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Microbial culturomics: paradigm shift in the human gut microbiome study

J.-C. Lagier[1,*], F. Armougom[1,*], M. Million[1], P. Hugon[1], I. Pagnier[1], C. Robert[1], F. Bittar[1], G. Fournous[1], G. Gimenez[1], M. Maraninchi[2], J.-F. Trape[3], E. V. Koonin[4], B. La Scola[1] and D. Raoult[1]

1) *Aix Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095,* 2) *Service de Nutrition, Maladies Métaboliques et Endocrinologie, UMR-INRA U1260, CHU de la Timone, Marseille, France,* 3) *IRD, UMR CNRS 7278-IRD 198, Route des Pères Maristes, Dakar, Sénégal and* 4) *National Centre for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA*

Abstract

Comprehensive determination of the microbial composition of the gut microbiota and the relationships with health and disease are major challenges in the 21st century. Metagenomic analysis of the human gut microbiota detects mostly uncultured bacteria. We studied stools from two lean Africans and one obese European, using 212 different culture conditions (microbial culturomics), and tested the colonies by using mass spectrometry and 16S rRNA amplification and sequencing. In parallel, we analysed the same three samples by pyrosequencing 16S rRNA amplicons targeting the V6 region. The 32 500 colonies obtained by culturomics have yielded 340 species of bacteria from seven phyla and 117 genera, including two species from rare phyla (*Deinococcus-Thermus* and *Synergistetes*, five fungi, and a giant virus (Senegalvirus). The microbiome identified by culturomics included 174 species never described previously in the human gut, including 31 new species and genera for which the genomes were sequenced, generating *c.* 10 000 new unknown genes (ORFans), which will help in future molecular studies. Among these, the new species *Microvirga massiliensis* has the largest bacterial genome so far obtained from a human, and Senegalvirus is the largest virus reported in the human gut. Concurrent metagenomic analysis of the same samples produced 698 phylotypes, including 282 known species, 51 of which overlapped with the microbiome identified by culturomics. Thus, culturomics complements metagenomics by overcoming the depth bias inherent in metagenomic approaches.

Keywords: Culturomics, gut microbiota, MALDI-TOF MS, metagenomic analysis, uncultured bacteria
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Corresponding author: D. Raoult, Aix-Marseille Université, URMITE, UMR CNRS 7278, IRD 198, INSERM U1095, Faculté de Médecine, 27 Bd Jean Moulin, Cedex 5, 13385 Marseille, France
E-mail: didier.raoult@gmail.com

*These authors contributed equally to this work.

Introduction

The composition of the human gut microbiome, the determination of which represents a major challenge in the 21st century [1], has been studied with different tools, leading to increasingly complex results [2–6]. The first approach used to study the gut microbiota employed microbial culture [2]. Subsequent studies that involved amplification and sequencing of 16S rRNA and later metagenomic analysis have dramatically expanded the known diversity of the human gut microbiome [4,5,7,8]. It is commonly accepted that *c.* 80% of the bacterial species found by molecular tools in the human gut are uncultured or even unculturable [1]. However, several drawbacks of the current metagenomic approaches, including major discrepancies among different studies, apparently reflect biases of the employed techniques. In particular, sequence-based techniques miss clinically relevant minority populations, including potentially pathogenic bacteria, such as *Salmonella* Typhi, *Tropheryma whipplei*, and *Yersinia enterocolitica*, that may be present at concentrations lower than 10^5/mL; this major problem is known as the depth bias.

Recently, there has been a renewed interest in culture methods for 'non-cultivable' species [3,9]. One of the gridlocks of the traditional bacteriological culture methods has

been recently overcome by advances in mass spectrometry (MS) techniques, which can accurately and rapidly identify microorganisms with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), allowing rapid screening of large numbers of colonies [10]. In this study, we combined the MS approach with extensive sequencing and exploration of the potential of numerous known and new culture methods to introduce culturomics as a major complement to metagenomics in the study of the human gut microbiome.

Materials and Methods

All of the data are detailed in the Supporting information. We used two African stools, both from healthy young males living in rural Senegal, and a stool from a French obese individual with a body mass index of 48.2 kg/m^2. Each patient's consent was obtained, and the study was approved by the local ethics committee of IFR48 (agreement number 09-022; Marseille, France). We designed 212 culture conditions, using variable physicochemical conditions, pre-incubation in blood culture bottles, rumen fluid and sterile stool extract to mimic the natural environment [9]. Moreover, with the aim of selecting a minority population, we used antibiotics, both active and passive filtration, and bacteriophages. We used MALDI-TOF MS to quickly identify a maximum of colonies. When the strains remained unrecognized, the 16S rRNA gene was sequenced. As previously described, a threshold similarity of >98.7% was chosen to define a new bacterial species [11]. The same three stool samples were tested by pyrosequencing of a 16S rRNA amplicon targeting the V6 region, the most variable region, as previously described [7] NCBI accession number=SRA049748. The new bacterial genera and species were sequenced with a paired-end strategy for high-throughput pyrosequencing on the 454-Titanium instrument. Senegalvirus was sequenced with the Roche 454 FLX-Titanium platform.

