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Taxonomic composition and variation in the gut microbiota of laboratory mice

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Abstract
The gut microbiota can affect host health, including humans. Mouse models have been used extensively to study the relationships between the host and the gut microbiota. With the development of cost-effective high-throughput DNA sequencing, several methods have been used to identify members of the gut microbiota of laboratory mice. In recent years, the amount of research and knowledge about the mouse gut microbiota has exploded, leading to significant breakthroughs in understanding of the taxonomic composition of and variation in this community. In addition, the rapidly increasing volume of data has allowed the development of public resources for exploring the mouse gut microbiota. In this review, we describe the concepts and pros and cons of basic methodologies that can be used to determine the gut bacterial profile in laboratory mice. We also present the key bacterial components of the mouse gut microbiota from the phylum to the species level and then compare them with those identified in other references. Additionally, we discuss variations in the mouse gut microbiota and their association with experiments using mice. Finally, we summarize the properties and functions of currently available public resources for exploring the mouse gut microbiota.

Introduction

The gut microbiota is the complex community of microorganisms that lives in the intestine of the host (Sommer and Bäckhed 2013). These microorganisms consist mainly of bacteria and some archaea, fungi, protozoa, and viruses, and they can be much greater in number than host somatic cells (Backhed 2009; Sommer and Bäckhed 2013). The human gut microbiota have received widespread attention because of its association with human health (Tremaroli and Bäckhed 2012). Differences in the composition of the human gut microbiota have been linked to various diseases, including Alzheimer's disease (Zhuang et al. 2018), depression (Naseribafrouei et al. 2014), inflammatory bowel disease (Ni et al. 2017), obesity (Ley et al. 2006), and Parkinson's disease (Scheperjans et al. 2015).

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Animal models of those diseases are likely to be affected by the gut microbiota through mechanisms similar to those in humans (Ericsson et al. 2015). In addition, animal models allow for the controlled experiments needed to identify causal relationships between the gut microbiota and associated diseases (Ericsson et al. 2015). Thus, animal models have been powerful tools in gut microbiota research (Heinritz et al. 2013; Nguyen et al. 2015; Stagaman et al. 2020). Among animal models, laboratory mice are used extensively in studies of the gut microbiota (Nguyen et al. 2015). There are numerous advantages of using mouse models (Nguyen et al. 2015): (1) Elements of their physiology and anatomical structures show similarities to those of humans. (2) Mice are complemented with extensive knowledge of gastroenterology, genetics, and immunology. (3) They have a high reproductive rate with a short life cycle, and their cost of maintenance is lower than that of other mammalian models.

To study the gut microbiota in laboratory mice, researchers had relied primarily on culture-based methods before the advent of next-generation sequencing (Gordon and Dubos 1970). The development of next-generation sequencing had revolutionized this field of research, allowing culture-based methods to be complemented by culture-independent methods, such as amplicon sequencing and shotgun sequencing (Misic et al. 2018). Dramatic advances in next-generation

sequencing have reduced sequencing costs (Metzker 2010), allowing large-scale analysis, even by individual laboratories (Shi et al. 2019). The amount of research and knowledge available on the mouse gut microbiota has exploded, leading to significant breakthroughs in understanding the taxonomic composition and variation in the gut microbiota of laboratory mice. In addition, the increase in available data has spurred the development of public resources to quickly navigate these datasets (Oliveira et al. 2018).

In this review, we describe the methods for determining the taxonomic profiles of bacterial communities in gastrointestinal samples from laboratory mice. We also present the core and pan microbiota of mice and discuss variations in the mouse gut microbiota that may affect reproducibility. Finally, we summarize the public resources for exploring profiles of the mouse gut microbiota.

Basic methodologies for mouse gut microbiota analysis

One of the major issues in microbiota analysis is the precise identification of the microbes that make up the microbiota (Ranjan et al. 2016). Given this consideration, we briefly explore three major approaches that can be used to determine the gut bacterial profile in mice: culture-based method, amplicon sequencing, and shotgun sequencing (Fig. 1).

Culture-based method

A culture-based method, also known as culturomics, detects the bacteria present in the sample using bacterial culture. In mouse gut microbiota analysis, the starting materials can be intestinal mucosal, luminal, or fecal samples collected from mice (Lagkouvardos et al. 2016b). The diluted samples are then plated on agar media and incubated under aerobic conditions or in an anaerobic chamber to simulate the intestinal environment (Lagkouvardos et al. 2016b). Single colonies of bacteria appear on agar media after incubation (Liu et al. 2020). To identify the bacteria, the pioneering work of the 1960s focused only on phenotypic features (Gordon and Dubos 1970; Lagkouvardos et al. 2016b). Recent studies have extracted DNA from the bacterial colonies and utilized the DNA sequence information as well as the phenotypic features for identification (Lagkouvardos et al. 2016b; Liu et al. 2020). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) can also be applied for identification (Lagier et al. 2018). The

