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

In 1675, Antoni Van Leeuwenhoek 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 18S rRNA 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.

* 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).

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*, *Archaea* 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 phylogenetic 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]; *Melainobacteria*, 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 *Bacteroidetes*), corresponding to species belonging to the *Ruminococcaceae*, *Clostridiaceae*, *Lachnospiraceae*, *Bacteroidaceae* families that contain a majority of

Fig. 1. Diversity of Prokaryotes, Eukaryotes and viruses in the human gut microbiota.

anaerobic species as yet uncultured. Moreover, several members of *Lachnospiraceae* family (*Eubacterium* spp., *Anaerostipes* spp., *Roseburia* spp., *Coprococcus* spp. for example) are butyrate-producing bacteria that are difficult to cultivate from faecal samples [34,35]. Butyrate-producing bacteria have recently been associated with health status in several diseases [36–38] as anti-inflammatory and anticarcinogenic properties have been identified [39]. These discrepancies between molecular and culture-dependant studies emphasize the need to improve the anaerobic culture to understand the specific role these species are playing in the human gut microbiota. Finally, species belonging to the highly diverse *Proteobacteria* phylum are commonly detected in molecular studies [26]. Due to the specific primers the phylum *Actinobacteria* needs to be amplified [40] and it often remains underrepresented by large sequencing studies [26], whereas its species are easily cultivated.

2.3. Culturomics: a third shift in understanding gut microbiota?

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) has emerged as a time- and cost-effective method for the identification of bacteria [41]. This efficient identification method has been the main key to developing high-throughput culture-dependent studies. Indeed, since 1995 with the development of culture-independent methods, notably 16S rRNA amplification and sequencing and more recently metagenomics studies, bacterial culture was considered as outdated. In the last four years, a new strategy named “culturomics” was

developed to study the human gut microbiota [24]. This method is based on the diversification of culture conditions (by varying atmosphere, temperature, incubation time, passive and active filtration or antibiotics in order to culture the minority populations) with the objective of mimicking the natural conditions within the digestive tract, using notably rumen fluid [24,42].

In their pioneering study, Lagier et al., using 212 culture conditions, testing 32,500 colonies by MALDI-TOF, identified 340 bacterial species, including 31 putative new species [24]. In addition, they demonstrated the complementarity of metagenomics and culturomics because only 15% of the bacterial species were concomitantly detected. A stringent analysis of the culture conditions tested in the first study showed that all the bacteria identified were cultured by testing 70 culture conditions [24]. These 70 culture conditions were applied for the study of supplementary stool samples from other patient populations such as those treated with antibiotics, suffering from anorexia nervosa or obesity, and healthy subjects from various geographic areas, extending the repertoire [24,42–45]. For the ongoing studies we selected 18 different culture conditions in liquid media with subcultures on solid media every 3 days [42]. This resulted in culturing 717 bacterial species from 7 phyla (*Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Synergistetes*) including several new bacterial species [42]. In order to describe these new isolates, we developed a polyphasic strategy combining phenotypic and genotypic characteristics obtainable and comparable by most laboratories, and named taxono-genomics [46]. The genome

