Diets Enriched in Oat Bran or Wheat Bran Temporally and Differentially Alter the Composition of the Fecal Community of Rats

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Abstract

A clear understanding of how diet alters gastrointestinal communities is important given the suggested link between gut community composition and a wide variety of disease pathologies. To characterize this link for commonly consumed dietary fiber sources, we investigated the change in the fecal community of rats fed diets containing 5% nonnutritive fiber (control), 3% (w:v) oat bran plus 2% nonnutritive fiber (OB), or 5% (w:w) wheat bran (WB) over a 28-d feeding trial using both molecular- and cultivation-based methodologies. Pooled fecal samples from 8 rats fed the same diet were analyzed at 4 time points. On d 28, bran-fed rats had approximately twice the total cultivable bacteria than rats fed the control diet. Over the course of feeding, the cultivable community was initially dominated by bacteroides, then by bifidobacteria, lactobacilli, enterococci, and various enterics. In contrast, molecular analysis revealed the appearance of new operational taxonomic units (phytotypes) that were both temporally and inequitably distributed throughout the fecal community. The majority of change occurred in 2 major lineages within the Firmicutes: the Clostridium coccoides group and the Clostridium leptum subgroup. The time course of change depended on the source of bran, with the majority of new phytotypes appearing by d 14 (OB) or d 28 (WB), although adaptation of the fecal community was slow and continued over the entire feeding trial. Bacterial community richness was higher in bran-fed rats than in those fed the control diet. Change within the C. coccoides and C. leptum lineages likely reflect their high abundance within the gut bacterial community and the role of clostridia in fiber digestion. The results illustrate the limitations of relying solely on cultivation to assess bacterial changes and illustrate that community changes are complex in an ecosystem containing high numbers of interdependent and competing species of bacteria. J. Nutr. 139: 2024–2031, 2009.

Introduction

Dietary fiber is composed predominantly of nonstarch polysaccharides and lignin (1) that pass into the colon, where they are fermented to various degrees by the resident bacteria (2), producing SCFA used as substrate by the epithelial cells lining the lower intestine (3). While it is recognized that healthy adult diets are naturally high in dietary fiber (4–6), the link between health and dietary fiber is not well established (4,7), although viscous fibers like β-glucans have been shown to lower cardiovascular disease risk factors (8).

The mammalian gastrointestinal tract contains a complex microbical community (9) that, in addition to producing SCFA, interacts with the host immune system (10) and has been implicated in pathogen exclusion (11), vitamin production in the lumen (12), the metabolism of suspected carcinogens (13), and the development of the gut architecture and function (14). Although it has long been recognized that material entering the colon can affect the resident community (15), the impact of dietary fiber on the bacterial community composition and structure remains poorly defined (16). Despite this, a wide variety of “purified” food components have appeared over the last decade claiming to improve gut health primarily through their “specific” effect on the gut microbial community (17,18).
The most well known are the fructans, fructooligosaccharides, and inulin, which are believed to stimulate the growth of indigenous colonic bifidobacteria resulting in a variety of health benefits to the host (19). More recently, others have shown that highly purified carbohydrate sources may elicit specific responses within the gastrointestinal bacterial community. For example, increases in rat cecal and fecal lactobacilli levels have been noted in response to β-glycans or mixed β-glucans and xylans (18) and stimulation of bacteria related to Ruminococcus bromii has been observed in humans following the ingestion of resistant starch (16).

Given the suggested links between gut community composition and a wide variety of disease pathologies (20,21), a clearer understanding of how dietary interventions alter gastrointestinal communities is important. We investigated the impact of 2 different naturally occurring dietary fibers (22) on the fecal community in a rat model. Unlike purified carbohydrate materials, wheat bran and oat bran are commonly consumed in relatively higher amounts on a daily basis and, therefore, are more representative of usual diets. Here, we compared the temporal and differential responses within fecal communities of rats that consumed oat bran or wheat bran.

