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Andreas Schwiertz *Editor*

Microbiota of the Human Body

Implications in Health and Disease

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Microbiota of the Human Body

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Preface

Microbes can now be found in nearly every niche the human body offers. However, the complexity of the microbiota of a given site depends on the particular environmental condition thereof. Only microbes that are able to grow under these conditions will prevail. Recent publications imply that the microorganisms do not only have multiple critical consequences for host physiological processes, such as postnatal development, immunomodulation and energy supply, but also effects on neurodevelopment, behaviour and cognition.

Within this book we will focus on the techniques behind these developments, epigenomics and on the various parts of the human body, which are inhabited by microorganisms, such as the mouth, the gut, the skin and the vagina. In addition, chapters are dedicated to the possible manipulations of the microbiota by probiotics, prebiotics and faecal transplantation.

I would like to express my gratitude to all chapters' authors for their contribution to this book and hope that it will be appreciated by readers as well as it occurred to me as an editor.

Herborn, Germany Andreas Schwiertz

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1 A Short Definition of Terms

Andreas Schwiertz and Volker Rusch

Keywords
Definition • Microbiota • Microbiome

We humans are colonized by myriads of microorganisms in various parts of the body, such as the skin, the mouth, the vagina and the gastrointestinal tract. Even the lung and other hitherto thought to be sterile parts, as the placenta, are now considered to be colonized. Furthermore, our microbiota is not only comprised of bacteria, but also of archaea and eukaryotes such as protozoa, fungi and nematodes. Even viruses, collectively termed the virome, can be found in the microbiota (Virgin 2014). It has been estimated that the human-associated microbiota, consists of at least 40,000 bacterial strains in 1800 genera (Luckey 1972; Frank and Pace 2008; Forsythe and Kunze 2013), which collectively harbor at least 9.9 million non-human genes (Li et al. 2014). They encode for approximately 500 times the human protein-coding genes which are currently annotated (http://www.ensembl.org). The estimated mass of the microbiota (1–2 kg in an adult body (Forsythe and Kunze 2013)) is comparable to the weight of the adult human brain (ca. 1.5 kg, Parent and Carpenter 1996).

As of today our knowledge on the human microbiota is due to the fast evolution of sequencing. On the 14th of April 2003 the completion of the human genome sequencing process was announced and in 2004 the quality assessment of the human genome sequence finally published. Since then huge efforts have been undertaken to sequence other important genomes like that of the rat (*rattus norvegicus*), the honey bee (*apis mellifera*) and even the Neanderthal. In 2008 the national institute of health decided to fund the Human Microbiome Project (HMP). Goal was the "*characterization of the human* ***microbiome*** *and analysis of its role in human health and disease*" (http://hmpdacc.org/) (Turnbaugh et al. 2007; Human Microbiome Project Consortium 2012). In parallel the MetaHIT project financed by the European Commission under the 7th FP program was launched. Its aim was to "*establish associations between the genes of the human intestinal* ***microbiota*** *and our health and disease*" (http://www.metahit.eu/) (Qin et al. 2010).

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Interestingly, as seen in the two mentioned projects the terms microbiome and microbiota are used analogical even if their scientific definition is dissimilar. The term "**microbiota**" describes the total collection of organisms of a geographic region or a time period. Searching Google scholar and Pubmed the first appearance of the term microbiota in connection with bacteria was a patent application by Alexander Goetz from 1945 (Goetz 1950). In the context of human health the term microbiota was first used to describe the gingival crevice (Socransky et al. 1953), while it did not appear before 1966 for the description of the biggest accumulation of bacteria within the human body the gastrointestinal microbiota (Dubos 1966). The term "microbiome" was originally used to refer to the collection of the genomes of the microbes in a particular ecosystem and

