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The role of the gut microbiome in the healthy adult status

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ABSTRACT

The gut microbiome, which hosts up to 1000 bacterial species that encode about 5 million genes, perform many of the functions required for host physiology and survival. Consequently, it is also known as "our forgotten organ". The recent development of next-generation sequencing technologies has greatly improved metagenomic research. In particular, it has increased our knowledge about the microbiome and its mutually beneficial relationships with the human host. Microbial colonization begins immediately at birth. Although influenced by a variety of stimuli, namely, diet, physical activity, travel, illness, hormonal cycles and therapies, the microbiome is practically stable in healthy adults. This suggests that the microbiome plays a role in the maintenance of a healthy state in adulthood. Quantitative and qualitative alterations in the composition of the gut microbiome could lead to pathological dysbiosis, and have been related to an increasing number of intestinal and extra-intestinal diseases. With the increase in knowledge about gut microbiome functions, it is becoming increasingly more possible to develop novel diagnostic, prognostic and, most important, therapeutic strategies based on microbiome manipulation.

1. The human microbiome: general facts and its interaction with the human host

A microbiota is defined as the community of microrganisms, including bacteria, archaea, viruses, and some unicellular eukaryotes, living in a specific environment. A microbiome, on the other hand, is the entire collection of all the genomic elements of a specific microbiota, whereas metagenomics is the field of molecular research that studies the complexity of microbiomes.

In this optics, and considering the human body as an environment, the human microbiota is the entire collection of microorganisms living on the surface and inside our body (Table 1) [1–4]. These communities are important for human physiology, immune system development, digestion and detoxification reactions. In fact, some of these microorganisms residing in the gut encode proteins involved in functions important for the host's health, such as enzymes required for the hydrolysis of otherwise indigestible dietary compounds, and the synthesis of vitamins [5,6]. Consequently, we have two genomes, one inherited from our parents and the other acquired, i.e., the microbiome. This concept is the basis of the definition of humans as "superorganisms" [7]. The most important difference between these two genomes is that, while the inherited genome remains almost stable during lifetime, the microbiome is extremely dynamic and can be influenced by a number of factors, among which, age [8], diet [9,10], hormonal cycles [11], travel [12], therapies [13], and illness [13].

Humans are born sterile and microbial colonization begins immediately at birth. The establishment of the infant microbiota appears to be mainly influenced by the type of delivery and the subsequent feeding practices [14–17]. In addition, a number of studies have identified a high intra-individual variability in the infant microbiota composition, especially during the first year of life; it assumes a more adult-like pattern when the host reaches 3 years of age [13–16]. A longitudinal microbiome analysis, carried out on different biological samples collected from the same healthy adults at different time points, has shown not only the presence of specific microbial signatures in the body sites evaluated, but also a great intra-individual variability over time [18]. Aging is associated with a number of physiological and biological modifications, and indeed, it has been recently reported that the microbiome composition differs between adults and the elderly [19].

Most of the human adult microbiota lives in the gut. Only in the human colon does microbial cell density exceed 10^{11} cells/g contents, being equivalent to 1–2 kg of body weight [20]. In addition, it has been estimated that the human gut microbiome accounts for more than 5 million different genes [21]. It is now known that over 1,000 different species colonize the human gut [22], all of which belong to a

Abbreviations: IBD, inflammatory bowel disease; NGS, next-generation sequencing; rRNA, ribosomal RNA.

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Table 1
Human microbiota composition across the five most extensively studied body sites. Interestingly, the oral and gut microbiota have the highest microbial diversity, while the urogenital tract has the smallest bacterial diversity [See references 1–4].

