Critical Reviews in Biotechnology

http://informahealthcare.com/bty
ISSN: 0738-8551 (print), 1549-7801 (electronic)

Crit Rev Biotechnol, Early Online: 1–12
 DOI: 10.3109/07388551.2013.819485

informa
healthcare

REVIEW ARTICLE

The human microbiota associated with overall health

Xiaofei Xu[1,2]*, Zhujun Wang[1]*, and Xuewu Zhang[1]

[1]College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, China and [2]Infinitus (China) Company Ltd, Guangzhou, China

Abstract

Human body harbors diverse microbes, the main components include bacteria, eukaryotes and viruses. Emerging evidences show that the human microbiota is intrinsically linked with overall health. The development of next-generation sequencing provides an unprecedented opportunity to investigate the complex microbial communities that are associated with the human body. Many factors like host genetics and environmental factors have a major impact on the composition and dynamic changes of human microbiota. The purpose of this paper is to present an overview of the relationship between human health and human microbiota (skin, nasal, throat, oral, vaginal and gut microbiota), then to focus on the factors modulating the composition of the microbiota and the future challenges to manipulate the microbiota for personalized health.

Keywords

Diseases, human microbiota, manipulation, personalized health

History

Received 27 July 2012
Revised 9 June 2013
Accepted 9 June 2013
Published online 2 August 2013

Introduction

All the microbes colonizing the human body constitute our microbiota, and the genes they encode are known as our microbiome. The human microbiota primarily contains bacteria, eukaryotes and viruses. Recently, culture-independent high-throughput sequencing technology provides a foundation for understanding the composition and variation of this complex community (Caporaso et al., 2011). In particular, some microarrays including PhyloChip, Microbiota Array and HITChip can simultaneously measure the presence and abundance of hundreds and thousands of phylotypes in a single sample (Paliy & Agans, 2012). Currently, scientists worldwide are beginning to explore the diverse human microbiomes from five different body areas: the skin, the nasopharyngeal, the oral cavity, the gastrointestinal and the female urogenital tracts.

The human microbiota is recognized to play vital roles in maintaining host health and has a profound effect on human diseases, including obesity, diabetes, cardiovascular diseases, inflammatory bowel diseases (IBD), cancers and other diseases. However, the discrimination of ''healthy microbiota'' from ''diseased microbiota'' is still a challenging problem. Many studies have demonstrated that there is a large variability in the composition of the microbial communities in healthy individuals, even twins share fewer viral sequences and less than 50% of species-level bacterial taxa (Clemente et al., 2012). More importantly, many factors (e.g. host genetics and various environmental factors) influence the composition and dynamic changes of the human microbiota (Sekirov et al., 2010). Defining the complex relationship between host and human microbiome and its role in health and disease will enhance the understanding of multiple pathological mechanisms and facilitate the development of novel diagnostic tools and therapeutic interventions. In this paper, we firstly provide an overview of human microbiota and its relationship with health and diseases (see Supplemental Materials), and then discuss the potential strategies and future perspectives to modulate human microbiota for personalized health.

*Both authors are identified as Co-First Author.

Address for correspondence: Dr XW Zhang, College of Light Industry and Food Sciences, South China University of Technology, 381 Wushan Road, Guangzhou 510640, China. Tel/Fax: 86 20 87113848. E-mail: snow_dance@sina.com

Who modulates the composition of human microbiota?

Modulating human microbiota by genetic factors

It has been demonstrated that the composition of the bacterial community is influenced by host genetics. For example, studies have indicated that black women are more likely to have bacterial vaginosis (BV) than non-Hispanic white women, this racial disparities are not explained by known risk factors and could be related to the fact that Blacks were more likely to have specific BV-related vaginal microflora, as well as gonococcal or chlamydial cervicitis (Ness et al., 2003). Benson et al. (2010) identified 18 host quantitative trait loci (QTL), which show significant linkage with relative abundances of specific microbial taxa and affect microbiota composition by controlling individual microbial species or groups of related taxa and pleiotropic effects on groups of distantly related organisms. This provides clear evidence for the importance of host genetic control in shaping individual microbiome diversity in mammals. The candidate gene

approaches (i.e. one gene is deleted or added to a model host system) show that a single host gene can have a tremendous effect on the diversity and population structure of the gut microbiota (Spor et al., 2011). Indeed, using PCR-denaturing gradient gel electrophoresis (DGGE) and qPCR methods and the fecal samples of 71 healthy individuals (14 were non-secretor individuals and 57 were secretors), Wacklin et al. (2011) showed that the diversity and composition of the human bifidobacterial population is strongly associated with the secretor genotype (FUT2 gene). Specifically, bifidobacterial diversity and abundance were significantly reduced in the non-secretor individuals compared with those in the secretor individuals. In particular, several genotypes related to *Bifidobacterium bifidum, B. adolescentis* and *B. catenulatum/pseudocatenulatum* were absent or rarely colonized in the non-secretor individuals. Reduced bifidobacterial abundance has been connected to intestinal disorders such as irritable bowel syndrome (Malinen et al., 2005) and inflammatory bowel disease (Seksik et al., 2003). This study suggests that the secretor status could affect the susceptibility to the diseases associated with the decreased bifidobacterial abundance in the intestine by effecting bifidobacterial diversity. Moreover, interesting research was conducted in 79 healthy Caucasian donors from Southern Finland to compare the effects of human blood group phenotype with the intestinal microbiota composition (Mäkivuokko et al., 2012). The results indicated that the composition of the microbiota in individuals with B-antigen is obviously different from that in non-B-individuals, which exhibited higher diversity of the *Eubacterium rectale-Clostridium coccoides* (EREC) and *Clostridium leptum* (CLEPT)-groups, compared with other blood groups. It is noted that all the subjects used were Caucasians from Finland, additional samples with broader genetic background are required to validate these findings, which will be very useful in the field of personalized nutrition and medicine.

Modulating human microbiota by antibiotics

The present evidence indicates that antibiotics cause major alterations in the composition of microbiota following treatment with antibiotics. For example, Dethlefsen et al. (2008) examined the distal gut bacterial communities of three healthy humans before and after treatment with ciprofloxacin. They found that the taxonomic composition of the community closely resembled its pretreatment state by the end of 4 weeks treatment, but some taxa failed to recover within 6 months. Further study also showed that ciprofloxacin had a profound and rapid effect on the gut microbiota, the loss of diversity and the shift in community composition occurred within 3-4 d of drug initiation, and the composition of the gut microbiota stabilized by the end of the experiment but was altered from its initial state (Dethlefsen & Relman, 2011). Similarly, Mangin et al. (2010) showed that amoxicillin treatment on 31 infants led to a significant decrease in *B. bifidum*, but did not affect *B. longum, B. pseudocatenulatum* and *B. catenulatum*, specially, induced a complete disappearance of *B. adolescentis* species. In general, antibiotics result in long-term decreases in bacterial diversity, and the recovery is incomplete, even some taxa like the *Bacteroides* community never returned to their original composition for up to 2 years post-treatment (Jernberg et al., 2007). Furthermore, a big issue is that the repeated use of antibiotics in humans could increase the reservoir of antibiotic-resistant genes in human microbiome (Sommer et al., 2009). In other words, antibiotics are beneficial in short-term use but their prolonged use may result in significant side-effects. However, it seems impossible for an individual living in a developed country to escape any exposure to antibiotics, which can be detected in water, agricultural products and meat. Further research should perform in-depth characterization of the resistance reservoir in the human microbiome with individuals from environments naïve to industrial exposure.

Modulating human microbiota by diet

Diet has been shown to influence the composition and activity of the human microbiota and is also easily manipulated. For the targeted increase of some beneficial bacteria, it is important to determine whether the consumption of specific food components are able to modify the composition of human microbiota, focusing on oral and gut microbiota.

Modulation of the oral microbiota by diet

Although the mouth constitutes the first passage of food after its ingestion, scientific background relating to the effect of diet on oral bacterial microbiota in humans is very scarce. For instance, it was observed that there was an increment in *Lactobacillus* together with a decrease in *Enterobacteriaceae*, after the administration of green tea extracts (60% catechins) to healthy and elderly human subjects (Goto et al., 1998; Okubo et al., 1992). Similarly, considering proanthocyanidin-rich diets, an increase in *Lactobacillus* and *Bifidobacterium* species was also observed after the intake of a grape seed supplement (2.4% monomers; 38.5% procyanidins) by humans (Yamakoshi et al., 2001). Signoretto et al. (2006) evaluated the effects of different diets (coffee, barley coffee, tea and wine) on the microbiological composition of saliva and dental plague, a total of 93 subjects were included in the study for the duration of roughly one month. The data indicated that each drinking habit subgroup showed significantly lower counts than the controls, in particular, *Streptococci* and *Lactobacilli* were significantly decreased in those subjects drinking coffee, tea, barley coffee. In a further study (Signoretto et al., 2010), the DGGE technique was applied to evaluate the composition of the microbiota of supra- and subgingival plaque samples collected from 75 adult subjects with different drinking habits (drinkers of coffee, red wine or water for at least 2 years). The results showed that the frequency of identification of anaerobes was significantly reduced when coffee and wine drinkers were compared with the control group, and distinct clusters of organisms from water, coffee and wine drinkers were formed. These results suggest that there is a correlation between consumption of specific foods as well as the drinking habits and oral microbiota and influence oral health.