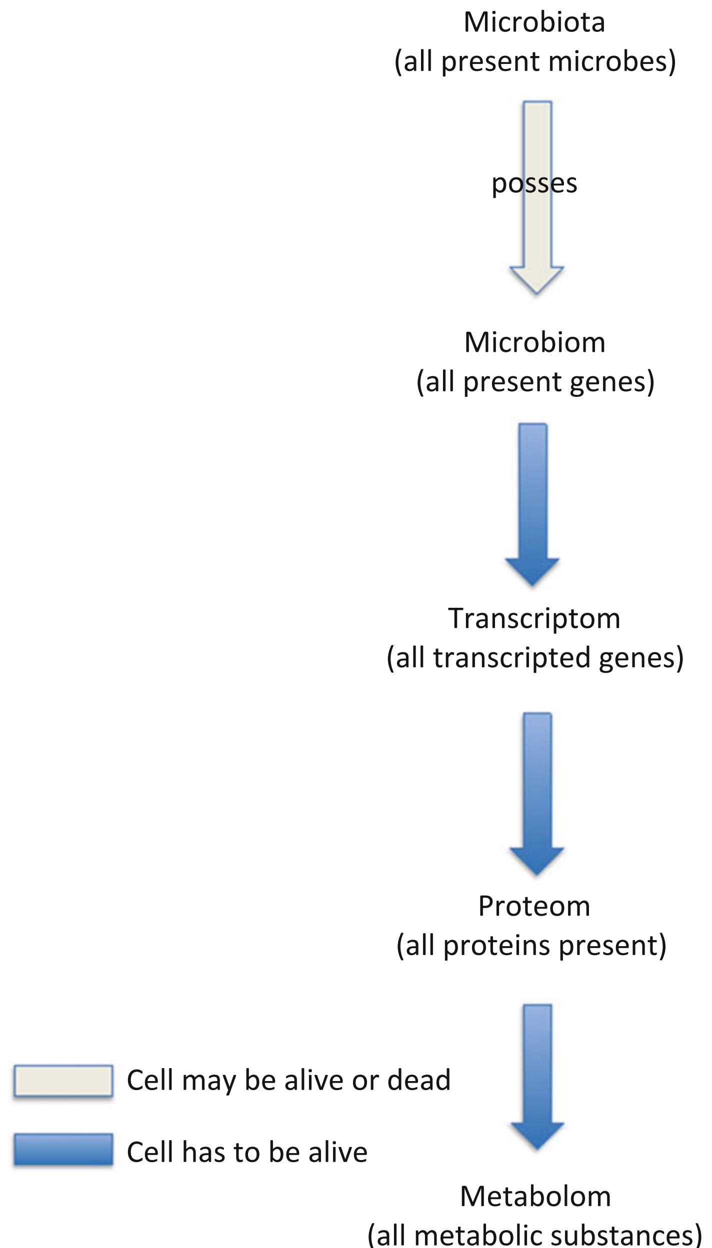

Fig. 1.1 Definitions of terms

termed by Nobel laureate Joshua Lederberg (1925–2008) (Hooper and Gordon 2001). Therefore, the term microbiota would be correct in the case of 16S rRNA studies and the term microbiome in genome studies. To study the microbiota of a given habitat and the therein present genes the microbes of interest may be dead or alive. However, the applied techniques do not allow for the discrimination between a living or a dead cell, in contrast to the determination of the transcriptom, proteome or the metabolom of a habitat (Fig. 1.1).

Only the determination of the latter three will allow us insights on the implication and importance of a specific microbe in a habitat and not only an ordinary number.

As we humans are not only determined by our genes, but by the transcribed proteins, so is not the sole microbe of importance, but its liaison to other microbes and us.

As Louis Pasteur once stated:"*Le microbe, c'est rien, le milieu, c'est tout! – The microbe is noting, it's the environment*".

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2 Studying the Human Microbiota

Alan W. Walker

Abstract
There are a range of methodologies available to study the human microbiota, ranging from traditional approaches such as culturing through to state-of-the-art developments in next generation DNA sequencing technologies. The advent of molecular techniques in particular has opened up tremendous new avenues for research, and has galvanised interest in the study of our microbial inhabitants. Given the dazzling array of available options, however, it is important to understand the inherent advantages and limitations of each technique so that the best approach can be employed to address the particular research objective. In this chapter we cover some of the most widely used current techniques in human microbiota research and highlight the particular strengths and caveats associated with each approach.