Results

Proof-of-concept

We studied stool samples from two young lean Africans from a rural environment in Senegal (Fig. 1) and one obese

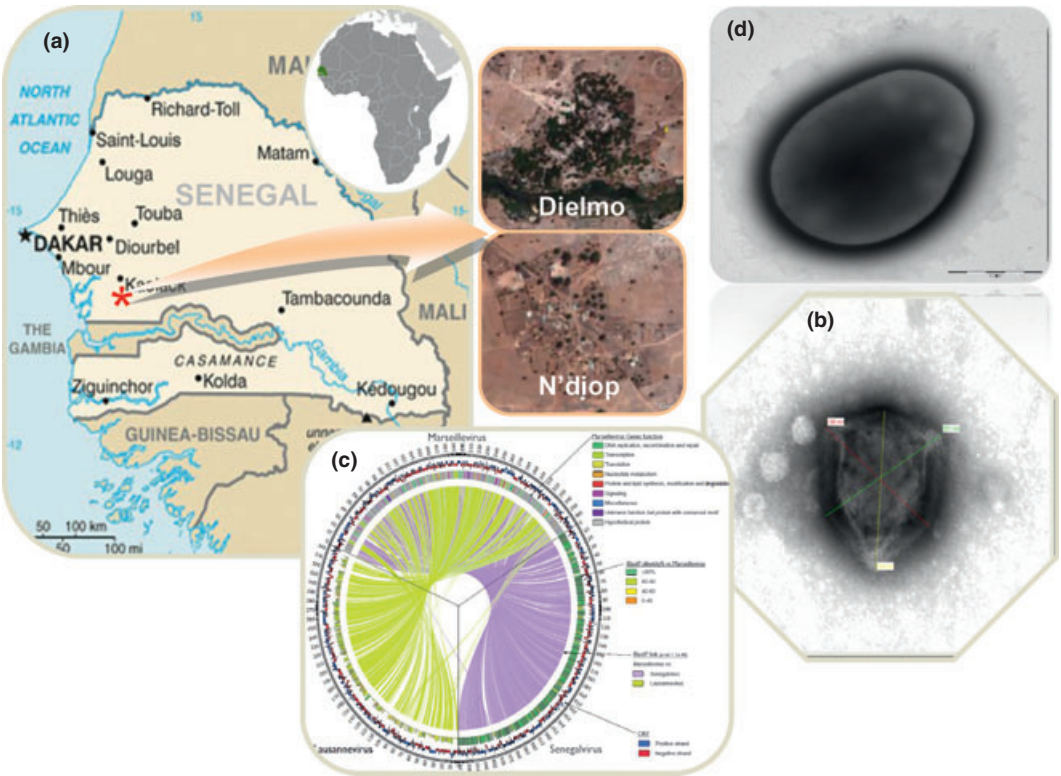

FIG. 1. The source of material for culturomics and the record-breaking virus and bacterium from the human gut. (a) The geographical locations of the Dielmo and N'diop villages (Sources: Wikitravel.org and Google Earth) from which the two African stool samples analysed in this work were obtained. (b) Electronmicrograph of the giant Senegalvirus, which was isolated from a stool sample of an individual from N'Diop. (c) Comparison of the Senegalvirus genome with the genomes of related giant viruses, Marseillevirus and Lausannevirus. (d) Electronmicrograph of *Microvirga massiliensis* (the bacterium with the largest genome ever isolated from humans), which was isolated from the Dielmo stool sample.

French individual, using 212 different conditions, including amoebal co-culture. For the first African sample, 56 different culture methods were applied, including different physico-chemical conditions and the addition of specific nutrients or inhibitors (Table S1). Using these approaches, we isolated 3000 colonies, which were subjected to MALDI-TOF MS analysis for rapid identification of microbial species [10]. This analysis resulted in the identification of 99 bacterial species, 42 of which had never been found in the human gut (Fig. S1), and two of which had never been described (Table 1 Fig. 2).