	Human microbiota (10 times more microbial than human cells: 10^{14} vs 10^{13})	
Human microbial habitats	Most represented Phyla and their relative abundance (%)	Number of species
Oral cavity	*Firmicutes* (36.7), *Bacteroidetes* (17.3), *Proteobacteria* (17.1), *Actinobacteria* (11.9), *Fusobacteria* (5.2)	>500
Skin	*Actinobacteria* (52), *Firmicutes* (24.4), *Proteobacteria* (16.5), *Bacteroidetes* (6.3)	~300
Airways	*Actinobacteria* (55), *Firmicutes* (15), *Proteobacteria* (8), *Bacteroidetes* (3)	>500
Gut	*Firmicutes* (38.8), *Bacteroidetes* (27.8), *Actinobacteria* (8.2), *Proteobacteria* (2.1)	>1000
Urogenital tract[a]	*Firmicutes* (83), *Bacteroidetes* (3), *Actinobacteria* (3)	~150

[a] Mainly female.

small number of phyla. The most abundant are *Firmicutes*, *Bacteroidetes* and *Actinobacteria*, while *Proteobacteria*, *Fusobacteria*, *Cyanobacteria* and *Verrucomicrobia* are usually less well represented [6]. Remarkably, given this high inter-individual variability in the gut microbiota composition, a core gut microbiome, shared by healthy adults, has been identified, which suggests that it plays a role in the maintenance of health status (Table 2) [23]. To date, a number of functions have been associated to the core microbiome, including polysaccharide digestion, immune system development, defense against infections, synthesis of vitamins, fat storage, angiogenesis regulation, and behavior development [5,6,24,25]. Interestingly, genes encoded by the human core microbiome encode proteins required for host survival, but not present in the human genome, this finding led to the definition of the microbiome as "our forgotten organ" [26].

In this scenario, alterations of the human gut microbiome can play a role in disease development. It is feasible that as we learn more about microbiome composition and functions in healthy individuals, and their modifications associated with specific disease, it will become possible to use the microbiome as a novel target for diagnostic and therapeutic applications. Here, we review the main techniques now available for metagenomic studies, and the association between microbial dysbiosis and the development of specific diseases.

Table 2
Human gut microbiota composition throughout life. In healthy conditions, microbial diversity and richness increase with age reach their highest complexity during adulthood. Despite inter- and intra-individual variations, the gut microbiome is practically stable in healthy adults. In the elderly, as in infants, the gut microbiome is more unstable and also has a lower diversity with respect to adults [49].

	Phylum level microbial composition (from the most to the less represented)	Modifying factors
Infant (up to 2–3 years)	*Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*	— Vaginal vs caesarian delivery — Gestational age — Infant hospitalization — Breast vs formula fed — Age at solid food introduction — Malnutrition — Antibiotic treatments
Adult	*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*	— Diet — Hormonal cycles — Travel — Therapies — Illness
Elderly (>70 years)	*Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*	— Lifestyle changes — Nutritional changes — Increased susceptibility to infections and inflammatory diseases — Use of more medications

2. Next-generation sequencing-based approaches for the study of the human microbiome

2.1. Background

The first microbial studies were based on the direct cultivation and isolation of microbes. Although these methodologies are currently used also for diagnostic purposes, they are somewhat limited because the growth conditions used may favor the selection of one or more species over the others. In addition, it is estimated that up to 99% of microbes are currently uncultivable [27]. Other methods, such as quantitative PCR and polyacrylammide gel electrophoresis separation, are also influenced by the use of specific probes for the detection of specific bacteria. Therefore, they are not suitable for the study of entire microbiomes.

Over the past ten years, the rapid development of next-generation sequencing (NGS) technologies, which increase the throughput of bases sequenced/run while reducing sequencing costs, has had a major impact on the field of metagenomics. In fact, a specific microbiome can be qualitatively and quantitatively characterized in-depth using NGS-based approaches without the selection bias and constraints associated with cultivation methods. These technologies are being used also in the Human Microbiome Project, the aim of which is to obtain a complete catalogue of the microbes living in the various districts of the human body and to define their functions [6,21,22].

Although NGS-based strategies have greatly improved our knowledge in the field of metagenomics, they have some limitations. In fact, some technical issues still need to be resolved, and NGS-based strategies depend largely on continuously updated databases, bioinformatic tools, and functional information. The combination of several analytic strategies, including traditional cultivation methods, to characterize the genomic and metabolic properties of specific bacteria will provide further insight into the role of the microbiota, and will also help to identify novel candidate targets for disease diagnosis and treatment.