Keywords
Microbiota • Techniques • Sequencing • PCR • FISH • Stable isotope • Metabolomics • Proteomics

2.1 Introduction

The Nobel prize winning biologist Sydney Brenner once remarked that "progress in science results from new technologies, new discoveries and new ideas, probably in that order" (Robertson 1980) and this sentiment has undoubtedly been well exemplified in the field of microbiota research. Study of the human microbiota can be traced back to Antonie van Leeuwenhoek's late Seventeenth Century description of "animalcules" in scrapings from the human mouth (Porter 1976), a discovery that was made possible by van Leeuwenhoek's ground-breaking work with microscopes. From the pioneering endeavours of Cohn, Pasteur, Koch and others in the Nineteenth Century, through to developments in anaerobic microbiology and molecular biology in the

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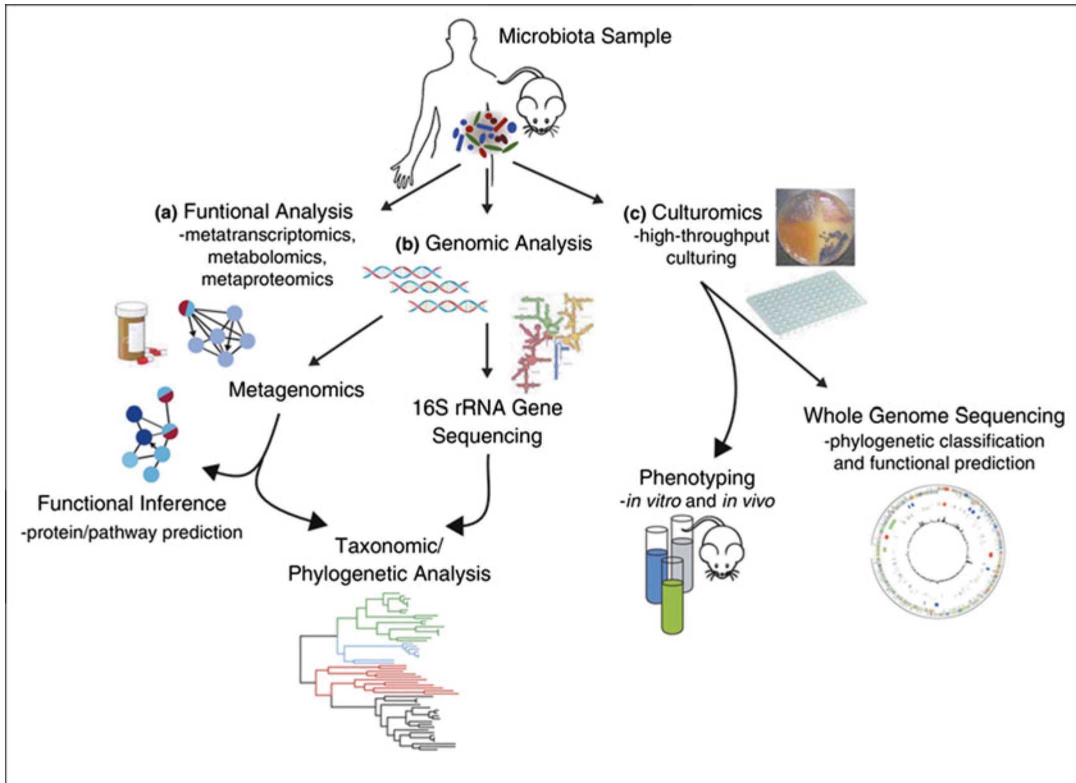

Fig. 2.1 Overview of some of the most common techniques used to study the human microbiota
(a) The functional activities of the microbiota can be studied by monitoring transcription (using RNA-seq/metatranscriptomics), protein production (metaproteomics) or metabolite production (metabolomics). **(b)** DNA sequence-based techniques are used to determine the composition of the microbiota (e.g. 16S rRNA gene surveys) and the functional encoding capabilities of the microbiome (shotgun metagenomics). **(c)** Culture remains highly relevant as cultured organisms can be studied in depth in the laboratory or in animal hosts. Recently, the term "culturomics" has been applied to high-throughput culturing of microbes in multi-welled plates containing highly nutritious growth media. Cultured organisms can also have their genomes sequenced, providing further information about their potential activities *in vivo*. These techniques can be used in combination to generate more comprehensive understandings of the human microbiota. Reprinted in unmodified form from: Pham and Lawley (2014) (Pham and Lawley 2014) under Creative Commons Attribution (CC BY) license

second half of the Twentieth Century, and the Twenty-first Century's own breakthroughs in genomics and DNA sequencing technologies (McPherson 2014), subsequent developments in the field of microbiota research have been similarly driven by successive waves of technological and methodological advances. As a result, today's microbiota researcher has the benefit of a staggering array of tools at their disposal (Fig. 2.1). This chapter gives a broad overview of the many techniques that are now available, and attempts to describe the inherent advantages and limitations of each of these techniques.

