Microbial Pathogenesis 106 (2017) 103–112

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Repertoire of human gut microbes

Perrine Hugon [a], Jean-Christophe Lagier [b], Philippe Colson [b], Fadi Bittar [b], Didier Raoult [b, c, *]

[a] Institut Pasteur, Unité de Biologie des Spirochètes, Paris, France
[b] Aix-Marseille Université URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, 27 Boulevard Jean Moulin, 13385, Marseille Cedex 5, France
[c] Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, 21589, Saudi Arabia

ARTICLE INFO

Article history:
Received 14 December 2015
Accepted 14 June 2016
Available online 16 June 2016

Keywords:
Gut microbiota
Culturomics
Taxonogenomics
Prokaryotes
Eukaryotes
Giant viruses
Megavirales
16SrRNA

ABSTRACT

In 1675, Antoni Van Leeuwenhoeck was the first to observe several forms using an optical microscope that he named “animalcules”, realizing later that these were microorganisms. The first classification of living organisms proposed by Ehrenberg in 1833 was based on what we could visualize. The failure of this kind of classification arises from viral culture, which preceded direct observations that were finally achieved during the 20th century by electron microscopy.

The number of prokaryotic species is estimated at approximately 10 million, although only 1800 were known in 1980, and 14,000 to date, thanks to the advent of 16S rRNA amplification and sequencing. This highlights our inability to access the entire diversity. Indeed, a large number of bacteria are only, known as Operational Taxonomic Units (OTUs) and detected as a result of metagenomics studies, revealing an unexplored world known as the “dark matter”. Recently, the rebirth of bacterial culture through the example of culturomics has dramatically increased the human gut repertoire as well as the 18SrRNA sequencing allowed to largely extend the repertoire of Eukaryotes. Finally, filtration and co-culture on free-living protists associated with high-throughput culture elucidated a part of the megavirome.

While the majority of studies currently performed on the human gut microbiota focus on bacterial diversity, it appears that several other prokaryotes (including archaea) and eukaryotic populations also inhabit this ecosystem; their detection depending exclusively on the tools used. Rational and comprehensive establishment of this ecosystem will allow the understanding of human health associated with gut microbiota and the potential to change this.

Contents

* Corresponding author. Aix-Marseille Université, URMITE – UMR 63, CNRS 7278, IRD 198, INSERM 1095, Faculté de Médecine, 27 Bd Jean Moulin, 13005, Marseille, France.
E-mail address: didier.raoult@gmail.com (D. Raoult).

http://dx.doi.org/10.1016/j.micpath.2016.06.020
0882-4010/

1. Introduction

The exploration of the human gut microbiota has exploded during the last decade. With the tremendous changes in molecular technologies and the new "omics" strategies developed, this ecosystem is now considered for its role in metabolism, immune system and human health [1]. Moreover, numerous metagenomic studies performed during the last years have suggested an association between the microbial composition of the human gut and various diseases including for instance obesity [2], Crohn's disease [3], or irritable bowel syndrome [4]. The gut microbiota harbours at least 10^{11} to 10^{12} bacteria per gram of faeces [5], and its composition varies with physiological factors [6] such as geographic provenance, age, dietary habits, malnutrition, and external factors can also imbalance the microbiota as probiotics or antimicrobial agents uses [7]. The relationship between the host and this complex ecosystem composed by prokaryotes, viruses, fungi and parasites is extremely complex. Recent significant efforts have been deployed to characterize the gut repertoire; however there is still a need to provide an efficient repertoire even for all microorganisms isolated or detected in the human gut [8]. Regarding viruses, giant ones have been recently showed being genuine members of the tree of life [9,10]. Thus, their tremendous gene repertoires contain genes with homologs in cellular organisms, among which those encoding DNA-dependent RNA polymerase. This represents a change of paradigm. Indeed, the predominant use of ribosomal genes to classify organisms that was introduced in the 1970s by C. Woese, who defined three domains of life, namely *Bacteria*, *Archea* and *Eukarya*, led to exclude viruses because they are devoid of such genes [11]. Apart from lacking ribosomes, giant viruses share many features with other intracellular microorganisms and can be considered as microbes. This led to propose in 2013 a new classification of microbes in four 'TRUC', an acronym for Things Resisting Uncompleted Classifications, that does not rely on ribosomal genes but takes into account giant viruses alongside with bacteria, archaea and eukaryotic microbes and should allow more comprehensive description of human gut microbiota [10]. In this review we are focusing on human gut components of the bacterial, fungal, parasites and archaeal diversity, as well as on the gut virome discovery.

