effects.

22 In a recent review spruce galactoglucomannan (GGM) was described as a potential wood-  
23 based food ingredient and the examples were given of plant-based polysaccharides that are  
24 already authorized for food purposes (Pitkänen et al., 2018). However, these examples are  
25 limited to food thickeners and stabilizers and large safety analyses would be needed to  
26 introduce GGMs as a mainstream food.

27 A change in the intestinal microbiota through diet modification can occur within a single day  
28 [Turnbaugh et al, 2009; Wu et al, 2011; Faith et al, 2013]. However, over the long-term,

1 microbial populations are generally stable; each individual's microbiota is most similar to their  
2 family members, with two unrelated individuals sharing ~30% of strains in their microbiota [Wu  
3 et al, 2011; Faith et al, 2013]. Thus, enrichment with *B. adolescentis* alone would not lead to  
4 long-term persistence of this bacteria within gut microbiota. In addition, it is thought that any  
5 strain that increases its numbers relative to that of other bacteria occupying the same  
6 ecological niche will quickly be eliminated by phage predation (nothing is known about phages  
7 activity against *B. adolescentis*). However, treatment with *B. adolescentis* applied together  
8 with glycans from the PP ethanol fraction may provide a long-term selective advantage,  
9 decreasing resilience of an unhealthy state. The addition of particular glycans might induce  
10 specific pathways utilization [Lammerts van Bueren et al, 2017]. Such synbiotic intervention  
11 consisting of the simultaneous use of probiotics and prebiotics might open novel prophylactic  
12 and therapeutic strategies to human health, such as the treatment of obesity or chronic  
13 inflammatory and immunity-related diseases.

14

## 15 **Conclusion**

16 Softwood hemicellulose is a rejected by-product of paper production from wood but our work  
17 suggest that high added value could be obtained for this by-product. Indeed, we showed that  
18 hemicellulose oligosaccharides obtained by autohydrolysis stimulate growth of beneficial gut  
19 bacteria species. Furthermore, in mice fed with these oligosaccharides we observed a  
20 reshaping toward a less obesogenic microbiome, and production of metabolites interesting for  
21 health. These results suggest that hemicellulose from softwood could be a source of important  
22 prebiotics for humans. Work is under investigation to further characterize the active  
23 oligosaccharide fractions and to optimize the production process.

