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Human Gut Bacterial Communities Are Altered by Addition of Cruciferous Vegetables to a Controlled Fruit- and Vegetable-Free Diet[1–3]

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Abstract

In the human gut, commensal bacteria metabolize food components that typically serve as energy sources. These components have the potential to influence gut bacterial community composition. Cruciferous vegetables, such as broccoli and cabbage, contain distinctive compounds that can be utilized by gut bacteria. For example, glucosinolates can be hydrolyzed by certain bacteria, and dietary fibers can be fermented by a range of species. We hypothesized that cruciferous vegetable consumption would alter growth of certain bacteria, thereby altering bacterial community composition. We tested this hypothesis in a randomized, crossover, controlled feeding study. Fecal samples were collected from 17 participants at the end of 2 14-d intake periods: a low-phytochemical, low-fiber basal diet (i.e. refined grains without fruits or vegetables) and a high ("double") cruciferous vegetable diet [basal diet + 14 g cruciferous vegetables/(kg body weight·d)]. Fecal bacterial composition was analyzed by the terminal restriction fragment length polymorphism (tRFLP) method using the bacterial 16S ribosomal RNA gene and nucleotide sequencing. Using blocked multi-response permutation procedures analysis, we found that overall bacterial community composition differed between the 2 consumption periods ($\delta = 0.603$; $P = 0.011$). The bacterial community response to cruciferous vegetables was individual-specific, as revealed by nonmetric multidimensional scaling ordination analysis. Specific tRFLP fragments that characterized each of the diets were identified using indicator species analysis. Putative species corresponding to these fragments were identified through gene sequencing as *Eubacterium hallii*, *Phascolarctobacterium faecium*, *Burkholderiales spp.*, *Alistipes putredinis*, and *Eggerthella spp.* In conclusion, human gut bacterial community composition was altered by cruciferous vegetable consumption, which could ultimately influence gut metabolism of bioactive food components and host exposure to these compounds. J. Nutr. 139: 1685–1691, 2009.

Introduction

Epidemiologic studies have shown that there is an inverse association between the consumption of cruciferous vegetables and risk of cancer, especially cancers of the digestive tract, bladder, prostate, and lung (1–3). In a meta-analysis, Kohlmeier et al. (4) concluded that cruciferous vegetables confer a protective benefit against cancer after controlling for the effects of overall vegetable intake. At least part of the protective effect of cruciferous vegetables is hypothesized to be due to their relatively high content of fiber and phytochemicals such as glucosinolates. Dietary fiber can be fermented by gut bacteria to yield SCFA and other metabolites that suppress the growth of tumor cells (5,6). Isothiocyanates (ITC),[6] one group of hydrolysis products of glucosinolates, have been shown to have anticarcinogenic properties (7–9). The enzyme myrosinase (EC 3.2.1.147), which is present in *Brassica* plant cells, catalyzes the hydrolysis of glucosinolates to ITC. Plant myrosinases can be deactivated by cooking; however, certain bacteria residing in the human gut have myrosinase-like activity and can metabolize glucosinolates. Thus, humans depend on gut bacteria to convert glucosinolates to ITC when cooked cruciferous vegetables are consumed. The importance of gut bacteria in producing ITC was elucidated in a previous feeding study that showed that urinary ITC excretion after cruciferous vegetable consumption decreased significantly when participants were pretreated with

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[6] Abbreviations used: ISA, indicator species analysis; ITC, isothiocyanate; MRBP, blocked multi-response permutation procedure; NMS, nonmetric multidimensional scaling ordination; rRNA, ribosomal RNA; tRFLP, terminal restriction fragment length polymorphism.

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antibiotics and bowel cleansing (10). In in vitro incubations of fecal or bacterial samples with glucosinolates, several gut bacteria species have been found to degrade glucosinolates (11–17). Thus, not only the amount of cruciferous vegetables consumed but also gut bacterial composition may determine exposure to bioactive ITC and ultimately affect cancer risk.

More than 800 species of bacteria reside in the human gut and 30–40 species dominate this community, comprising up to 99% of the total population (18). Additionally, individuals have their own distinct combination of predominant and subdominant bacteria species. This interindividual difference in community composition may ultimately contribute to differences in metabolism of dietary constituents and health status of the host. Several community fingerprinting techniques have been established to describe this interindividual difference in gut bacterial community profiles, because >70% of gut bacteria species are not cultivable (19). Most of these techniques are based on the sequence variation of the bacterial 16S ribosomal RNA (rRNA) gene, which is a phylogenetic marker (20). Terminal restriction fragment length polymorphism (tRFLP) analysis offers a rapid overview of interindividual differences in gut microbial communities (21). The method takes advantage of sequence variation of the 16S rRNA gene to generate sequence fragments. The pattern of the number and size of the sequence fragments is used to characterize the compositional differences in gut bacterial communities. Bacteria in fecal samples have long been used as a surrogate to study gut bacterial community, because the variation in gut bacterial community within a person has been shown to be less than the variation between individuals, although community composition differences do exist along the digestive tract and between different environments (e.g. between intestinal mucosa and lumen) (22).

