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Insight into the Animal Models for Microbiome Studies

13

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Abstract

The microbiomes, including bacteria, fungi, and viruses, exist within and on all the organisms, which is the current field of research. Particularly of interest are microbiome of human and its direct impact on human health. The health and fitness of animals, including humans, are influenced by the existence and composition of microbial communities of the host. To date, maximum microbiome research has been focused on the mouse as a model organism for studying the mechanisms of different processes occurring in the microbial communities. Mouse microbiome models have also been the primary choice for performing preclinical tests for studying relationships between the microbiomes and host physiological, metabolic, immune, and neurologic phenotypes. These were also used for developing methodologies to correct functional abnormalities in these communities that lead to disease. The mouse, however, is not a perfect model for studying different aspects of the microbiome and for studying the host stimuli and environmental responses. Hence, researchers have been conducting microbiome studies using other animals as well, for example, zebrafish, pigs, and *Drosophila*. This chapter summarizes the microbiome studies conducted using different models and an insight into its advantages.

Keywords

Microbiome · Animals · Models · Health · Microflora · Host–microbe interaction

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13.1 Introduction

Microbiomes including bacteria, fungi, and viruses live inside and on all organisms and are a growing area of research. Particularly of interest is the human microbiome because of its direct impact on the human health. Humans carry trillions of microbes inhabiting our bodies, resulting in creating complex, body-habitat specific, and adaptive ecosystems. These systems are finely adapted to continuously changing host physiology. The presence or absence of residential microbial communities has a direct impact on the health and fitness of the animal. Dysbiosis or dysbacteriosis is the microbial imbalance or maladaptation of the body, for example, the microbiota composition during a number of diseases such as inflammatory bowel disease, multiple sclerosis, types 1 and 2 diabetes, etc. (Berg 1996; Qin et al. 2010; Turnbaugh and Gordon 2009; Delzenne et al. 2011; Kau et al. 2011). The microbiota of humans plays an important role in possibly causing, spreading, and prevention of human illness (Lai et al. 2014; Norman et al. 2014; Palm et al. 2015). Gut microflora is now known as an important factor in etiology of a number of human diseases such as obesity (Turnbaugh and Gordon 2009; Delzenne et al. 2011), inflammation (Kau et al. 2011; Garrett et al. 2010), metabolic syndrome (Kau et al. 2011; Cani et al. 2012), and colorectal cancer (Arthur and Jobin 2013; Macdonald and Wagner 2012). Use of humanized mouse has brought great advancement in the field of gut microbiology and associated health outcomes (Turnbaugh et al. 2009; Goodman et al. 2011; Gootenberg and Turnbaugh 2011). Normally, these models are made by seeding germ-free mice with the bacteria derived from human. Therefore, they provide a solid system for studying different interactions between human microbiome and chronic diseases where use of humans as subject is not possible.

A number of potential features of healthy microbiome have been proposed. These potential features include prevalent organisms or molecular pathways (Cani et al. 2012) and usual ecological properties, such as diversity or stability (Garrett et al. 2010; Arthur and Jobin 2013). Normally, microbiomes show a great degree of diversity irrespective of the presence or absence of the diseased condition (Garrett et al. 2010; Macdonald and Wagner 2012). This characteristic of the microbiomes creates complication in identification of simple microbial agents causing disease or present in diseased state.

Most of the studies carried out regarding microbiome to date have focused on mouse as a model for studying how different mechanisms occur in the microbial communities. Mouse microbiome models have been first choice for carrying out not only preclinical tests for studying relationships between community and hosts metabolic, physiological, immune, and neurologic phenotypes but also for developing different methodologies to correct functional abnormalities in microbial communities, which results in causation of disease. Many reports have stated that mouse is not a perfect model for studying various aspects of microbiome and also for studying host stimuli and environmental response. Researchers are carrying out microbiome studies in other animals as well as in zebrafish, pigs, and *Drosophila.*

Development of different animal models for microbiota studies allows studying of microbiota subsets, i.e., causative vs. correlative factors in diseased states, and also offers a system to reveal putative therapeutics.

13.2 Mouse as the Animal Model for Human Microbiota Studies

The advantages of mouse models are numerous, and also, the expanse of research on the gastroenterology, genetics, and immunology of mice is much more than any other model. The genotypes and phenotypes range offered by mouse models out-do all other model organisms. Thus, mice models have played a very vital role in the research concerning human gut microbiota.

