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Review Article

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Current Status and Future Promise of the Human Microbiome

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The human-associated microbiota is diverse, varies between individuals and body sites, and is important in human health. Microbes in human body play an essential role in immunity, health, and disease. The human microbiome has been studies using the advances of next-generation sequencing and its metagenomic applications. This has allowed investigation of the microbial composition in the human body, and identification of the functional genes expressed by this microbial community. The gut microbes have been found to be the most diverse and constitute the densest cell number in the human microbiota; thus, it has been studied more than other sites. Early results have indicated that the imbalances in gut microbiota are related to numerous disorders, such as inflammatory bowel disease, colorectal cancer, diabetes, and atopy. Clinical therapy involving modulating of the microbiota, such as fecal transplantation, has been applied, and its effects investigated in some diseases. Human microbiome studies form part of human genome projects, and understanding gleaned from studies increase the possibility of various applications including personalized medicine. **(Pediatr Gastroenterol Hepatol Nutr 2013; 16: 71～79)**

Key Words: Human microbiome, Metagenome, Microbiota

INTRODUCTION

Microbes in the human body consists of archaea, bacteria, virus and eukaryotes; the number of bacteria in each host is estimated to reach trillions of cells [1], and their gene contents are more numerous than 100 times than the human genome [2]. These microbes play important roles in human health, including metabolism, homeostasis of the immune system, and in colonization resistance. However, most microbes cannot be cultured in the laboratory; the various conventional culture techniques are able to culture only limited species of the bacteria in nature including human body [3,4]. Metagenomics, defined as the study of the total genomes extracted from a complex mixture of microbes in a given environment [5], has been applied to various ecological approaches, including studies of the human microbiome, as it can be used to investigate various microbes simultaneously, without cultivation. This ap-

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proach has accelerated human microbiome studies and their clinical applications. Studies about diverse microbes in the human body, site-specific microbiota, relations between bacterial composition and disease have rapidly increased our understanding of the importance of the human microbiome and its roles in health [1,6-8]. This explosion of human microbiome data holds the promise of managing personal health based on individual genome and microbiome information. In this article, we review current strategies for studying the human microbiome and the possible future applications of microbiome data.

DEVELOPMENT OF TECHNIQUES

The introduction of next generation sequencing (NGS) changed the history of genomic research as it increased sequencing throughput, and did not require prior cloning steps. This revolutionary advance in sequencing technology led the way to study microbes with the new generation metagenomic research. Direct sequencing of microbial DNA extracted from a given environment by NGS eliminated the bottleneck of missed, unculturable microbes by supplying vast amounts of sequence data. However, genomic fragments from different origins within the metagenome could cause another obstruction. Bioinformatics should overcome this, thereby endowing these short reads with appropriate scientific value [9]. Metagenomics has generated new terms for use in microbial study such as 'microbiota' and 'microbiome'. Microbiota is defined as the collective microbial community inhabiting a specific environment, while the microbiome is the collective genomic contents of the microbiota [10]. The interaction between microbes or between microbes and host can also be analyzed by microbiome study. Various environmental microbiome studies including human microbiome have been performed using metagenomic analyses based on NGS. Other techniques in protein and metabolite analyses also improved, and they have been applied in microbiome studies. The comparison of these techniques is present in Table 1.

Table 1. Techniques in Microbiome Study

Technique	Characteristic	Limit
Target gene sequencing	Using 16S ribosomal RNA gene	Obtain only taxonomic information
	Observe bacterial composition	Amplification based approach
	Detect disease specific bacteria	Chimera production and PCR bias
Metagenomics	Using fragmented metagenome	Limited information of active bacteria and expressed gene contents
	Observe microbial composition without amplification	Bioinformatic bottleneck
	Detect gene contents of complex microbiota	
	Compare functional gene contents between samples	
Metatranscriptomics	Using enriched mRNA or synthesized cDNA from total RNA	Difficult to handle RNA sample
	Observe alive microbial composition in samples	Hard to extract RNA from environmental sample
	Detect gene expression profiles	Multiple purification steps needed
	Compare functional gene expressions between samples	
Metaproteomics	Using crude proteins or peptides	Insufficient information of proteins in database
	Observe microbial composition producing proteins	Difficult to extract total protein from environmental sample
	Detect protein profiles	
	Compare protein productionsbetween samples	
Metabolomics	Using liquid including complex metabolites	Insufficient information of metabolites in database
	Observe metabolic profiles	Mixed information of host and microbial metabolites
	Compare metabolites between samples	No unique protocol

RNA: ribonucleic acid, PCR: polymerase chain reaction.

