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Article

Concentrated Raw Fibers Enhance the Fiber-Degrading Capacity of a Synthetic Human Gut Microbiome

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Abstract: Consumption of prebiotic fibers to modulate the human gut microbiome is a promising strategy to positively impact health. Nevertheless, given the compositional complexity of the microbiome and its inter-individual variances, generalized recommendations on the source or amount of fiber supplements remain vague. This problem is further compounded by availability of tractable *in vitro* and *in vivo* models to validate certain fibers. We employed a gnotobiotic mouse model containing an *a priori* characterized 14-member synthetic human gut microbiome (SM) for their ability to metabolize a suit of fibers *in vitro*; the SM contains 14 different strains belonging to five distinct phyla. Since soluble purified fibers have been a common subject of studies, we specifically investigated the effects of concentrated raw fibers (CRFs)—containing fibers from pea, oat, psyllium, wheat and apple—on the compositional and functional alterations in the SM. We demonstrate that, compared to a fiber-free diet, CRF supplementation increased the abundance of fiber-degraders namely *Eubacterium rectale*, *Roseburia intestinalis* and *Bacteroides ovatus* and decreased the abundance of the mucin-degrader *Akkermansia muciniphila*. These results were corroborated by a general increase of bacterial fiber-degrading α-glucosidase enzyme activity. Overall, our results highlight the ability of CRFs to enhance the microbial fiber-degrading capacity.

Keywords: microbiota; microbiome; manipulation; fiber; diet; prebiotic; nutrition ; supplement

1. Introduction

Diets prevalent in industrialized countries are characterized not only by high amounts of protein and fat, but also by a deprivation of plant-derived fibers [1]. These so-called "Western-style" nutritional habits are linked to altered and potentially disease-promoting properties of the intestinal microbiome [2] further suggesting that supplementation of such diets with prebiotic fibers might be beneficial for the host. The intestinal microbiome has a remarkable impact on susceptibility and progression of various intra- and extra-intestinal pathologies [2]. Thus, targeted manipulation of the host's microbiome may alleviate this risk and has recently received considerable attention [3]. In this context, plant-derived fibers are considered to be promising host-beneficial dietary supplements for microbiota modulation [4]. Health-beneficial impacts of fibers are either mediated by general physiological influences, such as the intestinal pH value, maintaining integrity of the mucus layer or by microbial fermentation into host-beneficial metabolites, such as short-chain fatty acids (SCFA). SCFA play a crucial role in maintaining barrier integrity and immune homeostasis [5] and soluble fibers represent a major source of these microbially produced metabolites [2].

Previously, we reported a causal role of fiber deprivation, increasing susceptibility towards enteropathogenic infections in a gnotobiotic mouse model containing a 14-

member synthetic human gut microbiome (14SM) [6]. We demonstrated that a lack of dietary fiber resulted in a bloom of mucin-degrading commensals, such as *Akkermansia muciniphila,* leading to excess degradation of the intestinal mucus layer and subsequently facilitated infection with *Citrobacter rodentium* [6]. These results further strengthen the connection between dietary fiber and gut microbial modulation. Moreover, our 14SM gnotobiotic model provides an attractive approach to validate the modulation of the gut microbiota with fiber supplementation using the basal fiber-free diet [6]. However, due to the complexity of the intestinal microbiome and the resulting individual responses, general recommendations on quantity, source or combinations of fiber supplements for consumption for humans remain vague [7, 8].

Plant-derived fibers come in different chemical forms and structures, therefore providing distinct access for intestinal microbes to hydrolyze structure-specific glycosidic linkeages. Here, we used "concentrated raw fiber" (CRF) preparations from pea, oat, psyllium, wheat and apple to evaluate the detailed effects of fiber supplementation under strictly controlled conditions in our 14SM gnotobiotic mouse model. In contrast to purified fibers [9], CRF are fiber concentrates, which are extracted and isolated from skeletal substances in a non-chemical, thermophysical process, thus providing a diverse polysaccharide composition. Of note, the used wheat CRF was previously shown to increase fecal bulking in a randomized controlled human study [10], while the psyllium CRF was associated with an increased overall SCFA production [11] in an *in vitro* system. We evaluated the *in vivo* effects of these CRF on the relative abundances of the 14SM constituent strains, the emerging activities of bacterial glycan-degrading enzymes and the associated concentrations of different SCFA. Furthermore, we performed extensive correlation analyses to evaluate potential inter-microbial influences in response to fiber supplementation, and thus to better understand community-shaping properties of such dietary modulation.

2. Results

2.1. Experimental setup to study the specific effects of concentrated raw fibers on composition and function of a 14-member microbial community in mice

Germ-free (GF) C57BL/6N mice were raised and maintained under gnotobiotic conditions on a standard mouse chow (SC). At the age of six to eight weeks, mice were colonized via intragastric gavage with a synthetic microbiota consisting of 14 different human commensals (14SM) as described previously [6]. Strains of this 14SM community represent the five dominant phyla of the human intestinal microbiota and provide important core metabolic capabilities [6]. Five to sixteen days after initial gavage, mice were either switched to a fiber-free (FF) diet or a fiber-supplemented (FS) diet (**Fig. 1a**) containing concentrated raw fibers (CRFs) (VITACEL®, J. Rettenmaier und Söhne (JRS, Germany)) derived from wheat, oat, psyllium, pea and apple. As controls, some mice were maintained on a SC diet. Before diet switch, we confirmed proper colonization of all animals with the 14SM community by strain-specific qPCR from fecal samples as described previously [6]. 20 days after diet switch (feeding period), mice fed all three different diets were sacrificed and contents of cecum and colon were harvested for downstream analyses. As we aimed to determine the direct impact of fiber supplementation on microbiota composition and function in a tightly controlled gnotobiotic setting, we designed the FF and FS diets with the aim of providing an isocaloric composition as well as an identical formulation among these two diets, with the exception of the non-cellulose complex fiber amount (**Fig. 1b**).