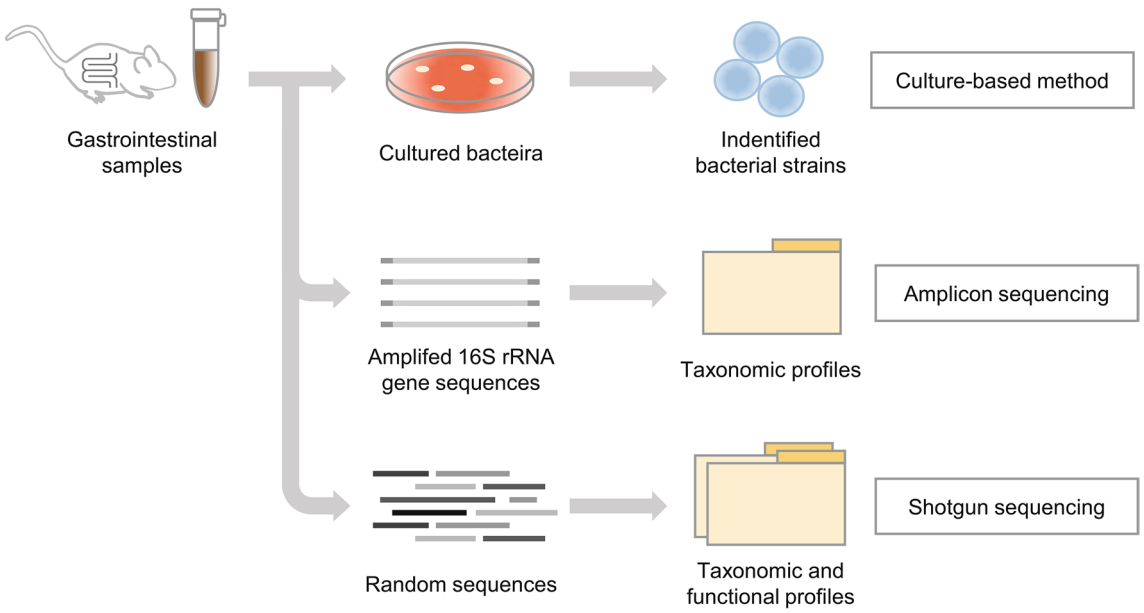

Fig. 1 There are three main approaches to determine the intestinal microbial composition of laboratory mice. In the culture-based method, bacterial cultures are used to detect the bacteria present in intestinal samples from the mice. Bacterial strains are identified by comparing the extracted 16S rRNA gene sequences to the 16S rRNA gene database, the extracted genomic sequences to the genome database, through matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) or through phenotypic features. New bacterial species can be discovered with this method. In amplicon sequencing, the amplified 16S rRNA gene sequences are sequenced and then compared to the 16S rRNA gene database to obtain taxonomic profiles. In shotgun sequencing, random sequences are sequenced and then compared against the genome database to obtain taxonomic profiles. Functional profiles can also be obtained from the shotgun sequencing

colonies can be preserved for further characterization and reference (Liu et al. 2020).

The main drawback of this method is that identifying the bacterial profile is biased toward microorganisms that grow rapidly on the agar media used, which may not reflect the actual abundance or significance in the gut (Boase et al. 2013; Koeller et al. 2018). Additionally, this method takes more time than culture-independent methods (McLain et al. 2016). Nevertheless, the value of the culture-based method should not be underestimated, as bacterial isolates are useful resources for studying phenotypic characteristics (McLain et al. 2016). Additionally, obtaining reference genomes of bacteria, which can be achieved with the culture-based method, is essential for later identification (Greenblum et al. 2015; Lagkouvardos et al. 2016b). In addition, the cultured bacterial genomes serve as references, facilitating the interpretation of metagenomic studies (Lagier et al. 2018).

Amplicon sequencing

Amplicon sequencing is a culture-independent method that is capable of analyzing the mouse gut microbiota with high resolution and outstanding throughput (Jovel et al. 2016). It is the most commonly used method of determining the microbiota profiles by sequencing amplified products of a phylogenetic marker (Ranjan et al. 2016; Tessler et al. 2017). The 16S ribosomal RNA (rRNA) gene is the most commonly used phylogenetic marker for bacteria (Fricker et al. 2019). This gene consists of highly conserved regions that can be recognized by primers for PCR amplification and taxon-specific hypervariable regions that are utilized for taxonomic classification (D'Amore et al. 2016; Fricker et al. 2019). Amplified sequences are clustered into operational taxonomic units (OTUs) based on sequence similarity, and representative sequences of OTUs are compared to 16S microbial databases for classification (Johnson et al. 2019). Meanwhile, the amplified sequences can also be classified using amplicon sequence variant (ASV) methods. The ASV methods distinguish between biological sequences and errors by assuming that the biological sequences are observed more frequently than the errors (Callahan et al. 2017). Callahan and colleagues compared ASVs to de novo OTUs, where OTU clusters are created only from observed reads, and closed-reference OTUs, where observed reads are recruited to the corresponding sequences in the reference database: ASVs can capture all biological variation in the sample, which cannot be performed with closed-reference OTUs, and ASVs can be compared validly between datasets, which cannot be performed with de novo OTUs (Callahan et al. 2017). Therefore, some studies recommended the use of ASVs instead of OTUs in targeted sequencing (Callahan et al. 2017; Caruso et al. 2019).

The downside of this method is that it poses some limitations on taxonomic and functional resolution. The taxonomic classification is often less accurate at the species level (Johnson et al. 2019; Ranjan et al. 2016). Functional profiles can be obtained by predictions using a tool such as PICRUSt2 (Douglas et al. 2020), but these predictions are less accurate than direct gene identification (Ranjan et al. 2016). However, amplicon sequencing is cost-effective, making it suitable for large-scale analysis (Jovel et al. 2016; Ranjan et al. 2016). Additionally, there is a large amount of well-curated sequence data that can be referenced in public databases such as EzBioCloud (Yoon et al. 2017), Greengenes (McDonald et al. 2012), RDP (Cole et al. 2014), and SILVA (Quast et al. 2013).

Shotgun sequencing

Shotgun sequencing is another culture-independent method. It randomly fragments the total DNA present in the sample and sequences the resulting millions of short reads instead of a single phylogenetic marker (Fricker et al. 2019; Sunagawa et al. 2013). Accordingly, the reads are sampled from all microorganisms, including bacteria, archaea, fungi, protozoa, and viruses (Fricker et al. 2019; Sommer and Bäckhed 2013). Taxonomic profiles can be obtained using reference databases based on marker genes (Truong et al. 2015) or bacterial core genes (Chalita et al. 2020).

