Rodent models to study the relationships between mammals and their bacterial inhabitants

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Laboratory rodents have been instrumental in helping researchers to unravel the complex interactions that mammals have with their microbial commensals. Progress in defining these interactions has also been possible thanks to the development of culture-independent methods for describing the microbiota associated to body surfaces. Understanding the mechanisms that govern this relationship at the molecular, cellular, and ecological levels is central to both health and disease. The present review of rodent models commonly used to investigate microbial-host "conversations" is focused on those complex bacterial communities residing in the lower gut. Although many types of pathology have been studied using gnotobiotic animals, only the models relevant to commensal bacteria will be described.

Introduction

Studies on host-microbe interactions have largely focused on understanding the molecular mechanisms of pathogenesis of infectious diseases, which is not surprising given the considerable public health and commercial interest in developing tools for the diagnosis of infections, as well as improving vaccination, pharmacologic, and antibiotic treatments. However, we now know that the acquisition of pathogenic bacteria does not always cause disease and that microbes classified as non-pathogenic can also cause disease in certain susceptible hosts. This is because the outcome of the bacteria-host interaction depends on the context of the communication with the specific host. In normal individuals, for instance, large numbers of microbes are found on most surfaces of the body, like the skin,[1,2] the oral cavity[3,4] and the gastrointestinal tract,[5,6] forming stable communities without causing disease. These communities are normally referred to as commensals (derived from Latin "cum mensa," meaning "eating at the same table"). In particular, the intestinal commensals, which are the focus of this review, have been demonstrated to play a pivotal role in aspects of host nutrition[7] and physiology such as development of adaptive lymphoid tissue,[8] innate immune response,[9,10] healing following mucosal injury,[11] development of intestinal

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angiogenesis,[12] energy extraction and storage,[13] and pathogenesis of autoimmune[14] and metabolic diseases.[15]

Most of the current knowledge of how mammals, including humans, interact with their microbial commensals has been obtained by way of animal experimentation. Although the critical role of microbes in human health was suggested as early as the 1880s,[16,17] it was not until recently that appropriate laboratory tools have been developed to directly characterize the fundamental mechanisms underpinning bacteria-host communication. Probably the two most significant achievements in facilitating our understanding of these interactions were the development of nucleic acid-based techniques for analyzing complex bacterial communities, and advances in germ-free technology to manipulate the composition of the microbial environment in experimental animals.

Like in other life science disciplines such as drug discovery, preclinical studies and toxicology, where animal models have been a mainstay of basic and applied research, rodent models have had a central place in revealing key features of the bacterial communities associated with vertebrates. The present review of rodent models commonly used to investigate microbial-host "conversations" is limited in scope to those complex bacterial communities residing in the lower gut. Although many types of pathology have been studied using gnotobiotic animals, only the models relevant to commensal bacteria will be described.

Gnotobiotic and Germ-Free Animals

The use of germ-free technology for investigating the interactions between the host and its associated microbiota has evolved substantially since the first conference on germ-free life in 1939.[18] By controlling the microbial composition of the environment in which the animals are reared, scientists have been able to obtain information about how microorganisms affect the normal physiological functions of the host.

Over the past decades, the terms "gnotobiotic," "axenic" and "germ-free" have been occasionally (and unfortunately) used interchangeably. "Gnotobiotic" (from the Greek gnosis meaning "knowledge" and bios meaning "life") was originally used to describe the biological status of animals used in germ-free research.[19] The terms "axenic" or "germ-free" (GF) refer to animals devoid of any other contaminating organism (also from Greek roots; a, "without", xenikos, "foreign").[20] Strictly speaking, the definition of GF requires the absence of any form of

life other than the subject animal, which is technically unrealistic. It may be more reasonable, however, to say that the animal is GF within the limitations of the current tests for microbial contaminants. As pointed out by Robert Fitzgerald, an animal may be demonstrably free of detectable known bacteria, yeast, fungi, and protozoa, but unless specific tests were also made for new viruses and rickettsiae, one would not be justified in concluding that the animal is, in fact, germ-free.[21] Perhaps because there will never be an unequivocal answer to the question of whether a GF animal is indeed free of every microorganism, the term gnotobiotic becomes more appropriate, meaning that they have known, or completely defined microbiota. Nevertheless, accurately defining the composition of associated microorganisms is not without limitations, as will be discussed in the next section.

"Gnotobiotic" has also been proposed to describe GF animals deliberately inoculated with one or several microbial species ("ex-GF": mono-associated, bi-associated, etc.).[22] The definition can be further stretched to include animals that lack one or more types of microorganisms but harbour an otherwise normal complex microbiota. For instance, using a combination of gnotobiotic technology and antibiotic treatment, it was possible to derive a colony of Balb/c mice that did not harbor lactobacilli in their gastrointestinal tracts but retained a complex collection of microbes, functionally equivalent to those of their conventional counterparts.[23] Animals that are guaranteed to exclude particular pathogens are called specific pathogen-free animals, and although not strictly gnotobiotic, this animals have been derived from GF ancestors and are normally kept under meticulous barrier conditions. In contrast to gnotobiotic animals, animals carrying the full (undefined) burden of microorganisms usually associated with their species are described as "conventional."[24]

The first GF animals were successfully produced at the end of the nineteenth century. Using aseptic caesarean section, Nuttal and Thierfelder generated GF guinea pigs and maintained them for two weeks under axenic conditions.[25] The rearing of GF rodents through successive generations in axenic conditions, however, was not achieved until the 1940s by Reyniers and coworkers at the University of Notre Dame[26] and by Gustafsson and coworkers at Lund University.[7,27,28] The approach to generate the original GF progenitor involved hand feeding pups with an artificial diet in a sterile isolator until maturity, after which a breeding GF colony was established from these progenitors.[29] The process was hampered by considerable logistical and technical challenges: it required an understanding of the composition of rodent milk, the development of a suitable dietary substitute for the pups, a method to sterilize the diet without affecting its nutritional value, and devising methods for hand-rearing the pups.[30]

Nowadays, colonies of GF rodents are generated and established through two experimental procedures. Some laboratories perform a Cesarean section on conventionally raised pregnant females at term (whose timing must be carefully calculated). Mothers are euthanized, their bodies passed through a germicidal bath, and the pups delivered inside a GF isolator (usually Trexler-type plastic isolators under positive pressure). After resuscitation, pups are placed with a GF foster mother that has recently delivered her litter, with the expectation she will accept the new pups. The GF status of the breeding colony must be confirmed and repeatedly tested. Another method, involves embryo transfer at the two-cell stage using a pseudo-pregnant GF female as recipient.[31,32] The donor female is superovulated by injecting gonadotrophin, and then mated. A few hours later, oviducts are dissected and embryos flushed out under the microscope. Fertilized two-cell stage embryos are washed with antibiotic-containing medium, and transferred into the oviducts of a GF recipient female that had been mated with a vasectomized GF male. Again, the GF conditions of the colony must subsequently be confirmed. This is generally achieved by culturing fecal pellets and skin swabs in universal culture media under both aerobic and anaerobic conditions, complemented with PCR on feces using bacterial-specific primers.