24

25

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## 1 References

- 2 Agus, A., Denizot, J., Thévenot, J., Martinez-Medina, M., Massier, S., Sauvanet, P., Bernalier-  
3 Donadille, A., Denis, S., Hofman, P., Bonnet, R., Billard, E., Barnich, N. (2016) Western diet  
4 induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive *E. coli*  
5 infection and intestinal inflammation. *Sci Rep*, 6:19032. Doi: 10.1038/srep19032
- 6 Al-Ghazzewi, F.H., Tester, R.F. (2012) Efficacy of cellulase and mannanase hydrolysates of  
7 konjac glucomannan to promote the growth of lactic acid bacteria. *J Sci Food Agric*, 92:2394-  
8 6. Doi: 10.1002/jsfa.5678.
- 9 Álvarez, C., Reyes-Sosa, F.M., Díez, B. (2016) Enzymatic hydrolysis of biomass from wood.  
10 *Microb Biotechnol*, 9:149-56. Doi: 10.1111/1751-7915.12346
- 11 Arora, T., Sharma, R., Frost, G. (2011) Propionate. Anti-obesity and satiety enhancing factor?  
12 *Appetite*, 56:511-5. Doi: 10.1016/j.appet.2011.01.016
- 13 Barcenilla, A., Pryde, S.E., Martin, J.C., Duncan, S.H., Stewart, C.S., Henderson, C., Flint,  
14 H.J. (2000) Phylogenetic relationships of butyrate-producing bacteria from the human gut.  
15 *Appl Environ Microbiol*, 66:1654-61. PubMed PMID: 10742256
- 16 Barker, H.,A. (1981). Amino acid degradation by anaerobic bacteria. *Ann. Rev Biochem*,  
17 50:23-40. Doi:10.1146/annurev.bi.50.070181.000323
- 18 Boulet, L., Faure, P., Flore, P., Montérémal, J., Ducros, V. (2017) Simultaneous determination  
19 of tryptophan and 8 metabolites in human plasma by liquid chromatography/tandem mass  
20 spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, 1054:36-43. Doi:  
21 10.1016/j.jchromb.2017.04.010.
- 22 Bultman, S.J. (2016) The microbiome and its potential as a cancer preventive intervention.  
23 *Semin Oncol*, 43:97-106. Doi: 10.1053/j.seminoncol.2015.09.001
- 24 Burokas, A., Arboleya, S., Moloney, R.D., Peterson, V.L., Murphy, K., Clarke, G., Stanton, C.,  
25 Dinan, T.G., Cryan, J.F. (2017) Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have  
26 Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice.  
27 *Biol Psychiatry*. 82:472-487. Doi: 10.1016/j.biopsych.2016.12.031
- 28 Carvalho, F.A., Koren, O., Goodrich, J.K., Johansson, M.E., Nalbantoglu, I., Aitken, J.D., Su,  
29 Y, Chassaing, B., Walters, W.A., González. A., Clemente, J.C., Cullender, T.C., Barnich, N.,  
30 Darfeuille-Michaud A., Vijay-Kumar, M., Knight, R., Ley, R.E., Gewirtz, A.T. (2012) Transient  
31 inability to manage proteobacteria promotes chronic gut inflammation in TLR5-deficient mice.  
32 *Cell Host Microbe*, 12:139-52. Doi:10.1016/j.chom.
- 33 Compare, D., Rocco, A., Sanduzzi Zamparelli M., Nardone, G. (2016) The Gut Bacteria-  
34 Driven Obesity Development. *Dig Dis*, 34:221-9. Doi: 10.1159/000443356
- 35 Conte, M.P., Aleandri, M., Marazzato, M., Conte, A.L., Ambrosi, C., Nicoletti, M., Zagaglia C,  
36 Gambarà, G., Palombi, F., De Cesaris, P., Ziparo, E., Palamara, A.T., Riccioli, A., Longhi, C.  
37 (2016). The Adherent/Invasive *Escherichia coli* Strain LF82 Invades and Persists in Human

- 1 Prostate Cell Line RWPE-1, Activating a Strong Inflammatory Response. *Infect Immun*,  
2 17;84:3105-3113. Doi: 10.1128/IAI.00438-16.
- 3 Corrêa-Oliveira, R., Fachi, J.L., Vieira, A., Sato, F.T., Vinolo, M.A. (2016) Regulation of  
4 immune cell function by short-chain fatty acids. *Clin Transl Immunology*, 22;5:e73. Doi:  
5 10.1038/cti.2016.17
- 6 Cummings, J.H., Macfarlane, G.T. (1991) The control and consequences of bacterial  
7 fermentation in the human colon. *J Appl Bacteriol*, 70:443-59. PubMed PMID:1938669
- 8 Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi,  
9 S.J., Berenjian, A., Ghasemi, Y. (2019) Prebiotics: Definition, Types, Sources, Mechanisms,  
10 and Clinical Applications. *Foods*. Mar 9;8(3). pii: E92. doi: 10.3390/foods8030092
- 11  
12 Deloule, V., Boisset, C., Chirat, C., Toussaint, B., inventors, INP Grenoble assignee, French  
13 Patent Fr. 1852334, 2016.
- 14 den Besten, G., van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.J., Bakker, B.M.  
15 (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and  
16 host energy metabolism. *J Lipid Res*, 54:2325-40. Doi: 10.1194/jlr.R036012
- 17 Derrien, M., Belzer, C., de Vos, W.M. (2017) *Akkermansia muciniphila* and its role in  
18 regulating host functions. *Microb Pathog*, 106:171-181. Doi: 10.1016/j.micpath.2016.02.005.
- 19 Derrien, M., Vaughan, E.E., Plugge, C.M., de Vos, W.M. (2004) *Akkermansia muciniphila* gen.  
20 nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol*,  
21 54:1469-76. Doi:10.1099/ijs.0.02873-0
- 22 Donia, M.S., Fischbach, M.A. (2015) Human microbiota. Small molecules from the human  
23 microbiota. *Science*, 349:1254766. Doi: 10.1126/science
- 24 El Kaoutari, A., Armougom, F., Gordon, J.I., Raoult, D., Henrissat, B. (2013) The abundance  
25 and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol*,  
26 11:497-504. Doi: 10.1038/nrmicro3050
- 27 Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., Maman, S.,  
28 Hernandez-Raquet, G., Combes, S., Pascal, G. (2018) FROGS: Find, Rapidly, OTUs with  
29 Galaxy Solution. *Bioinformatics*, 34:1287-1294. Doi: 10.1093/bioinformatics/btx791.
- 30 Faith, J.J., Guruge, J.L., Charbonneau, M., Subramanian, S., Seedorf, H., Goodman, A.L.,  
31 Clemente, J.C., Knight, R., Heath, A.C., Leibel, R.L., Rosenbaum, M., Gordon, J.I. (2013) The  
32 long-term stability of the human gut microbiota. *Science*. 5;341:1237439. Doi:  
33 10.1126/science.1237439
- 34 Fattore, E., Fanelli, R.. (2013) Palm oil and palmitic acid: a review on cardiovascular effects  
35 and carcinogenicity. *Int J Food Sci Nutr*, 64:648-59. Doi: 10.3109/09637486.2013.768213
- 36 Flint, H.J., Duncan, S.H., Scott, K.P., Louis, P. (2015) Links between diet, gut microbiota  
37 composition and gut metabolism. *Proc Nutr Soc*, 74:13-22. Doi 10.1017/S0029665114001463