Phylum	Family	
Actinobacteria	Actinomycetaceae	
	Bifidobacteriaceae	
	Bogoriellaceae	
	Brevibacteriaceae	
	Cellulomonadaceae	
	Coriobacteriaceae	
	Corynebacteriaceae	
	Dermabacteraceae	
	Dermacoccaceae	
	Dermatophilaceae	
	Dietziaceae	
	Geodermatophilaceae	
	Gordoniaceae	
	Intrasporangiaceae	
	Microbacteriaceae	
	Micrococcaceae	
	Micromonosporaceae	
	Mycobacteriaceae	
	Nocardiaceae	
	Nocardiodaceae	
	Promicromonosporaceae	
	Propionibacteriaceae	
	Streptomycetaceae	
	Bacteroidetes	Bacteroidaceae
		Flavobacteriaceae
		Porphyromonadaceae
		Prevotellaceae
Rikenellaceae		
Firmicutes	Sphingobacteriaceae	
	Acidaminococcaceae	
	Aerococcaceae	
	Bacillaceae	
	Carnobacteriaceae	
	Catabacteriaceae	
	Christensenellaceae	
	Clostridiaceae	
	Clostridiales	
	Enterococcaceae	
	Erysipelotrichaceae	
	Eubacteriaceae	
	Lachnospiraceae	
	Lactobacillaceae	
	Leuconostocaceae	
	Listeriaceae	
	Paenibacillaceae	
	Peptococcaceae	
	Peptostreptococcaceae	
	Planococcaceae	
	Ruminococcaceae	
	Staphylococcaceae	
	Streptococcaceae	
	Thermoactinomycetaceae	
	Veillonellaceae	
	Proteobacteria	Acetobacteraceae
		Aeromonadaceae
Alcaligenaceae		
Brucellaceae		
Burkholderiaceae		
Campylobacteraceae		
Caulobacteraceae		
Chromobacteriaceae		
Comamonadaceae		
Desulfovibrionaceae		
Enterobacteriaceae		
Francisellaceae		
Halomonadaceae		
Helicobacteraceae		
Legionellaceae		
Methylobacteriaceae		
Moraxellaceae		
Neisseriaceae		
Oxalobacteraceae		
Pasteurellaceae		
Pseudomonadaceae		

Phylum	Family
	Rhizobiaceae
	Rhodobacteraceae
	Salinisphaeraceae
	Shewanellaceae
	Sphingomonadaceae
	Succinivibrionaceae
	Sutterellaceae
	Vibrionaceae
	Xanthobacteraceae
	Xanthomonadaceae
Chlamydiae	Chlamydiaceae
Deinococcus-Thermus	Deinococcaceae
Fusobacteria	Fusobacteriaceae
Lentisphaerae	Victivallaceae
Spirochaetes	Brachyspiraceae
	Leptospiraceae
Synergistetes	Synergistaceae
Tenericutes	Mycoplasmataceae
Verrucomicrobia	Verrucomicrobiaceae
Euryarchaeota	Halobacteriaceae
	Methanobacteriaceae

3. The archaeal diversity

The *Archaea* community constitutes the third domain of life, separate from bacteria and eukaryote kingdoms [50], although archaea share many characteristics with both bacteria and eukaryotes [51]. Extremophile species, which live in extreme environments under high salt concentrations and extreme pH and temperatures, were the first to be discovered in the late 1970s [52]. However, we now know that archaeal mesophilic species also represents a large part of the diversity and inhabit non-extreme environments [53]. Moreover, the human gut microbiota include non-methanogenic and methanogenic species, with the latter representing up to 10^{10} cells per gram of faeces [54] and having a significant role in the gut microbiota ecosystem through methane production during anaerobic fermentations [55].

In total, eight archaeal species have been isolated in the human gut microbiota [26], all of which belong to the *Euryarchaeota* phylum (Fig. 1). *Methanobrevibacter ruminantium* [56] was the first species isolated from stool samples. This was probably a misidentification [26] as *Methanobrevibacter smithii* dominates in the human gut [57]. The atypical methanogenic *Methanosphaera stadtmanae* reduces the methanol [58] as does *Methanomassiliicoccus luminyensis*, discovered more recently, but is phylogenetically distant from *M. stadtmanae* [59].

Methanobrevibacter oralis was first isolated in the 1990s from human subgingival plaque [60]. Its genome was only sequenced recently from a stool sample strain [61]. *Methanomethylophilus alvus* [62] and *Methanomassiliicoccus intestinalis* [63], two species phylogenetically related to the *Thermoplasmatales*, were isolated and their genomes sequenced, highlighting a new order named *Methanomassiliicoccales* shared with *M. luminyensis* [55]. Very recently, *Methanobrevibacter arboriphilicus* has been isolated from the human gut [64]. Surprisingly it showed the lowest GC content in archaeal species [64].

Additionally, substantial diversity of archaeal species have been detected in the human gut microbiota [26,55] using 16S rRNA, mcrA or amoA gene sequences [55]. Two halophilic bacteria belonging to the *Euryarchaeota* phylum were detected in 2008 using the denaturing gradient gel electrophoresis (DGGE) [65] method, and several archaeal representatives associated with the human gut microbiota belonging to *Sulfolobales*, *Nitrososphaerales*, *Methanosarcinales*, *Methanomicrobiales* were identified [55].