Thus, to generate the FS diet, we reduced the dextrose content in the FF diet by an amount corresponding to 10% of the total weight and replaced it by the same amount of a concentrated fiber mix. The fiber mix in the FS diet consisted of equal amounts of CRF preparations obtained from pea, oat, psyllium, wheat and apple (2% (w/w) each). The five different used CRF preparations contained an average fiber length within a two-digit µm range and the fiber content (w/w) in these preparations ranged from 55% (apple) to 97%

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(wheat) (**Table 1**). Importantly, the ratio of insoluble-to-soluble fibers (I/S-ratio) differed significantly among the different preparations, with an I/S-ratio of 34 in the case of the pea preparation to an I/S-ratio of 0.2 for the psyllium preparation. Of note, the apple preparation contained 9% (w/w) pectin. The SC diet contained 3.9% fibers from naturally milled fibers, while contents of protein and fat were considerably lower compared to both, the FF and FS diets (**Fig. 1b**).

Figure 1. Experimental outline to study effects of concentrated raw fibers on a 14-member microbial community. a) Experimental outline. Germ-free (GF) C57BL/6N (n = 15), raised and maintained on a standard mouse chow (SC) were colonized with the 14SM community. 5-16 d after colonization, mice were either continued to be fed a SC diet (n = 7) or switched to a fiber-free (FF; n = 4) or a fiber-supplemented (FS, n = 4) diet. 20 d after diet switch, cecal and fecal samples were harvested for analyses. **b)** Composition in % (w/w) of the used diets; MVA = Minerals, vitamins and ash; NFE = Nitrogen-free extracts.

Table 1. Composition of the used concentrated fiber preparations in the FS diet. N/A = information not available; I/S-Ratio = Proportion of insoluble fiber compared to proportion of soluble fiber within the total dietary fiber content of the preparations.

Origin of Fiber Preparation	Total Dietary Fiber Content	I/S-Ratio	pH Value (10% Solution)	Pectin Content	Oxide Ash	Bulk Density	Average Fiber Length
Pea	~ 70%	~ 34	4.0 – 7.0	N/A	max. 5%	300 g/L – 620 g/L	N/A
Oat	~ 90%	N/A	5.5 – 7.5	N/A	max. 5%	260 g/L – 385 g/L	75 µm
Psyllium	~ 80%	~ 0.2	5.0 – 7.0	N/A	max. 3%	350 g/L – 700 g/L	N/A
Wheat	~ 97%	N/A	5.0 – 8.0	N/A	max. 3%	260 g/L – 355 g/L	50 µm
Apple	~ 55%	~ 4.5	3.0 – 5.0	~ 9%	max. 3%	255 g/L – 355 g/L	N/A

2.2. Increase in relative abundance of certain fiber-degrading commensals in response to dietary fiber supplementation

At the end of the 20 d feeding period with the three different diets, microbiota composition was analyzed in fecal samples using 16S rRNA gene sequencing, revealing different clustering of each of the three groups (**Fig. 2a**). These findings not only highlight the overall impact of diet on the microbiota composition, but also the specific and considerable effect of CRF supplementation. On a phylum level, we determined a significant increase in the abundance of Bacteroidetes ($p = 0.0046$; t-test) and a decrease in Firmicutes ($p = 0.0149$; t-test) (**Fig. 2b**) in FS-fed mice compared to FF-fed counterparts. On a strain-level, we detected significantly different relative abundances of 8 of the 14 community members in FS-fed mice compared to FF-fed control mice (**Fig. 2c, d**). Specifically, we detected significantly lower abundances of AM and MF (see **Fig. 2c** for strain abbreviations)

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in FS-fed mice compared to their FF-fed counterparts, while relative abundances of EC, DP, ER, BO, RI and BT were significantly increased (**Fig. 2d**) in response to CRF supplementation.

However, we also detected significant differences in the relative abundances of 9 strains when comparing FS-fed to SC-fed mice. Since the SC diet also contains natural fiber, albeit in a non-concentrated form and in lower amounts, these differences could not be rooted in presence of fibers alone and might be a result of different sources of fibers or the distinct protein and fat content. Since we were particularly interested in strain-specific changes in response to fiber supplementation and the emerging effects on microbiota function, we associated strain-specific changes between FS- and FF-fed mice with the metabolic potential of the respective strains to grow on a suit of mono- and polysaccharides as determined previously using carbohydrate *in vitro* growth assays [6] (**Fig. 2e**). This association revealed a decreased relative abundance of a mucin specialist, *Akkermansia muciniphila* (AM) (**Fig. 2d**), which is in line with previous findings that fiber deprivation results in overgrowth of this particular strain [6] resulting in decreased mucosal barrier integrity. This finding further highlights the inverse correlation between relative AM abundance in the colon and dietary fiber intake. Of note, increased abundances of AM were found to be associated with various pathologies in human studies [12-17], supporting the idea that, besides promoting microbiota-mediated SCFA production, fiber supplementation considerably contributes to maintenance of mucosal barrier integrity by preventing from excess mucus degradation.

Furthermore, fiber supplementation resulted in significantly increased relative abundances of BT, ER, RI and BO (**Fig. 2d**), which share the capability to metabolize a broad variety of plant-derived polysaccharides, such as starch, cellubiose and α- and/or β-glucans (**Fig. 2e**) as previously confirmed with a carbohydrate *in vitro* utilization assay [6]. Thus, the ability to degrade α- and/or β-glucans promoted commensal growth under CRF-supplemented conditions, probably due to such glucans being major components of the CRF preparations [18]. Importantly, not all of the strains capable of metabolizing complex polysaccharides, such as BU, were increased in response to supplementation with the selected fiber formulation (**Fig. 2d**), indicating strain-specific effects of supplementation with the chosen CRF supplements. In general, intensified fiber consumption by the microbiome is associated with increased intestinal H_2 levels, which can exhibit disadvantageous effects on the host [19]. Thus, the increased abundance of DP in FS-fed mice might be a counter-regulating effect given the H_2-consuming properties of this bacterium [20]. Since EC is not a fiber fermenter [6], its increased abundance is very likely a secondary effect due to changed abundances of microbes, which are directly affected by fiber supplementation, resulting in altered microenvironments or nutrient availability.