Materials and Methods

Feeding trial. This study was approved by the Health Canada Animal Care Committee. Twenty-four 28- to 42-d-old male BioBreeding control rats (Animal Resource Division, Health Canada), fed control diet for 2 wk prior to initiation of the study, were randomly assigned to receive either control (C),9 oat bran (OB), or wheat bran (WB) diets (n = 8; Table 1). Environmental enrichment (glass balls, gnawing sticks, and PVC pipe housing) was supplied. The rats were housed in mesh-bottomed stainless steel cages, held on a 12-h-light/-dark cycle (21°C and 40% humidity), and had free access to water (reverse osmosis-treated to 95% purity) and food. Diets were based on AIN-93G recommendations (23). Total protein, fat, carbohydrate, and dietary fiber were comparable across diets as calculated using bran proximate analyses. Calculated energy densities differed by 1% at most (Table 1).

Fecal samples were collected immediately prior to the start of the feeding trial (d 0) then on d 7, 14, and 28. To reduce inter-rat variability, fecal pellets within each diet group at each sampling time were pooled. Samples for cultivation analyses were processed immediately (see below). Other samples were stored frozen at −20°C.

Cultivation-based analysis. Fecal samples from d 0, 14, and 28 were analyzed for total colony-forming units (cfu/g wet weight feces) and species composition (bran diets only). Control samples were cultured using L-10 medium (24). OB or WB fecal samples were cultured using a modified L-10 medium containing 1% (wt/v) finely ground WB or OB as the sole carbohydrate source. All media were prepared under anaerobic conditions with the addition of 0.1% L-cysteine-HCl as a reductant. Previous work indicated that L-10 medium provides sufficient coverage to assess change in the cultivable community (25–27). The plating and culturing conditions as well as methods for obtaining isolates were as previously described (27). Isolates were identified using a combination of denaturing gradient gel electrophoresis (DGGE) and sequencing (27).

DNA isolation. Community fecal DNA was isolated and processed as previously described (26). Genomic DNA from individual bacteria was prepared from 5 mL overnight cultures using the Wizard Genomic DNA purification kit (Promega). DNA quality was assessed by gel electrophoresis (0.8% agarose), the concentration determined by spectrophotometry, and the samples stored at −20°C.

Fecal community DGGE analysis. The variable V2–3 region of the 16S ribosomal RNA (rRNA) community genes were amplified by PCR and analyzed by DGGE as previously described (27). Band cross-sectional areas were expressed as relative intensity (a fraction of the total area within lanes) and analyzed by cluster analysis followed by nonmetric multidimensional scaling analysis (NMMS) (28) using a Windows-based statistics program (Statistica).

DNA cloning and sequencing. Near full-length 16S rRNA genes from individual cultured bacteria were amplified from genomic DNA using the primers SD-Bact0008/R1492 (Supplemental Table 1). Libraries of near full-length 16S rRNA genes were amplified from community DNA samples using the primers F44/R1543 (Supplemental Table 1). Cloning and sequencing were as previously described (27).

Quantitative PCR. Total fecal bacteria 16S rRNA gene copy numbers were estimated using the 16S rRNA universal primer sets targeting the V2–3 region (HDA1/HDA2) and the V6–8 region (F940/R1422). The primer set BifidoF/BifidoR was used to target Bifidobacterium animalis. Primers and annealing temperatures are listed in Supplemental Table 1; all other conditions and standard curves were as previously described (27). Quantitative PCR (Q-PCR) was performed by creating 3 separate pools of fecal material from each diet condition at each time point. DNA was extracted from each of the 3 pooled samples (per day and diet) and PCR was performed in triplicate for each of these samples. Following amplification, melting temperature analysis of PCR products was performed to confirm the specificity of the reaction.

