Metatranscriptomic analysis of functional response of colonic microbiota to different dietary fibers in growing pigs

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Research Article

Keywords: carbohydrate active enzyme, colonic microbiota, dietary fiber, metatranscriptomics, pectin

Posted Date: July 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-49916/v1

Abstract

Background Dietary fibers are widely considered to be beneficial for health by producing nutrients by gut microbial fermentation while helping with weight management and gut health. So far, the gene expression profiles of CAZymes responsing to different type of fibers (raw potato starch, RPS; inulin, INU; pectin, PEC) in the gut microbes of pigs are not well understood. Therefore, we investigate the functional response of colonic microbiota to different dietary fibers in pigs based on metatranscriptomic analysis.

Results Results showed that the microbial composition and carbohydrate active enzymes (CAZymes) structure of three experimental groups changed significantly compared to the control group (CON). As determined by comparative analysis with the control diet, RPS increased the abundances of Parabacteroides, Ruminococcus, Faecalibacterium and Alloprevotella, and decreased Sutterella. INU increased the relative abundance of Fusobacterium and Rhodococcus, while decreased Bacillus. And pectin treatment increased the relative abundance of Streptococcus and Bacteroidetes group, while decreased the relative abundance of Clostridium, Clostridioides, Intestinibacter, Gemmiger, Muribaculum, and Vibrio. The gene expression of CAZymes GH8, GH14, GH24, GH38, GT14, GT31, GT77 and GT91 downregulated while GH77, GH97, GT3, GT10 and GT27 upregulated in the RPS diet group; AA4, AA7, GH14, GH15, GH24, GH26, GH27, GH38, GH101, GT26, GT27 and GT38 downregulated in the INU group; as to the PEC group, the gene expression of PL4, AA1, GT32, GH18, GH37, GH101, and GH112 downregulated while CE14, AA3, AA12, GH5, GH102 and GH103 upregulated. Compared to RPS and INU groups, the composition of colonic microbiota in the PEC group had more diverse changes with the variation of carbohydrate active enzymes and Streptococcus as the main contributor to CBM61, which promoted the digestion of pectin greatly.

Conclusions The results of this exploratory study displayed a comprehensive overview on the effects of different fibers on gut microbiota and CAZymes in pig colons, which will provide new insights into the impacts of the use of dietary fibers on animal or human health.

1. Background

Dietary fibers are defined as oligosaccharides, polysaccharides and the derivatives which can not be digested by the digestive enzymes to absorbable components in the small intestine, but can be partly fermented by bacteria in the large intestine [1]. Common dietary fibers include resistant starch, soluble and insoluble fiber as well as lignin [2]. As the dominant substrate for bacteria in the pig gastrointestinal tract, dietary fiber has shown to enhance bacterial growth, resulting in a higher fecal excretion of lipids, minerals and amino acids [3]. Dietary fibers are widely considered to be beneficial for health by producing vitamins, short chain fatty acids (notably butyrate) and other nutrients by microbial fermentation while helping with weight management and gut health [4]. Among the large number of genes that have been identified in the human gut microbiome, those that encode carbohydrate active enzymes (CAZymes) are of particular interest, as these enzymes are required to digest most of our complex repertoire of dietary

polysaccharides [5]. So far, the gene expression profiles of CAZymes in the gut microbes of pigs are not well understood.

Three dietary fibers, which differ in origin and composition, are commonly used to improve gut health. Inulin is effective prebiotic because its resist-digestion in the small intestine but it can be fermented within the colon. It plays pivotal roles in adjusting the composition of the intestinal microbiota, maintaining the normal intestinal environment, regulating intestinal function, and improving human health. It was showed that inulin stimulated the growth of *Bifidobacteria* but limited the growth of potential pathogenic bacteria such as *E. coli* and *Salmonella* [6]. Pectin is soluble non-starch polysaccharides that is more fermentable in the hindgut than insoluble non-starch polysaccharides, producing short-chain fatty acids that are beneficial to the organism. Most of the glycoside hydrolases (GHs) family enzymes associated with pectin. Raw potato starch is a kind of resistant starch, which is an insoluble dietary fiber. It is fermented by physiological bacteria in the large intestine to produce short chain fatty acids and gases, stimulate the growth of beneficial bacteria and increase the number of *Bifidobacteria*. So far, the mechanism of how different type of fibers affect microbiota metabolism in pig's colon is not clear.

Metatranscriptomics offers a means to predict the processes being mediated by microbes at a particular instant within an environmental sample, enabling insights into the functioning of microbial communities, and to potentially investigate their responses to environmental conditions [7]. In recent years, the application of metatranscriptomics in the analysis of microbial population increased sharply, but information on the comparison of microbial function responded to different dietary fibers based on metatranscriptomics is very limited. Therefore, we conducted a metatranscriptomics study to test the hypothesis that the colonic microbial composition and gene expression of CAZymes is responsive to different dietary fibers in pigs.

2. Results

2.1 Overview of the metatranscriptomes

On average, 100 million raw sequence reads were obtained from the metatranscriptome of each sample; and a total of 210.09 Gbp of high-quality sequences were generated from these 16 samples after removing the adapters and quality filtering. The Q20 and Q30 base percentages of each sample were above 98.95% and 96.30%, respectively.

A total of 1,081,814 contigs were identified after *de novo* assembling using MEGAHIT in these 16 samples, the length of these contigs ranged from 546 to 1,004,658 bp, with an average length of 1,623.05 bp. And 712,210 unigenes were clustered with CD-HIT (http://www.bioinformatics.org/cd-hit/) (95% identity and 90% coverage). Among these 16 samples, 19,264 core species were found in the all samples, and each sample had its own unique species (Fig. 1). In general, the PEC group had the highest numbers of specific species compared to other groups. Principal co-ordinate analysis (PCoA) based on unweighted

UniFrac distances showed that the colonic luminal digesta samples in the PEC group were clustered distinctly from those in other groups (Fig. 2).

2.2 Effect of different dietary fibers on colonic microbiota composition

The distribution of dominant bacteria in each group was shown in Fig. 3. In the CON group, bacteria detected in the proximal colonic luminal digesta samples belonging to 55 different phyla, and the most abundant phylum was Bacteroidetes, followed by Firmicutes and Proteobacteria, and 1,002 bacteria genera were observed in this group, *Prevotella, Bacteroides* and *Clostridium* were the three most abundant genera. The INU group contained 968 genera belonged to 57 phyla; the RPS group had 49 phyla made up of 918 genera and the PEC group contained 60 phyla and 1131 genera.

At the phylum level, the abundance of Verrucomicrobia in the RPS group was lower (fold change >2 or < 0.5; $q < 0.05$) than that in the CON group, and the abundance of Verrucomicrobia in the INU group was also lower (fold change >2 or < 0.5; $q < 0.05$) than that in the CON group, while the abundance of Fusobacteria, Actinobacteria and Cyanobacteria were greater (fold change >2 or < 0.5; $q < 0.05$) than those in the CON group, then the population of Proteobacteria, Spirochaetes and Verrucomicrobia phyla were greater (fold change >2 or < 0.5; $q < 0.05$) in the PEC group compared with those in the CON group colonic digesta samples (Additional file 1).

