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Metagenomic immunoglobulin sequencing reveals IgA coating Phyllobacterium myrsinacearum in gut contributing the high immunity of Honghe cattle

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Abstract

Background

The gastrointestinal microbiome plays a pivotal role in ruminants, and immunoglobulin A (IgA) coating bacteria directly provides the immune defence of hosts. The Honghe cattle (HC) are a breed native to Yunnan Province, China, exhibits remarkable environmental adaptability and disease resistance. However, research into its gut microbiota and IgA coating pattern remains at a nascent stage. In the present study, faecal samples from HC and commercial Simmental cattle (SC) that were subjected to identical grazing conditions were collected and subjected to a novel metagenomic immunoglobulin sequencing technique.

Results

This approach was employed to conduct a simultaneous investigation of the native (overall) and IgA-coated gut microbiota. Despite the absence of a statistically significant disparity in alpha-diversity between the HC and SC groups, beta-diversity analysis revealed a conspicuous separation in the composition of the gut microbiota. The native microbiota of both breeds was dominated by Bacillota and Bacteroidota, which were significantly more abundant in HC and SC, respectively. These compositional patterns were mirrored in the IgA coating gut microbiota. Furthermore, analysis revealed that multiple genes involved in the biosynthesis of SCFAs and innate immune-related metabolites were significantly enriched in the HC native microbiota. The utilisation of PHI-base for the annotation of pathogens has led to the identification of elevated levels of total pathogen abundance, as well as certain prominent pathogens, in SC when compared to HC. Moreover, serum immune indicators were found to be significantly higher in HC than in SC. By calculating and comparing the IgA coating levels for each stain recognised from the cattle gut microbiota, two strains, respectively belonging to *Phyllobacterium myrsinacearum* and *Arcanobacterium*, stood out with the highest IgA coating levels (193 and 182, respectively) and were significantly more highly coated in HC than SC. As indicated by the correlation analyses, a positive correlation was identified between these two strains and serum immune indicators, while a negative correlation was identified between the abundances of multiple major gut pathogens.

Conclusions

This study reveals distinct gut microbial and immunological characteristics between HC and commercial SC. The HC exhibits superior energy utilization, stronger mucosal immune function and lower intestinal pathogen load. Two core IgA-coated bacterial strains are closely linked to host immune homeostasis and pathogen inhibition, which suggest that high IgA-coated key strains may act as central nodes in the microbe–immune network, potentially contributing to enhanced humoral immunity and pathogen control and provides valuable microbial resources for the development of livestock probiotics and immune regulators.

1. Introduction

Within the mucosal immune system, secretory immunoglobulin A (IgA) is the most abundant antibody isotype in the intestinal lumen [1]. By selectively coating bacterial cells, IgA limits epithelial adherence and translocation of potential pathogens, while concomitantly promoting colonization and retention of beneficial commensals [2]. The dual function of this process is central to the concepts of "immune exclusion" and "immune inclusion", through which the host maintains a finely tuned balance between immune defence and tolerance at the mucosal surface [3]. Recent studies have demonstrated that the subset of IgA coating bacteria can vary significantly from the total microbiota and frequently provides a more direct indication of host–microbe interactions and immune selection [4]. In human models, IgA-coating patterns have been associated with disease states, metabolic phenotypes, and responsiveness to environmental challenges. This suggests that IgA-coated microbiota constitute a functionally critical and immunologically informative fraction of the gut ecosystem [5]. While IgA shapes the gut microbiota through selective microbial binding, conventional approaches to characterize IgA-microbe interactions are constrained by taxonomic resolution and throughput, 16S-based methods generally resolve bacteria only to the genus level and lack the genomic depth needed to investigate functional determinants of IgA binding, whereas sorting yields are often insufficient for shotgun metagenomic applications [6]. To overcome these limitations, we applied metagenomic immunoglobulin sequencing (MIG-Seq), an approach that combines magnetic-activated cell sorting of IgA-coated microbiota with high-throughput shotgun metagenomic sequencing [7]. MIG-Seq provides strain-level resolution of IgA coating and simultaneously retrieves comprehensive genomic information from both IgA-bound and unbound fractions, enabling systematic linking of coating status to functional gene content, replication rates, and host immunological parameters [8]. By delivering strain-specific resolution coupled with functional genomic insights into IgA binding, MIG-Seq reveals previously inaccessible layers of host-microbiota crosstalk that are essential for understanding how IgA selectively shapes intestinal microbial communities under both health and disease conditions [9]. Despite the rapid advances witnessed in the field of cattle microbiome research, the majority of studies conducted to date have focused on the composition of the gut or rumen microbiota as a whole [10–12]. In addition, there has been limited attention paid to the distal gut and its intricate relationship with mucosal immunity. A paucity of research has been conducted on the subject of comparative analyses of the native (overall) versus IgA coating gut microbiota in cattle.

The gastrointestinal microbiome plays a pivotal role in the nutrition, health, and immunity of ruminants, acting as a key interface between the host and its environment [13]. In the gastrointestinal tract of cattle, the fermentation of otherwise indigestible plant material by gut microorganisms results in a wide range of metabolites, including short-chain fatty acids (SCFAs) [14]. These SCFAs serve a dual role, functioning as energy sources and as signalling molecules that influence the health of the rumen and the intestines [15]. In particular, acetate, propionate, and butyrate have been demonstrated to regulate rumen barrier function and epithelial physiology [16]. Moderate increases in SCFAs have been demonstrated to support a healthier epimural microbiota and better epithelial integrity [17]. Beyond SCFAs, gut microbes synthesize a diverse range of innate immune-related metabolites, which collectively contribute to

shaping mucosal and systemic immune tone [18]. The microbial metabolism of dietary tryptophan has been demonstrated to generate indole-3-aldehyde, indole-3-propionic acid, and other aryl hydrocarbon receptor agonists [19]. These have been shown to upregulate antimicrobial peptides and strengthen mucosal defence.

Substantial disparities exist among different cattle breeds with regard to genetic background, production performance, and environmental adaptability [20]. Consequently, the composition and functional potential of their gut microbiota also display distinct breed-specific characteristics [21, 22]. The Honghe cattle (HC), a breed indigenous to the mountainous regions of Yunnan Province in southwestern China, have been sustained in grazing systems for an extended period. These animals are renowned for their robust constitution, environmental adaptability, and resistance to disease [23]. Conversely, Simmental cattle (SC) represent an introduced, high-production beef breed that is extensively utilised in commercial systems. It is postulated that their health and immune adaptation to local environments may deviate from that of indigenous breeds [24, 25]. As demonstrated in previous studies, the provision of housed feeding to ruminants has been shown to modify the composition of the rumen microbiota, enhance antioxidant status, and augment the expression of genes associated with rumen barrier function [26, 27]. However, research concerning the gut microbiota of HC remains inconclusive. Furthermore, in the context of the local HC breed, the extent to which its intestinal IgA coating exceeds that of introduced breeds remains to be elucidated. Additionally, it is imperative to ascertain which taxa are predominantly targeted by IgA and their contribution to the merits of this local breed.

