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ORIGINAL ARTICLE

Characteristics of gut microbiota in representative mice strains: Implications for biological research

Jianguo Guo | Chenchen Song | Yunbo Liu | Xuying Wu | Wei Dong | Hua Zhu | Zhiguang Xiang | Chuan Qin

Key Laboratory of Human Disease Comparative Medicine, Chinese Ministry of Health, Beijing Key Laboratory for Animal Models of Emerging and Remerging Infectious Diseases, Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Comparative Medicine Center, Peking Union Medical College, Beijing, China

Correspondence
Zhiguang Xiang and Chuan Qin, Key Laboratory of Human Disease Comparative Medicine, Chinese Ministry of Health, Beijing Key Laboratory for Animal Models of Emerging and Remerging Infectious Diseases, Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Comparative Medicine Center, Peking Union Medical College, Beijing, China.
Email: xiangzg@cnilas.org and qinchuan@pumc.edu.cn

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Abstract

Background: Experimental animals are used to study physiological phenomena, pathological mechanisms, and disease prevention. The gut microbiome is known as a potential confounding factor for inconsistent data from preclinical studies. Although many gut microbiome studies have been conducted in recent decades, few have focused on gut microbiota fluctuation among representative mouse strains.

Methods: A range of frequently used mouse strains were selected from 34 isolation packages representing disease-related animal (DRA), immunity defect animal (IDA), or gene-editing animal (GEA) from the BALB/c and C57BL/6J backgrounds together with normal mice, and their microbial genomic DNA were isolated from mouse feces to sequence for the exploration of gut microbiota.

Results: Mouse background strain, classification, introduced source, introduced year, and reproduction type significantly affected the gut microbiota structure ($p < 0.001$ for all parameters), with background strain contributing the greatest influence ($R^2 = 0.237$). In normal groups, distinct gut microbiota types existed in different mouse strains. Sixty-four core operational taxonomic units were obtained from normal mice, and 12 belonged to *Lactobacillus*. Interestingly, the gut microbiota in C57BL/6J was more stable than that in BALB/c mice. Furthermore, the gut microbiota in the IDA, GEA, and DRA groups significantly differed from that in normal groups ($p < 0.001$ for all). Compared with the normal group, there was a significantly higher Chao1 and Shannon index ($p < 0.001$ for all) in the IDA, GEA, and DRA groups. Markedly changed classes occurred with *Firmicutes* and *Bacteroidetes*. The abundances of *Helicobacter*, *Blautia*, *Enterobacter*, *Bacillus*, *Clostridioides*, *Paenibacillus*, and *Clostridiales* all significantly decreased in the IDA, GEA, and DRA groups, whereas those of *Saccharimonas*, *Rikenella*, and *Odoribacter* all significantly increased.

KEYWORDS
Bacteroidetes, BALB/c mice, C57BL/6J, disease-related animal, *Firmicutes*, gene-editing animal, gut microbiota, immunity defect animal, strains

1 | INTRODUCTION

Experimental animals are used as substitutes for human beings in studies of various physiological phenomena and pathological mechanisms as well as for disease prevention. According to the "reduction" principle in the "3 R" principle of animal experiments, we should reduce the number of animals used in experiments as much as possible and improve the utilization rate of experimental animals and the accuracy of experiments results. A study showed that approximately 10.5 million experimental animals were used in 2016 across the European Union, 2.3 million fewer than in 2014 (12.8 million).[1,2] Animal models are used in studies of tumor transplantation and immune treatments,[3] and nude mice, gnotobiotic animals,[4] and germ-free animals[5] are used in research on malignant tumors. Animal experiments also significantly contribute to understanding pathogenic mechanisms and treatment and recovery of various diseases, including diabetes,[6] obesity,[7] bronchial asthma,[8] and silicosis,[9] as well as Alzheimer's disease[10] and COVID-19.[11] This dependence on laboratory animals is even more pronounced in the pharmaceutical and chemical industries, where side effects (carcinogenic, pathogenic, teratogenic, toxic, mutagenic, disabling, and fatal) of drugs and chemical products are all evaluated using experimental animals.[12–14] With the development of space science, experimental animals are also used for the evaluation of the effects of weightlessness, radiation, and spaceflight on the physiological state of the body.[15,16] In addition, these experimental animals are widely used in animal husbandry[17] and agricultural science[18] and in investigating environmental issues.[19] Therefore, experimental animals are involved in many aspects of our lives.

However, the poor repeatability of animal models of human diseases has become a puzzling phenomenon in recent decades.[20] To address this, the environmental quality control and strain origin of experimental animals have become the focus of recent studies, including animal strain types[21]; commercial sources[22]; climatic factors such as temperature[23] and humidity[24]; physical and chemical factors such as light,[25] noise,[26] and harmful gases[27]; living and rearing environments such as rearing methods[27]; biological factors such as biting and fighting between experimental animals; and human influence. Several scholars identified[28] that the gut microbiota was also a potential confounding factor for inconsistent data from preclinical studies. Unfortunately, the composition of the gut microbiota varies greatly between individuals, reflecting the influence of the host genome and environmental factors. The gut microbiota is widely studied due to its potential involvement in the etiology of numerous gut-associated diseases. Furthermore, in recent years, the impact of the gut microbiota on host physiology and the onset of diseases, including metabolic[29] and neuronal disorders,[30] cancers,[31] gastrointestinal infections,[32] and chronic inflammation,[33] has become a focal point of interest. There is now evidence that understanding the gut microbiota of endangered animals can be used to target dietary supplements to maintain the health of endangered animals.[34] Many scholars believe that the gut microbiota is a new method to treat various diseases.[35] Yan Li et al.[35] found that cohousing of Rnf5$^{-/-}$ and wild-type (WT) mice abolishes antitumor immunity and the tumor inhibition phenotype, whereas the transfer of 11 bacterial strains, including *Bacteroides rodentium*, enriched in Rnf5$^{-/-}$mice, establishes antitumor immunity and restricts melanoma growth in germ-free WT mice. The composition of the gut microbiota may also be influenced by specific disease states[36,37] and may reciprocally affect the host and cause certain diseases.[38–40]

The relative balance of specific microbial metabolic activities in the gut and their interactions with the host may promote both health and disease. Thus, the composition and functional capacity of the gut microbiota may modulate risk positively or negatively to a wide variety of health and disease phenotypes. The number of genes in the gut microbiota is 150-fold greater than that of the host and is known as the "second human genome."[41] Gut commensal microbes make critical contributions to human health, and many elicit beneficial effects on the host. Many gut microbes are pioneer colonizers of the gut and have been associated with various health-promoting effects,[42,43] although the precise modes of action remain largely unknown. The importance of the gut microbiota in experimental animals has therefore been addressed in multiple studies.[44,45] Research has shown that γ radiation and pasteurization affected the nutritional composition of commercial animal feed[46] and that microbiome composition in both WT and disease model mice was heavily influenced by the mouse facility.[47] The composition of the gut microbiota is related to host biology,[48] and the dominant bacteria in mice are known to be similar to those in humans and are highly representative of the human gut microbiota.[49] Therefore, mice with a naturally complex microbiome are a good proxy for humans and their microbiomes.