At the genus level, compared with the CON group, 14 genera changed significantly in the relative abundance in the RPS group, and 17 and 25 genera in the INU and PEC groups (Table 1). The abundance of *Parabacteroides, Faecalibacterium, Ruminococcus* and *Alloprevotella* increased while *Sutterella* decreased in the RPS group. Inulin supplement increased the abundance of *Fusobacterium* and *Rhodococcus* while decreased the abundance of *Bacillus*. The abundance of *Streptococcus* and *Bacteroidetes_norank* increased, while *Clostridium, Clostridioides, Intestinibacter, Ruminococcaceae_norank, Gemmiger, Muribaculum, Enterococcus* and *Vibrio* decreased in the PEC group.

2.3 Effect of different dietary fibers on the activities of colonic CAZymes

In terms of CAZyme profiles, a total of 222 CAZymes families were detected, including 7 AAs (auxiliary activities), 36 CBMs (carbohydrate-binding modules), 15 CEs (carbohydrate esterases), 94 GHs (glycoside hydrolases), 57 GTs (glycosyl transferases) and 13 PLs (polysaccharide lyases). As shown in Fig. 4, GHs were the most abundant class in all 4 groups, but the distribution of CAZymes at class level had no significant change among four groups.

Compared with the CON group at the family level, some changes were found in the dietary fiber groups (Additional file 2). 30 CAZyme families changed significantly (fold change >2 or < 0.5; $q < 0.05$) in the RPS group, 9 CAZymes (CBM21, CBM74, GH128, GH77, GH85, GH97, GT10, GT27, and GT3) were upregulated in the mRNA expression, while 21 CAZymes (AA7, CBM26, CBM41, GH101, GH112, GH14, GH15, GH24, GH27, GH35, GH38, GH8, GH89, GT14, GT25,GT31, GT49, GT77, GT8, GT84, and GT91) were

downregulated. 14 CAZyme families were downregulated in the INU group. And 35 CAZyme families changed significantly in the PEC group, 13 CAZymes (AA12, AA3, CBM61, CBM9, CE14, GH102, GH103, GH16, GH5, GH85,GH88, GT1, and GT21) had higher abundances, while 22 CAZymes (AA1, AA2, AA6, CBM21, CBM26, CBM41, GH101, GH112, GH132, GH14, GH17, GH18, GH24,GH37, GH38, GT15, GT32, GT39, GT49, GT77, GT91, and PL4) were lower than that in the CON group. (Table 2). Among these altered CAZyme families, 4 CAZymes (AA7, GH15, GH27, and GT84) were downregulated in both INU and RPS groups; GH112 and GT91 decreased while GH85 increased in both RPS and PEC groups, and CBM21 increased in the RPS group while decreased in the PEC group. And 2 altered CAZymes (AA4 and GH26) were specific in the INU group, 14 (CBM74, GH128, GH35, GH77, GH8, GH89, GH97, GT10, GT14, GT25, GT27, GT3, GT31, and GT8) were specific in the RPS group, and 23 (AA1, AA12, AA2, AA3, AA6, CBM61, CBM9, CE14, GH102, GH103, GH132, GH16, GH17, GH18, GH37, GH5, GH88, GT1, GT15, GT21, GT32, GT39, and PL4) were specific in the PEC group.

2.4 Correlation between carbohydrate active enzymes and colonic microbiota

One of the most critical roles of the microbiota is its ability to utilize complex carbohydrate sources, the network of correlations analysis between CAZyme classes and microbiota (at the genus level) showed that *Prevotella* and *Tannerella* primarily contributed CAZyme encoding gene fragments of the GHs; GTs mainly produced by *Prevotellamassilia* and *Prevotella*; *Prevotellamassilia* and *Roseburia* primarily contributed to CBMs; *Lachnotalea* and *Butyricimonas* primarily contributed to CEs; *Butyricimonas* and *Mediterranea* primarily contributed to PLs; and AAs mainly produced by *Turicibacter* and *Chlamydia* in the growing pigs colon metatranscriptome among the significantly changed bacterial genera (Additional file 3).

Furthermore, 51 bacterial genera and 79 CAZymes families affected significantly by the dietary fiber treatment were used for the Spearman's rank correlation analysis (Fig. 5). The production of CBM21 and GT91 were negatively correlated with the abundance of Sutterella. Parabacteroides had a negative correlation with the production of GH14, GH24, GH8, GT14, GT31, and GT77, while contributed a proportion of GT10 and GT3; *Alloprevotella* and *Ruminococcus* contributed a proportion of GH77, GH97, GT10, GT27, and GT3; and *Faecalibacterium* was a contributor of CBM74 and GH38. *Streptococcus, Clostridioides, Intestinibacter, Vibrio, Clostridium, Gemmiger*, *Muribaculum* and *Enterococcus* altered significantly specific to the PEC vs. CON group, *Streptococcus* had a positive correlation with the production of AA12, AA3, CBM61, GH102, GH103, GH16 and GH5; *Clostridioides* contributed a proportion of AA1, CBM21, and PL4, and negatively correlated with the production of AA3, CBM61, GH102, and GH103; *Intestinibacter* had a negative correlation with the production of AA3, CE14, and GH102; *Vibrio* had a negative correlation with the production of AA12, AA3, CBM61, GH102, and GH103; *Clostridium* was a contributor of GH18 and GT32, and negatively correlated with the production of AA3; *Gemmiger* contributed a proportion of GH101 and GH112, and negatively correlated with the production of AA3 and CE14; *Muribaculum* was a contributor of CE14 and GH37.

According to the taxonomic distribution (about top 10 genera) of predicted CAZymes identifed from the metatranscriptomes in the PEC group, *Prevotella, Bacteroides, Mesorhizobium* and *Parabacteroides* were the largest genera in the predicated AAs, GHs, GTs, CEs, PLs and CBMs, and it's worth noting that *Streptococcus* was also the major microbial origin of the predicted CBMs (Fig. 6). Focus on the contributions of CAZymes from the major microbial communities in growing pigs colons in the PEC group compared to the CON group, it is noteworthy that *Prevotella* was the main contributor of GH5, *Mesorhizobium* was the main contributor of GH16, and *Streptococcus* was the main contributor of CBM61 in the PEC group (Fig. 7).

3. Discussion

In this study, three different dietary fibers were fed to pigs to comprehensively compare their effects on luminal microbiota composition and the activities of colonic CAZymes. Considerable microbiota variation existed in the proximal colonic luminal digesta samples of pigs in different dietary fiber groups. Compared with the INU and RPS groups, PEC group was characterized by more colonic microbiota changes, suggesting pectin enriched diet may have more widely impact on pig colonic microbiota.

A significant (fold change >2 or < 0.5; q < 0.05) increase in the *Parabacteroides*, *Ruminococcus*, *Faecalibacterium* and *Alloprevotella* was detected with a concurrent reduction in *Sutterella* in the RPS group. Potato starch fermented in the colon by the resident microbiota [8], the diet could enhance the growth of *Bifidobacteria* and *Lactobacilli* in rats, these bacteria are considered beneficial to the host due to their health promoting attributes such as immunomodulation and improving gut epithelial barrier function [9]. In addition, a previous study showed *Sutterella* increased greatly in the ceca of mice fed raw potato starch [10]. Consistent with these reports, RPS diet enhanced the growth of *Bifidobacteria* and *Lactobacilli* in our study. We found that the RPS group showed a decrease in *Sutterella*, and the result was opposite to the above study on mice, the different results may due to the different experimental animals, and larger individual variation of pigs should also be considered. Inulin diet could increase the abundance of *Bifidobacteria and Faecalibacteria*, while decrease the abundance of *Bacteroid*es [11, 12, 13]. In the diabetic rat gut microbiota, inulin treatment upregulated the abundance of probiotic bacteria *Lactobacillus*. Whereas downregulated the abundance of *Desulfovibrio,* which produce lipopolysaccharide [14]. In the present study, the abundance of *Bifidobacteria and Faecalibacteria* increased, then *Bacteroides* and *Desulfovibrio* decreased consistented with those literature mentioned before. And the relative abundance of *Fusobacterium* and *Rhodococcus* increased, while *Bacillus* decreased specific to the INU group. Previous reports suggested that pectin utilization was relatively common among Bacteroides [15, 16, 17], and neutral sugar-rich pectin was selectively metabolized to produce short-chain fatty acids and increase the beneficial Bifidobacteria population [18, 19]. Intriguingly, pectin treatment significantly increased the relative abundance of *Streptococcus* and *Bacteroidetes group,* while decreased the relative abundance of *Clostridium, Clostridioides, Intestinibacter, Gemmiger, Muribaculum,* and *Vibrio* significantly, and the relative abundance of *Bifidobacteria* had no significant change in the present study.

