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The intestinal microbiota in the rat model: major breakthroughs from new technologies

Julie Tomas, Philippe Langella and Claire Cherbuy*
Commensal and Probiotics–Host Interactions Laboratory, INRA, UMR1319 MICALIS, F-78350 Jouy-en-Josas, France

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Abstract

The mammalian intestine harbors a large and diverse community of micro-organisms, known as the intestinal microbiota. Recent developments in molecular profiling methods, mainly based on microbial 16S ribosomal RNA gene sequencing, have provided unprecedented insights into the make-up and diversity of intestinal microbial communities. Using these culture-independent analyses, gut microbiota of several mammals including laboratory rodents, have been revisited. The laboratory rat is one of the major species bred and kept for scientific research. Although this animal is bred in confined environments and subjected to procedures for satisfying health requirements that hamper natural colonization, some major features of mammalian gut microbiota are conserved. However, the gut microbiota varies according to the breeding conditions of the rats and this could impact reproducibility of the experimental models. Determining the non-pathogenic microbial community might be relevant in standards of quality control of laboratory animals. Molecular profiling techniques could be applied to document this information.

Keywords: 16S rRNA gene sequencing, Bacteroidetes, Firmicutes, Proteobacteria, diversity, altered schaedler flora, antimicrobials, diet

The intestinal microbiota: a life-partner for mammals

Mammals are colonized by a diverse collection of micro-organisms, most of which reside in the distal gut. The gut microbiota is acquired soon after birth and, in humans, evolves throughout the first year of life (Adlerberth and Wold, 2009). The gut microbial community reaches about 10^{14} bacteria and consists of at least 1000 species. The genes in the collective genomes of the intestinal microbiota, also called the microbiome, have been recently analyzed in humans; they outnumber those in the human genome by more than 100-fold (Gill *et al.*, 2006; Qin *et al.*, 2010). Analyses of the composition and function of the gut microbiome evidence its oddity compared with other microbial ecosystems and its adaptation to the host digestive environment (Ley *et al.*, 2006). In spite of stringent conditions in the gut, the intestine represents an attractive niche, rich and diverse in nutrients, where microbes can reside. In return, the gut microbiota provide benefits to their host, including the enzymatic capacity to break down a great range of plant polysaccharides, the synthesis of vitamins, and the establishment of a barrier against invasive pathogenic bacteria (Gill *et al.*, 2006). Vertebrate gut microbes further contribute to detoxification of xenobiotic compounds, angiogenesis, and the maturation of the immune system (Neish, 2009) and the colonic epithelium (Cherbuy *et al.*, 2004, 2010).

Bacteria dominate the gastrointestinal tract ecosystem, but some species from the archaeal domain and eukaryotic micro-organisms can also be found (Gerritsen *et al.*, 2011). Among the 70 known bacterial phyla, two dominate the human gut microbial community (Ley *et al.*, 2006): the Firmicutes and the Bacteroidetes phyla. The Firmicutes is currently the largest bacterial phylum and contains more than 200 genera. In humans, the majority of the Firmicutes in the gastrointestinal tract are the *Clostridium coccoides* group (also called *Clostridium* cluster XIVa) and the *Clostridium leptum* group (also called *Clostridium*

*Corresponding author. E-mail: Claire.Cherbuy@jouy.inra.fr

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cluster IV). The Bacteroidetes phylum includes *Bacteroides* and *Prevotella* groups. Members of other phyla, such as Proteobacteria (Enterobacteria), Actinobacteria (Bifidobacteria), Fusobacteria, Spirochaetes, and Verrucomicrobia have also been detected (Gerritsen *et al.*, 2011).

The same dominant bacterial phyla have been found across a wide range of vertebrates indicating that some features of the gut microbiome are conserved among mammals (Ley *et al.*, 2008). However, despite the similarities at the phyla level, the gut microbiota can greatly differ between and within mammals as it strongly diversifies at the bacterial species level (Ley *et al.*, 2006; Gerritsen *et al.*, 2011).

The composition of the microbial community reflects the co-evolution between hosts and their microbes. The traits that vertebrates have gained from their association with micro-organisms have likely played a crucial role in the development and performance of the hosts (Dethlefsen *et al.*, 2007). A survey of gut microbiota over a wide range of mammals suggests that diet, host morphology, and phylogeny influence bacterial diversity in the gut (Ley *et al.*, 2008). The evolutionary interactions between mammals and their microbes are so specific that some commensal bacteria become host adapted. This was shown with the characterization of the genomic repertoire of *Lactobacillus reuteri* isolated from various vertebrates, including humans. This study revealed that different subpopulations of *L. reuteri* have evolved a lifestyle adapted to the host from which they are derived (Frese *et al.*, 2011).