Perhaps the simplest strategy to understand microbial-host functional conversations is to study a particular host function in GF conditions, and then evaluate the consequences of adding a single or defined population of bacteria to the GF animal. Alternatively, the impact on a given host function could be investigated during the conventionalization of a GF animal. However, one has to be mindful that GF animals are functionally and physically immature in many physiological systems (immune and non-immune), which challenges comparisons of results obtained in GF conditions to those in natural settings. For example, mice raised in GF conditions show an immature intestinal pattern of high sialyltransferase and low fucosyltransferase activities relative to conventional mice;[33] the content of intestinal IgA-secreting plasma cells is reduced in GF animals[34] as is the size and number of Peyer's patches.[35] These differences are also observed systemically due to soluble bacterial structures being absent in GF animals.[36] A comprehensive summary of the multiple defects in structure and function of different organs in GF animals can be found elsewhere.[28,32] In most cases, the underpinning mechanisms of these alterations are not fully understood.

In addition, conventional animals have acquired the experience of living with their microbial communities since they were born[37] (or maybe even in utero[38-40]), so it is expected that the various systems that are affected directly or indirectly by this life-long history of commensalism would respond differently if the microbial stimulus is removed. In other words, because some functions in GF rodents have not achieved a sufficient level of development through life, simply inoculating bacteria into adult GF animals during a short period of time may reveal only partially how the microbes impact the physiology of the host.

Notwithstanding these limitations, gnotobiotic animals have been instrumental for researchers in understanding aspects of the mechanisms behind the assembly of the complex bacterial community, its implications in numerous diseases, and the evaluation of potential therapeutic solutions.

Simplified Microbiota Models

Gnotobiotic mice colonized with a pure bacterial culture (mono-association) represent the most reductionist approach for obtaining information about host-microbe specificity, the ecological

niche of that particular microbe, and mechanisms of pathogenicity, without competition from other species. Ex-GF NMRI mice mono-associated with *Bacteroides thetaiotaomicron*, a normal resident of the distal intestine of mice and humans, were used to demonstrate specific biochemical factors involved during bacterial colonisation.[41] This simple ecosystem reproduces the cellular, spatial, and kinetic features displayed by a complete microbiota with regards to the utilization of the host epithelial fucosylated glycans as a source of energy.[42] *B. thetaiotaomicron* senses the availability of fucose in the gut through the repressor FucR, and coordinates the expression of enzymes in the L-fucose pathway with those that regulate the production of fucosylated glycans in intestinal enterocytes. The authors speculated that during weaning, when nutrient accessibility becomes critical and fucose availability declines, *B. thetaiotaomicron* is capable of instructing the host to produce hydrolysable fucosylated glycans in order to ensure a sustained supply of carbohydrates. Similar studies by the same group have demonstrated that this organism can vary the expression pattern of its genes related to the utilization of polysaccharides as a function of the host's diet: during transition from milk to polysaccharide-rich chow at weaning,[43] or after switching from a diet rich in plant polysaccharides to a diet devoid of them but rich in simple sugars.[44,45] In addition, by simultaneously profiling the relative abundance of thousands of *B. thetaiotaomicron* mutants under various conditions, Goodman and colleagues were able to identify genes that are critical for the establishment and persistence of this bacterium in the human gut.[46] Strictly speaking, this is not a mono-associated model, but because all mutants belong to the same strain and individual mutants can be retrieved and analyzed, the model allows for the identification of various microbial functions, including adaptation to the host, in one single strain.

The metabolic interactions that occur in the large bowel have also been explored using defined microbial communities consisting of two or more representatives of microbes naturally occurring in humans. If sequenced representatives are chosen, predictions of their functions could be made after inspecting their genomes as well as how they modulate their gene expression in response to host stimuli or diet. Studies in a two-member human microbiota model (*B. thetaiotaomicron* and *E. rectale* in NMRI mice) demonstrated the ability of intestinal microbes to adapt their environment in the presence of neighboring bacteria. For instance, *B. thetaiotaomicron* upregulates the expression of a variety of polysaccharide utilization loci (PUL) to broaden its niche and degrade greater variety of glycan substrates, including those derived from the host that *E. rectale* is not able to access. In contrast, *E. rectale* became more selective in its harvest of sugars and other nutrients, downregulating a significant number of genes for carbohydrate metabolism in the presence of its neighbor, but increasing the expression of selected sugar and amino acid transporters.[47] *E. rectale* utilizes acetate produced by *B. thetaiotaomicron* to generate large amounts of butyrate, which in turn is used by the intestinal epithelium. A set of key metabolic genes relevant to energy conservation is also upregulated when *E. rectale* encounters *B. thetaiotaomicron*. Interestingly, the pathway for acetate metabolism observed in this model differs significantly from that in mice colonised with *B. thetaiotaomicron* and *Methanobrevibacter smithii*, a single predominant archeal methanogen in humans.[48] In this case, there is an increased production of acetate and no diversion to butyrate, indicating a specificity of the ecological dynamics in the intestinal tract. The quest for successful strategies to manipulate energy harvest from the diet has also been directed to the role of acetogens and sulfate-reducing bacteria. Using an elegant approach in bicolonized mice, Rey and colleagues characterized the niches of two acetogens in the mammalian gut: *Blautia hydrogenotrophica* and *Marvinbryantia formatexigens*. These microorganisms produce acetate from H_2 and CO_2 via the acetyl-CoA pathway in the distal colon, making an important contribution to the nutrition of the host.[49] The authors demonstrated through a combination of transcriptomics and mass spectrometry of metabolites that these two species occupy different niches in the intestinal tract with their own patterns of substrate utilization: *B. hyrogenotrophica* forages on complex oligosaccharides derived from the diet and the host, whereas *M. formatexigens* consumes mono- and oligo-saccharides resulting in a differential impact on energy balance.[50]