Colonic microbiome encoded a huge number of carbohydrate active enzymes to degrade polysaccharides beyond the capabilities of their host [20, 21], and these enzymes changed differently in the different three diet groups in this study. 30 CAZymes families altered significantly in the RPS diet group, 14 families changed significantly in the INU group, and 35 families changed significantly in the PEC group. Among these families, CBM26 and CBM41 downregulated in all three diet groups compared with the CON group, they can be considered as families having starch-binding domain (SBD) in the CAZymes database, an SBD is a special case of a carbohydrate-binding domain, it has no enzymatic activity but can attach the catalytic domain to the carbohydrate substrate to hold it and process it at the active site which gave the enzymes the ability to bind onto raw starch [22, 23], it may showed these three additive prevented the degradation of starch to a certain degree. And CBM61 increased in the PEC group compared with the CON group. As all CBMs, it can increase the local concentration of enzymes on the substrate, thereby enhancing catalytic activity [24]. Indeed, most pectin-degrading microbes have their own stock of enzymes that include avariety of hydrolases and lyases able to degrade the arabinan, rhamnogalacturonan, polygalacturonan and galactan "domains" in pectin, and the pectin binding CBMs are limited. Galactan could be recognized by CBM61, CBM61 bound with pectins with the greatest affinity, and especially to samples containing the beta-1,4-galactan side chain component of pectin and to beta-1,4-galactotetraose, indicating specificity for beta-1,4-linked galactose polymers. [25]. And according to the correlation analysis between CAZyme families and microbiota, *Streptococcus* was a main contributor of CBM61, the relative abundance of *Streptococcus* increased in the PEC group, this could slao explain the increase of CBM61 in the PEC group.

Except CBM families, there were more changes in other diverse CAZyme families in dietary fiber groups compared with the CON group, previous studies showed that GHs and PLs were the two key types of CAZymes to degrade different substrates. GHs cleave bonds by means of the insertion of a water molecule [26, 27], and PLs cleave complex carbohydrates with an elimination mechanism [28]. While CEs removed ester substituents from the glycan chains to facilitate the action of GHs and PLs, GTs assembled complex carbohydrates from activated sugar donors [29], AAs most involved in the process of cellulose and lignin degradation [30], they had little association with digestion of dietary fibers.

Focus on the changes about GHs and PLs, the abundance of family GH8, GH14, GH24 and GH38 downregulated while GH77 and GH97 upregulated in the RPS diet group; GH14, GH15, GH24, GH26, GH27, GH38,and GH101 downregulated in the INU group; as to the PEC group, PL4, GH18, GH37, GH101, and GH112 decreased while GH5, GH16, GH102 and GH103 increased.

To get insight into the effects of different dietary fibers on colonic microbiota and CAZymes, we found some correlations between the altered colonic microbiota and the changed CAZymes (Fig. 5). In these CAZymes families, GHs and PLs were the most prominent part that influenced the digestion of substrates, then we specific concentrated on the relationship between colonic microbiota and GHs together with PLs enzymes. Families GH13 and GH57 have alpha-amylase enzyme specificity, alpha-amylase represents the best known amylolytic enzyme, it catalyzes the hydrolysis of alpha-1,4-glucosidic linkages in starch and related alpha-glucans with the retaining reaction mechanism [31, 32]; PL9 could

produce two or more unsaturated galacturonates from pectic substrates confirming that it's endo-pectate lyase [33]; Enzymes in family GH32 could degrade fructans of the inulin type with an endo-cleavage mode [34]. In the present study, GH13 increased in the RPS group while decreased in the INU and PEC group, PL9 decreased in the INU group and increased in the RPS and PEC group. While GH57 decreased in the RPS and INU group while increased in the PEC group in a very small extent, GH32 increased in the RPS and PEC group while decreased in the INU group. These changes opposited to the studies mentioned before, maybe could explained as a comprehensive impact of many different types of subfamily enzymes in these families.

The abundance of GHs in pig colon shifted with the changes in the diet. GHs catalyzed the hydrolysis of O-glycosidic bonds in carbohydrates such as starch, and they catalyzed both hydrolysis and transglycosylation reactions, but the ratio varies enormously depending on the type of GHs, the substrate concentration and the reaction conditions. Various CAZymes originating from different taxonomic sources differ from each other significantly in their exact substrate preference. In the RPS group, the abundance of GH14 and GH8 decreased, GH14 was annotated as beta-amylase (EC 3.2.1.2), and beta-amylase is a crucial exo-hydrolase that contributes to the complete degradation of starch into metabolisable or fermentable sugar [35], GH8 was annotated as endohemicellulase, hemicellulose is an important anti-nutritional factor in feed, will hinder the digestion of nutrients and reduce the growth performance of animals, then enzymes in family GH8 could degrade hemicellulose and eliminate anti-nutritional effect [36], and cleave beta-1,4 linkages of beta-1,4 glucans, xylans (or xylooligosaccharides), chitosans, and lichenans (1,3-1,4-beta-D-glucan), they always show xylanase active on heteroxylans from various sources [37]. Different carbohydrate active enzymes changed correspond to different dominant bacteria. The production of GH14 and GH8 had negative correlations with Parabacteroides, and the abundance of the bacteria increased in the RPS group. CAZyme GH77 increased in the RPS group, it was annotated as debranching enzymes, and it contains only one enzyme specificity of 4-alpha-glucanotransferase (EC 2.4.1.25) [38], it involved in maltose metabolism in microorganisms [39]. Since *Alloprevotella* and *Ruminococcus* contributed a proportion of GH77*,* the enzyme increased along with the reduction of *Alloprevotella* and *Ruminococcus* in the RPS group. The enzyme GH38 dedicated for hydrolysis of oligosaccharides, it decreased in the RPS group along with the reduction of *Faeculibacterium*. The abundance of all significant changed GHs in the INU group decreased, this maybe the results of the negative effect of inulin on digestion.

