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Monotonous Diets Protect against Acute Colitis in Mice: Epidemiologic and Therapeutic Implications

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Abstract

OBJECTIVES—Multiple characteristics of industrialization have been proposed to contribute to the global emergence of inflammatory bowel diseases (IBDs: Crohn disease [CD] and ulcerative colitis [UC]). Major changes in eating habits during the past decades and the effectiveness of exclusive enteral nutrition (EEN) in the treatment of CD indicate the etiologic importance of dietary intake in IBDs. A uniform characteristic of nutrition in developing countries (where the incidence of IBD is low) and EEN is their consistent nature over prolonged periods. However, the potentially beneficial effect of dietary monotony in respect to mammalian intestinal inflammation has not been examined.

METHODS—The association between alternating (two different complete chows) and persistent regular diets, and dextran sulfate sodium (DSS) colitis susceptibility in C57BL/6J mice was studied. Colonic mucosal microbiota changes were investigated by high-throughput pyrosequencing of the *16S rRNA* gene.

RESULTS—The severity of colitis increased upon dietary alternation compared to consistent control feeding. The microbiota of the alternating nutritional group clustered discretely from both control groups.

CONCLUSIONS—Our findings highlight that monotonous dietary intake may decrease mammalian vulnerability against colitis in association with microbiota separation. The epidemiologic and therapeutic implications of our results are also discussed.

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Keywords

diet; colonic inflammation; colitis; microbiota; enteral nutrition; inflammatory bowel disease

INTRODUCTION

Inflammatory bowel diseases (IBDs), including Crohn disease (CD) and ulcerative colitis (UC), are chronic intestinal disorders affecting more than 4 million people (1). IBD is recognized to develop secondary to an exaggerated immune response against microbial components of the gut, which is transmitted by the mucosa and is modulated by genetic susceptibility and environmental triggers (2). The worldwide emergence of IBD (1) and the high monozygotic discordance rates for the disorders (3) suggests that environmental (including nutritional) factors may be even more or at least as important for their development than genetic susceptibility. The effectiveness of nutritional therapy in the treatment of CD further supports the relevance of dietary influences in IBD. Importantly, exclusive enteral nutrition (EEN) can induce partial or complete remission in up to 89% of newly diagnosed and in 50% of relapsed pediatric CD patients (4, 5). In the meantime, EEN has not been systematically examined in the treatment of UC, but some studies indicate its potential efficacy (6, 7).

Meta-analyses show that there is limited difference between EEN and first-line steroid therapy in pediatric CD trials (8). However, expanded investigations revealed that the effectiveness of steroid therapy in respect to clinical remission induction for active CD appears to be higher than for EEN, especially in adult studies (9, 10). In the meantime, EEN does not have major adverse effects compared to the frequently applied steroids in CD, and can even improve nutritional status and quality of life (11). This nutritional therapy has direct anti-inflammatory properties (12), but the mechanism of action of EEN is unknown. Multiple hypotheses have been generated to explain its efficacy including decreased dietary antigen exposure, overall nutritional repletion, correction of intestinal permeability, decreased inflammatory mediator production by reduced fat intake, and delivery of important micronutrients to the diseased intestine (13). However, the majority of these hypotheses are not supported by the clinical observations. Polymeric formulas are as effective as elemental (i.e. no difference in effectiveness with different antigen loads), fat content of diet does not influence efficacy (9), additives such as glutamine do not modify outcome (14), and significant improvement of inflammatory parameters precede changes in host nutritional parameters (i.e. independent efficacy from nutritional replenishment) (15). Interestingly, the possible beneficial effect of EEN arising from its monotonous (consistent) nature has not been entertained while this is a common feature of both the polymeric and elemental formulas utilized with success.

It is currently accepted that gut microbiota composition may play a major role in the development of IBD (16, 17). The influence of EEN on intestinal microbiota in CD has been shown to be prominent (18). Based on these findings the possibility for EEN to induce remission of CD through microbial composition modulation has been proposed. However, the details on how such modulation occurs have not been examined.

In this study, we investigated whether monotonous diets may provide protection against acute colitis in a murine model of IBD (dextran sulfate sodium [DSS] exposure). The effects of limited, but repetitive dietary variation on colonic mucosal microbiota composition were also studied. Our results indicate that the increased nutritional diversity of the developed world may contribute to the emergence of IBD.

