International Journal of Medical Microbiology 306 (2016) 316–327

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Mini review

The mouse gut microbiome revisited: From complex diversity to model ecosystems

Thomas Clavel[a,*], Ilias Lagkouvardos[a], Michael Blaut[b], Bärbel Stecher[c,d,**]

[a] ZIEL Institute for Food and Health, Technische Universität München, Freising, Germany
[b] Department of Gastrointestinal Microbiology, German Institute of Human Nutrition (DIfE), Nuthetal, Germany
[c] Max von Pettenkofer-Institute, Ludwig-Maximilians-Universität München, Munich, Germany
[d] German Center for Infection Research (DZIF), Partner Site Munich, Munich, Germany

ARTICLE INFO

Article history:
Received 29 January 2016
Received in revised form 1 March 2016
Accepted 2 March 2016

Keywords:
Host–microbe interactions
Mouse intestinal microbiota
Bacterial diversity
Anaerobic cultivation
Gnotobiology
Minimal microbiome

ABSTRACT

Laboratory mice are the most commonly used animal model in translational medical research. In recent years, the impact of the gut microbiota (*i.e.* communities of microorganisms in the intestine) on host physiology and the onset of diseases, including metabolic and neuronal disorders, cancers, gastrointestinal infections and chronic inflammation, became a focal point of interest. There is abundant evidence that mouse phenotypes in disease models vary greatly between animal facilities or commercial providers, and that this variation is associated with differences in the microbiota. Hence, there is a clear discrepancy between the widespread use of mouse models in research and the patchwork knowledge on the mouse gut microbiome. In the present manuscript, we summarize data pertaining to the diversity and functions of the mouse gut microbiota, review existing work on gnotobiotic mouse models, and discuss challenges and opportunities for current and future research in the field.

1. Introduction

Microbes are the dominating life form on earth. They can assemble into complex communities which are involved in numerous global biogeochemical conversion processes. Trillions of microbes, referred to as microbiota, colonize the skin and mucosal body surfaces of humans and other animals where they are engaged in a constant crosstalk with the host immune system and metabolism. Hence, these microbial communities are currently intensively studied in biomedical research. Although host-associated microbial communities include various microorganisms (bacteria, archaea, fungi, and protozoa) as well as viruses (Suhr and Hallen-Adams, 2015; Virgin, 2014), bacterial populations are dominant members of these ecosystems and are the primary focus of the present article.

The microbiota of the lower vertebrate gut forms one of the most dense and complex microbial ecosystems known to date, harboring several hundred different bacterial species (Berg, 1996; Qin et al., 2010). It has a major impact on host health, *e.g.* it breaks down indigestible dietary components, produces bioactive metabolites, influences immune system maturation and brain development, and protects against colonization by harmful pathogens [for recent reviews see (Buffie and Pamer, 2013; Flint et al., 2012; Hooper et al., 2012; Stecher et al., 2013)]. An abnormal microbiota structure and function, referred to as dysbiosis, is associated with numerous diseases, including chronic gut inflammation, cancer, metabolic and psychiatric disorders [for recent reviews see (Clavel et al., 2014; Louis et al., 2014; Sampson and Mazmanian, 2015; Wlodarska et al., 2015)].

To understand how microbial ecosystems function, it is essential to gain insights into the identity and physiology of their individual members. The classical tools to analyze taxonomic and functional diversity such as microscopy and cultivation have been gradually replaced by culture-independent approaches targeting for instance small subunit (16S) ribosomal RNA (rRNA) gene sequences (Zoetendal et al., 2006). In the last decade, next-generation sequencing technologies have revolutionized the field of microbial ecology by providing unprecedented insights into the diversity, composition, and function of various microbial ecosystems including the vertebrate gut (Acinas et al., 2004; Weinstock, 2012). Overall, meta-genomics, -transcriptomics, -proteomics, and metabolomics give overviews on community composition and diversity as well as activity of genes and metabolic pathways in a

* Corresponding author at: ZIEL Institute for Food and Health, Technische Universität München, Freising, Germany. Tel.: +49 81 61 71 55 34.
** Corresponding author at: Max von Pettenkofer-Institute, Ludwig-Maximilians-Universität München, Munich, Germany. Tel.: +49 89 21 80 72 948.
E-mail addresses: thomas.clavel@tum.de (T. Clavel), Stecher@mvp.uni-muenchen.de (B. Stecher).

http://dx.doi.org/10.1016/j.ijmm.2016.03.002
1438-4221/

given ecosystem. Yet, since all these analyses depend on the quality of databases that integrate information gained from genomic, chemical, and functional studies, they will benefit from future experiments carried out with cultured representatives. In many instances, comparative analysis of patient and healthy control cohorts revealed that shifts in the microbiome are associated with human diseases (Berry and Reinisch, 2013). However, to causally link microbiome signatures identified in clinical surveys, robust experimental *in vitro* and *in vivo* models are required to enable systematic experimental manipulation of the microbiota.

The laboratory mouse is currently the primary experimental model organism in preclinical research. A large variety of genetically-engineered strains and mouse models for human diseases exist (Eppig et al., 2015), and mouse strains can be relatively easily re-derived germ-free (Macpherson and McCoy, 2015), which has set the stage for the establishment of gnotobiotic models (*i.e.* mice colonized with defined bacterial strains or mixtures). Gnotobiology (Greek: *gnosis*: knowledge; *bios*: life; *logos*: study) has a long-standing tradition in this field of research because it is instrumental to test causal relationships between microbes and their host (Nuttal and Thierfelder, 1895; Orcutt et al., 1987; Trexler and Reynolds, 1957). Of course, a significant number of differences exist between human and mice that must be taken into account when interpreting data, in particular differences in gut physiology and the immune system (Mestas and Hughes, 2004), but also dietary habits, behavior and living environment. In addition, maintenance of germ-free mice is demanding and requires specialized equipment and experienced staff. Despite these challenges, gnotobiotic mice are widely used and can in combination with "omics" technologies and genetic engineering provide important mechanistic insights into the molecular mechanisms underlying microbe–microbe and microbe–host interactions.

Recently, the cultivation of bacteria experienced a great upswing. So far, effort has been focused on the establishment of culture collections of human- and plant-associated microbial ecosystems (Bai et al., 2015; Fodor et al., 2012; Hugon et al., 2015; Rettedal et al., 2014). These collections are essential to carry out microbial reconstitution experiments in germ-free model organisms. However, to investigate microbiota evolution and functions including host-specificity, the scientific community needs public bacterial collections derived from mice, which presently do not exist. In this review, we revisit current knowledge of the mouse gut microbiota, the relevance of mouse models for microbe–host interactions studies, and give an outlook on future challenges and opportunities in the field.

2. Humanized mice from a microbial perspective: Hybrid models of clinical relevance

A number of pioneering studies have employed human-derived *Escherichia coli* and *Bacteroides* spp. as model organisms to elucidate key principles of microbe–host interactions in gnotobiotic mice. These bacteria can be genetically manipulated and allow functional genetic analysis. *E. coli*, the workhorse of traditional and modern microbiology and genetics, is the most abundant facultative anaerobic commensal of the human gut (Bachmann, 1996). Over the last decade, the groups of Conway and Cohen (2015) studied *E. coli* carbohydrate utilization and competition in the intestine in detail. A seminal study by Hapfelmeier and colleagues used an auxotrophic *E. coli* mutant strain to generate a gnotobiotic mouse model for reversible bacterial colonization (Hapfelmeier et al., 2010). The growth of this mutant strain depends on exogenous supplementation of the amino acids D-alanine and meso-diaminopimelic acid, which are not provided by the germ-free murine host. Thus, the mutant only transiently colonizes the gut after which the mice re-establish a completely germ-free state. This model allows a number of fundamental principles underlying bacterial induction of mucosal immune responses to be addressed.

Using *Bacteroides* spp., several fundamental principles underlying host–microbe interactions have been elucidated. *Bacteroides thetaiotaomicron* (*B. theta*) and *Bacteroides fragilis* are highly abundant and ubiquitous obligate anaerobic members of the human gut microbiota (Moore and Holdeman, 1974; Qin et al., 2010). Their genomes encode a vast number of enzyme systems that mediate degradation of polysaccharides derived from the diet and mucosal secretions (Xu et al., 2003), which shapes the mutualistic relationship between *Bacteroides* spp. and their human host (Comstock, 2009). Mono-colonization of mice with *B. theta* induces pronounced changes in host mucosal gene expression (Hooper and Gordon, 2001). Specifically, *B. theta* can modify host epithelial differentiation in a way that supports its own growth: it promotes the production of fucosylated glycans by the host and in turn uses them as nutrient sources (Bry et al., 1996). Intriguingly, this process is dependent on the capacity of *B. theta* to utilize fucose, reflecting the mutualistic nature of commensal-host relationships. *B. fragilis* produces a polysaccharide with immune modulatory activities (Mazmanian et al., 2005) that prevents gut inflammation (Mazmanian, 2008) and promotes tolerogenic immune responses by signaling on Foxp3(+) regulatory T cells through TLR2 (Round et al., 2011).

In addition to mono-colonization experiments, interaction of *B. theta* with other strains has been studied in detail by transcriptome profiling. *B. theta* was shown to adapt to the presence of *Eubacterium rectale* or the probiotic strains *Bifidobacterium longum* and *Lactobacillus casei* by expanding the breadth of its carbohydrate utilization (Mahowald et al., 2009; Sonnenburg et al., 2006). Co-colonization with methanogenic, sulfate-reducing, and acetogenic bacteria also yielded valuable insights into the metabolism of these generally lowly abundant members of mammalian gut ecosystems (Rey et al., 2010, 2013; Samuel and Gordon, 2006). Taken together, these paradigm studies illustrate how members of the microbiota are able to adapt their substrate utilization in response to one another and engage in cross-feeding, which are fundamental principles also operating in complex ecosystems. Besides the species mentioned above, numerous other commensal bacteria (the majority of which is derived from humans) have been used to interrogate microbe–host interactions in gnotobiotic models (Table 1). Although models harboring only few bacteria have been very helpful, care must be taken when interpreting data. In simplified bacterial communities, the context of a fully diverse and competitive bacterial ecosystem is lacking. Several studies have therefore started using defined bacterial consortia of higher complexity (Table 1).

Germ-free mice can also be used to investigate functions of complex human-derived microbiota. Human fecal microbiota or culture collections can be stably transplanted into germ-free mice (Goodman et al., 2011; Kibe et al., 2005; Turnbaugh et al., 2009; Wos-Oxley et al., 2012). The microbiota in these models is complex and thus not fully characterized, and therefore these models are not gnotobiotic. Nevertheless, they allow mining the human microbiome for specific functions and address microbiota-specific effects on the immune system and metabolome and study inter-individual differences (Ahern et al., 2014; Marcobal et al., 2013). Moreover, mice with a humanized microbiota make it possible to test whether a complex human disease phenotype can be transmitted by microbiota transplantation (Ridaura et al., 2013; Subramanian et al., 2014). Hence, mice colonized with human gut microbiota are very helpful to test the clinical relevance of dysbiotic communities associated with diseases. However, it remains unclear how well different human gut bacterial taxa establish in the mouse intestinal milieu.

Table 1
Examples of human- and mouse-derived bacterial species and consortia use in gnotobiotic mice.