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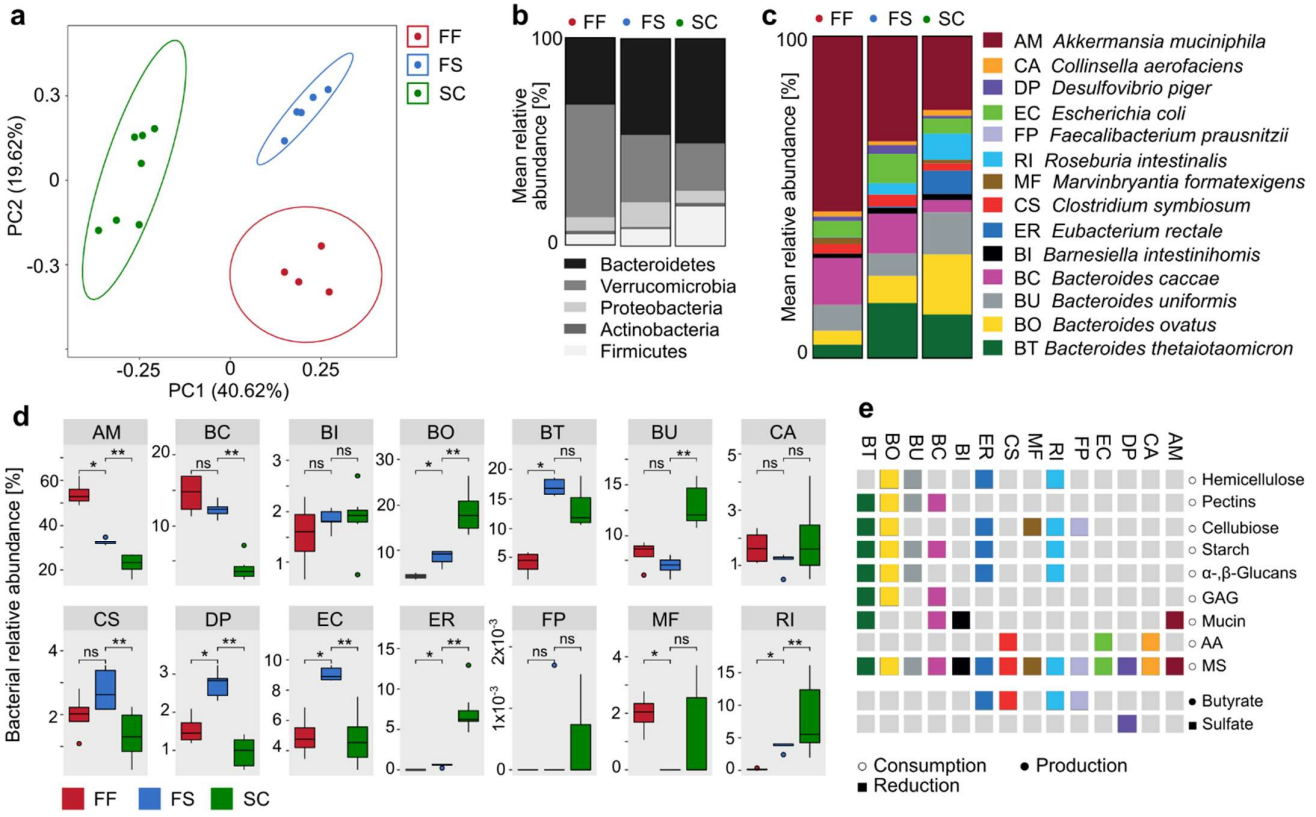

Figure 2. Compositional changes within a synthetic microbiota in response to dietary fiber supplementation. a) PCA plot of the microbiota composition as calculated with the "prcomp" function within the R package "stats", using 16S rRNA gene sequencing data from fecal samples. **b)** Mean relative abundances of the 14SM microbial community on a phylum level. **c)** Left panel: Mean relative abundance of each constituent strain of the 14SM community in mice fed either FF, FS or a SC diet. Right panel: Names of the 14SM-constituent strains and there 2-letter abbreviations. **d)** Tukey boxplots of the relative abundance of each strain; outliers shown as circles; statistics: Wilcoxon rank sum test performed with the "compare_means" function within the R package "ggpubr"; *:p< 0.05, **:p<0.01. **e)** Metabolic characteristics of each strain as determined in detail previously [6]; colored boxes represent metabolic activity related to the indicated substances; grey boxes represent no metabolic activity. GAG = glucosaminoglycans, AA = amino acids, MS = monosaccharides.

2.3. Inter-bacterial relations in relative abundance within the 14-member microbial community

To further investigate such potential inter-microbial influences and dependencies in response to CRF supplementation, we performed pairwise correlation analyses of all strains within each individual and across all groups (**Fig. 3a**). All correlation analyses were performed using the "rcorr" function within the R package "Hmisc" and visualized using the "corrplot" package. While the relative abundances of some strains, such as BI, FP and BT, provided little to no correlation with any of the other community members, certain bacteria, such as AM, BC, BO, BU, DP or ER significantly correlated with multiple other strains (**Fig. 3a**). These findings indicate a high inter-microbial dependency of AM, BC, BO, BU, DP or ER with other strains within the 14SM community, suggesting that the relative abundance of these strains were either strongly depending on the overall microbiota composition or, conversely, major influencers of the remaining microbiota in response to certain environmental changes, such as dietary supplementation. Besides BT, all other α-and β-glucan metabolizing microbes (BO, BU, ER and RI), provided significantly positive correlations with each other (**Fig. 3a**; Pearson correlation coefficient R>0), suggesting that there was no nutrient competition for these polysaccharides between these strains. Interestingly, BC, which is able to metabolize pectin, but not α- and β-glucans, provides a strong negative correlation to all of the α- and β-glucan degraders. Given

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the overall pectin concentration in the FS diet of roughly 2% (w/w) (**Table 1**), this suggested a potential competition for pectin with those α- and β-glucan degraders, which share the ability to metabolize pectin.