Shotgun sequencing is expensive and requires extensive comparisons between the references and the generated reads (Fricker et al. 2019; Ranjan et al. 2016). Additionally, there is much room for improvement in genome databases compared to the well-curated 16S databases (Chun et al. 2018; Tessler et al. 2017). However, shotgun sequencing provides better taxonomic and functional resolution than amplicon sequencing. It can more accurately detect bacterial species and even within-species variation if a reference database is available (Fricker et al. 2019; Garud et al. 2019; Ranjan et al. 2016). Therefore, shotgun sequencing may outperform amplicon sequencing as the reference databases continue to grow in size (Tessler et al. 2017). Additionally, direct gene identification through shotgun sequencing can provide functional information on the microbiota (Fricker et al. 2019).

Intestinal core- and pan-microbiota of laboratory mice

In this section, we analyzed the intestinal core- and pan-microbiota of laboratory mice to understand the taxonomic composition. We then discuss some of the notable bacterial taxa of the mouse gut microbiota at various levels.

Intestinal core- and pan-microbiota analysis

A study by Liu et al. defined the core- and pan-microbiota representing bacteria present in almost all mouse intestinal samples and subsets of mouse intestinal samples, respectively (Liu et al. 2020). We explored the core- and pan-taxa of the mouse gut microbiota using the same criteria: the core-taxa were those with a frequency of occurrence (FO) greater than 80% and an average relative abundance (RA) greater than 0.1%, and the pan-taxa were those with a FO greater than 5%. The FO is calculated as the number of samples that contain the taxon divided by the total number of samples, and the RA is the number of members of the taxon divided by the number of total reads at the same level in the sample. For example, the FO of Firmicutes is defined as 100% when the taxon is present in all samples, and the RA of Firmicutes is defined as 100% when Firmicutes is the only taxon at the phylum level.

Six mouse datasets (243 samples in total) were used in the analysis (Campbell et al. 2012; Casero et al. 2017; Ericsson et al. 2015; Rosshart et al. 2019; Tam et al. 2020; Zmora et al. 2018). We included cecal and fecal samples, which are the primary samples used in mouse studies (Gu et al. 2013), from mice of diverse backgrounds, as shown in Supplementary Table 1. The datasets consisted of the V4 hypervariable regions of the bacterial 16S rRNA genes. We determined the bacterial profiles using the EzBioCloud pipeline with 16S database version PKSSU4.0 (Yoon et al. 2017) and analyzed the gut core- and pan-microbiota (Table 1). We used EzBioCloud, because this database was composed of quality-controlled 16S rRNA gene sequences representing bacterial species and phylotypes (Yoon et al. 2017). The detailed methods are described in Supplementary Material 1. We classified the taxa into five different statuses: Valid name, Invalid name, *Candidatus*, Phylotype, and Group. The valid names are given to the standard type of taxa. The invalid names are similar to the valid names except that they are not published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM). The phylotypes are the taxa identified by DNA sequences that do not have enough supporting data to validate their name. *Candidatus* names are for the candidate taxa that cannot be cultivated as pure cultures. Lastly, as mentioned above, it is sometimes difficult to distinguish species by the 16S rRNA gene (Johnson et al. 2019; Ranjan et al. 2016); in these cases, we combined them under the Group label. Additionally, when members of the same group included different taxa at a higher taxonomic level, a new group was created at that level and labeled as Group.

Table 1 The number of bacterial core- and pan-taxa of the laboratory mouse gut

Taxonomic level	#Core-taxa	#Pan-taxa
Phylum	5	9
Class	6	17
Order	5	24
Family	10	44
Genus	45	287
Species	43	877
Total	114	1258

Notable bacterial taxa at various levels

At the phylum level, we found five core-taxa and nine pan-taxa (Table 1). Detailed information on these core- and pan-taxa, including the taxonomy, average RA, FO and status, can be found in Table 2 and Supplementary Table 2a. Firmicutes and Bacteroidetes were the two major phyla with the highest average RAs and FOs, as in other mammalian hosts (Ericsson et al. 2015). Proteobacteria, Tenericutes, and Actinobacteria were also identified as the core-taxa, in line with the previous studies (Wang et al. 2018; Zhang et al. 2018). Verrucomicrobia and Deferribacteres were the pan-taxa in our analysis and were known as phyla found in the mouse gut according to the previous results (Nagpal et al. 2018; Rosshart et al. 2017).

Six classes were recognized as the core classes, and 17 were recognized as the pan classes (Table 1). Detailed information on these core- and pan-taxa can be found in Table 3 and Supplementary Table S2b. Most previous studies showed that the dominant bacterial classes of the mouse gut microbiota were Clostridia and Bacilli, belonging to Firmicutes, and Bacteroidia, belonging to Bacteroidetes; these were also the major core classes in our results (Gorecki et al. 2019; Jin et al. 2015; Yu et al. 2016; Zhang et al. 2018). Mollicutes, Erysipelotrichi, and Gammaproteobacteria were the remaining core classes, which were also mentioned in the previous publications as members of the intestinal microbiota of mice (Gorecki et al. 2019; Jin et al. 2015; Zhang et al. 2018). The pan classes included Verrucomicrobiae and Epsilonproteobacteria, as in the previous studies (Jin et al. 2015; Yu et al. 2016).