With the two other stool samples, only those culture conditions that proved to be efficient with the first sample were used again, and many additional culture conditions were applied to maximize the chance of isolation of new species (Table S1). This optimized approach yielded 191 distinct bacterial isolates, including two new genera and six new species from the obese individual's stool sample; the largest number of bacteria ever identified in a single stool (219 bacteria and five fungi, including three new genera and 18 new bacterial species) were isolated from the second African sample (Tables 1 and 2; Fig. 2; Table S2) [12–16].

TABLE 1. Characteristics of the 23 new bacterial species and genera cultured from the Senegalese stools [12–16]

	Phylum	Initial culture conditions	Diameter (μm) (EM)	Genome size estimate (Mb)	ORFan (%)	Estimated GC content (%)	Genbank no.
N'Diop stool sample							
New species							
Oceanobacillus massiliensis	*Firmicutes*	Filtration brain–heart infusion 5% sheep blood 0.45-μm aerobe, 37°C	0.70	3.6	5.6	41	HQ586877
Bacillus timonensis	*Firmicutes*	Brain–heart infusion + sheep blood 5%, aerobe, 37°C	0.66	4.7	6.8	38.3	JF824810
Dielmo stool sample							
New species							
Kurthia massiliensis	*Firmicutes*	CNA aerobe 2.5% CO_2, 37°C	1.08	3.3	11.9	39.7	JF824795
Kurthia senegalensis	*Firmicutes*	Filtration 5% sheep blood agar 1.2-μm aerobe, 37°C	1.03	2.9	11.3	39.6	JF824796
Kurthia timonensis	*Firmicutes*	HTM, aerobe, 2.5% CO_2, 37°C	0.94	4.1	16.2	39	JF824797
Anaerococcus senegalensis	*Firmicutes*	*Brucella* anaerobe, 37°C	0.68	1.8	3	28.5	JF824805
Paenibacillus senegalensis	*Firmicutes*	Schaedler kanamycin vancomycin, aerobe, 37°C	0.66	5.7	10.7	48.3	JF824808
Bacillus massiliosenegalensis	*Firmicutes*	5% sheep blood agar, aerobe, 28°C	0.64	4.9	7.7	37.7	JF824800
Clostridium senegalense	*Firmicutes*	Inoculation in blood culture bottle for 5 days with 5 mL of sheep blood, 5% sheep blood agar, anaerobe, 37°C	1.05	3.9	11.5	29.3	JF824801
Peptoniphilus senegalensis	*Firmicutes*	Inoculation in blood culture bottle for 10 days with 5 mL of sheep blood, 5% sheep blood agar, anaerobe, 37°C	0.64	1.8	3.9	32.5	JF824803
Peptoniphilus timonensis	*Firmicutes*	Inoculation in blood culture bottle anaerobe for 14 days with 8 mL of rumen fluid, 5% sheep blood agar, anaerobe, 37°C	0.91	1.7	9.3	31	JN657222
Ruminococcus massiliensis[a]	*Firmicutes*	Inoculation in blood culture bottle anaerobe for 14 days with 8 mL of rumen fluid 5% sheep blood agar, anaerobe, 37°C	0.96	5.1	25	57	JN657221
Alistipes senegalensis	*Bacteroidetes*	Schaedler kanamycin vancomycin, anaerobe, 37°C	0.53	4	3.8	58.3	JF824804
Alistipes timonensis	*Bacteroidetes*	Inoculation in blood culture bottle anaerobe for 5 days, Schaedler kanamycin vancomycin, anaerobe 37°C	0.62	3.5	2.9	58.8	JF824799
Cellulomonas massiliensis	*Actinobacteria*	Passive filtration with Leptospira broth, 5% sheep blood agar, aerobic atmosphere, 37°C	0.48	3.4	7.9	73.9	JN657218
Aeromicrobium massiliense	*Actinobacteria*	5% sheep blood agar, aerobe, 37°C	1.04	3.3	10.5	72.6	JF824798
Brevibacterium senegalense	*Actinobacteria*	*Brucella*, aerobe, 37°C	0.68	3.4	9.6	69.9	JF824806
Enterobacter massiliensis	*Proteobacteria*	Phage T1 + T4, then 5% sheep blood agar, aerobe, 37°C	1.02	4.9	3	55.4	JN657217
Herbaspirillum massiliense	*Proteobacteria*	Passive filtration with Leptospira broth, 5% sheep blood agar, aerobic atmosphere, 37°C	0.44	4.2	8.1	59.7	JN657219
Microvirga massiliensis	*Proteobacteria*	MOD 2, aerobe, 37°C	2.28	9.35	24.1	59.2	JF824802
New genera							
Dielma fastidiosa	*Firmicutes*	Inoculation in blood culture bottle anaerobe for 10 days, brain–heart infusion, anaerobe, 37°C	0.59	3.6	10.5	40	JF824807
Senegalemassilia anaerobia	*Actinobacteria*	Inoculation in blood culture bottle anaerobe for 5 days. 5% sheep blood aga,r anaerobe, 37°C	0.70	2.3	6.3	61.8	JF824809
Timonella senegalensis	*Actinobacteria*	Inoculation in blood culture bottle anaerobe for 14 days with 8 mL of rumen fluid, 5% sheep blood agar, anaerobe, 37°C	0.59	3	11.9	61.3	JN657220