- 1 Floch, M.H. (2010) The effect of probiotics on host metabolism: the microbiota and  
2 fermentation. *J Clin Gastroenterol*, *44* Suppl 1:S19-21. Doi: 10.1097/MCG.0b013e3181dd4fb7
- 3 Fritsche, K.L. (2014) Linoleic acid, vegetable oils & inflammation. *Mo Med*, *111*:41-3. PubMed  
4 PMID:24645297
- 5 Fung, K.Y., Cosgrove, L., Lockett, T., Head, R., Topping, D.L. (2012) A review of the potential  
6 mechanisms for the lowering of colorectal oncogenesis by butyrate. *Br J Nutr*, *108*:820-31.  
7 Doi: 10.1017/S0007114512001948.
- 8 Gibson, G.R., Roberfroid, M.B. (1995) Dietary modulation of the human colonic microbiota:  
9 introducing the concept of prebiotics. *J Nutr*, *125*:1401-12. Review. PubMed PMID: 7782892.
- 10 Gu, Y., Wang, X., Li, J., Zhang, Y., Zhong, H., Liu, R., Zhang, D., Feng, Q., Xie, X., Hong, J.,  
11 Ren, H., Liu, W., Ma, J., Su, Q., Zhang, H., Yang, J., Wang, X., Zhao, X., Gu, W., Bi, Y., Peng,  
12 Y., Xu, X., Xia, H., Li, F., Xu, X., Yang, H., Xu, G., Madsen, L., Kristiansen, K., Ning, G.,  
13 Wang, W. (2017) Analyses of gut microbiota and plasma bile acids enable stratification of  
14 patients for antidiabetic treatment. *Nat Commun*, *8*:1785. Doi: 10.1038/s41467-017-01682-2
- 15 Haase, S., Haghikia, A., Wilck, N., Müller, D.N., Linker, R.A. (2018) Impacts of microbiome  
16 metabolites on immune regulation and autoimmunity. *Immunology*, *154*:230-238. Doi:  
17 10.1111/imm.12933.
- 18 Havenaar, R. (2011) Intestinal health functions of colonic microbial metabolites: a review.  
19 *Benef Microbes*, *2*:103-14. Doi: 10.3920/BM2011.0003
- 20 Henningsson, A.M., Björck, I.M., Nyman, E.M. (2002) Combinations of indigestible  
21 carbohydrates affect short-chain fatty acid formation in the hindgut of rats. *J Nutr*, *132*:3098-  
22 104. Doi:10.1093/jn/131.10.3098
- 23 Hoyles, L., Snelling, T., Umlai, U.K., Nicholson, J.K., Carding, S.R., Glen, R.C., McArthur, S.  
24 (2018) Microbiome-host systems interactions: protective effects of propionate upon the blood-  
25 brain barrier. *Microbiome*, *6*:55. Doi: 10.1186/s40168-018-0439-y
- 26 Illman, R.J., Topping, D.L., McIntosh, G.H., Trimble, R.P., Storer, G.B., Taylor, M.N., Cheng,  
27 B.Q. (1988) Hypocholesterolaemic effects of dietary propionate: studies in whole animals and  
28 perfused rat liver. *Ann Nutr Metab*, *32*:95-107. PubMed PMID:3214141
- 29 Insull, W. Jr. (2006) Clinical utility of bile acid sequestrants in the treatment of dyslipidemia: a  
30 scientific review. *South Med J*, *99*:257-73. PubMed PMID:16553100
- 31 Jacobs, A., Lundqvist, J., Stålbrand, H., Tjerneld, F., Dahlman, O. (2002) Characterization of  
32 water-soluble hemicelluloses from spruce and aspen employing SEC/MALDI mass  
33 spectroscopy. *Carbohydr Res*, *337*:711-17. Doi:10.1016/s0008-6215(02)00054-x
- 34 Jandhyala, S.M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., Nageshwar  
35 Reddy, D. (2015) Role of the normal gut microbiota. *World J Gastroenterol*, *21*:8787-803. Doi:  
36 10.3748/wjg.v21.i29.8787