Intervention studies have shown that diet can influence gut bacterial composition (23–28). A recent study demonstrated that Brussels sprouts, a cruciferous vegetable, altered the diversity and metabolic activities of gut bacteria in human fecal bacteria-associated rats (29). However, to our knowledge, the effect of cruciferous vegetable intake on gut bacterial composition in humans has not been studied to date. We hypothesized that cruciferous vegetables would have a selective effect on certain gut bacteria involved in metabolizing constituents of cruciferous vegetables (e.g. fiber, glucosinolates, etc.). The purpose of this study was to examine the extent to which cruciferous vegetable intake alters the gut bacterial composition in a randomized, crossover study of cruciferous vegetable supplementation.

Materials and Methods

Human subjects. This study was ancillary to a randomized, crossover, controlled feeding study, which was designed to test the response of selected biotransformation enzymes to cruciferous vegetable supplementation (30). Participants were healthy, nonsmoking men and women, 20–40 y old, and recruited on the basis of *GSTM1*, *GSTT1*, and *CYP1A2* genotypes. There was an extensive list of exclusion criteria that determined participant eligibility for the parent study (30). Those relevant to the current study included: medical history of gastrointestinal disorders; known allergies/intolerances to any foods used in the feeding trial; antibiotic use within the past 3 mo; current use of prescription and over-the-counter medications; and severe and frequent constipation necessitating treatment by a health care professional and/or frequent medication. Demographic information [age, gender, race, body weight, and height] was collected and genotypes were measured at screening. All activities were approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center, and informed, written consent was obtained from the study participants.

Dietary intervention. As part of the parent study, participants consumed 4 controlled diets in a randomized, crossover design: *1*) a low-phytochemical basal diet devoid of fruits and vegetables and whole-grain, high-fiber foods [major food items consumed included bagels, pasta, white rice, ready-to-eat cereal, dairy, etc.; menu details in (31)]; *2*) a "single-cruciferous" diet [i.e. basal diet + 7 g cruciferous vegetables/(kg body weight·d)]; *3*) a "double-cruciferous diet" [i.e. basal diet + 14 g cruciferous vegetables/(kg body weight·d)]; and *4*) a mixed diet [i.e. basal diet + 7 g cruciferous vegetables/(kg body weight·d) and 4 g apiaceous vegetables/(kg body weight·d)]. The supplemented cruciferous vegetables included broccoli (45.7% of the total cruciferous vegetables), cauliflower (34.6%), green and red cabbage (15.7%), and radish sprouts (4%). Cabbage and radish sprouts were provided raw whereas broccoli and cauliflower were fully cooked (one-half steamed, pureed, and prepared as a soup and the other one-half microwaved). We provided all food items to the participants during the diet periods and instructed them not to consume any other foods or beverages, except water. All diets were designed to provide similar proportions of macronutrients, except dietary fibers, by adjusting components of the basal diet to accommodate the addition of vegetables. Each diet period lasted 14 d. There was at least a 21-d washout period between each diet period.

Fecal sample collection. Fecal samples were collected from 17 participants at 1 time point at the end of the basal and double-cruciferous vegetable diet periods. Participants were provided with fecal collection tubes with scoop in the lid (76 × 20 mm; Sarstedt) containing 5 mL RNAlater (Ambion). They were instructed to collect 2 pea-sized aliquots of stool immediately at the time of defecation and place the stool into the collection tubes and mix well by shaking. The samples were delivered to the laboratory within 24 h and stored at −80°C.

Urinary ITC analysis. Twenty-four–hour urine collections were obtained from participants on d 13 of each diet period. The urinary total ITC excretion (estimated as total dithiocarbamates) was determined by HPLC as described previously (32).

Total fecal bacterial DNA extraction. Fecal samples in RNAlater were homogenized using an OMNI tissue homogenizer 115 and aliquoted into 300-μL aliquots. Fecal bacterial genomic DNA was extracted using a QIAamp DNA stool minikit (Qiagen) with 1 min of bead beating (21).

tRFLP. The tRFLP analysis was conducted using a modification of methods described previously (21). The bacterial 16S rRNA gene was amplified using a fluorescent-labeled primer. PCR products were purified and then treated with 0.025 mmol/L of each dNTP and 2 U of Klenow (exo^-) (New England BioLabs) to fill up the 5′-overhangs at 37°C for 30 min (33). The Klenow enzyme was deactivated at 70°C for 10 min and the DNA was digested overnight at 37°C with 5 U Alu I in a 20-μL reaction volume. Digested DNA (20 ng) from each sample was used for tRFLP analysis. Fragment analysis was conducted using capillary electrophoresis on an ABI 3100 (Applied Biosystems) at the Genomics Shared Resource of the Fred Hutchinson Cancer Research Center. GeneScan ROX-labeled GS500 (Applied Biosystems) was used as the internal size standard.

The tRFLP profiles were analyzed by DAx software (Van Mierlo Software Consultancy). Fragments that differed ≤2 base pairs in size were considered identical and were clustered together, being within the error of the instrument for fragment-size determination. Total peak area was summed across all fragments in each profile; peaks <1% of the total peak area were excluded as noise.