A step up in complexity, the association of GF animals with 5–15 species provides a more complex yet simple enough model to investigate host-microbe and microbe-microbe interactions. A simplified human intestinal microbiota consisting of seven bacterial species harbored in gnotobiotic rats[51] showed metabolic functions comparable to conventional rats with respect to previously proposed mucosa-associated characteristics:[52] production of short-chain fatty acids, conversion of bilirubin to urobilinogen, degradation of mucins and β-aspartylglycine, and inactivation of trypsin. Genomes of the selected bacterial community are publicly available, which has facilitated (as already mentioned) further studies at the molecular level. This approach can also be used to develop predictive models to speculate on the effect of various perturbations in the composition of the bacterial community. For instance, changes in species abundance and microbial gene expression in response to different diets were studied in a model community of 10 sequenced human intestinal bacteria in gnotobiotic mice. Transcript levels were used to develop a statistical model to identify dietary factors responsible for the changes in the microbial community and explain the interrelationship between diet and the structure of the gut microbiome.[53]

Occasionally, standard methods of inoculation of bacteria into GF animals do not result in a complete colonisation of a microbial set, even for a relatively simple 10-member community. However, it has been reported that almost the whole community could be successfully established for up to 70 days when single bacterial strains were inoculated into individual animals followed by grouping the animals to exchange their microbiotas.[54] Taking advantage of the coprophagic habits of the rodents, a high level of microbial colonisation was achieved. Moreover, when GF animals were introduced into the colony after a few weeks, they quickly acquired a similar microbiota to that of the donors. This suggests that the transferred microbiota had already achieved a significant level of stability and adaptation to the rodent gut environment. These observations suggest that the assembly of microbial

communities is governed, among other factors, by niche-related deterministic processes.

The Specific Pathogen-Free Animal

There is considerable evidence that infections and general wellbeing in laboratory animals influence a variety of biological parameters that in turn significantly affect the outcomes of scientific experiments. Over the years, governmental, academic, and professional organizations have recommended programs for health monitoring of breeding colonies with the intention to harmonize procedures,[55-57] and although local circumstances and historical practices may affect how these recommendations are actually applied, almost all commercial breeders are currently able to supply laboratory rodents with certified specific pathogen-free (SPF) status. This label is used only to indicate that the colony from which they originated tested negative for certain pathogens or perhaps opportunistic agents that are known to result in subclinical infections. SPF rodents are produced in barrier rooms in uncovered cages, and because of their exposure to microorganisms in the environment (air, food, humans and litter), they soon become colonized with commensal bacteria, the diversity of which is yet to be accurately defined.[31] The inadequate characterization of the "normal" microbial community structure in SPF rodents has considerable implications for the relevance of such animals as a standard model for the investigation of bacteria-host interactions.

SPF rodents currently available have been derived from previously GF ancestors that were associated with a few bacterial isolates originating from the feces of a healthy mouse. The original experiment was performed by Russell Schaedler and his colleagues during the mid-1960s.[58,59] They used pure cultures of four bacterial species (lactobacilli, anaerobic group N streptococci, bacteroides, and coliform bacilli) to inoculate GF mice in their laboratory at the Rockefeller Institute. The animals were maintained in plastic isolators where they were given food contaminated with the bacterial isolates. The experiment was initially conceived to assess the consequences of associating fecal isolates with GF mice, but given that the animals became protected against acquisition of opportunistic bacteria, the researchers subsequently supplied animal breeders with this "cocktail" of microorganisms for use in colonizing their rodent colonies.[60] A few other attempts to include extremely oxygen-sensitive fusiform (EOS) bacteria in defined microbiotas were later used for gnotobiotic studies. EOS bacteria constitute the predominant microbiota of mice but are technically challenging to manipulate in the laboratory.[61] One of those microbiotas, the altered Schaedler flora (ASF), reached great popularity in the late 70s and 80s when the National Cancer Institute decided to standardize the microbiota used in their rodent colonies and those of their contractors.[62] The ASF consisted of eight bacterial species: *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Bacteroides distasonis*, four extremely oxygen sensitive bacteria, and one spiral-shaped bacterium. Over the years, breeders of mice and rats[63] around the world have adopted the ASF and kept the animals for generations under strict barrier conditions to maintain their SPF status. However, animals harboring this microbiota have not evolved to a widespread model to study microbial-host interactions. Advances in ongoing genome projects coupled with current ease in cultivating strict anaerobes would certainly overcome previous technical hurdles to work with members of this microbial cocktail, although to date, this cocktail has not been deposited in any official culture repository.

The Human Flora-Associated (HFA) Rodent Model

The inoculation of GF mice or rats with fecal suspensions originating from human donors was conceived as a strategy to circumvent the variability introduced by environmental and genetic factors in human studies, or when ethical or practical reasons limited the study of the gastrointestinal communities directly on human volunteers. Investigators had hoped that HFA rodents would mimic the microbiota of the human intestinal tract, therefore being a more relevant model than their conventional counterparts for predicting the situation in humans. However, much controversy has been generated over the adequacy of HFA animals as surrogates for studying the ecology and metabolism of the human microbiota, with valid arguments on each side of the debate.[64]

The likelihood of successfully transferring human fecal bacteria into recipient rodents is questionable. Early reports indicated that the composition of the intestinal microbiota of HFA animals was similar to that of donor human inocula when classical microbiology techniques were employed.[65,66] We now know that microbial cultures largely underestimate the complexity and size of intestinal microbial communities;[67,68] therefore, conclusions from those observations may be biased, as they merely consider a subset of the full microbial load administered to the animals. But even with these technical limitations, scientists have noticed, in some cases, that not all members of the initial inocula could be implanted, in particular bifidobacteria and lactobacilli.[69-71] Recent publications adopt a more critical perspective by concluding that the composition of microbial communities from human donors resembles that of their corresponding HFA rodents only at the predominant species level.[72,73] Difficulties in achieving an exact match in microbial profiles between feces from recipient animals and human donors are not unexpected: reciprocal inoculation experiments in related vertebrates show that the host environment plays an important role in determining the microbial makeup.[74] This was challenged, however, when scientists from the Gordon group published the results of a transplantation study of human intestinal communities into GF animals.[75] Using multiplex pyrosequencing of the bacterial 16S rRNA genes and statistical models to compare the degree of similarity of the fecal bacterial communities, the authors demonstrated that human fecal microbiotas were successfully transplanted to GF mice with a significant preservation of their structure and diversity, even when the starting material was frozen feces. All bacterial phyla, 11 out of 12 bacterial classes, and 88% (58/66) of the genera detected in the donor sample were detected among the recipient mice, and this structure was stable for up to one month. Interestingly, the humanized mouse microbiota could be

transmitted to a second generation of mice without a significant reduction in diversity.