- 1 Keszthelyi, D., Troost, F.J., Masclee, A.A. (2009) Understanding the role of tryptophan and  
2 serotonin metabolism in gastrointestinal function. *Neurogastroenterol Motil*, 21:1239-49. Doi:  
3 10.1111/j.1365-2982.2009.01370.x
- 4 Kheirandish-Gozal, L., Peris, E., Wang, Y., Tamae Kakazu, M., Khalyfa, A., Carreras, A.,  
5 Gozal, D. (2014) Lipopolysaccharide-binding protein plasma levels in children: effects of  
6 obstructive sleep apnea and obesity. *J Clin Endocrinol Metab*, 99:656-63. Doi:  
7 10.1210/jc.2013-3327
- 8 Kim, C.H., Park, J., Kim, M. (2014) Gut microbiota-derived short-chain Fatty acids, T cells,  
9 and inflammation. *Immune Netw*, 14:277-88. Doi: 10.4110/in.2014.14.6.277.
- 10 Kim, Y., Lee, D., Kim, D., Cho, J., Yang, J., Chung, M., Kim, K., Ha, N. (2008) Inhibition of  
11 proliferation in colon cancer cell lines and harmful enzyme activity of colon bacteria by  
12 *Bifidobacterium adolescentis* SPM0212. *Arch Pharm Res*, 31:468-73. Doi: 10.1007/s12272-  
13 001-1180-y.
- 14 Kishani S, Vilaplana F, Xu W, Xu C, Wågberg L. (2018) Solubility of Softwood Hemicelluloses.  
15 *Biomacromolecules*, 19:1245-55. Doi:10.1021/acs.biomac.8b00088.
- 16 Klaassen, C.D., Cui, J.Y. (2015) Review: Mechanisms of How the Intestinal Microbiota Alters  
17 the Effects of Drugs and Bile Acids. *Drug Metab Dispos*, 43:1505-21. Doi:  
18 10.1124/dmd.115.065698
- 19 Klijn, A., Mercenier, A., Arigoni, F. (2005) Lessons from the genomes of bifidobacteria. *FEMS*  
20 *Microbiol Rev*, 29:491-509. DOI:10.1016/j.femsre.2005.04.010
- 21 La Rosa, S.L., Kachrimanidou, V., Buffetto, F., Pope, P.B., Pudlo, N.A., Martens, E.C.,  
22 Rastall, R.A., Gibson, G.R., Westereng, B. 2019 Wood-Derived Dietary Fibers Promote  
23 Beneficial Human Gut Microbiota. *mSphere*, 4. pii: e00554-18. Doi: 10.1128/mSphere.00554-  
24 18
- 25 Lammerts van Bueren, A., Mulder, M., Leeuwen, S.V., Dijkhuizen, L. (2017) Prebiotic  
26 galactooligosaccharides activate mucin and pectic galactan utilization pathways in the human  
27 gut symbiont *Bacteroides thetaiotaomicron*. *Sci Rep*, 7:40478. Doi: 10.1038/srep40478
- 28 Lawley, T.D., Walker, A.W. (2013) Intestinal colonization resistance. *Immunology*, 138:1-11.  
29 Doi: 10.1111/j.1365-2567.2012.03616.x
- 30 Lee DK, Kang JY, Shin HS, Park IH, Ha NJ. Antiviral activity of *Bifidobacterium adolescentis*  
31 SPM0212 against Hepatitis B virus. *Arch Pharm Res*. 2013 Dec;36(12):1525-32. Doi:  
32 10.1007/s12272-013-0141-3.
- 33 Lee, W.J., Hase, K. (2014) Gut microbiota-generated metabolites in animal health and  
34 disease. *Nat Chem Biol*, 10:416-24. Doi: 10.1038/nchembio.1535