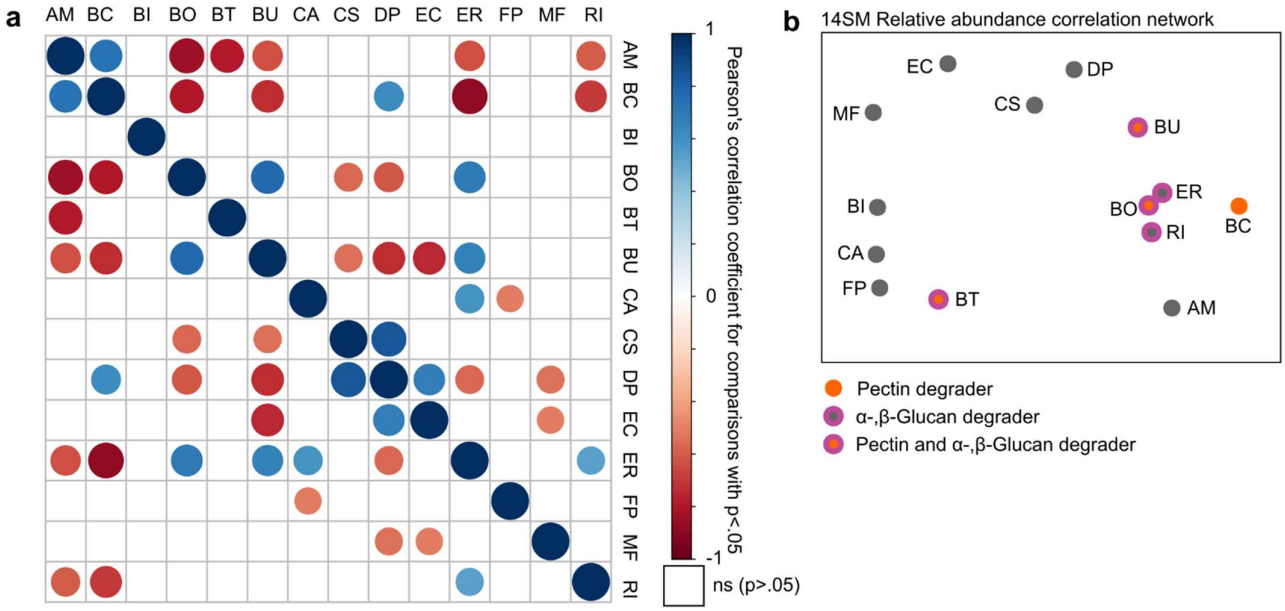

Figure 3. Correlations between relative abundances of 14SM community members. a) Pairwise correlation between relative abundances of all strains as determined by using 16S rRNA gene sequencing data from all mice across all groups; Analysis performed using the "rcorr" function of the R package "Hmisc" and visualized using the "corrplot" function. Colored circles depict statistically significant correlations ($p<0.05$); empty squares represent non-significant correlation independent of the determined correlation coefficient *R;* color intensity and circle size vary depending on the Pearson correlation coefficient *R,* with $R = 1$ (positive correlation) and $R = -1$ (negative correlation) displayed with maximal circle size and color intensity. **b)** Correlation network analysis using the "network" function in the R "igraph" package using the pairwise correlation matrix data as determined with "rcorr" for all 14 strains; shown inter-strain distances represent multidimensional correlation with smaller distances (clusters) representing stronger correlation of relative abundance changes.

To better illustrate such potential inter-microbial correlations, we performed a correlation network analysis (**Fig. 3b**), employing the "network" function in the R "igraph" package by using the correlation matrix data as determined with "rcorr". In this analysis, strains clustering together provide a higher "interdependency", meaning that the changes in the relative abundance of one strain is more likely to affect the relative abundance of a strain close-by than a more distant strain (**Fig. 3b**). As suggested by the data depicted in **Fig. 3a**, strains metabolizing α- and β-glucans are clustering strongly together. Interestingly, also BC (pectin-degrader) and AM (mucin-degrader) fall into the same correlation cluster (**Fig. 3b**) through their significant negative correlation with the glucan-degraders (**Fig. 3a**), suggesting that the decreased relative abundance of AM under fiber-supplemented conditions (**Fig. 2d**) is a secondary effect due to the bloom of fiber-fermenting microbes, while BC remains unaffected in its relative abundance in FS-fed mice (**Fig. 2d**), while it is strongly decreased in SC-fed mice.

2.4. Concentrated raw fiber supplementation is associated with changes in bacterial glycan-degrading enzymes

Given these fiber supplementation-mediated changes of microbial abundances (**Fig. 2** and **3**), we evaluated the functional outcomes of these compositional alterations. Thus, we determined the enzymatic activity of certain bacterial enzymes, which are involved in

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either fermentation of fiber-derived polysaccharides or the degradation of host-secreted mucin glycans, which were previously reported to be inversely associated with the amount of dietary fiber consumed [6]. The enzymes β-glucosidase (GLUC) and α-galactosidase (GAL) primarily target glycosidic linkages present in plant fiber-derived polysaccharides, with β-glucosidase being a crucial enzyme for hydrolyzing linkages in β-glucans [6]. Conversely, α-fucosidase (FUC), sulfatase (SULF) and β-*N*-acetylglucosaminidase (NAG) catalyze reactions involved in mucin glycan degradation [6]. While fecal activities of SULF and NAG remained unaffected by fiber supplementation, we detected significantly increased activities of GLUC and GAL in FS-fed mice compared to FF-fed mice, albeit FS-fed mice provided significantly lower GAL and GLUC activities as compared to SC-fed controls (**Fig. 4a**). This indicates that the source of fibers and their fine-scale composition seems to be more important to mediate functional outcomes of the microbiome than the amount of CRF alone. Surprisingly, we also detected a significant increase in FUC activities in FS-fed mice (**Fig. 4a**). This may be due to the presence of certain glycans in the FS diet harboring an alpha-1,6-linked fucose residue joined to the reducing end of an *N*-acetylglucosamine moiety, which is absent in the other diets.