Phylum	Species	References
Actinobacteria	*Bifidobacterium adolescentis*	Fukuda et al. (2011), Sefik et al. (2015), Wittmann et al. (2013)
	Bifidobacterium bifidum	Grimm et al. (2015)
	Bifidobacterium infantis	Fukuda et al. (2011)
	Bifidobacterium longum	Fukuda et al. (2011), Marcobal et al. (2015), Sonnenburg et al. (2006)
	Propionibacterium acnes	Naik et al. (2015)
	Propionibacterium granulosum	Sefik et al. (2015)
Bacteroidetes	*Alistipes shahii*	Devkota et al. (2012)
	Bacteroides acidifaciens	Berry et al. (2013)
	Bacteroides fragilis	Huang et al. (2011), Mazmanian et al. (2008), Sefik et al. (2015), Vetizou et al. (2015)
	Bacteroides thetaiotaomicron	Kamada et al. (2012), Kashyap et al. (2013), Li et al. (2015), Ng et al. (2013), Sefik et al. (2015), Sonnenburg et al. (2005)
	Bacteroides vulgatus	Bohn et al. (2006), Sefik et al. (2015), Waidmann et al. (2003)
	Bacteroides spp.	Wu et al. (2015)
	Bacteroides dorei, Bacteroides eggerthii, Bacteroides finegoldii, Bacteroides massiliensis, Bacteroides ovatus, Bacteroides salanitronis, Bacteroides uniformis	Sefik et al. (2015)
	Parabacteroides johnsonii, Parabacteroides merdae	Sefik et al. (2015)
Firmicutes	*Bacillus* sp.	Balish and Warner (2002)
	Blautia hydrogenotrophica	Rey et al. (2010)
	Clostridium butyricum	Takahashi et al. (2004)
	Corynebacterium pseudodiphtheriticum	Naik et al. (2015)
	Enterococcus faecalis	Balish and Warner (2002), Ocvirk et al. (2015), Steck et al. (2011)
	Enterococcus faecium	Sefik et al. (2015)
	Eubacterium rectale	Mahowald et al. (2009)
	Faecalibacterium prausnitzii (colonizes only together with *E. coli*)	Miquel et al. (2015)
	Lactobacillus casei	Balish and Warner (2002), Sefik et al. (2015)
	Lactobacillus reuteri, Lactobacillus acidophilus	Balish and Warner (2002)
	L. reuteri	Krumbeck et al. (2015)
	Lactobacillus rhamnosus, L. casei	Kozakova et al. (2015), Sefik et al. (2015)
	Lactobacillus spp.	Krumbeck et al. (2015), Sonnenburg et al. (2006)
	Lactococcus lactis	Balish and Warner (2002)
	Marvinbryantia formatexigens	Rey et al. (2010)
	Rothia nasimurium	Naik et al. (2015)
	Ruminococcus obeum	Hsiao et al. (2014)
	SFB (*Candidatus Arthromitus*)	Gaboriau-Routhiau et al. (2009), Ivanov et al. (2009)
	Staphylococcus aureus	Naik et al. (2015)
	Staphylococcus epidermidis, S. lentus, S. xylosus	Naik et al. (2015)
	Staphylococcus saprophyticus	Sefik et al. (2015)
	Veillonella spp., *Peptostreptococcus asaccharolyticus, Peptostreptococcus magnus, Lachnospiraceae* sp., *Clostridium perfringens, Clostridium histolyticum, Coprobacillus* sp., *Fusobacterium nucleatum, Fusobacterium mortiferum, Clostridium ramosum*	Sefik et al. (2015)
Proteobacteria	*Acinetobacter baumannii, Acinetobacter lwofii*	Sefik et al. (2015)
	Bilophila wadsworthia	Devkota et al. (2012)
	Desulfovibrio piger	Rey et al. (2013)
	Enterobacter cloacae	Macpherson and Uhr (2004)
	Escherichia coli	Balish and Warner (2002), De Paepe et al. (2011), Li et al. (2015), Lima-Filho et al. (2004), Schumann et al. (2012)
	E. coli	Waidmann et al. (2003)
Verrucomicrobia	*Akkermansia muciniphila*	Berry et al. (2013), Derrien et al. (2011), Ganesh et al. (2013)
Archaea	*Methanobrevibacter smithii*	Samuel and Gordon (2006)

Communities	Members	References
3-Member community	*E. coli, Lactobacillus johnsonii, B. longum*	Denou et al. (2009)
4-Member community	*Ruminococcus gnavus, B. thetaiotaomicron, Clostridium hathewayi, Clostridium orbiscindens*	Crost et al. (2010)
7-Member community	*B. longum* subsp. *longum, B. vulgatus, E. coli, E. faecalis, F. prausnitzii, Lactobacillus plantarum, Ruminococcus gnavus*	Eun et al. (2014)
8-Member community	*B. thetaiotaomicron, Bacteroides caccae, B. ovatus, Collinsella aerofaciens, Clostridium symbiosum, E. coli, E. rectale, M. formatexigens*	Rey et al. (2013)
8-Member community	*Anaerostipes caccae, B. thetaiotaomicron, B. longum, Blautia producta, C. butyricum, C. ramosum, E. coli, Lactobacillus plantarum*	Becker et al. (2011)
8-Member community	Altered Schaedler Flora	Dewhirst et al. (1999)
10-Member community	*B. thetaiotaomicron, B. ovatus, B. caccae, B. hydrogenotrophica, C. aerofaciens, Clostridium symbiosum, D. piger, E. coli, E. rectale, M. formatexigens*	Faith et al. (2011)
12-Member community	*Bacteroides caccae, B. cellulosyticus, B. ovatus, B. thetaiotaomicron, B. uniformis, B. vulgatus, Clostridium spiroforme, Clostridium scindens, C. aerofaciens, Dorea longicatena, Parabacteroides distasonis, R.obeum*	McNulty et al. (2013)

Table 1 (*Continued*)

Communities	Members	References
15-Member community	*B. caccae, B. ovatus, B. thetaiotaomicron, B. uniformis, B. vulgatus, B. cellulosilyticus, C. aerofaciens, C. scindens, C. spiroforme, D. longicatena, E. rectale, F. prausnitzii, P. distasonis, R. obeum, Ruminococcus torques*	McNulty et al. (2011)
17-Member community	Strains belonging to *Clostridium* clusters IV, XIVa and XVIII	Atarashi et al. (2013)
33-Member community	Bacterial strains isolated from human stool	Martz et al. (2015)
Unknown	*Clostridium* mix	Atarashi et al. (2011)

The table contains a non-comprehensive list of bacteria and corresponding references since 1999 (as it can be difficult to track the identity and origin of bacteria in earlier studies). Bacteria are sorted by phyla, alphabetic order, and complexity of communities. Readers should refer to the given references to obtain strain designation. The upper part of the table refers to experiments with single strains. Each bacterial species was used for mono-colonization whenever several species are listed for one given reference. Grey boxes highlight work performed using mouse-derived bacteria, which is clearly outnumbered by the number of colonization experiments done with human-derived bacteria.

3. *Perpetuum mobile*: The host shapes and is shaped by the microbiota

Whether a microorganism is capable of growing in a given environment mainly depends on the physicochemical conditions (*e.g.* pH, temperature, redox potential, oxygen tension) and substrate availability. Animals harbor a wide range of digestive systems for an efficient extraction of nutrients from their respective diet. Therefore, the intestinal tract of herbivores, carnivores, and omnivores display considerable anatomical and physiological differences. Gut microbial communities have adapted to these local conditions and co-evolved with their hosts, *e.g.* bacterial diversity was found to be higher in herbivores than in carnivores or omnivores (Ley et al., 2006). Reciprocal microbiota transplantation from fish into mice and vice versa led to drastic changes in the relative abundance of major community members: the donor microbiota changed in the recipient host in a way that it resembled the typical microbiota of the recipient host, as far as possible with the given inoculum (Rawls et al., 2006). A comprehensive series of "xenomicrobiota" transplantation experiments demonstrated that microbial consortia originating from the human, fish, and insect gut, and even from environmental samples are capable of colonizing the mouse intestine (Seedorf et al., 2014). Nonetheless, the composite "xenomicrobiota" established in recipient hosts were eventually out-competed by an autochthonous murine gut microbiota, indicating that the murine gut is a highly selective environment for bacterial colonization.

Several reports demonstrated that host-species phylogeny can be congruent with the relationship of their respective gut microbial communities (Ley et al., 2008b; Ochman et al., 2010). This illustrates that vertical transmission plays a role in community assembly, rather than being assembled strictly de novo from the environment. Evidence exists that this co-diversification results in the emergence of host specificity. Striking molecular evidence for microbial host specificity was provided by Jens Walter and colleagues who performed colonization experiments using different strains of the commensal species *Lactobacillus reuteri* (Frese et al., 2011). These authors showed that *L. reuteri* strains isolated from mice were capable of biofilm formation in the murine forestomach, while human, pig and chicken isolates failed to do so. This difference was also reflected in pronounced genomic differences among strains from distinct hosts. *L. reuteri* mutants in genes encoding cell surface structures exhibited reduced colonization efficiency, showing that the colonization phenotype was directly linked to the expression of specific bacterial signals involved in cellular adhesion.

Host factors including bile acids, mucin, antimicrobial peptides, and IgA can also influence colonization processes and microbiota architecture. For instance, bile acids are metabolites synthetized in the liver and secreted into the small intestine *via* the bile to help lipid absorption. Intricate interactions between bile acids and the gut microbiota are exemplified by anti-microbial properties of certain bile acids, and by their conversion *via* deconjugation, dehydroxylation, and dehydrogenation reactions catalyzed by specific bacterial species (Devlin and Fischbach, 2015; Ridlon and Bajaj, 2015). As bile acids undergo entero-hepatic circulation and reach substantial concentrations (up to hundreds of μM) in the intestinal lumen, variations in their concentrations and diversity can affect microbiota structure and function (Islam et al., 2011). Differences in bile acid pools between humans and mice exist, which certainly contributes to changes in their gut microbiota (Sayin et al., 2013; Seedorf et al., 2014).

The immune system is also an important modulator of gut microbial ecology. Antimicrobial peptides constitute an important arm of the innate immune barrier and are actively involved in shaping gut microbial communities. Altered production of Paneth cell-derived antimicrobial peptides can be an important mediator of a mouse-strain specific microbiota (Gulati et al., 2012). Lack of defensins was shown to alter microbiota composition and cause overgrowth of pathobionts (Salzman et al., 2010). Moreover, IgA, an effector molecule of adaptive immune responses, is induced in response to bacterial colonization and actively shapes the microbiota (Kubinak et al., 2015).

Mucus is another important host component that acts as a substrate for gut bacteria and can thereby shape microbial ecosystem architecture. Intestinal goblet cells secrete large quantities of mucins, which are heavily glycosylated, high molecular weight proteins that spread over the entire gut mucosa and thereby form a barrier to luminal microbes (Johansson et al., 2015). However, mucins also offer niches that favor colonization by specific bacteria able to metabolize glycoproteins (Berry et al., 2013). It has been demonstrated that changes in mucin composition (including their glycosylation) alter gut bacterial populations (Rausch et al., 2011; Staubach et al., 2012), and vice versa (Jakobsson et al., 2015).

These examples underscore the intricate interactions between gut bacterial communities and host-derived factors, which are per se influenced by host gene expression. Over the last decade, the impact of the host genome on gut microbiota composition has been a major subject of interest. Genome-wide mapping approaches, such as quantitative trait locus (QTL) mapping, have shown that specific genetic variants are linked to variation in certain bacterial populations (Benson et al., 2010; Wang et al., 2015). However, when investigating interactions between individual host genetic traits and the gut microbiome, the comparison of knockout animals and their wildtype counterparts can be affected by a number of confounding factors (*e.g.* cage effects, maternal transmission). This emphasizes the need for careful data interpretation and for the establishment of guidelines for proper experimental design, *e.g.* considering the use of littermate controls (Laukens et al., 2015).