- 1 Legrand-Poels, S., Esser, N., L'homme, L., Scheen, A., Paquot, N., Piette, J. (2014) Free fatty  
2 acids as modulators of the NLRP3 inflammasome in obesity/type 2 diabetes. *Biochem*  
3 *Pharmacol*, 92:131-41. Doi: 10.1016/j.bcp.2014.08.013
- 4 Ley, R.E., Turnbaugh, P.J., Klein, S., Gordon, J.I. (2006) Microbial ecology: human gut  
5 microbes associated with obesity. *Nature*, 444:1022-3. Doi:10.1038/4441022a
- 6 Liu, C., Deneris, E.S. (2011) Transcriptional control of serotonin-modulated behavior and  
7 physiology. *Neuropsychopharmacology*, 36:361-2. Doi: 10.1038/npp.2010.135
- 8 Lloyd-Price, J., Mahurkar, A., Rahnavard, G., Crabtree, J., Orvis, J., Hall, A.B., Brady, A.,  
9 Creasy, H.H., McCracken, C., Giglio, M.G., McDonald, D., Franzosa, E.A., Knight, R., White,  
10 O., Huttenhower, C. (2017) Strains, functions and dynamics in the expanded Human  
11 Microbiome Project. *Nature*, 550:61-66. Doi: 10.1038/nature23889
- 12 London, L.E., Kumar, A.H., Wall, R., Casey, P.G., O'Sullivan, O., Shanahan, F., Hill, C.,  
13 Cotter, P.D., Fitzgerald, G.F., Ross, R.P., Caplice, N.M., Stanton, C. (2014)  
14 Exopolysaccharide-producing probiotic Lactobacilli reduce serum cholesterol and modify  
15 enteric microbiota in ApoE-deficient mice. *J Nutr*, 144:1956-62. Doi: 10.3945/jn.114.191627
- 16 Lundqvist, J., Jacobs, A., Palm, M., Zacchi, G., Dahlman, O., Stalbrand, H. (2003)  
17 Characterization of galactoglucomannan extracted from spruce (*Picea abies*) by heat-  
18 fractionation at different conditions. *Carbohydr Polym*, 51:203-11. <https://portal.research.lu.se>
- 19 Mangin, I., Dossou-Yovo, F., Lévêque, C., Dessoy, M.V., Sawoo, O., Suau, A., Pochart, P.  
20 (2018) Oral administration of viable *Bifidobacterium pseudolongum* strain Patronus modified  
21 colonic microbiota and increased mucus layer thickness in rat. *FEMS Microbiol Ecol*, 94: 11.  
22 Doi: 10.1093/femsec/fiy177
- 23 Martinez-Medina, M., Garcia-Gil L.J. (2014) *Escherichia coli* in chronic inflammatory bowel  
24 diseases: An update on adherent invasive *Escherichia coli* pathogenicity. *World J Gastrointest*  
25 *Pathophysiol*, 5:213-27. Doi: 10.4291/wjgp.v5.i3.213
- 26 Marx, S.P., Winkler, S., Hartmeier, W. (2000) Metabolization of beta-(2,6)-linked fructose-  
27 oligosaccharides by different bifidobacteria. *FEMS Microbiol Lett*, 182:163-9.  
28 Doi:10.1111/j.1574-6968.2000.tb08891.x
- 29 Mazzarella, G., Perna, A., Marano, A., Lucariello, A., Rotondi Aufiero, V., Sorrentino, A.,  
30 Melina, R., Guerra, G., Taccone, F.S., Laquinto, G., De Luca, A. (2017) Pathogenic Role of  
31 Associated Adherent-Invasive *Escherichia coli* in Crohn's Disease. *J Cell Physiol*, 232:2860-  
32 2868. Doi: 10.1002/jcp.25717
- 33 McLaughlin, H.P., Motherway, M.O., Lakshminarayanan, B., Stanton, C., Paul Ross, R., Brulc,  
34 J., Menon, R., O'Toole, P.W., van Sinderen, D. (2015) Carbohydrate catabolic diversity of  
35 bifidobacteria and lactobacilli of human origin. *Int J Food Microbiol*, 203:109-21. Doi:  
36 10.1016/j.ijfoodmicro.2015.03.008