Modulation of the gut microbiota by diet

Dietary changes have been shown to have significant effects on the gut microbiota (Claesson et al., 2012; Wu et al., 2011). Using high-throughput 16S rDNA sequencing and

biochemical analyses, De Filippo et al. (2010) compared the fecal microbiota of European children (EU) and that of children from a rural African village of Burkina Faso. The results showed that the African children (the diet is low in fat and animal protein and rich in starch, fiber, plant polysaccharides) are colonized by members of *Prevotella* and *Xylanibacter*, in contrast, their European counterparts (a typical western diet low in fiber and high in animal protein, fat, starch and sugar) have high *Bacteroides* levels. This highlights the importance of preserving microbial diversity from ancient rural communities worldwide. Similarly, when shifting from a low-fat/high-fiber to a high-fat/low-fiber or a high-fat/high-fiber diet in mice (Turnbaugh et al., 2009) or humans (Wu et al., 2011), the notable changes in the gut microbiota were observed within 24 h. Importantly, diet was found to be related with enterotype: a diet high in animal protein and fat have a *Bacteroides*-dominated enterotype, while a high-carbohydrate and low-fat diet is associated with the *Prevotella*-dominated enterotype (Wu et al., 2011). In contrast, Minot et al. (2011) showed that human gut virome changed to a new state after dietary intervention, but the individuals on the same diet showed similar, although not identical, virome composition.

Furthermore, the diets from different cultures also impact the microbiota. Pieper et al. (2008) compared the intestinal microbial community composition in pigs fed four hulless barley varieties and two oat varieties. They observed the shifts in intestinal microbial communities among the hulless barley cultivars with a normal to high beta-glucan content. These hulless barleys had the lowest ($p < 0.05$) microbial diversity, whereas oats had intermediate diversity in comparison with low-beta-glucan hulless cultivars and hulled varieties. Moreover, hulless varieties favored xylan- and beta-glucan-degrading bacteria, whereas hulled barley favored *Lactobacilli*. Sonoyama et al. (2010) conducted a comparison of gut microbiota and allergic reactions in BALB/c mice fed different cultivars of rice: Yukihikari, rice A (common rice), rice B (brewery rice) and rice C (waxy rice). The results showed that the composition of fecal microbiota was different between mice fed Yukihikari and those fed rice A. The incidence of allergic diarrhea and the serum antibody levels by oral administration of ovalbumin were lower in mice fed Yukihikari, suggesting that the changes in the gut microbiota of mice fed Yukihikari could be advantageous in the prevention of food allergy. Thus, these results indicated that cultivar differences affect the gut microbiota in animal models. Whether or not the similar effects exist in the human microbiota warrants further exploration.

To systematically develop dietary strategies that modulate the human gut microbiome, it is necessary to evaluate the effects of different food components on the community structure and population dynamics of the gut microbiota.

Amino acids and protein. Faure et al. (2006) determined the effect of dietary amino acid supplementation (an amino acid cocktail containing L-threonine, L-serine, L-proline and L-cysteine) on the microbiota in dextran sulfate sodium (DSS)-treated rats. They found that *Bacteroides* counts were increased, *Enterobacteriaceae, Enterococci* and *Lactobacilli* counts were significantly decreased, in DSS-treated rats compared with controls. Amino acid supplementation can restore the levels of *Enterobacteriaceae, Enterococci* and *Lactobacilli* except for *Bacteroides* populations to those observed in controls, suggesting an increase in threonine, serine, proline and cysteine dietary supply can re-equilibrate the gut microbiota. Using continuous flow culture (CFC) models of the human colonic microbiota, inoculated with feces from ulcerative colitis (UC) and non-UC volunteers, Mills et al. (2008) investigated the effects of dietary glycated bovine serum albumin (BSA) on the composition of the colonic microbiota in UC patients. They found that, compared with native BSA, the glycated BSA modulated the gut microbiota of UC patients *in vitro* towards a more detrimental community structure, specifically, harmful bacteria (*Clostridia, Bacteroides* and sulfate-reducing bacteria (SRB)) were significantly increased and beneficial bacteria (*Eubacteria* and *Bifidobacteria*) were significantly decreased. Similarly, Świątecka et al. (2011) determined the impact of glycated pea proteins on the intestinal bacteria from a healthy human. The results indicated that *Lactobacilli* and *Bifidobacteria* were significantly increased, suggesting that glycated pea proteins modulated the gut microbiota of healthy human toward a beneficial community structure.

Prebiotics, probiotics and synbiotics. Prebiotics are non-digestible food ingredients (mostly oligosaccharides) that improve health by modulating the gut microbiota. Probiotics are defined as ''live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'' (Nakamura & Omaye, 2012). Most probiotic bacterial strains are lactic acid bacteria (LAB) belonging to the genera *Lactobacillus* and *Bifidobacterium*, and other microorganisms like yeasts and filamentous fungi can also be used as probiotics. Synbiotics are the combination of prebiotics and probiotics (Gourbeyre et al., 2011).

Ramirez-Farias et al. (2009) examined the changes in the fecal microbiota composition of 12 human volunteers after ingestion of inulin (10 g/d) for a 16-d period in comparison with a control period without ally supplement intake. The data revealed that *Faecalibacterium prausnitzii* exhibited a significant increase, and *B. adolescentis* showed the strongest response to insulin consumption, increasing from 0.89% to 3.9% of the total microbiota. Neyrinck et al. (2011) tested the ability of a water-extractable high molecular weight arabinoxylans (AX) from wheat to modulate the gut microbiota in high-fat-induced obese mice. They found that AX treatment can restore the number of bacteria that were decreased by high-fat feeding (i.e. *Bacteroides-Prevotella* spp. and *Roseburia* spp.) and markedly increased caecal content of *Bifidobacterium animalis lactis*. Martínez et al. (2010) assessed the effects of different forms of resistant starch (RS2 and RS4) on the community structure and population dynamics of the gut microbiota, 10 human subjects were included and they consumed crackers for three weeks each containing either RS2, RS4 or native starch. It was found that RS4, but not RS2, significantly increased *Actinobacteria* as well as *Bacteroidetes* and decreased *Firmicutes*. However, RS2 significantly raised the proportions of *Ruminococcus bromii* and *Eubacterium rectale* when compared to RS4. These findings imply that the chemical structure of RS

determines its accessibility by groups of colonic bacteria, the personalized health strategy could be achieved by selectively targeting specific bacterial populations with well-designed functional carbohydrates.

Murphy et al. (2013) investigated the impact of a bacteriocin-producing probiotic [*Lactobacillus salivarius* UCC118 Bac(+)] on diet-induced obesity in mice. Their data showed that the probiotic treatment had no significant impact on the proportions of *Firmicutes* but resulted in a relative increase in *Bacteroidetes* and *Proteobacteria* and a decrease in *Actinobacteria* compared with the non-bacteriocin-producing control. Using gas–liquid chromatography and 16S rRNA pyrosequencing, Wall et al. (2012) compared the effect of dietary supplementation for 8 weeks with human-derived *B. breve* strains on the composition of the gut microbiota in mice. The data revealed that both *B. breve* DPC 6330 and *B. breve* NCIMB 702258 supplementation resulted in the alteration of gut microbiota. Moreover, greater proportions of *Clostridiaceae* and lower proportions of *Eubacteriaceae* in mice supplemented with *B. breve* DPC 6330 were observed than in mice supplemented with *B. breve* NCIMB 702258 and unsupplemented controls. It demonstrates that the probiotic properties of *bifidobacteria* are strain-dependent and strain differences are important factors that influence modulation of the gut microbiota. Using a 3-stage continuous-culture system simulating the human colon, Maccaferri et al. (2012) investigated the effect of administration of 10(7) CFU/day of *Kluyveromyces marxianus* B0399 on the composition and metabolic activity of the human intestinal microbiota. They demonstrated that *K. marxianus* B0399 altered the colonic microbiota by increasing the bifidobacterial concentration. Furthermore, this strain was highly adhesive to human enterocyte-like Caco-2 cells and modulated the immune response by inducing proinflammatory cytokines. These results suggest that the gut microbiota is a realistic therapeutic target for improving human health.