More GHs changes were found by the regulation of PEC enriched diet. The abundance of GH5 increased along with the increase of *Streptococcus* and *Prevotella*, GH5 was mainly annotated as endo-glucanase, which is the main ingredient of cellulase [40]. and it also involved in the hydrolysis of galactose, some loops in GH5 enzymes could recognize galactosyl unit [41]. The abundance of GH16 increased along with the increase of *Streptococcus* and *Mesorhizobium*, and the degradation of galactose was predicted could be initiated by GH16 [42], this galactanases had substrate specificity acting on galactooligosaccharides [43]. And the abundance of GH103 decreased with the increase of Clostridioides and the decrease of Vibrio. The glycoside hydrolases of family GH103 are in fact lytic transglycosylases of bacterial origin [44], these enzymes cleave the beta-1,4 linkage between N-acetylmuramoyl and N-acetylglucosaminyl

residues in peptidoglycan. The abundance of GH102 increased with the increase of Intestinibacter. Along with the decrease of Gemmiger bacteria, the abundance of GH101 and GH112 decreased in the PEC group. The family GH101 is made up of endo-alpha-N-acetylgalactosaminidases and their homologues [45]. GH112 almost contains phosphorylases, such as lacto-N-biose phosphorylase, galacto-N-biose phosphorylase (EC 2.4.1.211), and D-galactosyl-1,4-L-rhamnose phosphorylase (EC 2.4.1.-). These GHs could promote the digestion of some polysaccharides.

4. Conclusions

In conclusion, the results of this exploratory study displayed a comprehensive overview on the effects of different fibers on gut microbiota and CAZymes in pig colons, especially showed the function of the *Streptococcus* along with CBM61 in the degradation of galactose in the PEC group, which will provide new insights into the impacts of the use of dietary fibers on animal or human health. This study shows a possibility to regulate selectively the abundance of the colon microbiota by means of dietary fiber, thus give us a better understanding of the role of different types of dietary fiber in regulating intestinal microbial metabolism.

5. Methods

5.1 Animal experiments and sample collection

Twenty-eight 35-day old pigs (Duroc×Landrace×Large White) with similar body weight (mean ± SEM, 8.79 ± 0.09 kg) were randomly allotted to 4 groups, each group consisting of 7 replicates (pens) with one pig per pen. Pigs in the four groups were fed control (CON) diet (a corn-soybean based diet), inulin (INU), raw potato starch (RPS), or pectin (PEC) enriched diets, respectively. As reported in the literatures, it is reasonable that the addition of different dietary fibers ranges from 3% to 10% (w/w). In this study, considering comparison of the effects of the three dietary fibers under the uniform condition, inulin, raw potato starch, or pectin were used to replace 8% (w/w) of corn starch in the CON diet, respectively (Additional file 4). The trial lasted 40 days, pigs had unlimited access to feed and water throughout the experimental period. At the age of 76 d, all pigs were anesthetized and euthanized with a jugular vein injection of 4% sodium pentobarbital solution (40 mg/kg body weight) after 12 h fasting. Proximal colonic luminal digesta samples were collected, snap-frozen using liquid nitrogen, and stored at - 80 °C condition until further analysis.

5.2 RNA extraction and metatranscriptomic sequencing

Total RNA was extracted from each proximal colonic luminal digesta samples of pigs with TRIzol reagent (Invitrogen, CA, USA) in accordance with the manufacturer's protocols and subjected to DNase I (TaKara, Dalian, China) digestion to remove contaminating DNA. Because there were 7 replicates in each group, 4 biological replicates were randomly selected for the RNA-Seq to reduce the experimental expense. Then, the total RNA quantity and purity were analysis of Bioanalyzer 2100 and RNA 6000 Nano Lab Chip Kit

(Agilent, CA, USA) with RIN number > 7.0. A total of 5 μg of RNA per sample was processed for rRNA depletion using a Ribo-Zero™ Magnetic kit (G+/G-Bacteria). A high-quality RNA sample (optical density (OD) 260/280 = 1.8-2.2) was used to construct the sequencing library. Following the TruSeq RNA preparation kit from Illumina (San Diego, CA, USA), the RNA was divided into small pieces and used as a template for cDNA synthesis. Apolymerase chain reaction (PCR) solution containing a mixture of dATP, dGTP, dCTP, and dUTP was used, and the PCR reaction amplified 15 cycles. In brief, libraries were size-selected for cDNA target fragments of 200–300 bp on 2% certified low range ultra agarose, followed by PCR amplification using Phusion DNA polymerase (NEB). After quantification with TBS380, the paired-end libraries were sequenced by Shanghai Biozeron Biothchology Co. Ltd. (Shanghai, China), and the read length was Illumina HiSeq PE 2×150 bp.

5.3 Metatranscriptome data analysis

The raw sequence reads were subjected to filtering of host reads, adapter sequences or poly-N and low-quality (Q < 20) sequences. (http://bio-bwa.sourceforge.net; https://github.com/jstjohn/SeqPrep; https://github.com/najoshi/sickle). The Q20, Q30 and GC content of the quality-filtered data were calculated. Ribosomal RNA sequences were removed by comparison with the NCBI rRNA, tRNA and SILVA databases. The remaining quality-filtered sequence reads were assembled de novo into transcripts using Megahit (https://github.com/voutcn/megahit) with the default parameters. Then, the transcripts of all sixteen samples were combined and clustered into unique classes with CD-HIT-EST at 95% identity. After the assembly and clustering of transcripts, the longest sequence of each class was treated as unigene. The expression of each unigene was evaluated as parts per million (PPM). The number of reads mapped to each unigene was counted, and the PPM of each gene was calculated based on the length of the gene and the read counts mapped to that gene. Then identify differentially expressed genes (DEGs) between 2 different samples according to the PPM. Genes with altered expression (fold change >2 or <0.5; $P < 0.05$) were selected for further study.

5.4 *Taxonomic annotation of unigenes*

BLASTP (BLAST version 2.2.28+, http: //blast.ncbi.nlm.nih.gov/Blast.cgi) was used to annotate the unigenes by comparing the gene against the NR database (e-value <$1e^{-5}$). The calculate the abundance of each taxonomic level according to the sum of corresponding gene abundance in each sample, and the abundance profile at the corresponding taxonomic level were measured, then the principal co-ordinates analysis of these samples based on unweighted UniFrac distances.

5.5 Functional annotation of CAZymes

CAZymes functional annotation was carried out using hmmscan (http://hmmer.janelia.org/search/hmmscan/)(e-value <$1e^{-5}$), and then obtain the annotation of the carbohydrate active enzyme corresponding to the gene.

5.6 Analysis of correlations between bacterial genera and CAZymes

Correlations between observed microbial taxa (bacterial genera) and CAZymes were explored using Spearman's rank correlation. Significant relationships (coefficient [p] of >0.5 or <-0.5 and *P* value of <0.05) between observed microbial taxa and CAZymes were selected for further study.

5.7 Analysis of microbiota distributed to CAZymes

The distribution of microbiota to CAZymes were visualized by Circos, evaluated the contribution of different bacteria to certain enzyme by analyzing the origin of the enzyme, the microbiota were ordered according to the distribution, we selected the top 10 and 15 genera respectively, then analysed the influence of the bacteria on the digestion of dietary fiber in follow-up studies.

Abbreviations

CAZymes: Carbohydrate active enzymes

CON: Control group

RPS: Raw potato starch group

INU: Inulin group

PEC: Pectin group

GHs: Glycoside hydrolases

PCoA: Principal co-ordinate analysis

AAs: Auxiliary activities

CBMs: Carbohydrate-binding modules

CEs: Carbohydrate esterases

GTs: Glycosyl transferases

PLs: Polysaccharide lyases

SBD: Starch-binding domain

Declarations

- **Ethics approval and consent to participate**

This study was carried out in accordance with the regulations of Animal Care and Use Guidelines of Nanjing Agricultural University.

- **Consent for publication**

Not applicable.