Extensive research on gut microbiota has been conducted in recent decades, and although most studies have focused on a variety of diseases, few have explored the bacterial community structures of representative mouse strains. There is abundant evidence that mouse phenotypes in disease models vary greatly between animal facilities and commercial providers[50] and that this variation is associated with differences in the microbiota.[50] Several studies have shown that genetically identical mice from the same litters share a more similar microbiota than those from different litters.[36,51] As mouse pups are born vaginally, the birth mothers' microbiota is their primary inoculum. In addition to environmental control, there are still several parameters, such as gender, background strain, introduced source, introduced year, and reproduction type, that should be considered in the formation of the gut microbiota and their effect on mouse gut microbiota as their contribution remains unclear. Furthermore, the characteristics of the mouse gut microbiota from animal studies need to be explored. Therefore, in this study, we studied the construction of the gut microbiota and the shaping factors, including gender, background strain, classification, introduced source, introduced year, and reproduction type.

2 | MATERIALS AND METHODS

2.1 | Detailed information on mice analyzed in this study

Thirty-four independent isolation packages were selected from the seed bank in the animal barrier facility of Beijing H.F.K. Biotechnology Co., Ltd. Isolation packages are barrier environments with positive pressure-independent ventilation systems used for animal feeding. Each isolation package contained only one mouse strain (Table 1). These included normal mice ($n = 11$), immunity defect animals (IDAs, $n = 9$), disease-related animals (DRAs, $n = 7$), and gene-editing animals (GEAs, $n = 7$). Between 9 and 10 animals (four or five females and five males) aged 8–10 weeks were selected for each strain. The mice in all isolation packages were fed with the same standard diet of food and water avoiding diet interference. Two to three grains (0.02–0.03 g) of fresh feces were collected and refrigerated for DNA extraction.

2.2 | Extraction and sequencing of DNA

Total genome DNA from two or three grains (0.02–0.03 g) of feces was extracted using the cationic hexadecyl trimethyl ammonium bromide/anionic sodium dodecyl sulfate (Sigma, Darmstadt, Germany) method.[52] DNA concentration and purity were monitored on 1% agarose gels and quantified using Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). DNA was diluted to 1 ng/μl using sterile water. 16S rRNA genes of distinct regions (16S V3–V4)[53] were amplified using a specific barcoded primer. All polymerase chain reactions (PCRs) were performed in 30-μl reactions using 15 μl of Phusion High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s, with a final elongation step at 72°C for 5 min. The PCR products were mixed with an equal volume of 1× loading buffer (contained SYB green) and separated using 2% agarose electrophoresis. PCR products were mixed in equal density ratios and then purified using the Gene JETTM Gel Extraction Kit (Thermo Scientific).

Sequencing libraries were generated using Ion Plus Fragment Library Kit 48 runs (Thermo Scientific) following the manufacturer's recommendations. Library quality was assessed using a Qubit@ 2.0 Fluorometer (Thermo Scientific). Finally, the library was sequenced on an Ion S5 TM XL platform, and 400 bp/600 bp single-end reads were generated.

2.3 | Sequencing data treatment

Single-end reads were assigned to samples based on their unique barcode and truncated by removing the barcode and primer sequence. Quality filtering on the raw reads was performed under specific filtering conditions to obtain high-quality clean reads according to the Cutadapt (V1.9.1, http://cutadapt.readthedocs.io/en/stable/). Reads were then compared with the reference database using the UCHIME algorithm (UCHIME algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) to detect chimera sequences, and then, the chimera sequences were removed to obtain clean reads. Sequence analyses were performed using Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/). Sequences with ≥97% similarity were assigned to the same OTUs. A representative sequence for each OTU was screened for further annotation by QIIME 1.9.1.

2.4 | Statistical analysis

Each sample was rarefied to 24 598 sequences for data analysis. Nonmetric multidimensional scaling (NMDS) was conducted based on the Bray–Curtis distance. Permutational multivariate analysis of variance (Adonis) was performed to evaluate the influence of the parameters on the bacterial community based on the Bray–Curtis distance using the package Vegan in R. We used the Mann–Whitney *U*-test to compare the abundance of taxa, Chao1, and Shannon index between distinct groups. A significance level of $p < 0.05$ was used for all analyses. A flower diagram was used to show the common OTUs in the normal strains. Network analysis was performed based on the abundance of the first 100 OTUs using Pearson's correlation analysis. Correlations between OTUs where the correlation coefficient was 0.8 and $p < 0.05$ are shown for the normal strains. Venn diagrams among groups were used to evaluate the stability of the gut microbiota. Adonis analysis and Mann–Whitney *U*-test were also used to compare the difference between the normal and diseased strains (IDA1, GEA1, and DRA1). The unweighted pair-group method with arithmetic mean (UPGMA) was conducted using the abundance data of the class level to show the similarity among the normal, IDA, GEA, and DRA groups. Finally, we screened several genera whose abundance significantly increased or decreased in the diseased groups (IDA, GEA, and DRA).