Construction of phylogenetic trees. Trees were generated as before (27) from near full-length 16S rRNA sequences and edited using the ARB software (29). Reference sequences were obtained from the Ribosomal Database Project II (RDPII, release 9.1). DOTUR (30) was used to assign sequences to operational taxonomic units (OTU; phylotypes) at a sequence diversity of <3% (31). Phylogenetic trees were constructed

<table>
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<td>Energy density, kJ/kg</td>
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1 Certified oat bran and hard red wheat bran were obtained from the American Association of Cereal Chemists (St. Paul, MN).
2 Diet ingredients total 1000 g.
3 Oat bran composition: 20.2% protein, 8.5% fat, 41.1% carbohydrate (nonfiber), 18.2% dietary fiber, 7% moisture, 5% ash.
4 Hard wheat bran composition: 15.6% protein, 4.3% fat, 21.8% carbohydrate (nonfiber), 42.7% dietary fiber, 9.9% moisture, 5.8% ash.
5 Vitamin and mineral mixes are as described for the AIN-93G rodent diet (23).

9 Abbreviations used: C, control diet; cfu, colony-forming unit; DGGE, denaturing gradient gel electrophoresis; FISH, fluorescent in situ hybridization; NMMS, nonmetric multidimensional scaling; OB, oat bran diet; OTU (phylotype), operational taxonomic unit; Q-PCR, quantitative PCR; rRNA, ribosomal RNA; WB, wheat bran diet.

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using Neighbor (32) and Phylo_win (33) using a Jukes Cantor correction (34). Bootstrap values were calculated using data resampled 1000 times (35).

**Statistical analyses.** Differences among the 16S rRNA gene libraries were assessed using Š-Libshuff (36) and UniFrac (37). A Bonferroni correction, provided by the Š-Libshuff software, was applied to adjust for multiple paired comparisons. TreeClimber (38) and LibCompare (39) were used to identify lineages responsible for significant differences over time. LibCompare identifies differentially represented taxa and TreeClimber compares the distribution of phylotypes within a phylogenetic tree using the parsimony statistic. Matrices of fractional divergence between OTU pairs (generated by ARB software) were used to create cluster diagrams using Sorensen distances (40) and subsequently used to generate NMMS diagrams using Statistica Software.

The impact of diet on overall community structure was tested indirectly, because the community 16S rRNA gene libraries represent pooled fecal samples from all rats within a diet at each time point; estimations of bacterial distributions were not performed for each rat within a diet group at each time point. Differences in OTU distributions among diets were tested by ANOVA (α = 0.05; Statistica Software) using the different time points as individual measures of OTU distributions for each diet to give an operational n = 4.

Q-PCR results were analyzed by ANOVA (α = 0.05) followed by Tukey’s Honestly Significant Differences test using the mean of 3 replicate measures for each of the extractions as 1 independent measure of the number of gene copies in the sample. The 3 separate extractions were then treated as 3 independent measures of the number of gene copies in the sample to give n = 3 for each time point for the 3 dietary conditions.

**Nucleotide accession numbers.** Nucleotide sequences have been deposited in GeneBank under accession numbers FJ87971-FJ881358. Phylotypes appearing in Supplemental Figure 1 have been designated as WBD-XXXX or OBD-XXXX to clarify dietary conditions but are listed in GeneBank simply as R-XXXX (no dietary indication) with the same corresponding numbering.

**Results**

**Analysis of change in the cultivable fecal community.** Total cultivable fecal anaerobes prior to the initiation of the feeding trial (d 0) were $1.3 \times 10^9$ cfu/g wet weight feces, similar to previous determinations (26). Anaerobic plate counts were ~1-log unit lower on each substrate ($1.9 \times 10^8$ and $8.1 \times 10^7$ cfu/g wet weight feces for OB and WB, respectively). By d 28, total counts on each respective substrate were higher. In addition, counts on OB ($7.6 \times 10^8$ cfu/g wet weight feces) and WB ($6.2 \times 10^9$ cfu/g wet weight feces) were approximately twice that of the control value ($3.5 \times 10^9$ cfu/g wet weight feces).

On d 0, the control community was dominated by various Bacteroides (70% of WB and 60% of OB isolates; Supplemental Table 2). However, by d 14, Bifidobacteria animalis dominated the WB and OB communities (70 and 50% of isolates, respectively). Feces from rats fed WB contained a higher proportion of Lactobacillus acidophilus, whereas the feces from rats fed OB contained various bacteroides, enterococci, and enterobacter species. By d 28, the dominant isolates in both diets were primarily enterics, with a single isolate Proteus mirabilis encompassing 40 and 50% of the total cultivable bacteria.

