

Clostridium butyricum and carbohydrate active enzymes contribute to the reduced fat deposition in pigs

Lingyan Ma¹, Shiyu Tao², Tongxing Song², Wentao Lyu¹, Ying Li³, Wen Wang¹,
Qicheng Shen¹, Yan Ni⁴, Jiang Zhu¹, Jiangchao Zhao⁵, Hua Yang¹, Yingping Xiao¹

¹Zhejiang Academy of Agricultural Sciences

²Huazhong Agricultural University

³Foshan University

⁴National Clinical Research Center for Child Health

⁵University of Arkansas

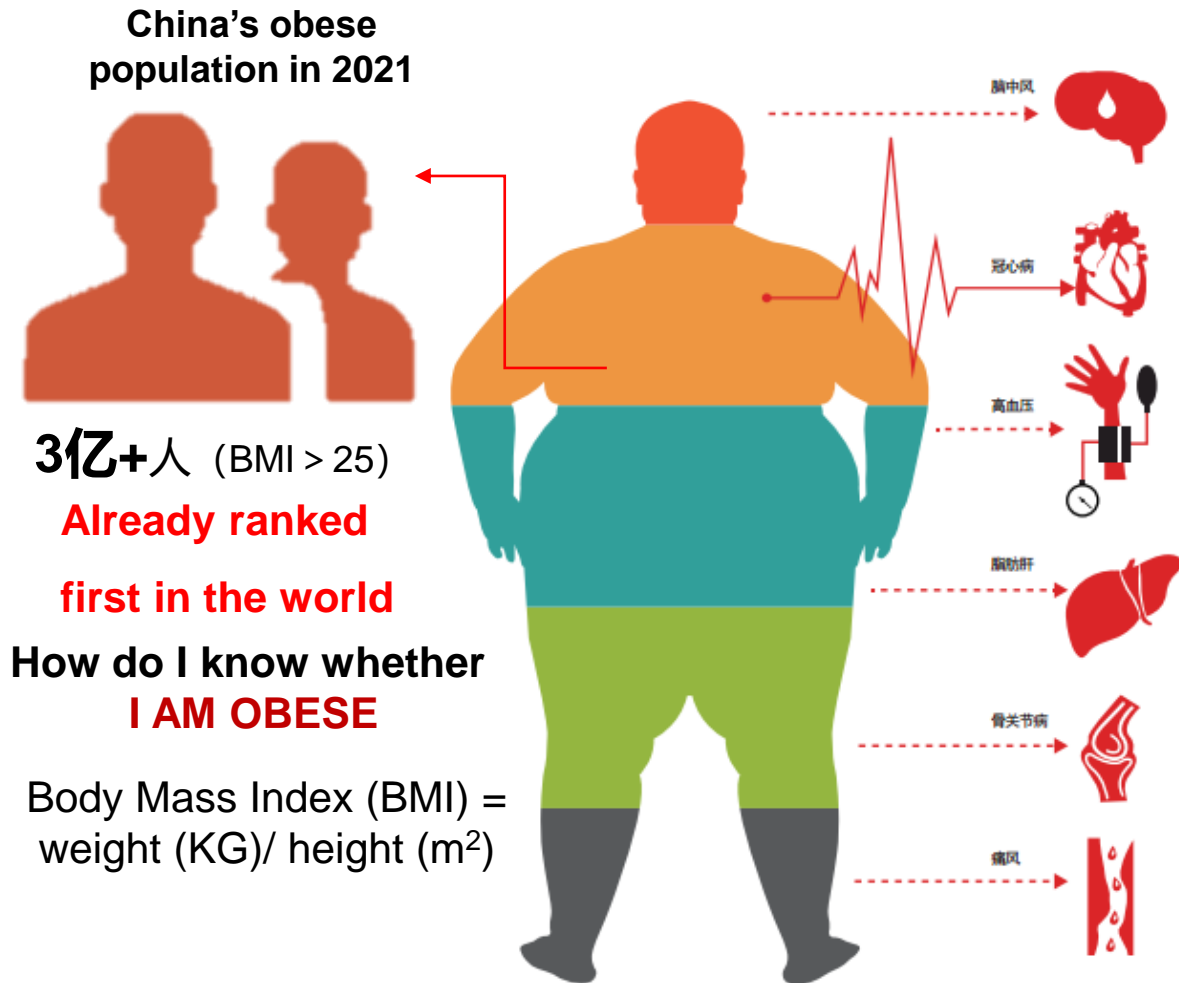


Ma, Lingyan, Shiyu Tao, Tongxing Song, Wentao Lyu, Ying Li, Wen Wang, Qicheng Shen, et al. 2023. “*Clostridium butyricum* and Carbohydrate Active Enzymes Contribute to the Reduced Fat Deposition in Pigs.” iMeta e160. <https://doi.org/10.1002/imt2.160>

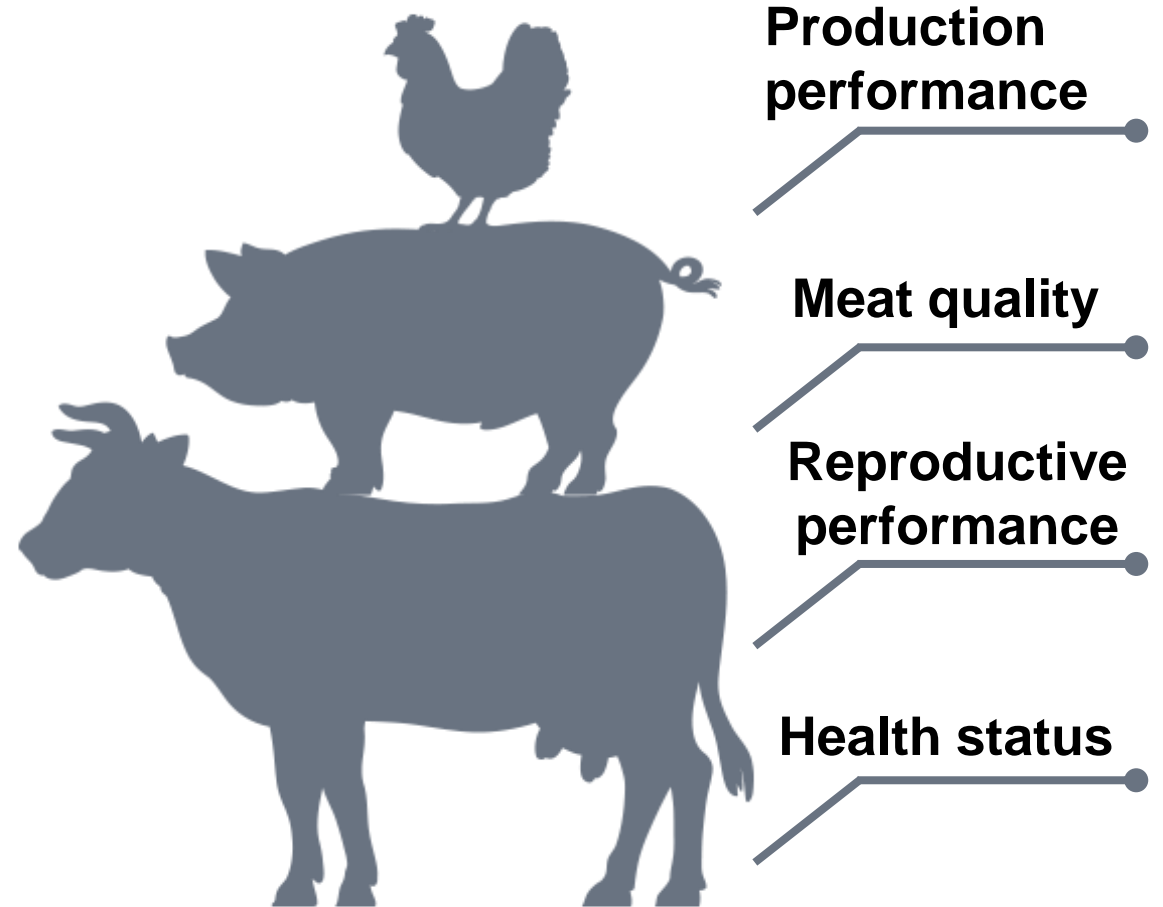


Introduction

Abnormal or excess fat accumulation that can harm health - **obesity/overweight**