- 1 Meijer, K., de Vos, P., Priebe, M.G. (2010) Butyrate and other short-chain fatty acids as  
2 modulators of immunity: what relevance for health? *Curr Opin Clin Nutr Metab Care*, 13:715-  
3 21. Doi: 10.1097/MCO.0b013e32833eebe5.
- 4 Messaoudi, S., Manai, M., Kergourlay, G., Prévost, H., Connil, N., Chobert, J.M., Dousset, X.  
5 (2013) *Lactobacillus salivarius*: bacteriocin and probiotic activity. *Food Microbiol*, 36:296-304.  
6 Doi: 10.1016/j.fm.2013.05.010.
- 7 Mezrich, J.D., Fechner, J.H., Zhang, X., Johnson, B.P., Burlingham, W.J., Bradfield, C.A.  
8 (2010) An interaction between kynurenine and the aryl hydrocarbon receptor can generate  
9 regulatory T cells. *J Immunol*, 185:3190-8. Doi: 10.4049/jimmunol.0903670
- 10 Miao, H., Chen, L., Hao, L., Zhang, X., Chen, Y., Ruan, Z., Liang, H. (2015) Stearic acid  
11 induces proinflammatory cytokine production partly through activation of lactate-HIF1 $\alpha$   
12 pathway in chondrocytes. *Sci Rep*, 5:13092. Doi: 10.1038/srep13092
- 13 Mitsuoka, Tm, Hidaka, Hm, Eida, T. (1987) Effect of fructo-oligosaccharides on intestinal  
14 microflora. *Nahrung*, 31:427-36. PubMed PMID: 3657917.
- 15 Moens, F., Weckx, S., De Vuyst, L. (2016) Bifidobacterial inulin-type fructan degradation  
16 capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium*  
17 *prausnitzii*. *Int J Food Microbiol*, 231:76-85. Doi: 10.1016/j.ijfoodmicro.2016.05.015
- 18 Moon, M.L., Joesting, J.J., Lawson, M.A., Chiu, G.S., Blevins, N.A., Kwakwa, K.A., Freund,  
19 G.G. (2014) The saturated fatty acid, palmitic acid, induces anxiety-like behavior in mice.  
20 *Metabolism*, 63:1131-40. Doi: 10.1016/j.metabol.2014.06.002
- 21 Moreno-Navarrete, J.M., Ortega F., Serino, M., Luche, E., Waget, A., Pardo, G., Salvador, J.,  
22 Ricart, W., Frühbeck, G., Burcelin, R., Fernández-Real, J.M. (2012) Circulating  
23 lipopolysaccharide-binding protein (LBP) as a marker of obesity-related insulin resistance. *Int*  
24 *J Obes (Lond)*, 36:1442-9. Doi: 10.1038/ijo.2011.256
- 25 Nakamura, K., Sato, T., Ohashi, A., Tsurui, H., Hasegawa, H. (2008) Role of a serotonin  
26 precursor in development of gut microvilli. *Am J Pathol*, 172:333-44. Doi:  
27 10.2353/ajpath.2008.070358
- 28 Nakamura, K., Sato, T., Ohashi, A., Tsurui, H., Hasegawa, H. (2008) Role of a serotonin  
29 precursor in development of gut microvilli. *Am J Pathol*, 172:333-44. Doi:  
30 10.2353/ajpath.2008.070358
- 31 Nauseef, W.M. (2014) Myeloperoxidase in human neutrophil host defence. *Cell Microbiol*,  
32 16:1146-55. Doi: 10.1111/cmi.12312
- 33 Neville, B.A., O'Toole, P.W. (2010) Probiotic properties of *Lactobacillus salivarius* and closely  
34 related *Lactobacillus* species. *Future Microbiol*, 5:759-74. Doi: 10.2217/fmb.10.35.
- 35 Ooi, C.P., Loke, S.C. (2014) Colesevelam for Type 2 diabetes mellitus: an abridged Cochrane  
36 review. *Diabet Med*, 31:2-14. Doi: 10.1111/dme.12295