Table 2 The gut core-microbiota of laboratory mice at the phylum level

Taxonomy	Name	Average RA (%)	FO (%)	Status
Firmicutes	Firmicutes	63.91	100.00	Valid name
Bacteroidetes	Bacteroidetes	24.41	100.00	Valid name
Proteobacteria	Proteobacteria	1.80	98.77	Valid name
Tenericutes	Tenericutes	2.89	93.83	Valid name
Actinobacteria	Actinobacteria	1.95	81.07	Valid name

There were five core orders and 24 pan orders in our results (Table 1). Detailed information on these core- and pan-taxa can be found in Table 4 and Supplementary Table 2c. Clostridiales, Bacteroidales, and Lactobacillales were described as the main bacterial orders in other studies and identified as the core orders (Nozu et al. 2016; Yu et al. 2016). Meanwhile, Campylobacterales, one of the pan orders, was referred to be included in the intestinal microbiota of mice in a study by Yu and colleagues (Yu et al. 2016).

The core- and pan-microbiota of the mouse gut belonged to 10 bacterial families and 44 bacterial families, respectively (Table 1). Detailed information on these core- and pan-taxa can be found in Table 5 and Supplementary Table S2d. A study by Hildebrand et al. classified laboratory mice into two enterotypes based on the bacterial composition of their intestinal microbiota: one dominated by *Lachnospiraceae* and *Ruminococcaceae* and the other dominated by *Bacteroidaceae* and *Enterobacteriaceae* (Hildebrand et al. 2013; Nguyen et al. 2015), all of which were the core families except for *Enterobacteriaceae* (belonging to the pan families). Members of *Muribaculaceae*, previously known as family S24-7, were major bacterial components of the mouse gut (Lagkouvardos et al. 2019), and *Muribaculaceae* was detected as the core family with high average RA and FO. The stomachs and small intestines of mice contain many facultative bacteria such as *Lactobacillaceae* due to high oxygen levels (Gu et al. 2013). However, members of the *Lactobacillaceae* are also found in the cecum and feces and were identified as the core-taxa in our results from cecal and fecal samples, probably because they can migrate from the forestomach to the cecum and feces (Nagpal et al. 2018; Walter 2008). The core families also included *Rikenellaceae*, consistent with a previous study (Gu et al. 2013). Several groups reported that the mouse gut was dominated by bacteria within *Porphyromonadaceae*, *Desulfovibrionaceae*, *Deferribacteraceae*, *Prevotellaceae*, and *Helicobacteraceae*, which were identified as the pan families in our results.

Table 3 The gut core-microbiota of laboratory mice at the class level

Taxonomy	Name	Average RA (%)	FO (%)	Status
Firmicutes;Clostridia	Clostridia	57.63	100.00	Valid name
Bacteroidetes; Bacteroidia	Bacteroidia	24.40	100.00	Valid name
Firmicutes; Bacilli	Bacilli	4.00	93.83	Valid name
Tenericutes; Mollicutes	Mollicutes	2.89	93.83	Valid name
Firmicutes; Erysipelotrichi	Erysipelotrichi	2.28	88.48	Valid name
Proteobacteria; Gammaproteobacteria	Gammaproteobacteria	0.27	85.60	Valid name

Table 4 The gut core-microbiota of laboratory mice at the order level

Taxonomy	Name	Average RA (%)	FO (%)	Status
Firmicutes; Clostridia; Clostridiales	Clostridiales	57.60	100.00	Valid name
Bacteroidetes; Bacteroidia; Bacteroidales	Bacteroidales	24.40	100.00	Valid name
Firmicutes; Bacilli; Lactobacillales	Lactobacillales	4.00	93.83	Valid name
Firmicutes; Erysipelotrichi; Erysipelotrichales	Erysipelotrichales	2.28	88.48	Valid name
Tenericutes; Mollicutes; PAC001057_o	PAC001057_o	0.67	84.36	Phylotype

Table 5 The gut core-microbiota of laboratory mice at the family level

Taxonomy	Name	Average RA (%)	FO (%)	Status
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*;	*Lachnospiraceae*	32.88	100.00	Valid name
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*	*Ruminococcaceae*	17.26	100.00	Valid name
Firmicutes; Clostridia; Clostridiales; *Christensenellaceae*	*Christensenellaceae*	6.57	100.00	Valid name
Bacteroidetes; Bacteroidia; Bacteroidales; *Muribaculaceae*	*Muribaculaceae*	13.13	99.18	Invalid name
Firmicutes; Clostridia; Clostridiales; Dehalobacterium_f	Dehalobacterium_f	0.13	98.77	Phylotype
Firmicutes; Clostridia; Clostridiales; Mogibacterium_f	Mogibacterium_f	0.34	90.95	Phylotype
Firmicutes; Bacilli; Lactobacillales; *Lactobacillaceae*	*Lactobacillaceae*	3.97	90.53	Valid name
Firmicutes; Erysipelotrichi; Erysipelotrichales; *Erysipelotrichaceae*	*Erysipelotrichaceae*	2.28	88.48	Valid name
Bacteroidetes; Bacteroidia; Bacteroidales; *Rikenellaceae*	*Rikenellaceae*	5.09	88.07	Valid name
Bacteroidetes; Bacteroidia; Bacteroidales; *Bacteroidaceae*	*Bacteroidaceae*	3.19	81.89	Valid name

The mouse gut had 45 core-genera and 287 pan-genera (Table 1). Detailed information on these core- and pan-taxa can be found in Table 6 and Supplementary Table S2e. A study by Wang et al. defined 37 core-genera, and a study by Xiao et al. identified the top 20 most abundant genus-level core bacteria from mice of diverse backgrounds (Wang