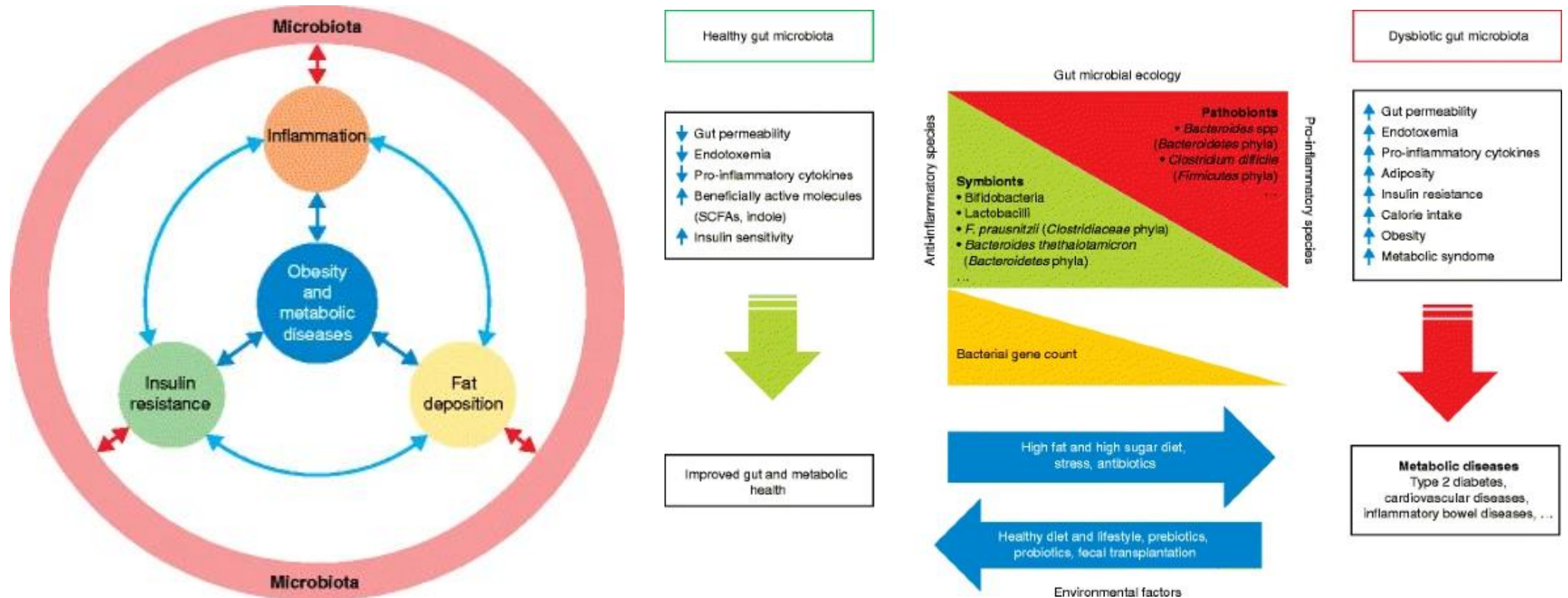
Fat deposition in livestock and poultry



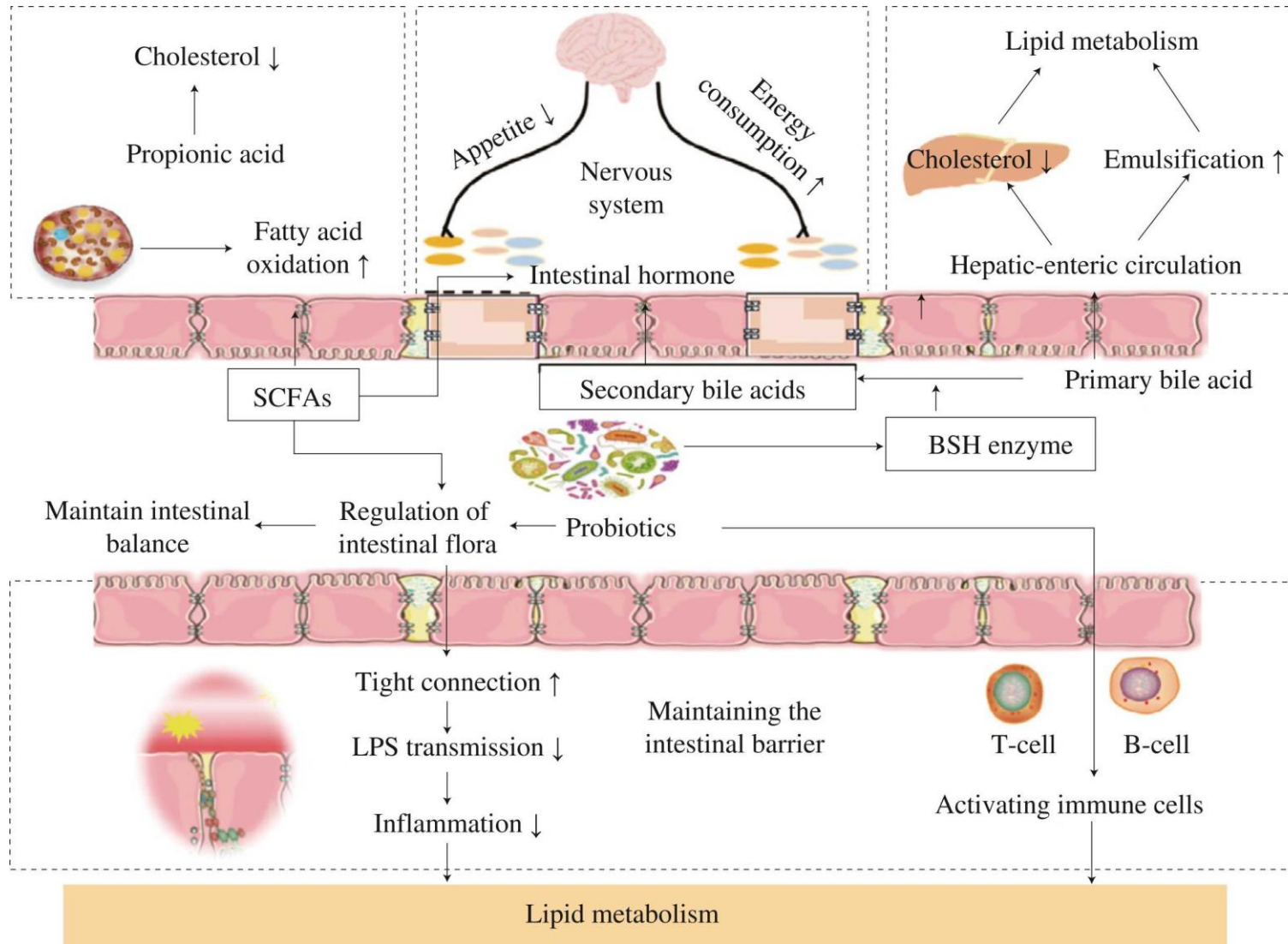
Introduction

Intestinal microbiota is closely related to host energy balance and metabolism.

Intestinal microbiota metabolites can serve as important regulators of lipid metabolism and participate in various aspects of lipid synthesis, transportation, storage and consumption through different pathways, and thus plays a key role in the regulation of lipid accumulation



Introduction



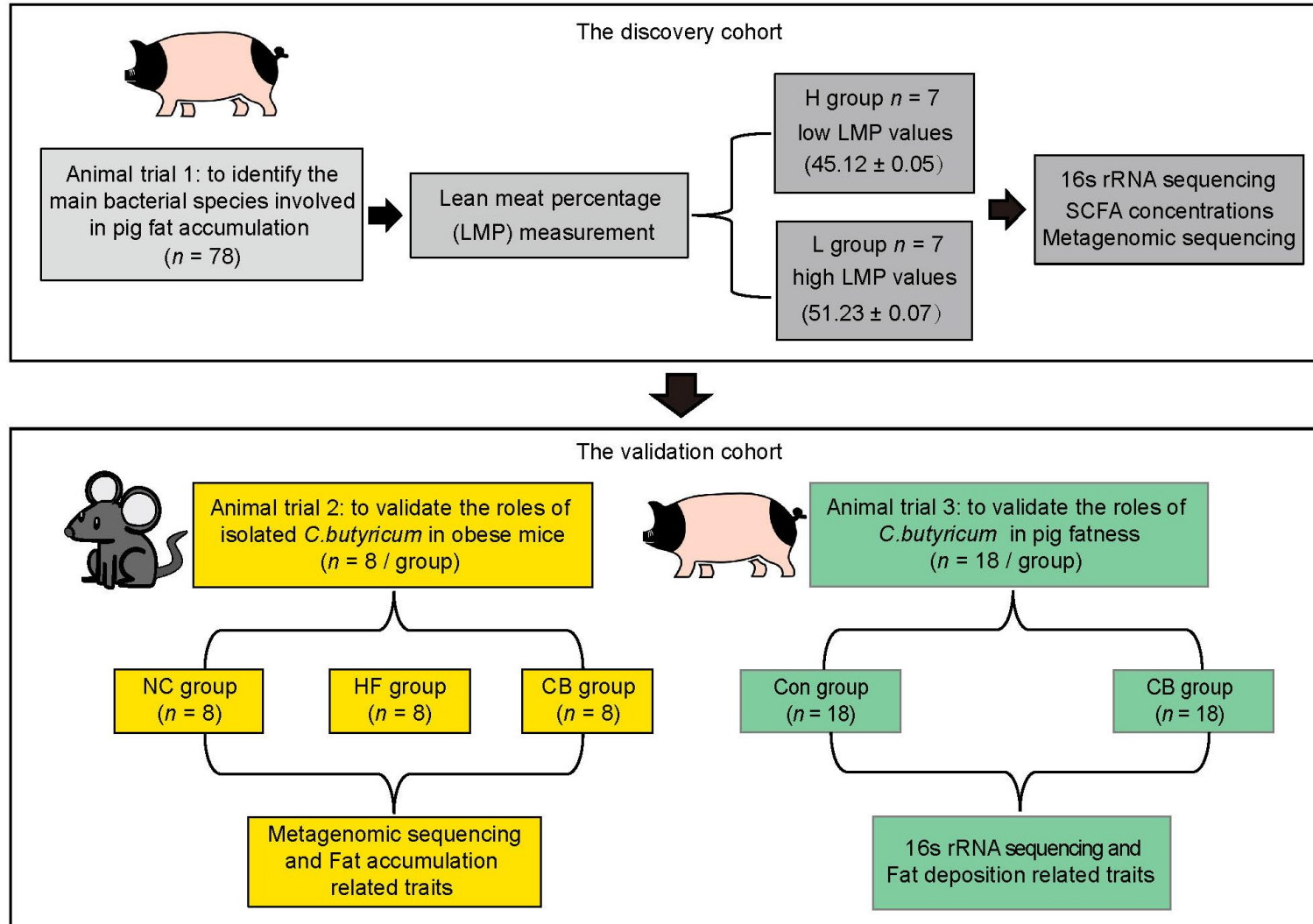
Ways of intestinal probiotics to regulate lipid metabolism:

- ① Probiotics can lower cholesterol by producing metabolites, mainly **short-chain fatty acids** and secondary bile acids;
- ② Metabolites can also stimulate the intestinal tract to secrete intestinal hormones and regulate lipid metabolism through the nervous system;
- ③ the inhibitory effect of probiotics on inflammation also plays an important role in regulating lipid metabolism.