- 1 Pawar, P.M., Koutaniemi, S., Tenkanen, M., Mellerowicz, E.J. (2013) Acetylation of woody  
2 lignocellulose: significance and regulation. *Front Plant Sci*, 4:118. Doi:  
3 10.3389/fpls.2013.00118.
- 4 Pitkänen L, Heinonen M, Mikkonen KS, (2018) Safety considerations of plant polysaccharides  
5 for food use: a case study on phenolic-rich softwood galactoglucomannan extract. *Food*  
6 *Funct*, 9:1931-1943. Doi: 10.1039/c7fo01425b.
- 7
- 8
- 9 Polari, L., Ojansivu, P., Mäkelä, S., Eckerman, C., Holmbom, B., Salminen, S. (2012)  
10 Galactoglucomannan extracted from spruce (*Picea abies*) as a carbohydrate source for  
11 probiotic bacteria. *J Agric Food Chem*, 60:11037-43. Doi: 10.1021/jf303741h.
- 12
- 13 Rajani, J., Dastar, B., Samadi, F., Karimi Torshizi, M.A., Abdulkhani, A., Esfandyarpour, S.  
14 (2016) Effect of extracted galactoglucomannan oligosaccharides from pine wood (*Pinus*  
15 *brutia*) on *Salmonella typhimurium* colonisation, growth performance and intestinal  
16 morphology in broiler chicks. *Br Poult Sci*, 57:682-692. Doi: 10.1080/00071668.2016.1200013
- 17 Reigstad, C.S., Salmonson, C.E., Rainey, J.F. 3rd, Szurszewski, J.H., Linden, D.R.,  
18 Sonnenburg, J.L., Farrugia, G., Kashyap, P.C. (2015) Gut microbes promote colonic serotonin  
19 production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J*,  
20 29:1395-403. Doi: 10.1096/fj.14-259598
- 21 Ridlon, JM, Alves, J.M., Hylemon, .PB., Bajaj, J.S. (2013) Cirrhosis, bile acids and gut  
22 microbiota: unraveling a complex relationship. *Gut Microbes*, 4:382-7. Doi:  
23 10.4161/gmic.25723
- 24 Rivas, S., Gullón, B., Gullón, P., Alonso, J.L., Parajó, J.C (2012) Manufacture and properties  
25 of bifidogenic saccharides derived from wood mannan. *J Agric Food Chem*, 60:4296-305. Doi:  
26 10.1021/jf300524s.
- 27 Rogier, E.W., Frantz, A.L., Bruno, M.E., Wedlund, L., Cohen, D.A., Stromberg, A.J., Kaetzel,  
28 C.S. (2014) Secretory antibodies in breast milk promote long-term intestinal homeostasis by  
29 regulating the gut microbiota and host gene expression. *Proc Natl Acad Sci U S A*, 111:3074-  
30 9. Doi: 10.1073/pnas.1315792111
- 31 Sampson, U.K., Fazio, S., Linton, M.F. (2012) Residual cardiovascular risk despite optimal  
32 LDL cholesterol reduction with statins: the evidence, etiology, and therapeutic challenges.  
33 *Curr Atheroscler Rep*, 14:1-10. Doi: 10.1007/s11883-011-0219-7
- 34 Simões, C.D., Maukonen, J., Kaprio, J., Rissanen, A., Pietiläinen, K.H., Saarela, M. (2013)  
35 Habitual dietary intake is associated with stool microbiota composition in monozygotic twins. *J*  
36 *Nutr*, 143:417-23. Doi: 10.3945/jn.112.166322