The physiology and anatomical structures of humans and mouse are quite similar, and thus, this is one of the reasons behind more use of mouse as a model organism in biomedical research. In the case of mice and humans, gastrointestinal tract is made of anatomically similar organs. The prominent differences in anatomy of both human and mice intestinal tract are because of their diets, patterns of feeding, metabolic requirements, and size of the body.

Mice and humans share the average ratio of intestinal surface area: body surface area (Casteleyn et al. 2010), but this ratio varies between different sections of gut between these two organisms. The average small intestine:colon length ratio is 2.5:7 in mice and humans, respectively (Treuting and Dintzis 2012), and the surface ratio of small intestine:colon is only 18 in mice as compared to 400 in humans (Casteleyn et al. 2010).

The cecum in mouse is relatively large in comparison to its total gastrointestinal tract. Cecum is recognized as the chief site for the fermentative decomposition of plant material and the synthesis of vitamin K and B, which is reabsorbed via coprophagy. The human cecum is small, having anatomically similar structure to colon and doesn't hold any clear function (Treuting and Dintzis 2012).

At microscopic level, there are numerous differences in the structure of intestinal tract of humans and mice (Treuting and Dintzis 2012). The colon of mouse consists of thin muscularis mucosae, whereas the human colon is covered with thicker mucosal wall. There are a number of transverse folds present along colonic mucosa in humans. In the case of mice, transverse folds are found only in cecum and proximal colon. These differences in the compartmentalization and structure of colon might contribute to creating different ecological microniches holding a variety of microbial communities.

The surface of mice intestinal crypts in mucin-producing goblet cells of proximal colon are abundantly present, whereas their number decreases at the base of crypt, in distal colon and rectum. In the case of humans goblet cells, they are profusely present from cecum to rectum.

There is a difference in the presence of another type of intestinal epithelial cell, i.e., the Paneth cell in mouse and human. The role of Paneth cells is to secrete antimicrobial components in the lumen of small intestine. In the case of humans,

Paneth cells are present in the cecum and proximal colon. Paneth cells are uniquely present in the cecum in mouse but are not present in entire colonic mucosa.

There is a difference in location and amount of defensins produced by Paneth cells, and their secretion and storage have been found to be different in human and mice (Cunliffe et al. 2001; Ghosh et al. 2002; Ouellette and Selsted 1996). The dissimilarities in location and amount of Paneth cells and goblet cells indicate difference in local immune responses, which might contribute to composition of intestinal microbiota.

Laboratory mice have been instrumental for understanding role of normal flora in many aspects of human physiology, which includes studies like angiogenesis by Stappenbeck et al. (2002) and Reinhardt et al. (2012), bone mineral density studies reported by Cho et al. (2012) and Sjogren et al. (2012a, b), and studies related to innate and adaptive immune function (Garrett et al. 2010; Littman and Pamer 2011; Hooper et al. 2012).

Mice are an important model system for studying host-microbiota interactions that are applicable to human biology (Spor et al. 2011) because of

1. Mice share around 99% genes with humans.
2. These genes have key similarities with human gut microbiome at phylum through family level.

There are a number of characters of mice genetics that mark the mice as a powerful model system for studying genetics of humans in the interactions between host and microbiota. The availability of both inbred and outbred strains, numerous collections of knockout, knock-in, and transgenic mutants (International Knockout Mouse Consortium [IKMC, http:// www.knockoutmouse.org]), available data and work done by Knockout Mouse Project [KOMP, https://www.komp.org], and data available at Mutant Mouse Regions Resource Center [MMRRC, http://www.mmrrc.org] also make mice a preferred study model.

13.3 Germ-Free and Antibiotics Treatment Models

Germ-free (GF) animals are devoid of any microorganism in its lifetime (Wostmann 1996; Yi and Li 2012). For studying the interactions between a host and its microbiota, germ-free animals are important experimental aids. They are colonized with specific microorganisms and are then referred to as gnotobiotic (Fritz et al. 2013; Smith et al. 2007). Germ-free mice are bred in isolators that prevent entry of any microorganisms. Hence, together with these special facilities, monitoring for contamination using different methods, along with the cost, labor, and skills required to maintain them, makes GF mice quite expensive (Fontaine et al. 2015; Nicklas et al. 2015).