**DGGE analysis.** Analysis of DGGE “fingerprint” profiles generated from the V2–3 region of community 16S rRNA genes (Fig. 1A) showed that all 3 fecal communities formed a single cluster, indicating a similar community composition at the initiation of feeding (d 0). After this time, the profiles from each of the 3 diet groups diverged and no clear relationships among the fecal communities were apparent by d 28. This showed that all 3 communities changed in response to dietary conditions and that all 3 communities differed from one another.

**Community-based comparative sequence analysis.** Š-Libshuff analysis of 16S rRNA gene library composition among diet groups over the experimental time course (Supplemental Table 3) showed that all 3 fecal communities were similar at d 0 but significantly differed by d 7 and this difference persisted until the termination of the trial (Supplemental Table 4). This conclusion was supported by UniFrac analysis ([37]; data not shown) and by NMMS analysis of 16S rRNA OTU frequencies (Supplemental Table 5; Fig. 1B). However, unlike the Š-Libshuff analysis, NMMS analysis suggested that C and OB communities were more similar to each other than to the WB community over time.

**Overall bacterial community change.** Overall community structure as a function of diet and time was examined by comparing the percentage of phylotypes (Supplemental Table 5) aligning within the major bacterial lineages (Fig. 2). In general, overall community structure remained constant; a comparison between diet groups using pooled time points (see Materials and Methods) showed no significant difference.

An examination of the phylotypes identified after d 0 showed that 81 phylotypes were found in the rat fecal communities of all 3 dietary groups (Fig. 3). More phylotypes were found in rats fed WB over the entire time course (192 OTU) than in rats fed OB or C diets (165 and 139 OTU, respectively). Estimates of the final community phylotype richness (total number of OTU per
few unique OTU were identified in the fecal communities from all diets on d 0, consistent with statistical analyses showing no significant differences at this time. By d 7, however, increased numbers of unique phylotypes in both the WB and OB fecal communities were observed. In the WB fecal community, these aligned predominately within the *Firmicutes*, whereas in the OB fecal community, these fell within the *C. leptum* subgroup and the *Bacteroides/Cytophaga* phylum. On d 14, the highest number of unique OTU occurred in the OB fecal community, aligning predominantly within the *C. leptum* subgroup but also within the *C. coccoides* group. Maximum richness in the WB fecal community occurred on d 28 and was associated predominantly with the *C. coccoides* group.

A large number of common OTU were shared among the fecal communities from rats fed WB and OB (36 in total; Fig. 3). However, unlike the uniquely occurring phylotypes, shared phylotypes were distributed randomly between both communities in a nontemporal manner (results not shown).

**Impact of substrate on total community bacteria.** Increased community richness (see above) combined with cultivation results suggested that bacterial numbers were higher in the fecal communities of rats fed OB and WB. This was tested indirectly by quantifying relative changes in 16S rRNA gene copy numbers using Q-PCR and 2 universal primer sets. Total 16S rRNA copy numbers (V2–3 universal primers) did not significantly differ across the experimental time course with the exception of the d 28 WB-fed community, where 16S rRNA gene copy number was significantly higher than that in the corresponding C- and OB-fed rats (Fig. 5). Similar results were obtained using the V6–8 universal primers (results not shown).

**Impact of diet on fecal bifidobacteria.** Results from the cultivation-based analysis suggested that OB and WB may have stimulated the growth of fecal bifidobacteria (Supplemental Table 2). However, no phylotypes homologous with *B. animalis* were found in any of the community libraries. Q-PCR estimates of fecal bifidobacteria 16S rRNA gene copy numbers showed...
significant increases only in the fecal community of rats fed WB (Fig. 5). Maximal bifidobacteria 16S rRNA copy numbers were <2% of those determined for the entire bacterial community.

Discussion

Studies investigating the response of gastrointestinal communities subjected to various dietary interventions often attempt to cultivate major components within the fecal community using a variety of selective media and assess change through alterations in total numbers or by the emergence of genera whose growth is stimulated. Previous work with this rat model has indicated that this approach is not useful, because the readily cultivable community poorly represents the overall community determined through phylogenetic analysis (26). However, cultivation is useful as an index of community change when carried out in parallel with phylogenetic-based analyses.