How do we investigate the gut microbial community?

For decades, information on the species composition within the gut microbiota was obtained from culture-based methods. Such studies have generated a wealth of useful data, but have limited our knowledge to the enumeration and characterization of cultivable micro-organisms. Hence, this approach failed to give a complete repertoire of the intestinal microbial species as most gut micro-organisms are resistant to the current culture-based methods. The development of culture-independent molecular techniques provides novel insights into the phylogenetic and functional characterization of the intestinal microbiota (Gerritsen *et al.*, 2011). Most of the techniques for molecular profiling [quantitative polymerase chain reaction (qPCR), temperature gradient gel electrophoresis (TGGE) or denaturing gradient gel electrophoresis (DGGE), terminal-restriction fragment length polymorphism (T-RFLP), and fluorescent *in situ* hybridization (FISH)] target the 16S ribosomal RNA (16S rRNA) gene sequence as a phylogenetic marker. Furthermore, recent developments in high-throughput sequencing technologies allow in-depth analyses of the phylogenetic diversity and of the genetic material obtained from the intestinal microbes (Qin *et al.*, 2010).

The culture-independent approaches reveal a biodiversity that traditional culture-dependent methods had not described and led to the identification of new bacterial species (Gill *et al.*, 2006). These techniques also allow revisiting gut ecosystems of non-human mammals for which few bacterial species have been isolated and characterized. The techniques have been applied to the intestinal microflora of farm animals such as cattle (Whitford *et al.*, 2001) and pigs (Leser *et al.*, 2002), wild animals such as the polar bear (Glad *et al.*, 2010), and laboratory rodents such as the mouse and the rat (Salzman *et al.*, 2002; Brooks *et al.*, 2003).

The rat: a long-standing model in biomedical research

The rat is the first mammalian species to be domesticated for scientific purposes. Initial foundation began in the early 1900s at the Wistar Institute of Philadelphia where one of the first breeding experiments was recorded by Helen King (Russel and Baker, 2006). Their small size, ease in handling, and high reproductive capacity in captivity have contributed to their widespread use as experimental models. Along with mice, rats have significantly contributed to the increase in knowledge in several areas of mammalian biology, including human and animal health (Abbott, 2009; Iannaccone and Jacob, 2009; Jacob, 2010). More than a million publications on studies in which rats were used have been inventoried, illustrating the substantial amount of data that have been gathered on this species (Jacob, 2010). The first rat genomic sequence was published in 2004 (Gibbs *et al.*, 2004) and was the third mammalian genome to be sequenced after those of the human and the mouse. It is estimated to be 2.75 gigabases (Gb) in size, smaller than the human (2.9 Gb) but larger than the mouse (2.5 Gb) genome.

Over time, numerous strains of rat have emerged and have been selected for traits of biomedical interest (Hedrich, 2006; Mashimo and Serikawa, 2009). Breeding programs have been used to produce rats designed for the study of specific human pathological conditions. Most of these models involve the study of diseases such as cardiovascular diseases, behavioral and neurological disorders, metabolic diseases, cancers and autoimmune diseases, whose incidence has increased substantially in Western countries in recent years (Mashimo and Serikawa, 2009). Rat resource centers currently list hundreds of strains and substrains of rat (Abbott, 2009; Iannaccone and Jacob, 2009; Mashimo and Serikawa, 2009; Jacob, 2010). Combined with the knowledge of the rat genome sequence these available strains facilitate the development of insights into mammalian genes that underlie diseases.

Since the 1980s, the mouse has become the favorite experimental model as the technology for genetic modification has been developed in this species, whereas

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it cannot be applied in the rat. However, mice and rats have their own advantages and are not commutable (Iannaccone and Jacob, 2009). Recently, methods such as traditional transgenesis through lentiviral transformation techniques, 'zinc finger nuclease' technology, and transposon insertional mutagenesis have been successfully employed to manipulate the rat genome (Jacob *et al.*, 2010). These technological advances will likely help rats to catch up with the 30-years head start in manipulation of mouse genetics and help in the generation of more valuable disease models in rats.