4. The existence of a human gut microbial virome

By contrast with the bacterial microbiota, only a few studies have explored the “human gut virome”, which represents a fairly new concept [66,67]. This appears paradoxical, as the existence of pathogenic viruses was discovered in human faeces a long time ago [68]. Studying viral diversity in the gut appears more difficult than for cellular organisms because they are, for most of them, not visible under a light microscope, and they are devoid of any conserved gene that is shared by all of them (that may be an equivalent to 16S-18S rRNA in cells) [69]. Thus, viruses have been sought in the human gut using cell culture, electron microscopy, PCR and finally metagenomics [67]. In current technology advances, high-throughput sequencing highlighted groups of viruses (or Virus Like Particle (VLPs)) associated with the human gut microbiota [70], equivalent to 10^9 VLPs per gram of faeces [71]. Both DNA and RNA viruses were investigated in human stools by metagenomics, from its very onset [66,72]. Recent work on the viral community provided a better understanding of the role viruses from the gut could play in its host [73,74], how it could interact with the complex gut ecosystem to influence food digestion [75], and also how it could actively participate in human health through the prevention of invasion by pathogens [67,76].

New sequencing strategies have shown that the gut virome was composed of both eukaryotic viruses and prokaryotic viruses (i.e. viruses that infect bacteria, known as bacteriophages), and that the latter dominate this virus community (Fig. 1) [67,75]. Tailed bacteriophages with double-stranded DNA (dsDNA) that belong to the order *Caudovirales* appear to be the most abundant in the gut with the single-strand DNA (ssDNA) bacteriophages belonging to the family *Microviridae* (Fig. 1) [70]. Many DNA or RNA viruses, including rotaviruses, caliciviruses, astroviruses or adenoviruses, have been associated with gastroenteritis [77]. In addition, several human viral pathogens such as enteroviruses, as well as viruses that are transmitted via the faecal-oral route and are excreted by the gut, including, for instance, hepatitis A and E viruses, can be found in stools. Many viruses were unexpectedly identified in human stools. This was also the case for plant viruses, among which *Pepper mild mottle virus* was found at the greatest titre, estimated to be up to 10^9 per gram of dried faeces, and whose presence seems to be directly correlated with ingested food [72]. These findings suggest that viral diversity in the gut is probably still largely untapped. Surprisingly, bacteriophages residing in the human gut have been estimated at having a titre of the same order of magnitude as that of their bacterial hosts (ratio 1:1) [67,74]. In contrast, in many habitats, oceans for example, bacteriophages are 10-fold more abundant

than their bacterial hosts (ratio 10:1) [75,78]. This could reflect the temperate lifestyle bacteriophages may adopt in the human gut, contrasting with the “kill the winner strategy” they use in marine habitats [75].

In the same way as for the gut microbiota community, our knowledge on viruses from the gut comes from evolving technologies, bearing in mind that each method provides benefits and has limitations [67]: culture-based techniques (phage isolation) and microscopic observations (TEM or fluorescence) were the first methods used, whereas PCR testing and Sanger sequencing form the current majority, and an increasing number of investigations conducted apply high-throughput sequencing to metagenomics [67]. Recently, the CRISPR (Cluster Regularly Interspaced Short Palindromic Repeats)/Cas system was exploited to target phage sequences in the gut microbiome in order to discover new phages associated with the human body; this shed lights on a common phage reservoir associated with the human gut microbiome. Thus, bacteriophage searches have opened a new field of interest and potential applications in therapeutics, diagnostics and biotechnology [67,79].