Table 6 The gut core-microbiota of laboratory mice at the genus level

Taxonomy	Name	Average RA (%)	FO (%)	Status
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Oscillibacter*	*Oscillibacter*	5.96	100.00	Valid name
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Pseudoflavonifractor*	*Pseudoflavonifractor*	4.62	100.00	Valid name
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159538_g	KE159538_g	4.08	100.00	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC000664_g	PAC000664_g	2.56	100.00	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001092_g	PAC001092_g	1.01	100.00	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001043_g	PAC001043_g	0.91	100.00	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Clostridium_g21	Clostridium_g21	0.63	100.00	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC000692_g	PAC000692_g	0.97	99.59	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001372_g	PAC001372_g	0.42	99.59	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; *Anaerotignum*	*Anaerotignum*	0.22	99.59	Valid name
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; *Frisingicoccus*	*Frisingicoccus*	0.22	99.59	Valid name
Firmicutes; Clostridia; Clostridiales; *Christensenellaceae*; PAC001360_g	PAC001360_g	4.43	99.18	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; LLKB_g	LLKB_g	2.00	99.18	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159571_g	KE159571_g	1.86	99.18	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159600_g	KE159600_g	0.52	99.18	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001165_g	PAC001165_g	0.33	99.18	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Clostridium_g24	Clostridium_g24	0.57	98.77	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; PAC001402_g	PAC001402_g	0.28	98.77	Phylotype
Firmicutes; Clostridia; Clostridiales; Dehalobacterium_f; PAC001221_g	PAC001221_g	0.12	98.35	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001524_g	PAC001524_g	0.15	97.94	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Anaerotruncus*	*Anaerotruncus*	0.22	97.53	Valid name
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Eubacterium_g6	Eubacterium_g6	3.51	97.12	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; *Acetatifactor*	*Acetatifactor*	0.72	97.12	Valid name
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; PAC000661_g	PAC000661_g	1.97	96.71	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Acutalibacter*	*Acutalibacter*	0.73	96.30	Valid name
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Eubacterium_g17	Eubacterium_g17	0.39	95.88	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159605_g	KE159605_g	0.33	95.88	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001199_g;	PAC001199_g	0.29	95.88	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001225_g	PAC001225_g	0.54	95.47	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001516_g	PAC001516_g	0.38	95.06	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001138_g	PAC001138_g	0.16	94.24	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Caproiciproducens*	*Caproiciproducens*	0.22	93.00	Valid name
Firmicutes; Bacilli; *Lactobacillales*; *Lactobacillaceae*; *Lactobacillus*	*Lactobacillus*	3.96	90.53	Valid name
Bacteroidetes; Bacteroidia; Bacteroidales; *Muribaculaceae*; PAC001068_g	PAC001068_g	3.10	90.53	Phylotype
Bacteroidetes; Bacteroidia; Bacteroidales; *Muribaculaceae*; PAC000186_g	PAC000186_g	2.63	90.12	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001091_g	PAC001091_g	0.36	90.12	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001385_g	PAC001385_g	0.12	89.71	Phylotype
Firmicutes; Clostridia; Clostridiales; *Christensenellaceae*; PAC001219_g	PAC001219_g	0.76	88.07	Phylotype
Bacteroidetes; Bacteroidia; Bacteroidales; *Rikenellaceae*; *Alistipes*	*Alistipes*	4.90	87.24	Valid name
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; Eubacterium_g23	Eubacterium_g23	0.66	86.83	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Agathobaculum*	*Agathobaculum*	0.24	86.83	Valid name
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; *Lachnospira*	*Lachnospira*	0.36	86.01	Valid name
Bacteroidetes; Bacteroidia; Bacteroidales; *Bacteroidaceae*; *Bacteroides*	*Bacteroides*	3.19	81.89	Valid name
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001228_g	PAC001228_g	0.36	81.89	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Eubacterium_g4	Eubacterium_g4	0.25	80.66	Phylotype

et al. 2019a; Xiao et al. 2015). We compared the core- and pan-genera identified in this work with the results of the two studies (Table 7). Five core-genera were identified in both studies: *Alistipes*, *Anaerotruncus*, *Bacteroides*, *Lactobacillus*, and *Pseudoflavonifractor*. Two core-genera were only detected in the study by Wang et al.: *Acetatifactor* and *Oscillibacter*. In the case of the pan-genera, both studies included seven genera: *Blautia*, *Marvinbryantia*, *Odoribacter*, *Parabacteroides*, *Prevotella*, *Roseburia*, and *Ruminococcus*. Ten pan-genera were only found in the study by Wang et al.: *Alloprevotella*, *Bifidobacterium*, *Eggerthella*, *Enterorhabdus*, *Gordonibacter*, *Helicobacter*, *Mucispirillum*, *Olsenella*, *Parasutterella*, and *Turicibacter*, and four pan-genera were only included in the study by Xiao et al.: *Clostridium*, *Coprobacillus*, *Desulfovibrio*, and *Enterococcus.* Moreover, several genera not found in our results were found in the Wang et al. and Xiao et al. studies. There were 13 genera in the former (*Allobaculum*, *Anaerofilum*, *Anaerostipes*, *Barnesiella*, *Clostridium* XIVa, *Clostridium* XlVb, *Erysipelotrichaceae_incertae_sedis*, *Flavonifractor*, *Intestinimonas*, *Lachnoanaerobaculum*, *Lachnospiracea_incertae_sedis*, *Rikenella*, and *Saccharibacteria_genera_incertae_sedis*) and four genera in the latter (*Butyrivibrio*, *Coprococcus*, *Eubacterium*, and *Faecalibacterium*).