- 1 Stilling, R.M., van de Wouw, M., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F. (2016) The  
2 neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis?  
3 *Neurochem Int*, 99:110-132. Doi: 10.1016/j.neuint.2016.06.011
- 4 Sugahara, H., Odamak,i T., Hashikura, N., Abe, F., Xiao, J.Z. (2015) Differences in folate  
5 production by bifidobacteria of different origins. *Biosci Microbiota Food Health*, 34:87-93. Doi:  
6 10.12938/bmfh.2015-003
- 7 The Lipid Research Clinics Coronary Primary Prevention Trial results I. Reduction in incidence  
8 of coronary heart disease. (1984) *JAMA*, 251:351-64. PubMed PMID:6361299
- 9 Teleman, A., Nordström, M., Tenkanen, M., Jacobs, A., Dahlman, O. (2003) Isolation and  
10 characterization of O-acetylated glucomannans from aspen and birch wood. *Carbohydr Res*,  
11 338:525-34. DOI:10.1016/s0008-6215(02)00491-3
- 12 Timmel, T.E. (1964) Wood hemicelluloses I. *Adv Carbohydr Chem.*;19:247-302. Review.  
13 PubMed PMID: 14272330
- 14 Tsujikawa, T., Andoh, A., Fujiyama, Y. (2003) Enteral and parenteral nutrition therapy for  
15 Crohn's disease. *Curr Pharm*, 9:323-32. PubMed PMID:12570824
- 16 Turnbaugh, P.J., Hamady, M., Yatsunenکو, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin,  
17 M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., Egholm, M., Henrissat, B., Heath, A.C., Knight, R.,  
18 Gordon, J.I. (2009). A core gut microbiome in obese and lean twins. *Nature*, 457:480-4. Doi:  
19 10.1038/nature07540
- 20 Ussar, S., Fujisaka, S., Kahn, C.R. (2016) Interactions between host genetics and gut  
21 microbiome in diabetes and metabolic syndrome. *Mol Metab*, 5:795-803. Doi:  
22 10.1016/j.molmet
- 23 van den Broek, L.A., Hinz, S.W., Beldman, G., Vincken, J.P., Voragen, A.G. (2008)  
24 Bifidobacterium carbohydrases-their role in breakdown and synthesis of (potential) prebiotics.  
25 *Mol Nutr Food Res*, 52:146-63. Doi:10.1002/mnfr.200700121
- 26 Verbeke, K.A., Boobis, A.R., Chiodini, A., Edwards, C.A., Franck, A., Kleerebezem, M., Nauta,  
27 A., Raes, J., van Tol, E.A., Tuohy, K.M. (2015) Towards microbial fermentation metabolites as  
28 markers for health benefits of prebiotics. *Nutr Res Rev*, 28:42-66. Doi:  
29 10.1017/S0954422415000037.
- 30 Vital, M., Howe, A.C., Tiedje, J.M. (Revealing the bacterial butyrate synthesis pathways by  
31 analyzing (meta)genomic data. *MBio*, 5:e00889. Doi: 10.1128/mBio.00889-14
- 32 Wall, R., Marques, T.M., O'Sullivan, O., Ross, R.P., Shanahan, F., Quigley, E.M., Dinan, T.G.,  
33 Kiely, B., Fitzgerald, G.F., Cotter, P.D., Fouhy, F., Stanton, C. 2012) Contrasting effects of  
34 Bifidobacterium breve NCIMB 702258 and Bifidobacterium breve DPC 6330 on the  
35 composition of murine brain fatty acids and gut microbiota. *Am J Clin Nutr*, 95:1278-87. Doi:  
36 10.3945/ajcn.111.026435

- 1 Wolever ,T.M., Fernandes, J., Rao, A.V. (1996) Serum acetate:propionate ratio is related to  
2 serum cholesterol in men but not women. *J Nutr*, 126:2790-7. Doi: 10.1093/jn/126.11.2790
- 3 Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., Keilbaugh, S.A., Bewtra, M.,  
4 Knights, D., Walters, W.A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R.,  
5 Nessel, L., Li, H., Bushman, F.D., Lewis, J.D. (2011) Linking long-term dietary patterns with  
6 gut microbial enterotypes. *Science*, 334:105-8. Doi: 10.1126/science.1208344
- 7 Yoo, H.D., Kim, D., Paek, S.H. (2012) Plant cell wall polysaccharides as potential resources  
8 for the development of novel prebiotics. *Biomol Ther (Seoul)*, 20:371-9. Doi:  
9 10.4062/biomolther.2012.20.4.371.
- 10 Yoshimoto, S., Loo, T.M., Atarashi, K., Kanda, H., Sato, S., Oyadomari, S., Iwakura, Y.,  
11 Oshima, K., Morita, H., Hattori, M., Honda, K., Ishikawa, Y., Hara, E., Ohtani, N. (2013)  
12 Obesity-induced gut microbial metabolite promotes liver cancer through senescence  
13 secretome. *Nature* ,499:97-101. Doi: 10.1038/nature12347