Giant viruses of amoebas have been discovered over the 12 last years, the first representative being Mimivirus [10,80]. To date, six new or putative viral families have been uncovered, which have been linked by phylogenomics to other double-stranded DNA viruses, including poxviruses, asfarviruses, irido-/ascoviruses, or phycodnaviruses [81,82]. These giant viruses were shown to be ubiquitous worldwide and common in the human environment, primarily water and soil [83]. Unlike other viruses, giant viruses are visible under a light microscope [84]. The size of giant virions is indeed similar to that of small parasitic prokaryotes. In addition, their genome is as large as those of these intracellular microorganisms and comprises a tremendous and unique gene repertoire among viruses. Therefore, they can be considered as microbes [11]. Most of these viruses have been isolated using co-culture on free-living amoebas from the genera *Acanthamoeba* [83]. Their detection as part of the virome has long been neglected due to their giant size, as samples were usually filtered prior to analysis to separate viruses from cellular organisms; through this procedure, giant viruses, in contrast with the majority of viruses, are not filtered and remain within the cellular fraction [85].

The first giant virus of amoeba isolated from the human gut, in 2012, was from the stools of a young healthy Senegalese man [24,85]. This was a serendipitous discovery as amoebal co-culture of this virus, which was named Senegalvirus, was triggered by the detection in the faeces of reads matching Marseillevirus, the prototype member of the second giant virus family identified [86]. In 2013, a second giant virus that is a close relative of Mimivirus named Shan virus, was isolated from the faeces of a Tunisian patient with pneumonia [87]. This strengthens the interest in expanding our knowledge of giant virus diversity and prevalence in human stools. Moreover, metagenomic reads generated from human stools were identified as matching sequences from virophages, which infect mimiviruses and were discovered in 2008 as the first viruses of viruses [88,89].

5. Eukaryotes

Research on this subdominant gut component is fairly recent, as evidenced by the first molecular study published in 2008 [90]. Eukaryotes, defined by the presence of a nucleus and organelles, represent the third domain of life besides Bacteria and Archaea [91]. The taxonomy of human gut eukaryotes is highly complex [91,92] and highlights five major groups (Amoebozoa, Opisthokonta, Excavata, Sar and Archaeplastida) based on recent molecular phylogenetic classification [92]. Fungal species including yeasts and

filamentous fungi are the most abundant group of eukaryotes in the human gut. However parasites, including unicellular organisms (protozoa) and multicellular species (helminths) (Fig. 1), could also be present in the human gut but in lower numbers. Their presence is mostly known to have a pathogenic consequence to the host [26].

5.1 Fungal diversity

Studying the human gut mycobiome consists of studying how the entire fungal community inhabits the human gastrointestinal tract. Very few studies have focused on the fungal diversity, however recent technological advances can provide deep understanding on this neglected component [93]. Both filamentous fungi and yeasts are cultivated on different solid media, and the majority of them can be identified using MALDI-TOF mass spectrometry [94,95]. As for bacteria, MALDI-TOF mass spectrometry has revolutionized the diagnosis of fungal infection in mycology laboratories, allowing both rapid and accurate identification [96]. New culture-independent methods, which remain under-developed for fungal population [97], are targeting mainly the Internal Transcribed Spacer ITS1 and ITS2 [91]. This region (i.e. ITS) appears to be more appropriate than 18S rDNA for analysing fungal population at the level of clades and species thanks to its high sequence variability [91]. However, many difficulties arise during fungal metagenomic analyses including the low presence of fungal species in the complex biota of the human intestinal tract, requiring the application of ultra-deep sequencing strategy, and the absence of a well established fungal ITS database [97].

The human gut mycobiome displays a very small fungal community divided into three major phyla (Ascomycota 63%, Basidiomycota 32%, Zygomycota 3%) [26], including 273 different species from more than 140 genera [91,93] (Table 2). Recently, a fourth phylum named Microsporidia has been reclassified as fungi [98]. It contains members associated with intestinal diseases [98] including *Encephalitozoon hellem*, *Encephalitozoon intestinalis*, *Enterocytozoon bieneusi* and *Enterocytozoon hellem*. Regarding the actual fungal repertoire, 216 different species were detected by molecular tools, 86 species were isolated by culture methods and 29 species were identified using both approaches [91,93]. Among the yeast community, *Candida* spp. is the most abundant species [26] and *Candida albicans* and *Crugosa rugosa* are in their natural environment. However, they can become pathogens and induce candidiasis under specific circumstances [26].