To remove the microbiota from the model animals, specific antibiotics having different mechanisms of action are utilized, such as antibiotic polymyxin B to target the Gram-negative bacteria and Vancomycin for Gram-positive bacteria (Atarashi

et al. 2011; Schubert et al. 2015). Thus, the composition of the animal gut may be manipulated by using the combination of antibiotics as per the requirements (Schubert et al. 2015; Zackular et al. 2016).

13.3.1 GF Mice as Experimental Models

GF animal models have been used to study and understand host-microbiota interactions in various fields of study, which include lipid metabolism (Nicholson et al. 2012), cardiology (Stepankova et al. 2010), neurogastroenterology (Diaz Heijtz et al. 2011; Al-Asmakh et al. 2012; Neufeld et al. 2011; McVey Neufeld et al. 2015), reproductive biology (Al-Asmakh et al. 2014; Shimizu et al. 1998), bone homeostasis (Sjogren et al. 2012a, b), and so on. Another interesting observation in the humanized rats was the maintenance of some metabolic activities in the gut microflora transferred from humans to rats. The activities such as production of equol (Bowey et al. 2003) and reduction of cholesterol (Gérard et al. 2004) have been reported to be maintained in the microflora.

Mice models are among the best preferred tools for studying microbiota-associated human diseases, by understanding the host-microbe interactions as monocolonization of single bacteria is possible. The mice are made germ-free and then inoculated with human gut microflora. These are termed the humanized gnotobiotic models (Goodman et al. 2011). This model thus helps in recapitalization of microbiota composition of the human gut.

The research findings on GF, however, cannot be directly utilized for treatment purposes. The reasons being that the bacterial species in mice gut are not found in humans, and this microflora is influenced by numerous factors involving anatomy, behavior, etc. (Gordon and Pesti 1971; Sommer and Backhed 2013; Kostic et al. 2013; Gootenberg and Turnbaugh 2011). In spite of these drawbacks, the GF mice is the most preferred model system for studying host-microbe interactions.

13.4 Other Models

There are numerous invertebrate model species, which are often used in the studies related to certain interactions between the host and its microbiota. The selection of these invertebrate species is dependent on two factors, namely, the innate immune system (Chu and Mazmanian 2013) and a highly restricted gut microbiota (Chaston and Goodrich-Blair 2010). 'Humanized' animals, i.e., models with human microflora, have been established to understand the human microbiome under controlled conditions, utilizing highly researched and genetically manipulable mice and rats, in addition to pigs, dogs, etc. (Hazenberg et al. 1981; Hirayama 1999; Bowey et al. 2003; Gérard et al. 2004; Kibe et al. 2005; Pang et al. 2007).

Some of the invertebrate models used are as follows:

Numerous systems have been studied, which include *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* and their respective symbionts,

Photorhabdus luminescens and *Xenorhabdus nematophila* (Clarke 2008; Wollenberg et al. 2016; Singh et al. 2015; Sicard et al. 2004), *Hirudo verbana* and *Aeromonas veronii* (Rio et al. 2009; Graf et al. 2006), and *Euprymna scolopes* and *Vibrio fischeri* (McFall-Ngai 2014; Schleicher and Nyholm 2011). The most commonly employed model organisms are, however, *Drosophila melanogaster* and *Caenorhabditis elegans*. The ease of rendering these models germ-free (Kietz et al. 2018; Berg et al. 2016), their small size, and freedom from regulatory concerns as in vertebrate models make these models advantageous over the other. The drawbacks are the gastrointestinal anatomical differences with the host, the differences in the microbiota of the gut, and inability to carry out certain studies in these systems such as adaptive immunity in humans.

The gut microflora of invertebrates has fewer microbial species, and the composition is dependent on the environment (McFall-Ngai 2007). In the case of vertebrates, adaptive immune response plays a role in the establishment and development of microbiota of the gut (McFall-Ngai 2007; Maynard et al. 2012). This leads to zebrafish *Danio rerio*, the simplest vertebrate system and having a diverse microbiota, being preferred as model system for host-symbiont relationship studies.