Using anaerobic fecal batch cultures, eight selected synbiotics (short-chain fructooligosaccharides or fructooligosaccharides, each combined with one of four probiotics, *Lactobacillus fermentum* ME-3, *Lactobacillus plantarum* WCFS1, *Lactobacillus paracasei* 8700:2 or *Bifidobacterium longum* 46) were tested for their effects on modulation of the fecal microbiota (Saulnier et al., 2008). The results indicated that synbiotic and prebiotics increased the *Bifidobacteria* and EREC group and decreased the levels of *Escherichia coli*, probiotics alone had little effect upon the groups, but higher levels of *Lactobacillus-Enterococcus* were observed when the probiotic was stimulated by the prebiotic component. It suggests that in modulating the gut microbiota toward a possible healthy composition synbiotics are more effective than prebiotics and probiotics alone. Vitali et al. (2010) investigated the impact of a synbiotic food, containing fructooligosaccharides and the probiotic strains *Lactobacillus helveticus* Bar13 and *Bifidobacterium longum* Bar33, on the gut microbiota composition and metabolic profiles. Although the synbiotic food did not modify the overall structure of the gut microbiome, the intake of the synbiotic food resulted in a shift of the fecal metabolic profiles, characterized by the significant increase of short chain fatty acids (SCFA), ketones, carbon disulfide and methyl acetate, suggesting potential health promoting effects. This indicates that further study needs to focus not only on the changes in the composition of gut microbiota but also on the serum or colonic metabolite profiles.

Phytochemicals. Phytochemicals are defined as bioactive non-nutrient plant compounds present in fruits, vegetables, grains and other plant foods, such as carotenoids, phenolics, alkaloids, organosulfur compounds. The studies of the effects of berries and berry phenolics on pathogenic intestinal bacteria revealed that anthocyanins from berries selectively inhibited the growth of pathogenic *Staphylococcus* spp., *Salmonella* spp., *Helicobacter pylori* and *Bacillus cereus* (Nohynek et al., 2006; Puupponen-Pimiä et al., 2005). Lee et al. (2006) revealed that tea phenolics significantly inhibited the growth of certain pathogenic bacteria such as *C. perfringens, C. difficile* and *Bacteroides* spp., while *Bifidobacterium* spp. and *Lactobacillus* sp. were less affected. This suggests that tea phenolics could be acted as metabolic prebiotics by modulation of the intestinal bacterial population. Tzounis et al. (2008) indicated that catechin significantly increased the growth of the EREC group, *Bifidobacterium* spp. and *E. coli*, as well as a significantly inhibited the growth of the *C. histolyticum* group, while epicatechin only significantly increased the growth of the EREC group. Resveratrol, a potent antioxidant found in wine, was found to increase *Lactobacilli* as well as *Bifidobacteria* and decrease *Enterobacteria*, in rats fed with 1 mg of resveratrol/kg/day (a human equivalent dose) for 25 days (Larrosa et al., 2009). Mao & Zhu (2011) investigated the impacts of six flavonoid compounds (baicalin, quercetin, icraiin, luteolin, amygdalin, naringin) on the composition of the human fecal microbiota. The results showed that incubation of six flavonoid compounds (40 or 160 mg/l) with fecal bacteria, led to a general increase in volatile fatty acids production (except baicalin), and improved the acetate, propionate (except baicalin and aringin) and butyrate (except baicalin) production. The number of the total bacteria were increased by naringin and quercetin addition, while there were no significant changes for the baicalin, icraiin, luteolin and amygdalin addition. This suggests that the flavonoid induced effects may be flavonid type-dependent and further insight into the potential of flavonoid monomers to act as prebiotics in the human large intestine *in vivo* is needed. Hidalgo et al. (2012) investigated the bacterial-dependent metabolism of malvidin-3-glucoside, gallic acid and a mixture of anthocyanins using an *in vitro* fermentation system. They demonstrated that all the anthocyanins tested enhanced significantly the growth of *Bifidobacterium* spp. and *Lactobacillus-Enterococcus* spp. These results showed that the intake of phytochemicals-rich foods can modify the composition of gut microbiota, exerting prebiotic-like effects.

Future challenges for personalized health

Manipulating early-life microbiota for healthy brain and immune systems

Increasing evidence has showed that gut microbiota may play important roles in the communication between gut and

brain (Figure 1), hence to impact behavior and mood. Using denaturing gradient gel electrophoresis and sequencing, Bercik et al. (2011) examined whether the intestinal microbiota affects behavior and brain biochemistry in mice. The data indicated that colonization of germ-free BALB/c mice with microbiota from NIH Swiss mice increased exploratory behavior, while colonization of germ-free NIH Swiss mice with BALB/c microbiota reduced exploratory behavior. Using measures of motor activity and anxiety-like behavior, Heijtz et al. (2011) showed that germ free (GF) mice exhibited increased motor activity and reduced anxiety, compared with specific pathogen free (SPF) mice with a normal gut microbiota. In particular, GF mice exposed to gut microbiota early in life displayed similar characteristics as SPF mice, but conventionalization of adult mice failed to normalize the behavior of GF mice, supporting the notion that there is a sensitive early-life period for gut microbiota to affect later-life brain and behavior. These observations suggest new frontiers for research and new therapeutic strategies in neurodevelopmental diseases.

On the other hand, accumulating evidences suggest that the commensal microbiota actively regulates the host immune system (Hooper et al., 2012) (Figure 1). Using germ-free (GF) and metagenomic approaches, it is reported that the composition of the gut microbiota influences the structure of gut-associated lymphoid tissues (GALTs) and the activation/differentiation status of immune and intestinal epithelial cells (IECs). Specifically, germ-free mice have reduced gut secretory IgA, defects in development of gut-associated lymphoid tissues, and smaller Peyer's patches and mesenteric lymph nodes (Round & Mazmanian, 2009). Furthermore, specific commensal bacterial species induce the accumulation of specific immune cell populations. For instance, O'Mahony et al. (2008) determined the influence of gut microbiota composition on T regulatory (Treg) activity. Mice consuming the commensal microbe *Bifidobacterium infantis* 35624 were infected with *S. typhimurium* or injected with LPS. They found that the consumption of a single commensal microbe *B. infantis* 35624 in mice drives the generation and function of Treg cells, which control excessive NF-kappa B activation

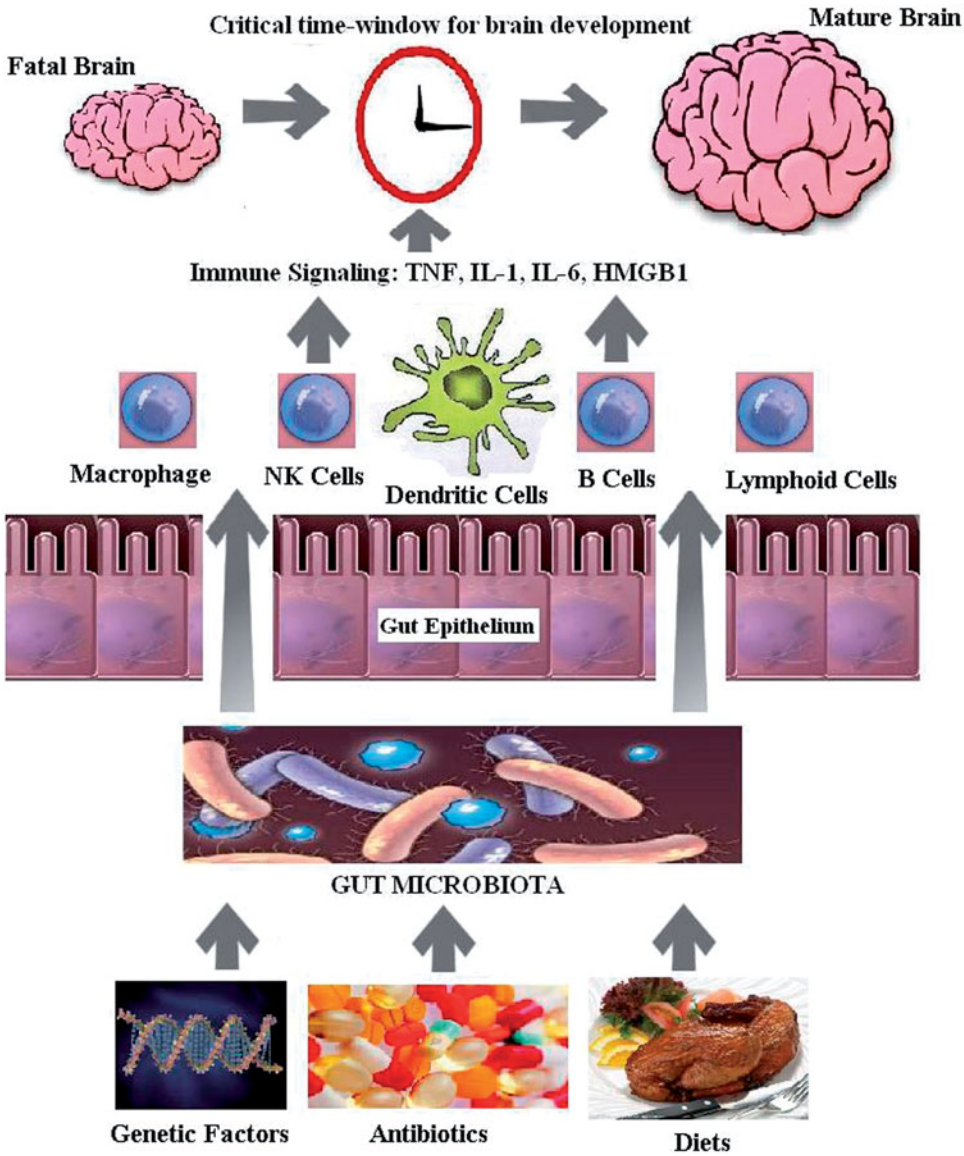

Figure 1. Interactions among gut microbiota and genetic factors, antibiotics, diets, immune system and brain.