From a metabolic standpoint, it may also be questioned whether the enzymatic activities tested or the metabolite profiles assessed represent valid readouts to determine a successful transfer of microbial activity from human donors to rodents. A limited set of gastrointestinal enzyme activities (β-glucosidase, β-glucuronidase, nitrate reductase, nitroreductase) are generally measured, and levels of some putrefactive products or short chain fatty acid are reported.[76-78] In any case, bacterial metabolism in the intestine of HFA mice reflected that of human feces only with respect to some metabolic activities, probably due to changes in the bacterial composition or a different intestinal environment in the recipient animals compared with that in the donors. Recent data indicate that it is possible to cluster microbial communities based on their gene content and infer pathways involved in their metabolism to better compare transmissibility of their function.[75] One report suggests that it is possible to reproduce the functions and composition of the human gut microbiota with remarkable similarity out of its readily culturable members using anaerobic culture conditions. When transplanted to gnotobiotic mice, the complete fecal set was comparable to the cultured community in its colonization dynamics, distribution and responsiveness to dietary changes.[79] It would be interesting to investigate how the metabolic functions of transplanted microbiotas compare with those developed by the microbiotas that co-evolved with their hosts.

Diet can have a profound effect on the resulting bacterial composition following inoculation of mice with fecal slurries. Since rodents and humans have different dietary habits, it should come as no surprise to find that failure to stabilize human-derived microbiotas in mice could be due to substantially different nutrient profiles. Standard chow diets normally administered to rodents are low in fat but rich in complex polysaccharides generally originating from plants; a stark difference to typical Western-like human diets, high in fat and simple sugars. Studies feeding rodents with human diets indicate that mice and rats show high adaptability to changes in their diets, although not without major impact on their metabolism.[80,81] Consequently, feeding HFA rodents with human diets as a strategy to stabilize their human-derived microbiota should be measured against how well the animals are able to maintain a normal metabolism.

It may be less debateable though, that the simulation of human gastrointestinal conditions in HFA mice represents a suitable and reliable approach for the investigation of colonisation resistance against pathogenic bacteria,[82,83] impact of the consumption of toxic compounds,[84] or carcinogens,[85] and the efficacy of therapeutic drugs. HFA animals have been used to assess the effects of antibiotics on human intestinal microbiota[86,87] and the risks associated with DNA transfer from food-borne genetically modified microorganisms.[88] Colon cancer biomarkers have also been studied in HFA animals.[89] The characterization of the microbiota configuration and its variations along the length of the gut, even when the gastrointestinal tract of rodents is not exactly the same as humans, is an example of a practical use of the model.[75]

Despite its limitations, the HFA mice model continues to stand as a useful tool for studying the ecosystem and metabolism of the human microbiota in conditions similar to those of the human intestinal tract. It is a useful substitute for human volunteers, especially when it is difficult to control for genetic, environmental, dietary and statistical factors that usually challenge conclusions from clinical studies.

The Microbiota of Conventionally Raised and Feral Animal

While gnotobiotic mice are colonised with simple, defined collections of microorganisms, conventional animals carry a full, usually undefined, community of microbes associated with their species. It has been discussed previously that even the most strict barrier conditions cannot prevent SPF animals from acquiring environmental bacteria, and that microbiological monitoring of these animals only aim at documenting that they are free of pathogens without providing an inventory of the actual microbial population. Given that the environmental conditions under which SPF animals are raised are considerably different to those used for conventional mice, it is highly likely that the microbiota that becomes adapted through successive generations in each type of animal is also different. Similarly, one could speculate that the microbiota of conventional laboratory mice do not truly represent the "normal" biota of mice. Husbandry is, therefore, an important consideration for host-microbe interactions.[90-92]

The composition of the fecal bacteria in feral or "wild" mice was determined by Wilson et al.[93] using 16S rRNA gene sequence analysis, and compared with that of SPF animals. Wild animals harbored a much larger proportion of bacteroides and lactobacilli, whereas the majority of sequences found in laboratory mice belonged to anaerobic clostridia. Although the study was not sufficiently powered to justify a broad conclusion (only 2 feral and 3 laboratory mice were used), these observations suggest that foreign microorganisms may have replaced the indigenous biota that co-evolved with mice in nature.

Understanding the evolutionary processes by which mammals have been interacting with their bacterial commensals and the factors that affect community makeup have been the aims of large programs, like the multinational MetaHIT[94,95] initiative and the Human Microbiome Project[96,97] supported by NIH. Although reviewing the scientific evidence that supports these concepts is out of the scope of this review, it is important to recognize their implications for the selection of an animal model for studying host-bacteria interactions. If we accept that mammals have co-evolved with microbes and therefore did not need to develop functions that are provided by bacteria, including the ability to extract energy and nutrients from the diet,[98] it is fair to assume that the physiological functions of laboratory mice are likely to differ from those naturally occurring in feral mice. Of course, this may be an oversimplified conclusion as we now know that even distantly related vertebrates share similar microbiotas, at least at a shallow phylogenetic level,[37] and that considerable functional redundancy of the gut microbiota has been reported.[99-101] Nevertheless, as more sophisticated metagenomic tools are becoming readily available, it

may be possible to detect even subtle functional differences in the host-microbe relationship, suggesting that selecting the appropriate model is, after all, not a trivial decision.

The identification and differentiation of "autochthonous" (resident) microbes from "allochthonous" (transient) microbial members of the intestinal community have been challenging tasks. The concept of autochthony has been extensively reviewed,[102,103] and raises fundamental questions concerning the ecological role of a given species in the complex intestinal environment. For instance, autochthonous species are specialized to occupy a defined physical niche and form stable populations during long periods of time, whereas transient species may behave more unpredictably depending on the endogenous or exogenous factors, such as the diet of the host. Native microorganisms can develop a remarkable host specialization, which has implications on the model of choice if the aim is to study these microorganisms in their native habitat: several reports indicate that some species of lactobacilli are not native to the human large bowel,[68,104,105] originating probably from ingested foods or other regions of the intestinal tract.[106] This is not the case for lactobacilli in rodents, where they can form stable and specialized communities; therefore, having a different impact on the host.