Cultivation of the fecal communities from bran-fed rats revealed 3 general trends. First, total cfu increased over time, most likely reflecting adaptation to the long-chain carbohydrate substrates found in the bran-containing diets. Secondly, the composition of the cultivable communities was similar in both WB- and OB-fed rats over time. However, the majority of isolates were not fiber-digesting species but represented fast-growing constitutents having the ability to utilize the simple sugars and oligosaccharides solubilized during autoclaving of the bran-containing growth media. *B. animalis* was typical of these isolates; it was a dominant cultured species at the midpoint of the feeding trial but represented ~2% of the total WB or OB community 16S rRNA gene pool. Finally, as previously observed (26), the composition of the cultivable community did not correspond to that determined through phylogenetic analysis.

Molecular-based analysis demonstrated that changes in the fecal community in response to bran diets were both temporally and inequitably distributed throughout the fecal community. New phylotypes, stimulated by OB and WB feeding, fell primarily within 2 major lineages within the *Firmicutes*, a phylum containing a wide variety of carbohydrate polymer-digesting species. The appearance of new phylotypes was substrate specific, probably reflecting differences in the carbohydrate polymer composition of OB and WB dietary fiber. More importantly, these phylotypes were not closely related to previously cultivated species or to previously reported OTU (Supplemental Fig. 1) and represent additional richness within the fecal community of this animal model. There have been few molecular-based studies examining the impact of OB or WB on changes within the fecal communities of monogastric animals. Using fluorescent in situ hybridization (FISH), Hughes et al. (42) found that β-glucans induced changes primarily in the *Clostridium histolyticum* group and to a lesser degree cluster IX (*Sporomusa*) and the *Bacteroides/Prevotella* lineage in fecal slurries, but they also observed no stimulation of bifidobacteria. Using the same methodology, Costabile et al. (43) found that wheat germ but not WB was bifidogenic. However, these differences likely reflect differences in the composition and structure of the fecal community in humans and in rodents (9,44) and the chosen methodologies.

Much attention has focused on the bifidogenic effects of various dietary substrates and it is widely believed that this is beneficial to host health (45). Our cultivation results suggested that OB and WB diets were bifidogenic; *B. animalis* were clearly dominant by d 14, an observation consistent with many previous culture-based reports examining other sources of dietary fiber. These results are, however, at odds with our determinations of bifidobacteria 16s rRNA gene copy numbers. We found higher bifidobacteria 16s rRNA gene copy numbers only in the WB-fed rat fecal community. In addition, the maximal growth stimulation was observed on d 28 (Fig. 5). However, the increase in the d 28 community is of little consequence, considering that total bacterial load also increased with this diet; the final ratio increased from ~0.003 to 0.017 copies of bifidobacterial rRNA per copy of V2–3 rRNA. In any case, bifidobacteria maximally represented ~2% of the overall community 16s rRNA sequence load and phylotypes homologous with bifidobacteria were not identified among the 2400 sequenced 16s rRNA clones. Given the specificity of the bacterial community response to WB and OB and the increased community richness in these communities, the finding that WB may also have stimulated the growth of bifidobacteria is likely of little consequence. Whereas bifidobacteria have the genetic capacity to ferment a wide variety of carbohydrates (46), they are generally not very effective fiber digesters (25,47) and the moderate increases in their numbers likely reflects increased availability of simple carbohydrates resulting from the activities of fibrolytic species within the community during the adaptation to WB.

WB and OB feeding significantly increased community richness (Supplemental Table 6), probably as a result of the increased diversity of carbohydrate polymers entering the cecum. This finding contrasts with previous reports where WB tended to lower diversity in swine feeding trials (48,49). A major difference between our experiments and the swine feeding trials is that the C and WB swine diets contained a high amount of
corn meal; this means that the total amount of fermentable substrate was similar in C and WB. In contrast, we replaced very poorly fermented cellulose (~10% (50)) with a more readily fermented WB (~40% (51)) or with completely fermentable OB, both of which contain a wide diversity of carbohydrate polymer types.