The laboratory rat: a model with distinct microbial status

Laboratory rodent production must conform to criteria of health quality and reproducibility required by users. The production and maintenance of disease-free laboratory animals are achieved through stringent controlled breeding conditions including gnotobiotic procedures and strict hygiene. The pioneering development of gnotobiology techniques by Gustafsson and Reyniers in the 1950s led to the incorporation of gnotobiotic procedures into rat breeding for elimination of pathogens (Carter and Foster, 2006). These two scientists established germ-free or gnotobiotic rat colonies that were available for the first time for stocking breeding colonies for scientists or commercial firms. According to the breeding conditions laboratory rodents have varying sanitary and microbial status.

Gnotobiotic animals are derived from stocks or strains obtained by hysterectomy or embryo transfer and are continuously maintained under aseptic conditions in sterile housing systems such as isolators (Carter and Foster, 2006). The microbial status of gnotobiotic rats is fully defined and includes germ-free, which have no micro-organism living in or on the animal, and 'defined microbiota' animals. Specific pathogen-free (SPF) animals are recommended for biomedical research by agencies such as the American Association for Laboratory Animal Science (AALAS) and the Federation of Laboratory Animal Science Associations (FELASA). These animals are specified as 'animals that are free of specific microorganisms and parasites but otherwise containing an undefined microbiota' (Carter and Foster, 2006). They are defined on the basis of a negative screen for specific known pathogens. SPF colonies need to be renewed repeatedly in order to eliminate specified bacteria or viruses or because the recommendations for SPF housing change. Stock colonies of SPF animals are mostly established by seeding caesarean-derived offspring of rodents with well-defined micro-organisms. Subsequently the animals are introduced into an established SPF unit, where they obtain other microbes through association with other animals or by acquisition naturally from the environment. These SPF animals can be kept for generations under strict barrier conditions. Conventional animals are reared in a breeding room without microbiological controlled environment and have an unknown microbiota and unknown disease status.

The altered Schaedler flora (ASF): a tool for standardizing the rodent microbiota

When stock colonies of SPF are established, transfer of a defined microbiota is often used to provide colonization resistance against pathogens and to reverse germ-free-associated characteristics. Schaedler was the first scientist to inoculate rodents with a cocktail of commensal bacteria. The first Schaedler flora, described in 1965, consisted of eight aerobic and aerotolerant bacterial species isolated from mice (Schaedler *et al.*, 1965): *Escherichia coli*, *Streptococcus faecalis*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, Group N *Streptococcus*, *Bacteroides distasonis*, a *Clostridium* sp., and a fusiform bacterium. As the extremely oxygen-sensitive bacteria (EOS), which dominate the microbiota, were not included due to technical difficulties for their isolation, this flora was revised in 1987 under the National Cancer Institute proposal (Orcutt *et al.*, 1987). Four bacteria from the original Schaedler flora (*E. coli*, *S. faecalis*, anaerobic group N *Streptococcus* and an unidentified *Clostridium*) were replaced with three species of fusiform-shaped anaerobes and one anaerobic spirochete, all of which had also been isolated from mice. This new defined microbiota, now known as the altered Schaedler flora (ASF), consisted of four members of the original Schaedler flora (the two lactobacilli, *B. distasonis*, and the fusiform bacterium), a spiral-shaped bacterium, and three new fusiform EOS bacteria.

Since the 1980s the ASF has been widely used by commercial breeders and animal facilities to provide defined, limited, and balanced colonization of SPF mice and rats. The eight bacteria in the ASF have been phylogenetically characterized on the basis of their 16S rRNA gene sequences (Dewhirst *et al.*, 1999). As previously reported, three strains were identified as *L. acidophilus* (strain ASF 360), *L. salivarius* (strain ASF 361), and *B. distasonis* (strain ASF 519), based on phenotypic criteria. ASF 360 clusters with *L. acidophilus* and with *Lactobacillus lactis* and is likely a novel *Lactobacillus*, while the 16S rRNA gene sequence of ASF 361 is identical to that of *Lactobacillus murinis* and *Lactobacillus animalis*. ASF 519 is related to an unnamed group containing *B. distasonis*. Among the four EOS fusiform anaerobes (ASF 356, ASF 502, ASF 492, and ASF 500), three (ASF 356, ASF 502, and ASF 492) fall into *Clostridium* cluster XIV, with the 16S rRNA gene sequence of ASF 492 being identical to that of *Eubacterium plexicaudatum*. ASF 500 is not closely related to any sequences in databases and clusters with the low GC-content Gram-positive bacteria. The spiral-shaped

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bacterium (ASF 457) clusters with the *Flexistipes* group (Dewhirst *et al.*, 1999). In mice the ASF remains stable through generations (Stehr *et al.*, 2009). Moreover, several members of the ASF are detected in the intestinal microbiota of mice (Salzman *et al.*, 2002) or rats (Brooks *et al.*, 2003) bred in conventional or SPF units, suggesting that even when the microbiota diversifies some members of the simplified microbiota persist.