3 | RESULTS

3.1 | Mice gut microbiota–shaping factors

We performed Adonis analysis according to the gender, background strain, classification, introduced source, introduced year, and reproduction type of the strains in the 34 isolated packages to analyze the contribution of these factors to the fecal bacterial community structure. We found that background strain, classification, introduced source, introduced year, and reproduction type all significantly affected the bacterial community structure (Table 2). We then analyzed the contribution of various factors within four separated classes: the normal group and IDA1, GEA1, and DRA1 (IDA, GEA,

TABLE 1 Detailed information on mice analyzed in this study

Package name	Strain name	*N* (female/male)	Background strains	Classification	Sort type	Introduced source	Introduced year	Reproduction type	Coat color
B.C.S	BALB/c	10 (5/5)	BALB/c	Normal	–	SDZX	2017	Inbred	White
BALB.c	BALB/c	10 (5/5)	BALB/c	Normal	–	JAX	1999	Inbred	White
BALB.cJ	BALB/c	10 (5/5)	BALB/c	Normal	–	JAX	2017	Inbred	White
C57B.J	C57BL/6J	10 (5/5)	C57BL/6J	Normal	–	JAX	2017	Inbred	Black
C57BL6J	C57BL/6J	10 (5/5)	C57BL/6J	Normal	–	SDZX	2009	Inbred	Black
C57BLS	C57BL/6J	9 (4/5)	C57BL/6J	Normal	–	SDZX	2017	Inbred	Black
CBAOLA	CBA/Ola	10 (5/5)	CBAOLA	Normal	–	SDZX	2014	Inbred	Brown
DBA.1	DBA/1	10 (5/5)	DBA/1	Normal	–	SDZX	2009	Inbred	Gray
DBA.2J	DBA/2	10 (5/5)	DBA/2	Normal	–	JAX	2009	Inbred	Gray
ICR	ICR	10 (5/5)	Swiss	Normal	–	SDZX	2017	Outbreed	White
KM	KM	10 (5/5)	Swiss	Normal	–	SDZX	2017	Outbreed	White
BALBcAnu	BALB/cA-nu	10 (5/5)	BALB/c	IDA	nu/nu	SDZX	2009	Inbred	White
BALBcnu	BALB/cA-nu	10 (5/5)	BALB/c	IDA	nu/nu	HFK	2016	Inbred	White
NF	NSIG	10 (5/5)	BALB/c	IDA	NODscid	HFK	2017	Inbred	White
NODLtJH	NOD/LtJ	10 (5/5)	ICR/Jcl	IDA	NODLtJ	JAX	2009	Inbred	White
NODltJJ	NOD/LtJ	10 (5/5)	ICR/Jcl	IDA	NODLtJ	JAX	2017	Inbred	White
NODscid	NOD SCID	10 (5/5)	BALB/c	IDA	NODscid	JAX	2014	Inbred	White
NODscidJ	NOD SCID	10 (5/5)	BALB/c	IDA	NODscid	JAX	2017	Inbred	White
scid	SCID	10 (5/5)	BALB/c	IDA	scid	JAX	2014	Inbred	White
scid.j	SCID	10 (5/5)	BALB/c	IDA	scid	JAX	2017	Inbred	White
APOE	B6-ApoE tm1	9 (4/5)	C57BL/6J	GEA	KO	HFK	2011	Inbred	Black
IFNar1	B6-IFNαr1tm1	10 (5/5)	C57BL/6J	GEA	KO	HFK	2017	Inbred	Black
INTS10	INTS10	10 (5/5)	C57BL/6J	GEA	KO	HFK	2017	Inbred	Black
NTCP	B6-Ntcp$^{tm1(hNTCP)}$	10 (5/5)	C57BL/6J	GEA	TG	HFK	2016	Inbred	Black
PAP	PAP	10 (5/5)	C57BL/6J	GEA	TG	HFK	2015	Inbred	Black
Rag2	B6-Rag2 tm1	10 (5/5)	C57BL/6J	GEA	KO	HFK	2015	Inbred	Black
RAg2gray	B6-Rag2 tm1	10 (5/5)	C57BL/6J	GEA	KO	HFK	2015	Inbred	Gray
FVB	FVB	10 (5/5)	Swiss	DRA	–	HFK	2016	Inbred	White
kkcgAYJ	KK/Upj-Ay	10 (5/5)	Yellow KK	DRA	–	HFK	2010	Inbred	Yellow
KKUpjAyJ	KK/Upj-Ay	10 (5/5)	Yellow KK	DRA	–	HFK	2016	Inbred	Yellow
MRLMpJ	MRL/MpJ	10 (5/5)	LG, AKR, C3H, C57BL/6	DRA	–	HFK	2012	Inbred	White
ob.ob	ob/ob	10 (5/5)	C57BL/6J	DRA	–	HFK	2011	Inbred	Black

TABLE 1 (Continued)

Package name	Strain name	N (female/male)	Background strains	Classification	Sort type	Introduced source	Introduced year	Reproduction type	Coat color
SAMP1	SAMP1	10 (5/5)	AKR/J	DRA	-	JAX	2016	Inbred	White
SAMP8	SAMP8	10 (5/5)	AKR/J	DRA	-	JAX	2011	Inbred	White

Note: The "introduced source" represents the companies from which mice were purchased, and the "introduced year" represents the time when the mice entered the company.

Abbreviations: DRA, disease-related animal; GEA, gene-editing animal; HFK, Beijing HFK Bioscience Co., Ltd; IDA, immunity defect animal; JAX, the Jackson laboratory; KI, knock-in; KO, knock-out; SDZX, Shanghai Lab Animal Research Center; TG, transgenic.

and DRA group–associated background strains). We found that gender had an important influence on GEA1 and introduced years significantly affected the gut microbiota in all four classes (Table 2). Introduced sources also significantly contributed to the normal, IDA, and DRA groups (Table 2).

The other strain groups (IDA1, GEA1, and DRA1) exhibited significantly different bacterial community structures compared with those in the normal group (Table 2). For the normal group, background strain was the most important factor ($R^2 = 0.499$), whereas for the IDA and GEA groups, sort type was the most contributing factor, and for the DRA group, the introduced source was the most contributing factor (Table 2). In particular, the immunodeficiency type for IDA1 and the gene-modified type for GEA1 also play a key role in shaping the gut bacterial community structure (Table 2).

3.2 | Bacterial community structures of normal mouse strains

A total of 110 mice were analyzed from 11 isolation packages, including 7 background strains of mice (BALB/c, C57BL/6J, CBA/Ola, DBA/1, DBA/2, KM, and ICR). NMDS analysis showed significant differences in the gut microbiota structure of each mouse strain (Figure 1A). Chao1 index represented bacterial richness, and Shannon index represented community diversity. Chao1 and Shannon index in the normal mice strains drastically fluctuated (Figure 1B). There is relatively lower Chao1 and Shannon index in KM and ICR strains (Figure 1B). Furthermore, there was a similar result in the abundances of taxa at the phylum and genus levels (Figure 1C,D). However, the main phyla of mice gut microbiota were determined to be *Firmicutes* and *Bacteroidetes*, and the main genera were *Lactobacillus*, *Blautia*, *Bacteroides*, *Ruminococcaceae*, and *Clostridioides* (Figure 1C,D).