Mutualistic relationships between host species and their gut microbe are the result of long-term co-evolution driven by bacterial, host-derived, and environmental factors. As outlined above, specific bacterial functions such as the expression of adhesion proteins or the induction of host-derived substrates used subsequently for growth can confer colonization advantages (Bry et al., 1996; Frese et al., 2011). Also, the host immune system can shape the microbiota and specific bacteria can shape the immune system, resulting in a complex cross-talk equilibrium (Atarashi et al., 2013; Chung et al., 2012; Hooper et al., 2012; Ivanov et al., 2009). Mouse models have been very useful for dissecting molecular mechanisms underlying these microbe–host interactions. However, it is now clear that many host and bacterial factors that influence these interactions are host species-specific. In contrast to the well-known differences in gut physiology between humans and mice (*e.g.* intestinal architecture, physicochemical properties, immune system, diet), there is to date surprisingly little knowledge on the specificity of their gut microbiomes.

4. Composition of the mouse gut microbiota

Ley et al. (2008a) were the first to compare fecal microbiota of numerous animal species using next generation sequencing technologies, but mice were not included in this study. Earlier work by the same authors reported high similarities at the phylum level between the murine and human gut microbiota (Ley et al., 2005, 2006). In order to provide a comprehensive picture of bacterial phyla composition in the mouse gut, we processed 6034 mouse 16S rRNA amplicon datasets retrieved from the Sequence Read Archive, one of the public repositories for high-throughput sequencing data (Leinonen et al., 2011). Seven phyla (*Actinobacteria*, *Bacteroidetes*, *Deferribacteres*, *Firmicutes*, *Proteobacteria*, *Tenericutes*, and *Verrucomicrobia*) were found in more than 10% of samples (4797 after filtering) and were thus considered as common inhabitants of the mouse intestine (Fig. 1). *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were most dominant, with median relative abundance of 47.8, 28.8, and 2.0% total reads, respectively. Members of the phylum *Firmicutes* are clearly the most prevalent murine gut bacteria, displaying relative abundances of more than 20% reads in 84.5% of the samples analyzed. In contrast, *Verrucomicrobia* and *Proteobacteria* showed a much lower prevalence at this relative abundance threshold of 20% reads (approximately 10% of samples). The prevalence of *Actinobacteria*, *Deferribacteres*, and *Tenericutes* was <1%. The approach used in the present large-scale analysis may be followed up in future studies and lead to a more precise definition of a prototypic mouse microbiota.

With respect to comparative analysis, there is still little knowledge on specific traits that distinguish the human from the mouse gut microbiota. Recently, Nguyen et al. (2015) compared existing shotgun metagenomic datasets (four from human subjects and five from mouse feces) and reported that lactobacilli and *Turicibacter* spp. (family *Erysipelotrichaceae* within *Firmicutes*) are characteristic of mice, whereas *Prevotella*, *Ruminococcus*, and *Faecalibacterium* spp. showed higher relative abundances in human feces. The most comprehensive investigation published so far on the mouse intestinal microbiota using next generation sequencing also looked at mouse- and human-specific taxa (Xiao et al., 2015). In this shotgun metagenomic study establishing the first microbial gene catalog from the mouse gut, the genera *Coprobacillus* (family *Erysipelotrichaceae*), *Anaerotruncus* (*Ruminococcaceae*), *Marvinbryantia* (*Lachnospiraceae*), and *Pseudoflavonifractor* (unclassified *Clostridiales*) were characterized by higher relative abundances in mice, compared with higher abundances of *Oscillibacter* (*Ruminococcaceae*) and *Klebsiella* (*Enterobacteriaceae*) in the human gut.

The fact that a bacterial strain is better adapted to a given host species may have various reasons. The ability of microbes to optimally adapt to the living conditions prevailing in the intestinal tract or parts of it is certainly of major importance. For example, lactobacilli adhere to the squamous epithelium in the forestomach of mice reaching concentrations of 10^8 cells per g of organ content whereas the concentration of lactobacilli in the human stomach is $<10^4$ cells per g of organ content (Tannock, 1995). An entire bacterial phylum, the *Deferribacteres*, is prevalent in the gut of wild (Linnenbrink et al., 2013) and laboratory mice but to the current state of knowledge absent from humans. *Mucispirillum schaedleri*, the only member of this phylum described so far in the mouse intestine (Robertson et al., 2005), is part of the Altered Schaedler Flora (Dewhirst et al., 1999). Segmented filamentous bacteria (SFB), which are conspicuous because they form long filaments, are another example of bacteria frequently described in mice, while they may exhibit age-dependent colonization kinetics in humans (Yin et al., 2013). In mice, these bacteria are found in the terminal ileum, where they are in close contact with the intestinal epithelium (Klaasen et al., 1992) and may depend on absorptive epithelial cells for growth and differentiation (Schnupf et al., 2015). SFB colonization induces formation of IL-17-producing T-helper (Th17) cells and thereby modulates the balance between Th17 cells and Foxp3-(+) regulatory T-cells (Ivanov et al., 2009). All these studies provide evidence that the mouse and human gut microbiota can be differentiated by the presence of specific bacterial taxa that dominate microbial communities as assessed by sequencing. However, these findings require additional investigations at higher taxonomic resolution and also need to be confirmed using methods that allow precise quantification of target bacterial groups.

In summary, despite the relatively high number of existing high-throughput sequencing datasets on the mouse gut microbiota, description of the ecosystem and its microbial members is still in its infancy. It will require concerted actions with larger sets of samples collected worldwide combined with the improvement of reference databases (*via* reinforced cultivation efforts and detailed genetic studies) to make further progress towards a representative, specific, and refined picture of the mouse gut microbiota. Moreover, the host factors mentioned above and the impact of diet discussed below contribute to substantial variations in the gut microbiota. This highlights the need to work with minimal communities of defined microorganisms which to a certain extent represent complex ecosystems in order to perform detailed ecological and mechanistic studies.

5. Gnotobiotic mouse models for gut microbiota research

Phenotypes may vary greatly in mouse models of diseases depending on the genetic background of the mice and differences in hygiene standard between animal facilities. The gut microbiota is also a prominent variable (Laukens et al., 2015). On the one hand, such discrepancies led to seminal discoveries: for instance the importance of SFB for the induction of Th-17 immune responses was discovered by comparing mucosal T-cell subsets from C57BL/6 mice obtained from different vendors (Ivanov et al., 2009). On the other hand, this condition caused a high number of non-reproducible mouse disease phenotypes. Pioneering investigations in the 1960's already reported that various colonies of mice harboring different gut bacterial populations displayed different susceptibility to infections (Dubos and Schaedler, 1960). Lack of reproducibility remains a problem that precludes proper investigation of the molecular mechanisms underlying diseases if body microbiomes are not the primary research target. For example, upon import into a new animal facility *via* embryo transfer, mice

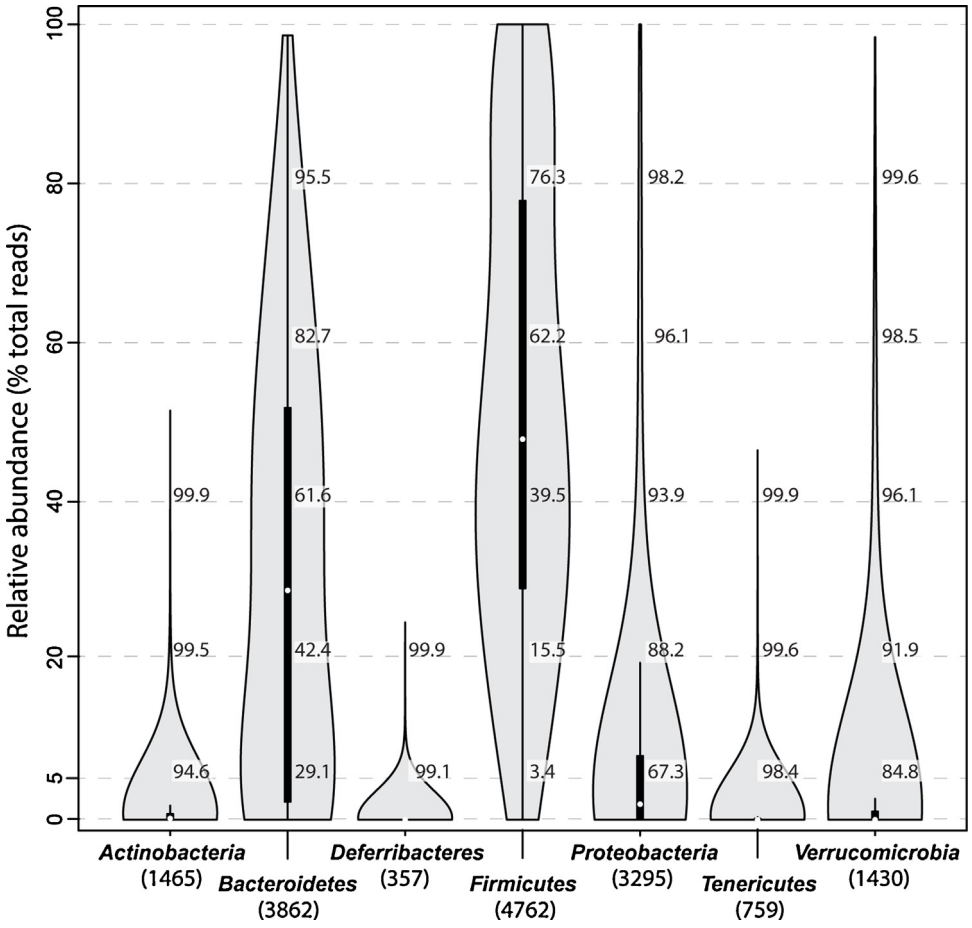

Fig. 1. Relative sequence abundance of common phyla in the mouse gut. The violin plots show the distribution of common phyla based on 16S rRNA gene studies across 4797 mouse gut samples. Data were calculated on the basis of Operational Taxonomic Units (ca. 250 nucleotides) classified by the Ribosomal Database Project from a total of 6034 mouse gut samples available in the Sequence Read Archive. Sequences were processed by and outputs downloaded from IMNGS (www.imngs.org). The term common phyla refers to those found in >10% of all samples. In order to avoid including analysis of unrepresentative samples, those for which the sum of abundances of common phyla was <80% or those with <1000 sequences classified as bacteria were discarded, resulting in 4797 samples. For each phylum, the percentage of samples with abundance below the selected thresholds (horizontal dashed lines) is shown along the violin plots. The total number of positive samples (>0.5% relative abundance per sample) to which these percentages refer is shown in brackets below the phylum names.

are usually associated with a different gut microbial community which can change susceptibility to colitis (Yang et al., 2013). For the same reason, wildtype littermate animals should be used as control groups for *Salmonella* infection experiments to test phenotypes of mutant mouse lines (Kaiser et al., 2012).

The observed differences in hygiene status and microbiota of laboratory rodents were the trigger for establishment of the so called Schaedler Flora to create "isobiotic" mouse lines and improve standardization in mouse breeding facilities by colonizing germ-free mice with a defined mixture of cultivable bacterial species (see below). Hence, the rationale for using minimal microbiota (*i.e.* defined communities of microorganisms to mimic a complex ecosystem) is not novel, but recent technical advances in molecular tools and cultivation approaches can enhance research in the field (Macpherson and McCoy, 2015). In the next paragraphs, we give a short overview of existing minimal microbiota that have been used and illustrate urgent needs in the field of gnotobiology.