Results

□ Experimental animals and study design



Three animal trails were included in this study:

Animal trail 1:

to explore the correlation between the gut microbiome and pig fatness;

Animal trail 2:

to validate the roles of isolated *C. butyricum* in fat accumulation in high fat (HF)-diet-induced obese mice;

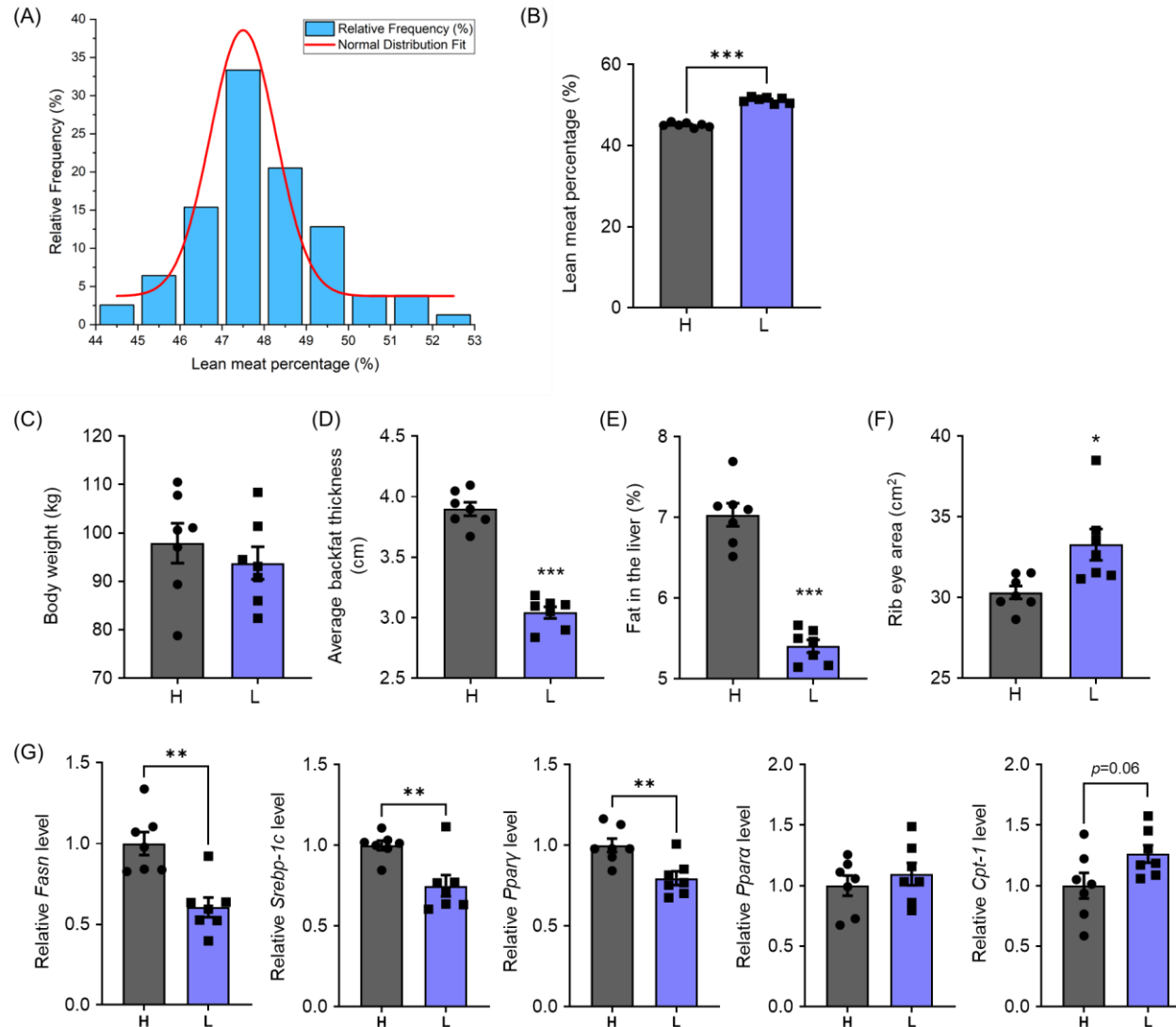
Animal trail 3:

to validate roles of *C. butyricum* in pig fatness.



Results

□ The fatness phenotypes of pigs



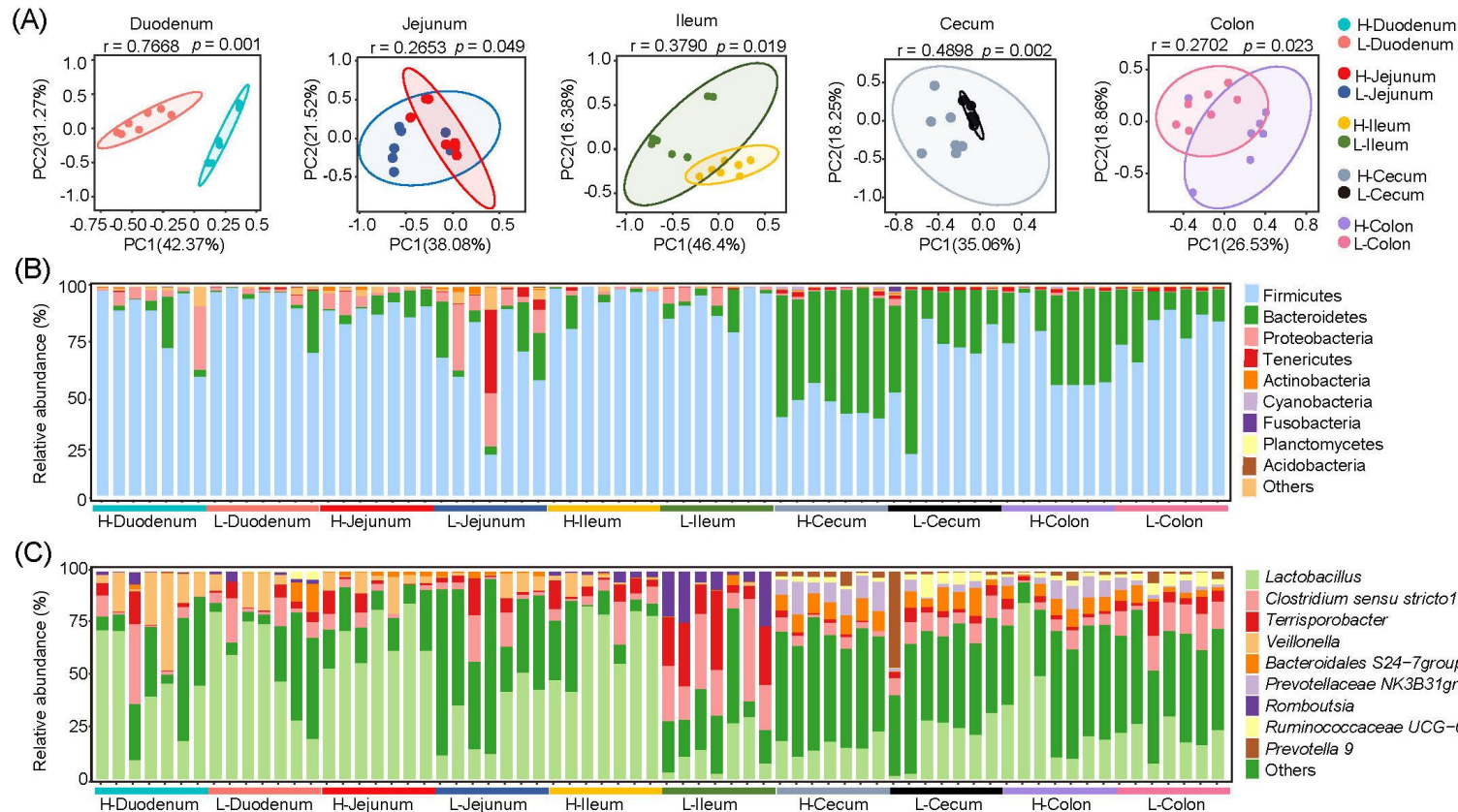
We used the lean meat percentage (LMP) as an index to divide 14 pigs into high (H) and low (L) fatness groups:

- No significant differences in body weight were found between the groups
- pigs in the L group had an almost 21% lower backfat thickness
- the thickness of the ribeye area in low-fatness pigs was significantly higher, with an average increase of 9.7%



Results

□ The gut microbiota composition along the gastrointestinal tract of pigs



- PCA results showed significant differences in bacterial communities;
- **Phylum levels:**
 - In the duodenum, jejunum, and ileum, the phyla Firmicutes, Proteobacteria, and Bacteroidetes were dominant, making up more than 70%, 10%, and approximately 10% of the microbiota;
 - In the colon and cecum, Firmicutes and Bacteroidetes were the most abundant phyla, with a combined average abundance of over 90%;