Final Remarks

Human health could be thought of as the collective property of human-associated microbiota, and experimental tools to decorticate the mechanisms that govern this interaction are becoming increasingly necessary. Gnotobiology coupled with molecular genetics provide an excellent technology to create and manipulate bacterial ecosystems to investigate fundamental questions about us and our intestinal symbionts. Although one single model may not suffice to unravel the complexity of these interactions, thorough consideration of the limitations inherent to each model will certainly allow us to articulate the right questions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci U S A 2008; 105:17994-9; PMID:19004758; http://dx.doi.org/10.1073/pnas.0807920105.

2. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al.; NISC Comparative Sequencing Program. Topographical and temporal diversity of the human skin microbiome. Science 2009; 324:1190-2; PMID:19478181; http://dx.doi.org/10.1126/science.1171700.

3. Nasidze I, Li J, Quinque D, Tang K, Stoneking M. Global diversity in the human salivary microbiome. Genome Res 2009; 19:636-43; PMID:19251737; http://dx.doi.org/10.1101/gr.084616.108.

4. Human Microbiome Project Consortium. A framework for human microbiome research. Nature 2012; 486:215-21; PMID:22699610; http://dx.doi.org/10.1038/nature11209.

5. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005; 308:1635-8; PMID:15831718; http://dx.doi.org/10.1126/science.1110591.

6. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al.; MetaHIT Consortium. Enterotypes of the human gut microbiome. Nature 2011; 473:174-80; PMID:21508958; http://dx.doi.org/10.1038/nature09944.

7. Gustafsson BE. Vitamin K deficiency in germfree rats. Ann N Y Acad Sci 1959; 78:166-74; PMID:13830426; http://dx.doi.org/10.1111/j.1749-6632.1959.tb53101.x.

8. Bouskra D, Brézillon C, Bérard M, Werts C, Varona R, Boneca IG, et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature 2008; 456:507-10; PMID:18987631; http://dx.doi.org/10.1038/nature07450.

9. Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 2004; 303:1662-5; PMID:15016999; http://dx.doi.org/10.1126/science.1091334.

10. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol 1997; 159:1739-45; PMID:9257835.

11. Ismail AS, Behrendt CL, Hooper LV. Reciprocal interactions between commensal bacteria and γ δ intraepithelial lymphocytes during mucosal injury. J Immunol 2009; 182:3047-54; PMID:19234201; http://dx.doi.org/10.4049/jimmunol.0802705.

12. Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci U S A 2002; 99:15451-5; PMID:12432102; http://dx.doi.org/10.1073/pnas.202604299.

13. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004; 101:15718-23; PMID:15505215; http://dx.doi.org/10.1073/pnas.0407076101.

14. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 2011; 108(Suppl 1):4615-22; PMID:20660719; http://dx.doi.org/10.1073/pnas.1000082107.

15. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 2010; 328:228-31; PMID:20203013; http://dx.doi.org/10.1126/science.1179721.

16. Escherich T. Die Darmbakterien des Neugeborenen und Sauglings. The intestinal bacteria of the neonate and breast-fed infant. Fortschr Med 1885; 3:515-22.

17. Schottelius M. Die Bedeutung der Darmbakterien fur die Ehrnahrung. II Arch Hyg 1902; 42:48-70.

18. Reyniers JA, Trexler PC. The design of micrurgical machines for use in bacteriology. In: Thomas CC, ed. Micrurgical and Germfree Techniques. Springfield, 1943:5-25.

19. Reyniers JA, Trexler PC, Ervin RF, Wagner M, Luckey TD, Gordon HA. The need for a unified terminology in germfree life studies. Lobund Reports 1949; 2:151-62.

20. Baker JA, Ferguson MS. Growth of platyfish (*Platypoecilus maculatus*) free from bacteria and other microorganisms. Proc Soc Exp Biol Med 1942; 51:116-9.

21. Fitzgerald RJ. Gnotobiotic contribution to oral microbiology. J Dent Res 1963; 2:549-52; PMID:13945366; http://dx.doi.org/10.1177/00220345630420016601.

22. Trexler PC, Orcutt RP. Development of gnotobiotics and contamination control in laboratory animal science. In: McPherson CW, Mattingly SF, eds. 50 Years of Laboratory Animal Science. Memphis, TN: Am Assoc Lab Anim Sci, 2000:121-8.

23. Tannock GW, Crichton C, Welling GW, Koopman JP, Midtvedt T. Reconstitution of the gastrointestinal microflora of lactobacillus-free mice. Appl Environ Microbiol 1988; 54:2971-5; PMID:2975941.

24. Coates ME. Gnotobiotic animals in research: their uses and limitations. Lab Anim 1975; 9:275-82; PMID:1107656; http://dx.doi.org/10.1258/002367775780957296.

25. Nuttal G, Thierfelder H. Thierisches Leben ohne Bacterien im Verdauungskanal. Z Phys Chem 1896; 21:109-21; http://dx.doi.org/10.1515/bchm2.1896.21.2-3.109.

26. Reyniers JA. The pure culture concept and gnotobiotics. Ann N Y Acad Sci 1959; 78:3-16; http://dx.doi.org/10.1111/j.1749-6632.1959.tb53091.x.

27. Gordon HA. The germ-free animal. Its use in the study of "physiologic" effects of the normal microbial flora on the animal host. Am J Dig Dis 1960; 5:841-67; PMID:13707167; http://dx.doi.org/10.1007/BF02232187.

28. Wostmann BS. The germfree animal in nutritional studies. Annu Rev Nutr 1981; 1:257-79; PMID:6764717; http://dx.doi.org/10.1146/annurev.nu.01.070181.001353.

29. Reyniers JA, Sacksteder MR. Observations on the survival of germfree C3H mice and their resistance to a contaminated environment. Proc Anim Care Panel 1958; 8:41-53.

30. Gustafsson BE. Germ free rearing of rats. General technique. Acta Pathol Microbiol Scand 1948; 73:1-130.

31. Faith JJ, Rey FE, O'Donnell D, Karlsson M, McNulty NP, Kallstrom G, et al. Creating and characterizing communities of human gut microbes in gnotobiotic mice. ISME J 2010; 4:1094-8; PMID:20664551; http://dx.doi.org/10.1038/ismej.2010.110.

32. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol 2007; 19:59-69; PMID:17118672; http://dx.doi.org/10.1016/j.smim.2006.10.002.