in vivo. By systematic analysis of genotobiotic mice, Gaboriau-Routhiau et al. (2009) indicated that colonization by a whole mouse microbiota induced a broad spectrum of proinflammatory T helper 1 (Th1), Th17, and regulatory T cell responses, while most tested complex microbiota and individual bacteria failed to efficiently stimulate intestinal T cell responses, which seems to be related to segmented filamentous bacterium, a non-culturable *Clostridia*-related species. Based on the unique role of this bacterium in the postnatal maturation of gut immune functions, its changes in the infant flora may influence the development of host immune responses. Atarashi et al. (2011) showed that the intestinal microbiota *Clostridium* species, belonging to clusters XIVa and IV, promoted T(reg) cell accumulation in mice. The oral administration of *Clostridium* during the early life of conventionally reared mice resulted in resistance to colitis in adult mice, suggesting a new therapeutic approach to colitis.

Recent research shows that gut microbiota are most likely one of the drivers of immune development and, in turn, the immune system shapes the composition of the microbiota. In particular, the early microbial exposure of the gut is thought to play an important role in regulating and fine-tuning the immune system throughout life (Kelly et al., 2007). Mulder et al. (2009) used the pig as a model to evaluate the impact of early-life environment on microbial diversity of the adult gut and subsequent immune processes. They found that pigs, housed in a natural outdoor environment, showed a dominance of *Firmicutes*, in particular *Lactobacillus*, while animals housed in a hygienic indoor environment had reduced *Lactobacillus* and higher numbers of potentially pathogenic phylotypes. Inman et al. (2010) assessed the effects of rearing under low- (farm, sow) and high-hygiene (isolator, milk formula) conditions on intestinal microbiota and immune development in neonatal piglets. The data indicated that although microbiota in both groups was similar between 2 and 5 days, piglets reared on the mother had more diverse flora than siblings reared in isolators by 12–28 days, and dendritic cells accumulated in the intestinal mucosa more rapidly in isolator piglets. It supports the note that the establishment and development of the normal gut microbiota requires continuous microbial exposure during the early stages of life (Schmidt et al., 2011). Hansen et al. (2012) analyzed the effects of early gut colonization patterns on changing the composition of the resident microbiota and future immune system reactivity. They revealed that a single oral inoculation of GF mice with a cecal microbiota suspension at three weeks of age permanently changed the gut microbiota composition, and permanently changed the levels of systemic regulatory T cells, NK and NKT cells, and cytokine production. Olszak et al. (2012) showed that exposure to microbes during early childhood has persistent effects on natural killer T cell function. Colonization of neonatal-but not adult-GF mice with a conventional microbiota protected the animals from mucosal iNKT accumulation and related pathology. These results highlight that a time window is required for the artificial microbial colonization of GF mice, and the delayed colonization of the gut could cause permanent changes in the immune system.

Thus, it is very important to manipulate human microbiota by early-life exposure. Although it is not realistic to modify human microbiota through altering human genetics, we can select a suitable delivery and feeding mode as well as the external environment to modify early-life human microbiota. Babies are rapidly colonized, immediately upon birth, by the microbes from their mother's vagina, from skin microbes or from the surrounding environments, depending on the delivering mode (Dominguez-Bello et al., 2010). Vaginally born infants acquire bacterial communities resembling their own mother's vaginal microbiota, while babies delivered by Caesarean section harbor bacterial communities similar to the skin microbiota of their mothers with a prevalence of *Staphylococcus* and *Propionibacterium* spp. (Biasucci et al., 2010; Dominguez-Bello et al., 2010). Using the Human Oral Microbe Identification Microarray (HOMIM) in healthy three-month-old infants (38 infants born by C-section, and 25 infants delivered vaginally), Lif Holgerson et al. (2011) revealed that significantly more bacterial taxa were detected in the infants delivered vaginally (79 species/species clusters) compared with infants delivered by Caesarian section (54 species/species clusters), while *Slackia exigua* was detected only in infants delivered by Caesarian section. In addition, breast-fed newborns dominate by *Bifidobacteria* when compared to the formula-fed newborns (Bezirtzoglou et al., 2011; Le Huërou-Luron et al., 2010). These works confirm the differences in the microbiota in infants due to the mode of delivery and feeding. In addition, the environment at delivery and hygiene measures can also affect the composition of the neonate's microbiota (Putignani et al., 2010). The key point of further research is to determine the exact time-window for the establishment of healthy late-life microbiota (Figure 1).

Linking gut virome and bacterial communities with complex diseases

Currently, most studies focus on the relationship between gut bacterial communities and human diseases. However, there is a close relationship between gut bacterial communities and gut virome, i.e. the gut virome is primarily composed of prokaryotic viruses that prey on gut bacteria, thus a key question arises: given that the gut bacteria are an important player in human health and diseases, how the viral and bacterial communities are interacted to maintain host health? A few studies have begun to explore these complex interactions. For example, although herpes virus is pathogenic and can cause acute disease, Barton et al. (2007) showed that latent herpes virus infection has capacity to protect mice against the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis* by enhancing innate immunity. Similarly, Yager et al. (2009) showed that murine gamma-herpesvirus-68 (gammaHV68) or murine cytomegalovirus (mCMV) increased resistance to infection with *Listeria monocytogenes*, confirmed the existence of virus latency-mediated protection against bacterial infection. Moreover, *Helicobacter pylori* has been shown to be associated with different clinical outcomes, including acute and chronic gastritis, peptic ulcer disease and gastric cancer (Talebi et al., 2011). However, Perry et al. (2010) found that the monkeys infected with *H. pylori* were less likely to develop tuberculosis than uninfected animals, demonstrated the protection of *H. pylori* against tuberculosis.

Such an immunity to TB produced by exposure to other microbes could be very implicated in vaccine development and disease control. Overall, these results revealed that there is a symbiotic relationship among host, virome and bacteria. An emerging central concept in evolutionary biology suggests that symbiosis is a universal characteristic of living organisms (Ottaviani et al., 2011). We can hypothesize that one pathogen could induce a continuous innate immune response from the host and provides incidental protection against other pathogens, although the detailed mechanism is unclear. If this hypothesis is true, maybe we need to reconsider the ''classic immune strategy'' that eliminating pathogens to protect the host, alternatively, somehow allowing oriented toward co-existence of the commensal microbiota for active immune response in the steady-state intestine.

Recomposing gut microbiota for potential therapy of diseases

As described above, human disease states are often associated with imbalances in the gut microbiota. In order to restore a healthy microbial community, one valuable strategy is transplantation of a foreign gut microbiota from a healthy to a diseased individual for the treatment of certain diseases. Such as, Khoruts et al. (2010) demonstrated after 2 weeks of transplanting the microbial community from a healthy donor to a patient suffering from *C. difficile*-associated disease (CDAD), the microbiota of the recipient was dramatically changed from a *Firmicutes*- and *Bacteroidetes*-deficient configuration to a community highly similar to that of the donor, dominated by *Bacteroides* spp. Importantly, the symptoms associated with CDAD in the patient disappeared. Recently, Lawley et al. (2012) showed that the treatment of *C. difficile* 027/BI infected mice with feces from healthy mice rapidly restored a diverse and healthy microbiota, and resolved *C. difficile* disease and contagiousness. In particular, they found that a simple mixture of six phylogenetically diverse intestinal bacteria can re-establish a health-associated microbiota and clear *C. difficile* 027/BI infection from mice. These results demonstrated the therapeutic potential of health-associated microbial communities to treat intestinal dysbiosis.

However, how to realize a successful transfer of an exogenous microbiota is still a challenging problem. Even the use of antibiotics prior to transplantation could not facilitate the colonization process by reducing bacterial load. In fact, it may be more difficult to achieve the establishment of transplanted microbiota. For example, using deep sequencing and phylogenetic clustering, Manichanh et al. (2010) evaluated the long-term effects of exogenous microbiota transplantation combined with, and without, an antibiotic pretreatment in a rat model. The results indicated that the reduced bacterial diversity by antibiotic intake prior to transplantation did not increase establishment of the donor phylotypes, even after 3 months of treatment, although some dominant lineages were still transferred successfully.