In order to address these issues, a novel metagenomic immunoglobulin sequencing was applied to faeces of HC and SC to simultaneously investigate the native and IgA coating gut microbiota [7]. The specific aims of this study were to: The objectives of this study are threefold: firstly, to assess breed-specific differences in the genetic potential of cattle gut microbiota for SCFAs and innate immune factor biosynthesis; secondly, to compare the composition and diversity of native and IgA coating gut microbiota between HC and SC; and thirdly, to characterise the burden and composition of gut pathogens between HC and SC. Moreover, the key taxa with elevated IgA coating levels in HC were identified, and their associations with relative high immunity were explored. This study provides a comprehensive analysis of the differences between native and IgA-coated gut microbiota in local and introduced cattle breeds. The study identifies candidate bacterial strains that may underlie breed-specific immune advantages, reveals the critical contributions of the gut microbiota to the immune system, metabolism and animal health, and has significant potential for the development of microbiome-based interventions.

2. Materials and methods

2.1 Experiment animals and sample collection

The cattle utilised in the present study were fed in Yuanyang County, Honghe Hani and Yi Autonomous Prefecture, Yunnan Province, China (23.17° N and 102.89° E) (Fig. 1). Fifteen HC and fifteen SC were

selected for the purpose of sample collection (see Table S1 for further details). Fecal and serum samples were collected from each cattle subject in August 2025. In order to circumvent the possibility of contamination by environmental factors, the surface feces were meticulously removed, and solely the inner feces that were not directly exposed to the air were collected. Blood samples were collected from the jugular vein using venipuncture, which were collected into evacuated tubes containing coagulants (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Serum was separated by centrifugation at 3500 ×g for 15 min. All samples were subjected to rapid freezing in liquid nitrogen, then transported to the laboratory and stored at − 80°C for subsequent DNA extraction.

2.2 Measurements of serum immune indicators

Serum levels of immunoglobulin A (IgA, #MM-3343201), immunoglobulin G (IgG, # MM-069401), immunoglobulin M (IgM, #MM-097401), interferon-gamma (IFN-γ, #MM-3306901), tumor necrosis factor-alpha (TNF-α, #MM-156801), interleukin-10 (IL-10, #MM-3695201) were analyzed with ELISA kits (Jiangsu Meimian Industrial Co., Ltd, China). All the above biochemical parameters were determined according to the manufacturer's instructions. Absorbance was measured with a microplate reader (Multiskan FC; Thermo Fisher, New York, NY).

2.3 Sample processing and IgA coating bacterial sorting

The fecal samples were then processed under sterile conditions to isolate bacterial cells. In summary, samples were homogenised in phosphate-buffered saline (PBS) and filtered through 100 μm cell strainers to remove large debris. The bacterial suspension thus obtained was then subjected to incubation with fluorescein isothiocyanate (FITC)-conjugated anti-cow IgA antibody (abcam) at a temperature of 4°C for a period of 30 minutes under conditions of darkness. Following a wash with Phosphate Buffered Saline (PBS) to remove unbound antibodies, IgA coating bacteria were enriched using magnetic-activated cell sorting with anti-FITC magnetic beads (Miltenyi Biotec) in accordance with the manufacturer's protocols. Subsequent analysis was performed on both IgA coating-enriched fractions and native (unsorted) bacterial fractions [7].

2.4 DNA extraction and metagenomic sequencing

Total genomic DNA was extracted from both IgA + and native bacterial fractions using the E.Z.N.A.® Stool DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. A high-quality DNA sample, characterised by an OD260/280 ratio of 1.8–2.2 and an OD260/230 ratio of at least 2.0, was utilised in the construction of the sequencing library. The construction and subsequent sequencing of metagenomic shotgun libraries was conducted at Shanghai Biozeron Biological Technology Co. Ltd. In summary, for each sample, metagenomic libraries were prepared in accordance with standard genomic DNA library preparation procedures. The concentration of all libraries was then measured using a High Sensitivity Double Stranded DNA Kit on a Qubit Fluorometer (Thermo Fisher Scientific). The libraries were finally subjected to sequencing using the MGI-T7 sequencing platform (Shanghai BIOZERON Biotech. Co., Ltd) with PE150 mode in accordance with the standard protocols.

2.5 Data processing

Subsequently, raw sequence reads were subjected to a quality trimming process utilising the Fastp software [28], with the objective of eradicating adaptor contaminants and low-quality reads. Subsequently, the quality control readings were mapped against the cattle genome (NCBI: GCA_002263795.3) by means of the BWA mem algorithm (parameters: -M -k 21 -t 16, http://bio-bwa.sourceforge.net/bwa.shtml). The gut microbiota composition of both IgA coating and native fractions was annotated using clean reads that were aligned to the GTDB-Tk [29], with the Genome Taxonomy Database serving as the reference.

In order to annotate the function of gut microbiota, contigs were assembled from metagenomic reads using MEGAHIT, in accordance with the default settings [30]. For the purposes of subsequent analyses, only contigs exceeding 500 base pairs in length were considered. The potential function genes were forecasted from contigs by MetaGeneMark with the default parameters [31]. Subsequent to this, redundant sequences were filtered out across the samples using CD-hit [32]. The clean reads from each sample were then mapped back to the predicted genes using BBMap, enabling precise quantification of gene abundance [33]. The functional gene annotation was performed by aligning the predicted genes to the KEGG database using DIAMOND (evalue = 1e-5, id ≥ 70) [34]. The biosynthesis-related functional analyses focused on the SCFAs included formate, acetate, propionate, butyrate, and valerate. The innate immune factors included indole-3-acetic acid (IAA), indole-3-aldehyde (IAId), kynurenine, secondary bile acids (SBAs), succinate, and trimethylamine N-oxide (TMAO). The genetic potential of the gut microbiota was analysed following the marker genes based on the KEGG database for sorting. The detailed marker genes and abundance calculation processes utilised in the present study have been previously documented in a separate report [35]. Furthermore, the pathogens present within the native gut microbiota of cattle were annotated by the PHI-base [36].

The investigation into the IgA coating of gut microbiota at the strain level was conducted using MetaWRAP v1.3 [37]. This software was utilised to analyse metagenome assembled genomes (MAGs) from the metagenomic datasets. The taxonomy of the remaining metagenomic assemblies (MAGs) was annotated through GTDB-Tk, with reference to the Genome Taxonomy Database, and a maximum-likelihood phylogenetic tree was constructed for the MAGs utilising the 120 bacterial marker genes [38]. Prodigal v2.6.3 was utilised to identify genes within each MAG, which were then aligned against the KEGG database using DIAMOND [39]. Subsequently, the abundance of each MAG in the native and IgA coating fractions was calculated using TPM (Transcripts Per Kilobase of Taxonomy per Million mapped reads) to eliminate biases from sequence length and sequencing depth. IgA coating levels of each MAGs were calculated by comparing microbial abundance between IgA coating and native fractions [7]. IgA coating levels were classified into three categories: The categorisation of samples as "uncoated", "low coating" or "high coating" is based on the log-transformed IgA+ probability scores (thresholds at -1.3 and 1).

2.6 Statistics analysis

All statistical analyses were performed in the R v4.4.2 platform and visualized by the "ggplot2" package [40, 41].