Bacterial 16S rRNA gene clone library. A bacterial 16S rRNA gene clone library was established based on the bacterial genomic DNA from 1 participant during the double-cruciferous vegetable intake period to relate the tRFLP fragments to their taxonomic information. This participant was chosen because the fecal bacterial community as examined by tRFLP analysis showed a substantial difference between the 2 controlled diets and included 3 of the 4 tRFLP fragments that were significantly associated with cruciferous vegetable consumption. The library was created using Invitrogen TOPO-TA cloning kit (Invitrogen)

following the manufacturer's protocol. A total of 96 white bacterial colonies were picked randomly and grown in Luria-Bertani broth (Invitrogen). The inserted 16S rRNA genes were partially sequenced at the Marine Biological Laboratory (Woods Hole, MA) using an ABI 373 sequencer (Applied Biosystems), dye terminator chemistry, and the 700r primer (34).

16S rRNA sequence alignment, BLAST, and phylogenetic inference. 16S rRNA gene sequences were edited and assembled into consensus sequences using PHRED and PHRAP software packages (CodonCode) based on PHRAP quality scores of 20. The final data set containing 79 sequences was compared with the GenBank sequences by using the program BLAST (35). Consensus sequences were aligned to the 16S rRNA sequence database in the ARB software package (36). Phylogenetic relationships of bacterial 16S rRNA gene sequences (based on the *Escherichia coli* 16S rRNA gene nucleotide position 68–644; the rest of the positions were masked) were inferred using the neighbor joining method with Kimura 2-parameter genetic distances (2:1 transition:transversion ratio) with bootstrap proportions calculated using PAUP 4.0 from 100 resampled data sets on the aligned sequences (37). Selected bacteria species were included in the phylogenetic tree for reference. These sequences were further digested in silico by Fragment Finder (38) to match the specifically sized tRFLP fragments.

Statistical analysis. Paired *t* tests were used to compare any differences in urinary ITC excretion and dietary fiber intake for the participants between the basal and double-cruciferous vegetable diet periods.

Peak area data from tRFLP profiles were exported from DAx. For each tRFLP fragment identified, the arcsin transformed square root of the peak area ratio (i.e. $\sin^{-1}\sqrt{P_i}$; P_i = individual peak area:total peak area) was calculated and used for further statistical analysis. The mean and SD of transformed P_i for individual peaks of each triplicate extraction were calculated after the binning process. Nonmetric multidimensional scaling ordination (NMS) analysis, blocked multi-response permutation procedures (MRBP), indicator species analysis (ISA), and cluster analysis were performed based on the transformed P_i values using PC-ORD (MjM Software Design) to assess the overall bacterial community composition and fragment abundance differences due to cruciferous vegetable consumption compared with the basal diet (39). To assess the number of dimensions that explained the most variation in the multivariate dataset, a preliminary NMS analysis was performed by requesting a 6-D solution stepping down to a 1-D solution with a randomly generated starting point. The instability criterion was set at 0.0005 with 500 iterations. Fifty runs were performed on the real data whereas 20 runs were performed on the randomized data. The criteria for the best solutions were: *1*) a dimension number was selected beyond which additional dimensions provide only small reductions in stress (using Monte Carlo test); *2*) the final stress was <15; and *3*) the stress reached stability as the iterations proceeded. We then reran the analysis starting from the same random number of the preliminary analysis. Pearson correlations of each NMS ordination axes and individual tRFLP fragments and other anthropometry and dietary variables (e.g. age, body weight, BMI, total cruciferous vegetables consumed per day, daily fiber intake, and urinary total ITC/24 h) were tested for significance. Categorical variables such as gender and race were also plotted against ordination axes to investigate any patterns. Cluster analysis was used to construct a hierarchical tree based on Ward's group linkage method and Euclidean distances. The hierarchical dendrogram was scaled by Wishart's objective function and percent of information remaining at the centroids, which was then translated into percent of community similarity. MRBP was used to test whether there was an overall difference between the gut bacterial community structures of the basal diet period and the double-cruciferous vegetable diet period in all participants. ISA using a randomization technique was performed to detect any individual fragments differentiating dietary conditions. Indicator values were determined for the tRFLP fragments to identify any fragments that significantly differed between the 2 dietary periods. *P*-values were calculated from Monte Carlo tests of significance using 1000 randomizations. Type I errors ($\alpha = 0.01$) indicated the proportion of these 1000 times an indicator values was equal to or greater than the indicator value from the actual data. The identified fragments were compared with the in silico digestion results of the cloned 16S rRNA gene sequences from the established clone libraries in our laboratory and their possible taxonomic affiliations were determined.

Stata 9.0 (StataCorp) was used for all statistical analysis unless specified. Significance was set at $P < 0.05$ except as noted. Values in the text are the mean ± SD unless otherwise noted.

Results

Demographic and dietary information. The age of the 17 participants (12 women and 5 men) was 28.5 ± 4.8 y. There were 6 Asians, 10 Caucasians, and 1 Asian-Caucasian participant. Body weight and BMI at baseline were 66.0 ± 12.6 kg and 23.2 ± 2.9 kg/m^2, respectively. Because cruciferous vegetables were fed on the basis of body weight, cruciferous vegetable intake during the double-cruciferous vegetable diet period was 819 ± 144 g/d and ranged from 660 to 1080 g/d. The diets were designed so that daily energy, carbohydrate, fat, and protein intakes were similar during the basal and the double-cruciferous vegetable diet periods; however, fiber intake was higher during the latter ($P < 0.01$; **Table 1**).