Except for transplantation, another strategy is the delivery of specific probiotics. Generally, it is now believed that probiotics exert beneficial effects by improving the characteristics of the gut microbiota, as discussed above. In addition, growing evidence has also suggested that the microbiota manipulation properties of probiotics can be applicable to other human-resident microbial communities. For example, genetically modified *Streptococcus* mutants have potential for the prevention of dental caries (Hillman, 2002). Some bacteria could be not intravaginally instilled, but are also orally delivered for vaginal colonization. Using rhesus macaques, Lagenaur et al. (2011) verified that a GusA-producing strain *L. jensenii* 1153–1646 can traverse the gastrointestinal tract and colonize the vagina, with potential benefit in preventing infections of the urogenital tract. Although the role of probiotics in the treatment of atopic dermatitis (AD) remains controversial, Gerasimov et al. (2010) showed that the administration of a probiotic mixture containing *L. acidophilus* DDS-1, *B. lactis* UABLA-12, and fructo-oligosaccharide displayed significant clinical improvement in 90 children aged 1–3 years with moderate-to-severe AD. This is supported by another study in 40 pediatric AD patients (23 males and 17 females) aged $1 \sim 13$ years. This study indicated that a combination of probiotics (*B. bifidum, L. acidophilus, L. casei* and *L. salivarius* strains) was found to effectively reduce the SCORing Atopic Dermatitis index and serum cytokines interleukin (IL)-5, IL-6, interferon (IFN)-γ, and total serum IgE levels (Yeşilova et al., 2012). However, the efficacy of probiotic therapy in adults with AD requires further investigation. Similarly, Bouilly-Gauthier et al. (2010) revealed that a dietary supplement combining a specific probiotic *L. johnsonii* (La1) and nutritional doses of carotenoids can reduce early UV-induced skin damage.

However, the normal microflora in healthy humans displays remarkable quantitative and qualitative stability, and host genetics could be a major determinant for such a high intrinsic stability of the indigenous microbiota, as discussed in a previous section. If the genetic background does not match well, even the already released probiotics could not survive for an extended time. For example, based on 16S rRNA gene genotyping, Buhnik-Rosenblau et al. (2011) found that *L. johnsonii* (a potentially probiotic bacterium) appeared at significantly higher levels in C57BL/6J versus BALB/c mouse feces, their offspring presented similar *L. johnsonii* levels. However, after oral administration, the *L. johnsonii* level decreased rapidly in the BALB/c gut, suggesting that some selective force does not allow its persistence at higher levels. This suggests that the genetic background of the host should be considered when delivering a probiotic bacterium for personalized health.

Thus, it presents a major obstacle to modify its composition by the whole microbiota transplantation or the introduction of specific probiotic strains. In order for successful and permanent colonization, many key issues need to be addressed and assessed, including how to facilitate transplantation, what donors are compatible with the recipient, what genetic backgrounds are required for the recipient, what risks might be involved in this procedure, or which beneficial strains are strongly competitive?

Characterizing human microbiota-metabolized products

The main microbial exposure in the oral cavity and gastrointestinal tract is through the ingestion of food and drinks. The

mechanical and enzymatic digestion of food begins in the mouth and continues in the stomach as a substrate and then into the oral and intestinal microbiota. Growing evidence shows that the nutritional value of food is dependent not only on itself but importantly on its metabolites produced by oral or gut microbiota.

The diet itself is not toxic, but oral or gut microbiota could metabolize it into toxic compounds. For example, ethanol (alcohol) itself is not strongly carcinogenic, but oral bacteria (the commonly encountered oral *Streptococci*) have the capacity to convert ethanol to acetaldehyde, which is an *in vitro* and *in vivo* genotoxin and recognized human carcinogen (Kurkivuori et al., 2007; Langevin et al., 2011). Besides, some yeasts also possess metabolic pathways for this biotransformation (Nieminen et al., 2009; Uittamo et al., 2009), leading to direct carcinogenic acetaldehyde exposure of the oral and gastrointestinal tract after alcohol use. Furthermore, some diets are not only non-toxic but are also functional, i.e. so called ''functional foods'', for instance, high-protein and low-carbohydrate (HPALC) weight-loss diets. These diets can help overweight people achieve weight loss (Johnstone et al., 2008). However, a 4 weeks of experiment on 17 obese men showed that HPALC diets increased the protein fermentation and decreased the carbohydrate fermentation by gut microbiota, and caused a significant decrease in fecal cancer-protective metabolites (e.g. butyrate) and the greatest formation of hazardous metabolites like N-nitroso compounds (NOCs), and promoted metabolite profiles likely to be detrimental to colonic health (Russell et al., 2011). But, the long-term effect of such diets on microbiota-derived metabolites that influence colonic health requires further study.

On the other hand, even the diet contains toxins and certain oral or intestinal microbes could metabolize them into less toxic compounds by the release of specific enzymes. For instance, Zhao et al. (2011) showed that *Myxococcus fulvus* ANSM068 from deer gut can degradate aflatoxin by releasing an enzyme into the culture supernatant. Upadhayaa et al. (2012) indicated that one anaerobic bacteria (named MM11, having 97% sequence similarity with *E. biforme*) from swine gut microbiota, can completely degradate ochratoxin A. In particular, Gao et al. (2011) found that *B. subtilis* ANSB060 from fish gut can detoxify aflatoxin B1, M1 and G1 simultaneously in the culture supernatant. These results suggest that it is crucial to evaluate in detail the metabolic functionality of human microbiota rather than composition-focused studies, and to know how human microbiota might alter the release of either beneficial or harmful compounds from food, which may provide key clues to better understand the relationship between diet, microbiota and host health.

Omics technologies for the mechanism of host-human microbiota interactions

Currently, the molecular mechanisms underlying the interaction between microorganisms and humans are largely unknown. For this purpose, the novel omics technologies like metagenomics, metatranscriptomics, metaproteomics and metabonomics provide great opportunities (Leser & Mølbak, 2009; Nicholson et al., 2012).

Recently, based on the genome sequences of nine strains representing four species of *Bifidobacterium*, a comparative genome analysis of these genomes reveals different genomic potentials to adapt to their habitats. For example, *B. longum* subsp. *infantis* contains more genes involved in the utilization of human milk oligosaccharides, consistent with its infant gut habitats; while *B. longum* subsp. *longum* contains more genes for utilization of plant-derived complex carbohydrates and polyols, consistent with its adult gut habitats (Lee & O'Sullivan, 2010). In particular, the loss of much of this genome potential is observed when strains are adapted to pure culture environments. For instance, when comparing *B. longum* DJO10A (a minimally cultured in the laboratory) with *B. longum* NCC2705 (a culture collection strain), seven regions have been precisely deleted from strain NCC2705. The subsequent experiment validated this targeted loss of genomic regions, when growth of the intestinal *B. longum* in the laboratory for 1000 generations, a lantibiotic encoding region was deleted, which resulted in a significantly reduced competitive ability of this deletion strain against *C. difficile* and *E. coli* (Lee et al., 2008). These studies provide directions to understand how the gut bacteria adapt to the intestinal habitat/culture environment or why are some bacteria uncuturable. To date, not too much knowledge about the molecular mechanisms of proposed probiotic benefits for *bifidobacteria* is available, which greatly weakens the scientific credibility of health claims. The bifidobacterial genome sequences have provided new opportunity to understand the biology of these bacteria in the human GI tract, such as how they survive in the large intestine through complex carbohydrate utilization and competition with bacteriophages and other bacteria.

In addition to health-promoting taxa, the genus *Bifidobacterium* also includes *B. dentium*, an opportunistic cariogenic pathogen. Through global transcriptome analysis, Ventura et al. (2009) identified specific genetic adaptations of a *Bifidobacterium* taxon, *B. dentium* Bd1, from the oral cavity. Some genes encoding tissue adhesion, acid tolerance and the metabolism of human saliva-derived compounds and carbohydrates were highly expressed, suggesting that the genome of this opportunistic cariogen has evolved through a very limited number of horizontal gene acquisition events for adapting to the oral environment. Using whole-genome microarrays and biological pathway reconstructions, van Baarlen et al. (2009) found there existed striking differences in modulation of NF-κB-dependent pathways for the expression profiles of human mucosa, after consumption of living *L. plantarum* bacteria in different growth phases. Moreover, using the transcriptomes from the mucosa of the proximal small intestine of healthy volunteers, van Baarlen et al. (2011) revealed that three *Lactobacilli* (*L. acidophilus, L. casei* and *L. rhamnosus*) induced differential gene-regulatory networks and pathways. The large person-to-person variation in response to transcriptomes observed can help to explain why probiotic supplementation may lead to measurable effects on some people but not in others.