5.1. Lessons learnt from studies using minimal gut microbiota

Various bacterial consortia used for the inoculation of germ-free mice have been described in the literature. The first community, termed Schaedler Flora, was developed by Schaedler et al. (1965). It consisted of two *Lactobacillus* strains and one of each *Streptococcus*, *Bacteroides*, *Enterococcus*, and *E. coli* strain. This first version was later modified by Orcutt et al. (1987) and termed Altered Schaedler Flora (ASF). The ASF encompasses eight different strains, which were identified based on their 16S rRNA gene sequences (Dewhirst et al., 1999), and for which genome information is now available (Wannemuehler et al., 2014). Two members of the community are lactobacilli (ASF 360 and 361) and one strain is related to *Parabacteroides distasonis* (ASF 519). The forth strain is *M. schaedleri* (ASF 457), and the remaining four members display a fusiform shape and have an extreme oxygen sensitivity in common. Three of these are affiliated with *Clostridium* species (ASF 356, 500, 502) and one strain was identified as *Eubacterium plexicaudatum* (ASF 492) (Dewhirst et al., 1999). Mice colonized with the ASF display a certain degree of "normalization" when compared with germ-free mice (Wymore Brand et al., 2015). However, it is unknown whether all ASF strains are representative members of dominant bacterial communities in the mouse intestine. Moreover, mice associated with ASF do not differ substantially from germ-free mice with respect to microbiota-associated characteristics such as the induction of mucosal immune responses, degradation of trypsin, mucin, and β-aspartylglycine as well as conversion of bilirubin and formation of short-chain fatty acids, which are usually re-established in germ-free mice after colonization with a complex microbiota (Bouskra et al., 2008; Geuking et al., 2011; Midtvedt et al., 1985).

Several other defined microbial communities have been described, which are all based on human-derived strains (Table 1).

Faith et al. employed a community of ten sequenced bacterial strains to investigate the response of this community to four different diets (Faith et al., 2011). The study aimed to predict the diet-dependent abundance of each community member and obtain information on substrate preferences and phenotypic flexibility of the community members. The consortium included *B. thetaiotaomicron*, *Bacteroides ovatus* and *Bacteroides caccae*, which are capable of degrading complex polysaccharides; *E. rectale*, *Marvinbryantia formatexigens*, *Collinsella aerofaciens*, and *E. coli*, which preferentially utilize oligosaccharides and simple sugars; *Clostridium symbiosum* and *E. coli*, which convert amino acids; the sulfate reducer *Desulfovibrio piger* and the acetogen *Blautia hydrogenotrophica*, both of which are capable of oxidizing H_2 formed in the course of carbohydrate and amino acid fermentation by the other community members. Using a linear model, the authors demonstrated that the variation in abundance of each organism could largely be explained based on knowing which organisms were present and what the concentrations of the dietary ingredients were fed to the mice.

In a more recent study, a model community consisting of twelve different species was used (McNulty et al., 2013) (Table 1). Community members were selected on the basis of several criteria: (1) high prevalence in the intestinal microbiota of humans; (2) membership in one of the three major phyla present in the human intestine; (3) availability of their genome sequences; and (4) their ability to grow *in vitro*. Following colonization, one group of mice was first fed a diet low in fat and high in plant polysaccharides (LF/HPP) for two weeks, switched to a diet high in fat and sugar (HF/HS) for two more weeks and back to LF/HPP for additional two weeks. The mice in the second group were also fed the two diets but in reverse order. This is one of the first examples where community and transcriptome profiling in combination with high-resolution metaproteomics were applied in parallel to a defined microbiota to analyze the response to dietary changes. The study led to a number of interesting findings: the relative abundance of some community members (*B. caccae*, *B. ovatus* and *B. cellulosilyticus*) changed in response to the alternating diets whereas that of other members such as *B. thetaiotaomicron* did not. Moreover, whereas the proportion of *B. caccae* increased on LF/HPP and decreased on HF/HS, the relative abundance of *B. ovatus* and *B. cellulosilyticus* decreased. Diet was found to explain the variance in bacterial population size. *B. cellulosilyticus* was identified as the most versatile carbohydrate utilizer within this community. Transcriptome analysis revealed strongly diet-dependent expression patterns and indicated that this organism is highly efficient in adapting the expression of genes/proteins to its needs. However, this work also demonstrates that the sensitivity of metatranscriptomic and metaproteomic methods may become limited if the relative proportion of a community member is low. Hence, such limitations are not restricted to complex microbiomes but may even occur when well-defined and less complex microbial communities are analyzed.

For the establishment of another model community, the simplified human intestinal microbiota (SIHUMI), bacterial strains were selected based on their reported presence in the human intestine and their *in vitro* fermentation capabilities (Becker et al., 2011) (Table 1). Germ-free rats were first associated with seven bacterial species (*Anaerostipes caccae*, *B. thetaiotaomicron*, *B. longum*, *Blautia producta*, *Clostridium ramosum*, *E. coli* and *Lactobacillus plantarum*) and fecal concentrations of short-chain fatty acids were compared with those of germ-free animals. To increase the fecal butyrate concentration, which was low with this original consortium, the community was complemented with the butyrate producer *Clostridium butyricum*. The complemented community was capable of degrading mucins, β-aspartylglycine, and bilirubin but to a lower extent than conventional rats. Dietary changes in fermentable fiber and fat content affected the abundance of community members. The relative increase of one member of this community, *C. ramosum*, in response to a high-fat diet reflected the situation reported for obese mice and human subjects. This model community was subsequently also established in mice where these results could be reproduced and extended (Woting et al., 2014). The community was stable over time, was vertically transmitted to the offspring, and has also been used as a background microbiota in gnotobiotic mice to investigate the role of mucin-degrading *Akkermanisa muciniphila* in *Salmonella enterica Typhimurium* infection-driven inflammation (Ganesh et al., 2013). Surprisingly, *A. muciniphila* exacerbated the intestinal inflammation caused by *Salmonella* in this animal model.

In the context of inflammatory bowel disease (IBD), Eun et al. (2014) associated germ-free wildtype and colitis-prone interleukin-10-deficient (IL-$10^{-/-}$) mice on different genetic background with a seven-member community (Table 1) to investigate mouse-strain effects on intestinal inflammation and on microbial community composition. Criteria for strain selection were reports about different abundances in IBD patients and healthy subjects, effects in experimental colitis, human origin, availability of genome sequence, and formation of a stable community in mice. This consortium induced colitis in all IL-$10^{-/-}$ mice, but the phenotype was more aggressive in mice with the 129S6/SvEv than the C57BL/6 background. The abundance of community members was affected by host genetic background and presence of colonic inflammation.

5.2. The unknown majority should become minority: Cultivation is worth the effort!

As outlined in the previous paragraphs, model microbial consortia have provided valuable insights into microbe–host interactions. However, they show some important limitations. The ASF is the most established consortium of bacteria originating from the mouse intestine, but the strains are not publicly available (Wymore Brand et al., 2015). As discussed above, gnotobiotic models colonized by human gut bacteria are suitable for testing clinical relevance, but they may be problematic for establishment of mouse models with defined microbiota in terms of colonization efficacy and regulation of host physiology (Chung et al., 2012; Frese et al., 2011). Most of all, one problem common to all minimal microbiomes used so far relates to our very limited view of mouse gut bacterial diversity and composition, which prevents establishment of representative defined communities.

In order to provide an overview of the diversity of common and dominant bacterial taxa in the mouse intestine from currently available datasets, we followed the same universal 16S rRNA amplicon approach as described in Fig. 1 and generated a dendrogram showing bacterial taxonomy (down to the family level) with prevalence and abundance information (Fig. 2). The most prevalent families were the *Lachnospiraceae* and *Ruminococcaceae* within the phylum *Firmicutes*, and the *Porphyromonadaceae* within *Bacteroidetes*. Of note, the Ribosomal Database Project (Wang et al., 2007) classifies members of family S24-7, known to be dominant in the mouse intestine but without cultivable members yet available (Seedorf et al., 2014), as *Porphyromonadaceae*, which has inflated values for this family in the present analysis. Most importantly, these data based on 4712 samples shows that 12 of the 37 taxa listed at the family level are still unknown, *i.e.* either they do not have even a single representative strain in culture or the taxonomic classification of corresponding cultured bacteria is ambiguous. Reaching average prevalence and relative abundance values of up to 60% samples and >9% total reads, these unknown taxa may be considered as the most wanted bacteria from the mouse gut. Six of these taxa were even unknown at the class or order level. In total, 3747 samples (79.5%) contained sequences corresponding to these unknown taxa, with an average cumulative abundance of 21.1% total reads. This clearly demonstrates that a substantial part of the

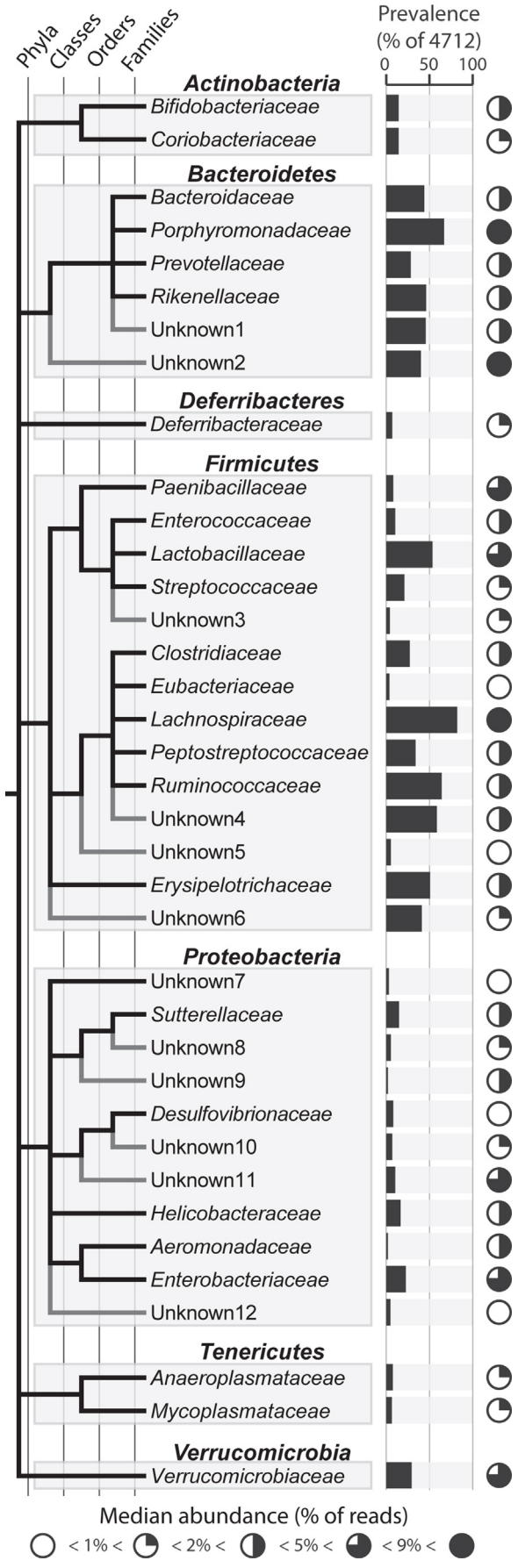

Fig. 2. Taxonomic tree of common bacterial families in the mouse gut. The analysis refers to 4712 samples derived from the Sequence Read Archive and analyzed using IMNGS (www.imngs.org). Families belonging to common phyla (as defined in Fig. 1) and detected (>0.5% relative sequence abundance) in >100 samples are shown. Their prevalence (percentage of positive samples) and median abundance are indicated with histograms and pie charts, respectively. Samples where the cumulative abundance of all common families was below 80% total reads were excluded.

bacterial communities in the mouse gut remains to be characterized.