Urinary ITC excretion after dietary intervention. Daily total urinary ITC excretions on d 13 of the basal diet and double-cruciferous vegetable diet periods were 0.546 ± 1.41 and 200 ± 148 μmol/24 h, respectively ($P < 0.01$). There was substantial individual variation in ITC excretion after cruciferous vegetable consumption (range, 1.1–378 μmol/24 h).

NMS analysis of bacterial community structure. Using NMS analysis of the tRFLP profiles in the fecal samples after 500 iterations, the stress of the final solution was 14.9 and stable. The results from the Monte Carlo test showed that the solutions for each axis differed from solutions found by chance ($P = 0.02$). The final solution showed that 3 axes explained a cumulative variation in the data set of 82%, with 45, 15, and 22% explained by axes 1, 2, and 3, respectively. Pearson correlations of each ordination axis and selected anthropometry and dietary variables (age, body weight, BMI, total cruciferous vegetable consumed per day, daily fiber intake, and urinary total ITC/24 h) were not significant. NMS analysis of tRFLP profiles showed differences in bacterial community structure among individuals and within individuals for the 2 dietary periods (**Supplemental Fig. 1**). Two patterns emerged from the data. First, each participants had a unique gut bacterial community pattern. Most triplicate points originating from 1 stool sample clustered together in the plot, which indicated good reproducibility of the technique. Second, within a person, the community structure

TABLE 1 Daily energy and macronutrient intake by participants when they consumed a basal diet devoid of fruits and vegetables and the basal diet supplemented with cruciferous vegetables (double-cruciferous vegetable diet)[1]

Macronutrient	Basal diet	Double-cruciferous vegetable diet
Total energy, *kJ/d*	9577 ± 2318	9556 ± 1858
Carbohydrate, *g/d*	319 ± 75	330 ± 68
Fat, *g/d*	74 ± 19	72 ± 14
Protein, *g/d*	87 ± 22	93 ± 17
Fiber, *g/d*	8.9 ± 2.3	28.6 ± 5.8*

[1] Data are means ± SD, $n = 17$. *Different from basal diet, $P < 0.01$.

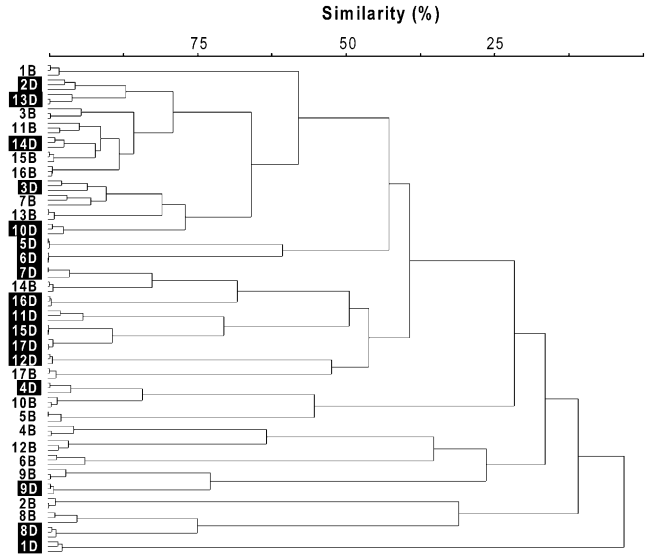

FIGURE 1 Cluster analysis of the participants' gut bacterial community tRFLP profiles after the basal diet (B) and the double-cruciferous vegetable diet (D) periods. The number before B or D indicates participant number (1–17). Three replicates within each sample are shown as 3 branches in each centroid. The Wishart's objective function was used to measure the bacterial community difference in the hierarchical dendrogram and was rescaled as percent similarity.

shifted noticeably after cruciferous vegetable consumption. Additionally, the bacterial community structure differences between the basal diet and double-cruciferous vegetable diet periods varied from individual to individual.

MRBP, cluster analysis, and ISA. MRBP analysis showed that there was a difference between the gut bacterial community structure in an individual consuming the basal diet compared with consuming the double-cruciferous vegetable diet in all 17 participants ($\delta = 0.603$; $P = 0.011$).

Cluster analysis showed that whereas triplicates of the same sample always clustered together with >90% similarity, the samples from the basal and the double-cruciferous vegetable diet periods from the same participant did not always cluster closely. Gut bacterial community structure of the same participant consuming the basal and the double-cruciferous vegetable diets varied considerably (**Fig. 1**).

The ISA suggested that there were 5 tRFLP fragments significantly associated with the diet periods (**Table 2**). Four of them were significantly associated with cruciferous vegetable consumption and one was associated with the basal diet. Through the in silico digestion of the sequenced bacterial 16S rRNA genes, we identified the putative taxonomic affiliation and closest relatives of these tRFLP fragments (Table 2).

Phylogenetic analysis of the gut bacterial 16S rRNA gene clone library. Sequence data obtained from the fecal bacterial 16S rRNA gene clone library have been submitted to the GenBank databases under accession numbers FJ 227596 to FJ227683. Members of the Firmicutes, Bacteroidetes, and Actinobacteria were represented in this clone library (**Fig. 2**). A large proportion (74%) were in the Clostridia class, with 54% in Cluster XIVa and 19% in Cluster IV. Another 6% of the clones belonged to the class Bacilli of the Firmicutes. The other 19% of the clones were Bacteroidetes, whereas the Actinobacteria clone represented only 1% of the clones in the library.