Verberkmoes et al. (2009) developed a non-targeted, shotgun mass spectrometry-based metaproteomics approach for the first deep proteome measurements in human fecal samples. They found that a high proportion of proteins were from common gut bacteria (*Bacteroides, Bifidobacterium* and

Clostridium). Almost 30% of all identified proteins were human, which is related to digestive enzymes, structural cell adhesion, cell–cell interaction proteins and human innate immunity proteins, demonstrating a novel complex interaction between the human host and its associated microbes. Rudney et al. (2010) used a novel three-dimensional peptide separation method to conduct the first metaproteomic analysis of the human salivary microbiota. The peptides identified were distributed among five bacterial phyla (61%), archea (0.5%) and viruses (0.8%), which were linked to translation, glycolysis, amino acid metabolism and energy production. Recently, using a novel high-throughput approach based on denaturing polyacrylamide gel electrophoresis and liquid chromatography-tandem mass spectrometry, Kolmeder et al. (2012) investigated the composition and temporal stability of the intestinal metaproteome based on fecal samples collected from three healthy subjects over a period of six to twelve months. They recognized a stable common core of approximately 1000 proteins in each of the subjects, although the fecal metaproteome is subject-specific, suggesting the existence of a common functional core that is mainly involved in carbohydrate transport and degradation.

Using a broad MS-based metabolomics, Wikoff et al. (2009) showed that the gut "microbiome" exhibited a surprisingly large effect on mammalian blood metabolites. The results showed that many organic acids containing phenyl groups were greatly increased in the presence of gut microbes and amino acid metabolites were particularly affected, such as the production of the antioxidant indole-3-propionic acid (IPA) is completely dependent on the presence of gut

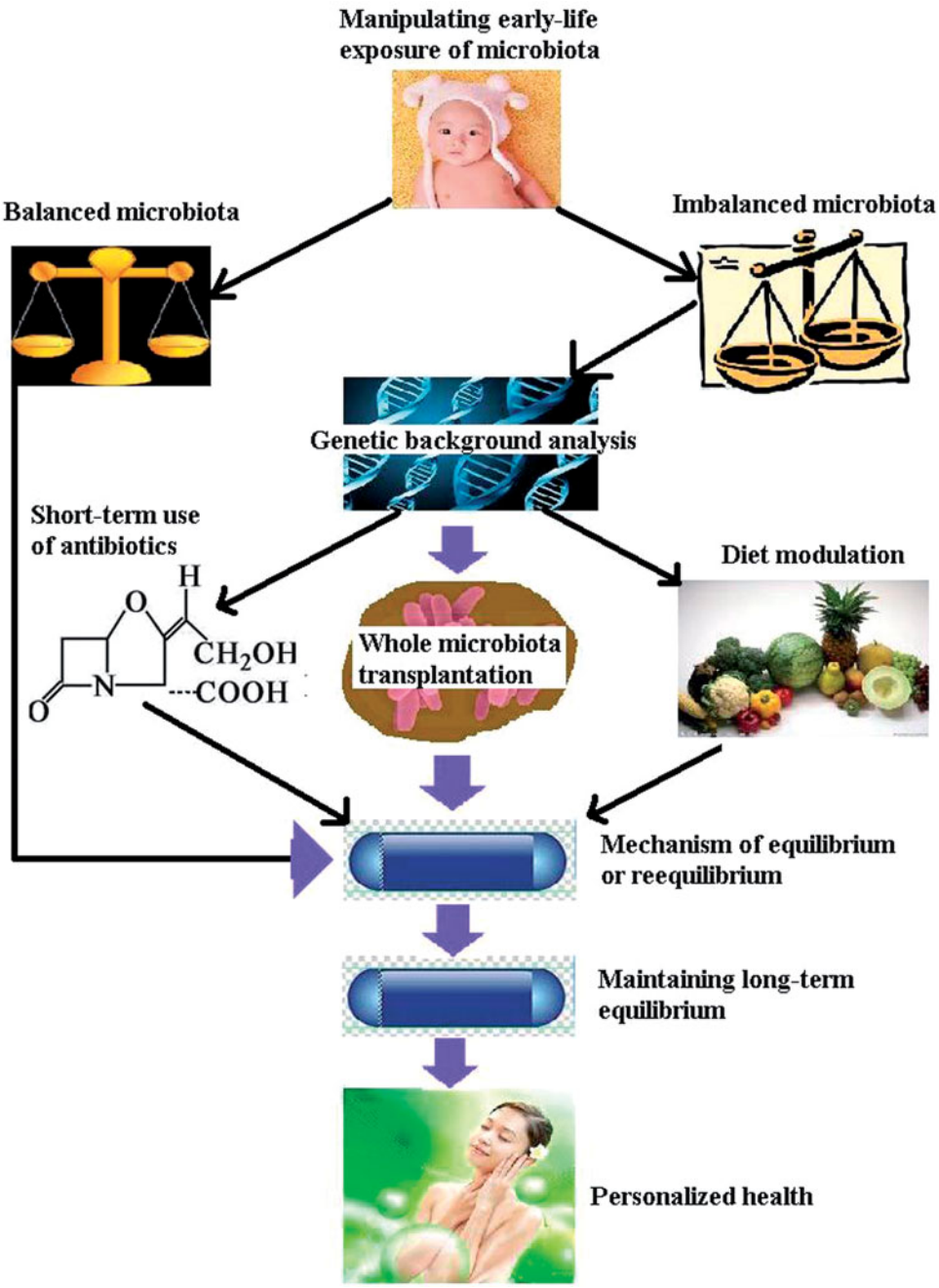

Figure 2. Schematic representation of manipulating human microbiota for personalized health.

microflora, suggesting a significant interplay between bacterial and mammalian metabolism. Based on a NMR spectroscopy of aqueous fecal extracts, Le Gall et al. (2011) investigated differences in metabolic activity of gut microbiota in patients with ulcerative colitis (UC) ($n = 13$), irritable bowel syndrome (IBS) ($n = 10$) and healthy controls (C) ($n = 22$). They detected a trend toward increased taurine and cadaverine levels in UC with increased bile acid and decreased branched chain fatty acids in IBS relative to controls, demonstrating a correlation between the gut microbiota profile and metabolite composition. Using a combination of genomics and metabolomics, Fukuda et al. (2011) investigated the molecular mechanisms of *Bifidobacteria* in protecting against infectious diseases. They found that the genes encoding an ATP-binding-cassette-type carbohydrate transporter in certain *Bifidobacteria* could increase the production of acetate to protect mice against death induced by *E. coli* O157:H7. In order to determine the effect of gut microbiota on energy metabolism in mouse models, Mestdagh et al. (2012) characterized the metabolic phenotype of germ-free (GF) ($n = 20$) and conventional mice ($n = 20$). The data indicated that in GF animals the lower lactate and circulating very-low-density lipoprotein (VLDL) levels as well as higher levels of (D)-3-hydroxybutyrate were observed in liver, plasma and brown adipose tissue (BAT) of GF animals compared to normal mice. It suggests that the gut microbiota modulates the lipid metabolism in BAT. In order to better understand the role of gut microbiota in energy metabolism, further characterization of other metabolic profiles like amino acids and carbohydrates metabolism in the liver is also required, except for lipid metabolism.

Conclusions

There exist large interpersonal variations of human microbiota within a given body habitat, although the individual microbiota is relatively stable over time. The present data indicated that there is a link between human microbiota and human diseases (Table S1), but it is noted that major issues remain in allowing medicine to draw any simplistic conclusions about the microbiome and disease. For example, some results come from mouse models, while mouse models poorly mimic human status (Seok et al., 2013); and even from human models, it is not sure how much noise impacts these studies (Caporaso et al., 2011). However, the ultimate goal would be to prevent the development of diseases by interfering with human microbiome, i.e. the targeted manipulation of the microbiota could be used to change host susceptibility to human diseases and decrease the incidence and severity of diseases. The homeostasis of human microbiota is dependent on genetic factors and environmental conditions (e.g. antibiotics, diets) (Figure 1). We are unable to modify human microbiota through altering human genetics, while the long-term use of antibiotics will lead to adverse effects, thus, other factors rather than genetic factors and antibiotics should be selected to modify the colonization of human microbiota for personalized health (Figure 2). For example, the available methods include the optimization of human microbiota early-life exposure, and the manipulation of day-to-day diets like the release of prebiotics, probiotics and synbiotics. In particular, the nutritional value of food is dependent not only on itself but importantly on its metabolites produced by oral or gut microbiota, there is an urgent need to evaluate in detail the metabolic functionality of human microbiota rather than composition-focused studies. With the emerging omics technologies, such as metagenomics, metatranscriptomics, metaproteomics and metabonomics, it will be possible to understand the activities, functions and interactions of human microbiota from a systems biology perspective. It can be envisaged that targeting specific microbiota could be applied to disease diagnosis and therapy in the near future.

Declarations of interest

The authors report no declarations of interest.

References

Atarashi K, Tanoue T, Shima T, et al. (2011). Induction of colonic regulatory T cells by indigenous *Clostridium* species. Science, 331, 337–41.

Barton ES, White DW, Cathelyn JS, et al. (2007). Herpesvirus latency confers symbiotic protection from bacterial infection. Nature, 447, 326–9.

Benson AK, Kelly SA, Legge R, et al. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc Natl Acad Sci USA, 107, 18933–8.