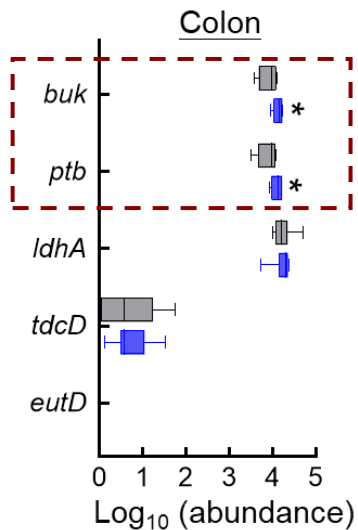
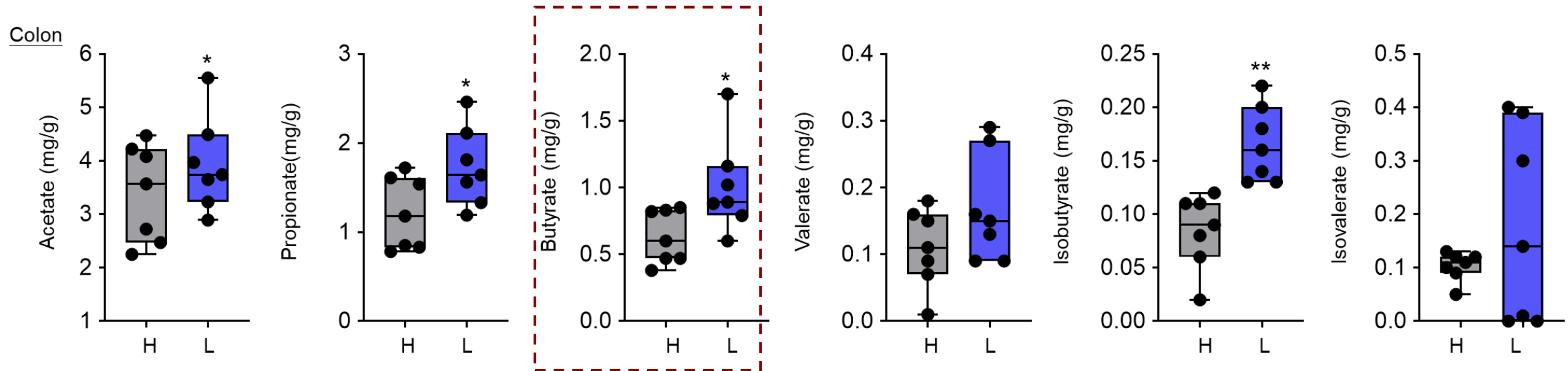
Genus levels:

- At the genus level, *Lactobacillus*, *Veillonella*, and *Terrisporobacter* were more abundant in areas of the small intestine, such as the duodenum and jejunum, while *Bacteroidales S24-7* group and *Prevotella 9* were more enriched in the colon and cecum sections



Results

□ Association of gut microbiota and SCFAs with fatness in pigs



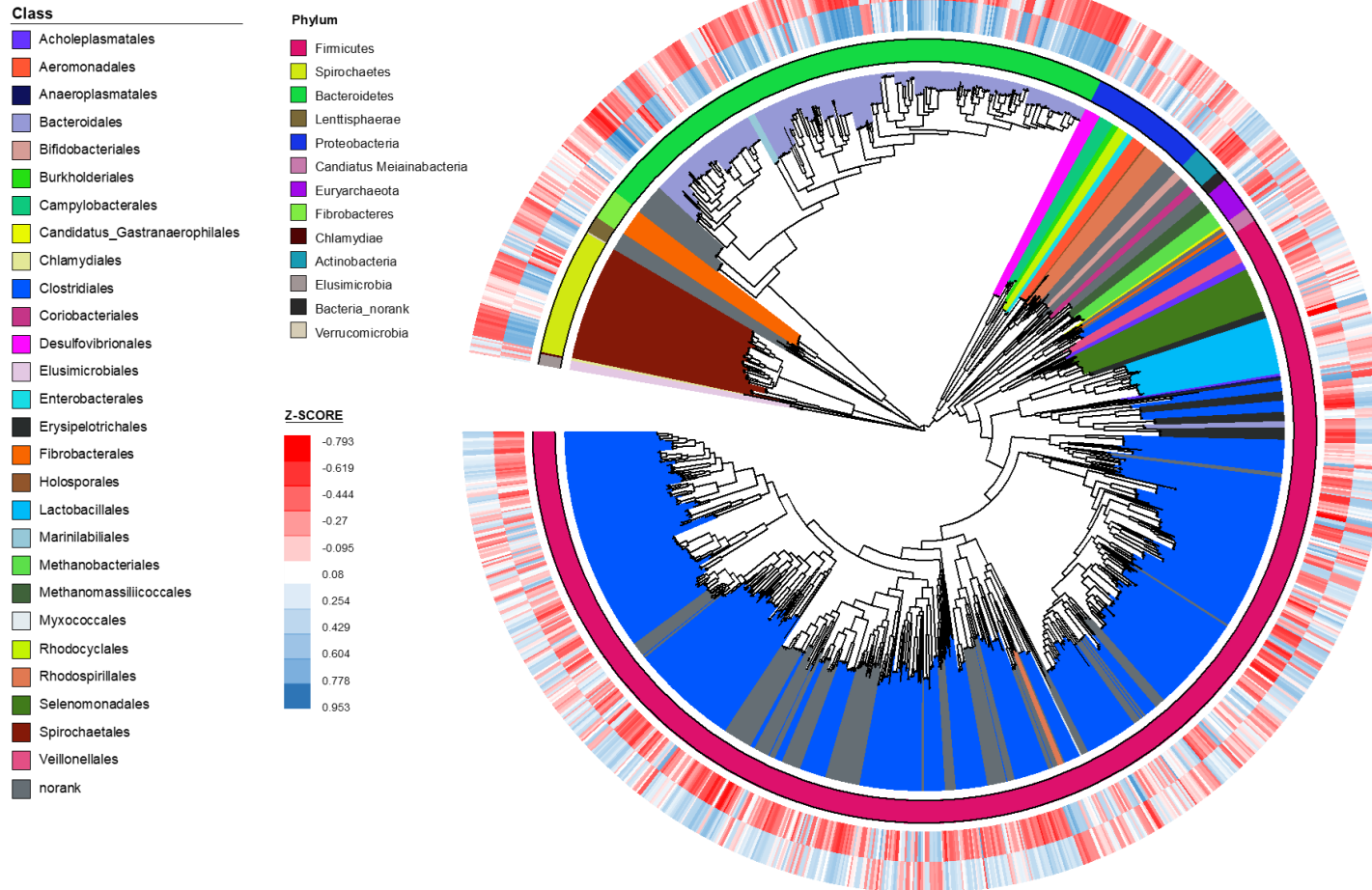
SCFAs levels:

- Greater concentrations of acetate, propionate, butyrate, and isobutyrate were found in the colon of the L group;
- **A notable difference emerged in the levels of *buk* and *ptb* in the L group, particularly in the colon**



Results

□ Metagenome-assembled bacterial genomes



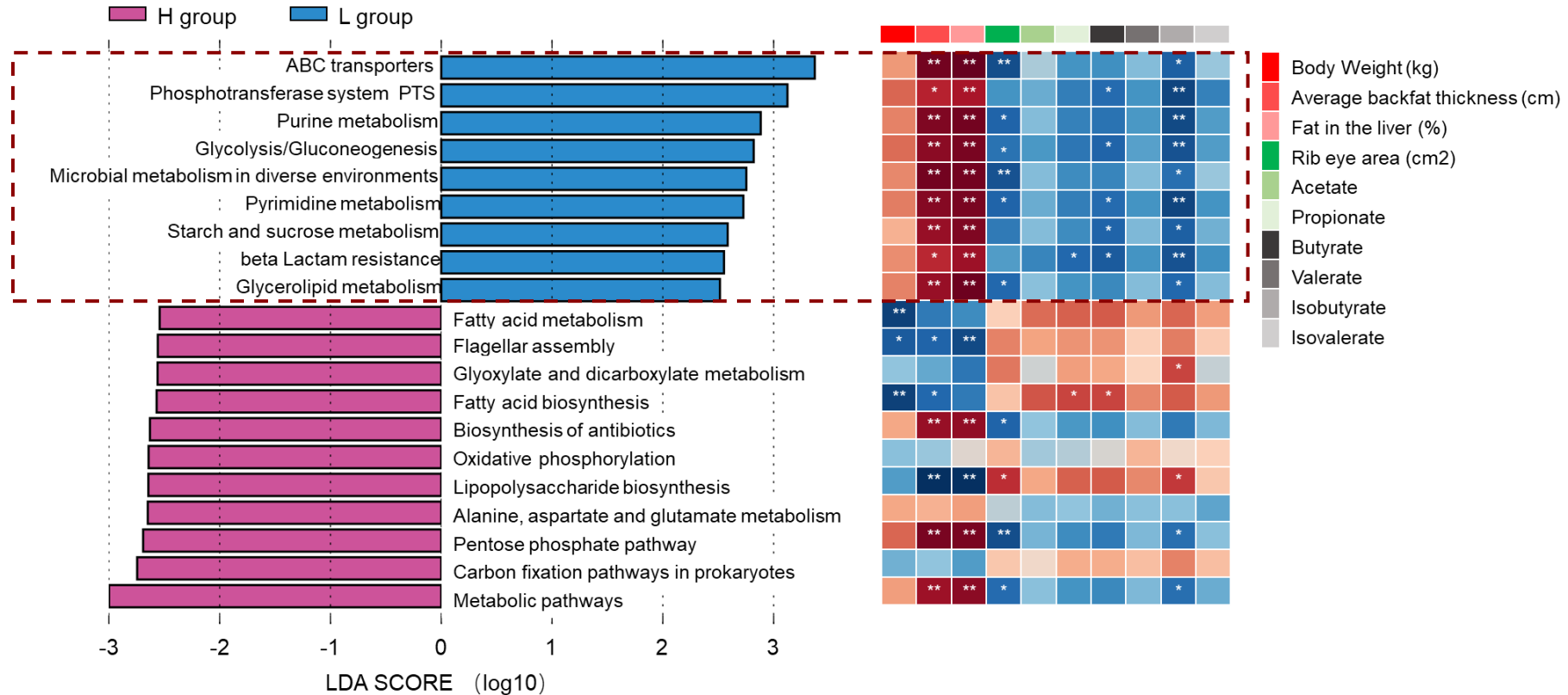
Reconstruction of bacterial genomes from metagenomic sequence data generated a total of 1,288 MAGs:

- 782 MAGs were identified as Firmicutes, 291 as Bacteroidetes, and 68 as Spirochaetes. Furthermore, 64 MAGs were identified as Proteobacteria, 18 as Euryarchaeota, and only 16 MAGs were assigned to Actinobacteria;
- **The MAGs enriched in the L group were mostly assigned to Firmicutes; conversely, the MAGs identified as Bacteroidetes were more abundant in the H group**



Results

Functional profiling of the gut microbiome related to fatness based on metagenomic sequencing



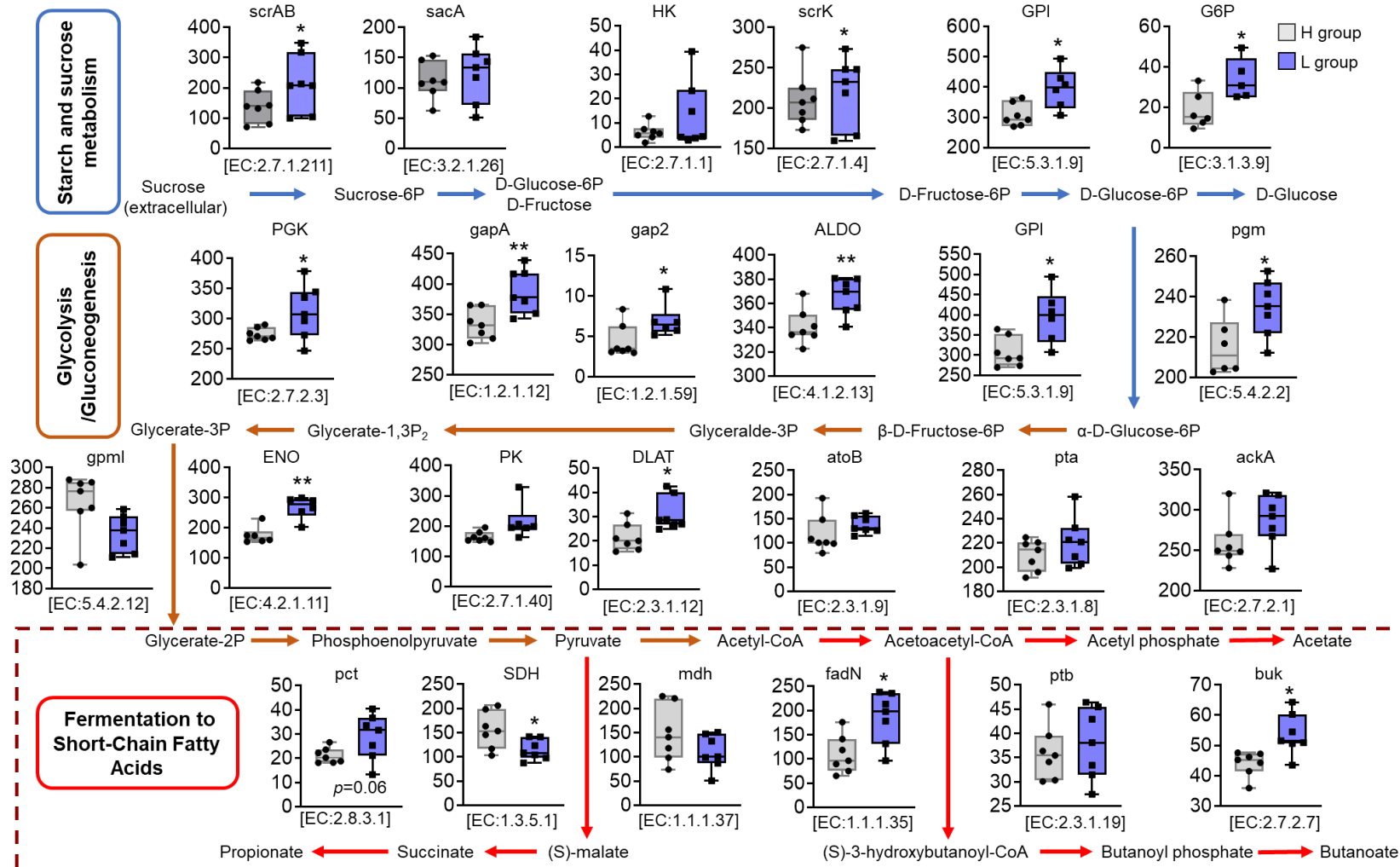
KEGG levels:

- Those L group-enriched pathways were negatively correlated with body weight, average backfat thickness, and liver fat but positively associated with rib eye area and SCFA levels;



Results

Functional profiling of the gut microbiome related to fatness based on metagenomic sequencing

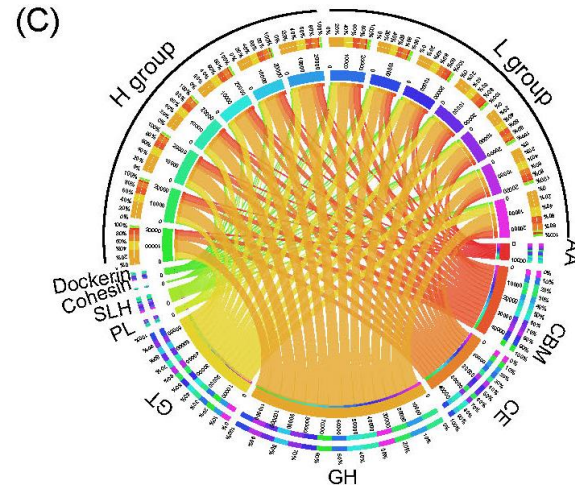
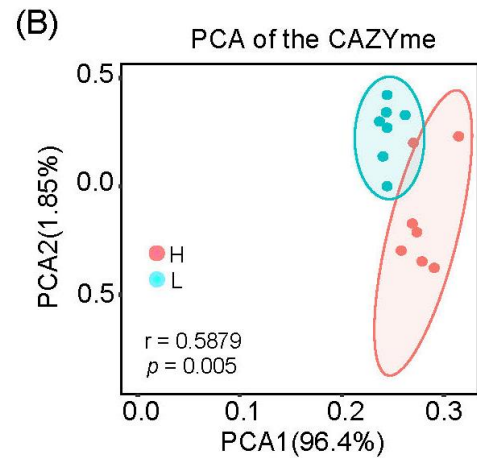
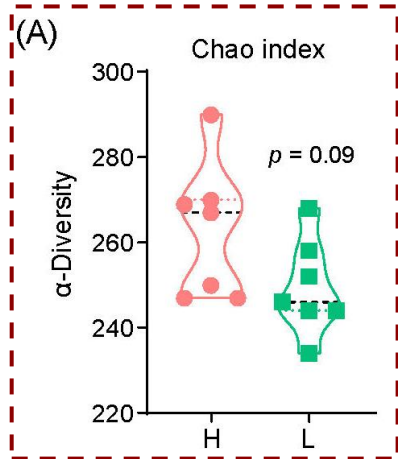


- The metagenome sequencing data indicated a higher abundance of these genes in the L group:
- including the **fermentation pathway responsible for metabolizing sucrose into acetate, butyrate, and propionate**