Discussion

In this study, we examined the effects of a high-cruciferous vegetable diet and a diet devoid of fruits and vegetables on the gut bacterial community profile as part of a randomized, crossover feeding study. We showed that: *1*) each participant had a unique gut bacterial composition even when all individuals received the same controlled diet; *2*) the gut bacterial composition differed significantly when participants consumed a basal diet devoid of fruits and vegetables compared with that diet supplemented with cruciferous vegetables; *3*) specific bacteria species were associated with cruciferous vegetable intake; and *4*) the response of the gut bacterial community to cruciferous vegetable consumption was unique for each individual but not directly related to the amount of cruciferous vegetables consumed.

Generally, the gut bacterial community of healthy adults is relatively stable with minor fluctuation over a short period of time (40). However, short-term dietary shifts, such as those found in controlled dietary interventions, have been shown to alter this community (23–28). Using MRBP, we showed that there was a significant difference in the overall gut bacterial community structure between the basal diet and the cruciferous vegetable diet periods in the 17 participants. Cruciferous

TABLE 2 tRFLP fragments that are significantly associated with the 2 controlled diets in the ISA[1]

tRFLP fragments, base pair	Indicator value[2]	Associated diet	Taxonomic affiliation[3]	Clone name	Closest relatives	Similarity to closest relatives
159	40.1	Double[4]	Firmicutes	E06, F05, G01	*Eubacterium hallii*	98%[5]
219	17.6	Double	Firmicutes	C04, C08	*Phascolarctobacterium faecium*	99%
222	32.8	Basal[4]	Proteobacteria	N[6]	*Burkholderiales*	N
241	48.3	Double	Bacteroidetes	B03	*Alistipes putredinis*	98%
468	23.5	Double	Actinobacteria	N	*Eggerthella spp.*	N

[1] ISA showed that 5 tRFLP fragments were significantly associated with either basal or double-cruciferous diet ($P < 0.01$). *P*-value of each fragment was calculated from Monte Carlo tests of significance using 1000 randomizations. Type I error (0.01) indicates the proportion of these 1000 times an indicator values was equal or larger than the indicator value from the actual data.
[2] Indicator values showed how well the specific fragments pointed to a particular dietary period within the data set. The values range from zero (no indication) to 100 (perfect indication).
[3] Taxonomic affiliations of the bacteria corresponding to some of these tRFLP fragments were identified through the 96 sequenced clones and from other established clone libraries.
[4] Basal, Basal diet; Double, double-cruciferous vegetable diet.
[5] Percentages of sequence similarity between the cloned 16S rRNA gene sequences and those of their closest relatives using BLAST.
[6] N, No match found in the 96 sequenced clones of this study; fragment was matched with sequences from other studies.

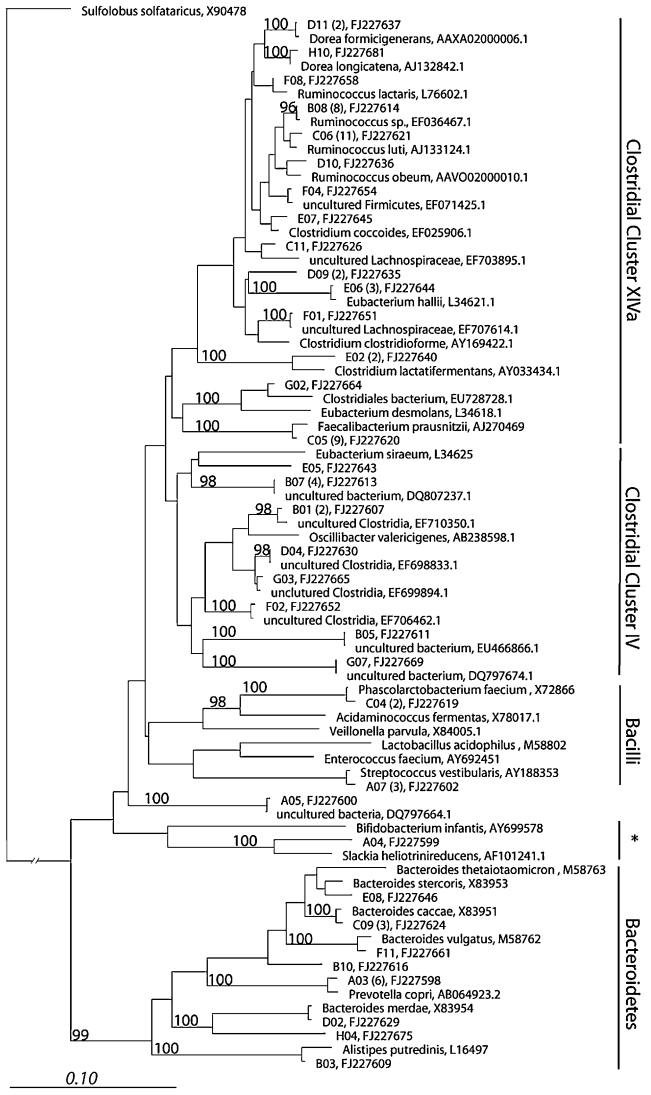

FIGURE 2 Phylogenetic analysis of the 16S rRNA gene amplified from the fecal bacterial community of 1 participant after cruciferous vegetable intake. Selected bacteria species were included in the phylogenetic tree for reference. Bootstrap values > 95 are indicated on the tree and the number of clones is shown in parentheses if there is more than one. *, Actinobacteria group.