The genus *Bacteroides* dominates one of the two enterotypes identified in wild mice (Wang et al. 2014). The genus was detected as the core genus in our results, but *Robinsoniella*, which dominates the other enterotype, was not found. *Mucispirillum*, one of the pan-genera, colonizes only the mucus layer of the laboratory mouse gut but does not colonize the human gut (Krych et al. 2013; Robertson et al. 2005). "*Candidatus* Arthromitus" was also included in the pan-genera. "*Candidatus* Arthromitus" had been a candidate genus name for segmented filamentous bacteria (SFB) designated recently as "*Candidatus* Dwaynesavagella" (Oren et al. 2020; Snel et al. 1995; Thompson et al. 2012). SFB have intrasegmental bodies and reside primarily in the terminal ileum of mice (Hedblom et al. 2018; Ivanov et al. 2009). They are known to potentially trigger host immune responses, such as the accumulation of T helper 17 (Th17) cells, a subset of CD4 + T cells that produce the cytokine interleukin-17 (Ivanov et al. 2009; Wang et al. 2019b). SFB have positive effects in mediating protective immunity but have adverse effects in promoting autoimmune diseases (Lee et al. 2011; Talham et al. 1999; Thompson et al. 2013; Wu et al. 2010).

There were 43 core-taxa and 877 pan-taxa identified at the species level in our survey (Table 1). Detailed information on the core- and pan-taxa can be found in Table 8 and Supplementary Table 2f. Most taxa were classified as either Phylotype or Group. The pan species included *Akkermansia muciniphila*, an intestinal mucin-degrading bacterium (Derrien et al. 2004). It is present in healthy humans and

Table 7 Bacterial core-genera of the laboratory mouse gut identified by Wang et al. and Xiao et al.

Name	Feature	Wang et al. 2019	Xiao et al. 2015
Alistipes	Core/Pan	O	O
Anaerotruncus	Core/Pan	O	O
Bacteroides	Core/Pan	O	O
Lactobacillus	Core/Pan	O	O
Pseudoflavonifractor	Core/Pan	O	O
Acetatifactor	Core/Pan	O	X
Oscillibacter	Core/Pan	O	X
Blautia	Pan	O	O
Marvinbryantia	Pan	O	O
Odoribacter	Pan	O	O
Parabacteroides	Pan	O	O
Prevotella	Pan	O	O
Roseburia	Pan	O	O
Ruminococcus	Pan	O	O
Alloprevotella	Pan	O	X
Bifidobacterium	Pan	O	X
Eggerthella	Pan	O	X
Enterorhabdus	Pan	O	X
Gordonibacter	Pan	O	X
Helicobacter	Pan	O	X
Mucispirillum	Pan	O	X
Olsenella	Pan	O	X
Parasutterella	Pan	O	X
Turicibacter	Pan	O	X
Clostridium	Pan	X	O
Coprobacillus	Pan	X	O
Desulfovibrio	Pan	X	O
Enterococcus	Pan	X	O
Allobaculum	Not included	O	X
Anaerofilum	Not included	O	X
Anaerostipes	Not included	O	X
Barnesiella	Not included	O	X
Clostridium XIVa	Not included	O	X
Clostridium XlVb	Not included	O	X
Erysipelotrichaceae_incertae_sedis	Not included	O	X
Flavonifractor	Not included	O	X
Intestinimonas	Not included	O	X
Lachnoanaerobaculum	Not included	O	X
Lachnospiracea_incertae_sedis	Not included	O	X
Rikenella	Not included	O	X
Saccharibacteria_genera_incertae_sedis	Not included	O	X
Butyrivibrio	Not included	X	O
Coprococcus	Not included	X	O
Eubacterium	Not included	X	O
Faecalibacterium	Not included	X	O

The features indicate whether the taxon was assigned to the core-microbiota or pan-microbiota in the present work

mice (Belzer and de Vos 2012; Dingemanse et al. 2015). *Akkermansia muciniphila* may serve as a potential candidate to modulate intestinal tumor development and prevent metabolic disorders (Depommier et al. 2020; Dingemanse et al. 2015). Other pan species, such as *Acetatifactor muris*, *Acutalibacter muris*, *Mucispirillum schaedleri*, *Muribaculum intestinale*, and *Turicimonas muris*, were previously found to exist in the intestines of mice (Lagkouvardos et al. 2016b; Pfeiffer et al. 2012; Robertson et al. 2005).

Variations in the mouse gut microbiota and reproducibility

Even genetically similar laboratory mice differ in the composition of the intestinal microbiota. Additionally, the gut microbiota of mice is not fixed permanently but instead can be easily changed. Since the gut microbiota is associated with the host phenotype, variations in the mouse gut microbiota may induce different host phenotypes and affect experimental reproducibility.

Several vendors sell mice including Charles River Laboratories (CRL), Envigo, Taconic Biosciences (TAC), and The Jackson Laboratory (JAX). Mice purchased from different vendors differ in their gut microbiota composition and resulting phenotypes. This is because the vendor is one of the most influential factors affecting the gut microbiota (Ericsson et al. 2015; Yang et al. 2019).