This microbiota is far simpler than that of conventional experimental animals and does not recapitulate all the functions carried out by the intestinal microbiota. Some germ-free animal characteristics are not reversed in rats or mice associated with the ASF (Norin and Midtvedt, 2010). However, it has recently been shown that members of the ASF trigger a gut immune response through activation and generation of colonic CD4+ regulatory T-cells in mice (Geuking *et al.*, 2011). Other simplified microbiota were also proposed for rats and were defined as more representative of the functions of a whole complex microbiota (Yanabe *et al.*, 2001).

What is the composition of the 'normal' intestinal microbiota in the rat?

Culture-independent approaches, based on comparative sequence analyses of 16S rRNA gene, have revolutionized our understanding of bacterial species inhabiting the gut of mammals. The adult rat gastrointestinal tract microbiota has been surveyed using various techniques such as 16S rRNA gene genotyping (Brooks *et al.*, 2003; Manichanh *et al.*, 2010), qPCR (Delroisse *et al.*, 2008), FISH (Dinoto *et al.*, 2006), and PCR-DDGE/TTGE. These analyses reveal that the richness of bacterial species in the rat intestine is of the same order of magnitude or even higher than in the human gut microbiota (Brooks *et al.*, 2003; Manichanh *et al.*, 2010). Hence, the rat gut microbiota strongly diversifies in conventional or in SPF breeding units following the removal of the animals from isolators and introduction into barrier rooms. This may sound surprising as breeding rooms are confined environments and as the diets given to rodents are poorly diversified. However, such diversity could contribute to the harvesting of more nutrients from this basic diet. Analyses from the suckling period to maturity indicate that the rat intestinal microbiota is poorly diversified during the suckling period and that the diversity increases remarkably at the time of weaning or during the maturation of the immune system (Inoue and Ushida, 2003a, b). This is illustrated by the molecular profile of the gut microbiota obtained by TTGE. Indeed eight to ten amplicon bands are obtained at the end of the suckling period and the number of bands significantly increases after weaning (Inoue and Ushida, 2003a, b). Most of the bacterial species of the offspring are derived from their mothers and are still detected when the rats are mature (Inoue and Ushida, 2003a, b).

Bacterial phyla described in the rat gut microbiota are similar to the phyla present in human and mouse intestines as Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria are detected. These results have been obtained through a large-scale survey of bacterial 16S rRNA genes in cecal or fecal contents using pyrosequencing or clone sequencing methods (Brooks *et al.*, 2003; Manichanh *et al.*, 2010). This microbial diversity is also described for the microbiota associated with the intestinal mucosa (Dalby *et al.*, 2006). As reported for the human microbiota (Turnbaugh *et al.*, 2006) the Firmicutes and the Bacteroidetes phyla dominate (Brooks *et al.*, 2003; Manichanh *et al.*, 2010). Sequencing study of 16S rRNA gene clones from a random library also reveals that Verrucomicrobiaceae represented 5% of the total sequences in intestinal contents (Karlsson *et al.*, 2011).

The *Clostridium* superfamily is widely represented and is distributed among the *C. coccoides* and the *C. leptum* groups, and other members of the low GC Gram-positive bacteria. This is shown by 16S rRNA gene library analyses or by quantitative method targeted bacterial groups or species such as FISH (Brooks *et al.*, 2003; Dinoto *et al.*, 2006). However, the rat gut microbiota has several unusual features when compared with that of humans. In contrast to humans (Joly *et al.*, 2010), *Lactobacillus species* represent a significant proportion of the rat microbiota and can reach 10–15% of the total sequences read (Brooks *et al.*, 2003; Dalby *et al.*, 2006; Manichanh *et al.*, 2010). The high level of lactobacilli in the rat microbiota is confirmed by qPCR (Delroisse *et al.*, 2008). Furthermore, analyses of sequence similarities of the 16S rRNA gene clones indicate that only a few Gram-negative phylotypes are related to previously identified *Bacteroides* and *Prevotella*. Instead, the majority of Gram-negative phylotypes fall into a separate lineage within the *Bacteroides–Cytophaga* group (Brooks *et al.*, 2003). As in adult humans *Bifidobacterium* spp. is not dominant in the rat gut microbiota as revealed by qPCR (Dinoto *et al.*, 2006; Delroisse *et al.*, 2008).