MATERIALS AND METHODS

Animals and Tissue Collection

C57BL/6J male mice (Jackson Laboratories, Bar Harbor, ME, USA) were utilized in the study. Mice were randomly assigned at 50 days postnatal age (P50) to two different standard rodent diets *ad libitum*: regular chow (R) and NIH-31 diet (2920X and 7017, Harlan-Teklad, Madison, WI, USA). These chows have limited composition differences (supplemental Table 1, <http://links.lww.com/MPG/A180>). A third group of mice received both diets in an alternating fashion. Our primary goal was to diversify the nutrition of the mice receiving the alternating diet without increasing antigen exposure. In the meantime, we cannot absolutely rule out that the animals receiving the alternating diet were not exposed to increased amounts of antigen, although the protein source of the two chows was the same from the same company, and the same batches of the chows were utilized throughout the experiments. The dietary alternation was done every 2–3 days from P50 to P70 (total of 9 switches). Total body weight was followed sequentially during the feeding protocol as well (supplemental Fig. 1, <http://links.lww.com/MPG/A181>). There were no significant differences in body weight gain between the groups from P50 to P70. Three weeks after the initiation of the dietary regimens, the mice were either exposed to DSS or euthanized by CO₂ asphyxiation for the collection of colonic mucosal scrapings. The colons were placed on ice, transected longitudinally, cleansed from feces, washed with ice cold normal saline, followed by the collection of colonic mucosa with a microscope slide (19) (excluding the cecum). The mucosal scrapings were flash frozen on dry ice, and stored at –80°C as earlier described (20). The protocol was approved by the Institutional Animal Care Committee for Baylor College of Medicine.

Dextran Sulfate Sodium (DSS) Exposure

The standard rodent diets and the dietary alternation were continued for the mice during the DSS challenge. Susceptibility to colitis was tested by administering 3% DSS (MW=36,000–50,000, MP Biomedicals, LLC, Solon, OH, USA) in drinking water and provided *ad libitum* for 5 days, followed by regular water exposure for an additional 4 days. This molecular weight of DSS has been shown to induce colonic inflammation in previous work (21). The animals were weighed daily and colons were collected following CO₂ asphyxiation at day 9.

Colons were longitudinally transected and processed for standard hematoxylin-eosin staining after fixation in 10% formaldehyde. Histological severity of inflammation was determined by a blinded pathologist based upon a colitis scoring system modified from Albert et al (22) (including the percentage of the damaged area) (Table 1).

DNA Extraction for Microbial Studies

Mucosal scrapings were centrifuged at 14,000 rpm for 30 seconds and re-suspended in 500µl RLT buffer (Qiagen, Valencia, CA) (with β- mercaptoethanol). Sterile 5mm steel beads (Qiagen, Valencia, CA) and 500µl sterile 0.1mm glass beads (Scientific Industries, Inc., NY, USA) were added for complete bacterial lyses in a Qiagen Tissue Lyser (Qiagen, Valencia, CA), run at 30Hz for 5min. Samples were centrifuged briefly and 100µl of 100% ethanol was added to a 100µl aliquot of the sample supernatant. This mixture was added to a DNA spin column, and DNA recovery protocols were followed as instructed in the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) starting at step 5 of the Tissue Protocol. DNA was eluted and diluted to a final concentration of 20ng/µl.

Massively Parallel bTEFAP

Bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTEFAP) was performed as described previously (23). bTEFAP utilizes Titanium reagents and procedures, and a one-step PCR, mixture of Invitrogen AccuPrime Taq Polymerase, and amplicons originating from the 357R to 926F region numbered in relation to *E. coli* 16S rRNA. The bTEFAP procedures were performed at the Petrosino lab. We considered sequences associated with at least genus information to be well-classified. Sequences with identity scores (to known or well characterized 16S rRNA sequences) greater than 97% (<3% divergence) were resolved at the species level, between 95% and 97% at the genus level, between 90% and 95% at the family and between 85% and 90% at the order level, between 80% and 85% at the class level and below this to the phylum. Reads that could not be assigned with a bootstrap confidence above 80%, they were placed into an artificial “unclassified” taxon.