We then analyzed the number of common and unique OTUs among the different groups. We observed 64 common OTUs in 11 isolation packages (Figure 1E), and the dominant was *Lactobacillus* (Figure 1F). Ten gut microbiota belonging to *Firmicutes*, *Bacteroides*, and *Proteobacteria* were analyzed. Furthermore, it was found that the correlation between different OTUs from *Firmicutes* was stronger (Figure 1G).

C57BL/6J and BALB/c mice are the most commonly used experimental animal models.[54–56] Therefore, we focused on detailing the characteristics of the gut microbiota in normal mice from these strains. Three isolated packages of each background strain were used for analysis. According to the NMDS results (Figure 2A), the gut microbiota of C57BL/6J and BALB/c strains was significantly separated ($p < 0.001$). Compared with BALB/c mouse strains, the inner Bray–Curtis distance of the C57BL/6J mouse strains was significantly decreased ($p < 0.001$) (Figure 2B). The Chao1 and Shannon index in BALB/c mouse strains both exhibited greater fluctuation than that in C57BL/6J mouse strains (Figure 2C,D). The dispersion and trend of the diversity index supported the hypothesis that the gut microbiota in C57BL/6J mouse strains is more stable than that

TABLE 2 Adonis analysis of gut bacterial communities according to variable types in different groupings

	All mice		Normal		IDA1[a]		GEA1[b]		DRA1[c]	
Variable	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value
Gender	0.005	0.073.	0.004	0.969	0.004	0.924	0.021	0.027*	0.004	0.657
Background strain	0.237	0.001***	0.499	0.001***	–	–	–	–	–	–
Classification	0.128	0.001***	–	–	0.102	0.001***	0.232	0.001***	0.117	0.001***
Sort type	–	–	–	–	0.329	0.001***	0.273	0.001***	–	–
Introduced source	0.105	0.001***	0.119	0.001***	0.181	0.001***	–	–	0.139	0.001***
Introduced year	0.032	0.001***	0.108	0.001***	0.088	0.001***	0.085	0.001***	0.064	0.001***
Reproduction type	0.075	0.001***	0.159	0.001***	–	–	–	–	–	–

[a]This analysis contained IDA and normal animals with BALB/c background (groups B.C.S., BALB.c, and BALB.cJ).

[b]This analysis contained GEA and normal animals with C57BL/6J background (groups C57B.J, C57BL6J, and C57BLS).

[c]This analysis contained DRA and normal animals with BALB/c background and C57BL/6J background.

*$p < 0.05$, ***$p < 0.001$.

in BALB/c mouse strains. Finally, we compared the common OTUs among isolated packages raised from the same background strain. We observed that 264 OTUs of gut microbiota coexisted in the BALB/c mouse strains and 432 OTUs coexisted in the C57BL/6J mouse strains (Figure 2E,F), indicating that compared with the BALB/c mouse strains, a more complex network was present in C57BL/6J mice, and the gut microbiota had better stability in these strains.

3.3 | Changes in gut microbiota in diseased strains

After the analysis of the gut microbiota in normal mice, we addressed changes in the gut microbiota in diseased strains. NMDS analysis showed that the gut microbiota community of mice in the normal group was significantly different from those in the IDA, GEA, and DRA groups ($p < 0.001$) (Figure 3A). The Bray–Curtis distance dissimilarity for IDA, GEA, and DRA was significantly less than that of the normal group (Figure 3B). Bacterial diversity was also changed. The Chao1 and Shannon indexes were both significantly higher in the IDA, GEA, and DRA groups than in the normal group (Figure 3C,D). There were significant differences in the phylogenetic diversity index of the gut microbiota in the IDA, GEA, and DRA groups in comparison with those in the normal group (Figure 3E). There was also greater similarity in bacterial composition at the phylum level in the IDA, GEA, and DRA groups than in the normal group based on the UPGMA result (Figure 3F). The abundance of *Firmicutes* was also significantly lower in the IDA, GEA, and DRA groups than in the normal group, whereas this contrast was reversed with the abundance of *Bacteroidetes* (Figure 3G).

Finally, we also explored the changes at the genus level. The abundances of many genera distinctly increased or decreased in the disease model groups. We screened for several genera of interest. Compared with the normal group, the abundances of *Helicobacter*, *Blautia*, *Enterobacter*, *Bacillus*, *Clostridioides*, *Paenibacillus*, and *Clostridiales* all significantly decreased in the IDA, GEA, and DRA groups, whereas those of *Saccharimonas*, *Rikenella*, and *Odoribacter* significantly increased in DRA group (Figure 3H).

4 | DISCUSSION

The use of murine models in biomedical research is often an important and necessary step in developing better understanding of disease pathology, and there is a growing concern regarding the reproducibility of the results from murine-based studies.[57] The mouse gut microbiota makes an important contribution to the explanation of these research results. Therefore, we designed a study to examine the differences in gut microbiota in various kinds of mouse models obtained from the same commercial sources. We sought to identify the influencing factors that affect the bacterial community structure and study the microbiota population characteristics of the normal and IDA, GEA, and DRA mouse models.

Previous studies addressed changes in gut microbiota in mice from different suppliers or studied the diet and living environment, temperature, humidity, light, sound, and barrier facilities.[58–61] We used mice that were raised in 34 isolated packages at the same supplier facilities, thereby excluding the interference of diet, living environment, barrier facilities, temperature, humidity, light, and sound.