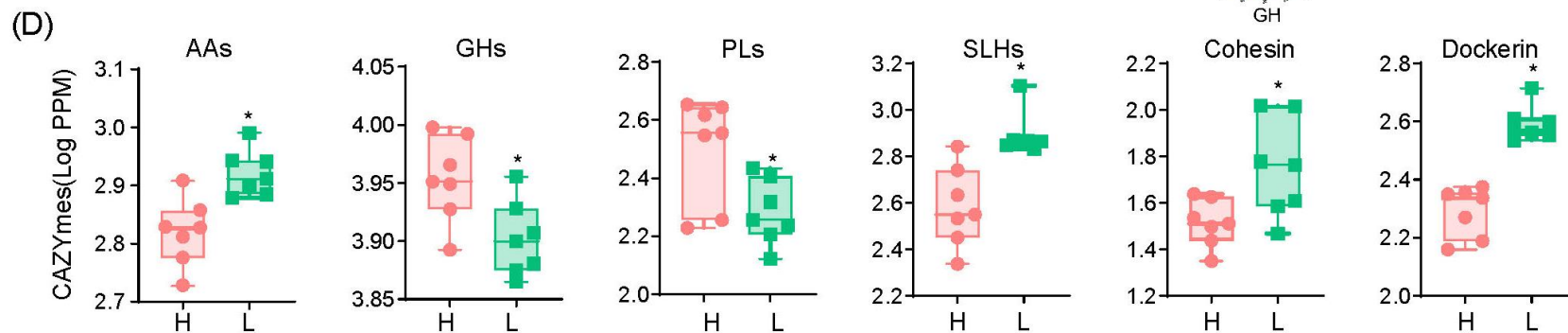


Results

Comparisons of the CAZymes genes encoded by the gut microbiome in pigs

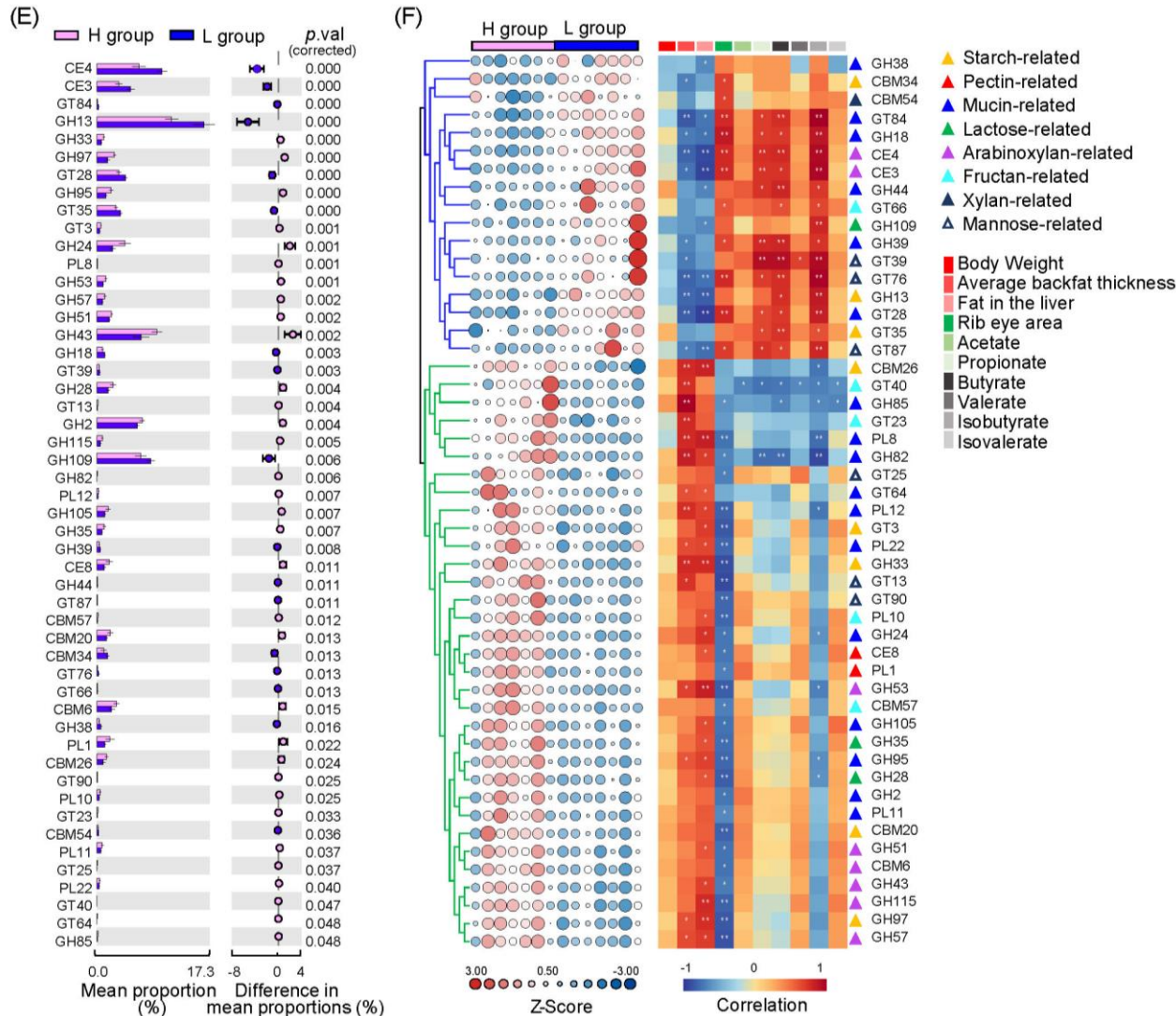


- The L group exhibited a **lower diversity** of CAZymes than the H;
- PCA further highlighted a **significant difference** in CAZYme profiles between the two ;
- The L group displayed significantly lower abundances of genes encoding GHs and PLs, while exhibiting higher levels of AAs, SLHs, cohesin, and dockerin



Results

□ The changes of CAZymes linked to SCFA production and fatness traits in pigs

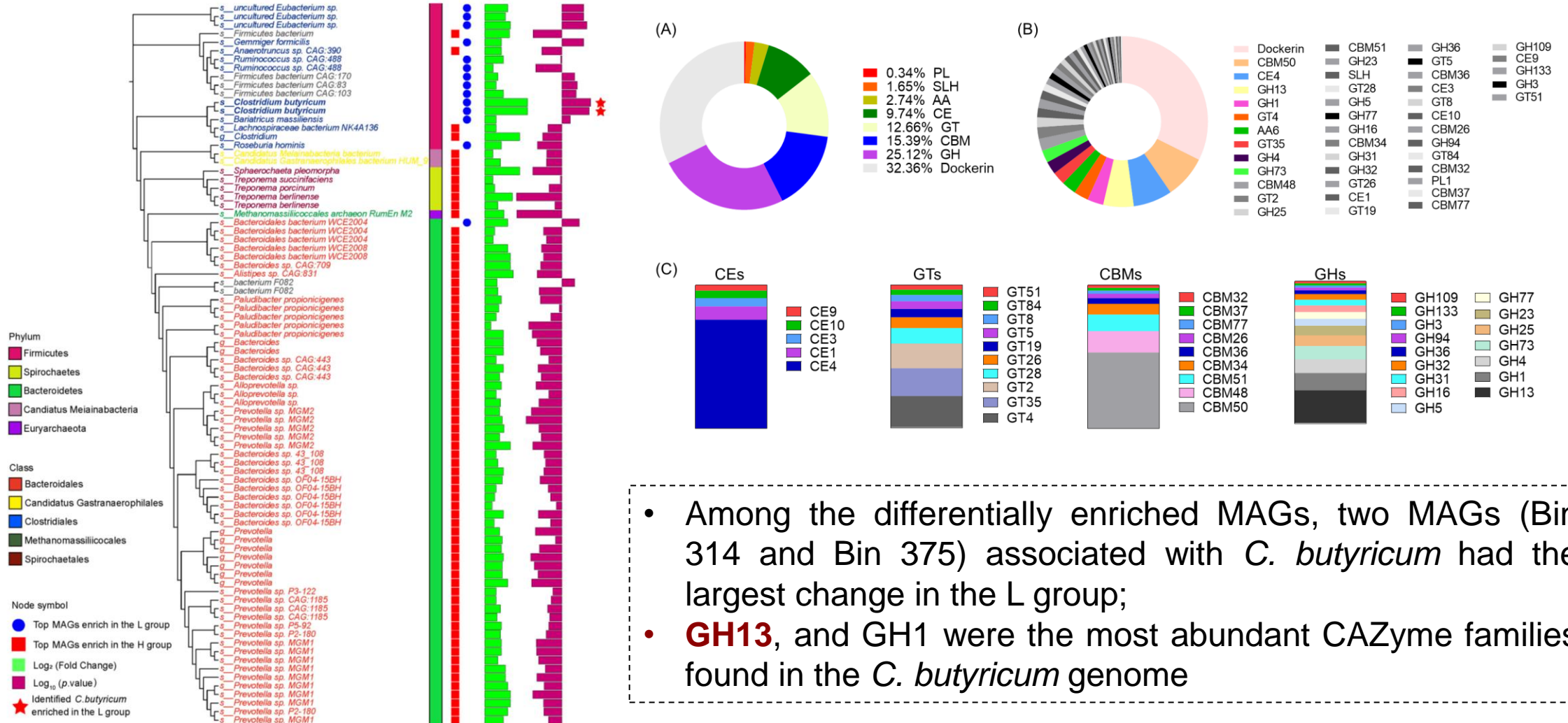


- The CAZymes enriched in the L group showed a positive correlation with SCFA production and ribeye area while exhibiting a negative correlation with average backfat thickness and liver fat;
- **These findings strongly suggest that microbiota-mediated CAZymes play a crucial role in regulating SCFA productions and host phenotypes.**



Results

Identifying a significant change of *C. butyricum* in pigs with low fatness and its association with CAZymes

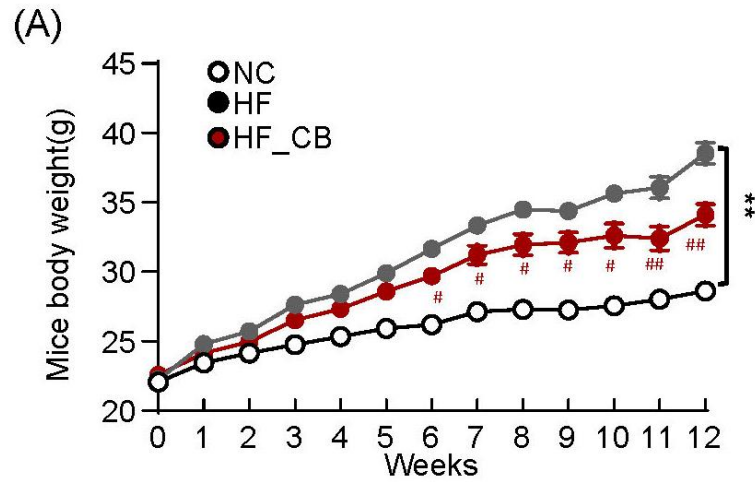


- Among the differentially enriched MAGs, two MAGs (Bin 314 and Bin 375) associated with *C. butyricum* had the largest change in the L group;
- **GH13**, and GH1 were the most abundant CAZyme families found in the *C. butyricum* genome