Bacterial Diversity Data Analysis

Raw 454 sequence data was processed using a combination of software tools. Multiplexed reads were assigned to their originating samples, quality trimmed (average ≥ 25 , no barcode mismatches allowed), and filtered (minimum length = 200, no homopolymers > 10bp, maximum number of ambiguous bases per read = 1) using mothur (24). Trimmed reads were then normalized across all samples using an in-house script designed to randomly choose a user-specified number of reads from each sample. In this experiment, 15,000 reads per sample were chosen. These normalized read sets were then subjected to OTU (operational taxonomic unit) -based analysis utilizing CloVR (25). CloVR is an application that integrates multiple state-of-the-art analysis tools into a single program. Chimeric sequences were detected and removed via UCHIME (26), phylogenetic distance metrics were generated by QIIME (OTU defined as 97% identity) (27), and statistics were generated by Metastats (28). Metastats utilizes the nonparametric t-test, Fisher’s exact test, and the false discovery rate (FDR) to generate a prioritized list of OTUs that define observed differences between two user-defined populations. The q-value is the adjusted p-value based on FDR calculation. Machine learning was performed using the Genboree Metagenomics Toolset, a suite of bioinformatics tools put together by the Bioinformatics Research Lab at Baylor College of Medicine. This toolkit utilizes Random Forrest (29) and Boruta (30) to identify the features responsible for driving the separation of two distinct communities.

Statistical and Bioinformatic Analysis

For the bioinformatic analysis of the microbiota data, please see the paragraphs above. Unpaired, two tailed t-tests were utilized in the group comparisons where statistical significance was declared at $p < 0.05$. Error bars represent standard error of the mean (SEM).

RESULTS

Increased Severity of DSS Colitis on Alternating Diet

The effect of alternating two different control diets on chemically induced (DSS) colitis in C57BL/6J mice was examined. Weight loss is usually a reliable measure of colitis severity in this model. Nine days after initiating DSS, the alternating group lost significantly more weight compared to one of the controls (regular chow: R) ($p < 0.05$). The other control group (NIH-31) diet showed improvement in body weight as well compared to the switching group, but this did not reach statistical significance (Figure 1). Mice receiving the alternating diet had significantly more tissue damage than the controls (Figure 2; SW vs. R: $p = 0.0046$; SW vs. NIH-31: $p < 0.0001$).

Dietary Alternation Reduced Microbiota Diversity

Studies suggest that microbiota diversity is reduced in CD and UC (see Discussion). Therefore, we examined the effect of the dietary alternation on murine microbiota diversity in independent experiments from the DSS challenge. There was a tendency for decreased colonic mucosal microbiota diversity in the switching group (Figure 3, SW vs. R: $p = 0.055$; SW vs. NIH-31: $p = 0.125$).

Microbiota Separation following Dietary Alternation

Principal component analysis showed separation between the three different diet groups. The dietary alternation microbiota clustered discretely from both control groups (Figure 4). Four phyla (*Archaea-Other*, *Bacteria-Actinobacteria*, *Bacteria-Other*, *Bacteria-Tenericutes*) differed by T test between the switching and the R group. Meanwhile, there was one (*Bacteria-Bacteroidetes*) difference between the switching (SW) and the NIH-31 dietary group ($p < 0.05$, Table 2; supplemental Fig. 2 [<http://links.lww.com/MPG/A181>]).

There was a trend for an increase in Tenericutes in SW compared to both control dietary groups (SW vs. R: $p = 0.0477$; SW vs. NIH-31: $p = 0.055$; supplemental Fig. 2 [<http://links.lww.com/MPG/A181>]). Eight genera (*Lachnospiraceae-Bryantella*, *Archaea-Other*, *Bifidobacteriales-Bifidobacteriaceae*, *Erysipelotrichaceae-Allobaculum*, *Staphylococcaceae-Staphylococcus*, *Erysipelotrichaceae-Other*, *Erysipelotrichaceae-Coprobaecillus*, *Bacteria-Other*) differed between SW and R, and 10 genera (*Incertae Sedis XIII_Anaerovorax*, *Ruminococcaceae-Other*, *Lachnospiraceae-Hespellia*, *Staphylococcaceae-Staphylococcus*, *Porphyromonadaceae-Tannerella*, *Clostridiales-Other*, *Bacteroidales-Other*, *Deferribacteraceae-Mucispirillum*, *Lachnospiraceae-Oribacterium*, *Lachnospiraceae-Bryantella*) between SW and NIH-31. *Lachnospiraceae-Bryantella* and *Staphylococcaceae-Staphylococcus* both increased in the alternating dietary group compared to controls ($p < 0.05$, Table 2; supplemental Fig. 3 [<http://links.lww.com/MPG/A181>]).