The majority of 16S rRNA sequences found in the rat intestinal microbiota are from unknown bacteria (Brooks *et al.*, 2003; Dinoto *et al.*, 2006). These unidentified sequences are related to those that have been previously detected in mice feces (Salzman *et al.*, 2002). In particular, the gut microbiota of rats and mice are rich in an uncharacterized group called 'fusiform-shaped anaerobic bacteria' (Dinoto *et al.*, 2006). Some bacterial species are abundant in the rat gut microbiota but not in the human intestinal ecosystem: this is the case for the segmented filamentous bacteria (SFB), which have been detected in high abundance by qPCR in the microbiota associated with the intestinal mucosa (Dalby *et al.*, 2006). SFB have also been described in mice and play a major role in the maturation of T-cell responses in the gut (Gaboriau-Routhiau *et al.*, 2009). Comparison of fecal bacterial 16S rRNA gene sequences between rat and human samples

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also indicates that *Turicibacter* and an uncharacterized member of the Porphyromonadaceae family are also found in higher amounts in the rat microbiota (10–15% of the total reads) than in the human microbiota (Manichanh *et al.*, 2010). In contrast, *Faecalibacterium* is scarce in the rat gut microbiota but abundant in the human gut microbiota (Manichanh *et al.*, 2010).

Taken together these data indicate that, although bred in confined environment and with procedures for satisfying health requirement that can hamper the natural colonization, some major features of the mammalian gut microbiota are conserved. However, as previously shown for other mammals, this microbiota diversifies at the level of group or bacterial species and contains numerous unidentified bacteria. The development of culture-independent approaches allows pointing out relevant factors that modify the composition of the rat microbiota.

Effect of the host genetic background

The effect of the genetic background on gut microbiota composition is particularly relevant for laboratory rodents as several hundred strains of outbred or inbred rats and mice are available. The effect of the genetic background on the composition of the microbiota has been mostly studied in mice and less documented in rats. In mice, the genotype has a strong effect on the gut microbial community as its composition differs between inbred strains raised in the same conditions (Vaahtovuo *et al.*, 2003; Jussi *et al.*, 2005). Molecular profiling based on 16S rRNA genes shows that the microbiota is more similar between individuals when they are derived from inbred strains than when they are derived from outbred strains (Hufeldt *et al.*, 2010).

In rats, the interplay between genotype and the intestinal microbiota is investigated through indirect observation. Metabolic parameters (glucose, lipid, amino acid, and energy metabolisms) differ for four inbred rat strains [Brown Norway, Lewis, Wistar Kyoto, and Fischer F344], but the divergences exceed that explainable by genetic polymorphisms (Pontoizeau *et al.*, 2011). This indicates that metabolic variations cannot be entirely reduced to their genetic component. Environmental influences play a role in these variations, including the strong contributions of host–gut microbiota interactions. Such data suggest that these host–gut microbial interactions in the rat can be strain-specific (Pontoizeau *et al.*, 2011). Another metabonomic study performed on different rat strains also reveals the interplay between the host genome and the microbiome and its impact on the host metabolic profile (Dumas *et al*, 2007).

Data suggest that the effect of the genetic background is different in mice and rats. For example, the lactobacilli population is different between BALB/c and C57BL/6J mouse lines. In particular, high levels of *Lactobacillus johnsonii* are measured in C57BL/6J mice, whereas this bacterium fails to colonize the gut of BALB/c mice (Buhnik-Rosenblau *et al.*, 2011). In contrast, genetically different rats have the same dominant population of lactobacilli (de Waard *et al.*, 2002). Moreover, when a rat gut microbiota is transferred to another strain of rat previously treated with broad-spectrum activity antibiotics, the microbiota of the recipient evolves to that of the donor (Manichanh *et al.*, 2010), suggesting here again a minor effect of the genetic background on gut microbiota composition.