- **Availability of data and material**
- **Competing interests**

The authors declare that they have no competing interests.

- **Funding**

This research has received funding from the the National Key R&D Program of China (2017YFD0500505) and National Natural Science Foundation of China (31872362).

- **Authors' contributions**

YS conceived the ideas. YS designed the study. JX and RX carried out the experiments. JX, RX, MJ and YS analyzed the data, and JX and YS wrote the manuscript. All authors read and gave approval for the manuscript.

- **Acknowledgements**

The authors acknowledge members of Shanghai Biozeron Biotechnology Co., Ltd. for their technical support, and the Laboratory of Gastrointestinal Microbiology of Nanjing Agricultural University for providing relevant experimental equipment.

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Tables

Table 1 Relative abundances (percentage) of microbial genera significantly affected by different dietary fibers in pigs colon.

Genus	Group[1]		FC[2]	Q-value
	CON	RPS		
Parabacteroides	2.852±0.549	8.218±2.173	3.268	0.000
Acinetobacter	0.934±0.334	0.337±0.162	0.371	0.000
Bacteria norank	0.846±0.092	1.830±0.880	2.101	0.000
Coprococcus	0.816±0.368	0.289±0.060	0.410	0.003
Anaerofilum	0.789±0.333	0.228±0.122	0.306	0.000
Faecalibacterium	0.712±0.082	1.986±0.683	2.925	0.000
Duodenibacillus	0.333±0.109	0.113±0.017	0.389	0.000
Fusicatenibacter	0.281±0.261	0.069±0.032	0.319	0.000
Terrisporobacter	0.265±0.081	0.120±0.032	0.492	0.017
Ruminococcus	0.250±0.048	0.728±0.250	3.125	0.000
Prevotellaceae unclassified	0.172±0.138	0.050±0.021	0.386	0.000
Alloprevotella	0.161±0.030	0.273±0.109	2.052	0.000
Tannerella	0.128±0.031	0.058±0.005	0.469	0.041
Sutterella	0.116±0.030	0.037±0.004	0.349	0.000
	CON	INU		
Bacteria unclassified	4.619±1.100	2.559±0.885	0.362	0.005
Lactobacillus	2.660±1.535	0.875±0.596	0.216	0.000
Clostridiales unclassified	0.976±0.200	0.468±0.097	0.348	0.001
Acinetobacter	0.934±0.334	0.436±0.231	0.344	0.000
Bacteria norank	0.846±0.092	4.424±0.493	4.002	0.000
Coprococcus	0.816±0.368	0.165±0.038	0.163	0.000
Flavonifractor	0.757±0.173	0.390±0.067	0.382	0.005
Fusobacterium	0.460±0.148	3.773±3.612	4.560	0.000
Peptostreptococcaceae unclassified	0.443±0.102	0.249±0.100	0.416	0.014
Phascolarctobacterium	0.419±0.059	0.251±0.082	0.397	0.018
Duodenibacillus	0.333±0.109	0.085±0.017	0.229	0.000
Fusicatenibacter	0.281±0.261	0.029±0.013	0.069	0.000
Terrisporobacter	0.265±0.081	0.132±0.068	0.378	0.002
Bariatricus	0.227±0.074	0.080±0.019	0.272	0.000
Bacillus	0.116±0.030	0.057±0.023	0.380	0.003
Pseudoflavonifractor	0.115±0.018	0.055±0.005	0.375	0.008
Rhodococcus	0.100±0.037	0.502±0.212	3.488	0.000
	CON	PEC		
Clostridium	7.144±2.012	2.907±0.744	0.329	0.000
Bacteria unclassified	4.618±1.100	0.550±0.103	0.093	0.000
Lactobacillus	2.660±1.535	1.183±0.405	0.446	0.000
Streptococcus	1.627±0.452	4.268±0.869	2.228	0.000

Clostridiales unclassified	0.976±0.200	0.398±0.075	0.388	0.000
Acinetobacter	0.934±0.334	0.081±0.015	0.078	0.000
Bacteria norank	0.846±0.092	2.115±0.533	2.178	0.000
Coprococcus	0.816±0.368	0.253±0.030	0.281	0.000
Anaerofilum	0.789±0.333	0.017±0.002	0.020	0.000
Flavonifractor	0.757±0.173	0.290±0.085	0.300	0.000
Clostridioides	0.499±0.297	0.098±0.008	0.176	0.000
Intestinibacter	0.498±0.136	0.171±0.037	0.297	0.000
Peptostreptococcaceae unclassified	0.443±0.102	0.074±0.006	0.149	0.000
Phascolarctobacterium	0.419±0.059	0.195±0.026	0.405	0.000
Ruminococcaceae norank	0.401±0.064	0.190±0.074	0.408	0.000
Terrisporobacter	0.265±0.081	0.016±0.002	0.060	0.000
Bariatricus	0.227±0.074	0.072±0.028	0.238	0.000
Bacteroidetes norank	0.227±0.122	4.862±2.371	17.152	0.000
Gemmiger	0.199±0.059	0.092±0.013	0.414	0.000
Prevotellaceae unclassified	0.172±0.138	0.081±0.043	0.447	0.000
Muribaculum	0.145±0.128	0.056±0.013	0.352	0.000
Enterococcus	0.142±0.057	0.077±0.026	0.409	0.001
Tannerella	0.128±0.031	0.073±0.012	0.486	0.001
Vibrio	0.119±0.061	0.021±0.006	0.134	0.000
Pseudoflavonifractor	0.115±0.018	0.053±0.014	0.367	0.000

[1] CON, a control diet; RPS, a raw potato starch enriched diet; INU, an inulin enriched diet; PEC, a pectin enriched diet. The data are expressed as mean ± SEM, n =4.

[2] FC=fold change (for relative abundances of microbial genera derived from the treatment diet compared with the control diet group).

Table 2 Gene expression of CAZymes significantly affected by different dietary fibers in pigs colon.

Group[1]	Family[2]	FC[3]	Q-value
RPS vs. CON	AA7	0.394	0.001
	CBM21	2.137	0.013
	CBM26	0.156	0.000
	CBM41	0.127	0.002
	CBM74	7.488	0.000
	GH101	0.117	0.000
	GH112	0.262	0.010
	GH128	2.281	0.035
	GH14	0.088	0.000
	GH15	0.116	0.016
	GH24	0.083	0.000
	GH27	0.375	0.002
	GH35	0.431	0.003
	GH38	0.201	0.000
	GH77	2.084	0.004
	GH8	0.391	0.004
	GH85	3.429	0.001
	GH89	0.305	0.000
	GH97	2.008	0.002
	GT10	5.674	0.039
	GT14	0.423	0.017
	GT25	0.323	0.018
	GT27	2.297	0.029
	GT3	2.453	0.000
	GT31	0.103	0.044
	GT49	0.246	0.000
	GT77	0.088	0.013
	GT8	0.488	0.015
	GT84	0.143	0.003
	GT91	0.099	0.044
INU vs. CON	AA4	0.234	0.029
	AA7	0.194	0.000
	CBM26	0.170	0.000
	CBM41	0.159	0.016
	GH101	0.288	0.017
	GH14	0.027	0.000
	GH15	0.101	0.016
	GH24	0.067	0.000