33. Nanthakumar NN, Dai D, Newburg DS, Walker WA. The role of indigenous microflora in the development of murine intestinal fucosyl- and sialyltransferases. FASEB J 2003; 17:44-6; PMID:12475916.

34. Glaister JR. Factors affecting the lymphoid cells in the small intestinal epithelium of the mouse. Int Arch Allergy Appl Immunol 1973; 45:719-30; PMID:4749414; http://dx.doi.org/10.1159/000231071.

35. Gordon HA. Morphological and physiological characterization of germfree life. Ann N Y Acad Sci 1959; 78:208-20; PMID:13851201; http://dx.doi.org/10.1111/j.1749-6632.1959.tb53104.x.

36. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122:107-18; PMID:16009137; http://dx.doi.org/10.1016/j.cell.2005.05.007.

37. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, et al. Evolution of mammals and their gut microbes. Science 2008; 320:1647-51; PMID:18497261; http://dx.doi.org/10.1126/science.1155725.

38. Jiménez E, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, et al. Is meconium from healthy newborns actually sterile? Res Microbiol 2008; 159:187-93; PMID:18281199; http://dx.doi.org/10.1016/j.resmic.2007.12.007.

39. Satokari R, Grönroos T, Laitinen K, Salminen S, Isolauri E. *Bifidobacterium* and *Lactobacillus* DNA in the human placenta. Lett Appl Microbiol 2009; 48:8-12; PMID:19018955; http://dx.doi.org/10.1111/j.1472-765X.2008.02475.x.

40. Pettker CM, Buhimschi IA, Magloire LK, Sfakianaki AK, Hamar BD, Buhimschi CS. Value of placental microbial evaluation in diagnosing intra-amniotic infection. Obstet Gynecol 2007; 109:739-49; PMID:17329528; http://dx.doi.org/10.1097/01.AOG.0000255663.47512.23.

41. Hooper LV, Xu J, Falk PG, Midtvedt T, Gordon JI. A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proc Natl Acad Sci U S A 1999; 96:9833-8; PMID:10449780; http://dx.doi.org/10.1073/pnas.96.17.9833.

42. Bry L, Falk PG, Midtvedt T, Gordon JI. A model of host-microbial interactions in an open mammalian ecosystem. Science 1996; 273:1380-3; PMID:8703071; http://dx.doi.org/10.1126/science.273.5280.1380.

43. Bjursell MK, Martens EC, Gordon JI. Functional genomic and metabolic studies of the adaptations of a prominent adult human gut symbiont, *Bacteroides thetaiotaomicron*, to the suckling period. J Biol Chem 2006; 281:36269-79; PMID:16968696; http://dx.doi.org/10.1074/jbc.M606509200.

44. Sonnenburg JL, Xu J, Leip DD, Chen CH, Westover BP, Weatherford J, et al. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 2005; 307:1955-9; PMID:15790854; http://dx.doi.org/10.1126/science.1109051.

45. Martens EC, Chiang HC, Gordon JI. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 2008; 4:447-57; PMID:18996345; http://dx.doi.org/10.1016/j.chom.2008.09.007.

46. Goodman AL, McNulty NP, Zhao Y, Leip D, Mitra RD, Lozupone CA, et al. Identifying genetic determinants needed to establish a human gut symbiont in its habitat. Cell Host Microbe 2009; 6:279-89; PMID:19748469; http://dx.doi.org/10.1016/j.chom.2009.08.003.

47. Mahowald MA, Rey FE, Seedorf H, Turnbaugh PJ, Fulton RS, Wollam A, et al. Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proc Natl Acad Sci U S A 2009; 106:5859-64; PMID:19321416; http://dx.doi.org/10.1073/pnas.0901529106.

48. Samuel BS, Gordon JI. A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. Proc Natl Acad Sci U S A 2006; 103:10011-6; PMID:16782812; http://dx.doi.org/10.1073/pnas.0602187103.

49. Breznak JA, Switzer JM. Acetate synthesis from H2 plus CO2 by termite gut microbes. Appl Environ Microbiol 1986; 52:623-30; PMID:16347157.

50. Rey FE, Faith JJ, Bain J, Muehlbauer MJ, Stevens RD, Newgard CB, et al. Dissecting the in vivo metabolic potential of two human gut acetogens. J Biol Chem 2010; 285:22082-90; PMID:20444704; http://dx.doi.org/10.1074/jbc.M110.117713.

51. Becker N, Kunath J, Loh G, Blaut M. Human intestinal microbiota: Characterization of a simplified and stable gnotobiotic rat model. Gut Microbes 2011; 2:1-9; PMID:21637012.

52. Midtvedt T, Bjørneklett A, Carlstedt-Duke B, Gustafsson BE, Høverstad T, Lingaas E, et al. The influence of antibiotics upon microflora-associated characteristics in man and mammals. Prog Clin Biol Res 1985; 181:241-4; PMID:4022981.

53. Faith JJ, McNulty NP, Rey FE, Gordon JI. Predicting a human gut microbiota's response to diet in gnotobiotic mice. Science 2011; 333:101-4; PMID:21596954; http://dx.doi.org/10.1126/science.1206025.

54. Rezzonico E, Mestdagh R, Delley M, Combremont S, Dumas ME, Holmes E, et al. Microbial and metabolic characterization of a simplified microbiota mouse model. Gut Microbes 2011; 2:307-18.

55. Recommendations for the health monitoring of mouse, rat, hamster, guineapig and rabbit breeding colonies. Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Animal Health accepted by the FELASA Board of Management November 1992. Lab Anim 1994; 28:1-12; PMID:8158962; http://dx.doi.org/10.1258/002367794781065933.

56. Laboratory Animal Breeders Association Accreditation Scheme (LABAAS) Manual 1993.

57. Nicklas W, Baneux P, Boot R, Decelle T, Deeny AA, Fumanelli M, et al.; FELASA (Federation of European Laboratory Animal Science Associations Working Group on Health Monitoring of Rodent and Rabbit Colonies). Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. Lab Anim 2002; 36:20-42; PMID:11831737; http://dx.doi.org/10.1258/0023677021911740.

58. Schaedler RW, Dubs R, Costello R. Association of germfree mice with bacteria isolated from normal mice. J Exp Med 1965; 122:77-82; PMID:14325475; http://dx.doi.org/10.1084/jem.122.1.77.