Additionally, *Saccharomyces cerevisiae* and *Galactomyces geotrichum* [90] are two yeasts detected frequently in human faecal samples. The second has been cultivated recently from two different stool samples [99,100]. Evidence has shown that many fungal species are directly associated with food ingestion prior to sampling. This may explain the presence of *G. geotrichum* and *S. cerevisiae* in human stools due to their use in food production [26]. However, determining the role fungi play in digestion remains to be illustrated. Finally, several filamentous species belonging to *Penicillium* and *Aspergillus* genera have been detected [90] and cultured more recently [99,101]. It is likely that these fungi could represent food contamination, or could just come from food ingestion [26]. Although they have been found in many studies [18,99,102], it is not certain that they play any significant role in the human gut microbiota.

5.2 Other eukaryote diversity

More than 50 helminthic genera (complex and multicellular organisms) belonging to nematodes, trematoda and cestoda groups are known to parasitize the human gut [103] and cause infections in millions of people worldwide. In addition, 15 different commensal

Table 2
Eukaryotes cultivated in the human gut microbiota.

Taxa	Species
Fungi	Ascomycota
	<i>Acremonium falciforme</i> <i>Acremonium strictum</i> <i>Aspergillus niger</i> <i>Aspergillus flavipes</i> <i>Aspergillus flavus</i> <i>Aspergillus fumigatus</i> <i>Aspergillus ruber</i> <i>Aspergillus</i> spp. <i>Aspergillus sydowii</i> <i>Aspergillus versicolor</i> <i>Beauveria bassiana</i> <i>Blastoschizomyces capitatus</i> <i>Candida albicans</i> <i>Candida famata</i> <i>Candida glabrata</i> <i>Candida guilliermondii</i> <i>Candida kefyr</i> <i>Candida krusei</i> <i>Candida lambica</i> <i>Candida lusitanae</i> <i>Candida norvogensis</i> <i>Candida parapsilosis</i> <i>Candida pararugosa</i> <i>Candida rugosa</i> <i>Candida</i> sp. <i>Candida sphaerica</i> <i>Candida tropicalis</i> <i>Candida utilis</i> <i>Candida zeynaloides</i> <i>Cladosporidium bruhnei</i> <i>Cladosporium</i> spp. <i>Clavispora lusitanae</i> <i>Davidiella</i> sp. <i>Davidiella tassiana</i> <i>Debaryomyces hansenii</i> <i>Fusarium</i> sp. <i>Galactomyces candidum</i> <i>Galactomyces geotrichum</i> <i>Geotrichum</i> spp. <i>Geotrichum candida</i> <i>Geotrichum candidum</i> <i>Hansenula anomala</i> <i>Hypocrea lixii</i> <i>Isaria farinosa</i> <i>Kluyveromyces marxianus</i> <i>Penicillium allii</i> <i>Penicillium brevicompactum</i> <i>Penicillium camemberti</i> <i>Penicillium citrinum</i> <i>Penicillium decumbens</i> <i>Penicillium dipodomycicola</i> <i>Penicillium marneffeii</i> <i>Penicillium notatum</i> <i>Penicillium solitum</i> <i>Penicillium</i> sp. <i>Penicillium steckii</i> <i>Saccharomyces cerevisiae</i> <i>Saccharomyces</i> sp. <i>Torulaspora delbrueckii</i> <i>Yarrowia lipolytica</i> <i>Zygosaccharomyces bisporus</i>
Fungi	Basidiomycota
	<i>Climacocystis</i> sp. <i>Cryptococcus albidus</i> <i>Cryptococcus luteolus</i> <i>Cryptococcus</i> spp. <i>Cystofilobasidium capitatum</i> <i>Exophiala dermatitidis</i> <i>Malassezia globosa</i> <i>Malassezia pachydermatis</i> <i>Malassezia restricta</i> <i>Malassezia</i> sp. <i>Pityrosporum</i> sp. <i>Rhodotorula glutinis</i> <i>Rhodotorula rubra</i>

Table 2 (continued)