Below we briefly review the NGS-based strategies that can be used for metagenomic purposes (Fig. 1).

2.2. Shotgun sequencing

Shotgun sequencing is the analysis of an entire microbial community. It is based on the extraction of genomic DNA directly from an environmental sample; this DNA is used to prepare an NGS library for downstream high-throughput sequencing. Subsequent data analysis, performed with specific bioinformatic tools, is required to assign the obtained reads to both the host and its microbial components, and to perform genome assembly. The great advantage of this method is that it avoids both the cultivation and PCR steps since the DNA is directly analyzed. It can also identify bacteria up to species level (the complete, or almost complete, genome can be assembled), and is also used for virome analysis (there is no universal tag for virus analysis). However, the correct assignment of sequencing reads is often difficult due to limitations in the databases currently available as reference. Moreover, genome assembly could be flawed especially in the case of less abundant and/or closely related species. Function assignment may be difficult, and could also be ambiguous. Finally, some biases could be related to the method used for DNA extraction [28].

2.3. 16S rRNA sequencing

Targeted sequencing of specific genes enables one to study the microbiome in all its complexity in an easy and cost-effective manner. All bacteria host the 16S rRNA gene, which is generally used for

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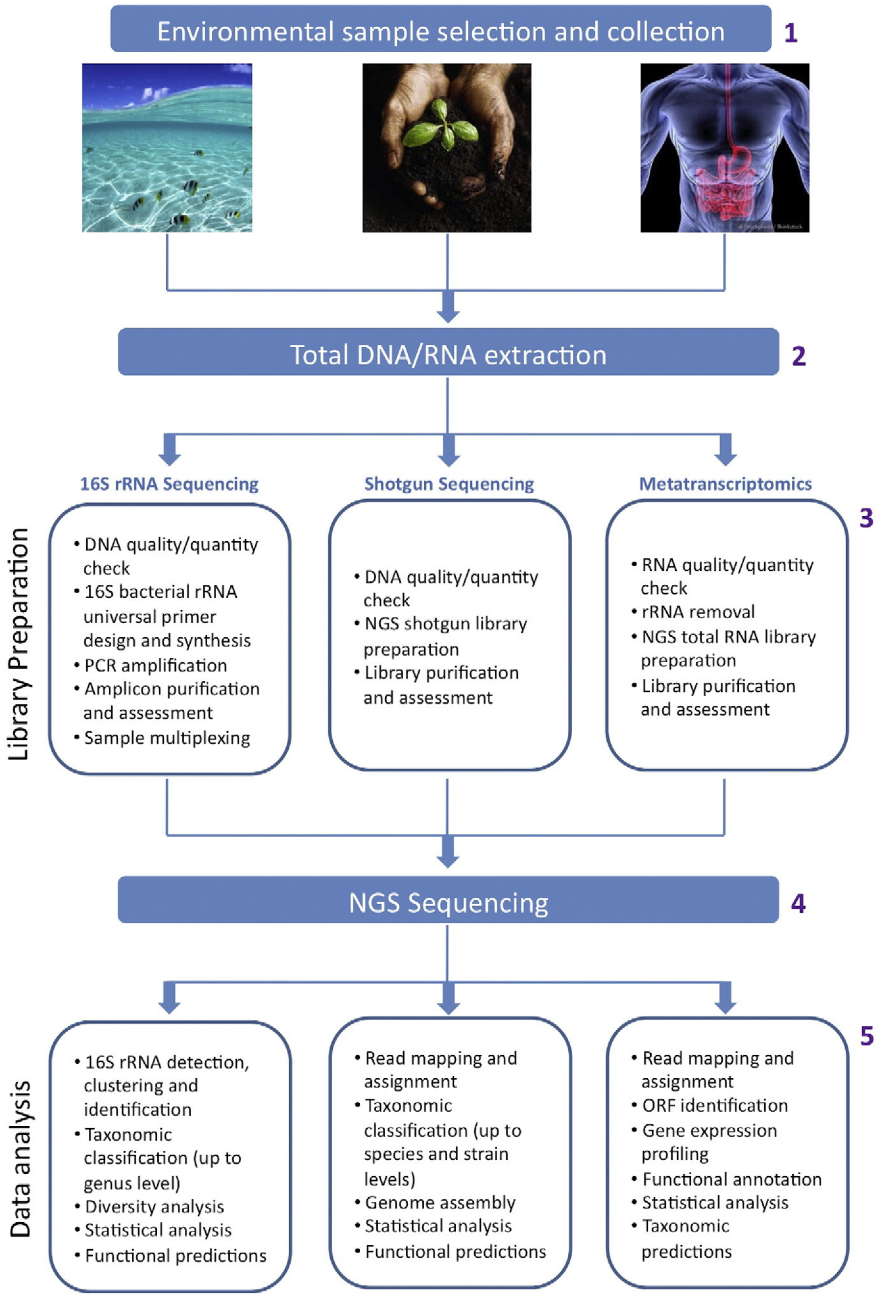