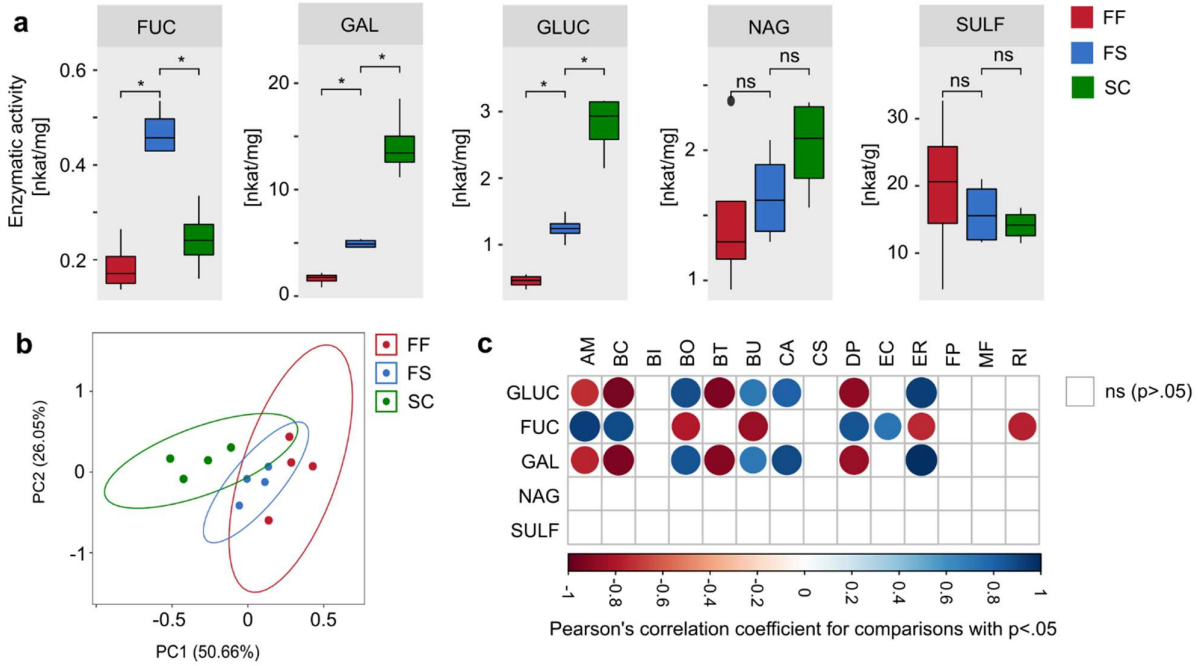

Figure 4. Concentrated raw fiber supplementation results in increased activity of bacterial fiber-degrading enzymes. **a)** Tukey boxplots of enzymatic activities of bacterial glycan-degrading enzymes in fecal samples, normalized on the amount of total fecal protein; FUC = α-fucosidase, GLUC = β-glucosidase, GAL = α-galactosidase, NAG = β-*N*-acetylglucosaminidase, SULF = sulfatase; statistics: Wilcoxon rank sum test performed with the "compare_means" function within the R package "ggpubr". *:p<0.05. **b)** PCA plot of the glycan-degrading enzyme activity pattern of FF-, FS- and SC-fed mice as calculated with the "prcomp" function within the R package "stats", using data sets shown in a). **c)** Pairwise correlation between glycan-degrading enzyme activities and the relative abundance of 14SM strains from all mice across all groups. Analysis performed using the "rcorr" function of the R package "Hmisc" and visualized using the "corrplot" function. Colored circles depict statistically significant correlations (p<0.05); empty squares represent non-significant correlation; color intensity and circle size vary depending on the Pearson correlation coefficient *R*, with *R* = 1 (positive correlation) and *R* = -1 (negative correlation) displayed with maximal circle size and color intensity.

The overall glycan-degrading enzyme activity pattern, as determined by principle components analysis (PCA) using activity data of all determined enzymes, revealed a different clustering between FF- and SC-fed mice only, while FS-fed mice provided an intermediate activity pattern (**Fig. 4b**). The activity of GLUC and GAL exhibited a strong

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positive correlation with the relative abundance of the fiber-degrading strains BO, BU and ER, but also with CA (**Fig. 4c**), which might benefit from released monosaccharides from polysaccharide degradation catalyzed by other strains. As expected, FUC activity exhibited a strong positive correlation with the relative abundance of mucin-glycan degrading AM, but also with DP, BC and EC (**Fig. 4c**). While BC is mucin generalist, meaning that it is also capable of mucin degradation (**Fig. 2e**), based on our previous work [6], EC is neither a mucin glycan-, nor a fiber-degrading commensal (**Fig. 2e**). Although correlation analyses suggest that increased FUC activity in FS-fed mice are associated with EC, we could, so far, not confirm FUC expression in EC and this finding might be a non-causal, correlative artifact due to secondary effects.

2.5. Changes of bacterial glycan-degrading enzyme activities are interlinked with a specific short-chain fatty acid production profile

Besides other features, microbe-mediated fiber degradation results in production of short-chain fatty acids (SCFA) [21]. Since SCFA provide important beneficial effects for the host [5, 22], we next investigated whether the fiber degradation-associated enzyme activity pattern in FS-fed mice resulted in altered SCFA production. While concentrations of acetate and formate did not differ significantly between FF- and FS-fed mice, propionate concentrations in cecal contents were significantly lower in FS-fed mice compared to their FF-fed counterparts (**Fig. 5a**). In line with this, PCA analysis of the overall SCFA production pattern between the three groups revealed that CRF supplementation did not result in a significantly different SCFA production compared to FF-fed mice, while the SC chow-mediated SCFA production was significantly different from the FF diet (**Fig. 5b**). In contrast to the SC-fed control group, the concentration of the main host-modulatory SCFA, butyrate [23], did not increase in FS-fed mice compared to FF-fed mice (**Fig. 5a**). The most important butyrate producers within the 14SM community are FP, RI, CS and ER [6] (**Fig. 2e**).