The calculation of two alpha-diversity indices, Chao1 and Shannon, and the beta-diversity distance (Bray-Curtis) of native and IgA coating gut microbiota was performed utilising the "vegan" package [42]. The Wilcoxon rank-sum test was employed to assess the disparities in alpha-diversity indices and the relative abundances of microbial phyla, species, and MAGs in the native or IgA coating gut microbiota between the SC and HC groups, respectively. Principal coordinate analysis (PCoA) and adonis test based on the Bray-Curtis distance were further performed using the "ape" packages to evaluate the variations in the native or IgA coating gut microbiota composition between the SC and HC groups [43]. Furthermore, the Wilcoxon rank-sum test was employed to assess the disparities in the abundances of genes implicated in the synthesis of SCFAs and innate immune factors between the native gut microbiota of the SC and HC groups. Concurrently, the Wilcoxon rank-sum test was utilised to estimate the disparities in the concentrations of total pathogens and individual pathogens, in addition to the serum immune indicator profiles, between the SC and HC groups. Furthermore, the Wilcoxon rank-sum test was utilised to evaluate the disparities in IgA coating levels among distinct microbial taxa between the SC and HC groups. Finally, Spearman correlation was performed using the "psych" package to investigate the correlation between the IgA coating level of key taxa with the abundances of pathogens and the contents of serum immune indicators [44].

3. Results

3.1 Differences in the native gut microbiota

An investigation into the alpha-diversity of the native gut microbiota of cattle of the SC and HC breeds revealed no significant difference (Wilcoxon rank-sum test, $P > 0.05$, Fig. 2a). Conversely, PCoA based on the Bray-Curtis distance demonstrated distinct clusters of the native gut microbiota between the SC and HC (Fig. 2b). A significant discrepancy in the native gut microbiota composition was also obtained between the SC and HC (Adonis test, $P < 0.05$, Fig. 2b). The phyla of Bacillota and Bacteroidota dominated the native gut microbiota of both SC and HC, followed by the Verrucomicrobiota phylum (Fig. 2c). The relative abundance of Bacillota was found to be significantly higher in the HC compared to that in the SC, while Bacteroidota was more abundant in the native gut microbiota of SC (Wilcoxon rank-sum test, $P < 0.05$, Fig. 2c). The analysis revealed that three bacterial species (*Methanocorpusculum* sp017555685, *Cryptobacteroides* sp017631175, and *Akkermansia* sp947303185) exhibited a marked increase in abundance within the native gut microbiota of HC subjects when compared to SC subjects (Wilcoxon rank-sum test, $P < 0.05$, Fig. 2d). In contrast, bacterial species that exhibited significantly higher relative abundances in the SC samples were predominantly affiliated with the genera *Physcousia*, *Onthomorpha*, *Alloprevotella*, and *Alistipes* (Wilcoxon rank-sum test, $P < 0.05$, Fig. 2d).

3.2 Differences in the potentials of SCFAs synthesis for native gut microbiota

In general, the native gut microbiota of HC exhibited significantly stronger capacities for synthesising SCFAs (Fig. 3). In summary, a number of genes (*pflD*, *pdh*, *acdAB*, *pct*, and *ACSS1_2*) which are involved in the synthesis of acetate by pyruvate directly through the acetyl-CoA were found to be more abundant in the native gut microbiota of HC than in those of SC (Wilcoxon rank-sum test, $P < 0.05$, Fig. 3a). For propionate synthesis, the genes involved in the initial (*sucCD* and *pct*) and ultimate (*ACSS1_2*, *acdAB*, and *pct*) processes exhibited significantly higher abundances in the native gut microbiota of HC compared to those of SC (Wilcoxon rank-sum test, $P < 0.05$, Fig. 3b). Furthermore, the gene responsible for synthesising butyrate directly through butanoyl-CoA (*atoAD*) exhibited a marked increase in abundance in the native gut microbiota of HC compared to those of SC (Wilcoxon rank-sum test, $P < 0.05$, Fig. 3c).

3.3 Differences in the immunity

In order to compare the immunity of SC and HC, the total abundance of pathogens was investigated in the native gut microbiota of the two groups. It was found that the total abundance of pathogens was more abundant in the native gut microbiota of SC compared to HC counterparts (Wilcoxon rank-sum test, $P < 0.05$, Fig. 4a). In particular, several pathogens, including *Porphyromonas gingivalis*, *Salmonella enterica*, *Flavobacterium psychrophilum*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes*, exhibited significantly higher abundances in the native gut microbiota of SC than in HC samples (Wilcoxon rank-sum test, $P < 0.05$, Fig. 4b). Furthermore, the overall abundance of functional genes associated with the synthesis of innate immune factors in the native gut microbiota of HC was higher than that in SC (Fig. 4c). Specifically, this encompasses nitrilase for indole-3-acetic acid synthesis, kynB for kynurenine synthesis, and sucCD and gabD for succinate synthesis (Wilcoxon rank-sum test, $P < 0.05$, Fig. 4c). Furthermore, the serum levels of IgA, IgG, and IgM in the HC group exhibited a marked increase in comparison to the SC group (Wilcoxon rank-sum test, $P < 0.05$, Fig. 4d).

3.4 Differences in the IgA coating gut microbiota

In order to initiate the investigation into the IgA coating of gut microbiota, it is first necessary to recognise the MAGs present within the gut microbiota of cattle. A total of 2,556 MAGs were obtained (Fig. 5a). Analogous to the native gut microbiota, the alpha-diversity of IgA coating gut microbiota between the SC and HC groups exhibited a non-significant variation (Wilcoxon rank-sum test, $P > 0.05$, Fig. 5b), whilst their composition demonstrated a significant difference (Adonis test, $P < 0.05$, Fig. 5c). Furthermore, analysis of the gut microbiota revealed that Bacillota and Bacteroidota were the most prevalent bacterial phyla in the two cattle breeds (Fig. 5d). In comparison with the native gut microbiota, the relative abundance of IgA coating Bacillota was found to be significantly increased, while that of IgA coating Bacteroidota was markedly decreased in SC (Wilcoxon rank-sum test, $P < 0.05$, Fig. 5d). In HC, the relative abundances of these two major phyla in IgA coating gut microbiota demonstrated no

significant differences when compared with their proportions in the native gut microbiota (Wilcoxon rank-sum test, $P > 0.05$, Fig. 5d). Furthermore, in both cattle breeds, the relative abundance of IgA coating Verrucomicrobiota (the third dominant bacterial phylum) was significantly reduced compared with that in the native gut microbiota (Wilcoxon rank-sum test, $P < 0.05$, Fig. 5d).

At the species level, the abundances of IgA coating *Physcousia* sp017559845 and *Phyllobacterium myrsinacearum* (*P. myrsinacearum*) were significantly enriched in HC (Wilcoxon rank-sum test, $P < 0.05$, Fig. 5e). Conversely, the presence of multiple IgA coating Alistipes species was observed to be more prevalent in SC (Wilcoxon rank-sum test, $P < 0.05$, Fig. 5e). At the strain level, the predominantly enriched IgA coating microbial strains in SC included HH_F9-IgA.semibin2.Bin624, XM_F7-IgA.metabat2.Bin68, and HH_F3-IgA.semibin2.Bin494 (Wilcoxon rank-sum test, $P < 0.05$, Fig. 5f). Meanwhile, the major enriched IgA coating microbial strains in HC were HH_F16-IgA.semibin2.Bin225, HH_F4-IgA.semibin2.Bin12, and HH_F1-IgA.metabat2.Bin55 (Wilcoxon rank-sum test, $P < 0.05$, Fig. 5f).