Fig. 1. Next Generation sequencing-based approaches for metagenomics. Starting from an environmental sample of interest (1), total DNA and/or RNA are extracted (2). Three different sample preparation strategies can be used depending on the project aims: 16 s rRNA Sequencing, Shotgun Sequencing, and Metatranscriptomics (3). Usually, the 16S rRNA procedure allows sample multiplexing while a higher coverage is required for the others. After sequencing (4), specific bioinformatic pipelines are used for data analysis (5).

phylogenetic purposes. The 16S rRNA gene has a peculiar structure characterized by hypervariable regions spaced by ultra-conserved regions [29]. Therefore, universal primers (by annealing on the conserved regions) can be used to amplify, in a single PCR reaction, virtually all the bacteria present in a target environment, and to unequivocally identify them at the end of sequencing [30]. Although 16S rRNA sequencing has the advantage of being easy to perform, fast and relatively inexpensive, DNA extraction may be biased and the PCR methodology may confound the analysis and produce ambiguous results [28,31]. Also in this case, the assignment of reads depends on the accuracy of the reference databases used, and the procedure does not produce data about bacterial functions.

2.4. Metatranscriptomics

Metatranscriptomics serves to analyze the entire transcriptome of an environmental site to obtain a comprehensive view of gene expression profiles and functional data. Several factors affect the large-scale application of metatranscriptomics, namely, technical issues related to RNA extraction and storage procedures, RNA quality and quantity and, most important, the enrichment procedure used to remove rRNAs. In addition, host contaminations may affect the procedure, and cannot be removed by the currently available rRNA purification methods [32]. Finally, bioinformatic tools for metatranscriptomic data analysis are still being developed.