There have been many studies of the gut microbiota using genetically similar mice from different vendors. SFB were first found, starting with the discovery that C57BL/6 mice from JAX and TAC showed different Th17 immune responses (Ivanov et al. 2009, 2008). SFB are found in most mice from large commercial vendors but rarely in mice from JAX (Ericsson et al. 2015). Commensal *Bifidobacterium* was more abundant in C57BL/6 mice from JAX than C57BL/6 mice from TAC, which led to increased antitumor immunity in JAX mice compared with TAC mice (Sivan et al. 2015). SFB and *Bifidobacterium* were included in the pan-genera in our results. Mice from different vendors also exhibited differences in susceptibility to malaria (Villarino et al. 2016), skin grafts (McIntosh et al. 2018), and *Salmonella* infection (Velazquez et al. 2019). Villarino and colleagues found increased abundances of *Lactobacillus* and *Bifidobacterium* in malaria-resistant C57BL/6 mice from JAX and TAC compared to malaria-susceptible C57BL/6 mice from other vendors (Villarino et al. 2016). When comparing the rejection kinetics of skin transplants, C57BL/6 mice from TAC rejected skin grafts more rapidly than C57BL/6 mice from JAX, and *Alistipes* was selected as a significant factor in the graft rejection phenotype (McIntosh et al. 2018). *Alistipes* and *Lactobacillus* were included in the core-genera. *Enterobacteriaceae*, one of the pan families, was less abundant in C57BL/6 mice from JAX than in C57BL/6 mice from CRL, Envigo, and TAC, making JAX mice susceptible to *Salmonella* infection (Velazquez et al. 2019). Brown and colleagues recently reported that the abundance of *Akkermansia*, one of the pan-genera, was significantly increased in C57BL/6 mice from JAX compared with C57BL/6 mice from TAC (Brown et al. 2020).

Meanwhile, the gut microbiota is not fixed but can be easily altered. The gut microbiota of co-housed mice become synchronized through the cage effect, which may explain a large portion of the variation in the mouse gut microbiota (Hildebrand et al. 2013). The cage effect leads to similar microbiota-related phenotypes between co-housed mice and can affect study results (Elinav et al. 2011; Hildebrand et al. 2013; Stecher et al. 2010). The cage effect is due to coprophagy, a behavioral trait of mice that eat feces for nutrition (Deloris Alexander et al. 2006).

This effect can also be utilized experimentally. Researchers can co-house mice before using them in experiments to minimize any possible confounding. This technique has several drawbacks: there are limitations on the number of mice per cage, and co-housing experimental and control groups is not always possible for practical reasons (Witjes et al. 2020). Additionally, the co-housing method is less effective than the littermate method in standardizing microbiota in mouse models (Robertson et al. 2019). However, it is the most popular method to minimize variation in the mouse microbiota (Basson et al. 2020), probably because of its simplicity. Researchers can also use this effect as well as fecal microbiota transplantation to test whether phenotype-inducing microbiota in one group can be transferred to another group. For example, Velazquez and colleagues co-housed CRL and JAX mice and found that a fraction of JAX mice had increased resistance to *Salmonella* after co-housing (Velazquez et al. 2019).

Variations in the mouse gut microbiota may affect experimental reproducibility. Whether to minimize variations for robustness or to embrace variations for generalization depends on the purpose of the experiment; the important issue is to be aware that the mouse microbiota may affect the outcome (Witjes et al. 2020). These variations can also lead researchers to accidentally discover microbiota-dependent phenotypes when experiments cannot be reproduced (Eberl et al. 2019).

Public resources for mouse gut microbiota

As data on the mouse gut microbiota have increased, public resources for these data have appeared on the web. Researchers can use these public resources to explore the composition of the mouse gut microbiota. In the last section, we summarize some of the significant public resources (Table 9).

There are public resources available for finding information about bacterial strains cultured from laboratory mouse

Table 8 The gut core-microbiota of laboratory mice at the species level

Taxonomy	Name	Average RA (%)	FO (%)	Status
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Oscillibacter*; PAC001188_s	PAC001188_s	1.69	100.00	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Pseudoflavonifractor*; *Flintibacter butyricus* group	*Flintibacter butyricus* group	0.85	100.00	Group
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Oscillibacter*; PAC001502_s group	PAC001502_s group	0.57	98.77	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC000664_g; PAC001104_s	PAC001104_s	0.43	98.77	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001372_g; PAC001372_s	PAC001372_s	0.40	98.77	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Pseudoflavonifractor*; PAC001574_s group	PAC001574_s group	0.34	98.77	Group
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Pseudoflavonifractor*; PAC001131_s group	PAC001131_s group	1.12	97.53	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001043_g; PAC002511_s group	PAC002511_s group	0.18	97.12	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001524_g; PAC001524_s	PAC001524_s	0.14	96.71	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001165_g; PAC001384_s group	PAC001384_s group	0.25	95.47	Group
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Pseudoflavonifractor*; PAC002474_s group	PAC002474_s group	0.21	95.47	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Clostridium_g24; PAC001374_s	PAC001374_s	0.21	95.47	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Oscillibacter*; KE159714_s group	KE159714_s group	0.96	94.65	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159600_g; EU511112_s	EU511112_s	0.24	94.24	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Pseudoflavonifractor*; PAC001549_s	PAC001549_s	0.21	94.24	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Anaerotruncus*; KE159677_s	KE159677_s	0.15	93.83	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; *Acetatifactor*; PAC001801_s group	PAC001801_s group	0.23	93.42	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Clostridium_g21; KE159781_s	KE159781_s	0.17	93.42	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159538_g; KE159538_s	KE159538_s	2.25	92.59	Phylotype
Firmicutes; Clostridia; Clostridiales; *Christensenellaceae*; PAC001360_g; PAC001371_s	PAC001371_s	1.35	91.36	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Pseudoflavonifractor*; PAC001786_s group	PAC001786_s group	0.32	91.36	Group
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Oscillibacter*; EU454374_s	EU454374_s	0.15	90.95	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Oscillibacter*; PAC001522_s	PAC001522_s	0.60	90.12	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001043_g; PAC001752_s	PAC001752_s	0.14	90.12	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001225_g; PAC001225_s	PAC001225_s	0.16	89.71	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; *Anaerotignum*; PAC001540_s	PAC001540_s	0.14	89.71	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001385_g; PAC001727_s group	PAC001727_s group	0.12	89.71	Group

Table 8 (continued)