Most of published human microbiome studies have focused on sequencing of the small subunit 16S ribosomal ribonucleic acid (RNA), target gene sequencing, as a phylogenetic marker for determining the composition of bacteria or archaea in samples, because of the efficiency with which 16S ribosomal RNA gene sequencing allows identification of bacteria within a community. Although amplification provides advances in the efficiency of experiments and reduction in cost, it can generate chimeric products and obscure the real level of diversity and microbial composition of a given sample [11]. Metagenomic shotgun sequencing could avoid these problems without amplification step and the inherent primer-related pitfalls. This technique can provide microbial community without amplification, and detect various functional genes in sample. However, functional gene analysis of complex microbial community requires millions of sequences in each sample, and this makes bioinformatic bottlenecks. Although metagenomic shotgun sequencing poses many bioinformatic challenges in the identification and improvement of the accuracy of information derived from the relatively short gene fragments generated by NGS, most scientists are now using this technique to study the microbiome in various environments. Other limitation of metagenomic fragment sequencing is based on DNA; it cannot provide evidence of the expression of each functional gene in a sample. To address the activity of genes in an environmental microbial community, metatranscriptomic sequencing has been used in microbiome studies (Table 1). Metatranscriptomics has already been applied to the analysis of 10 healthy fecal microbiomes; this study indicated that functions of carbohydrate metabolism, energy production, and synthesis of cellular components are the most expressed functions by microbes [12]. Although metatranscriptomic analysis is able to characterize the activity of functional genes in a sample, the improvement of efficient protocols for the extraction of RNA and the construction of sequencing library are requisite, given the instability of mRNA and the difficulties involved in RNA extraction from samples. Moreover, analyses of proteins and metabolites are important for the understanding of microbial functions. Identifications of crude proteins and liquids including metabolites from samples have been applied to study microbial function in the human body [13,14]. Combining results from these different approaches can help to elucidate the ecological roles of microbes in our body.

Table 2. Summary of Human Microbiome Consortiums

Microbiome consortium	Summary
Human Microbiome Project (HMP) (http://commonfund.nih.gov/hmp/)	US National Institutes of Health (NIH) funded To characterize the microbial communities at several different sites on the human body Microbiome analysis using metagenome Whole genome sequencing of reference strains 15-18 human body sites The project numbers of gastrointestinal tract, oral, urogenital tract, skin are higher than other sites
Metagenomics of the human intestinal tract (Meta-HIT) (http://www.metahit.eu/)	European commission funded To establish associations between the genes of the human intestinal microbiota and our health and disease Focus on inflammatory bowel disease (IBD) and obesity Microbial gene analysis using metagenome Projects usually focus on intestinal microbiota Gut bacterial gene catalog
International Human Microbiome Consortium (IHMC) (http://www.human-microbiome.org/)	To work under a common set of principal and policies to study the role of the human microbiome in the maintenance of health and causation of disease To use these information to improve the ability to prevent and treat disease Focus on generating a shared comprehensive data resource

HUMAN MICROBIOME CONSORTIUMS

Improvements in NGS and the accompanying bioinformatic development have attracted the interests of basic and industrial researchers investigating the human microbiome. The US National Institutes of Health funded the Human Microbiome Project (HMP) consortium to the tune of $171 million, and the European Commission provided €22 million in funds to the metagenomics of the human intestinal tract (Meta-HIT), aimed at understanding the role of the microbiome in human health (Table 2). The purpose of the HMP was to characterize microbial communities in human body and their relationship to human health and disease. The HMP provided baseline microbiome data obtained from 'healthy' adults for comparison them with that of patients. They investigated the microbial communities in 15-18 body sites of 242 adults (129 males and 113 females), aged 18-40 years old. Bacteria were isolated from the human body, and their genomes sequenced to establish reference genome sequences (800 genomes are available from public database). The consortium published the results of their past 5 years' studies involving 16S rRNA sequencing, whole genome sequencing of isolates, and metagenomic sequencing of total DNA in 2012 [15,16] and released 'phase I' datasets on Data Analysis and Coordination Center (http://hmpdacc.org/).