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2.5. Bioinformatic tools

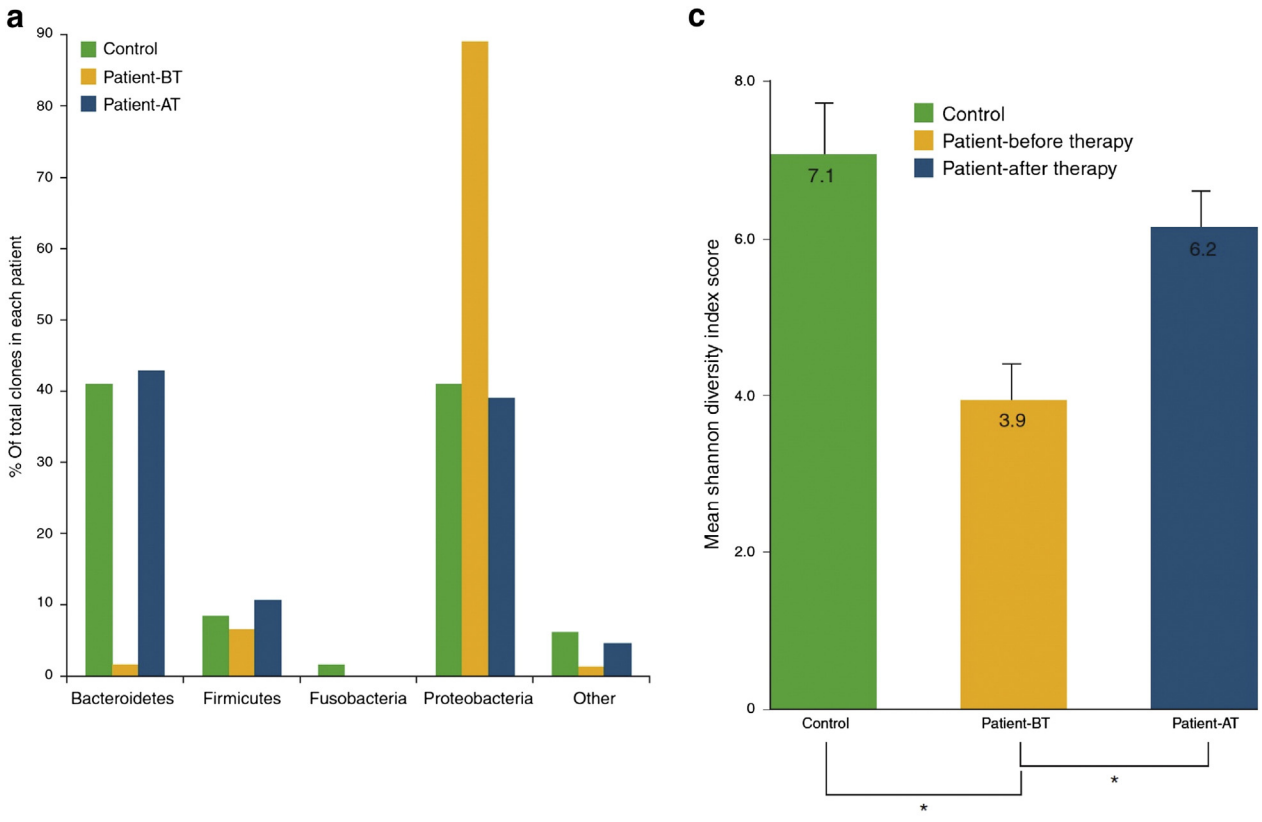
The widespread use of the above-mentioned metagenomic strategies has increased the need for specific data analysis pipelines able to interpret the complex NGS data generated, as reviewed elsewhere [33,34].

Various tools for the analysis of 16S NGS reads have been developed in recent years, and we previously evaluated the performances of two of them in the same dataset [35]. Most of these methods include both taxonomic identification and diversity analysis [36–38]. Taxonomic identification accuracy is variable and depends not only on the specific pipeline used, but also on the portion of the 16S rRNA sequenced, PCR biases and the availability of updated databases. In general, taxonomic assignment is from phylum to genus, while species identification is more difficult [39]. In fact, the 16S rRNA sequencing approach gives a picture of the entire qualitative and quantitative composition of a microbiome, but does not provide information about a specific bacteria genome and/or functions. *In silico* tools for the functional prediction of the most well studied microbiomes, such as the human gut microbiome have recently become available [40].

Shotgun DNA sequencing, which is the NGS sequencing of the whole DNA isolated from an environmental sample, generates the sequence of the host and of all the microbes present in the studied environment, including bacteria, archaea, fungi and viruses, without culture-related and PCR-related biases. Specific tools are available to obtain taxonomic identification up to species and strain levels [41–43]. Besides providing a more accurate identification, with these tools it is possible to make predictions about functional properties. Various pipelines are available for this procedure although they still have computational limitations [44–49]. Metatranscriptomics promises to largely overcome the lack of knowledge and also the ambiguity related to assignment. Indeed, a few tools are already available for this kind of analysis although most depend on the availability of genome reference sequences [50–52].