2.2 Classical Microbiological Methods

2.2.1 Culture

For well over a 100 years microbiologists have used the classical approaches of cultivating microbes in the laboratory, isolating individual colonies and then studying these isolated strains in order to describe their phenotypic characteristics and metabolic capabilities (see Lagier et al. (2015a) for a recent overview of the techniques used). As a result of these extensive efforts, it has

been estimated that over 1000 distinct microbial species have been cultured from the human gastrointestinal tract alone (McPherson 2014), and characterisation of microbes and gene function discovery in the laboratory remains the bedrock upon which many of the more modern molecular techniques that will be described in later sections of this chapter rely upon. A further advantage of having a strain in culture is that it allows potential exploitation for therapeutic purposes should it turn out to have beneficial properties (Walker et al. 2014).

The simplest form of microbial cultivation is to incubate samples or individual strains in batch culture in nutritious or selective growth media. Batch culture studies allow selective enrichment of bacterial groups of interest, comparisons to be made between growth rates and metabolite production on different substrates, and interactions between specific species to be observed and measured (Belenguer et al. 2006). Many microbial inhabitants of humans are obligately anaerobic and therefore exquisitely sensitive to oxygen. As a result, some species can be killed by even very brief exposure to air (Flint et al. 2007), making them much more difficult to grow. To permit laboratory cultivation of these species, culturing must therefore be carried out under strictly anaerobic conditions, for example by using anaerobic cabinets or Hungate roll tubes (Eller et al. 1971). Cultivation of particularly fastidious gut species can also be enhanced by using media containing rumen fluid, filtered stool extracts, or mixtures of short chain fatty acids, which can be utilised by some gut bacteria as growth substrates (Duncan et al. 2002; Lagier et al. 2015b).

A limitation of batch culture is that results can only be obtained over relatively short periods of time before the supply of nutrients in the growth medium is exhausted or toxic by-products accumulate and lead to cessation of microbial growth (Ferenci 1999). A further, and key, disadvantage to using culture is that it is highly labour intensive, and a range of complex growth media are typically required to recover as wide a diversity of organisms from a sample as possible. It is also known that many of the microbial species that inhabit the human body have yet to be grown in the laboratory (Rajilic-Stojanovic et al. 2007). This problem is particularly acute for bodily sites such as the colon, where the majority of the constituent bacteria are strict anaerobes. As such, culture alone cannot address the sheer complexity of the human microbiota.

Nonetheless, there are many reasons to be optimistic that cultured coverage of the human microbiota can be greatly improved. DNA-sequence based surveys of the gut microbiota, for example, commonly show that many of the most abundant sequences map to cultured species, and that it is the rarer sequences that are less likely to be derived from a cultured isolate (Walker et al. 2014). This suggests that it is insufficient culturing effort rather than an inherent "unculturability" that is the main barrier to successful novel isolations. Furthermore, unlike environments such as soil, which can harbour very slow growing microbes, bacteria living in the human body are often provided with relatively stable environmental conditions, and a generally reliable supply of growth nutrients, and must therefore be capable of multiplying quickly or else face being rapidly outcompeted. Provided the correct conditions can be supplied in artificial growth media it can be assumed therefore that these species will be relatively more amenable to culture. Indeed, novel species continue to be regularly isolated from the human microbiota, and there have been some impressive recent examples of successful high-throughput culturing programmes (Lagier et al. 2015b; Goodman et al. 2011). Such efforts have been dubbed "culturomics", and have contributed to a reinvigorated interest in the use of culture-based techniques to better characterise the human microbiota. Information gleaned from modern genomics methods can also be used to design improved culture media that support the growth of previously uncultivated species (Bomar et al. 2011).