Our results showed that gender, background strains, introduced year, introduced source, and reproduction type all significantly affected the gut microbiota in mice (Table 2). Gender-dependent effects on the microbiome have been suggested in various animal models,[62,63] and we found gender as an important influencing factor for the GEA group (Table 2). Prior studies showed that *Bmal1* (a gene encoding a core molecular clock component) deletion induced alterations in bacterial abundances in feces, with differential effects based on sex.[64] Chin-Hee Song et al.[65] investigated the changes in mouse gut microbiome composition based on sex, NAOM/DSS-induced colorectal cancer (CRC), and Nrf2 genotype. Their results

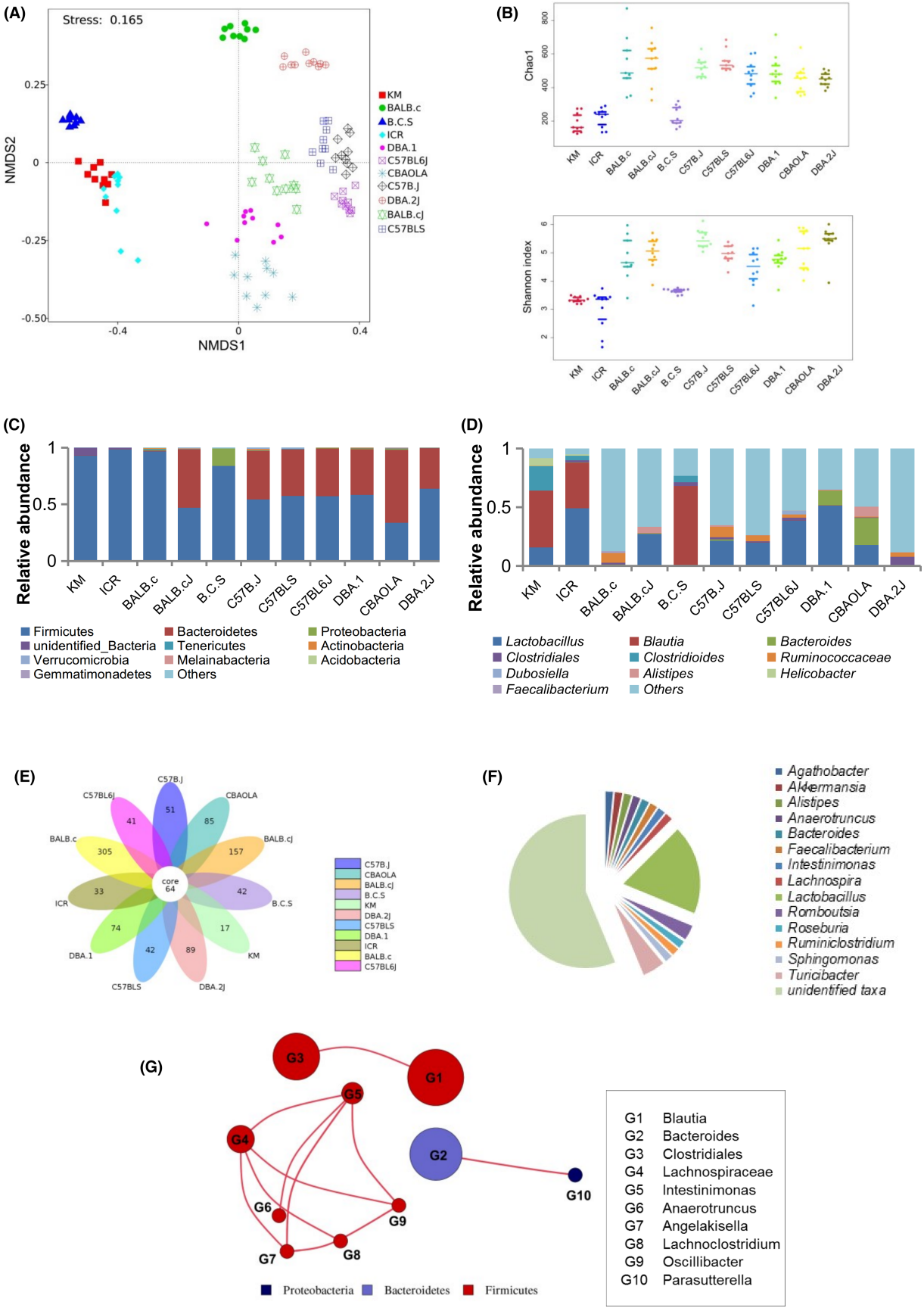


FIGURE 1 Bacterial community structures in normal mouse strains. (A) NMDS analysis of gut microbiota composition in normal mouse strains. (B) Chao1 and Shannon index in normal mouse strains. (C) Relative abundance of gut bacteria at the phylum level in normal mice strains. (D) Relative abundance of gut bacteria at the genus level in normal mice strains. (E) Flower plot. (F) 64 core of OTUs. (G) Correlation of 10 filtered key OTUs.

