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.