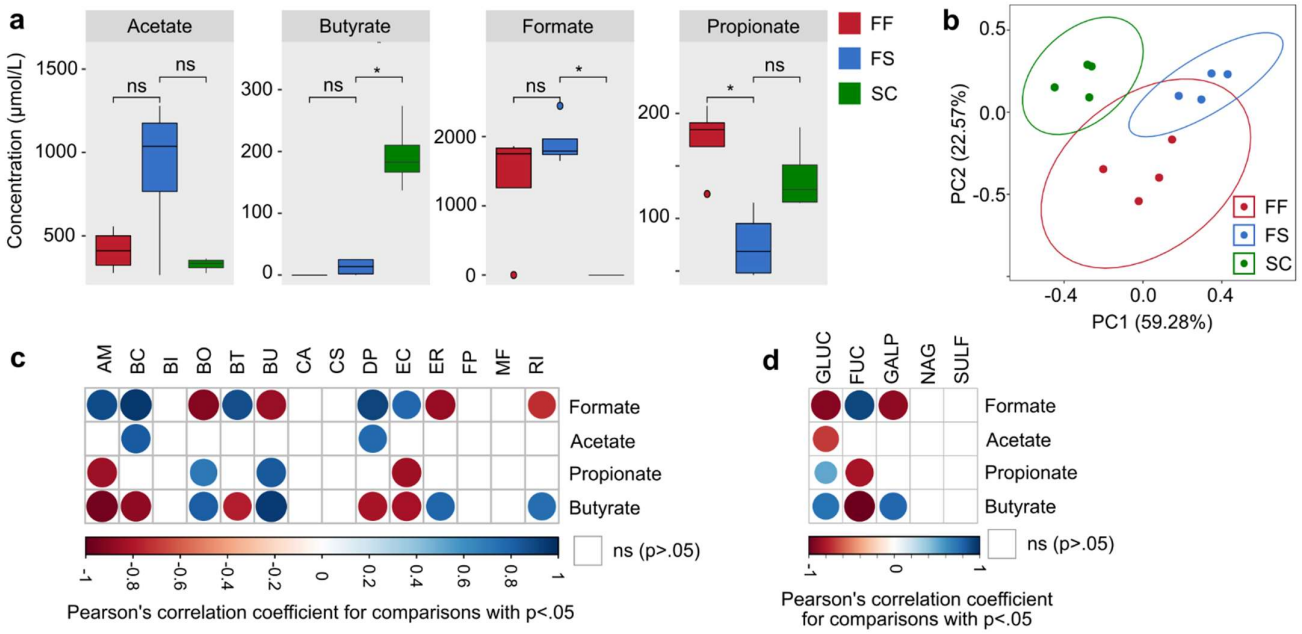

Figure 5. Functional changes of bacterial enzyme activity are interlinked with a specific short-chain fatty acid profile. **a)** Tukey boxplots of short-chain fatty acid (SCFA) concentrations in cecal contents, normalized on the amount of cecal matter; statistics: Wilcoxon rank sum test, *:p<0.05. **b)** PCA plot of the cecal SCFA concentration pattern of FF-, FS- and SC-fed mice as obtained by mathematical transformation of data sets shown in d). **c)** Pairwise correlation between cecal SCFA concentrations and the relative abundance of 14SM strains **d)** Pairwise correlation between cecal SCFA

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concentrations and glycan-degrading enzyme activities from all mice across all groups; characteristics of correlation matrix are identical to panel a).

Although relative abundances of RI and ER were significantly higher in FS-fed mice compared to FF-fed mice (**Fig. 2d**), the abundances of these strains were significantly lower compared to SC-fed control mice, which provided the highest butyrate concentrations among the three groups. Thus, the relative abundance of RI and ER appear to be predictors for butyrate concentrations in 14SM-colonized mice, due to their strong positive correlation with corresponding butyrate levels (**Fig. 5c**) and their relative abundances in FS-fed mice was probably not elevated enough to translate into significant increases in butyrate and propionate concentrations compared to FF-fed mice. Additionally, the relative abundance of BO also correlated positively with butyrate concentrations (**Fig. 5c**). However, this correlation is probably rooted in non-butyrate related inter-microbial interactions, since this strain is not known to be a main butyrate producer within the 14SM community. Furthermore, while butyrate concentrations exhibited positive correlation with GLUC and GALP activities, propionate only correlated positively with GLUC and formate with FUC activities (**Fig. 5d**).

In summary, given the strong effects of fiber supplementation on relative abundances of certain fiber degraders (**Fig. 2c, d**) and the associated increase in bacterial fiber-degrading enzyme activities (**Fig. 4a**), the non-significant SCFA levels compared to FF-fed mice (**Fig. 5a**) were somewhat unexpected but in line with the relatively decent increase of GLUC and GALP activities compared to FF-fed mice. Combining data from Fig. 2 to Fig. 5 suggest the presence of two independent functional correlation pathways connecting the 14SM community with glycan-degrading enzyme activities and SCFA production (**Fig. 6**). While we found a strong positive correlation between GLUC and GALP activity with butyrate and propionate levels, FUC activity correlated with the production of acetate and formate. Besides the ability of the host to metabolize certain amino acids into formate, it can also be produced as a by-product of metabolic activities of intestinal commensals [24]. Importantly, elevated concentrations of formate were previously reported to be a signature feature of inflammation-associated microbiome dysbiosis in a mouse model of colitis [25] and was associated with increased abundances of commensal *E. coli* strains, which is in line with our correlation analyses (**Fig. 5c, Fig. 6**).

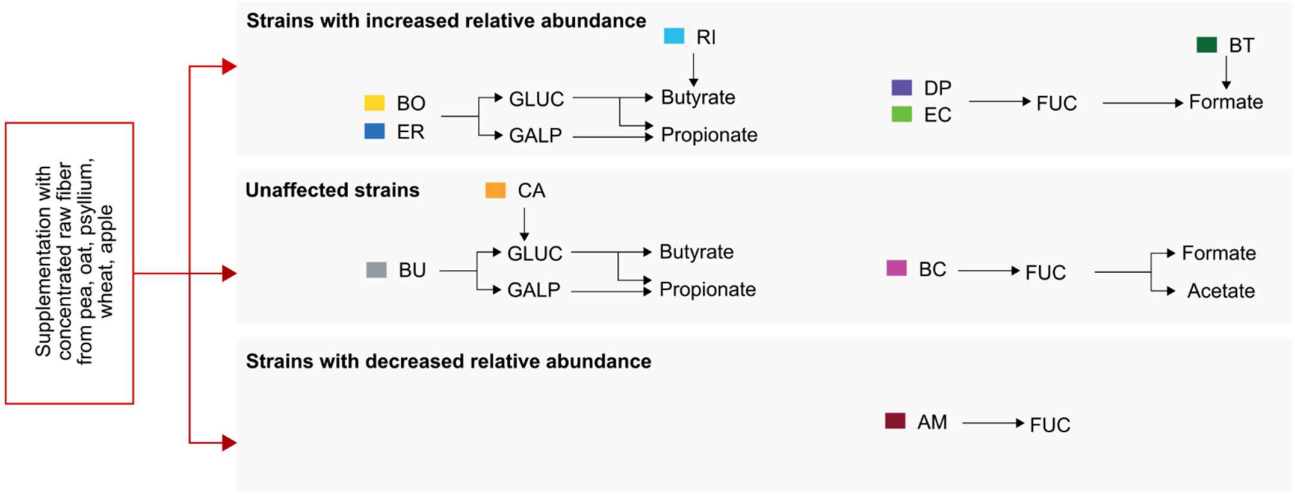

Figure 6. Summary of correlations between relative abundances of 14SM strains, glycan-degrading enzyme activity and SCFA production in response to fiber supplementation. Strains, which are significantly increased in FS-fed mice compared to FF-fed mice are depicted in the upper panel, while unaffected strains are shown in the middle panel and strains with decreased relative abundance in the lower panel. Arrows only show significant ($p<0.05$) positive ($0<R<1$) correlations as determined in Fig. 3 to Fig. 5.