3. The human microbiome, healthy status and diseases

Various reviews have extensively covered the link between the human microbiome and state of health [see e.g., 53,54]. In this section we will only briefly mention some of the most relevant data related to



b

Consensus Lineage from phylum to family	Control	Patient-BT	Patient-AT
P->Bacteroidetes;C->Bacteroidia;O->Bacteroidales;F->Bacteroidaceae	2449	69	2462
P->Bacteroidetes;C->Bacteroidia;O->Bacteroidales;F->Porphyromonadaceae	10		367
P->Firmicutes;C->Clostridia;O->Clostridiales;F->Lachnospiraceae	5	434	579
P->Proteobacteria;C->Betaproteobacteria;O->Burkholderiales;F->Alcaligenaceae	158		
P->Proteobacteria;C->Gammaproteobacteria;O->Enterobacteriales;F->Enterobacteriaceae	1661	7610	2639
P->Proteobacteria;C->Gammaproteobacteria;O->Pasteurellales;F->Pasteurellaceae	7	441	6
P->Proteobacteria;C->Gammaproteobacteria;O->Pseudomonadales;F->Pseudomonadaceae	106		61

Fig. 2. Ileum-associated microbiome composition in a Crohn's disease patient before (patient-BT) and after (patient-AT) nutritional therapy and in a control subject, as characterized by 16S rRNA next-generation sequencing. (a) Note the reduction of Bacteroidetes and the significantly higher prevalence of Proteobacteria in the patient-BT vs the patient-AT and vs the control patient. (b) The heatmap table shows in brownish red the most significant alterations; the numerical figures indicate the number of bacterial families sequenced in each patient. (c) The mean Shannon Diversity Index Score shows significantly lower values in the patient-BT compared with values obtained in the patient-AT and in the control subject (*$P < 0.05$) [69].

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this issue. In fact, as the gut core microbiome seems to be almost stable during healthy adulthood, its qualitative and quantitative alterations, which lead of course to functional modifications, has been reported in a number of human diseases [55–58]. In particular, microbial richness, intended as bacterial diversity, is usually considered an indicator of a healthy status: reduced bacterial diversity has been related to obesity and immune-related and inflammatory diseases [55–64]. In addition, as a healthy microbiome composition is required for a number of physiological functions [5,6,24,25], qualitative alterations, especially at level of the core microbiome, can lead to the development of disease. This phenomenon has been mostly studied in inflammatory bowel diseases (IBD), including both Crohn's disease and ulcerative colitis [65–69].

Inflammatory bowel diseases are chronic recurrent disease of the gastrointestinal tract, and are caused by a combination of genetic and environmental factors, including the gut microbiota. A number of studies have reported significant alterations of gut microbial composition in IBD patients compared with not affected individuals [65–69]. Not only do IBD patients have an altered rate of *Bacteroidetes*, *Proteobacteria* and *Firmicutes* colonization, but the bacterial diversity of their microbiome is generally lower than that of controls. In this context, we characterized the 16S rRNA ileum-associated microbiome of a Crohn-affected patient at diagnosis and after nutritional therapy that reduces gut inflammation, and found that the microbial balance was restored by nutritional therapy; in fact, at follow-up the microbial balance did not differ between the patient and control (Fig. 2) [69].

Differences in the gut microbiome between obese and not obese subjects have been also reported and extensively reviewed [62–64]. Although it is well recognized that alterations of gut microbiota and gut permeability can induce the inflammatory status typical of obesity, the specific microbial changes and the mechanisms by which they act, are still unclear. The same applies to an increasing number of intestinal and extra-intestinal diseases [55–58]. The more we learn about the gut microbiota and its functions, the better we will understand the mechanisms underlying a number of diseases, and how to use the microbiota itself as diagnostic and/or prognostic biomarker. Similarly, greater insight into the gut microbiota will enable us to develop novel therapeutic strategies also in the context of an ever more personalized medicine [70].

4. Conclusions and perspectives

Metagenomics has shed considerable light on microbiomes, including the human microbiome, and on the complex relationships between microbes and their hosts. Today, we know that the gut microbiome plays a role in functions required for the physiology and correct development of our organs, and that its composition is related to aging, environmental factors (diet, physical activity, etc.), and pathological conditions. Understanding the role of specific microbes will open the way to novel strategies for disease diagnosis, monitoring and therapy. The modulation of the gut microbiota to gain a healthy status is the challenge facing metagenomic research in coming years.

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