Effect of the breeding facilities

The environment also affects the composition of the intestinal microbiota. In mice, implantation of a female with genetically distinct embryos results in microbial profiles that are similar between mother and offspring (Friswell *et al.*, 2010). For the same strain of laboratory rodents, microbiota may vary between breeding centers. Molecular profiling by DDGE reveals that strains of mice purchased from different laboratory animal vendors have distinct composition of gut microbiota (Hufeldt *et al.*, 2010). Phylogenetic analyses of *Lactobacillus* strains from Wistar rats showed that the indigenous *Lactobacillus* composition is determined more by environmental influences, i.e. the animal facility, than by host genetics (de Waard *et al.*, 2002).

The gut microbiota of laboratory rodents differs not only according to the suppliers but also within an individual breeding center, as rodents can be raised in multiple rooms. The gut microbiota of mice maintained in different rooms show a cluster-room effect (Fushuku and Fukuda, 2008; Hufeldt *et al.*, 2010). A study conducted in a Charles River Raleigh facility identified two distinct phenotypes of Sprague–Dawley rats, which differed in their urinary metabolite profiles (Rohde *et al.*, 2007). These phenotypes were derived from different initial stock colonies of rats associated with the ASF, which were started 2 years apart. The phenotypic differences appear to be due to alterations in the gut microbiota diversification, most likely due to a slow rate of microbiota conventionalization in the more recent colony. The metabolomic phenotype of these rats can be reversed by cross-housing with rats coming from the former colony. Hence, the metabolomic profile of rats initially seeded with the ASF varies according to the environment to which they are exposed.

Another study investigated the intestinal commensal bacteria under different breeding conditions of the rats: (i) under microbiologically controlled conditions (barrier), (ii) under standard conditions (conventional), and (iii) in barrier animals adapted to standard conditions (barrier/conventional) (Teran-Ventura *et al.*, 2010). The barrier group showed a higher number of strictly anaerobic bacteria (*Bacteroides* spp. and *Clostridium* spp.), while

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Bifidobacterium spp. were scarce. The conventional group had higher numbers of *Lactobacillus* and B*ifidobacterium* spp. and a slight reduction in *Bacteroides* spp. The microbiota of the barrier/conventional group adapted to standard conditions showed characteristics that were intermediate between the barrier and conventional groups.

These data indicate that exposure to various environmental factors, such as different caretakers, other animals, and deviations in treatment of food and water may influence the composition of the gut microbiota, including within an individual breeding facility.

Effect of antimicrobials

Although antimicrobials are not recommended, and ill rats are often euthanized, antimicrobial agents can be used to treat rats if the animals are valuable to an ongoing study or are not being used to generate antimicrobial sensitive data. In one study, Manichanh *et al*. (2010) used 16S rRNA gene pyrosequencing to investigate the changes in microbiota composition following treatment for 3 days with a broad-spectrum antibiotic cocktail (vancomycin and imipenem) administered in drinking water. The antibiotic treatment had a profound and long-lasting effect on the composition of the intestinal microbiome. At the end of the treatment the bacterial diversity was reduced ten-fold with the average phylotype richness changed from 217 to 21 operational taxonomic units (OTUs). The two major phyla (Bacteroidetes and Firmicutes) were markedly reduced. In contrast, two minor phyla (Proteobacteria and Tenericutes) had increased, likely because they took advantage of the empty niches created by the antibiotic treatment (Manichanh *et al*., 2010). One month after the treatment, Bacteroidetes and Firmicutes were recovered as the two major phyla and Proteobacteria and Tenericutes decreased to their initial low levels (Manichanh *et al*., 2010). However, large-scale 16S rRNA gene analyses showed that 3 months after the end of the antibiotic treatment the microbiota did not return to its original composition. In particular, Bacteroidetes diversity was lower in samples obtained after antibiotic treatment than in samples taken before treatment (Manichanh *et al*., 2010).