3.5 Recognizing the key strains in the gut microbiota of HC

The mean overall IgA coating level of the gut microbiota was found to be significantly higher in HC than in SC counterparts (Wilcoxon rank-sum test, $P < 0.05$, Fig. 6a). The major IgA coating bacterial phyla included Bacillota, Bacteroidota, Pseudomonadota, Actinomycetota, and Patescibacteriota (Fig. 6a). Among them, the IgA coating levels of Bacillota, Actinomycetota, and Pseudomonadota in the gut microbiota of HC were significantly higher than those in SC (Wilcoxon rank-sum test, $P < 0.05$, Fig. 6b). Furthermore, one bacterial species (*P. myrsinacearum*) and two strains (HH_F11-IgA.metabat2.Bin89 and HH_F1-IgA.metabat2.Bin55) were observed to have a significantly higher IgA coating level in the gut microbiota of HC than in SC counterparts (Wilcoxon rank-sum test, $P < 0.05$, Fig. 6b). The IgA coating level of MAGs was found to be a useful indicator of the type of MAGs, with 49 strains assigned to the high IgA coating type, accounting for 1.92% of the total MAGs (Fig. 6c). Among them, HH_F11-IgA.metabat2.Bin89 and HH_F1-IgA.metabat2.Bin55 were strains with the top 2 IgA coating levels, which were 182 and 193, respectively (Table S2).

Furthermore, correlation analysis demonstrated a significant positive correlation between the IgA coating level of HH_F1-IgA.metabat2.Bin55 (*Arcanobacterium*) and serum IgM concentration in cattle. In addition, the IgA coating level of HH_F11-IgA.metabat2.Bin89 (*P. myrsinacearum*) exhibited a significant positive correlation with serum IgG levels (Spearman correlation, $P < 0.05$, Fig. 6e). Furthermore, the levels of IgA coating in these two key strains exhibited a significant negative correlation with the abundances of multiple major pathogenic bacteria in the cattle gut microbiota (Spearman correlation, $P < 0.05$, Fig. 6f).

4. Discussion

This study applied a novel metagenomic immunoglobulin sequencing technique to identify IgA-coated microorganisms at the strain level and quantify their coating levels within the gut microbiomes of HC and SC. The two cattle breeds investigated in this study exhibited distinct taxonomic assemblages,

despite similar overall richness and evenness. The gut microbiota of HC was found to contain a greater abundance of Bacillota (formerly known as Firmicutes), while Bacteroidota (formerly known as Bacteroidetes) were present in smaller numbers. This indicated a higher Firmicutes/Bacteroidetes (F/B) ratio in HC. The F/B ratio in animal gut microbiota has been demonstrated to reflect the balance between these dominant phyla and to influence energy extraction, health, and environmental adaptation. Higher ratios have been shown to correlate with increased caloric harvest from diet, aiding survival in nutrient-poor conditions [45]. Furthermore, the IgA coating level of Bacillota exhibited a significant increase in HC compared to SC counterparts. This phenomenon could be interpreted as a form of "corrective" immune selection, whereby the host attempts to reinforce beneficial or less harmful populations (Bacillota) and constrain potentially problematic ones (such as Bacteroidota) [46]. The advantage of HC in F/B ratio may serve as an important microbiological basis for maintaining favourable health status and production performance of HC under local extensive grazing conditions [47].

Furthermore, the results of this study indicated that the gut microbiota of HC exhibited a greater genetic capacity for SCFA production in comparison to that of SC. In view of the well-established roles of SCFAs in providing energy to colonocytes, reinforcing epithelial barrier function, modulating local and systemic immune responses, and inhibiting pathogen overgrowth, this enhanced SCFAs synthesis capacity is likely to contribute to improved gut health and immune status in HC [48]. A secondary substantial line of evidence pointing to breed-specific immune differences was the contrasting pathogen load and functional potential for innate immune factor biosynthesis in the native microbiota of HC and SC. SC was found to harbour a gut microbiota with higher pathogenic pressure under the same environmental and management conditions, which may translate into greater risk for enteric and systemic infections [49]. Conversely, the enrichment of biosynthetic genes associated with innate immune factors in HC indicated the presence of a microbial community that was not only more capable of producing SCFAs but also better equipped to generate immunomodulatory metabolites that support mucosal and systemic immune equilibrium [50]. In conjunction with the diminished pathogen burden and augmented immune-related metabolite biosynthetic capacity, the heightened serum immunoglobulin levels in HC suggest a synchronised microbe-immune axis that promotes heightened resistance and resilience [51]. The findings of this study suggested that HC might constitute a more advantageous immune defence system compared to SC via a plausible "beneficial microbiota-metabolite-immune effect" pathway. However, it should be noted that the observed associations represent correlative evidence only. Rigorous causal validation through subsequent mechanistic studies is therefore required to confirm this hypothesis.

A substantial body of research has been dedicated to the study of cattle and other livestock, with a predominant focus on the composition of gut microbiota or the characteristics of rumen fermentation. However, limited attention has been paid to the systematic comparison of IgA-coated microbiota between indigenous breeds and those that have been introduced [52]. This study revealed a substantial increase in the overall IgA coating level of gut microbiota in HC compared to that in SC. This result indicated a robust immune response targeting specific bacteria in HC, which shaped microbial composition by promoting beneficial symbionts or marking potentially harmful ones for control [53]. The tight coupling of high IgA coating, elevated serum immunoglobulins, and reduced pathogen burden

suggests a plausible mechanism for the superior immune phenotype of HC, in which a small set of IgA-targeted strains helps maintain an immunologically vigilant yet controlled environment [54]. Two key strains were identified with extremely elevated IgA coating levels and significantly enriched in the gut microbiota of HC. In contrast to the findings in human and murine studies, where IgA-coated bacteria are predominantly associated with inflammatory taxa [55, 56], the highly IgA-coated strains identified in this study were predominantly correlated with enhanced SCFA metabolic potential and reduced pathogen abundance. This finding indicates that under natural grazing conditions, IgA coating exerts a more protective immune tolerance effect [57]. These strains are promising candidates for development as next-generation probiotics or microbial consortium members aimed at improving gut health and immunity in more susceptible breeds, such as SC [58].

Of the two key strains identified, the strain belonging to *P. myrsinacearum* merits particular attention. *Phyllobacterium myrsinacearum* is a Gram-negative, rod-shaped bacterium in the genus *Phyllobacterium*, family Phyllobacteriaceae, and order Hyphomicrobiales within the class Alphaproteobacteria [59]. It was first isolated from sugar-beet roots and has since been identified in other plant species, including Ardisia crispa, as well as in other rhizosphere environments [60]. Within the plant rhizosphere and root systems, it functions as a diazotroph (nitrogen-fixing) and plant-growth-promoting bacterium, thereby enhancing nitrogen availability and fostering plant growth in certain contexts [61]. This particular strain of bacteria in the gut microbiota of Honghe cattle may have originated from symbiotic microorganisms present in their diet [62]. At the present time, there is an absence of any evidence that *P. myrsinacearum* is utilised or endorsed as a probiotic in the context of livestock farming. Whilst the results of the present study lend support to this hypothesis, further feeding experiments are required in order to verify the actual effects of *P. myrsinacearum* as a probiotic for cattle cultivation.