	GH26	0.468	0.029
	GH27	0.264	0.001
	GH38	0.115	0.000
	GT49	0.087	0.000
	GT77	0.183	0.033
	GT84	0.193	0.029
PEC vs. CON	AA1	0.001	0.005
	AA12	4190.444	0.001
	AA2	0.005	0.000
	AA3	52416.837	0.000
	AA6	0.093	0.000
	CBM21	0.007	0.000
	CBM26	0.145	0.000
	CBM41	0.169	0.001
	CBM61	2.818	0.007
	CBM9	2.265	0.000
	CE14	76.073	0.000
	GH101	0.015	0.000
	GH102	17.157	0.005
	GH103	63.183	0.000
	GH112	0.098	0.000
	GH132	0.004	0.000
	GH14	0.271	0.003
	GH16	2.577	0.000
	GH17	0.002	0.000
	GH18	0.396	0.023
	GH24	0.137	0.000
	GH37	0.114	0.043
	GH38	0.256	0.000
	GH5	2.183	0.000
	GH85	6.695	0.000
	GH88	3.186	0.000
	GT1	11.835	0.001
	GT15	0.004	0.000
	GT21	4471.475	0.001
	GT32	0.474	0.018
	GT39	0.033	0.027
	GT49	0.140	0.000

GT77	0.137	0.037
GT91	0.001	0.043
PL4	0.149	0.000

[1] RPS, a raw potato starch enriched diet; CON, the control group; INU, an inulin enriched diet; PEC, a pectin enriched diet.

[2] The carbohydrate active enzymes families are classifed according to the CAZy database. AA, Auxiliary Activities; CBM, Carbohydrate-Binding Modules; CE, Carbohydrate Esterases; GH, Glycoside Hydrolases; GT, Glycosyl Transferases; PL, Polysaccharide Lyases.

[3] FC=fold change (for gene expression of CAZymes derived from the treatment diet compared with the control diet group).

Figures

Figure 1

Species Venn analysis. Different colors represent different samples, and the common area where two circles of different colors overlap is marked with a number to indicate the number of species shared by the two samples.

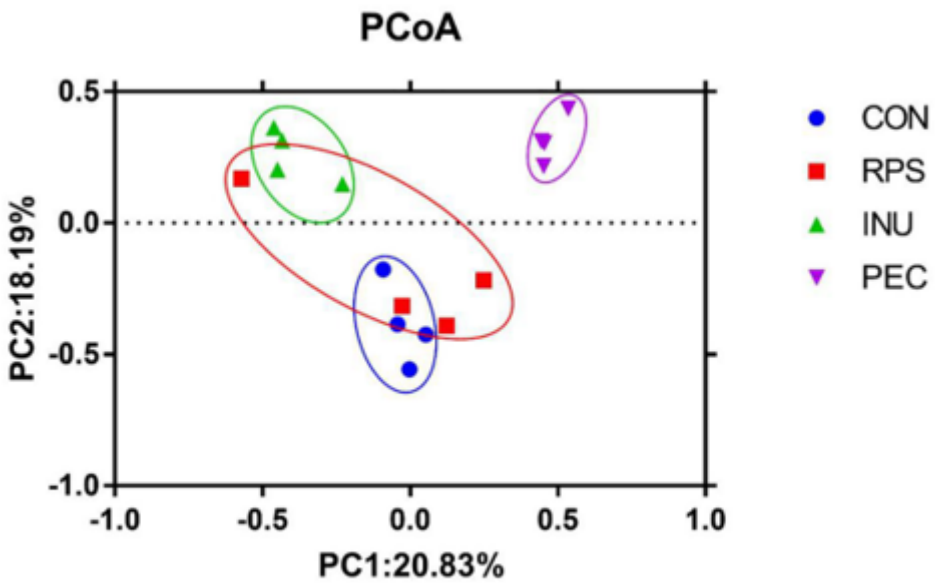
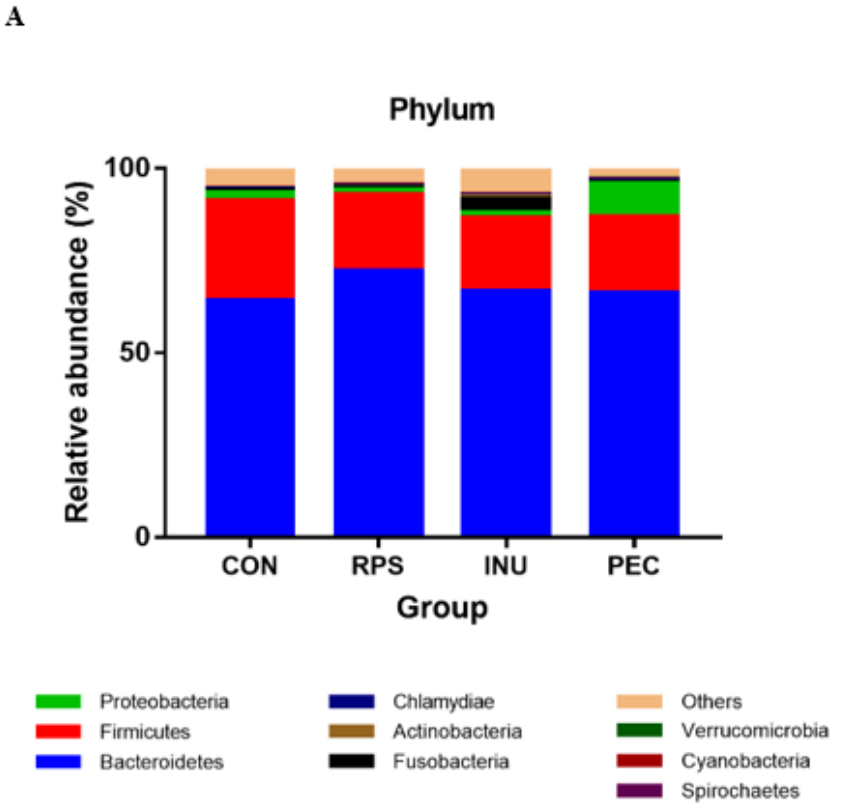


Figure 2

Unweighted unifrac PCoA plot of colonic bacteria of the abundance of genes. The percentages in the axis labels represent the percentages of variation explained by the principal components. The closer proximity of dots indicates higher similarity. CON, a control diet; RPS, a raw potato starch enriched diet; INU, an inulin enriched diet; PEC, a pectin enriched diet.



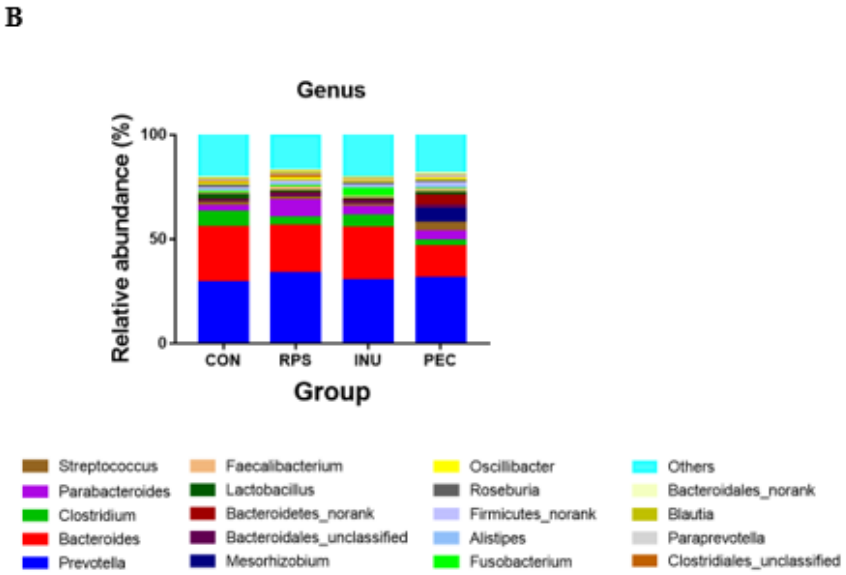


Figure 3

The distribution of dominant bacteria at the phylum (A) and genus (B) levels. CON, a control diet; RPS, a raw potato starch enriched diet; INU, an inulin enriched diet; PEC, a pectin enriched diet.