EM : Electron Microscopy.
[a]The characterization of this bacterial species was not performed, because the impossibility of subculture.

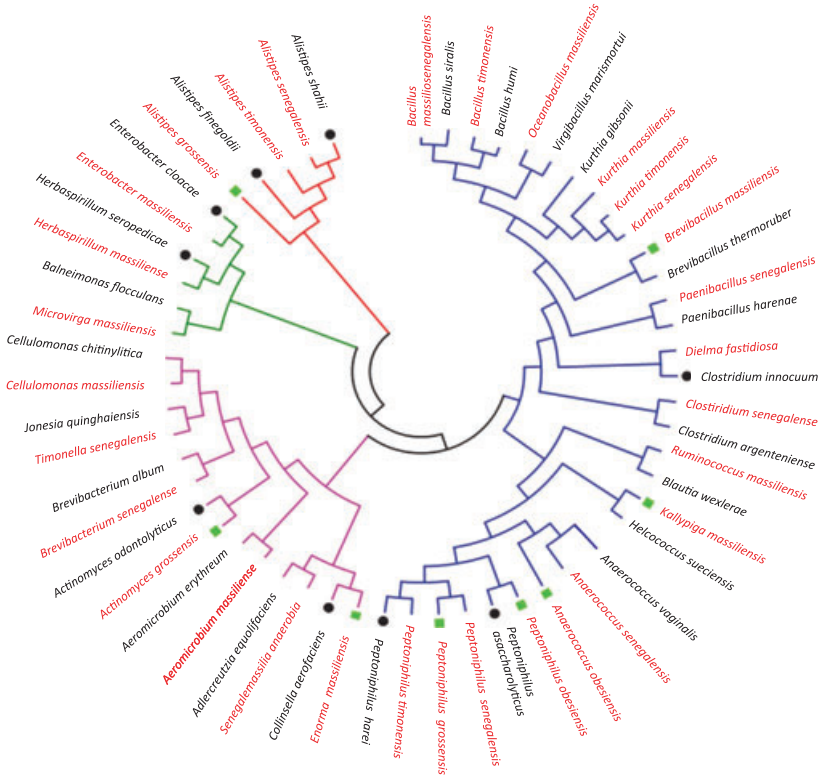

FIG. 2. Phylogenetic tree representing the new bacterial species and genera obtained by culturomics. Red labels indicate the new species found in the Senegalese patients and obese patient. Dark labels indicate the closest neighbour species defined as Isolates and Type in the RDP-II database. Tree branches in red, dark green, purple and blue represent the phyla *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, respectively. Green squares denote new species found in the obese patient. Dark circles indicate that the genome sequence is available for the closest neighbour species.

TABLE 2. Characteristics of the eight new bacterial species and genera cultured from the stools of the obese individual

	Phylum	Initial culture conditions	Diameter (μm) (EM)	Genome size estimate (Mb)	ORFan (%)	Estimated GC content (%)	Genbank no.
New species							
Anaerococcus obesiensis	*Firmicutes*	Inoculation in blood culture bottle with thioglycolate for 4 days, 5% sheep blood agar, anaerobe, 37°C	0.71	2.05	3.7	30.1	JN837490
Brevibacillus massiliensis	*Firmicutes*	M17, aerobe, 37°C	0.73	5.1	7.2	53	JN837488
Peptoniphilus grossensis	*Firmicutes*	Inoculation in blood culture bottle for 26 days with rumen and sheep blood, 5% sheep blood agar, anaerobe, 37°C	0.77	2.1	5.5	34.5	JN837491
Peptoniphilus obesiensis	*Firmicutes*	Inoculation in blood culture bottle for 26 days with rumen and sheep blood, 5% sheep blood agar, anaerobe, 37°C	0.85	1.77	4.7	30.4	JN837495
Alistipes obesiensis	*Bacteroidetes*	Inoculation in blood culture bottle for 11 days with rumen, 5% sheep blood agar, anaerobe, 37°C	0.61	3.1	7.3	58.5	JN837494
Actinomyces grossensis	*Actinobacteria*	Inoculation in blood culture bottle with thioglycolate for 4 days, 5% sheep blood agar, anaerobe, 37°C	0.49	1.87	5.2	56	JN837492
New genera							
Enorma massiliensis	*Actinobacteria*	Inoculation in blood culture bottle with thioglycolate for 4 days, 5% sheep blood agar, anaerobe, 37°C	0.57	2.3	7.9	61.8	JN837493
Kallipyga massiliensis	*Firmicutes*	Inoculation in blood culture bottle for 26 days with rumen and sheep blood, 5% sheep blood agar, anaerobe, 37°C	0.67	1.77	6.3	51.4	JN837487