Hence, it is obvious that gnotobiology research and the development of minimal microbiomes require better technical foundations. More cultivation-based projects are urgently needed to isolate novel bacteria from the mouse gut, as done for other ecosystems (Bai et al., 2015; Lagier et al., 2015; Vartoukian et al., 2016). Additional effort is also required to bridge the gap between novel cultivation strategies and their lack of use in the field (Gich et al., 2012; Zengler et al., 2002). Future studies should also include the complementary use of metagenomics, single-cell approaches, and cultivation of novel bacteria (Dichosa et al., 2014; Pope et al., 2011). Most importantly, novel bacteria must be also thoroughly described taxonomically, which is essential to improve resolution of 16S rRNA amplicon and metagenomic studies. Dominant bacteria in the gut, including important families such as the *Lachnospiraceae* and *Ruminococcaceae*, must rapidly be re-classified to avoid misinterpretations of data (Lawson and Rainey, 2015). Moreover, all strains should be made available to the entire scientific community to avoid losing them over the years. Pioneering studies in the 1960s and 1970s generated large numbers of isolates, but very few are still available. A comprehensive collection of representative murine gut bacteria will facilitate the design of future colonization studies in germ-free mice. These public collections should be linked to well-curated databases of gene and genome sequences to eventually help interpretation of omics datasets (Kim et al., 2012). This will require thorough genetic studies for proper analysis of novel bacterial genomes.

6. Challenges pertaining to mouse microbiome research and the establishment of model gut microbiota

Typical chow fed to mice in an animal laboratory differs considerably from human diets, which per se display a high degree of variability among human populations and individuals, whose preferences are influenced by availability, culture, tradition, price and individual taste. However, it may even be asked to which extent chow diets fed to laboratory mice really reflect the diets typically consumed by mice living in the wild, and whether they are really appropriate despite very broad usage (Clavel et al., 2014). It is reasonable to assume that the diet of wild mice is influenced by season and location and therefore more varied (Maurice et al., 2015). Laboratory mice differ from wild mice not only in their diets, environmental conditions, bacterial and parasitic exposure but also in their genotypes. This is in accordance with differences in intestinal microbiota composition observed between laboratory mice and wild mice (Wang et al., 2014). Differences in microbiota composition even exist between mice from different laboratories (Campbell et al., 2012), highlighting the fact that it is still difficult to tell which members of the microbiota are mouse-specific and, if yes, whether specificity extends to all mouse strains or is restricted to certain mouse strains. The most comprehensive study to date of the gut microbiota in laboratory mice included samples from 184 animals originating from six facilities (Xiao et al., 2015). In the future, gaining additional knowledge of the mouse gut microbiota will inevitably be linked to the necessity of including many more samples than so far analyzed.

The variability in many parameters affecting the gut microbiota in laboratory animals indicates that no matter which

microorganisms are selected for creating an artificial community to associate germ-free mice, it will hardly be possible to develop a unique model that is suitable for investigating all conceivable questions. This is amplified by the fact that our knowledge of the mouse gut microbiota is constantly evolving, thereby influencing our view of what a standard healthy mouse microbiota is. Research in the field will benefit from current efforts in depicting the mouse gut microbiota in greater detail and in describing and archiving community members. A high resolution view of the mouse gut microbiome combined with well-curated strains and genomes collections will offer unprecedented opportunities towards the establishment of standardized mouse models and towards the study of specific bacterial functions based on the association of mice colonized by a model microbiota with one strain carrying the given function, which is less artificial than monocolonization of germ-free mice. In this respect, the development of tools for targeted detection of each member of model communities will also be crucial.

How should a defined microbial community in gnotobiotic mice be composed to serve as a useful model for investigating the principles underlying host–microbiota interactions? This certainly depends on the question to be investigated. Before establishing a gnotobiotic mouse model, it is important to consider its purpose, *i.e.* the underlying research question. In general, the model community should reproduce as many effects as possible that are typical of conventional mice but not observed in germ-free mice. Examples include normalization of cecum size and of other microbiota-associated characteristics such as degradation of trypsin, mucin, and β-aspartylglycine, conversion of bilirubin and formation of short-chain fatty acids (Midtvedt et al., 1985). These microbiota-associated characteristics can be conferred to the host by intestinal bacteria that do not necessarily have to be specific to the host species (Becker et al., 2011). Immunity-related interactions may likely display a higher degree of host specificity than microbial metabolic activities that are common to both humans and mice. This leads to the question whether only bacterial strains that have been isolated from mice should be used or whether strains of human origin can also be used. As already pointed out, this will depend on the particular research question and whether a strain of human origin can establish itself in the intestinal tract of a mouse. This decision could be facilitated by the establishment of a comprehensive catalogue of genomes from multiple isolates of the same bacteria obtained from different host species. This catalogue could lead to the identification of species core- and pan-genomes, which will allow assigning species into "generalists" (*i.e.* same strains are isolated from different host species) and "specialists" (*i.e.* strains from clusters depending on host species, as in case of *L. reuteri*). Using single strains or mixtures isolated from human subjects is relevant for testing clinical relevance and causal relationships, whereas it seems appropriate to use mouse strains for the purpose of generating isobiotic mouse lines and standardizing models between different animal facilities. Practical aspects also have to be kept in mind. For example, it must be possible to grow the selected strains in the laboratory, genome sequences should be available for functional analyses, and the community should be stable over time and be transferred to the offspring.

In summary, creating a defined minimal microbiota that reproduces all effects observed in conventional mice or in humans will not be possible, simply because a less complex microbiota is less stable and because of the high inter-individual variability in the prevalence and abundance of bacterial taxa in human and mouse populations (Qin et al., 2010; Xiao et al., 2015). Nevertheless, gnotobiotic mouse models are very useful to dissect mechanisms underlying microbe–host interactions, and technical advances of the last decades in molecular and culture-based characterization of complex microbial communities open promising avenues toward future development of refined minimal microbiomes.

Acknowledgment

The authors acknowledge support through the priority program SPP-1656 of the German Research Foundation (DFG).

References

Acinas, S.G., Klepac-Ceraj, V., Hunt, D.E., Pharino, C., Ceraj, I., Distel, D.L., Polz, M.F., 2004. Fine-scale phylogenetic architecture of a complex bacterial community. Nature 430, 551–554.

Ahern, P.P., Faith, J.J., Gordon, J.I., 2014. Mining the human gut microbiota for effector strains that shape the immune system. Immunity 40, 815–823.

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., Kim, S., Fritz, J.V., Wilmes, P., Ueha, S., Matsushima, K., Ohno, H., Olle, B., Sakaguchi, S., Taniguchi, T., Morita, H., Hattori, M., Honda, K., 2013. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500, 232–236.

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, I.I., Umesaki, Y., Itoh, K., Honda, K., 2011. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 331, 337–341.

Bachmann, B., 1996. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12. In: Neidhardt, F.C., Curtiss, R. (Eds.), *Escherichia coli* and Salmonella: Cellular and Molecular Biology. ASM Press, Washington, DC, pp. 2460–2488.

Bai, Y., Muller, D.B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., Dombrowski, N., Munch, P.C., Spaepen, S., Remus-Emsermann, M., Huttel, B., McHardy, A.C., Vorholt, J.A., Schulze-Lefert, P., 2015. Functional overlap of the Arabidopsis leaf and root microbiota. Nature 528, 364–369.

Balish, E., Warner, T., 2002. *Enterococcus faecalis* induces inflammatory bowel disease in interleukin-10 knockout mice. Am. J. Pathol. 160, 2253–2257.

Becker, N., Kunath, J., Loh, G., Blaut, M., 2011. Human intestinal microbiota: characterization of a simplified and stable gnotobiotic rat model. Gut Microbes 2, 25–33.

Benson, A.K., Kelly, S.A., Legge, R., Ma, F., Low, S.J., Kim, J., Zhang, M., Oh, P.L., Nehrenberg, D., Hua, K., Kachman, S.D., Moriyama, E.N., Walter, J., Peterson, D.A., Pomp, D., 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc. Natl. Acad. Sci. U.S.A. 107, 18933–18938.

Berg, R.D., 1996. The indigenous gastrointestinal microflora. Trends Microbiol. 4, 430–435.

Berry, D., Reinisch, W., 2013. Intestinal microbiota: a source of novel biomarkers in inflammatory bowel diseases? Best Pract. Res. Clin. Gastroenterol. 27, 47–58.

Berry, D., Stecher, B., Schintlmeister, A., Reichert, J., Brugiroux, S., Wild, B., Wanek, W., Richter, A., Rauch, I., Decker, T., Loy, A., Wagner, M., 2013. Host-compound foraging by intestinal microbiota revealed by single-cell stable isotope probing. Proc. Natl. Acad. Sci. U.S.A. 110, 4720–4725.

Bohn, E., Bechtold, O., Zahir, N., Frick, J.S., Reimann, J., Jilge, B., Autenrieth, I.B., 2006. Host gene expression in the colon of gnotobiotic interleukin-2-deficient mice colonized with commensal colitogenic or noncolitogenic bacterial strains: common patterns and bacteria strain specific signatures. Inflamm. Bowel Dis. 12, 853–862.

Bouskra, D., Brezillon, C., Berard, M., Werts, C., Varona, R., Boneca, I.G., Eberl, G., 2008. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature 456, 507–510.

Bry, L., Falk, P.G., Midtvedt, T., Gordon, J.I., 1996. A model of host–microbial interactions in an open mammalian ecosystem. Science 273, 1380–1383.

Buffie, C.G., Pamer, E.G., 2013. Microbiota-mediated colonization resistance against intestinal pathogens. Nat. Rev. Immunol. 13, 790–801.

Campbell, J.H., Foster, C.M., Vishnivetskaya, T., Campbell, A.G., Yang, Z.K., Wymore, A., Palumbo, A.V., Chesler, E.J., Podar, M., 2012. Host genetic and environmental effects on mouse intestinal microbiota. ISME J. 6, 2033–2044.

Chung, H., Pamp, S.J., Hill, J.A., Surana, N.K., Edelman, S.M., Troy, E.B., Reading, N.C., Villablanca, E.J., Wang, S., Mora, J.R., Umesaki, Y., Mathis, D., Benoist, C., Relman, D.A., Kasper, D.L., 2012. Gut immune maturation depends on colonization with a host-specific microbiota. Cell 149, 1578–1593.

Clavel, T., Desmarchelier, C., Haller, D., Gerard, P., Rohn, S., Lepage, P., Daniel, H., 2014. Intestinal microbiota in metabolic diseases: from bacterial community structure and functions to species of pathophysiological relevance. Gut Microbes 5, 544–551.

Comstock, L.E., 2009. Importance of glycans to the host-bacteroides mutualism in the mammalian intestine. Cell Host Microbe 5, 522–526.

Conway, T., Cohen, P.S., 2015. Commensal and pathogenic *Escherichia coli* metabolism in the gut. Microbiol. Spectr. 3, http://dx.doi.org/10.1128/microbiolspec.MBP-0006-2014.