In addition to increased community richness, WB also increased the total bacteria load in the d 28 fecal community. It is unclear whether this increase is the result of increased substrate availability in fiber-adapted animals or an increased surface area for colonization, because a relatively large amount of undigested WB is found in the feces. One other factor is an increase in the amount of nitrogen entering the cecum because of the protein covalently attached to the carbohydrate polymers of the cell wall matrix (52,53). WB material entering the cecum may contain higher amounts of protein, because it is more highly lignified and lignin is thought to limit protein degradation in the small intestine by acting as a physical barrier to enzyme penetration. This protein will stimulate bacterial growth in an environment that is probably nitrogen poor.

The overall structure of the fecal communities from C and bran-fed rats remained constant over the course of the feeding trial. This finding agrees with a previous analysis of community change in swine, measured by FISH using various genera and group-specific probes (48). However, it is in contrast to other studies that noted changes in specific genera or in the relative proportions of major cecal and/or fecal groups in response to various substrates (16,18,43,54,55). The reason for the discrepancy probably lies in methodological differences. Our approach (16S rRNA sequence comparative analysis) only allows an examination of the dominant constituents within the fecal community, whereas FISH can be used to target specific genera or groups of bacteria. Although the community structure was similar under all dietary conditions, the percentage distribution of phylotypes among the major lineages appeared different from what we have previously observed (26,27). Statistical testing of the d 0 communities across all 3 studies showed no significant differences (results not shown), which suggests that community structure may not be of prime importance when assessing the effects of dietary fiber.
It is apparent that a lengthy time period is required for the gut bacterial community to stabilize after the initiation of OB or WB feeding. Bran-related changes in the fecal communities, assessed by NMMS analysis, demonstrated none had formed stable communities by d 28; stable communities would have been indicated by a clustering of points in the NMMS diagrams. This was unexpected, because previous results obtained with rats fed a commercial rat unpurified diet containing ground corn, beet pulp, and oats showed a stable control fecal community for up to 56 d (27). In addition, we have fed fructooligosaccharides to rats maintained on commercial rat nonpurified diet and observed a stable community after ~14 d (S. P. J. Brooks and M. Kalmokoff, unpublished data). The relative instability of the community in C-fed rats may be due to the absence of readily fermentable material in the AIN-93G diet; wood cellulose is poorly fermented by rats (50). A daily cycling of bacterial output has been implied in humans (56), but with no attempt at strict diet control these results are difficult to interpret. Nevertheless, even though it is poorly fermented, wood cellulose appears to impact community composition, because the NMMS analysis of the Sorenson distance matrices for each 16S gene library showed that C and OB diets (containing 5 and 2% wood cellulose, respectively) were more closely related than either was to the WB diet (Fig. 1B). It is difficult to quantify these differences using NMMS, because only dimensionless distances are obtained and these reflect only the groups being tested at any given time. Thus, the relative proximity of the OB and C diets in the NMMS analysis may be due to a much larger difference in the WB diets.

Overall, our findings demonstrate that natural dietary fiber sources like WB or OB affect the composition of the fecal community. It is impossible, at the present time, to ascribe a functional importance to this change (either metabolic or physiological), given the paucity of data on host-specific bacteria interaction and the large genetic distance between newly identified phylotypes and cultured species. A larger question is whether diet-induced community change can ultimately be identified phylotypes and cultured species. A larger question is whether diet-induced community change can ultimately be correlated with the health state of the host (21). Although it is certain that bacteria participate in gut development and interact with the immune system (13), the specific role played by individual bacteria, naturally present in significant numbers within the gastrointestinal tract, remains to be determined.

Acknowledgment
K.A., J.K., F.M., and M.T. conducted research; K.A., M.K., J.G.-J. and S.P.J.B. designed the research; K.A., M.K., and S.P.J.B. analyzed the data and wrote the paper; M.K., B.S., J.G.-J., and S.P.J.B. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