In another study, Fak *et al*. (2008) administered a different broad-spectrum antimicrobial cocktail (metronidazole, neomycin, and polymyxin B) in drinking water to pregnant rats, starting 3–4 days before the expected date of parturition and ending on the day of birth or 1 day after the birth of pups. The researchers then determined the effects on lactobacilli and Enterobacteriaceae. At the end of administration of the antibiotics, there was a significant decrease in the numbers of lactobacilli in the feces of the dams, but their levels returned to pre-treatment levels at 2 weeks post-treatment. Changes in the concentration of Enterobacteriaceae in the feces of the dams were not significant. There were perturbations of the intestinal microbiota in the offspring that remained for the 2-weeks duration of the study; these consisted of elevated levels of Enterobacteriaceae; however, no change in the numbers of lactobacilli were detected (Fak *et al*., 2008). These authors further showed that, compared with pups from untreated dams, pups from antibiotic-treated mothers had decreased stomach growth and function, lower pancreatic protein levels, higher intestinal permeability, and increased plasma levels of the acute phase protein, haptoglobin.

These studies highlight the marked influence that treatment with broad spectrum antimicrobials may have on the intestinal microflora of rats. The reduction in diversity during the period of disruption of the microflora may have significant consequences for the host in metabolism as well as protection against enteric pathogens, although no adverse effects were noted in the studies.

Modulation by diet

As shown in several other species, the composition of the laboratory rat microbiota is modified by the composition of the diet. Carbohydrate (CHO) content of the diet influences the intestinal composition of the rat, with a specific pattern for each CHO (Licht *et al*., 2006). *Bifidobacteria* and lactobacilli are mainly increased on raffinose or isomalto-oligosaccharides supplemented diets (Dinoto *et al*., 2006; Ketabi *et al*., 2011). The quality of protein also modifies the composition of the rat gut microbiota. Here again there is a specific pattern for each of the proteins studied, with modulation occurring mainly with the *Lactobacillus* and *Bifidobacterium* groups (Qi *et al*., 2011). It has been shown that the consumption of apple pectin increases the population of butyrate-producing Clostridiales, and decreases the population of specific species within the Bacteroidetes (Licht *et al*., 2010).

The effect of a high fat diet on gut microbiota composition has been extensively investigated, given its potential link with obesity (Turnbaugh *et al*., 2006). In rats, a high-fat diet induces changes in the composition of the microbiota as it increases the relative proportions of both Bacteroidales and Clostridiales orders (de La Serre *et al*., 2010). The effect of bile salt on gut microbiota has been recently studied in rats as its secretion is stimulated by a high-fat diet. A diet supplemented with cholic acid induces a decrease in the diversity of the microbiota, associated with severe changes in the rat gut microbiota composition at the phylum level. In that case changes include an increase in Firmicutes and a decrease in Bacteroidetes (Islam *et al*., 2011), i.e. similar to the shift described in mice with a high fat diet. This model can be used to better understand the role of bile salts and the bacterial systems used to tolerate bile acids.

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Impacts of iron deficiency and repletion on the rat gut microbiota were analyzed as iron is involved in many biological processes for the host and for nearly all gut bacteria (Dostal *et al.*, 2012). The abundance of dominant species is strongly modified by iron deficiency. The changes include greater numbers of lactobacilli and Enterobacteriaceae and a large significant decrease in some of the major producers of butyrate (*Roseburia* spp./ *E. rectale* group). Repletion with iron partially restores bacterial populations (Dostal *et al.*, 2012).

These data indicate that changes in laboratory rat gut microbiota composition occur even when dietary components are not typically those given to laboratory rodents. This suggests that, although the diet is restricted to rat chow, the gut microbiota retains the capacity to diversify along with the diet.

Human microbiota-associated rodents

As experimental animals can be bred and kept in an isolator, the microbiota can be modified 'to infinity'. Besides being a tool to provide 'clean' rodents, gnotobiotic technology is increasingly used to study the interactions between commensal bacteria and their host (Rul *et al.*, 2011; Thomas *et al.*, 2011). As only 15% of bacterial species are common to mice and humans (Spor *et al.*, 2011), models of human microbiota-associated rats and mice have been developed (Gootenberg and Turnbaugh, 2011). Hence, the dominant human fecal microbiota, coming from adult or infant, can be transferred and maintained in initially germ-free rats (Edwards *et al.*, 2003; Alpert *et al.*, 2008). Similar observations have been made for key microbial activities, as the production of equol or the conversion of cholesterol can be transferred from the donor to the recipient rat (Bowey *et al.*, 2003; Gerard *et al.*, 2004). However, great variability and fluctuations with time have been reported in these models (Bernbom *et al.*, 2006). Furthermore, as some bacteria coming from humans, such as *L. reuteri*, fail to colonize rats or mice, the transferred human microbiota is likely to be incomplete (Frese *et al.*, 2011). Recently, a 'mini human gut microbiota' has been defined and consists of eight bacterial strains chosen according to their occurrence in humans, their metabolic activities and the availability of their genomic sequences. This bacterial community is stable over time and is easily transferred to the offspring (Becker *et al.*, 2011).