Supplementation of the FF diet with the chosen mix of CRF derived from wheat, oat, psyllium, pea and apple did result in an increase of some, but not all strains, which were found to correlate with the GLUC/GALP-associated pathway, while also some strains correlating with the FUC-associated pathway were increased. It is worth noting, that such identified correlations do not necessarily equate with causal connections within these pathways. I.e., BU strongly correlated with GLUC and GALP activities as well as with cecal butyrate concentrations, although this strain is not a known butyrate producer. However, this strain might be important to support the butyrate production of other strains via yet unknown mechanisms.

3. Discussion

A lack of fiber intake is commonly associated with decreased microbial diversity in the gut [26] as well as with increased concentrations of metabolites, which can be harmful to the host [27]. Thus, supplementing a fiber-deficient Western diet [2], appeared as a reasonable strategy to restore or even boost host-beneficial properties of a given individual's indigenous microbiome. Although this approach seems trivial at first glance, it launches several challenges concerning the dose or source of fiber to be consumed by a certain individual, fitting the preconditions and needs of a specific microbiome composition. Although various human studies demonstrated beneficial effects of general fiber supplementation in most, but not all, study participants (reviewed in [28]), personalized and more tailored approaches are rare. Additionally, in-depth analyses of microbiome-specific effects of fiber supplementation on inter-microbial interactions and the resulting functional outcomes are difficult to conduct due to the complexity of the microbiome and the multitude of potential inter-microbial influences.

Thus, we aimed to investigate such interconnections and functional outcomes in a gnotobiotic mouse model with a standardized microbiome consisting of 14 human commensal bacteria strains. These commensals comprise the five most abundant bacterial phyla in the human host and provide all core metabolic functions. Importantly, their ability to consume certain poly- and monosaccharide, as well as their capacity to produce SCFA has been assessed previously [6]. We could demonstrate that most, but not all, fiber-degrading commensals within this community provided increased relative abundances in response to supplementation of a fiber-deprived diet with a mix of CRF obtained from pea, oat, psyllium, wheat and apple. This was particularly the case for α- and β-glucan degrading commensals, while commensals capable of hydrolyzing pectin, which is prevalent in the added apple preparation, were not positively affected. Although the relative abundances of fiber-degrading commensals and the activities of enzymes involved in bacteria-mediated fiber degradation increased significantly in response to fiber supplementation, this did not translate into a more host-beneficial SCFA production pattern. Our correlation network analyses suggest dynamic interconnections in response to fiber supplementation between the 14 constituent strains and reveal the importance of yet unidentified inter-microbial interactions to exhibit beneficial properties. Previous studies have routinely used purified fiber supplements in rodent systems to show a positive impact on the generation of SCFAs [29]. However, one needs to be cautious about the amount of fibers used in the rodent diets and the translatability of such amounts to human hosts.

Given the far more complex microbiome composition and associated microbial interconnections and dependences in the human gut, this highlights the challenges in designing personalized dietary recommendations for the benefit of the host. A pioneering study to address these points investigated the effects of four different fiber supplements on microbial diversity and emerging SCFA production in a human trial [30]. Among other findings, applied dietary fiber interventions were specific and limited to a few taxa within each participant, which, however, translated into a relatively consistent SCFA production pattern across the participants receiving the same supplements [30]. However, only ten participants were recruited for each cohort, which seems insufficient to make generalized statements on suitability of these supplements for a larger pool of individuals.

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In summary, our findings demonstrate that supplementation of a given diet with a mix of CRF resulted in significant changes of the intestinal microbiome. While some, but not all, fiber-degrading commensals provided increased abundances in response to fiber supplementation, abundances of mucin specialist *Akkermansia muciniphila* were significantly decreased. Given the reported disease-associated properties of *A. muciniphila*, this finding further encourages to avoid over-focusing on emerging SCFA production when assessing host-influencing properties of the microbiome in response to dietary fiber supplementation and calls for greater attention to other beneficial properties of a fiber-modulated microbiome.

4. Materials and Methods

Mouse experiments: Germ-free female C57BL/6N mice were originally purchased from Taconic Biosciences, Germany. The animals were bred and housed inside the local germ-free facility of the University of Luxembourg. For ethical aspects of the performed animal experiments, see "Institutional Review Board Statement" below. Mice were raised and maintained under gnotobiotic conditions on a standard mouse chow (SC). The animals were kept in ISO-cages with a maximum of 5 mice per cage and colonized at the age of six to eight weeks via intragastric gavage with a synthetic microbiota consisting of 14 different human commensals (14SM) as described previously [6]. Five to sixteen days after initial gavage after 14SM colonization confirmation via qPCR, mice were either switched to a fiber-free (FF) diet, a fiber-supplemented (FS) diet or remained on the SC diet as a control group. All diets and water were provided in sterile conditions *ad libitum*. The well-being of all animals was evaluated and fecal samples were collected once per week. 20 days after diet switch, mice fed all three different diets were sacrificed and contents of cecum and colon were harvested for downstream analyses.