2.2.2 Continuous Culture

A more sophisticated method to cultivate microbes in the laboratory is the use of continuous culture model systems such as fermentors

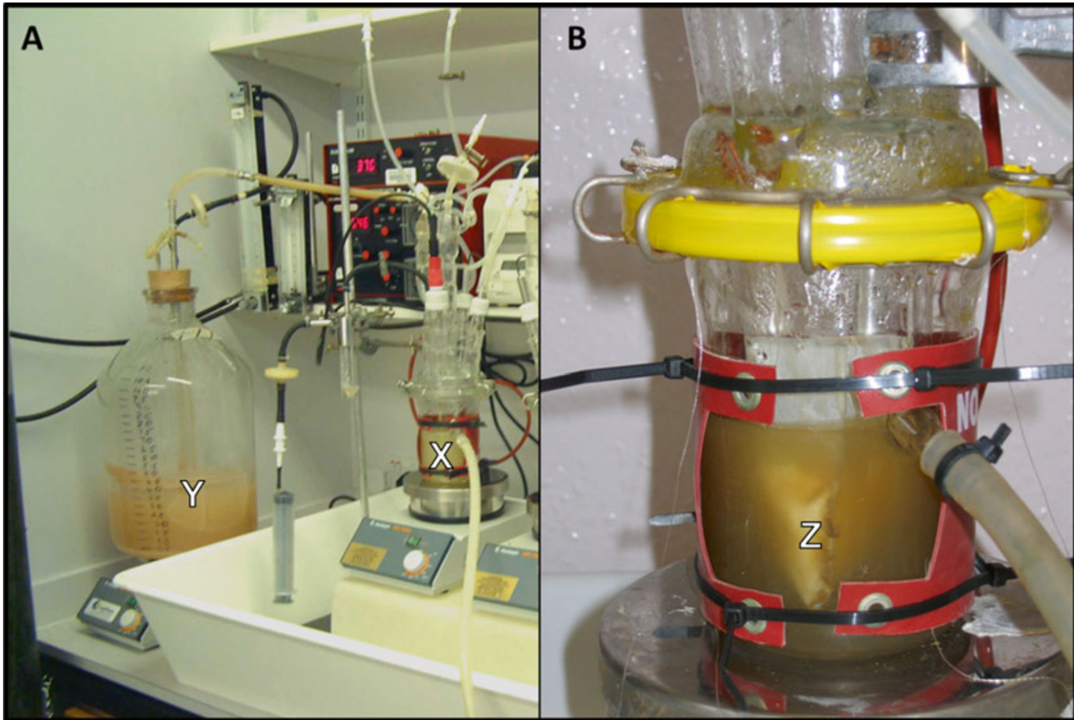

Fig. 2.2 Continuous culture fermentor system Fermentors are continuous culture model systems, which allow long term cultivation of microbes. **(A)** An example of a single vessel fermentor system (the culture vessel is labelled with "X"), inoculated with human faeces and fed a constant supply of nutritious growth medium (labelled "Y"). The contents of the culture vessel are gassed with CO_2 or N_2 to ensure that they remain anaerobic, and can be maintained at defined pH and temperatures, which are constantly monitored. **(B)** a modified fermentor vessel, incorporating a nylon bag containing insoluble particulate substrates (labelled "Z"), developed to identify fibre-degrading gut bacteria

(Fig. 2.2). In contrast to the batch approach, continuous culture is carried out in an open system, which is continually supplied at one end with fresh growth medium/nutrients, and overflow is allowed to drain from the vessel at the other end, diluting out toxic metabolic by-products and dead cells. Systems such as these reach a "steady state" equilibrium, allowing the researcher to exert an enhanced level of control over prevailing environmental conditions within the culture vessel, and can therefore be run over relatively long time periods (Miller and Wolin 1981). These sort of systems have been commonly used to study colonic microbes, and a number of research groups have made fermentors more advanced by incorporating distinct sequential stages, which aim to mimic the sort of environmental changes microbes are exposed to as they pass along the length of the gastrointestinal tract (Van den Abbeele et al. 2010). While these model systems are an advance over simple batch culture it should be noted, however, that they still have important limitations. For example, they lack an immune system, and metabolites such as short chain fatty acids (SCFAs) produced by the bacteria are not absorbed, meaning results may not necessarily be directly translatable to the situation *in vivo*.

2.2.3 Animal Models

Microbes of interest can also be cultivated and maintained in animal models. Until relatively recently, for example, the only way to grow segmented filamentous bacteria, which have been shown to have important pro-inflammatory