Bercik P, Denou E, Collins J, et al. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology, 141, 599–609.

Bezirtzoglou E, Tsiotsias A, Welling GW. (2011). Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). Anaerobe, 17, 478–82.

Biasucci G, Rubini M, Riboni S, et al. (2010). Mode of delivery affects the bacterial community in the newborn gut. Early Hum Dev, 86, 13–15.

Bouilly-Gauthier D, Jeannes C, et al. (2010). Clinical evidence of benefits of a dietary supplement containing probiotic and carotenoids on ultraviolet-induced skin damage. Br J Dermatol, 163, 536–43.

Buhnik-Rosenblau K, Danin-Poleg Y, Kashi Y. (2011). Predominant effect of host genetics on levels of *Lactobacillus johnsonii* bacteria in the mouse gut. Appl Environ Microbiol, 77, 6531–8.

Caporaso JG, Lauber CL, Costello EK, et al. (2011). Moving pictures of the human microbiome. Genome Biol, 12, R50.

Claesson MJ, Jeffery IB, Conde S, Power SE. (2012). Gut microbiota composition correlates with diet and health in the elderly. Nature, 488, 178–84. DOI: 10.1038/nature11319.

Clemente JC, Ursell LK, Parfrey LW, Knight R. (2012). The impact of the gut microbiota on human health, an integrative view. Cell, 148, 1258–70.

De Filippo C, Cavalieri D, Di Paola M, et al. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA, 107, 14691–6.

Dethlefsen L, Huse S, Sogin ML, Relman DA. (2008). The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol, 6, e280.

Dethlefsen L, Relman DA. (2011). Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci USA, 108, 4554–61.

Dominguez-Bello MG, Costello EK, Contreras M, et al. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA, 107, 11971–5.

Faure M, Mettraux C, Moennoz D, et al. (2006). Specific amino acids increase mucin synthesis and microbiota in dextran sulfate sodium-treated rats. J Nutr, 136, 1558–64.

Fukuda S, Toh H, Hase K, et al. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature, 469, 543-U791.

Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, et al. (2009). The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity, 31, 677–89.

Gao X, Ma Q, Zhao L, et al. (2011). Isolation of *Bacillus subtilis*, screening for aflatoxins B1, M1, and G1 detoxification. Eur Food Res Technol, 232, 957–62.

Gerasimov SV, Vasjuta VV, Myhovych OO, Bondarchuk LI. (2010). Probiotic supplement reduces atopic dermatitis in preschool children, a randomized, double-blind, placebo-controlled, clinical trial. Am J Clin Dermatol, 11, 351–61.

Goto K, Kanaya S, Nishikawa T, et al. (1998). The influence of tea catechins on faecal flora of elderly residents in long-term facilities. Annals of Long Term Care, 6, 43e48.

Gourbeyre P, Denery S, Bodinier M. (2011). Probiotics, prebiotics, and synbiotics, impact on the gut immune system and allergic reactions. J Leukocyte Biol, 89, 685–95.

Hansen CH, Nielsen DS, Kverka M, et al. (2012). Patterns of early gut colonization shape future immune responses of the host. PLoS One, 7, e34043.

Heijtz RD, Wang S, Anuar F, et al. (2011). Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA, 108, 3047–52.

Hidalgo M, Oruna-Concha MJ, Kolida S, et al. (2012). Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. J Agric Food Chem, 60, 3882–90.

Hillman JD. (2002). Genetically modified *Streptococcus mutans* for the prevention of dental caries. Antonie Van Leeuwenhoek, 82, 361–6.

Hooper LV, Littman DR, Macpherson AJ. (2012). Interactions between the microbiota and the immune system. Science, 336, 1268–73.

Inman CF, Haverson K, Konstantinov SR, et al. (2010). Rearing environment affects development of the immune system in neonates. Clin Exp Immunol, 160, 431–9.

Jernberg C, Löfmark S, Edlund C, Jansson JK. (2007). Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J, 1, 56–66.

Johnstone AM, Horgan GW, Murison SD, et al. (2008). Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. Am J Clin Nutr, 87, 44–55.

Kelly D, King T, Aminov R. (2007). Importance of microbial colonization of the gut in early life to the development of immunity. Mutat Res, 622, 58–69.

Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ. (2010). Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea. J Clin Gastroenterol, 44, 354–60.

Kolmeder CA, de Been M, Nikkilä J, et al. (2012). Comparative metaproteomics and diversity analysis of human intestinal microbiota testifies for its temporal stability and expression of core functions. PLoS One, 7, e29913. Epub 2012 Jan 18.

Kurkivuori J, Salaspuro V, Kaihovaara P, et al. (2007). Acetaldehyde production from ethanol by oral streptococci. Oral Oncol, 43, 181–6.

Lagenaur LA, Lee PP, Hamer DH, Sanders-Beer BE. (2011). Demonstration of vaginal colonization with GusA-expressing *Lactobacillus jensenii* following oral delivery in rhesus macaques. Res Microbiol, 162, 1006–10.

Langevin F, Crossan GP, Rosado IV, et al. (2011). Fancd2 counteracts the toxic effects of naturally produced aldehydes in mice. Nature, 475, 53–8.

Larrosa M, Yañéz-Gascón MJ, Selma MV, et al. (2009). Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. J Agric Food Chem, 57, 2211–20.

Lawley TD, Clare S, Walker AW, et al. (2012). Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing *Clostridium difficile* disease in mice, PLos Pathog, 8, e1002995. DOI: 10.1371/journal.ppat.1002995.

Le Gall G, Noor SO, Ridgway K, et al. (2011). Metabolomics of fecal extracts detects altered metabolic activity of gut microbiota in ulcerative colitis and irritable bowel syndrome. J Proteome Res, 10, 4208–18.

Le Huërou-Luron I, Blat S, Boudry G. (2010). Breast- v. formula-feeding, impacts on the digestive tract and immediate and long-term health effects. Nutr Res Rev, 23, 23–36.

Lee HC, Jenner AM, Low CS, Lee YK. (2006). Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. Res Microbiol, 157, 876–84.

Lee JH, Karamychev VN, Kozyavkin SA, et al. (2008). Comparative genomic analysis of the gut bacterium *Bifidobacterium longum* reveals loci susceptible to deletion during pure culture growth. BMC Genomics, 9, 247.

Lee JH, O'Sullivan DJ. (2010). Genomic insights into bifidobacteria. Microbiol Mol Biol Rev, 74, 378–416.

Leser TD, Molbak L. (2009). Better living through microbial action, the benefits of the mammalian gastrointestinal microbiota on the host. Environ Microbiol, 11, 2194–206.

Lif Holgerson P, Harnevik L, Hernell O, et al. (2011). Mode of birth delivery affects oral microbiota in infants. J Dent Res, 90, 1183–8.

Maccaferri S, Klinder A, Brigidi P, et al. (2012). Potential probiotic *Kluyveromyces marxianus* B0399 modulates the immune response in Caco-2 cells and peripheral blood mononuclear cells and impacts the human gut microbiota in an in vitro colonic model system. Appl Environ Microbiol, 78, 956–64.

Mäkivuokko H, Lahtinen SJ, Wacklin P, et al. (2012). Association between the ABO blood group and the human intestinal microbiota composition. BMC Microbiol, 12, 94. DOI: 10.1186/1471-2180-12-94.

Malinen E, Rinttilä T, Kajander K, et al. (2005). Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. Am J Gastroenterol, 100, 373–82.

Mangin I, Suau A, Gotteland M, et al. (2010). Amoxicillin treatment modifies the composition of *Bifidobacterium* species in infant intestinal microbiota. Anaerobe, 16, 433–8.

Manichanh C, Reeder J, Gibert P, et al. (2010). Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. Genome Res, 20, 1411–19.

Mao SY, Zhu WY. (2011). Effects of six flavonoid compounds addition on short-chain fatty acids production and human fecal microbial community change during in vitro fermentation. African J Microbiol Res, 5, 4484–91.

Martínez I, Kim J, Duffy PR, et al. (2010). Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. PLoS One, 5, e15046.

Mestdagh R, Dumas ME, Rezzi S, et al. (2012). Gut microbiota modulate the metabolism of brown adipose tissue in mice. J Proteome Res, 11, 620–30.

Mills DJ, Tuohy KM, Booth J, et al. (2008). Dietary glycated protein modulates the colonic microbiota towards a more detrimental composition in ulcerative colitis patients and non-ulcerative colitis subjects. J Appl Microbiol, 105, 706–14.

Minot S, Sinha R, Chen J, et al. (2011). The human gut virome, inter-individual variation and dynamic response to diet. Genome Res, 21, 1616–25.

Mulder IE, Schmidt B, Stokes CR, et al. (2009). Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biol, 7, 79. doi: 10.1186/1741-7007-7-79.

Murphy EF, Cotter PD, Hogan A, et al. (2013). Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. Gut, 62, 220–6.