vegetables are rich in several dietary components, e.g. dietary fibers such as cellulose, hemicelluloses, and pectin (41), and other compounds such as lignans (42) and glucosinolates (43) that can serve as metabolic substrates for certain human gut bacteria. For example, cellulose can be converted to SCFA by *Bacteroidetes* (44). Lignans such as secoisolariciresinol can be metabolized in vitro by certain *Peptostreptococcus* and *Eggerthella* isolated from feces (45). Glucosinolates have been shown in in vitro studies to be utilized by several gut bacteria species, including *E. coli*, *Bacteroides thetaiotaomicron*, *Enterococcus faecalis*, *Enterococcus faecium*, certain *Peptostreptococcus spp.*, and *Bifidobacterium spp.* (11,12,14,16). Thus, constituents of high-cruciferous vegetable diets have the potential to influence the growth of certain bacteria in the human gut bacterial community and ultimately modify the community composition. The community shift in this study may have been due to some of these specific components but was most likely due to the contributing effects of all of them.

Using ISA and sequencing of 16S rRNA genes, we were able to identify putative species that significantly differed between the 2 intake periods. The tRFLP fragments identified possibly as *Eubacterium hallii*, *Phascolarctobacterium faecium, Alistipes putredinis*, and *Eggerthella spp.* were associated with cruciferous vegetable intake and a fragment identified possibly as *Burkholderiales* was associated with the basal diet (Table 2). Some of these species are phylogenetically related to bacteria described previously as able to metabolize cruciferous vegetable components such as fiber, lignan, and glucosinolates. For example, *Eggerthella* was found to metabolize lignans; several species in the order of Bacteroidales, to which *Alistipes putredinis* belongs, can degrade fiber and glucosinolates (11,12,14,16,44,45). This further strengthens the effectiveness of the applied molecular approaches in examining bacterial community responses to diet.

We showed that there is a substantial difference in the bacterial community structure among individuals (Fig. 1; Supplemental Fig. 1). This uniqueness of gut bacterial community composition has been reported in other studies (22,46). We also observed that the difference in bacterial composition associated with cruciferous vegetable intake varied among individuals. For example, the direction of community difference from basal diet to cruciferous vegetable diet in the NMS plot varied for different participants (Supplemental Fig. 1). There are several possible explanations for these different responses. First, the variation among individuals after 2 feeding periods probably reflects the initial variation of gut bacterial community composition. Second, given that bacteria species belonging to different phylogenetic groups were modified in different participants, it is possible that bacteria species that have the same metabolic functions associated with cruciferous vegetable consumption may not be related closely phylogenetically. For example, in vitro incubations with glucosinolates showed that bacteria able to hydrolyze glucosinolates come from several different phylogenetic families, including Actinobacteria, Firmicutes, and Bacteroidetes (11,12,14,16). Phylogenetic analysis of the glycosidase gene family shows that the activity of hydrolyzing glucosinolates is not conserved within 1 discrete phylogenetic group of bacteria (47). Thus, if different individuals harbor different types of distantly related bacteria having myrosinase-like activity, it is possible that different species in the gut bacterial community are triggered or suppressed upon glucosinolate ingestion. This may partially explain the different response of gut bacterial community composition to cruciferous vegetable intake in this study. Further, considering the variety of cruciferous vegetable components that may affect gut bacterial populations, the capacity to contribute to this individual variation is extensive.

Our study has several strengths. We measured gut bacterial community differences in response to diet in a controlled feeding study. Several studies have used this approach (23–28); however, to our knowledge, none have measured the relationship between cruciferous vegetable consumption and gut bacterial community profile. The amounts of cruciferous vegetable and other dietary components consumed were precisely controlled. The intakes of major dietary nutrients (total energy, carbohydrate, fat, and protein) were kept constant across the diet periods (Table 1). We used a high throughput molecular fingerprinting approach based on the 16S rRNA gene to characterize the response of the gut bacterial community to cruciferous vegetable intake. Although methods based on isolation of individual organisms can be useful to identify species involved in metabolism of cruciferous vegetable components (11,12,14,16,44,45), they represent only a simplified view of the multiple interactions of gut bacteria

associated with the community changes during dietary interventions. Additionally, after combining the clone library data and in silico digestion predictions of tRFLP fragments based on sequence data, we were able to link specific candidate bacteria species to specific tRFLP fragments (Table 2). Although 16S rRNA gene sequencing can be considered the gold standard to determine bacterial taxonomy, it is very costly, which limits its application in large-scale epidemiologic studies. tRFLP analysis applied in this study offered rapid yet reliable data for a picture of overall community composition in response to cruciferous vegetable consumption.