13.4.1 Zebrafish (*Danio rerio*)

Over the last few decades, Zebrafish use in research has increased progressively. Zebrafish is advantageous owing to the limited requirements of less space, availability in large numbers, cost effectiveness, and high prolificacy. The preference for zebrafish is also due to many similarities to its mammalian hosts (Trede et al. 2004; Norton et al. 2008; Alsop and Vijayan 2009; Wong et al. 2013). Homology is seen in the adaptive immune system and in the digestive system. Organs similar to mammals Zebrafish are pancreas, gall bladder, liver, and intestine. Also, the intestinal epithelial cells consist of absorptive enterocytes, goblet cells, and enteroendocrine cells and share similarity to mammals.

Rawls et al. (2007) focused on the transparent nature of the zebrafish, which allows real-time visualization of fluorescently labeled microbes lining the gut, throughout. The external fertilization of Zebrafish is followed by development of the embryo. Transparency of the embryo and larvae permits for the visualization of developing cells and the successive development of microflora using time-lapse microscopy. Studies concerning the host genes or signaling pathways that are regulated by the gut microbiota can be performed (Patton and Zon 2001). Genetic screening methods include mutagenesis, retrovirus-based insertional mutagenesis, zinc finger nucleases, morpholino-based gene knockdown, role of RNAi in function loss (Amacher 2008; Nasevicius and Ekker 2000; De Rienzo et al. 2012), and genome editing using TALEN system (Bedell et al. 2012). Thus, the zebrafish has a numerous features that make it an attractive experimental system.

Pham et al. (2008) have reported studies on early postembryonic development of Zebrafish using relatively simple methods for the development of green fluorescent (GF) and gnotobiotic zebrafish. GF zebrafish larvae are obtained by surface

sterilization of embryos with various antibiotics (Bates et al. 2006; Davis et al. 2016a, b). These GF larvae may be labeled, or the microflora of the larvae may be fluorescently labeled in the larva and visualized, through the transparent body (Bates et al. 2007; Russo et al. 2015; Singer et al. 2010). By using these techniques, the role of microbiota can be determined in various disease conditions such as inflammatory bowel disease (IBD) (Yang et al. 2014; Geiger et al. 2013; Brugman et al. 2009), effect of probiotic bacteria on stress- and anxiety-related behavior (Davis et al. 2016a, b), metabolism and reproduction (Qin et al. 2014; Giorgini et al. 2010), and immunity and pathogen resistance (Wang et al. 2016; Qin et al. 2017). Limitations in the use of zebrafish as study model for microbiota-related research are the differences in environmental conditions and exposures.

13.4.2 Fruit Fly (Drosophila)

Numerous studies have been carried out on the gut microflora of Drosophila in order to understand the host-microbe interaction. The composition of gut microflora has been studied, and many studies have shown that the microbial community is less complex as compared to those found in mammalian gut (Corby-Harris et al. 2007; Cox and Gilmore 2007; Ren et al. 2007; Ryu et al. 2008; Chandler et al. 2011; Wong et al. 2011). Drosophila is therefore being looked upon as a model for host microbial interaction studies.

It was observed in a couple of studies that the gut microflora composition was highly subjective to diet and bacteria belonging to families Acetobacteraceae, Lactobacillales, and Enterobacteriaceae were most dominant (Corby-Harris et al. 2007; Chandler et al. 2011). The microflora of the gut is aerobic in nature and is easily cultured in the laboratory, which has made possible to have microbial stocks of the microbiota available for studies on host-microbial interaction (Chandler et al. 2011; Charroux and Royet 2012; Shin et al. 2011). Thus, these factors have made Drosophila, a model for studies on symbiosis, with huge potential to disclose new insights into host–symbiont interactions.

13.4.3 Dogs (*Canis familiaris*)

There are a number of factors that make dogs the preferred models over other models. Gastrointestinal tract of the dogs with respect to size and structure is fairly identical to humans, in that dogs are monogastric like humans, cecum more developed than human cecum (Song et al. 2013; Misic et al. 2015), and both suffer from diet-induced periodontal disease (Gorrel 1998; Harvey 1998). Common oral flora includes *Streptococcus, Staphylococcus, Pseudomonas, Actinomyces, Pasteurella, Neisseria*, and *Porphyromonas* spp. (Dewhirst et al. 2010; Sakamoto et al. 2005), while common lung microflora includes *Pseudomonas, Streptococcus, Prevotella,* and *Fusobacterium* (Ericsson et al. 2016; Erb-Downward et al. 2011). Thus, dogs are an ideal model species for finding the microbiota present in other internal organ

systems such as the respiratory tract, intestinal tract, skin, etc. Also, studies on beneficial effects of the gut microflora and probiotic benefits are being carried out using canine models. There are numerous limitations to the usage of dogs as study models. Like rats, dogs are costly and require housing facilities.