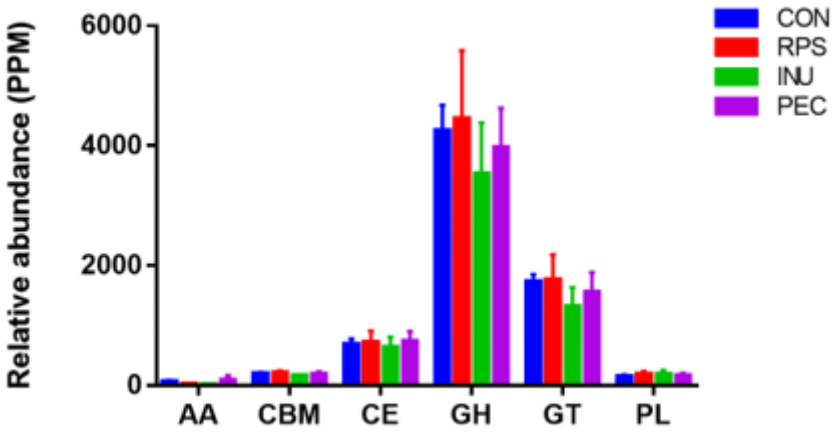


Figure 4

The distribution of CAZyme at the class level in each group. AA, auxiliary activities; CBM, carbohydrate-binding modules; CE, carbohydrate esterases; GH, glycoside hydrolases; GT, glycosyl transferases; PL, polysaccharide lyases. CON, a control diet; RPS, a raw potato starch enriched diet; INU, an inulin enriched diet; PEC, a pectin enriched diet.

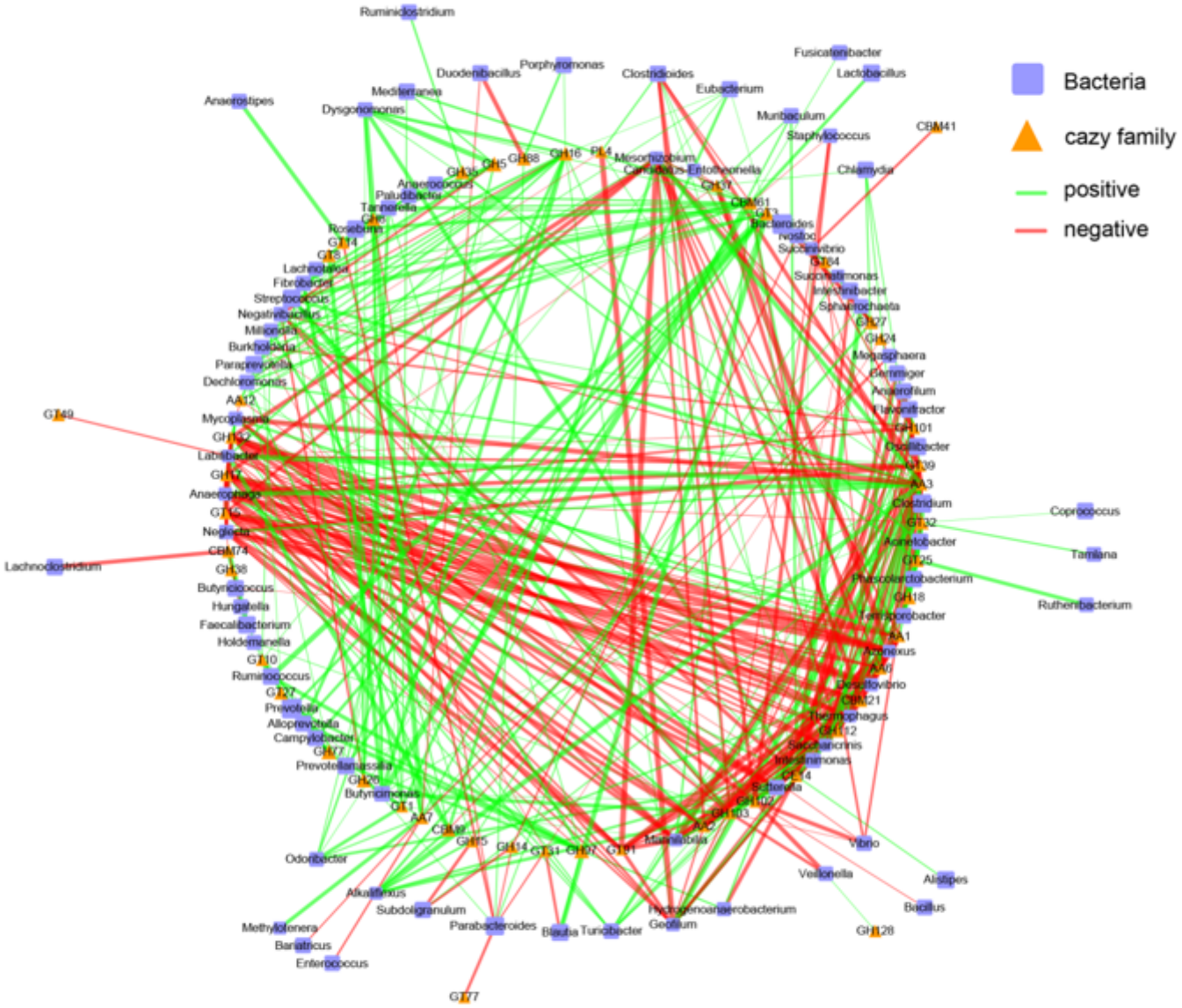


Figure 5

The network of correlations between bacteria and CAZyme families changed significantly by the dietary fiber. Green lines represent positive correlations and red lines represent negative correlations.