There are also limitations in this study. The small sample size limited our power to stratify by demographic or genetic factors in statistical tests. Each participant consumed different amounts of cruciferous vegetables based on their body weight, an approach that was used to normalize intakes for body weight. Therefore, the intake of fiber and glucosinolates varied from person to person during the double-cruciferous vegetable diet period. Nonetheless, we observed significant overall effects of the controlled diets despite not detecting significant correlations among the NMS axes and daily cruciferous vegetable intake, fiber intake, or urinary ITC excretion when participants consumed the cruciferous diet. Because many factors influence glucosinolate metabolism (e.g. cooking method, gut transit time, ITC absorption rate, further gut bacterial degradation of ITC, *GST* genotype, etc.), the lack of an association between the NMS axes and urinary ITC excretion is not surprising. Each diet period lasted 2 wk; therefore, the bacterial community changes detected reflected short-term effects of intensive cruciferous vegetable consumption. Similarly, other studies have shown that 1–4 wk of dietary intervention was sufficient to elicit a gut microbiota shift (23–28). However, whether this community shift is steady for a longer period of time is unknown. There are also limitations of the tRFLP technique in analyzing bacterial community structure, which has been discussed previously (21). We had only 96 rRNA genes sequenced, which were derived from 1 fecal sample after cruciferous vegetable intake. Considering the hundreds of bacteria species residing in the human gut, it was possible that only the dominant species in the community were selected for the phylogenetic analysis, plus species presented in other participants might not exist in this person. This could limit our ability to confirm the relationship between tRFLP fragments and bacteria species.

In summary, we showed that there was a significant difference in gut bacterial community after 14 d of consuming a cruciferous vegetable-rich diet compared with a fruit- and vegetable-free basal diet. This bacterial community difference was unique to individuals. We also identified several putative gut bacteria species that were associated with the specific diets, offering additional evidence that gut bacteria can be modified rapidly by dietary components. This study also showed that tRFLP analysis is a useful approach for evaluating gut bacterial community composition and to monitor community structure changes due to dietary intervention. Further studies are needed to identify the in vivo gut bacteria response to specific cruciferous vegetable components, such as glucosinolates.

Literature Cited

1. Verhoeven DT, Goldbohm RA, van Poppel G, Verhagen H, van den Brandt PA. Epidemiological studies on brassica vegetables and cancer risk. Cancer Epidemiol Biomarkers Prev. 1996;5:733–48.
2. Murillo G, Mehta RG. Cruciferous vegetables and cancer prevention. Nutr Cancer. 2001;41:17–28.
3. Higdon JV, Delage B, Williams DE, Dashwood RH. Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. Pharmacol Res. 2007;55:224–36.
4. Kohlmeier L, Su L. Cruciferous vegetables consumption and colorectal cancer risk: meta-analysis of the epidemiological evidence. FASEB J. 1997;11:A369.
5. Bingham SA. Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. Proc Nutr Soc. 1990;49:153–71.
6. Beyer-Sehlmeyer G, Glei M, Hartmann E, Hughes R, Persin C, Bohm V, Rowland I, Schubert R, Jahreis G, et al. Butyrate is only one of several growth inhibitors produced during gut flora-mediated fermentation of dietary fibre sources. Br J Nutr. 2003;90:1057–70.
7. Myzak MC, Dashwood RH. Chemoprotection by sulforaphane: keep one eye beyond Keap1. Cancer Lett. 2006;233:208–18.
8. Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett. 2008;269:291–304.
9. Holst B, Williamson G. A critical review of the bioavailability of glucosinolates and related compounds. Nat Prod Rep. 2004;21:425–47.
10. Shapiro TA, Fahey JW, Wade KL, Stephenson KK, Talalay P. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. Cancer Epidemiol Biomarkers Prev. 1998;7:1091–100.
11. Brabban AD, Edwards C. Isolation of glucosinolate degrading microorganisms and their potential for reducing the glucosinolate content of rapemeal. FEMS Microbiol Lett. 1994;119:83–8.
12. Rabot S, Guerin C, Nugon-Boudon L, Szylit O. Glucosinolate degradation by bacterial strains isolated from a human intestinal microflora. Proceedings from the 9th Internation Rapeseed Congress, organized by GCIRC (Group Consultatif International de Recherches sur le Colza. In English: International Group of Research on Rapeseed); 1995 July 4–7; Cambridge, UK.
13. Getahun SM, Chung FL. Conversion of glucosinolates to isothiocyanates in humans after ingestion of cooked watercress. Cancer Epidemiol Biomarkers Prev. 1999;8:447–51.
14. Elfoul L, Rabot S, Khelifa N, Quinsac A, Duguay A, Rimbault A. Formation of allyl isothiocyanate from sinigrin in the digestive tract of rats monoassociated with a human colonic strain of Bacteroides thetaiotaomicron. FEMS Microbiol Lett. 2001;197:99–103.
15. Krul C, Humblot C, Philippe C, Vermeulen M, van Nuenen M, Havenaar R, Rabot S. Metabolism of sinigrin (2-propenyl glucosinolate) by the human colonic microflora in a dynamic in vitro large-intestinal model. Carcinogenesis. 2002;23:1009–16.
16. Cheng DL, Hashimoto K, Uda Y. In vitro digestion of sinigrin and glucotropaeolin by single strains of Bifidobacterium and identification of the digestive products. Food Chem Toxicol. 2004;42:351–7.
17. Rouzaud G, Young SA, Duncan AJ. Hydrolysis of glucosinolates to isothiocyanates after ingestion of raw or microwaved cabbage by human volunteers. Cancer Epidemiol Biomarkers Prev. 2004;13:125–31.
18. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307:1915–20.
19. Tannock GW. Analysis of the intestinal microflora using molecular methods. Eur J Clin Nutr. 2002;56: Suppl 4:S44–9.
20. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173:697–703.
21. Li F, Hullar MA, Lampe JW. Optimization of terminal restriction fragment polymorphism (TRFLP) analysis of human gut microbiota. J Microbiol Methods. 2007;68:303–11.
22. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. Science. 2005;308:1635–8.
23. Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology. 1995;108:975–82.
24. Tuohy KM, Kolida S, Lustenberger AM, Gibson GR. The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides–a human volunteer study. Br J Nutr. 2001;86:341–8.
25. Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, Flourie B, Brouns F, Bornet FR. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized,