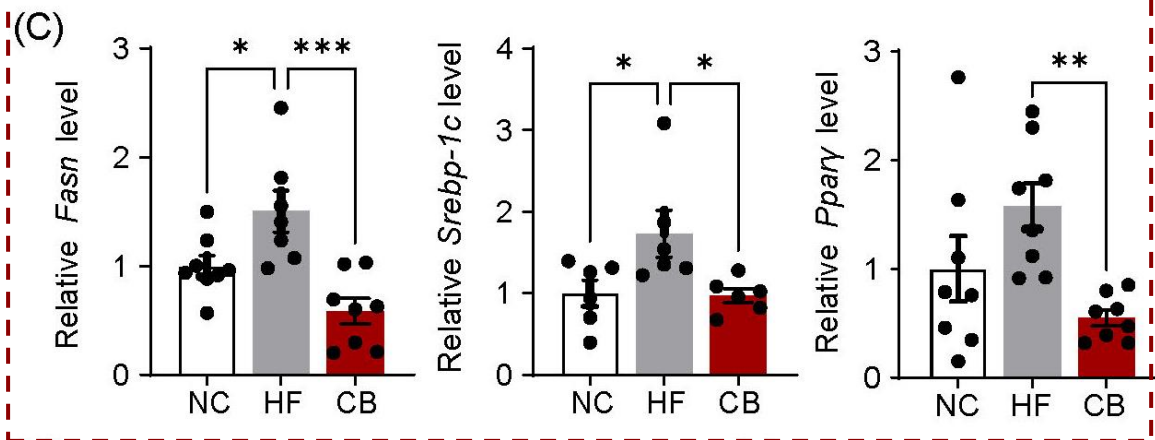
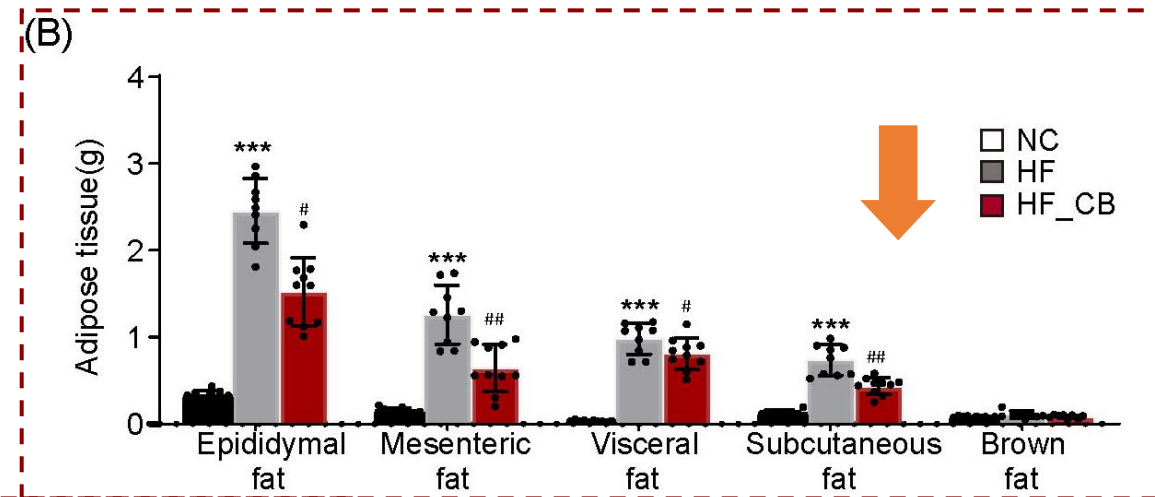


Results

Isolated *C. butyricum* alleviated fat accumulation in obese mice



significantly decreased body fat accumulation



lipolysis

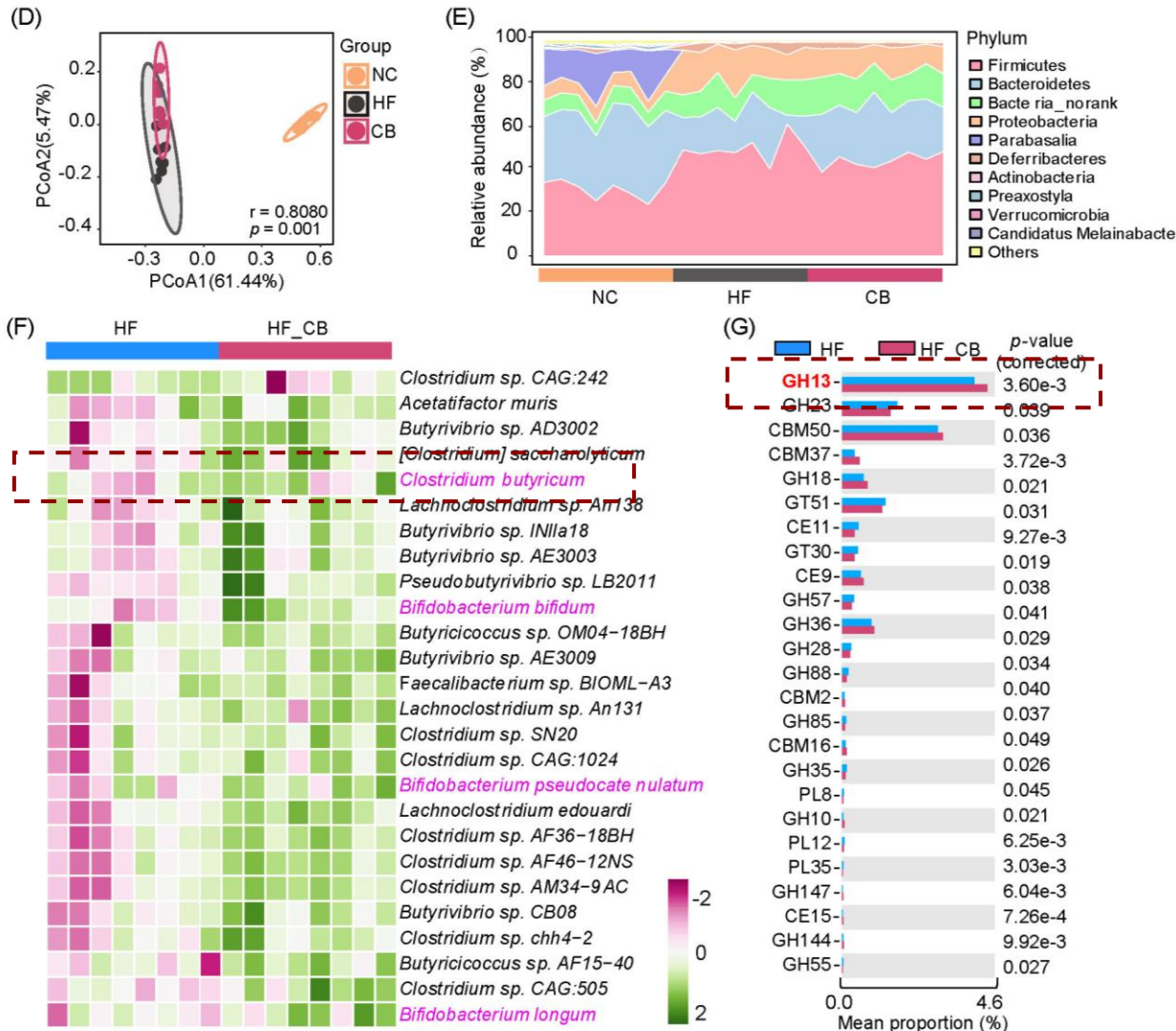


lipogenesis



Results

□ Isolated *C. butyricum* increased the enrichment of GH13 in obese mice

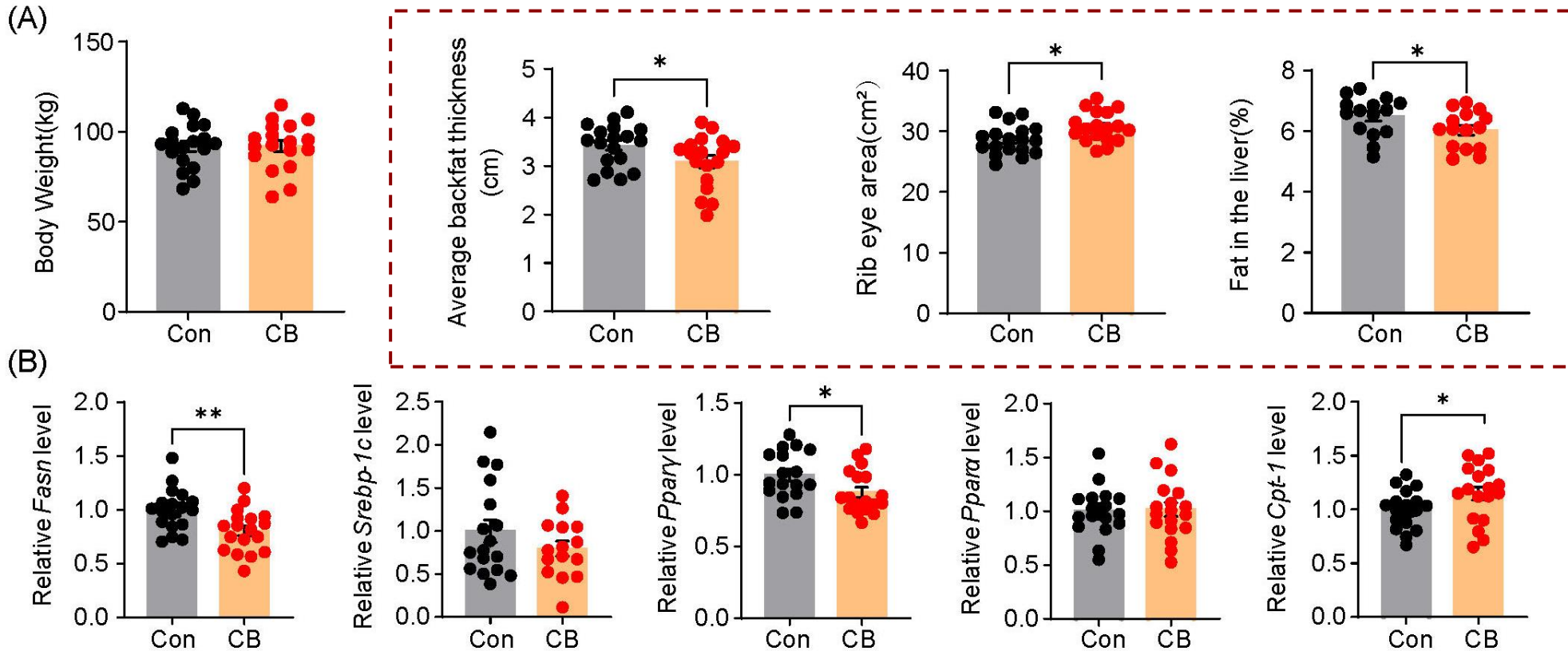


- β -diversity value could effectively differentiate between lean mice fed a normal chow diet and obese mice fed a high-fat diet;
- At the phylum level, the overall composition of the gut microbiota revealed an increase in Firmicutes and a decrease in Bacteroidetes in high-fat diet-induced obese mice, which were restored by *C. butyricum* treatment;
- **Importantly, the abundance of *C. butyricum* increased after *C. butyricum* treatment.**