A significant strength of this study is the reconstruction and analysis of 2,556 metagenomic assemblies (MAGs) from cattle gut metagenomes, which facilitated a strain-resolved perspective of IgA binding. This study systematically compared the composition and functions of the native and IgA coating gut microbiota between HC and SC for the first time. Furthermore, the study characterised the key highly IgA-coated strains at the MAGs level and explored their relationships with host immune phenotypes. However, it is important to acknowledge the limitations of this approach. Firstly, the present study was conducted in a single geographic location and at a single time point. However, it should be noted that seasonal, dietary, and environmental variations were not captured in this study and may therefore influence both microbiota and immunity [63]. Secondly, functional inferences are based on gene abundance rather than direct metabolite measurements; integrating fecal and serum metabolomics would strengthen causal links between microbial pathways and host phenotypes [64]. Thirdly, although the correlations between IgA-coating levels, serum immunoglobulins, and pathogen abundances are compelling, they do not establish causality. In order to ascertain whether these microbes directly modulate host immunity and pathogen resistance, experimental validation is required. Such validation may take the form of the colonization of germ-free or antibiotic-treated animal models with candidate high IgA-coated strains [65]. Future work should include multi-centre validation with larger cohorts,

alongside gnotobiotic animal models or in vitro co-culture systems, to functionally validate the immunomodulatory roles of the identified strains and to clarify their causal contributions to host health.

5. Conclusions

In this study, we applied MIG-Seq for the first time to systematically compare the gut microbial composition, functional potential, pathogen burden, IgA coating patterns, and host immune status between HC and SC, thereby establishing multi-dimensional ecological and immunological distinctions between a locally adapted breed and a commercial breed. The study found that the F/B ratio was significantly higher in the HC, indicating enhanced energy acquisition capacity. Additionally, the level of IgA coating on the surface of gut bacteria was markedly elevated in HC, reflecting stronger mucosal immune surveillance. Moreover, HC harboured a gut ecosystem enriched in SCFA synthesis pathways and reduced pathogenic loads compared with SC. The identification of two high IgA-coating core strains, *Phyllobacterium myrsinacearum* (Bin89) and *Arcanobacterium* (Bin55), which are tightly linked to host humoral immunity and pathogen suppression, provides a microbial basis for the superior immune robustness of the local breed and offers concrete candidates for next-generation probiotics or immune modulators.

Abbreviations

HC
Honghe cattle
IgA
immunoglobulin A
MIG
metagenomic immunoglobulin sequencing
PCoA
Principal coordinate analysis
SC
Simmental cattle
SCFA
short-chain fatty acids

Declarations

Ethics approval and consent to participate

All animal experimental protocols were approved by the Animal Care Committee at the Faculty of Animal Science and Technology, Yunnan Agricultural University (Kunming, P. R. China).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

Ye Yu: conceptualization, methodology, data curation, formal analysis, investigation, and writing-original draft. Qian Li: methodology, formal analysis and investigation. Runqi Fu: writing-original draft. Chunjia Jin: methodology and writing-reviewing and editing. Binlong Fu: formal analysis and methodology. Yuyan Wang: formal analysis, methodology. Qianxi li: data curation, investigation. Rui Ma: formal analysis and methodology. Yanfen Cheng: methodology, writing-reviewing and editing. Jing Leng: conceptualization, methodology, investigation, supervision, project administration, funding acquisition, validation, and writing-reviewing and editing. All the authors have agreed to the published version of the manuscript.

Data availability

Sequence data that support the findings of this study have been deposited in the NCBI Sequence Read Archive database with the accession number PRJNA1470076.

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Figures

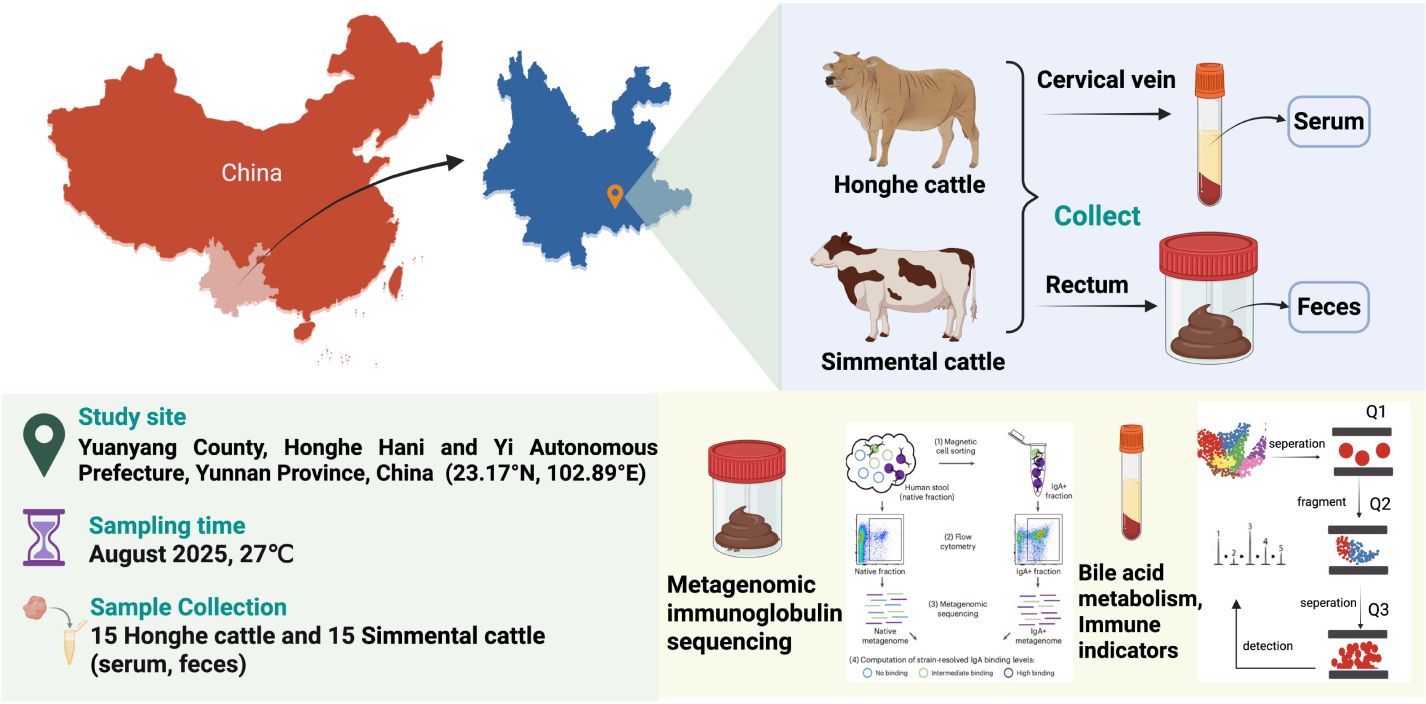


Figure 1

Blueprint for sampling and sequencing.