Animal diets: The fiber-free (FF) diet and the fiber-supplemented (FS) diet were manufactured by SAFE diets (Augy, France) and were synthesized according to a modified version of the Harlan TD.08810 diet. The composition of the FF diet was (per 1000 g of total diet weight): 269 g casein, 4 g L-cystine, 444.235 g dextrose monohydrate, 75 g corn oil, 75 g lard, 80 g cellulose, 35 g mineral mix (AIN-93G-MX (94046)), 15 g vitamin mix (AIN-93-VX (94047)), 2.75 g cholin bitatrate and 15 mg TBHQ. The composition of the FS diet was (per 1000 g): 269 g casein, 4 g L-cystine, 344.235 g dextrose monohydrate, 75 g corn oil, 75 g lard, 80 g cellulose, 35 g mineral mix (AIN-93G-MX (94046)), 15 g vitamin mix (AIN-93-VX (94047)), 2.75 g cholin bitatrate, 15 mg TBHQ, 20 g VITACEL pea fiber EF 150, 20 g VITACEL oat fiber, HF401-30, 20 g VITACEL psyllium husk fiber P-95, 20 g VITACEL wheat fiber WF-101 and 20 g VITACEL organic apple fiber AF400-30. All VITACEL fibers were kindly provided by J. RETTENMAIER & SÖHNE GMBH + CO KG, Rosenberg, Germany). The standard chow is the standard mouse chow used in the local gnotobiotic facility, which was also manufactured by SAFE diets (version A04, product code U8233G10R). All diets were sterilized using 9 kGy gamma irradiation.

Culturing and colonization of germ-free mice with synthetic microbiota: Culturing of all 14 bacterial strains of the synthetic microbiota (SM) and subsequent colonization of germ-free C57BL/6 mice was performed as published previously [6].

Illumina 16S rRNA gene sequencing and data analysis: Murine fecal samples were stored at –20 °C until processing for bacterial DNA extraction, as previously described [6]. This protocol uses dual-index primers to amplify the V4 region of the 16S rRNA gene [31]. For each plate, ZymoBIOMICS™ Microbial Community DNA Standard (D6305) and an internal 16S mock bacterial community control (DNA QC 16S) from 10 genomic DNAs obtained from DSMZ (Lot No: 2019-1) were also run in quadruplicate. Libraries were prepared using Quick-16S™ NGS Library Prep Kit (Zymo Research, Irvine, CA), according to the manufacturer protocol. The final pooled library was quantified with and Qubit® and the amplicons were sequenced on an Illumina MiSeq with MiSeq®Reagent Kit v2 (500-cycle) (Illumina, USA). The raw sequencing data have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under the study accession number

PRJEBPRJEB45381. Sequences were processed with the program mother (v1.44.3) [32] according to the MiSeq SOP, which can be found on the mother website [31, 33].

Intestinal fatty acid analysis: 30-100 mg of flash-frozen cecal content was homogenized using 1.4 mm ceramic beads (5 beads per tube). Per 50 mg of cecal content, 500 µl of stock solution (2-Ethylbutyric acid, 20 mmol/L) was used (VK05 Tough Micro-Organism Lysing Kit). Cecal content was homogenized for 30 sec at 4 500 × g at 10 °C (Precellys24 Homogenizer) and centrifuged at 21 000 x g for 5 min and 4 °C. Sample homogenate was further processed and measurements of SCFA was performed was previously described using high-performance liquid chromatography (HPLC) [34].

Detection of glycan-degrading enzyme activities: Enzymatic activities of β-glucosidase, α-galactosidase, α-fucosidase, β-N-acetylglucosaminidase and sulfatase were detected as described previously [35].

Data analysis: Data transformation and analysis was performed in *R Studio*, version 4.0.2 (2020-06-22) using the following packages: readxl (version 1.3.1)[36], ggplot2 (version 3.3.3) [37], ggpubr (version 0.4.0) [38], ggfortify (version 0.4.11) [39], Hmisc (version 4.5.0) [40], xfun (version 0.23) [41], corrplot (version 0.84) [42], corrr (version 0.4.3) [43], stats (version 4.0.2) and igraph (version 1.2.6) [44]. For multiple comparisons of non-parametrical data sets, a Wilcoxon rank sum test was used with a significance level of 0.05. For pairwise comparison of two groups with normally distributed values, a parametric t-test was used with a significance level of 0.05.

Author Contributions: Conceptualization, A.S., M.N., and M.S.D.; methodology, A.S. and M.N.; software, A.S.; validation, M.S.D.; formal analysis A.S.; investigation, A.S., M.N., J.D.T and M.S.D.; resources, M.S.D.; data curation, A.S.; writing—original draft preparation, A.S. and M.N.; writing—review and editing, A.S., M.N., J.D.T, and M.S.D.; visualization, A.S.; supervision, M.S.D; project administration, M.S.D.; funding acquisition, M.S.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the Directive 2010/63/EU of the European Union, incorporated into local law as the "Règlement grand-ducal du 11 janvier 2013 relatif à la protection des animaux utilisés à des fins scientifiques". The study was approved by the Animal Experimentation Ethics Committee (AEEC) of the University of Luxembourg and by the Luxembourgish Ministry of Agriculture, Viticulture and Rural Development (national authorization number: LUPA2019/51 approved 27th August 2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw sequencing data have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under the study accession number PRJEBPRJEB45381.

Conflicts of Interest: The authors declare no conflict of interest

List of abbreviations

AM	*Akkermansia muciniphila*
BC	*Bacteroides caccae*
BI	*Barnesiella intestinihominis*
BO	*Bacteroides ovatus*
BT	*Bacteroides thetaiotaomicron,*
BT	*Bacteroides thetaiotaomicron*
BU	*Bacteroides uniformis*

CA	*Collinsella aerofaciens*
CRF	Concentrated raw fiber
CS	*Clostridium symbiosum*
DP	*Desulfovibrio piger*
EC	*Escherichia coli*
ER	*Eubacterium rectale*
FF	Fiber-Free
FP	*Faecalibacterium prausnitzii*
FUC	α-fucosidase
GAL	α-galactosidase
GF	Germ-free
GLUC	β-glucosidase
HPLC	High-performance liquid chromatography
I/S-ratio	Insoluble-to-soluble fibers
MF	*Marvinbryantia formatexigens*
MVA	Minerals, vitamins and ash
NAG	β-N-acetylglucosaminidase
NFE	Nitrogen-free extracts
PCA	Principal Components Analysis
RI	*Roseburia intestinalis*
SC	Standard mouse chow
SCFA	Short chain-fatty acid
SM	Synthetic microbiota
SULF	Sulfatase

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