Results

□ *Clostridium butyricum* treatment alleviated fat accumulation in Jinhua pigs

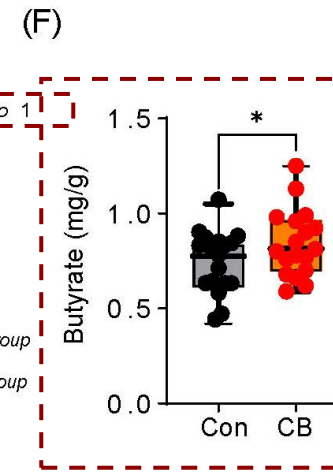
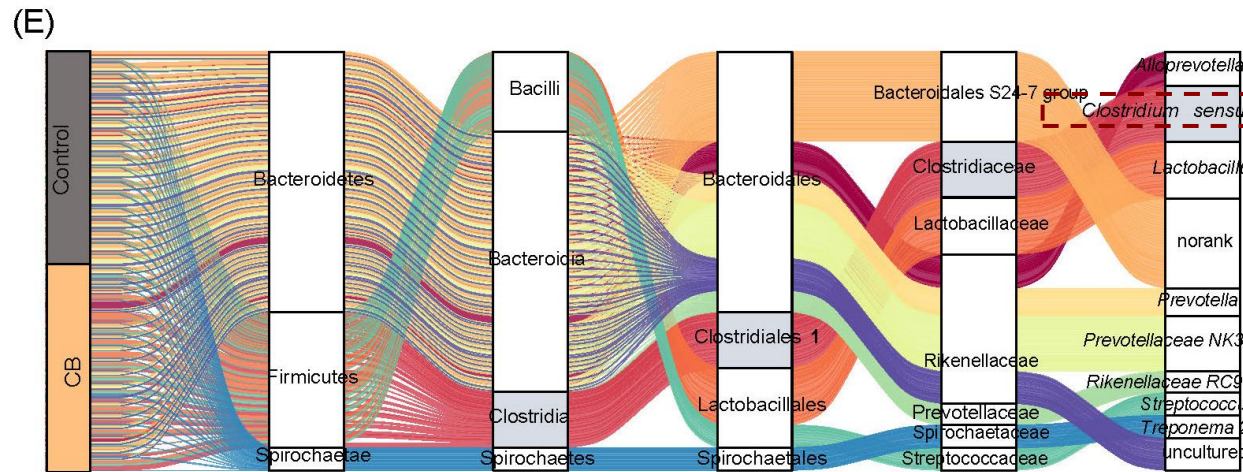
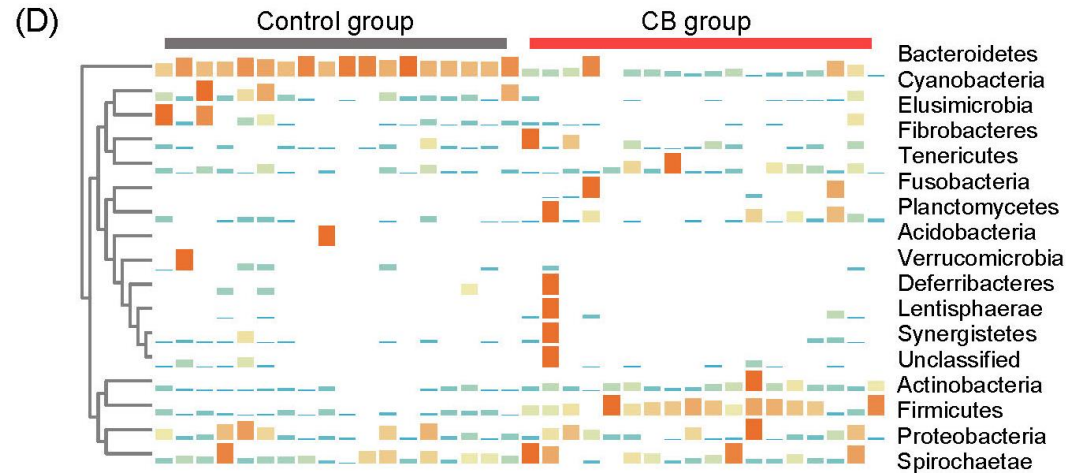
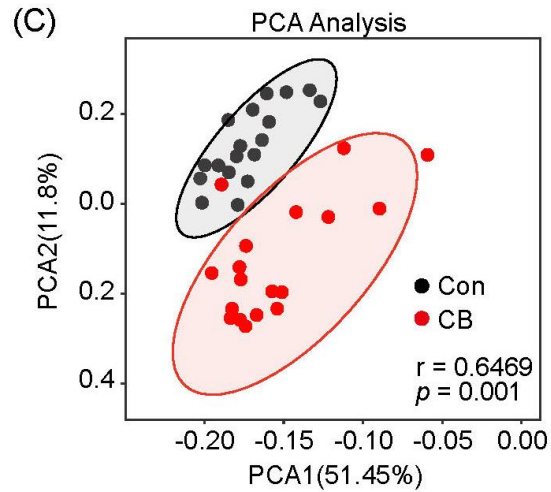


- Compared with the control group, pigs fed *C. butyricum* had a significant increase in ribeye area and lower average backfat thickness and liver fat.
- RT-PCR results further showed that *Fasn* and *Pparγ* were significantly increased with *C. butyricum* treatment, while the mRNA level of *Cpt-1* was downregulated in the CB group



Results

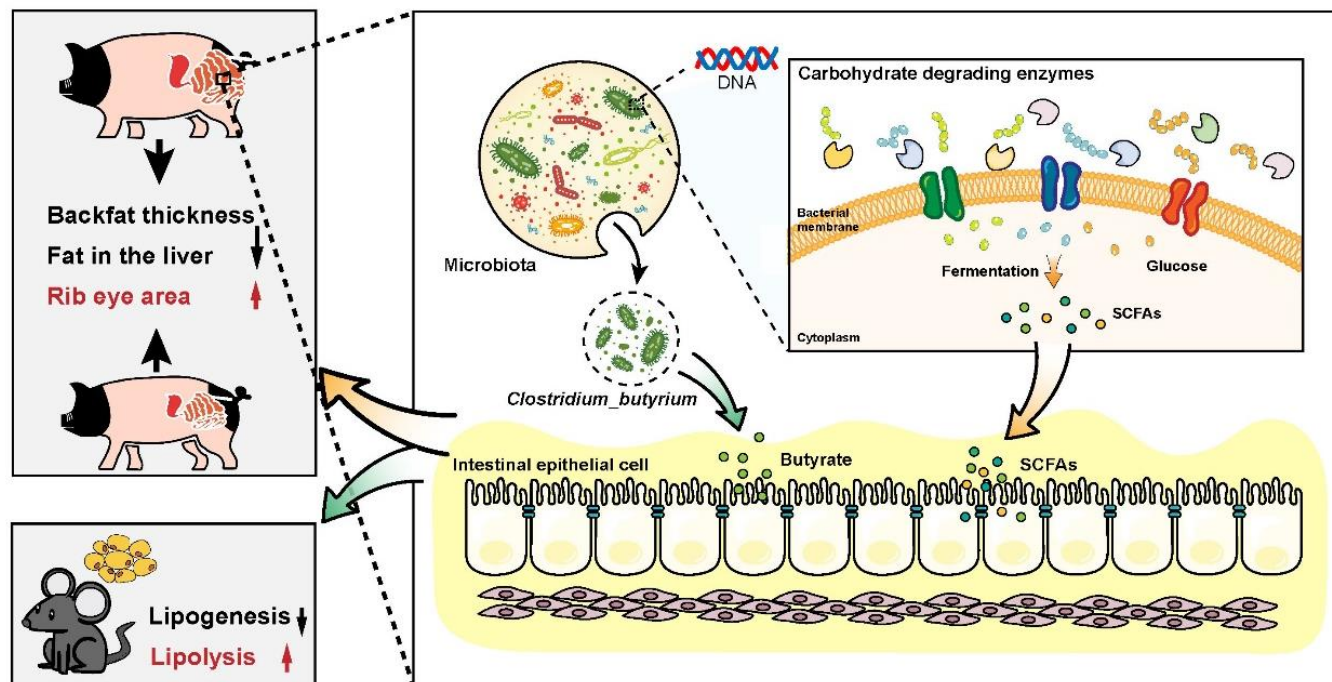
□ *Clostridium butyricum* treatment alleviated fat accumulation in Jinhua pigs



- PCA plot showed a clear separation of the gut microbiota between the control and CB groups;
- Importantly, the abundance of *Clostridium sensu stricto* 1 was significantly increased after *C. butyricum* treatments, which was similar to the trends in the lower-fatness Jinhua pigs;



Summary



- Significant differences in microbial composition and potential functional capacity among different gut locations were found in Jinhua pigs with distinct fatness phenotypes.
- Jinhua pigs with lower fatness exhibited higher levels of short-chain fatty acids in the colon, highlighting their enhanced carbohydrate fermentation capacity.
- *Clostridium butyricum* might be a representative bacterial species from Jinhua pigs with lower fatness, and a significantly higher percentage of its genome was dedicated to CAZyme glycoside hydrolase family 13 (GH13).
- Isolated *C. butyricum* alleviated fat accumulation and increased the enrichment of GH13 in obese mice.

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