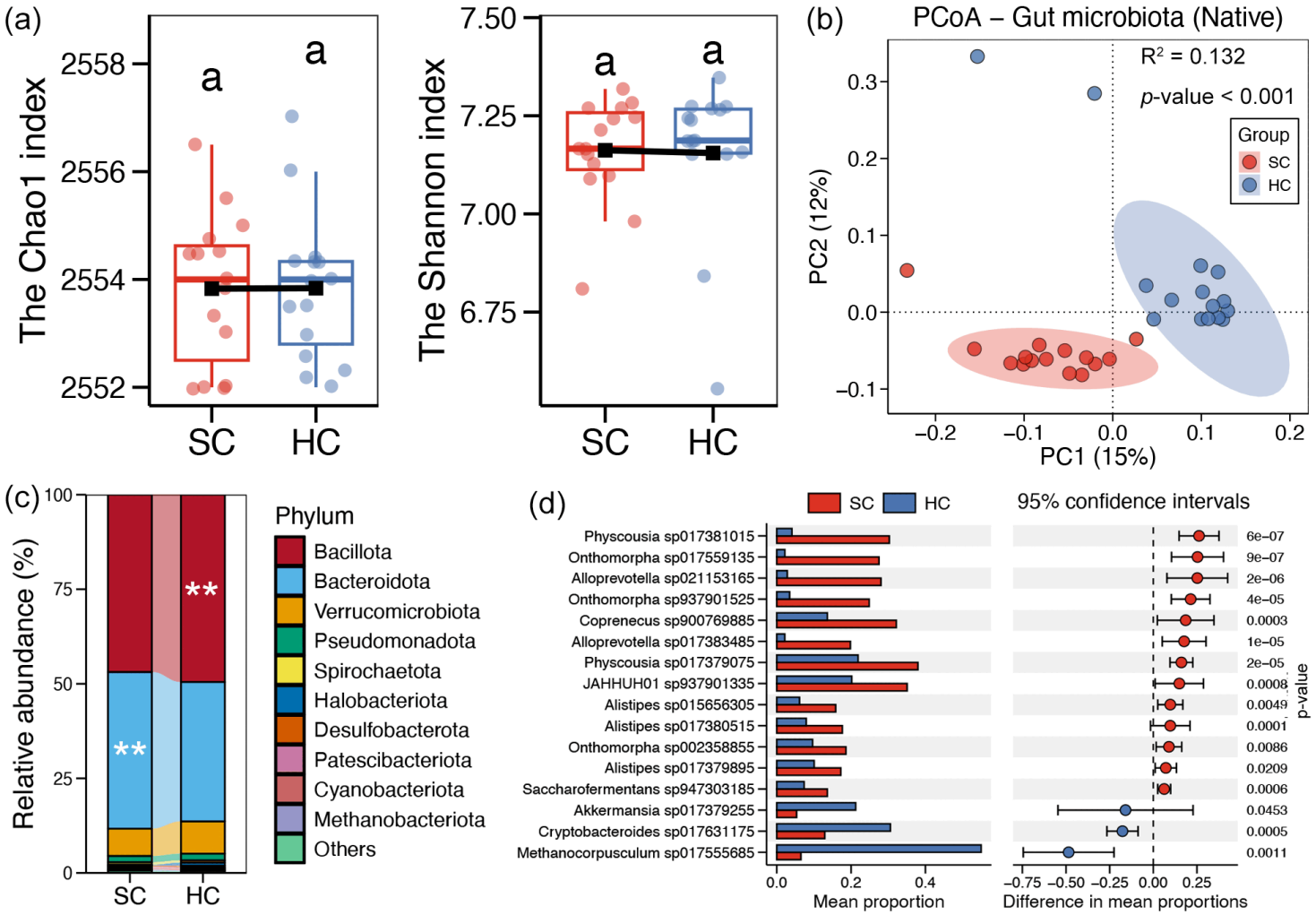


Figure 2

Differences in the native gut microbiota. (a) Differences in the alpha-diversity indices of native gut microbiota between the SC and HC groups. Black square represents the average values of corresponding index and different lowercases letters above boxes in each sub-figure represent significant differences between different groups (Wilcoxon rank-sum test, $P< 0.05$). (b) PCoA and adonis test based on the Bray-Curtis distance of the native gut microbiota between the SC and HC groups. (c) Relative abundances of dominant bacterial phyla in the native gut microbiota of SC and HC groups. ** represents significant higher relative abundance of corresponding phylum in corresponding group (Wilcoxon rank-sum test, $P < 0.05$). (d) Bacterial species with significantly different relative abundances between the native gut microbiota of SC and HC groups (Wilcoxon rank-sum test, $P < 0.05$).

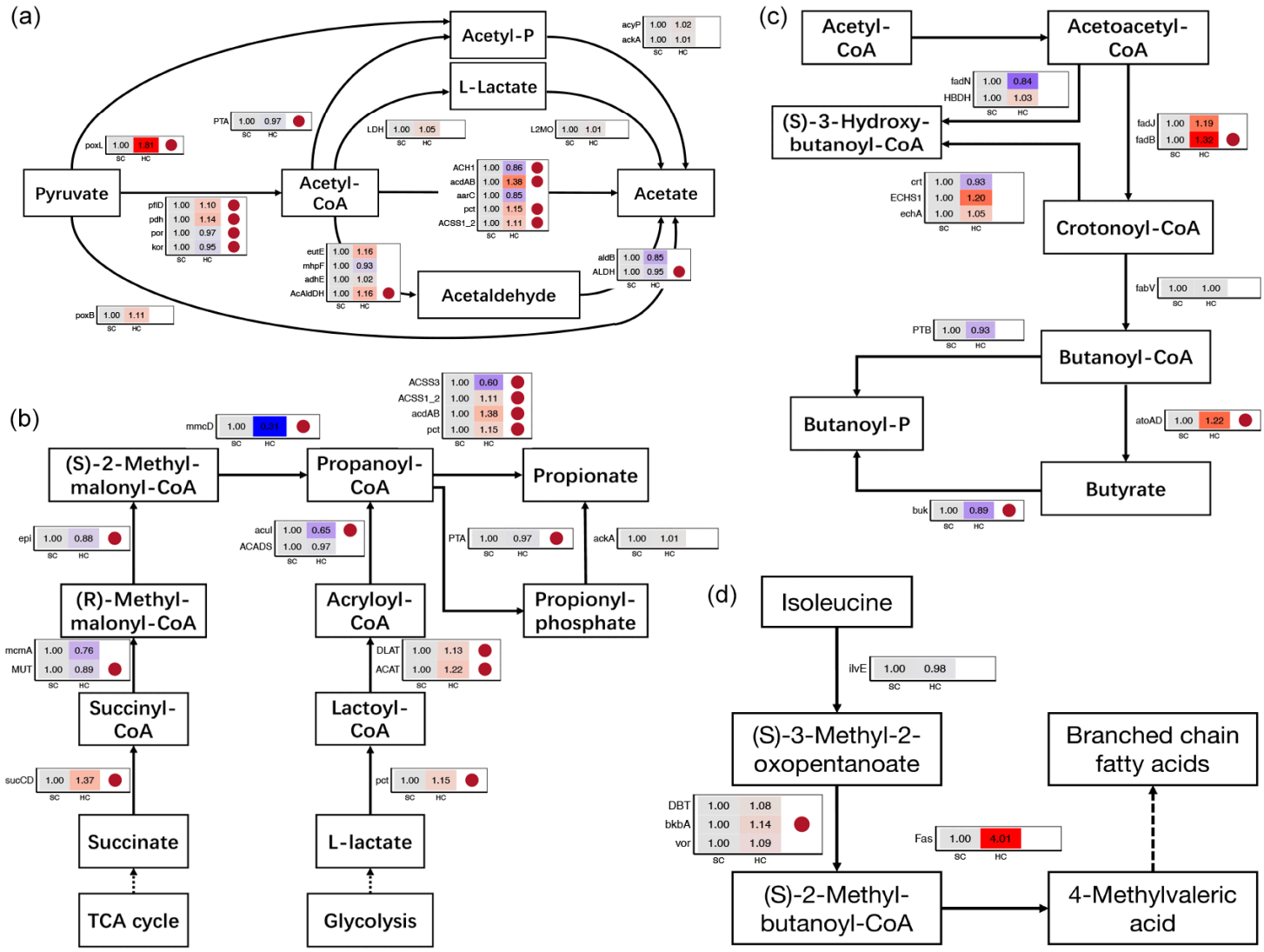


Figure 3

The relative abundance of each gene in the SCFA biosynthesis pathway was compared between SC and HC. Pathways for synthesis of acetate (a), propionate (b), butyrate (c), and valerate (d) mediated by gut microbiota of cattle. Heatmaps showed the differences in the abundances of SCFA biosynthesis genes in the native gut microbiota of SC and HC groups. Colors in each block represent the average abundance (TPM) of each gene in the SC or HC groups. Values in blocks of wild groups represent the fold changes in average abundance of each gene in HC samples compared to those in SC counterparts. The red point to the right of each row represents significant differences in the abundances of the corresponding genes between the SC and HC groups (Wilcoxon rank-sum test, $P < 0.05$).