In contrast to HMP, the European consortium of the Meta-HIT focused on the human intestinal tract, since the human intestinal tract contain the majority of human microbiota and plays critical roles in human health [17-19]. The objectives of the Meta-HIT are to understand the relationship between the genes of the human intestinal microbiota and human health and disease. This project focused on inflammatory bowel disease (IBD) and obesity, which are of increasing importance in Europe. They extensively investigated microbial genes in the human intestine and their relationships with disease, in order to understand the mechanisms of disease development. They used metagenomic analyses in all their studies, and published the catalogue of microbial genes in the gut, as well as distinct enterotypes based on microbial composition, and the functions of microbial genes [20-24].

Data generated by these consortiums provide an improved understanding of the relationships between the microbiome and host physiology. The International Human Microbiome Consortium was establish to work under a common principal and policies to study the role of the human microbiome in human health and disease. This consortium focused on generating a shared data resource for use by investigators into the human microbiome. These consortiums have spearheaded extensive developments in the bioinformatic tools necessary for study of the microbiome using large amounts of genomic data.

THE HUMAN MICROBIOME

The human microbiome is defined as the genomes of the microbiota in the human body. Increasing understanding of the coevolution microbes with their human hosts has highlighted the importance of the human microbiome in health. The microbiome plays essential roles in the maintenance and development of the immune system, metabolism, and homeostasis. The phyla Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Cyanobacteria, and Fusobacteria are predominant among bacteria in the human body. The relative abundance of these phyla varies between different sites in the body, and also varies among individuals depending on age, diet, and geographical distribution [25,26].

Among body sites, the highest numbers of taxonomic units and genetic contents have been observed in stool samples [15]. Stool represents the intestinal tract, and most microbes residing here are anaerobes (about 4,000 bacterial species), with an estimated cell number of about 10^{12} cells per gram of contents [27]. Bacteria inhabiting the intestinal tract participate in maintenance of homeostasis in the human body; an imbalance in their composition could generate disease states. In newborns, the delivery mode influences the composition of the intestinal microbiota, and initiates the colonization of microbiota in the intestine after this exposure [28]. Diet is

another significant impact factor in the development of infant microbiota during the first year [29]. The genus of *Bifidobacteria* is predominant in breast-fed infants, and its abundance decreases along with increasing diversity of other genera in formula-fed infants; an adult-like microbiota is developed with the introduction of solid foods. The dominance of *Bifidobacteria* is associated with utilization of human milk oligosaccharides and the developing microbiota is related to diet type [29,30]. Differences in the development of the initial microbiota could influence development of subsequent microbiota and of the immune system. For instance, there are some reports indicating that early antibiotic exposure, which influences the composition of the microbiota, increases the risk of development of allergic asthma and IBD, and interaction with the immune system has also been reported [31-33]. Two phyla, bacteroidetes and firmicutes, are predominant in the normal intestinal microbiota, and they are able to digest complex dietary polysaccharides [25]. The human gut microbiota has been classified into 3 enterotypes, depending on the dominant presence of *Bacteroides*, *Prevotella*, or *Ruminococcus* genera [21]. However, the composition of the intestinal microbiota varies between healthy individuals (there are no core species), and microbiota in a Korean cohort was found to be significantly different from that in a US cohort [16,34]. These inter-individual variations could be associated with the susceptibility to development of a specific disease.