2. The prokaryotes

2.1. Culture-based methods as the pioneer strategy for human gut microbiota research

The first discrepancy arose from initial culture studies [5]. At that time, the 1970s, gram-staining and microscopic examination performed directly on stool samples were the techniques used to study gut microbiota composition [12–14]. While such techniques revealed the predominance of gram-negative bacteria in stool samples [12], culture counts identified a majority of gram-positive bacteria [14] and anaerobes dominated the community. The second discrepancy was named few years later by Staley and Konopka as the "great plate count anomaly" [15]. It was the difference between "what we can see" on direct microscopic observation and "what's growing in our plate". This was indeed confirmed 20 years later as only 1% of bacteria can be easily grown *in vitro* [16].

Anaerobes were considered to be the major component of gut microflora [13], however this seems biased as a great majority of studies concentrated their efforts on these specific bacteria [5]. In 1969, Hungate revolutionized the anaerobic culture in developing the roll tube technique [17], thus allowing isolation of extremely oxygen-sensitive (EOS) bacteria. Several species (within genera *Bacteroides*, *Clostridium*, *Veillonella*, *Ruminococcus*, *Eubacterium*, *Bifidobacterium*, *Lactobacillus*, *Fusobacterium*, *Peptococcus* and *Peptostreptococcus*) were considered to dominate the gut microbiota. Finally, before molecular tools were incorporated, it was estimated that 400–500 different species composed the gut microflora [13,18], which remained partially characterized due to the technical limitations.

2.2. The molecular revolution: how improved technologies enhanced our knowledge of prokaryotic diversity

Introduced fifteen years ago, 16S rDNA sequence analysis is still the basic tool for studying bacterial taxonomy and phylogenic relationships between microorganisms. In the 2000s, the introduction of high-throughput sequencing techniques based on the amplification of the 16S rRNA gene improved understanding of bacterial diversity from complex microbiota, and demonstrated that 80% of bacteria detected with molecular tools were uncultured [5]. However, it is important to understand the biases and limitations of 16S rRNA gene profiling. Firstly, 16S rRNA gene lacks sensitivity within specific genera and cannot delineate between two species with high interspecies similarity [19]. Secondly, gene sequence heterogeneity can be encountered in species having more than one copy [20]. Regarding DNA extraction kits [21,22], the hypervariable region targeted in 16S rRNA gene and primer choices [23], the depth bias [24], several studies reported the serious impact on the microbiota abundance and diversity these factors could play. More recently, a study performed on 16 stool samples revealed that pyrosequencing performed on the V6 region on 16S rRNA gene has neglected some of the gram-negative bacteria detected using transmission electron microscopy [25].

Regarding prokaryotic diversity in humans, more than 120 different prokaryotic phyla have been identified and only 31 phyla included cultured species [8]. Moreover, 12 bacterial phyla with cultured representatives have been recorded in humans (*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Chlamydiae*, *Deinococcus-Thermus*, *Fusobacteria*, *Tenericutes*, *Lentisphaerae*, *Spirochaetes*, *Synergistetes* and *Verrucomicrobia*) (Fig. 1, Table 1) [8], where each phylum represents species that have also been isolated in the human gut. Moreover, the majority of species isolated in the gut belong to four phyla, *Firmicutes*, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* and dominant species from the families *Bacillaceae*, *Enterobacteriaceae*, *Corynebacteriaceae* and *Bacteroidaceae* respectively [8]. In addition to cultured bacteria, several phyla have only been detected in the gut and remain as yet uncultured [26]: species belonging to TM7 have been detected in both healthy persons and patients suffering from inflammatory bowel disease [27]; *Melainabacteria*, a new candidate phylum sibling to *Cyanobacteria* [28], and the *Gemmatimonadetes* phylum [26].

High-throughput sequencing studies performed the last ten years [29–33] showed a majority of reads belonging to two dominant phyla (*Firmicutes* and *Bacteroides*), corresponding to species belonging to the *Ruminococcaceae*, *Clostridiaceae*, *Lachnospiraceae*, *Bacteroidaceae* families that contain a majority of