Culture independent analyses and rat models of disease: a winning combination

As previously reported, the rat offers many advantages in biomedical research and several rat strains have been selected and bred to have traits of specific disorders. The laboratory rat is also widely used in pharmaceutical research, especially for testing drugs used in human diseases. Some of these tests include the impact of drugs on gut microbiota, as done for the non-steroidal anti-inflammatory drug indomethacin (Dalby *et al.*, 2006), or as for the atypical antipsychotic drug, olanzapine (Davey *et al.*, 2012).

Biobreeding (BB) rats are well established animal models of spontaneously developing autoimmune diabetes. Sublines of diabetes-prone (BB-DP) and diabetes-resistant (BB-DR) rats have been developed and the intestinal microbiota have been investigated in both sublines (Roesch *et al.*, 2009). At the time of onset of diabetes in BB-DP and BB-DR rats, the bacterial communities in these two rat strains differ. *Lactobacillus* and *Bifidobacterium* populations are higher in BB-DR than in BB-DP rats. According to these data, *Lactobacillus* and *Bifidobacterium* are beneficial because they delay or prevent the onset of diabetes. A *L. johnsonii* strain has been isolated from the stool of BB-DR rats. This strain of *L. johnsonii* prevents diabetes when fed to BB-DP rats (Valladares *et al.*, 2010). In a rat model of colonic hypersensitivity, mimicking the irritable bowel syndrome, the gut microbial community was analyzed using a high-density DNA microarray for phylogenetic analysis (Nelson *et al.*, 2011). There is a dramatic change in the ratio of Firmicutes relative to Bacteroidetes. Bacteroidetes increase and the composition of species within this phylum is different (Nelson *et al.*, 2011). Similar investigation by pyrosequencing has been used to evaluate the effect of bariatric surgery (Li *et al.*, 2011). These data suggest that the laboratory rat can be a useful model for better understanding of the underlying mechanisms between gut microbiota and diseases.

The gastrointestinal microbiota: a parameter for checking laboratory rodents?

Today, quality and reproducibility of laboratory rodents are mostly based on eliminating and securing the absence of pathogens. Scientists are aware of the rodent pathogens for which the animals are routinely tested, but do not get information about the intestinal bacterial diversity of the experimental animals that they use. The composition of the microbiota, particularly the anaerobes, is not often checked, and new species can be introduced, either from human caretakers or from other animals as breeding facilities frequently house several thousand animals.

It is often assumed that the microbiota remains stable while subsequent SPF generations of animals are bred. However, the intestinal microbiota varies according to the ASF-associated rat generation from which they come, with a strong impact on the metabolic parameters (Rohde *et al.*, 2007). In the early 2000s scientists observed alterations of some animal models for immunological studies and failed to reproduce previous data. In that case, alterations of the experimental animal models

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followed the renewal of the production units of commercial breeders and were likely attributable to degeneration of the non-pathogenic microbial environment (Berard 2004).

Increasing data show a positive correlation between the composition of the intestinal microbiota and diseases (Tlaskalova-Hogenova *et al.*, 2011). According to the correlation between the diversity of the intestinal microbiota and the expression of disease, the quality of SPF housing facilities can influence model diseases. This has been shown for a rodent model of type 1 diabetes. Clean conditions, antibiotic decontamination, and renewal of production units are environmental factors that increase the incidence of type 1 diabetes (Tlaskalova-Hogenova *et al.*, 2011). The impact of such an environment can also be suggested for other pathologies associated with the gut microbiota, such as inflammatory bowel disease (Hansen *et al.*, 2007; Lauritsen *et al.*, 2010).

Given the substantial impact of the intestinal microbiota on mammalian biology, which is increasingly documented, deeper analyses of the intestinal microbiota composition might be relevant in standards of quality control of laboratory animals. Such information on this parameter should improve reproducibility and predictability of animal models (Hansen *et al.*, 2007; Lauritsen *et al.*, 2010). As both mice and rats are relevant in biomedical research, such procedures and studies should not be limited to mice but should also be extended to rats. The major breakthroughs in high-throughput sequencing and molecular profiling techniques could support a robust tool that may enable breeders to document this information.

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