Taxonomy	Name	Average RA (%)	FO (%)	Status
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Pseudoflavonifractor*; NFKI_s group	NFKI_s group	0.37	89.30	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001043_g; PAC001557_s group	PAC001557_s group	0.13	88.48	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC000692_g; PAC001666_s	PAC001666_s	0.11	88.48	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Clostridium_g24; PAC001376_s	PAC001376_s	0.13	88.07	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159605_g; KE159605_s	KE159605_s	0.11	87.24	Phylotype
Bacteroidetes; Bacteroidia; Bacteroidales; *Muribaculaceae*; PAC001068_g; PAC001071_s	PAC001071_s	2.65	86.42	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; PAC001402_g; PAC001561_s	PAC001561_s	0.14	86.42	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159538_g; PAC001708_s	PAC001708_s	0.55	86.01	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; *Lachnospira*; PAC002369_s	PAC002369_s	0.36	86.01	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159571_g; KE159641_s group	KE159641_s group	0.82	85.19	Group
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001199_g; PAC001746_s	PAC001746_s	0.15	84.77	Phylotype
Firmicutes; Clostridia; Clostridiales; *Ruminococcaceae*; *Acutalibacter*; PAC001519_s	PAC001519_s	0.13	84.36	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001043_g; PAC001552_s	PAC001552_s	0.20	83.95	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; KE159605_g; KB822463_s	KB822463_s	0.16	81.89	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; Eubacterium_g4; PAC001174_s	PAC001174_s	0.26	80.66	Phylotype
Firmicutes; Clostridia; Clostridiales; *Lachnospiraceae*; PAC001092_g; PAC001742_s	PAC001742_s	0.12	80.25	Phylotype

intestinal samples: miBC (Lagkouvardos et al. 2016b) and mGMB (Liu et al. 2020). A study from Lagkouvardos et al. used the previously mentioned culture-based method to isolate 100 bacterial strains representing 76 species from the guts of laboratory mice (Lagkouvardos et al. 2016b). The miBC includes information such as the cultivation conditions and isolation sources of these strains. Researchers can also check the GenBank accession numbers of the 16S rRNA genes and draft genomes. Meanwhile, the mGMB was released four years after the miBC (Liu et al. 2020). Liu et al. isolated 244 bacterial strains representing 126 species from the cecal contents of laboratory mice using the

Table 9 Public resources for exploring the mouse gut microbiota

Name	Related methodology	Data source	URL
miBC	Culture-based method	Mouse gut	https://www.dsmz.de/collection/catalogue/microorganisms/special-groups-of-organisms/dzif-sammlung/maus-mikrobiomliste
mGMB	Culture-based method	Mouse gut	http://www.cgmcc.net/english/mgmb/
MMDB	Amplicon sequencing	Mouse gut	http://leb.snu.ac.kr/mmdb
IMNGS	Amplicon sequencing	Various hosts & environments	https://www.imngs.org/
MGnify	Amplicon sequencing & Shotgun sequencing	Various hosts & environments	https://www.ebi.ac.uk/metagenomics/
MG-RAST	Amplicon sequencing & Shotgun sequencing	Various hosts & environments	https://www.mg-rast.org/

culture-based method. The mGMB contains the metadata of the cultured strains and the GenBank accession numbers of the 16S rRNA genes. Both repositories allow researchers to obtain strains of interest via web pages.

MMDB is a public resource for exploring the gut microbiota based on the taxonomic profiles obtained from 16S rRNA gene amplicon data from laboratory mice (Yang et al. 2019). The MMDB was created from 554 well-curated samples from mice of diverse backgrounds. The MMDB contains information on 7,502 bacterial taxa ranging from the phylum to the species level that make up the mouse gut. This database can be searched for whether bacteria of interest are contained in the mouse gut microbiota. In addition, the distribution of the bacteria according to inbred mouse strain, sampling location, and vendor can be viewed in the search results, which informs the relationships between the factors and the bacteria.

The mouse gut microbiota can also be explored by searching the mouse gut in databases containing microbiota data from various hosts and environments: IMNGS (Lagkouvardos et al. 2016a), MGnify (Mitchell et al. 2020), and MG-RAST (Meyer et al. 2008). IMNGS was built from all available 16S rRNA gene amplicon data in the NCBI SRA database (Lagkouvardos et al. 2016a). The bacteria of interest can be searched within a specific group of samples, such as intestinal samples from laboratory mice, to determine the number of reads of the bacteria present in that group. The advantage of this database is its enormous size (searching for samples with the origin of *Mus musculus* yielded 4,825 results at the time of writing), but no manual curation has been done on the metadata. MGnify and MG-RAST include the 16S rRNA gene amplicon datasets and the shotgun sequencing datasets (Meyer et al. 2008; Mitchell et al. 2020). Searching for the mouse gut returns the analysis results, including metadata and taxonomic profiles. MG-RAST can also be searched for samples with specific bacteria (Meyer et al. 2008).

Conclusions

This review aims to guide the basic methodologies, relevant studies, and public resources for the mouse gut microbiota to support researchers in this field. It is important to understand what methods are available and use appropriate methods before conducting an analysis of the microbiota. In addition, we show the core- and pan-taxa of the laboratory mouse gut. Knowing the major components of the gut microbiota will significantly help in understanding the mouse gut. We also describe the variations in the mouse gut microbiota associated with the vendors and the cage effect and introduce their association with experimental reproducibility. Finally, we summarize the public resources that are available to help researchers quickly navigate the explosively growing mouse gut microbiota profiles. Overall, we describe the microbiota of the laboratory mouse gut, which is expected to help studies involving the gut microbiota in other hosts, especially humans.

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Declarations

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