Traditionally, it has been understood that disease is generated after infection with bacteria; however, this concept is changing to an understanding that imbalances in the indigenous microbial composition causes disease to present, and many studies have reported the relationship between gut microbiota, host metabolism, and disease [2,3,10,35,36]. The role of dysbiosis, an imbalance in the microbial composition and a shift in their function from normal to disease [37], has been investigated in several diseases, including IBD, obesity, metabolic syndrome, type 1 and type 2 diabetes, and allergy. Given the inter-individual variation in intestinal microbiota, no single species act as a biomarker of dysbiosis [38]. Nonetheless, monitoring of dysbiosis (alterations in the microbial community) can be used diagnostically, and to assess the effects of treatment. Some bioinformatic tools have already been developed and used to identify microbes, genes, proteins, and transcripts associated with disease [39]. A reduction in bacterial diversity and decrease in the population of the anaerobic firmicutes has been reported to be associated with disease [40-42]. Similarly, a reduction in anaerobes with increase in facultative anaerobes, mostly proteobacteria, including *Salmonella*, *Shigella*, *Klebsiella*, *Proteus*, and *Escherichia coli* have been reported in disease states [43-45]. Changes in the microbial composition influenced the amount of metabolites produced by microbes in the gut. A shift in short chain fatty acid (SCFA) production has been associated with diarrhea, by reducing sodium and water absorption [46], and is related to a delay in the re-establishment of the indigenous microbiota composition. Butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, have been reported to be related to inflammation in IBD [47]. Furthermore, bacteria within *Enterobacteriaceae* are able to survive in the inflamed gut than are members of firmicutes and bacteroidetes [43,44,48].

Moreover, the development of various immune-related cells is influenced by gut microbiota [49]. The gut mucosal immune system is the largest lymphoid site in body, and gut bacteria interact with lymphoid follicles of the gut mucosa, and regulatory and effector T cells. Dysbiosis changes the immune regulatory systems that normally manage inflammation in the gut, and is associated with immune-mediated disorders [2]. The changes in community function brought about by dysbiosis have also been investigated and compared using metagenomic analyses. Most functional studies on the microbiome of IBD and colon disorders have been performed by the Meta-HIT consortium. The metagenome shared between healthy persons and patients were lower (25%) than that shared between healthy persons (38%). In addition, the gene numbers in IBD patients was significantly lower than

those in healthy persons [20]. This indicates that microbiota in IBD patients are unable to produce some functional genes that are generated by healthy microbiota. This could cause disorders of the human intestinal tract, and holds promise for the development of diagnostic tools for disorder.

Although metagenomic and metatranscriptomic approaches provide ways to discover functional genes of the predominant microbes in the gut, the extraction methods are important due to their significant effects on the downstream results [50]. Recently, most microbiome studies have been focused on the variation in functional genes produced by microbiota in each person, in order to understand their effects on individual health. However, further development of bioinformatic tools is necessary for accurately interpreting information from vast amount of sequence data.

APPLICATIONS OF HUMAN MICROBIOME

Extensive microbiome studies and accumulation of information is opening the possibility of clinical application of this research in modulation of the microbiome. Antibiotics, probiotics, and prebiotics related to the human microbiome have already been used in clinical therapy and for controlling infectious disease. Although antibiotics are the most effective treatment for bacterial infections, their use poses risks of increasing resistance to antibiotics and indirect impact on non-target bacteria [51]. In addition, incomplete recovery of the gut microbiota after exposure to antibiotics could influence the homeostasis of the gut [51,52]. This indicates that care should be taken in clinical treatment of patients. Probiotics are generally a mixture of lactic acid-producing bacteria, such as *Lactobacilli* and *Bifidobacteria*, which are beneficial to health; yeasts, such as *Saccharomyces*, are also used. Probiotics play a role in host immune responses by direct interactions between microbes [53]. Recent analyses demonstrated some effects of probiotics in dysbiosis, such as in necrotizing enterocolitis in infants and in antibiotic-associated diarrhea [54,55]. Prebiotics are non-digestible food ingredients that beneficially affect the host by activating beneficial microbes. For instance, the ingestion of oligosaccharides could stimulate growth of *Bifidobacterium* species and protect against *Clostridium difficile* infection [56]. The effects and mechanisms of these treatments have been studied by investigating the human microbiome.