Only the Archaea phyla changed significantly when corrected for multiple testing that likely relates to the relatively low sample numbers in our group comparisons. Therefore, these individual taxa results only represent trends with respect to bacterial dysbiosis secondary to increased dietary diversity.

DISCUSSION

Multiple characteristics of the tremendously changing environment (including nutrition) have been implicated as potential causes of the rising IBD incidence over the past 5–6 decades. Among these are pollution (31), refrigeration (32), increased hygiene (33), decreased infection with pathogenic organisms (34), and increased consumption of total fats, omega-6 fatty acids, and meat with a decreased intake of fruits, vegetables, and fiber (35). Interestingly, the increase in dietary diversity resulting from large scale refrigeration and augmented world-wide trade (36) along with enhancing out-of-home eating habits (37) has not been blamed for the emergence of IBD. In the meantime, the success of exclusive enteral nutrition as a therapeutic modality in CD (see introduction) emphasizes the potential etiologic importance of the latter dietary characteristic of industrialization. Some studies have suggested that higher socioeconomic status (average family income, family size, urbanization, education) may lead to a higher incidence rate of IBD (38, 39). Such demographics usually associate with increased dietary diversity as well (40).

To support these epidemiologic observations we examined the associations between agricultural import and IBD prevalence in a Middle-Eastern European country (Hungary, Figure 5). The increase in agricultural import (41) paralleled a striking rise in the prevalence of both UC and CD in this region (42), indicating a possible connection between dietary diversity and IBD. Naturally, other correlates of agricultural import increase (increased antigen load, etc.), concomitant lifestyle and environment changes may also contribute to this observation emphasizing the difficulties in drawing direct association between nutrition and disease etiology. Nevertheless, epidemiologic observations support our theory that nutritional monotony, which characterizes rural populations consuming locally produced seasonal products (43) may be protective against immune mediated chronic forms of intestinal inflammation. The findings of this work on increased severity of chemically induced colitis in mice receiving rather modestly alternating diets further sustain this possibility.

The commensal microbiota is a major communicator of dietary modification towards the intestinal immune system of the host and can rapidly change its composition upon nutritional challenges (44, 45). A modest level of microbiota composition disturbance (or dysbiosis) and decreased diversity has been observed in IBD (46, 47). Therefore, it is commonly accepted that the intestinal microbiota plays an important role in the pathogenesis of these disorders (2). In spite of the success of EEN for the treatment of CD there is surprisingly limited information about its effects on the intestinal microbiota. Temperature gradient gel electrophoresis (TGGE) revealed significant fecal bacterial composition changes following EEN therapy in children with CD (18). Similarly, denaturing gel electrophoresis (DGGE) indicated marked shifts in mucosal bacterial populations upon EEN (48). Consistent with these observations, EEN significantly decreased fecal *Enterobacteria*,

Bifidobacteria, *Bacteroides*, *Clostridium coccoides* and *C. leptum* diversity in pediatric CD (49) and *Faecalibacterium prausnitzii* in adult CD (50). On the contrary, EEN (control formula) in critically ill children did not influence bacterial diversity in stool following 7 days of therapy (by DGGE), but induced a trend for increase in *Lactobacillus* and *Enterococcus* species (sp) and a decrease in *Bifidobacterium* sp. and Enterobacteriaceae by standard culture methods (51). Therefore, it is currently difficult to make convincing conclusions about the effects of EEN on human intestinal microbiota composition. It is also unknown whether EEN has different microbiota effects in people without intestinal inflammation and in patients with IBD where dysbiosis can be present. Importantly, if approached with the concept presented here, traditional mouse models of IBD are already maintained on EEN since those receive the same composition diet (i.e. monotonous diet) day-by-day. In light of this concept, mice consuming the monotonous diets (control and NIH-31) represented two different forms of EEN “therapy” and the switching group corresponds to animals on a modest form of “industrialized” (augmented diet diversity) nutrition.