Crost, E.H., Pujol, A., Ladire, M., Dabard, J., Raibaud, P., Carlier, J.P., Fons, M., 2010. Production of an antibacterial substance in the digestive tract involved in colonization-resistance against *Clostridium perfringens*. Anaerobe 16, 597–603.

De Paepe, M., Gaboriau-Routhiau, V., Rainteau, D., Rakotobe, S., Taddei, F., Cerf-Bensussan, N., 2011. Trade-off between bile resistance and nutritional

competence drives *Escherichia coli* diversification in the mouse gut. PLoS Genet. 7, e1002107.
Denou, E., Rezzonico, E., Panoff, J.M., Arigoni, F., Brussow, H., 2009. A Mesocosm of *Lactobacillus johnsonii*, *Bifidobacterium longum*, and *Escherichia coli* in the mouse gut. DNA Cell Biol. 28, 413–422.
Derrien, M., Van Baarlen, P., Hooiveld, G., Norin, E., Muller, M., de Vos, W.M., 2011. Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader *Akkermansia muciniphila*. Front. Microbiol. 2, 166.
Devkota, S., Wang, Y., Musch, M.W., Leone, V., Fehlner-Peach, H., Nadimpalli, A., Antonopoulos, D.A., Jabri, B., Chang, E.B., 2012. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in $Il10^{-/-}$ mice. Nature 487, 104–108.
Devlin, A.S., Fischbach, M.A., 2015. A biosynthetic pathway for a prominent class of microbiota-derived bile acids. Nat. Chem. Biol. 11, 685–690.
Dewhirst, F.E., Chien, C.C., Paster, B.J., Ericson, R.L., Orcutt, R.P., Schauer, D.B., Fox, J.G., 1999. Phylogeny of the defined murine microbiota: altered Schaedler flora. Appl. Environ. Microbiol. 65, 3287–3292.
Dichosa, A.E., Daughton, A.R., Reitenga, K.G., Fitzsimons, M.S., Han, C.S., 2014. Capturing and cultivating single bacterial cells in gel microdroplets to obtain near-complete genomes. Nat. Protoc. 9, 608–621.
Dubos, R.J., Schaedler, R.W., 1960. The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. J. Exp. Med. 111, 407–417.
Eppig, J.T., Blake, J.A., Bult, C.J., Kadin, J.A., Richardson, J.E., Mouse Genome Database Group, 2015. The Mouse Genome Database (MGD): facilitating mouse as a model for human biology and disease. Nucleic Acids Res. 43, D726–D736.
Eun, C.S., Mishima, Y., Wohlgemuth, S., Liu, B., Bower, M., Carroll, I.M., Sartor, R.B., 2014. Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-$10^{-/-}$ mice. Infect. Immun. 82, 2239–2246.
Faith, J.J., McNulty, N.P., Rey, F.E., Gordon, J.I., 2011. Predicting a human gut microbiota's response to diet in gnotobiotic mice. Science 333, 101–104.
Flint, H.J., Scott, K.P., Louis, P., Duncan, S.H., 2012. The role of the gut microbiota in nutrition and health. Nat. Rev. Gastroenterol. Hepatol. 9, 577–589.
Fodor, A.A., DeSantis, T.Z., Wylie, K.M., Badger, J.H., Ye, Y., Hepburn, T., Hu, P., Sodergren, E., Liolios, K., Huot-Creasy, H., Birren, B.W., Earl, A.M., 2012. The "most wanted" taxa from the human microbiome for whole genome sequencing. PLoS ONE 7, e41294.
Frese, S.A., Benson, A.K., Tannock, G.W., Loach, D.M., Kim, J., Zhang, M., Oh, P.L., Heng, N.C., Patil, P.B., Juge, N., Mackenzie, D.A., Pearson, B.M., Lapidus, A., Dalin, E., Tice, H., Goltsman, E., Land, M., Hauser, L., Ivanova, N., Kyrpides, N.C., Walter, J., 2011. The evolution of host specialization in the vertebrate gut symbiont *Lactobacillus reuteri*. PLoS Genet. 7, e1001314.
Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, J.M., Topping, D.L., Suzuki, T., Taylor, T.D., Itoh, K., Kikuchi, J., Morita, H., Hattori, M., Ohno, H., 2011. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 469, 543–547.
Gaboriau-Routhiau, V., Rakotobe, S., Lecuyer, E., Mulder, I., Lan, A., Bridonneau, C., Rochet, V., Pisi, A., De Paepe, M., Brandi, G., Eberl, G., Snel, J., Kelly, D., Cerf-Bensussan, N., 2009. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity 31, 677–689.
Ganesh, B.P., Klopfleisch, R., Loh, G., Blaut, M., 2013. Commensal *Akkermansia muciniphila* exacerbates gut inflammation in *Salmonella* Typhimurium-infected gnotobiotic mice. PLoS ONE 8, e74963.
Geuking, M.B., Cahenzli, J., Lawson, M.A., Ng, D.C., Slack, E., Hapfelmeier, S., McCoy, K.D., Macpherson, A.J., 2011. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity 34, 794–806.
Gich, F., Janys, M.A., Konig, M., Overmann, J., 2012. Enrichment of previously uncultured bacteria from natural complex communities by adhesion to solid surfaces. Environ. Microbiol. 14, 2984–2997.
Goodman, A.L., Kallstrom, G., Faith, J.J., Reyes, A., Moore, A., Dantas, G., Gordon, J.I., 2011. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. Proc. Natl. Acad. Sci. U.S.A. 108, 6252–6257.
Grimm, V., Radulovic, K., Riedel, C.U., 2015. Colonization of C57BL/6 mice by a potential probiotic *Bifidobacterium bifidum* strain under germ-free and specific pathogen-free conditions and during experimental colitis. PLoS ONE 10, e0139935.
Gulati, A.S., Shanahan, M.T., Arthur, J.C., Grossniklaus, E., von Furstenberg, R.J., Kreuk, L., Henning, S.J., Jobin, C., Sartor, R.B., 2012. Mouse background strain profoundly influences Paneth cell function and intestinal microbial composition. PLoS ONE 7, e32403.
Hapfelmeier, S., Lawson, M.A., Slack, E., Kirundi, J.K., Stoel, M., Heikenwalder, M., Cahenzli, J., Velykoredko, Y., Balmer, M.L., Endt, K., Geuking, M.B., Curtiss 3rd, R., McCoy, K.D., Macpherson, A.J., 2010. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. Science 328, 1705–1709.
Hooper, L.V., Gordon, J.I., 2001. Commensal host–bacterial relationships in the gut. Science 292, 1115–1118.
Hooper, L.V., Littman, D.R., Macpherson, A.J., 2012. Interactions between the microbiota and the immune system. Science 336, 1268–1273.
Hsiao, A., Ahmed, A.M., Subramanian, S., Griffin, N.W., Drewry, L.L., Petri Jr., W.A., Haque, R., Ahmed, T., Gordon, J.I., 2014. Members of the human gut microbiota involved in recovery from *Vibrio cholerae* infection. Nature 515, 423–426.
Huang, J.Y., Lee, S.M., Mazmanian, S.K., 2011. The human commensal *Bacteroides fragilis* binds intestinal mucin. Anaerobe 17, 137–141.
Hugon, P., Dufour, J.C., Colson, P., Fournier, P.E., Sallah, K., Raoult, D., 2015. A comprehensive repertoire of prokaryotic species identified in human beings. Lancet Infect. Dis. 15, 1211–1219.
Islam, K.B., Fukiya, S., Hagio, M., Fujii, N., Ishizuka, S., Ooka, T., Ogura, Y., Hayashi, T., Yokota, A., 2011. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology 141, 1773–1781.
Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V., Tanoue, T., Imaoka, A., Itoh, K., Takeda, K., Umesaki, Y., Honda, K., Littman, D.R., 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139, 485–498.
Jakobsson, H.E., Rodriguez-Pineiro, A.M., Schutte, A., Ermund, A., Boysen, P., Bemark, M., Sommer, F., Backhed, F., Hansson, G.C., Johansson, M.E., 2015. The composition of the gut microbiota shapes the colon mucus barrier. EMBO Rep. 16, 164–177.
Johansson, M.E., Jakobsson, H.E., Holmen-Larsson, J., Schutte, A., Ermund, A., Rodriguez-Pineiro, A.M., Arike, L., Wising, C., Svensson, F., Backhed, F., Hansson, G.C., 2015. Normalization of host intestinal mucus layers requires long-term microbial colonization. Cell Host Microbe 18, 582–592.
Kaiser, P., Diard, M., Stecher, B., Hardt, W.D., 2012. The streptomycin mouse model for *Salmonella diarrhea*: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response. Immunol. Rev. 245, 56–83.
Kamada, N., Kim, Y.G., Sham, H.P., Vallance, B.A., Puente, J.L., Martens, E.C., Nunez, G., 2012. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science 336, 1325–1329.
Kashyap, P.C., Marcobal, A., Ursell, L.K., Smits, S.A., Sonnenburg, E.D., Costello, E.K., Higginbottom, S.K., Domino, S.E., Holmes, S.P., Relman, D.A., Knight, R., Gordon, J.I., Sonnenburg, J.L., 2013. Genetically dictated change in host mucus carbohydrate landscape exerts a diet-dependent effect on the gut microbiota. Proc. Natl. Acad. Sci. U.S.A. 110, 17059–17064.
Kibe, R., Sakamoto, M., Yokota, H., Ishikawa, H., Aiba, Y., Koga, Y., Benno, Y., 2005. Movement and fixation of intestinal microbiota after administration of human feces to germfree mice. Appl. Environ. Microbiol. 71, 3171–3178.
Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., Won, S., Chun, J., 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int. J. Syst. Evol. Microbiol. 62, 716–721.
Klaasen, H.L., Koopman, J.P., Poelma, F.G., Beynen, A.C., 1992. Intestinal, segmented, filamentous bacteria. FEMS Microbiol. Rev. 8, 165–180.
Kozakova, H., Schwarzer, M., Tuckova, L., Srutkova, D., Czarnowska, E., Rosiak, I., Hudcovic, T., Schabussova, I., Hermanova, P., Zakostelska, Z., Aleksandrzak-Piekarczyk, T., Koryszewska-Baginska, A., Tlaskalova-Hogenova, H., Cukrowska, B., 2015. Colonization of germ-free mice with a mixture of three lactobacillus strains enhances the integrity of gut mucosa and ameliorates allergic sensitization. Cell. Mol. Immunol., http://dx.doi.org/10.1038/cmi.2015.09.
Krumbeck, J.A., Marsteller, N.L., Frese, S.A., Peterson, D.A., Ramer-Tait, A.E., Hutkins, R.W., Walter, J., 2015. Characterization of the ecological role of genes mediating acid resistance in *Lactobacillus reuteri* during colonization of the gastrointestinal tract. Environ. Microbiol., http://dx.doi.org/10.1111/1462-2920.13108.
Kubinak, J.L., Petersen, C., Stephens, W.Z., Soto, R., Bake, E., O'Connell, R.M., Round, J.L., 2015. MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health. Cell Host Microbe 17, 153–163.
Lagier, J.C., Hugon, P., Khelaifia, S., Fournier, P.E., La Scola, B., Raoult, D., 2015. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin. Microbiol. Rev. 28, 237–264.
Laukens, D., Brinkman, B.M., Raes, J., De Vos, M., Vandenabeele, P., 2015. Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. FEMS Microbiol. Rev. 40, 117–132.
Lawson, P.A., Rainey, F.A., 2015. Proposal to restrict the genus Clostridium (Prazmowski) to *Clostridium butyricum* and related species. Int. J. Syst. Evol. Microbiol., http://dx.doi.org/10.1099/ijsem.0.000824.
Leinonen, R., Sugawara, H., Shumway, M., 2011. The sequence read archive. Nucl. Acids Res. 39, D19–D21.
Ley, R.E., Backhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., Gordon, J.I., 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. U.S.A. 102, 11070–11075.
Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M.L., Tucker, T.A., Schrenzel, M.D., Knight, R., Gordon, J.I., 2008a. Evolution of mammals and their gut microbes. Science 320, 1647–1651.
Ley, R.E., Lozupone, C.A., Hamady, M., Knight, R., Gordon, J.I., 2008b. Worlds within worlds: evolution of the vertebrate gut microbiota. Nat. Rev. Microbiol. 6, 776–788.
Ley, R.E., Peterson, D.A., Gordon, J.I., 2006. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124, 837–848.
Li, H., Limenitakis, J.P., Fuhrer, T., Geuking, M.B., Lawson, M.A., Wyss, M., Brugiroux, S., Keller, I., Macpherson, J.A., Rupp, S., Stolp, B., Stein, J.V., Stecher, B., Sauer, U., McCoy, K.D., Macpherson, A.J., 2015. The outer mucus layer hosts a distinct intestinal microbial niche. Nat. Commun. 6, 8292.
Lima-Filho, J.V., Vieira, L.Q., Arantes, R.M., Nicoli, J.R., 2004. Effect of the *Escherichia coli* EMO strain on experimental infection by *Salmonella enterica* serovar Typhimurium in gnotobiotic mice. Braz. J. Med. Biol. Res. 37, 1005–1013.