A direct modulation of microbiota at an ecological scale is fecal transplantation (bacteriotherapy). This therapy replaces the intestinal microbiota of patients with *Clostridium difficile* infection (CDI) with the microbiota from a healthy person. Meta-analyses of the effects of this therapy have reported around 90% resolution rates in CDI patients [57,58]. Several reports on fecal transplantation have highlighted the potential use of CDI treatment in recalcitrant conditions that have not responded to long-term use of broad-spectrum antibiotics [59,60]. Although fecal transplantation is mostly used in CDI, it has been attempted in other conditions, including IBD, irritable bowel syndrome, and metabolic disorders [61]. However, there are some considering issues for fecal transplantation including host immune response with introducing new pathogen or microbiota to recipient. Other consider issues are the determination of suitable donors for treat, management of the donor feces as preparing, freezing and storing samples before treatment. Although more efforts will be needed to improve the fecal transplantation, it is clear the therapy is successful to CDI patient.

CONCLUSION

For human health, it is essential to consider the role of microbiota in clinical fields. International human microbiome studies using metagenomics have highlighted the functional role of the microbiota in the body, and it promises new clinical applications. The development of sequencing technology and bioinformatic algorithm for metagenomes ensures easy access to vast amount of data in the near future. However, clinical applications of this data will require studies to clarify, for example, the disease that

are caused by dysbiosis of the microbiota, the beneficial effects of microbiome modulation, and the extent of inter- and intra-individual microbiome variations. Increased understanding of the role of the human microbiome in disease is expected to result in novel microbiome-related clinical treatments and the development of personalized medicine.

REFERENCES

1. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449:804-10.
2. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. Nature 2012;489:231-41.
3. Krznarić Z, Vranešić Bender D, Kunović A, Kekez D, Stimac D. Gut microbiota and obesity. Dig Dis 2012;30:196-200.
4. Aslam Z, Yasir M, Khaliq A, Matsui K, Chung YR. Too much bacteria still unculturable. Crop Environ 2010;1: 59-60.
5. Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 1998;5:R245-9.
6. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005;308:1635-8.
7. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature 2009;457:480-4.
8. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science 2009;326:1694-7.
9. Scholz MB, Lo CC, Chain PS. Next generation sequencing and bioinformatic bottlenecks: the current state of metagenomic data analysis. Curr Opin Biotechnol 2012;23:9-15.
10. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature 2012;489:242-9.
11. von Wintzingerode F, Göbel UB, Stackebrandt E. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 1997;21:213-29.
12. Gosalbes MJ, Durbán A, Pignatelli M, Abellan JJ, Jiménez-Hernández N, Pérez-Cobas AE, et al. Metatranscriptomic approach to analyze the functional human gut microbiota. PLoS One 2011;6:e17447.
13. Verberkmoes NC, Russell AL, Shah M, Godzik A, Rosenquist M, Halfvarson J, et al. Shotgun metaproteomics of the human distal gut microbiota. ISME J 2009;3:179-89.
14. Turnbaugh PJ, Gordon JI. An invitation to the marriage of metagenomics and metabolomics. Cell 2008;134:708-13.
15. Methé BA, Nelson KE, Pop M, Creasy HH, Giglio MG, Huttenhower C, et al; Human Microbiome Project Consortium. A framework for human microbiome research. Nature 2012;486:215-21.
16. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, et al; Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
17. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005;307:1915-20.
18. Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr 2002;22:283-307.
19. Ley RE. Obesity and the human microbiome. Curr Opin Gastroenterol 2010;26:5-11.
20. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010;464:59-65.
21. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature 2011;473:174-80.
22. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006;444:1022-3.
23. Lepage P, Leclerc MC, Joossens M, Mondot S, Blottière HM, Raes J, et al. A metagenomic insight into our gut's microbiome. Gut 2013;62:146-58.
24. Manichanh C, Chapple CE, Frangeul L, Gloux K, Guigo R, Dore J. A comparison of random sequence reads versus 16S rDNA sequences for estimating the biodiversity of a metagenomic library. Nucleic Acids Res 2008;36: 5180-8.
25. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature 2012;486:222-7.
26. Ursell LK, Clemente JC, Rideout JR, Gevers D, Caporaso JG, Knight R. The interpersonal and intrapersonal diversity of human-associated microbiota in