13.4.4 Rabbits (*Oryctolagus cuniculus*)

Rabbits are used not so commonly as animal models, more so due to the cost factor. Rats or mice are preferred to rabbits. The same is true for research concerning GF models. Rabbits have been used for research on infectious diseases caused by GI tract pathogens (De and Chatterje 1953). The rabbits have well-developed system, and hand-rearing cesarean-born rabbits are maintained as GF models to be used in the studies (Lanning et al. 2000; Schousboe et al. 2001). A part of the small intestine is reported being used in experiments on studying the effects of pathogens like *Vibrio cholerae*, etc. (Taylor et al. 1958; Duncan et al. 1968; Arm et al. 1965; Sanyal et al. 1995; Melling et al. 1976). The technique involves ligation of small intestine and inoculation of pathogen under study in the portion of intestine and placing it back in the abdomen (De and Chatterje 1953). There are, of course, limitations to the use of rabbits as model species in host-microbiota interactive research mainly including their cost, relative to rodents.

13.4.5 Pigs (*Sus scrofa domesticus*)

Pigs are omnivorous and have anatomical and physiological similarities with human gut. Their gut microflora has been well-characterized (Zhao et al. 2015), and the composition is similar to human gut microflora (Panasevich et al. 2018; Pedersen et al. 2013; Ji et al. 2018). Microbial community is similar to the human donor developed in germfree piglets. Hence, these GF animals are excellent models for studies dealing with the effect of dietary changes on the establishment of the gut microbiome. Apart from these, other features that make pigs a model of choice for studies are size, physiology, developmental stages relative to humans, and ability to manipulate their genome (Perleberg et al. 2018; Ryu et al. 2018). Hence, they have been used in a number of studies such as gastrointestinal immuno-ontogeny (Sinkora and Butler 2016), diet-induced obesity (Turnbaugh et al. 2006), xenotransplantation (Vodicka et al. 2005), gastrointestinal physiology (Roura et al. 2016), and cardiovascular physiology (Hughes 1986; Gallo et al. 2017). Effects of resistant starch (Haenen et al. 2013), high- and low-fat diets (Heinritz et al. 2016), antibiotics (Looft et al. 2012; Allen et al. 2011), prebiotics (Berding et al. 2016), probiotics (Barszcz et al. 2016; Riboulet-Bisson et al. 2012; Shen et al. 2010; Zhang et al. 2014; Wen et al. 2014), and myriad other compounds (Liu et al. 2012) on the GM of pigs. Pigs have been successfully colonized with human microflora (Wang and Donovan 2015). The housing and feeding costs as well as the size of the pig models make them undesirable as study models.

13.5 Conclusions

The microbiome field has undergone a big change in recent years. The studies have mainly focused our attention on the role played by the host microbiota in the maintenance of host health. The role of the microflora of the host in the initiation and propagation of disease has also been highlighted by this study. These interactions are studied using model systems. Fundamental discoveries in microbiome research can be made using the most controllable animal systems, including nonmammal vertebrates such as the zebrafish and invertebrates such as Drosophila and *Caenorhabditis elegans*. The early studies of humanized animal models have permitted assessment of the human microbiome that would be challenging to achieve using customary human cohort studies or in vitro model systems.

Each one of the animal models described in this chapter displays some resemblance to the physiology of the human digestive system, thus providing valuable knowledge from diverse angles about the gut microbiota in health and disease. The information obtained from these studies has diversified our understanding of the human gut microbiota in general. Although much of the research to date has focused on the human microbiome, similar metagenomic studies can be applied to understand better animals of agricultural importance as well as pets.

It can be concluded that depending on the study, the animal model may be selected. Dogs may be used for studies related to host-associated microbiota and interactions between host and microbes, while for research concerning nutrition, omnivorous animals such as pigs may be used. The fruit fly offers the advantage of microbial manipulability in the perspective of a genetically manipulable host, while the zebrafish is good for experiments requiring a greater degree of genetic tractability than that of complex vertebrates. Thus, depending on the line of research, the most appropriate model for the study may be selected.

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Conflict of Interest The authors declare no conflict of interests.

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