59. Schaedler RW, Dubos R, Costello R. The development of the bacterial flora in the gastrointestinal tract of mice. J Exp Med 1965; 122:59-66; PMID:14325473; http://dx.doi.org/10.1084/jem.122.1.59.

60. Baker DE. The commercial production of mice with a specified flora. Natl Cancer Inst Monogr 1966; 20:161-6; PMID:5948524.

61. Dewhirst FE, Chien CC, Paster BJ, Ericson RL, Orcutt RP, Schauer DB, et al. Phylogeny of the defined murine microbiota: altered Schaedler flora. Appl Environ Microbiol 1999; 65:3287-92; PMID:10427008.

62. Orcutt RP, Gianni FJ, Judge RJ. Development of an "Altered Schaedler Flora" for the generation of NCI gnotobiotic rodents. Microecol Ther 1987; 17:59.

63. Rohde CM, Wells DF, Robosky LC, Manning ML, Clifford CB, Reily MD, et al. Metabonomic evaluation of Schaedler altered microflora rats. Chem Res Toxicol 2007; 20:1388-92; PMID:17900170; http://dx.doi.org/10.1021/tx700184u.

64. Silley P. Human flora-associated rodents—does the data support the assumptions? Microb Biotechnol 2009; 2:6-14; PMID:21261878; http://dx.doi.org/10.1111/j.1751-7915.2008.00069.x.

65. Hazenberg MP, Bakker M, Verschoor-Burggraaf A. Effects of the human intestinal flora on germ-free mice. J Appl Bacteriol 1981; 50:95-106; PMID:7014548; http://dx.doi.org/10.1111/j.1365-2672.1981.tb00874.x.

66. Pecquet S, Andremont A, Tancrède C. Selective antimicrobial modulation of the intestinal tract by norfloxacin in human volunteers and in gnotobiotic mice associated with a human fecal flora. Antimicrob Agents Chemother 1986; 29:1047-52; PMID:3729360; http://dx.doi.org/10.1128/AAC.29.6.1047.

67. Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD, et al. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl Environ Microbiol 1999; 65:4799-807; PMID:10543789.

68. Tannock GW, Munro K, Harmsen HJM, Welling GW, Smart J, Gopal PK. Analysis of the fecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR20. Appl Environ Microbiol 2000; 66:2578-88; PMID:10831441; http://dx.doi.org/10.1128/AEM.66.6.2578-2588.2000.

69. Ducluzeau R, Ladire M, Raibaud P. Effect of bran ingestion on the microbial faecal floras of human donors and of recipient gnotobiotic mice, and on the barrier effects exerted by these floras against various potentially pathogenic microbial strains. Ann Microbiol (Paris) 1984; 135A:303-18; PMID:6326642.

70. Raibaud P, Ducluzeau R, Dubos F, Hudault S, Bewa H, Muller MC. Implantation of bacteria from the digestive tract of man and various animals into gnotobiotic mice. Am J Clin Nutr 1980; 33(Suppl):2440-7; PMID:7001883.

71. Hirayama K, Kawamura S, Mitsuoka T. Development and stability of human faecal flora in the intestine of ex-germ-free mice. Microb Ecol Health Dis 1991; 4:95-9; http://dx.doi.org/10.3109/08910609109140269.

72. Gérard P, Béguet F, Lepercq P, Rigottier-Gois L, Rochet V, Andrieux C, et al. Gnotobiotic rats harboring human intestinal microbiota as a model for studying cholesterol-to-coprostanol conversion. FEMS Microbiol Ecol 2004; 47:337-43; PMID:19712322; http://dx.doi.org/10.1016/S0168-6496(03)00285-X.

73. Kibe R, Sakamoto M, Yokota H, Ishikawa H, Aiba Y, Koga Y, et al. Movement and fixation of intestinal microbiota after administration of human feces to germfree mice. Appl Environ Microbiol 2005; 71:3171-8; PMID:15933018; http://dx.doi.org/10.1128/AEM.71.6.3171-3178.2005.

74. Rawls JF, Mahowald MA, Ley RE, Gordon JI. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell 2006; 127:423-33; PMID:17055441; http://dx.doi.org/10.1016/j.cell.2006.08.043.

75. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 2009; 1:ra14; PMID:20368178; http://dx.doi.org/10.1126/scitranslmed.3000322.

76. Hirayama K, Itoh K, Takahashi E, Mitsuoka T. Comparison of composition of faecal microbiota and metabolism of faecal bacteria among "human-flora-associated" mice inoculated with faeces from six different human donors. Microb Ecol Health Dis 1995; 8:199-211; http://dx.doi.org/10.3109/08910609509140098.

77. Hirayama K. Ex-germ free mice harbouring intestinal microbiota derived from other animal species as an experimental model for ecology and metabolism of intestinal bacteria. Exp Anim 1999; 48:146-52; http://dx.doi.org/10.1538/expanim.48.219.

78. Mallett AK, Bearne CA, Rowland IR, Farthing MJ, Cole CB, Fuller R. The use of rats associated with a human faecal flora as a model for studying the effects of diet on the human gut microflora. J Appl Bacteriol 1987; 63:39-45; PMID:2820914; http://dx.doi.org/10.1111/j.1365-2672.1987.tb02415.x.

79. Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, Dantas G, et al. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. Proc Natl Acad Sci U S A 2011; 108:6252-7; PMID:21436049; http://dx.doi.org/10.1073/pnas.1102938108.

80. Gallou-Kabani C, Vigé A, Gross MS, Rabès JP, Boileau C, Larue-Achagiotis C, et al. C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. Obesity (Silver Spring) 2007; 15:1996-2005; PMID:17712117; http://dx.doi.org/10.1038/oby.2007.238.

81. Simmgen M, Knauf C, Lopez M, Choudhury AI, Charalambous M, Cantley J, et al. Liver-specific deletion of insulin receptor substrate 2 does not impair hepatic glucose and lipid metabolism in mice. Diabetologia 2006; 49:552-61; PMID:16404553; http://dx.doi.org/10.1007/s00125-005-0084-4.

82. Edwards CA, Rumney C, Davies M, Parrett AM, Dore J, Martin F, et al. A human flora-associated rat model of the breast-fed infant gut. J Pediatr Gastroenterol Nutr 2003; 37:168-77; PMID:12883304; http://dx.doi.org/10.1097/00005176-200308000-00016.

83. Henteges DJ, Marsh WW, Petschow BW, Rahman ME, Dougherty SH. Influence of human milk diet on colonization resistance mechanisms against *Salmonella typhimurium* in human faecal bacteria-associated mice. Microb Ecol Health Dis 1995; 8:139-49; http://dx.doi.org/10.3109/08910609509140092.