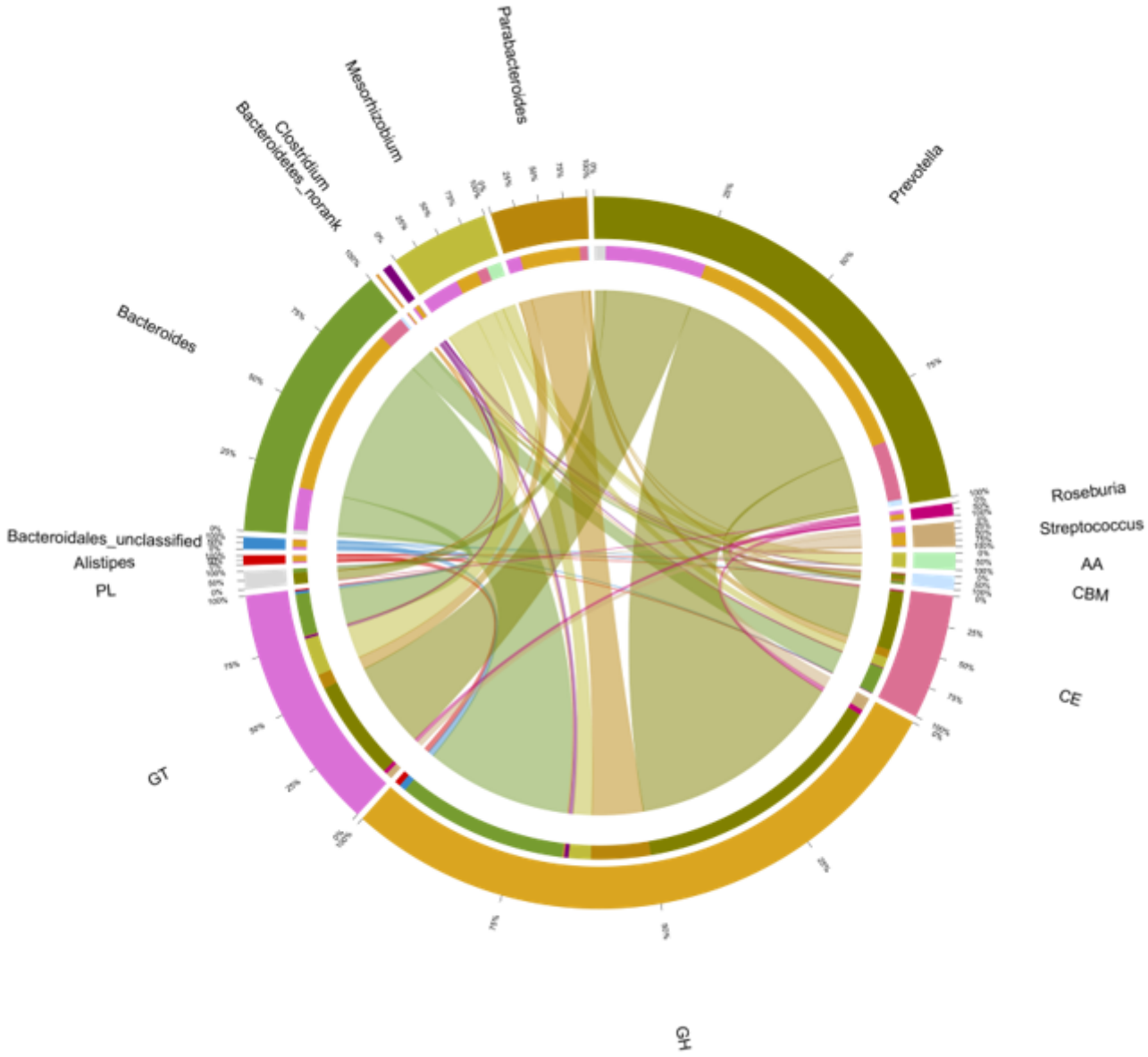


Figure 6

Taxonomic distribution (at genus level) of predicted CAZymes identifed from the metatranscriptomes in PEC group. CAZyme families and the corresponding genus are shown on the above and below sides, respectively. The inner ring designates the total number of unigenes encoding a given CAZyme class (below) and the total number of CAZymes associated with the given genus, the outermost ring designates the relative abundance of a given CAZyme family (below) and the relative abundance of unigenes from a given genus (above).The width of the bars between a given genus and a given CAZyme family indicates their relative abundance compared to that in the other genera.

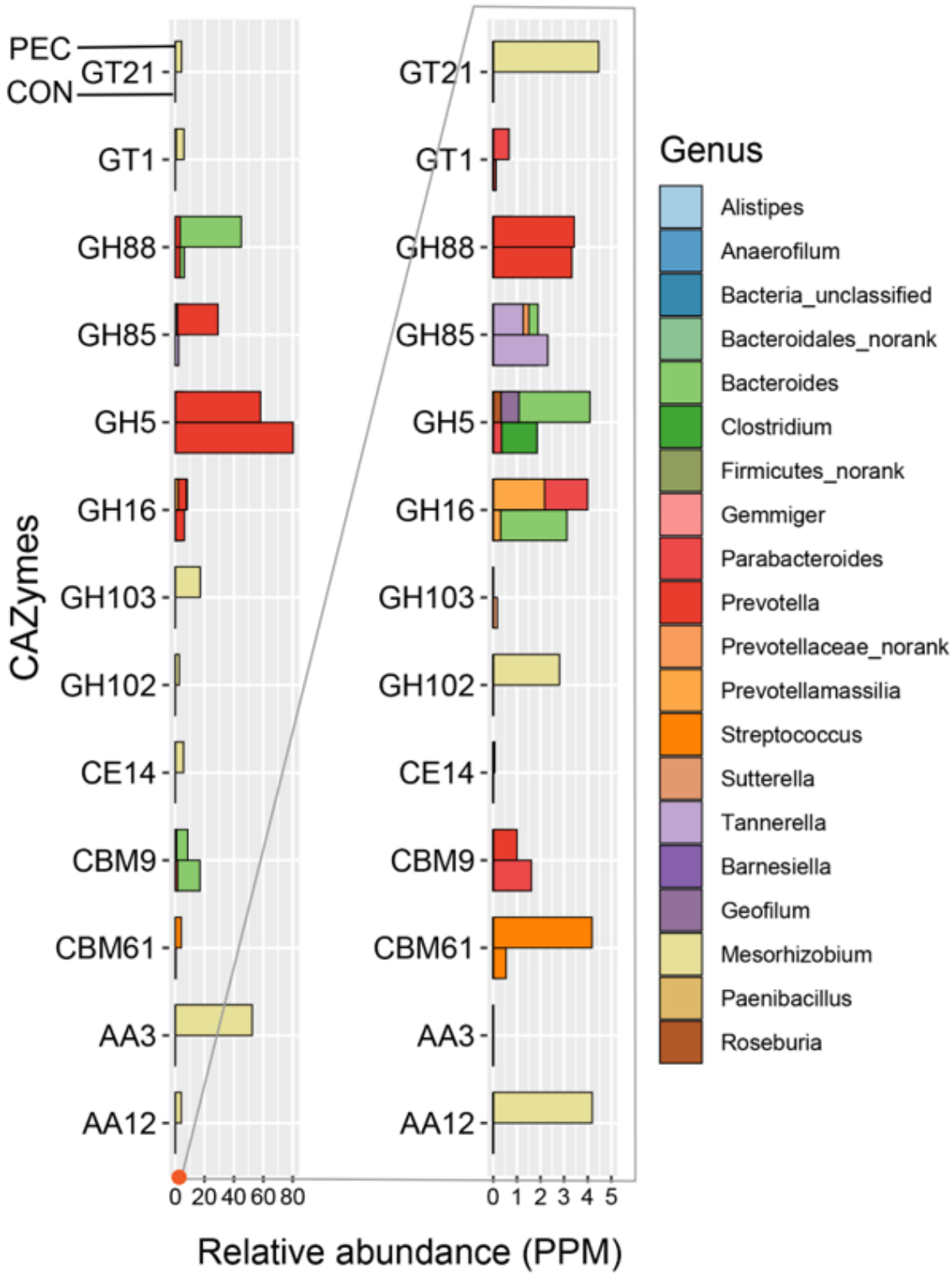


Figure 7

Contributions of CAZymes from the major microbial communities in growing pigs colons (PEC vs. CON). Graphs show the abundance of top 15 genera that are the major contributors of CAZymes to the growing pig colon ecosystem. AA, auxiliary activities; CBM, carbohydrate-binding modules; CE, carbohydrate esterases; GH, glycoside hydrolases; GT, glycosyl transferases. PEC, a pectin enriched diet; CON, a control diet.

Supplementary Files

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