Interestingly, microbiota diversity decreased upon the alternating nutrition in this study. This would indicate increased susceptibility to inflammation in light of the decreased microbiota diversity observed in IBD (46, 47). However, EEN (i.e. monotonous) appears to further reduce microbiota diversity in CD patients according to the limited (not high-throughput) investigations already discussed above (49, 50). These later observations would contradict our theory that monotonous (but complete: containing all required micro- and macronutrients) diets may protect against IBD by increasing diversity and optimizing microbiota composition. In the meantime, repetitive nutrition may have differing microbiota effects under inflamed and normal intestinal conditions as supported by observations in critically ill (but devoid of intestinal inflammation) children where EEN had both diversifying and simplifying effects on bacterial taxa (51). In fact, two-species model microbiota experiments in gnotobiotic mice indicate that modification in host diet can induce selective pressure on bacterial species depending on their fermentative capacity (52). Therefore, our findings indicate that dietary alternation may decrease microbiota diversity in mammals by providing selective advantage to bacterial strains with a broad range of metabolic competence under normal (non-inflamed) conditions.

Tenericutes increased in abundance on the switching diet. There are conflicting results on this phyla in regards to murine models of IBD where Tenericutes decreased during DSS challenge (53), but increased at some point of *Citrobacter* (C.) *rodentium* infection (54). Similar to microbiota diversity, it is difficult to determine from these observations how the abundance of Tenericutes may influence colitis susceptibility under non-inflamed (“normal”) conditions. Our findings would suggest that an increase in this phylum might promote vulnerability to mammalian colitis.

We found an increased abundance of the genera *Bryantella* and *Staphylococcus* upon dietary alternation. *Bryantella* has been observed to decrease during active DSS colitis (55). However, it has also been shown to increase upon murine *C. rodentium* infection (56). As for *Staphylococcus*, our recent study revealed that it was overrepresented in mice with

augmented susceptibility to DSS (57). Consequently, a higher abundance of *Bryantella* and *Staphylococcus* may induce more severe colitis upon noxious stimuli.

This manuscript includes the first high-throughput analysis to test the effects of alternating and monotonous dietary intake on commensal microbiota composition and associated susceptibility to acute colitis in mice. Our results implicate that a consistent (monotonous) diet may have preventative effects against intestinal inflammation in mammals. The relevance of our findings in regards to the emergence of IBD upon industrialization, and for nutritionally based therapeutic modalities for the disease group will have to be further explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ANOVA	analysis of variance
bTEFAP	bacterial tag-encoded FLX amplicon pyrosequencing
CD	Crohn disease
DSS	dextran sulfate sodium
EEN	exclusive enteral nutrition
IC	inflammatory cell
IBD	inflammatory bowel disease
NIH	NIH-31 control diet
OTU	operational taxonomic unit
P50/70	50/70 days postnatal age
PCA	principal component analysis
R	regular chow
SW	switching diet
UC	ulcerative colitis

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Figure 1.

The percentage of total body weight changes during DSS challenge. The animals lost weight similarly up to day 8 of the experiment, but the control (persistent diets) groups started to gain weight by day 9 while the switching group did not. ● regular chow: R; ■ NIH-31 diet; ▲ switching diet group:SW (# $p < 0.05$ R vs. SW)

Figure 2. Histological severity of colitis in the experimental groups. Colitis severity was significantly higher in the alternating (SW) dietary group. ● regular chow: R; ■ NIH-31 diet: NIH-31; ▲ switching diet group: SW. p values represent two tailed non-paired T test.

Figure 3.

Microbiota diversity in the different groups of the study. There was a trend for decreased diversity in the alternating (switching diet) diet group compared to consistent control chow feeding. p values represent two tailed non-paired T test.

Figure 4. Principal component analysis (PCA) of the colonic mucosal bacterial communities by *16S rRNA* analysis. Unweighted Unifrac analysis. The alteration of control chows induced a unique microbiota composition separating from both consistent feeding groups. ● regular chow; ■ NIH-31 diet; ▲ switching diet group

Figure 5.