Linnenbrink, M., Wang, J., Hardouin, E.A., Kunzel, S., Metzler, D., Baines, J.F., 2013. The role of biogeography in shaping diversity of the intestinal microbiota in house mice. Mol. Ecol. 22, 1904–1916.
Louis, P., Hold, G.L., Flint, H.J., 2014. The gut microbiota, bacterial metabolites and colorectal cancer. Nat. Rev. Microbiol. 12, 661–672.
Macpherson, A.J., McCoy, K.D., 2015. Standardised animal models of host microbial mutualism. Mucosal Immunol. 8, 476–486.
Macpherson, A.J., Uhr, T., 2004. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 303, 1662–1665.
Mahowald, M.A., Rey, F.E., Seedorf, H., Turnbaugh, P.J., Fulton, R.S., Wollam, A., Shah, N., Wang, C., Magrini, V., Wilson, R.K., Cantarel, B.L., Coutinho, P.M., Henrissat, B., Crock, L.W., Russell, A., Verberkmoes, N.C., Hettich, R.L., Gordon, J.I., 2009. Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proc. Natl. Acad. Sci. U.S.A. 106, 5859–5864.
Marcobal, A., Kashyap, P.C., Nelson, T.A., Aronov, P.A., Donia, M.S., Spormann, A., Fischbach, M.A., Sonnenburg, J.L., 2013. A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. ISME J. 7, 1933–1943.
Marcobal, A., Yusufaly, T., Higginbottom, S., Snyder, M., Sonnenburg, J.L., Mias, G.I., 2015. Metabolome progression during early gut microbial colonization of gnotobiotic mice. Sci. Rep. 5, 11589.
Martz, S.L., McDonald, J.A., Sun, J., Zhang, Y.G., Gloor, G.B., Noordhof, C., He, S.M., Gerbaba, T.K., Blennerhassett, M., Hurlbut, D.J., Allen-Vercoe, E., Claud, E.C., Petrof, E.O., 2015. Administration of defined microbiota is protective in a murine Salmonella infection model. Sci. Rep. 5, 16094.
Maurice, C.F., Knowles, S.C., Ladau, J., Pollard, K.S., Fenton, A., Pedersen, A.B., Turnbaugh, P.J., 2015. Marked seasonal variation in the wild mouse gut microbiota. ISME J. 9, 2423–2434.
Mazmanian, S.K., 2008. Capsular polysaccharides of symbiotic bacteria modulate immune responses during experimental colitis. J. Pediatr. Gastroenterol. Nutr. 46 (Suppl. 1), E11–E12.
Mazmanian, S.K., Liu, C.H., Tzianabos, A.O., Kasper, D.L., 2005. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 122, 107–118.
Mazmanian, S.K., Round, J.L., Kasper, D.L., 2008. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 453, 620–625.
McNulty, N.P., Wu, M., Erickson, A.R., Pan, C., Erickson, B.K., Martens, E.C., Pudlo, N.A., Muegge, B.D., Henrissat, B., Hettich, R.L., Gordon, J.I., 2013. Effects of diet on resource utilization by a model human gut microbiota containing *Bacteroides cellulosilyticus* WH2, a symbiont with an extensive glycobiome. PLoS Biol. 11, e1001637.
McNulty, N.P., Yatsunenko, T., Hsiao, A., Faith, J.J., Muegge, B.D., Goodman, A.L., Henrissat, B., Oozeer, R., Cools-Portier, S., Gobert, G., Chervaux, C., Knights, D., Lozupone, C.A., Knight, R., Duncan, A.E., Bain, J.R., Muehlbauer, M.J., Newgard, C.B., Heath, A.C., Gordon, J.I., 2011. The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. Sci. Transl. Med. 3 (106), ra106.
Mestas, J., Hughes, C.C., 2004. Of mice and not men: differences between mouse and human immunology. J. Immunol. 172, 2731–2738.
Midtvedt, T., Bjorneklett, A., Carlstedt-Duke, B., Gustafsson, B.E., Hoverstad, T., Lingaas, E., Norin, K.E., Saxerholt, H., Steinbakk, M., 1985. The influence of antibiotics upon microflora-associated characteristics in man and mammals. Prog. Clin. Biol. Res. 181, 241–244.
Miquel, S., Leclerc, M., Martin, R., Chain, F., Lenoir, M., Raguideau, S., Hudault, S., Bridonneau, C., Northen, T., Bowen, B., Bermudez-Humaran, L.G., Sokol, H., Thomas, M., Langella, P., 2015. Identification of metabolic signatures linked to anti-inflammatory effects of *Faecalibacterium prausnitzii*. MBio 6, e00300–e00315.
Moore, W.E., Holdeman, L.V., 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl. Microbiol. 27, 961–979.
Naik, S., Bouladoux, N., Linehan, J.L., Han, S.J., Harrison, O.J., Wilhelm, C., Conlan, S., Himmelfarb, S., Byrd, A.L., Deming, C., Quinones, M., Brenchley, J.M., Kong, H.H., Tussiwand, R., Murphy, K.M., Merad, M., Segre, J.A., Belkaid, Y., 2015. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. Nature 520, 104–108.
Ng, K.M., Ferreyra, J.A., Higginbottom, S.K., Lynch, J.B., Kashyap, P.C., Gopinath, S., Naidu, N., Choudhury, B., Weimer, B.C., Monack, D.M., Sonnenburg, J.L., 2013. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature 502, 96–99.
Nguyen, T.L., Vieira-Silva, S., Liston, A., Raes, J., 2015. How informative is the mouse for human gut microbiota research? Dis. Model Mech. 8, 1–16.
Nuttal, G.H.F., Thierfelder, H., 1895. Thieresches Leben ohne Bakterien im Verdauungskanal. Z. Physiol. Chem. 21, 109–121.
Ochman, H., Worobey, M., Kuo, C.H., Ndjango, J.B., Peeters, M., Hahn, B.H., Hugenholtz, P., 2010. Evolutionary relationships of wild hominids recapitulated by gut microbial communities. PLoS Biol. 8, e1000546.
Ocvirk, S., Sava, I.G., Lengfelder, I., Lagkouvardos, I., Steck, N., Roh, J.H., Tchaptchet, S., Bao, Y., Hansen, J.J., Huebner, J., Carroll, I.M., Murray, B.E., Sartor, R.B., Haller, D., 2015. Surface-associated lipoproteins link *Enterococcus faecalis* virulence to colitogenic activity in IL-10-deficient mice independent of their expression levels. PLoS Pathog. 11, e1004911.
Orcutt, R.P., Gianni, F.J., Judge, R.J., 1987. Development of an "Altered Schaedler Flora" for the generation of NCI gnotobiotic rodents. Microecol. Ther. 17, 59.
Pope, P.B., Smith, W., Denman, S.E., Tringe, S.G., Barry, K., Hugenholtz, P., McSweeney, C.S., McHardy, A.C., Morrison, M., 2011. Isolation of Succinivibrionaceae implicated in low methane emissions from Tammar wallabies. Science 333, 646–648.
Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D.R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J.M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H.B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Jian, M., Zhou, Y., Li, Y., Zhang, X., Qin, N., Yang, H., Wang, J., Brunak, S., Dore, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J., Bork, P., Ehrlich, S.D., 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59–65.
Rausch, P., Rehman, A., Kunzel, S., Hasler, R., Ott, S.J., Schreiber, S., Rosenstiel, P., Franke, A., Baines, J.F., 2011. Colonic mucosa-associated microbiota is influenced by an interaction of Crohn disease and FUT2 (Secretor) genotype. Proc. Natl. Acad. Sci. U.S.A. 108, 19030–19035.
Rawls, J.F., Mahowald, M.A., Ley, R.E., Gordon, J.I., 2006. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell 127, 423–433.
Rettedal, E.A., Gumpert, H., Sommer, M.O., 2014. Cultivation-based multiplex phenotyping of human gut microbiota allows targeted recovery of previously uncultured bacteria. Nat. Commun. 5, 4714.
Rey, F.E., Faith, J.J., Bain, J., Muehlbauer, M.J., Stevens, R.D., Newgard, C.B., Gordon, J.I., 2010. Dissecting the in vivo metabolic potential of two human gut acetogens. J. Biol. Chem. 285, 22082–22090.
Rey, F.E., Gonzalez, M.D., Cheng, J., Wu, M., Ahern, P.P., Gordon, J.I., 2013. Metabolic niche of a prominent sulfate-reducing human gut bacterium. Proc. Natl. Acad. Sci. U.S.A. 110, 13582–13587.
Ridaura, V.K., Faith, J.J., Rey, F.E., Cheng, J., Duncan, A.E., Kau, A.L., Griffin, N.W., Lombard, V., Henrissat, B., Bain, J.R., Muehlbauer, M.J., Ilkayeva, O., Semenkovich, C.F., Funai, K., Hayashi, D.K., Lyle, B.J., Martini, M.C., Ursell, L.K., Clemente, J.C., Van Treuren, W., Walters, W.A., Knight, R., Newgard, C.B., Heath, A.C., Gordon, J.I., 2013. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341, 1241214.
Ridlon, J.M., Bajaj, J.S., 2015. The human gut sterolbiome: bile acid-microbiome endocrine aspects and therapeutics. Acta Pharm. Sin., B 5, 99–105.
Robertson, B.R., O'Rourke, J.L., Neilan, B.A., Vandamme, P., On, S.L., Fox, J.G., Lee, A., 2005. *Mucispirillum schaedleri* gen. nov., sp. nov., a spiral-shaped bacterium colonizing the mucus layer of the gastrointestinal tract of laboratory rodents. Int. J. Syst. Evol. Microbiol. 55, 1199–1204.
Round, J.L., Lee, S.M., Li, J., Tran, G., Jabri, B., Chatila, T.A., Mazmanian, S.K., 2011. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332, 974–977.
Salzman, N.H., Hung, K., Haribhai, D., Chu, H., Karlsson-Sjoberg, J., Amir, E., Teggatz, P., Barman, M., Hayward, M., Eastwood, D., Stoel, M., Zhou, Y., Sodergren, E., Weinstock, G.M., Bevins, C.L., Williams, C.B., Bos, N.A., 2010. Enteric defensins are essential regulators of intestinal microbial ecology. Nat. Immunol. 11, 76–83.
Sampson, T.R., Mazmanian, S.K., 2015. Control of brain development, function, and behavior by the microbiome. Cell Host Microbe 17, 565–576.
Samuel, B.S., Gordon, J.I., 2006. A humanized gnotobiotic mouse model of host–archaeal–bacterial mutualism. Proc. Natl. Acad. Sci. U.S.A. 103, 10011–10016.
Sayin, S.I., Wahlstrom, A., Felin, J., Jantti, S., Marschall, H.U., Bamberg, K., Angelin, B., Hyotylainen, T., Oresic, M., Backhed, F., 2013. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab. 17, 225–235.
Schaedler, R.W., Dubs, R., Costello, R., 1965. Association of germfree mice with bacteria isolated from normal mice. J. Exp. Med. 122, 77–82.
Schnupf, P., Gaboriau-Routhiau, V., Gros, M., Friedman, R., Moya-Nilges, M., Nigro, G., Cerf-Bensussan, N., Sansonetti, P.J., 2015. Growth and host interaction of mouse segmented filamentous bacteria in vitro. Nature 520, 99–103.
Schumann, S., Alpert, C., Engst, W., Loh, G., Blaut, M., 2012. Dextran sodium sulfate-induced inflammation alters the expression of proteins by intestinal *Escherichia coli* strains in a gnotobiotic mouse model. Appl. Environ. Microbiol. 78, 1513–1522.
Seedorf, H., Griffin, N.W., Ridaura, V.K., Reyes, A., Cheng, J., Rey, F.E., Smith, M.I., Simon, G.M., Scheffrahn, R.H., Woebken, D., Spormann, A.M., Van Treuren, W., Ursell, L.K., Pirrung, M., Robbins-Pianka, A., Cantarel, B.L., Lombard, V., Henrissat, B., Knight, R., Gordon, J.I., 2014. Bacteria from diverse habitats colonize and compete in the mouse gut. Cell 159, 253–266.
Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A.M., Burzyn, D., Ortiz-Lopez, A., Lobera, M., Yang, J., Ghosh, S., Earl, A., Snapper, S.B., Jupp, R., Kasper, D., Mathis, D., Benoist, C., 2015. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. Science 349, 993–997.
Sonnenburg, J.L., Chen, C.T., Gordon, J.I., 2006. Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol. 4, e413.
Sonnenburg, J.L., Xu, J., Leip, D.D., Chen, C.H., Westover, B.P., Weatherford, J., Buhler, J.D., Gordon, J.I., 2005. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 307, 1955–1959.
Staubach, F., Kunzel, S., Baines, A.C., Yee, A., McGee, B.M., Backhed, F., Baines, J.F., Johnsen, J.M., 2012. Expression of the blood-group-related glycosyltransferase B4galnt2 influences the intestinal microbiota in mice. ISME J. 6, 1345–1355.
Stecher, B., Maier, L., Hardt, W.D., 2013. 'Blooming' in the gut: how dysbiosis might contribute to pathogen evolution. Nat. Rev. Microbiol. 11, 277–284.