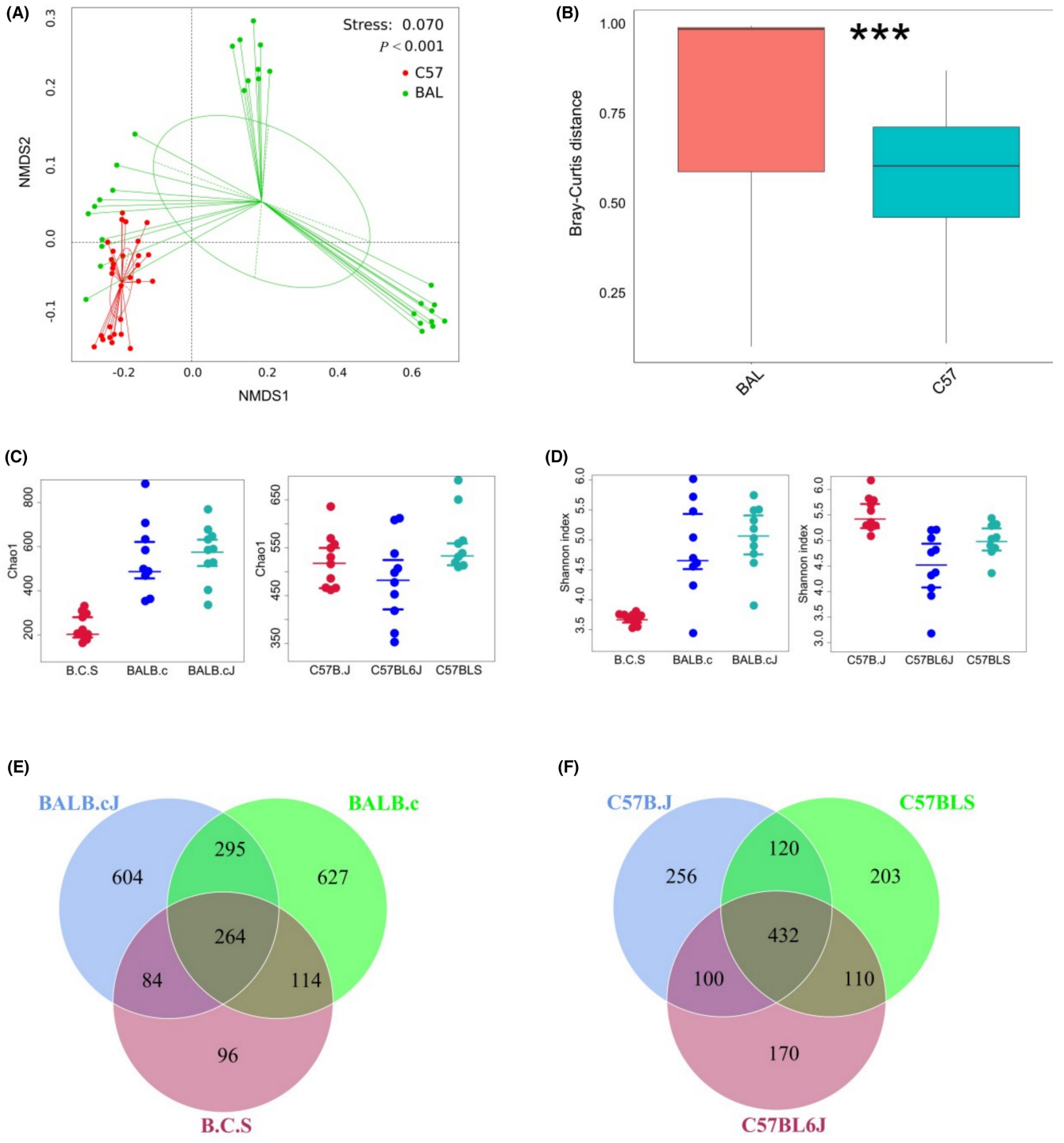
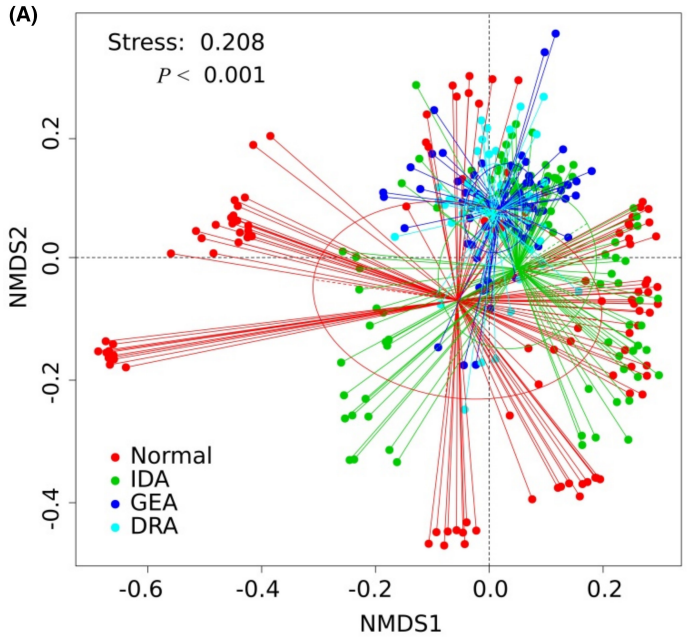
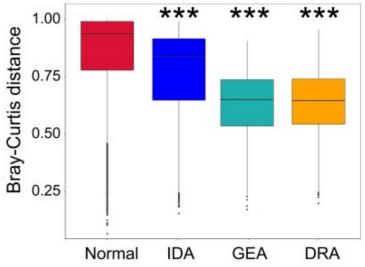
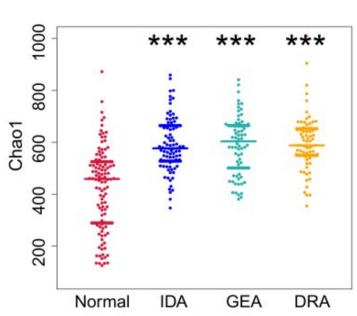


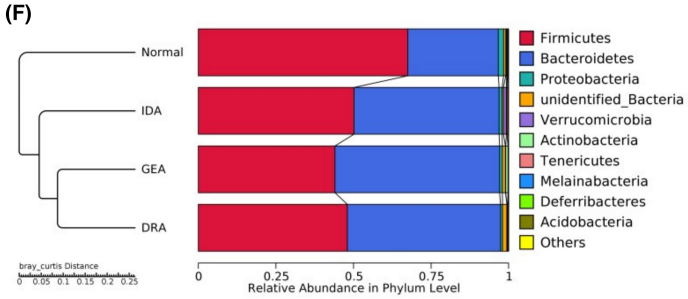
FIGURE 2 Robustness of the bacterial community in BALB/c (including packages BALB.cJ, BALB.c, and B.C.S.) and C57BL/6J (C57 including packages C57B.J, C57BLS, and C57BL6J) strains. (A) NMDS analysis of gut microbiota communities in BAL and C57 mice. (B) Comparison of inner Bray–Curtis distance in BAL and C57 mice. (C) Chao1 index of gut microbiota in three packages belonging to BAL and C57 mice. (D) Shannon index of gut microbiota in three packages belonging to BAL and C57 mice. (E,F) Venn diagram of three individual packages in BAL and C57 mice, $^{***}p < 0.001$.

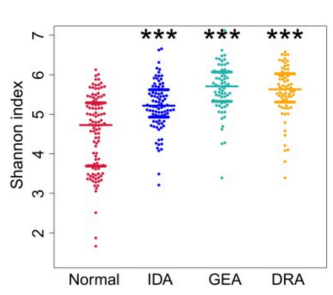
FIGURE 3 Characteristics of the bacterial community in diseased animal models of different types of disease. (A) NMDS analysis of gut microbiota communities. (B) Comparison of inner Bray–Curtis distance in normal, DRA, IDA, and GEA groups. (C) Chao1 index. (D) Shannon index. (E) Phylogenetic diversity (PD) index. (F) Cluster analysis based on the relative abundance of taxa at the phylum level. (G) Relative abundance of *Firmicutes* and *Bacteroidetes*. (H) Heatmap of changed genus in the DRA, IDA, and GEA groups compared with that in the normal group. Red: significantly decreased in all DRA, IDA, and GEA groups compared with the normal group; green: significantly increased in all DRA, IDA, and GEA groups compared with the normal group.