Correlation between agricultural import and IBD prevalence in Middle-East Europe (Hungary). Between 2001 and 2006, the agricultural import increased in Hungary, with a parallel increase in the prevalence of both ulcerative colitis (UC) and Crohn disease (CD). See Discussion for further details.

Table 1

Histological scoring system for assessing the severity of colonic inflammation. The tissue damage grade and inflammatory cell (IC) infiltration was multiplied by the percent of the affected area (i.e. 100% = a multiplication factor of 1, and 50% = 0.5, for example). Therefore, the maximal severity score is 8 by this scoring system.

Tissue damage grade	
0	normal
1	damage in epithelium
2	focal ulceration limited to the mucosa
3	focal transmural inflammation and ulceration
4	extensive, but patchy transmural inflammation and ulceration bordered by normal mucosa
5	extensive transmural inflammation and ulceration involving large sections
Inflammatory cell (IC) information	
0	<3 IC/field (40×) in lamina propria
1	>3 IC in lamina propria
2	confluence of IC extending into the submucosa
3	confluence of IC in all tissue layers

Table 2

Significant bacterial abundance differences at the phyla and genera level between the dietary groups (SW: switching diet, R:regular diet and NIH:NIH-31 diet). P and q values represent probability (q= p adjusted for multiple testing) of non-parametric T test comparisons between the switching and the respective control groups (see Materials and Methods). *Lachnospiraceae-Bryantella* and *Staphylococcaceae-Staphylococcus* both increased in the alternating dietary group compared to controls (labeled with **bold**).

	Mean (SW)	Mean (R)	Mean (NIH)	P value	Q value
Phyla					
<i>Archaea_Other</i>	0.016205%	0.000000%		0.0016	0.0153
<i>Bacteria_Actinobacteria</i>	0.032730%	0.223003%		0.0167	0.0798
<i>Bacteria_Other</i>	0.226881%	0.464843%		0.0406	0.1139
<i>Bacteria_Tenericutes</i>	11.428959%	4.670854%		0.0477	0.1139
<i>Bacteria_Bacteroidetes</i>	9.474281%		19.143173%	0.0140	0.1596
Genera					
<i>Lachnospiraceae_Bryantella</i>	5.940634%	1.283595%		0.0029	0.2728
<i>Archaea_Other</i>	0.016205%	0.000000%		0.0038	0.2728
<i>Bifidobacteriales_Bifidobacteriaceae</i>	0.003231%	0.169279%		0.0045	0.2728
<i>Erysipelotrichaceae_Allobaculum</i>	1.711511%	0.050534%		0.0150	0.6726
<i>Staphylococcaceae_Staphylococcus</i>	0.044288%	0.013048%		0.0207	0.7436
<i>Erysipelotrichaceae_Other</i>	1.984042%	0.388114%		0.0406	0.8516
<i>Erysipelotrichaceae_Coprobacillus</i>	0.029645%	0.005018%		0.0420	0.8516
<i>Bacteria_Other</i>	0.226881%	0.464843%		0.0498	0.8516
<i>Incertae Sedis XIII_Anaerovorax</i>	0.024478%		0.148641%	0.0014	0.4181
<i>Ruminococcaceae_Other</i>	3.349796%		6.385434%	0.0045	0.6898
<i>Lachnospiraceae_Hespellia</i>	0.032474%		0.015711%	0.0150	1.0000
<i>Staphylococcaceae_Staphylococcus</i>	0.044288%		0.008980%	0.0153	1.0000
<i>Porphyromonadaceae_Tannerella</i>	9.220930%		18.684872%	0.0236	1.0000
<i>Clostridiales_Other</i>	9.006892%		17.338370%	0.0257	1.0000
<i>Bacteroidales_Other</i>	0.106804%		0.217520%	0.0268	1.0000
<i>Deferribacteraceae_Mucispirillum</i>	0.011283%		0.109429%	0.0294	1.0000
<i>Lachnospiraceae_Oribacterium</i>	0.068125%		0.005168%	0.0329	1.0000
<i>Lachnospiraceae_Bryantella</i>	5.940634%		2.675154%	0.0476	1.0000