placebo-controlled, parallel-group, dose-response relation study. Am J Clin Nutr. 2004;80:1658–64.
26. Langlands SJ, Hopkins MJ, Coleman N, Cummings JH. Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. Gut. 2004;53:1610–6.
27. Smith SC, Choy R, Johnson SK, Hall RS, Wildeboer-Veloo AC, Welling GW. Lupin kernel fiber consumption modifies fecal microbiota in healthy men as determined by rRNA gene fluorescent in situ hybridization. Eur J Nutr. 2006;45:335–41.
28. Costabile A, Klinder A, Fava F, Napolitano A, Fogliano V, Leonard C, Gibson GR, Tuohy KM. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. Br J Nutr. 2008;99:110–20.
29. Humblot C, Bruneau A, Sutren M, Lhoste EF, Dore J, Andrieux C, Rabot S. Brussels sprouts, inulin and fermented milk alter the faecal microbiota of human microbiota-associated rats as shown by PCR-temporal temperature gradient gel electrophoresis using universal, Lactobacillus and Bifidobacterium 16S rRNA gene primers. Br J Nutr. 2005;93:677–84.
30. Navarro SL, Peterson S, Chen C, Makar KW, Schwarz Y, King IB, Li SS, Li L, Kestin M, et al. Cruciferous vegetable feeding alters UGT1A1 activity: diet- and genotype-dependent changes in serum bilirubin in a controlled feeding trial. Cancer Prev Res. 2009;2:345–52.
31. Chang JL, Bigler J, Schwarz Y, Li SS, Li L, King IB, Potter JD, Lampe JW. UGT1A1 polymorphism is associated with serum bilirubin concentrations in a randomized, controlled, fruit and vegetable feeding trial. J Nutr. 2007;137:890–7.
32. Liebes L, Conaway CC, Hochster H, Mendoza S, Hecht SS, Crowell J, Chung FL. High-performance liquid chromatography-based determination of total isothiocyanate levels in human plasma: application to studies with 2-phenethyl isothiocyanate. Anal Biochem. 2001;291:279–89.
33. Egert M, Friedrich MW. Post-amplification Klenow fragment treatment alleviates PCR bias caused by partially single-stranded amplicons. J Microbiol Methods. 2005;61:69–75.
34. Hullar MA, Kaplan LA, Stahl DA. Recurring seasonal dynamics of microbial communities in stream habitats. Appl Environ Microbiol. 2006;72:713–22.
35. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10.
36. Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar, Buchner A, Lai T, Steppi S, et al. ARB: a software environment for sequence data. Nucleic Acids Res. 2004;32:1363–71.
37. Swofford D. PAUP. Phylogenetic analysis using parsimony. Sunderland (MA): Sinauer Associates; 2000.
38. Kaplan L, Hullar MA, Sappelsa L, Stahl DA, Hatcher P, Frazier S. The role of organic matter in structuring microbial communities. London: IWA Publishing; 2005.
39. McCune B, Grace JB, editors. Analysis of ecological communities. 1st ed. Glendenen Beach (OR): MjM Software; 2002.
40. Delgado S, Suarez A, Otero L, Mayo B. Variation of microbiological and biochemical parameters in the faeces of two healthy people over a 15 day period. Eur J Nutr. 2004;43:375–80.
41. Bourquin LD, Titgemeyer EC, Fahey GC Jr. Vegetable fiber fermentation by human fecal bacteria: cell wall polysaccharide disappearance and short-chain fatty acid production during in vitro fermentation and water-holding capacity of unfermented residues. J Nutr. 1993;123:860–9.
42. Milder IE, Arts IC, van de Putte B, Venema DP, Hollman PC. Lignan contents of Dutch plant foods: a database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. Br J Nutr. 2005;93:393–402.
43. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 2001;56:5–51.
44. Robert C, Chassard C, Lawson PA, Bernalier-Donadille A. Bacteroides cellulosilyticus sp. nov., a cellulolytic bacterium from the human gut microbial community. Int J Syst Evol Microbiol. 2007;57:1516–20.
45. Clavel T, Henderson G, Alpert CA, Philippe C, Rigottier-Gois L, Dore J, Blaut M. Intestinal bacterial communities that produce active estrogen-like compounds enterodiol and enterolactone in humans. Appl Environ Microbiol. 2005;71:6077–85.
46. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444:1022–3.
47. Mian IS. Sequence, structural, functional, and phylogenetic analyses of three glycosidase families. Blood Cells Mol Dis. 1998;24:83–100.