84. Scheepers PTJ, Velders DD, Steenwinkel MJST, van Delft JHM, Driessen W, Stratemans MME, et al. Role of intestinal microflora in the formation of DNA and haemoglobin adducts in rats treated with 2-nutroflutorene and 2-aminofluorene by gavage. Carcinogenesis 1994; 15:1422-41; http://dx.doi.org/10.1093/carcin/15.7.1433.

85. Gordon HA, Pesti L. The gnotobiotic animal as a tool in the study of host microbial relationships. Bacteriol Rev 1971; 35:390-429; PMID:4945725.

86. Barc MC, Bourlioux F, Rigottier-Gois L, Charrin-Sarnel C, Janoir C, Boureau H, et al. Effect of amoxicillin-clavulanic acid on human fecal flora in a gnotobiotic mouse model assessed with fluorescence hybridization using group-specific 16S rRNA probes in combination with flow cytometry. Antimicrob Agents Chemother 2004; 48:1365-8; PMID:15047545; http://dx.doi.org/10.1128/AAC.48.4.1365-1368.2004.

87. Andremont A. Raibaud, Tancrede C. Effect of erythromycin on microbial antagonism: A study in gnotobiotic mice associated with a human faecal flora. J Infect Dis 1983; 148:579-87; PMID:6413596; http://dx.doi.org/10.1093/infdis/148.3.579.

88. Tuohy K, Davies M, Rumsby P, Rumney C, Adams MR, Rowland IR. Monitoring transfer of recombinant and nonrecombinant plasmids between *Lactococcus lactis* strains and members of the human gastrointestinal microbiota in vivo--impact of donor cell number and diet. J Appl Microbiol 2002; 93:954-64; PMID:12452951; http://dx.doi.org/10.1046/j.1365-2672.2002.01770.x.

89. Hambly RJ, Rumney CJ, Fletcher JME, Rijken PJ, Rowland IR. Effects of high- and low-risk diets on gut microflora-associated biomarkers of colon cancer in human flora-associated rats. Nutr Cancer 1997; 27:250-5; PMID:9101554; http://dx.doi.org/10.1080/01635589709514534.

90. Schellinck HM, Cyr DP, Brown RE. Chapter 7: How many ways can mouse behavioral experiments go wrong? Confounding variables in mouse models of neurodegenerative diseases and how to control them. In: Brockmann HJ, Roper TJ, Naguib M, Wynne-Edwards KE, Mitani J, Simmons LW, eds. Advances in the study of behavior. Burlington MA: Academic Press Inc. 2010: 255-365.

91. Hufeldt MR, Nielsen DS, Vogensen FK, Midtvedt T, Hansen AK. Variation in the gut microbiota of laboratory mice is related to both genetic and environmental factors. Comp Med 2010; 60:336-47; PMID:21262117.

92. Bleich A, Hansen AK. Time to include the gut microbiota in the hygienic standardisation of laboratory rodents. Comp Immunol Microbiol Infect Dis 2012; 35:81-92; PMID:22257867; http://dx.doi.org/10.1016/j.cimid.2011.12.006.

93. Wilson KH, Brown RS, Andersen GL, Tsang J, Sartor B. Comparison of fecal biota from specific pathogen free and feral mice. Anaerobe 2006; 12:249-53; PMID:17070078; http://dx.doi.org/10.1016/j.anaerobe.2006.09.002.

94. Dusko Ehrlich S; MetaHIT consortium. [Metagenomics of the intestinal microbiota: potential applications]. Gastroenterol Clin Biol 2010; 34(Suppl 1):S23-8; PMID:20889001; http://dx.doi.org/10.1016/S0399-8320(10)70017-8.

95. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al.; MetaHIT Consortium. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010; 464:59-65; PMID:20203603; http://dx.doi.org/10.1038/nature08821.

96. Xu J, Mahowald MA, Ley RE, Lozupone CA, Hamady M, Martens EC, et al. Evolution of symbiotic bacteria in the distal human intestine. PLoS Biol 2007; 5:e156; PMID:17579514; http://dx.doi.org/10.1371/journal.pbio.0050156.

97. Martiny JB, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL, et al. Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 2006; 4:102-12; PMID:16415926; http://dx.doi.org/10.1038/nrmicro1341.

98. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006; 124:837-48; PMID:16497592; http://dx.doi.org/10.1016/j.cell.2006.02.017.

99. Kolmeder CA, de Been M, Nikkilä J, Ritamo I, Mättö J, Valmu L, et al. Comparative metaproteomics and diversity analysis of human intestinal microbiota testifies for its temporal stability and expression of core functions. PLoS One 2012; 7:e29913; PMID:22279554; http://dx.doi.org/10.1371/journal.pone.0029913.

100. Tannock GW. Probiotics: time for a dose of realism. Curr Issues Intest Microbiol 2003; 4:33-42; PMID:14503687.

101. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature 2009; 457:480-4; PMID:19043404; http://dx.doi.org/10.1038/nature07540.

102. Savage DC. Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol 1977; 31:107-33; PMID:334036; http://dx.doi.org/10.1146/annurev.mi.31.100177.000543.

103. Walter J, Ley R. The human gut microbiome: ecology and recent evolutionary changes. Annu Rev Microbiol 2011; 65:411-29; PMID:21682646; http://dx.doi.org/10.1146/annurev-micro-090110-102830.

104. Walter J, Hertel C, Tannock GW, Lis CM, Munro K, Hammes WP. Detection of *Lactobacillus, Pediococcus, Leuconostoc,* and *Weissella* species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. Appl Environ Microbiol 2001; 67:2578-85; PMID:11375166; http://dx.doi.org/10.1128/AEM.67.6.2578-2585.2001.

105. Dal Bello F, Walter J, Hammes WP, Hertel C. Increased complexity of the species composition of lactic acid bacteria in human feces revealed by alternative incubation condition. Microb Ecol 2003; 45:455-63; PMID:12704557; http://dx.doi.org/10.1007/s00248-003-2001-z.

106. Bunte C, Hertei C, Hammes WP. Monitoring and survival of *Lactobacillus paracasei* LTH 2579 in food and the human intestinal tract. Syst Appl Microbiol 2000; 23:260-6; PMID:10930079; http://dx.doi.org/10.1016/S0723-2020(00)80013-2.