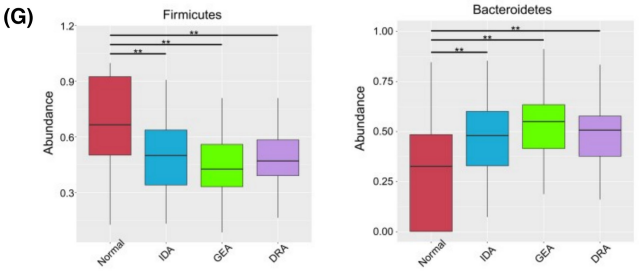

(A)
Stress: 0.208
P < 0.001
NMDS2
NMDS1
Normal
IDA
GEA
DRA

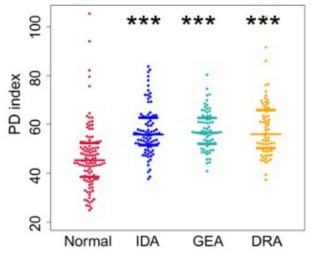

(B)
Bray-Curtis distance
***
Normal
IDA
GEA
DRA

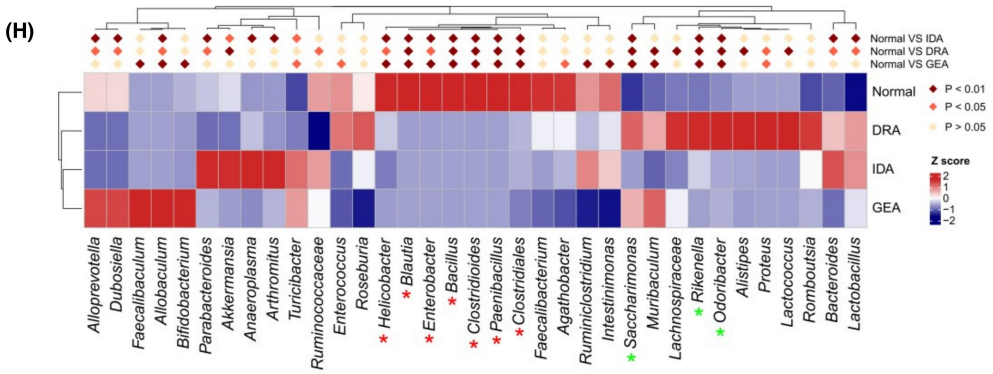

(C)
Chao1
***
Normal
IDA
GEA
DRA


(F)
Normal
IDA
GEA
DRA
Firmicutes
Bacteroidetes
Proteobacteria
unidentified_Bacteria
Verrucomicrobia
Actinobacteria
Tenericutes
Melainabacteria
Deferribacteres
Acidobacteria
Others
Relative Abundance in Phylum Level


(D)
Shannon index
***
Normal
IDA
GEA
DRA


(G)
Firmicutes
Bacteroidetes
Abundance
Normal
IDA
GEA
DRA


(E)
PD index
***
Normal
IDA
GEA
DRA


(H)
Normal VS IDA
Normal VS DRA
Normal VS GEA
Normal
DRA
IDA
GEA
P < 0.01
P < 0.05
P > 0.05
Z score
Alloprevotella
Dubosiella
Faecalibaculum
Allobaculum
Bifidobacterium
Parabacteroides
Akkermansia
Anaeroplasma
Arthromitus
Turicibacter
Ruminococcaceae
Enterococcus
Roseburia
Helicobacter
Blautia
Enterobacter
Bacillus
Clostridioides
Paenibacillus
Clostridiales
Faecalibacterium
Agathobacter
Ruminiclostridium
Intestinimonas
Saccharimonas
Muribaculum
Lachnospiraceae
Rikenella
Odoribacter
Alistipes
Proteus
Lactococcus
Romboutsia
Bacteroides
Lactobacillus

showed that the abundance of *Bacteroides vulgatus* was higher in WT CRC groups than in WT controls in both males and females. The abundance of *Akkermansia muciniphila* was not altered by Nrf2 KO. In contrast, the abundances of *Lactobacillus murinus* and *B. vulgatus* were changed differently by Nrf2 KO depending on sex and CRC. This suggested that gender is closely related to the composition of gut bacteria in transgenic animals. Our results also showed that with different introduced years, there was a significant difference in gut bacteria among all four classes. Several reports have shown that a comparative analysis of gut community composition from 19 different laboratory mice based on denaturing gradient gel electrophoresis showed significant differences in this composition in individual mice over the course of a few weeks,[66] and the composition of fecal metabolites from laboratory mice has also been shown to vary over time.[67] The gut microbiota of mice may therefore adapt to a new gut environment and interact with the host to form a new community. The introduced source significantly contributed to the normal, IDA, and DRA groups (Table 2). Our findings also revealed that the gut microbiota was different in mice with different introduced sources, and the possible interference of diet, water quality, and residential environment has been ruled out. Interestingly, the background strain (Figure 1A–D) contributed most to the differences in gut microbiota in experimental animals, and studies have shown that the mouse genetic component can play a role in determining gut microbiota composition and that a proportion of bacterial taxa are heritable.[68] Differences within the introduced years and introduced sources may have also influenced the changes in the gut microbiota in different mouse strains. However, background strains are the focus of experimental studies using different strains of mice. The IDA1 and GEA1 strains were developed by gene deletion, knockout, modification, or insertion, and therefore, in comparison with normal mice, gene changes were the most contributing factors. For the DRA1 strain, the introduced sources were the most contributing factors, and this may be due to the different interference factors of gut bacteria in different laboratories or experimental environments.

The gut microbiota community fluctuated widely in normal mice, and the microbiota community of each strain was almost completely separated (Figure 1A). A previous study has shown that the composition of the gut microbiota is influenced by inheritance.[69] For example, compared with the mouse strains from C57BL6J, C57B.J, and C57BLS isolation packages, those from BALB.C, BALB.CJ, and B.C.S. isolation packages had higher differential isolation. The Chao1 and Shannon results also corroborated this analysis (Figure 1B). C57BL/6J was bred in 1921, and the biggest characteristic of this inbred strain is genetic stability. The BALB/c strain is another widely used experimental mouse that is characterized by albino immune deficiency, easy reproduction, small weight difference between males and females, and extreme sensitivity to carcinogens. Therefore, BALB/c is often used to establish experimental animal models of immune deficiency diseases and of lung and kidney cancers.[70,71] In comparison, the genetic background of C57BL/6J mice seems to be purer, and the gut microbiota of C57BL/6J strain mice is more stable. Our results showed that the background strains were a significant factor affecting the composition of gut microbiota. From Figure 1E,F, we observed that among the 64 core OTUs (Figure 1E), the dominant microbiota was *Lactobacillus* from *Firmicutes* (Figure 1F). Meanwhile, according to Figure 1G, we observed stronger correlation of gut bacteria from *Firmicutes* in normal mice. This suggested that gut bacteria from *Firmicutes* in normal mice were the focus of the study, and gut bacteria from *Firmicutes* should be considered when analyzing the experimental results.