Taxa		Species
Fungi	Zygomycota	<i>Rhodotorula</i> sp.
		<i>Trichosporon asahii</i>
		<i>Trichosporon beigelii</i>
		<i>Trichosporon</i> spp.
		<i>Basidiobolus ranarum</i>
Helminths	Nematodes	<i>Mucor racemosus</i>
		<i>Mucor</i> spp.
		<i>Rhizopus</i> spp.
		<i>Ancylostoma duodenale</i>
		<i>Necator americanus</i>
Protozoa	Amoebozoa	<i>Strongyloides stercoralis</i>
		<i>Endolimax nana</i>
		<i>Entamoeba coli</i>
		<i>Entamoeba dispar</i>
		<i>Entamoeba histolytica</i>
Protozoa	Parabasalia	<i>Iodamoeba bütschlii</i>
		<i>Dientamoeba fragilis</i>
Protozoa	Stemenopile	<i>Pentatrichomonas hominis</i>
		<i>Blastocystis hominis</i>

or parasitic protozoa genera [91] have also been described. Only a few of intestinal protozoa (defined as unicellular eukaryotes) playing a role in disease in humans, while the majority of protozoa exhibit a free-living lifestyle in water and soil environments [91]. *Giardia intestinalis* (flagellates), *Cryptosporidium parvum*, *Cyclospora cayetanensis* and *Isopora belli* (Coccidia), *Blastocystis* sp. (stemenopile), *Entamoeba histolytica* (Amoeba) and *Balantidium coli* (ciliates) are the most abundant protozoa parasitizing the human gut through a cyst and trophozoite life cycle [91] (Table 2).

Despite the drawbacks and the poor sensitivity associated with the microscopic observation of stool samples, it remains the gold standard used by most parasitological diagnostic laboratories [104]. Depending on the microscopist's skill, diagnosis will target the presence of protozoan cysts and trophozoites, as well as microscopic identification of helminthic eggs [91]. Because there are sometimes too few eggs in samples, this tool must be replaced by other methods such as serology-based ones including ELISA (Enzyme-Linked Immunosorbent Assay) [105] and DFA (Direct Fluorescent Antibody) assays [106]. Parasitic coproculture remains a challenging technique [91], although it demonstrates good efficiency for some helminths [107] and protozoa [108,109]. Molecular approaches based on conventional PCR, Real-Time-PCR [110], PCR-restriction fragment length polymorphism (RFLP) [111] and more recently the Loop-mediated isothermal amplification (LAMP) assay [112], are alternative and promising methods for differentiating parasites [113]. Finally, next generation sequencing using pyrosequencing methods has been used to detect and genotype several protozoans [114,115]. However, more metagenomic and culturomic studies targeting the eukaryome are required at this time to determine the occurrence of both fungi and parasites in the human gut [91].

6. Perspectives

As described by Robert Koch, "a pure culture remains the foundation of all research in microbiology" [42]. The rebirth of the culture through the example of culturomics due to the efficient, cost-effective and rapid MALDI-TOF identification method opens broad perspectives for the study of complex ecosystems [42]. This revolution now largely recognized in bacterial culture is relevant for eukaryote identification [91].

However, we have a major lack of tools that can list both prokaryotes and eukaryotes isolated in humans. The recent repertoire proposed by Hugon et al. is an important first step, providing

researchers and clinicians with the bacterial species associated with humans [8]. This may be implemented with eukaryotes.

Finally, this rebirth of culture highlighted a major discrepancy, that the taxonomic description is not currently adapted to this dramatic increase of new prokaryotic species [8]. Taxono-genomics has proposed for three years modification of this taxonomic classification. This new concept is based on current microbiological tools such as MALDI-TOF (spectra and comparison with the closest bacterial species) and genome sequencing (including genome comparison) to be in accordance with this reemerging field [42,46,116]. Moreover, the taxonomic classification remains unclear for fungi and a few explored fields for the eukaryotes. Finally, the emerging recognition of giant viruses of amoebas as inhabitants of environments and humans still expands the range of micro-organisms to be characterized and enumerated from human gut, and this would involve new high-throughput strategies that have started being implemented [11]. Thinking beyond that, amoebal viruses are an incentive to readdress microbe classification [11].

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