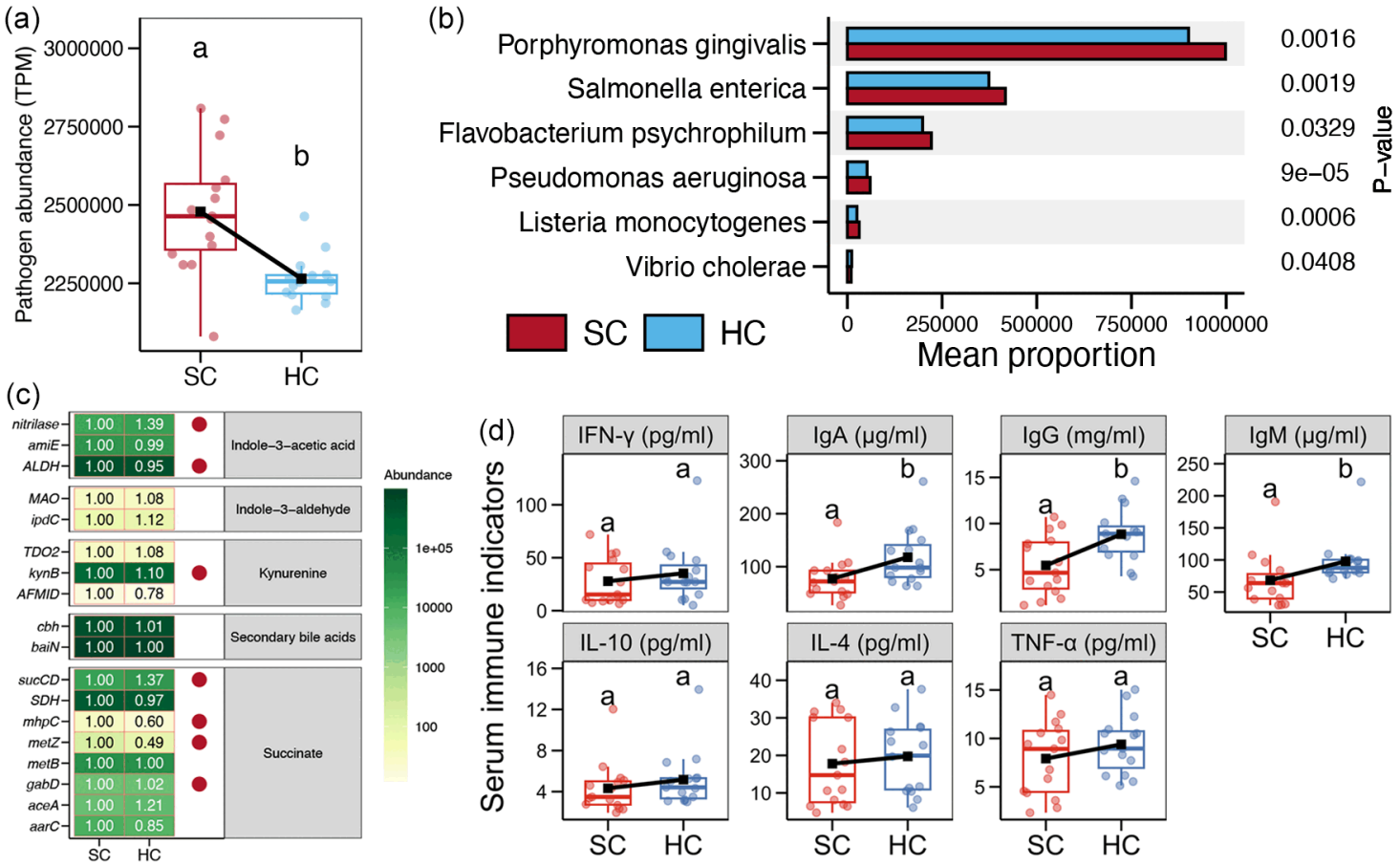


Figure 4

Differences in the immunity. (a) Differences in the total abundance of pathogens between the gut microbiota of SC and HC groups. (b) Pathogens with significantly different relative abundances between the native gut microbiota of SC and HC groups (Wilcoxon rank-sum test, $P < 0.05$). (c) Heatmap revealing the differences in the abundances of genes involving in the synthesis of innate immune factors in the native gut microbiota of SC and HC groups. Colors in each block represent the average abundance (TPM) of each gene in the SC or HC groups. Values in blocks of wild groups represent the fold changes in average abundance of each gene in HC samples compared to those in SC counterparts. The red point to the right of each row represents significant differences in the abundances of the corresponding genes between the SC and HC groups (Wilcoxon rank-sum test, $P < 0.05$). (d) Differences in the serum immune indicates between the SC and HC groups. Black square represents the average values of corresponding index and different lowercases letters above boxes in each sub-figure represent significant differences between different groups (Wilcoxon rank-sum test, $P < 0.05$).