Thus, our results suggested that gut bacteria composition is more stable in mice with a stable genetic background (Figure 2A,B). In general, compared with normal BALB/c mice, the composition of the gut microbiota in C57BL/6J mice was more stable. Therefore, we believed that the composition of the gut microbiota in mice was influenced by multiple parameters rather than a single factor. Thus, experimental mice with stable gut bacteria may have lower feeding requirements and stronger resilience via more stable symbiosis with their gut bacteria. The gut bacteria of BALB/c mice vary greatly, and thus, BALB/c mice may be a closer model to humans, where the gut bacterial community can vary from person to person through multiple variables, including regional, dietary, and genes.[72] Therefore, BALB/c may play a greater role in clinical research.

At the phylum and genus levels, there were significant differences between the species and quantity of gut microbiota of normal mice and those in the different background strains (Figure 1C,D). Sixty-four common OTUs (Figure 1E) were measured, and the dominant gut microbiota belonging to *Lactobacillus* (Figure 1E,F) was identified. *Lactobacillus* is a large group of miscellaneous gram-positive bacteria that ferment sugars into lactic acid. In humans, many *Lactobacillus* are essential and perform important physiological functions. Studies have shown that lactic acid bacteria can improve gastrointestinal function and immunity, maintain the balance of gut bacteria, and provide nutrients and other functions. For example, a study in lupus-nephritis mice showed that *Lactobacillus* spp. in the gut microbiota exert anti-inflammatory effects by repairing the damaged gut barrier, suppressing proinflammatory factors in the lymphatic circulation, and improving the ratio of regulatory versus pathogenic T cells, thereby attenuating kidney inflammation.[73]

In this study, we performed association analysis on common microbiota in healthy mice (Figure 1G). Compared with the OTUs for *Bacteroidetes* and *Proteobacteria*, the OTUs of *Firmicutes* were more closely related to each other. Studies have shown that polysaccharides, including inulin oligosaccharides or galactose oligosaccharides, can be metabolized by *Bifidobacterium* or *Lactobacillus* into lactic or acetic acid, and these metabolites can then be metabolized by *Anaerostipes*. These metabolites can be converted to butyrate by *Eubacterium* (the nearest neighbor of *Lachnospiraceae*-incertae-sedis), *Allobaculum*, and *Roseburia*,[74] suggesting that the gut microbiota interacts to help in maintaining host health. A decrease in the abundance of the gut bacteria can be compensated by supplementation with other intestinal symbiotic bacteria to maintain normal life activities. Our results indicated that recovering the network of OTUs of *Firmicutes* may be an important route to maintaining health.

Bacteroidetes and *Firmicutes* were the most abundant bacteria in the four groups studied here. Our results showed that compared with that in normal mice, the ratio of *Firmicutes* to *Bacteroidetes* in mice of diseased strains significantly decreased (Figure 3F,G). In clinical research, the relative contents of *Bacteroidetes* and *Firmicutes* are often used to explore the impact on the organism. The fermentable fiber in feed has been shown to change the microbial composition in the gut and lungs, especially by altering the proportion of *Bacteroidetes* and *Firmicutes*.[75] In autoimmune diseases, the ratio of *Firmicutes* to *Bacteroidetes* in immunodeficient patients can be lower than that in healthy subjects,[76] and this ratio was changed in diabetic mice with NLRP3-KO,[77] These results suggest that when experimental animals are used, the ratio of *Firmicutes* and *Bacteroidetes* in these strains may affect the experimental results, and the number of *Firmicutes* and *Bacteroidetes* should be considered during subsequent analysis. In this study, we observed decreased abundances of *Helicobacter*, *Blautia*, *Enterobacter*, *Bacillus*, *Clostridioides*, *Paenibacillus*, and *Clostridiales* and increased abundances of *Saccharimonas*, *Rikenella*, and *Odoribacter* in the DRA group (Figure 3H), suggesting that when various types of mice are used to study diseases, the content of and changes in dominant gut microbiota should be included in the detection focus.

Understanding the status of gut microbiota in mice and using high-quality experimental animals can greatly improve the accuracy of the experimental results. Recent research on gut microbiota has focused on the contribution of the gut microbiota in various diseases, as well as the role of environments and facilities or/and between different manufacturers. By contrast, the bacterial community structure of representative mouse strains from different backgrounds of the same manufacturer has rarely been investigated, and our study helps in compensating for this deficiency. Future work will focus on observing the changes in the gut microbiota in experimental animals in the process of biological research and clinical translations studies and on emphasizing the influence of the gut microbiota on the repeatability and accuracy of experimental animals in studies of different types of diseases.

5 | CONCLUSION

This study was designed to determine the composition, structure, and gut bacteria in different strains of mice provided by the same commercial sources. Our findings argue that eliminating the interference of diet, manufacturer, facility, and feeding environment, the background strains had the most effect on the gut microbiota of experimental mice. The most representative changes in bacterial abundance were found with *Bacteroidetes* and *Firmicutes* in the normal, IDA, GEA, and DRA groups. This study suggests that it is necessary to assess the composition of gut bacteria in mice in future basic and clinical studies and provides biological implications of differences between different mouse strains. In future studies, the influence of gut microbiota in experimental animals should be considered.

AUTHOR CONTRIBUTIONS

Jianguo Guo, Zhiguang Xiang, and Chuan Qin designed the study; Yunbo Liu, Xuying Wu, Wei Dong, and Hua Zhu performed the experiments; Jianguo Guo and Chenchen Song analyzed the data; Jianguo Guo and Chenchen Song wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest. Jianguo Guo and Chuan Qin are editorial board members of Animal Models and Experimental Medicine and a coauthors of this article. To minimize bias, they were excluded from all editorial decision making related to the acceptance of this article for publication.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Animal Ethics is IACUC-20220415.

ORCID

Jianguo Guo https://orcid.org/0000-0002-9811-2590
Zhiguang Xiang https://orcid.org/0000-0002-9317-7766
Chuan Qin https://orcid.org/0000-0002-6261-1232

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