Nakamura YK, Omaye ST. (2012). Metabolic diseases and pro- and prebiotics, mechanistic insights. Nutr Metab (Lond), 9, 60. DOI: 10.1186/1743-7075-9-60.

Ness RB, Hillier S, Richter HE, Soper DE, et al. (2003). Can known risk factors explain racial differences in the occurrence of bacterial vaginosis? J Natl Med Assoc, 95, 201–12.

Neyrinck AM, Possemiers S, Druart C, et al. (2011). Prebiotic effects of wheat arabinoxylan related to the increase in bifidobacteria, *Roseburia* and *Bacteroides/Prevotella* in diet-induced obese mice. PLoS One, 6, e20944. doi: 10.1371/journal.pone.0020944.

Nicholson JK, Holmes E, Kinross J, et al. (2012). Host-gut microbiota metabolic interactions. Science, 336, 1262–7.

Nieminen MT, Uittamo J, Salaspuro M, Rautemaa R. (2009). Acetaldehyde production from ethanol and glucose by non-*Candida albicans* yeasts in vitro. Oral Oncol, 45, e245–8.

Nohynek LJ, Alakomi HL, Kähkönen MP, et al. (2006). Berry phenolics, antimicrobial properties and mechanisms of action against severe human pathogens. Nutr Cancer, 54, 18–32.

O'Mahony C, Scully P, O'Mahony D, et al. (2008). Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kappaB activation. PLoS Pathog, 4, e1000112. doi: 10.1371/journal.ppat.1000112.

Okubo T, Ishihara N, Oura A, et al. (1992). In vivo effects of tea polyphenols intake on human intestinal microflora and metabolism. Biosci Biotechnol Biochem, 56, 588e591.

Olszak T, An D, Zeissig S, et al. (2012). Microbial exposure during early life has persistent effects on natural killer T cell function. Science, 336, 489–93.

Ottaviani E, Ventura N, Mandrioli M, et al. (2011). Gut microbiota as a candidate for lifespan extension, an ecological/evolutionary perspective targeted on living organisms as metaorganisms. Biogerontology, 12, 599–609.

Paliy O, Agans R. (2012). Application of phylogenetic microarrays to interrogation of human microbiota. FEMS Microbiol Ecol, 79, 2–11.

Perry S, de Jong BC, Solnick JV, et al. (2010). Infection with *Helicobacter pylori* is associated with protection against tuberculosis. PLoS ONE, 5, e8804.

Pieper R, Jha R, Rossnagel B, et al. (2008). Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. FEMS Microbiol Ecol, 66, 556–66.

Putignani L, Carsetti R, Signore F, Manco M. (2010). Additional maternal and nonmaternal factors contribute to microbiota shaping in newborns. Proc Natl Acad Sci USA, 107, E159.

Puupponen-Pimiä R, Nohynek L, Hartmann-Schmidlin S, et al. (2005). Berry phenolics selectively inhibit the growth of intestinal pathogens. J Appl Microbiol, 98, 991–1000.

Ramirez-Farias C, Slezak K, Fuller Z, et al. (2009). Effect of inulin on the human gut microbiota, stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. Br J Nutr, 101, 541–50.

Round JL, Mazmanian SK. (2009). The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol, 9, 313–23.

Rudney JD, Xie H, Rhodus NL, et al. (2010). A metaproteomic analysis of the human salivary microbiota by three-dimensional peptide fractionation and tandem mass spectrometry. Mol Oral Microbiol, 25, 38–49.

Russell WR, Gratz SW, Duncan SH, et al. (2011). High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. Am J Clin Nutr, 93, 1062–72.

Saulnier DM, Gibson GR, Kolida S. (2008). In vitro effects of selected synbiotics on the human faecal microbiota composition. FEMS Microbiol Ecol, 66, 516–27.

Schmidt B, Mulder IE, Musk CC, et al. (2011). Establishment of normal gut microbiota is compromised under excessive hygiene conditions. PLoS One, 6, e28284.

Sekirov I, Russell SL, Antunes LC, Finlay BB. (2010). Gut microbiota in health and disease. Physiol Rev, 90, 859–904.

Seksik P, Rigottier-Gois L, Gramet G, et al. (2003). Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. Gut, 52, 237–42.

Seok J, Warren HS, Cuenca AG, et al. (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci USA, 110, 3507–12.

Signoretto C, Bianchi F, Burlacchini G, et al. (2010). Drinking habits are associated with changes in the dental plaque microbial community. J Clin Microbiol, 48, 347–56.

Signoretto C, Burlacchini G, Bianchi F, et al. (2006). Differences in microbiological composition of saliva and dental plaque in subjects with different drinking habits. New Microbiol, 29, 293–302.

Sommer MO, Dantas G, Church GM. (2009). Functional characterization of the antibiotic resistance reservoir in the human microflora. Science, 325, 1128–31.

Sonoyama K, Ogasawara T, Goto H, et al. (2010). Comparison of gut microbiota and allergic reactions in BALB/c mice fed different cultivars of rice. Brit J Nutr, 103, 218–26.

Spor A, Koren O, Ley R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. Nat Rev Microbiol, 9, 279–90.

Świątecka D, Narbad A, Ridgway KP, Kostyra H. (2011). The study on the impact of glycated pea proteins on human intestinal bacteria. Int J Food Microbiol, 145, 267–72.

Talebi Bezmin Abadi A, Rafiei A, Ajami A, et al. (2011). *Helicobacter pylori* homB, but not cagA, is associated with gastric cancer in Iran. J Clin Microbiol, 49, 3191–7.

Turnbaugh PJ, Ridaura VK, Faith JJ, et al. (2009). The effect of diet on the human gut microbiome, a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med, 1, 6ra14. doi: 10.1126/scitranslmed.3000322.

Tzounis X, Vulevic J, Kuhnle GG, et al. (2008). Flavanol monomer-induced changes to the human faecal microflora. Br J Nutr, 99, 782–92.

Uittamo J, Siikala E, Kaihovaara P, et al. (2009). Chronic candidosis and oral cancer in APECED-patients, production of carcinogenic acetaldehyde from glucose and ethanol by *Candida albicans*. Int J Cancer, 124, 754–6.

Upadhayaa SD, Songa JY, Park MA, et al. (2012). Isolation, screening and identification of swine gut microbiota with ochratoxin A biodegradation ability. Asian-Aust J Anim Sci, 25, 114–21.

van Baarlen P, Troosta F, van der Meera C, et al. (2009). Differential NF-kappaB pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. Proc Natl Acad Sci USA, 106, 2371–6.

van Baarlen P, Troost F, van der Meer C, et al. (2011). Human mucosal in vivo transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. Proc Natl Acad Sci USA, 108, 4562–9.

Ventura M, Turroni F, Zomer A, et al. (2009). The *Bifidobacterium dentium* Bd1 genome sequence reflects its genetic adaptation to the human oral cavity. PLoS Genet, 5, e1000785.

Verberkmoes NC, Russell AL, Shah M, et al. (2009). Shotgun metaproteomics of the human distal gut microbiota. ISME J, 3, 179–89.

Vitali B, Ndagijimana M, Cruciani F, et al. (2010). Impact of a synbiotic food on the gut microbial ecology and metabolic profiles. BMC Microbiol, 10, 4. doi: 10.1186/1471-2180-10-4.

Wacklin P, Mäkivuokko H, Alakulppi N, et al. (2011). Secretor genotype (FUT2 gene) is strongly associated with the composition of Bifidobacteria in the human intestine. PLoS One, 6, e20113.

Waligora-Dupriet AJ, Campeotto F, Romero K, et al. (2011). Diversity of gut *Bifidobacterium* species is not altered between allergic and non-allergic French infants. Anaerobe, 17, 91–6.

Wall R, Marques TM, O'Sullivan O, et al. (2012). Contrasting effects of *Bifidobacterium breve* NCIMB 702258 and *Bifidobacterium breve* DPC 6330 on the composition of murine brain fatty acids and gut microbiota. Am J Clin Nutr, 95, 1278–87.

Wikoff WR, Anfora AT, Liu J, et al. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci USA, 106, 3698–703.

Wu GD, Chen J, Hoffmann C, et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. Science, 334, 105–108.

Yager EJ, Szaba FM, Kummer LW, et al. (2009). Gamma-Herpesvirus-induced protection against bacterial infection is transient. Viral Immunol, 22, 67–72.

Yamakoshi J, Tokutake S, Kikuchi M, et al. (2001). Effect of proanthocyanidin-rich extract from grape seeds on human fecal flora and faecal odor. Microbial Ecol Health Disease, 13, 25e31.

Yeşilova Y, Çalka Ö, Akdeniz N, Berktaş M. (2012). Effect of probiotics on the treatment of children with atopic dermatitis. Ann Dermatol, 24, 189–93.

Zhao LH, Guan S, Gao X, et al. (2011). Preparation, purification and characteristics of an aflatoxin degradation enzyme from *Myxococcus fulvus* ANSM068. J Appl Microbiol, 110, 147–55.