Steck, N., Hoffmann, M., Sava, I.G., Kim, S.C., Hahne, H., Tonkonogy, S.L., Mair, K., Krueger, D., Pruteanu, M., Shanahan, F., Vogelmann, R., Schemann, M., Kuster, B., Sartor, R.B., Haller, D., 2011. *Enterococcus faecalis* metalloprotease compromises epithelial barrier and contributes to intestinal inflammation. Gastroenterology 141, 959–971.

Subramanian, S., Huq, S., Yatsunenko, T., Haque, R., Mahfuz, M., Alam, M.A., Benezra, A., DeStefano, J., Meier, M.F., Muegge, B.D., Barratt, M.J., VanArendonk, L.G., Zhang, Q., Province, M.A., Petri Jr., W.A., Ahmed, T., Gordon, J.I., 2014. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature 510, 417–421.

Suhr, M.J., Hallen-Adams, H.E., 2015. The human gut mycobiome: pitfalls and potentials-a mycologist's perspective. Mycologia 107, 1057–1073.

Takahashi, M., Taguchi, H., Yamaguchi, H., Osaki, T., Komatsu, A., Kamiya, S., 2004. The effect of probiotic treatment with *Clostridium butyricum* on enterohemorrhagic *Escherichia coli* O157:H7 infection in mice. FEMS Immunol. Med. Microbiol. 41, 219–226.

Tannock, G.W., 1995. Normal Microflora. Chapman & Hall, London.

Trexler, P.C., Reynolds, L.I., 1957. Flexible film apparatus for the rearing and use of germfree animals. Appl. Microbiol. 5, 406–412.

Turnbaugh, P.J., Ridaura, V.K., Faith, J.J., Rey, F.E., Knight, R., Gordon, J.I., 2009. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci. Transl. Med. 1, 6ra14.

Vartoukian, S.R., Adamowska, A., Lawlor, M., Moazzez, R., Dewhirst, F.E., Wade, W.G., 2016. In vitro cultivation of 'unculturable' oral bacteria, facilitated by community culture and media supplementation with siderophores. PLoS ONE 11, e0146926.

Vetizou, M., Pitt, J.M., Daillere, R., Lepage, P., Waldschmitt, N., Flament, C., Rusakiewicz, S., Routy, B., Roberti, M.P., Duong, C.P., Poirier-Colame, V., Roux, A., Becharef, S., Formenti, S., Golden, E., Cording, S., Eberl, G., Schlitzer, A., Ginhoux, F., Mani, S., Yamazaki, T., Jacquelot, N., Enot, D.P., Berard, M., Nigou, J., Opolon, P., Eggermont, A., Woerther, P.L., Chachaty, E., Chaput, N., Robert, C., Mateus, C., Kroemer, G., Raoult, D., Boneca, I.G., Carbonnel, F., Chamaillard, M., Zitvogel, L., 2015. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 350, 1079–1084.

Virgin, H.W., 2014. The virome in mammalian physiology and disease. Cell 157, 142–150.

Waidmann, M., Bechtold, O., Frick, J.S., Lehr, H.A., Schubert, S., Dobrindt, U., Loeffler, J., Bohn, E., Autenrieth, I.B., 2003. *Bacteroides vulgatus* protects against *Escherichia coli*-induced colitis in gnotobiotic interleukin-2-deficient mice. Gastroenterology 125, 162–177.

Wang, J., Kalyan, S., Steck, N., Turner, L.M., Harr, B., Kunzel, S., Vallier, M., Hasler, R., Franke, A., Oberg, H.H., Ibrahim, S.M., Grassl, G.A., Kabelitz, D., Baines, J.F., 2015. Analysis of intestinal microbiota in hybrid house mice reveals evolutionary divergence in a vertebrate hologenome. Nat. Commun. 6, 6440.

Wang, J., Linnenbrink, M., Kunzel, S., Fernandes, R., Nadeau, M.J., Rosenstiel, P., Baines, J.F., 2014. Dietary history contributes to enterotype-like clustering and functional metagenomic content in the intestinal microbiome of wild mice. Proc. Natl. Acad. Sci. U.S.A. 111, E2703–E2710.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Wannemuehler, M.J., Overstreet, A.M., Ward, D.V., Phillips, G.J., 2014. Draft genome sequences of the altered schaedler flora, a defined bacterial community from gnotobiotic mice. Genome Announc. 2, e00214–e00287.

Weinstock, G.M., 2012. Genomic approaches to studying the human microbiota. Nature 489, 250–256.

Wittmann, A., Autenrieth, I.B., Frick, J.S., 2013. Plasmacytoid dendritic cells are crucial in *Bifidobacterium adolescentis*-mediated inhibition of *Yersinia enterocolitica* infection. PLoS ONE 8, e71338.

Wlodarska, M., Kostic, A.D., Xavier, R.J., 2015. An integrative view of microbiome-host interactions in inflammatory bowel diseases. Cell Host Microbe 17, 577–591.

Wos-Oxley, M., Bleich, A., Oxley, A.P., Kahl, S., Janus, L., Smoczek, A., Nahrstedt, H., Pils, M., Taudien, S., Platzer, M., Hedrich, H.J., Medina, E., Pieper, D., 2012. Comparative evaluation of establishing a human gut microbial community within rodent models. Gut Microbes 3, 234–249.

Woting, A., Pfeiffer, N., Loh, G., Klaus, S., Blaut, M., 2014. *Clostridium ramosum* promotes high-fat diet-induced obesity in gnotobiotic mouse models. MBio 5, e01514–e01530.

Wu, M., McNulty, N.P., Rodionov, D.A., Khoroshkin, M.S., Griffin, N.W., Cheng, J., Latreille, P., Kerstetter, R.A., Terrapon, N., Henrissat, B., Osterman, A.L., Gordon, J.I., 2015. Genetic determinants of in vivo fitness and diet responsiveness in multiple human gut Bacteroides. Science 350, aac5992.

Wymore Brand, M., Wannemuehler, M.J., Phillips, G.J., Proctor, A., Overstreet, A.M., Jergens, A.E., Orcutt, R.P., Fox, J.G., 2015. The altered schaedler flora: continued applications of a defined murine microbial community. ILAR J. 56, 169–178.

Xiao, L., Feng, Q., Liang, S., Sonne, S.B., Xia, Z., Qiu, X., Li, X., Long, H., Zhang, J., Zhang, D., Liu, C., Fang, Z., Chou, J., Glanville, J., Hao, Q., Kotowska, D., Colding, C., Licht, T.R., Wu, D., Yu, J., Sung, J.J., Liang, Q., Li, J., Jia, H., Lan, Z., Tremaroli, V., Dworzynski, P., Nielsen, H.B., Backhed, F., Dore, J., Le Chatelier, E., Ehrlich, S.D., Lin, J.C., Arumugam, M., Wang, J., Madsen, L., Kristiansen, K., 2015. A catalog of the mouse gut metagenome. Nat. Biotechnol. 33, 1103–1108.

Xu, J., Bjursell, M.K., Himrod, J., Deng, S., Carmichael, L.K., Chiang, H.C., Hooper, L.V., Gordon, J.I., 2003. A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science 299, 2074–2076.

Yang, I., Eibach, D., Kops, F., Brenneke, B., Woltemate, S., Schulze, J., Bleich, A., Gruber, A.D., Muthupalani, S., Fox, J.G., Josenhans, C., Suerbaum, S., 2013. Intestinal microbiota composition of interleukin-10 deficient C57BL/6J mice and susceptibility to *Helicobacter hepaticus*-induced colitis. PLoS ONE 8, e70783.

Yin, Y., Wang, Y., Zhu, L., Liu, W., Liao, N., Jiang, M., Zhu, B., Yu, H.D., Xiang, C., Wang, X., 2013. Comparative analysis of the distribution of segmented filamentous bacteria in humans, mice and chickens. ISME J. 7, 615–621.

Zengler, K., Toledo, G., Rappe, M., Elkins, J., Mathur, E.J., Short, J.M., Keller, M., 2002. Cultivating the uncultured. Proc. Natl. Acad. Sci. U.S.A. 99, 15681–15686.

Zoetendal, E.G., Vaughan, E.E., de Vos, W.M., 2006. A microbial world within us. Mol. Microbiol. 59, 1639–1650.