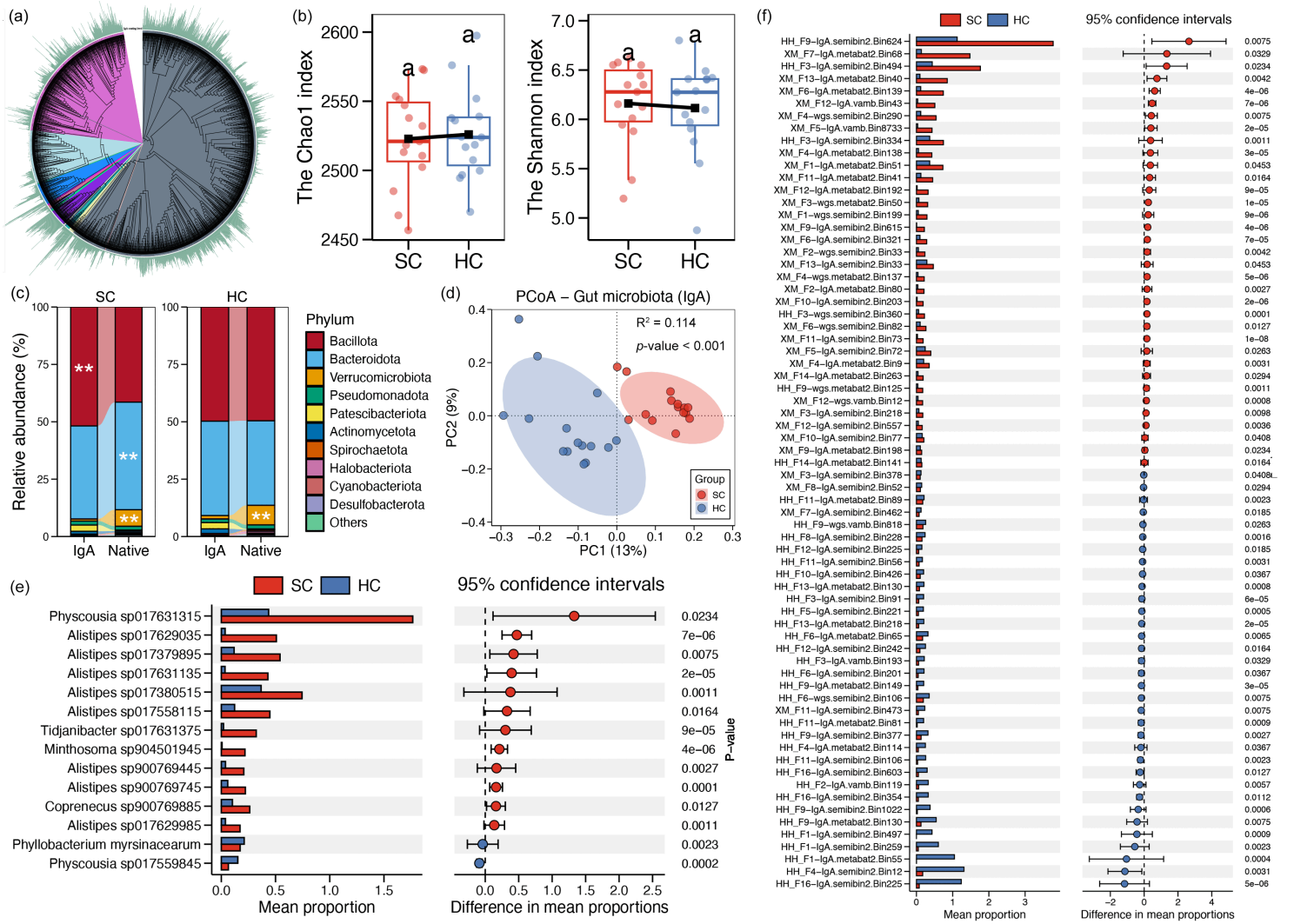


Figure 5

Differences in the IgA coating gut microbiota. (a) Phylogeny for MAGs in the gut microbiota of SC and HC. (b) Differences in the alpha-diversity indices of IgA coating gut microbiota between the SC and HC groups. Black square represents the average values of corresponding index and different lowercases letters above boxes in each sub-figure represent significant differences between different groups (Wilcoxon rank-sum test, $P < 0.05$). (c) Principal coordinates analyses (PCoA) and adonis test based on the Bray-Curtis distance of IgA coating gut microbiota between the SC and HC groups. (d) Relative abundances of dominant bacterial phyla in the IgA coating gut microbiota of SC and HC groups. ** represents significant higher relative abundance of corresponding phylum in corresponding group (Wilcoxon rank-sum test, $P < 0.05$). Bacterial species (e) and strains (f) with significantly different relative abundances between the IgA coating gut microbiota of SC and HC groups (Wilcoxon rank-sum test, $P < 0.05$).

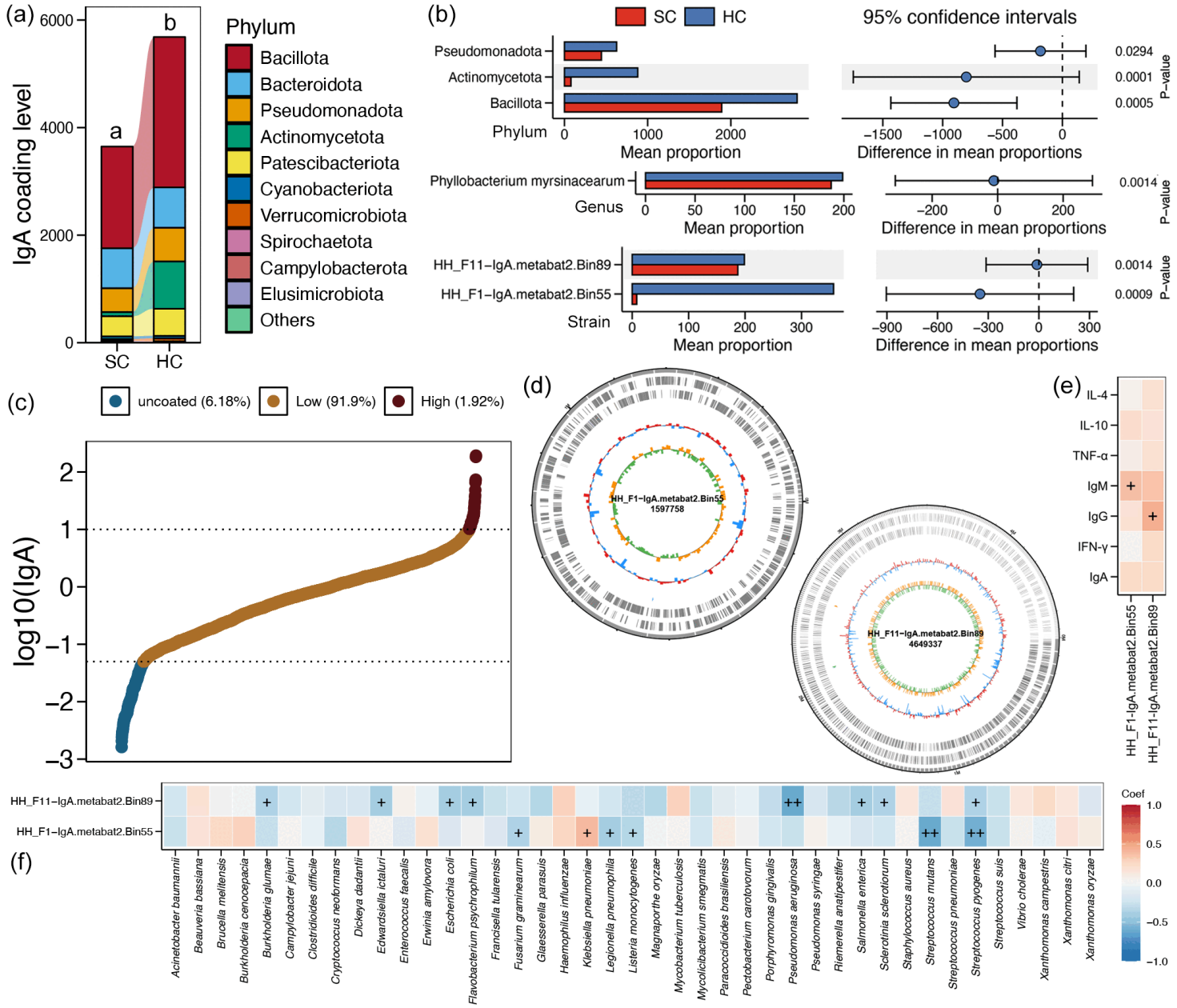


Figure 6

IgA coating of gut microbiota in SC vs HC. (a) Differences in the IgA coating level between the gut microbiota of HC and SC. Different lowercases letters above bars represent significant differences between different groups (Wilcoxon rank-sum test, $P < 0.05$). (b) Bacterial phyla, genera, and strains with significantly different IgA coating levels between the gut microbiota of SC and HC groups (Wilcoxon rank-sum test, $P< 0.05$). (c) Distribution of the IgA coating levels among all strains. (d) Circle plots revealing the gene composition of key strains. Heatmaps exhibiting the associations between the IgA coating levels of key strains with serum immune indicates (e) and gut pathogen abundances (f). Colors in each block represent correlation coefficients, and "+" and "++" represent the *p*-value lower than 0.05 and 0.01, respectively (Spearman correlation).

Supplementary Files

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