key body sites. J Allergy Clin Immunol 2012;129:1204-8.
27. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008;134:577-94.
28. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107:11971-5.
29. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4578-85.
30. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 2000;30:61-7.
31. Kozyrskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. Chest 2007;131:1753-9.
32. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Rep 2012;13:440-7.
33. Hviid A, Svanström H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut 2011;60:49-54.
34. Lee S, Sung J, Lee J, Ko G. Comparison of the gut microbiotas of healthy adult twins living in South Korea and the United States. Appl Environ Microbiol 2011;77:7433-7.
35. Young VB. The intestinal microbiota in health and disease. Curr Opin Gastroenterol 2012;28:63-9.
36. DuPont AW, DuPont HL. The intestinal microbiota and chronic disorders of the gut. Nat Rev Gastroenterol Hepatol 2011;8:523-31.
37. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. Gut 2004;53:1-4.
38. Blumberg R, Powrie F. Microbiota, disease, and back to health: a metastable journey. Sci Transl Med 2012;4:137rv7.
39. Kuczynski J, Lauber CL, Walters WA, Parfrey LW, Clemente JC, Gevers D, et al. Experimental and analytical tools for studying the human microbiome. Nat Rev Genet 2011;13:47-58.
40. McLaughlin SD, Walker AW, Churcher C, Clark SK, Tekkis PP, Johnson MW, et al. The bacteriology of pouchitis: a molecular phylogenetic analysis using 16S rRNA gene cloning and sequencing. Ann Surg 2010;252:90-8.
41. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 2006;55:205-11.
42. Lepage P, Häsler R, Spehlmann ME, Rehman A, Zvirbliene A, Begun A, et al. Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. Gastroenterology 2011;141:227-36.
43. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2007;2:119-29.
44. Garrett WS, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, et al. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe 2010;8:292-300.
45. Mai V, Young CM, Ukhanova M, Wang X, Sun Y, Casella G, et al. Fecal microbiota in premature infants prior to necrotizing enterocolitis. PLoS One 2011;6:e20647.
46. Ramakrishna BS, Mathan VI. Colonic dysfunction in acute diarrhoea: the role of luminal short chain fatty acids. Gut 1993;34:1215-8.
47. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, et al. Low counts of Faecalibacterium prausnitzii in colitis microbiota. Inflamm Bowel Dis 2009;15:1183-9.
48. Carvalho FA, Koren O, Goodrich JK, Johansson ME, Nalbantoglu I, Aitken JD, et al. Transient inability to manage proteobacteria promotes chronic gut inflammation in TLR5-deficient mice. Cell Host Microbe 2012;12:139-52.
49. Biasucci G, Rubini M, Riboni S, Morelli L, Bessi E, Retetangos C. Mode of delivery affects the bacterial community in the newborn gut. Early Hum Dev 2010;86 Suppl 1:13-5.
50. Ó Cuív P, Aguirre de Cárcer D, Jones M, Klaassens ES, Worthley DL, Whitehall VL, et al. The effects from DNA extraction methods on the evaluation of microbial diversity associated with human colonic tissue. Microb Ecol 2011;61:353-62.
51. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 2010;156:3216-23.
52. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4554-61.

53. Reid G, Younes JA, Van der Mei HC, Gloor GB, Knight R, Busscher HJ. icrobiota restoration: natural and supplemented recovery of human microbial communities. Nat Rev Microbiol 2011;9:27-38.
54. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics 2010;125: 921-30.
55. Kale-Pradhan PB, Jassal HK, Wilhelm SM. Role of Lactobacillus in the prevention of antibiotic-associated diarrhea: a meta-analysis. Pharmacotherapy 2010;30: 119-26.
56. Hopkins MJ, Macfarlane GT. Nondigestible oligosaccharides enhance bacterial colonization resistance against Clostridium difficile in vitro. Appl Environ Microbiol 2003;69:1920-7.
57. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol 2013;108:500-8.
58. Kachrimanidou M, Malisiovas N. Clostridium difficile infection: a comprehensive review. Crit Rev Microbiol 2011;37:178-87.
59. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, et al; Fecal Microbiota Transplantation Workgroup. Treating Clostridium difficile infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol 2011;9:1044-9.
60. Borody TJ, Brandt LJ, Paramsothy S, Agrawal G. Fecal microbiota transplantation: a new standard treatment option for Clostridium difficile infection. Expert Rev Anti Infect Ther 2013;11:447-9.
61. Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol 2011;9:88-96.