EM : Electron Microscopy.

Optimization of rapid screening by MALDI-TOF MS

With a low level of operator training, MALDI-TOF MS allows rapid discrimination between identified bacteria (present in the current data bank) and unidentified bacteria. Indeed, subsequent to the analysis of the first sample, with the two other tested samples we were able to identify a significantly greater number of colonies: without automatic colony picking, we tested a total of 29 500 different colonies from these two samples, as compared with the 3000 colonies tested from the first sample. In addition to the better training of the operators, as and when necessary, each previously unknown bacterial spectrum was added to our data bank, facilitating the screening for further studies. MS, which obviates the need for both the time-consuming Gram-staining procedure and the usual biochemical tests, seems to be the current method of choice for the identification of microorganisms, and has the potential to supplant traditional microbiological methods [10]. Indeed, with the third stool sample, we tested, under eight different culture conditions, 50–100 colonies that were indistinguishable in appearance. This experiment allowed us to identify several species, notably those from the genus *Enterococcus*, for which identification in routine bacteriology is mainly based on colony morphology.

Eliminating the predominant population

The high concentration of bacteria in the human gut (10^{12} to 10^{11} bacteria per gram of stools) [1] hampers non-selective culture analysis. Therefore, we used antibiotics in culture media to eliminate sensitive organisms and thus facilitate the identification of resistant ones. We developed different strategies to extend the use of 'classic selective media'. First, to identify new proteobacteria, we had to develop alternative strategies, because *Escherichia coli* is the overwhelmingly dominant bacterial species in the human gut under aerobic conditions. We used a cocktail of *E. coli* lytic bacteriophages [17] that allowed us to clear the culture of *E. coli* and to identify an unknown enterobacterial species (*Enterobacter massiliensis*) that was not detected by classic axenic culture.

Otherwise, an effective method to remove the major bacterial population was active filtration with successive membranes (from 5 to 0.2 μm); this procedure allowed us to identify eight new bacterial species. Finally, using the physical characteristics of certain bacteria, we applied passive filtration, which resulted in the identification, in the second African stool sample, of three motile bacteria that have not been previously detected in the human gut, including two new species (Table 1 and Table S1).

Enrichment of samples in blood culture bottles

Incubation of clinical samples in blood culture bottles is known to promote the growth of *Kingella kingae* in osteoarticular infections. Therefore, for the second and the third samples, we developed an enrichment culture technique involving several days of direct pre-incubation of stools in an aerobic or anaerobic blood culture bottle, allowing the growth of 29 bacterial species that were not detected by standard axenic culture (including 24 anaerobic species). This approach yielded three new genera and three new species. Addition of sheep blood to the blood culture bottle allowed us to identify three additional species.

New culture conditions

To increase the growth of bacteria under culture conditions that mimic their natural environment, and drawing from previous studies on environmental bacteria [9], we used sterile rumen fluid [3] (Fig. S2) and sterile fresh human stools with or without pre-incubation in blood culture bottles. This approach allowed us to isolate 17 strains that were not recovered in classic axenic conditions, including two new genera, three new species, and one species of the *Deinococcus-Thermus* phylum that has not been previously cultured from human clinical samples [18].

Finally, in an effort to obtain fastidious bacteria by amoebal co-culture with *Acanthamoeba polyphaga* [19], we identified from the three stool samples four additional bacterial species that have not been detected by axenic culture. Serendipitously, we also isolated, from the first African stool, a new giant virus strain, which we named Senegalvirus and that has the largest genome among the viruses isolated from humans (Figs 1 and S3), with the exception of two reports of mimivirus detection [19]. The isolation of a non-filterable giant virus from a human stool indicates that giant viruses could constitute a component of the gut microbiome that is missed by metagenomic studies with 0.22-μm filters [6,20].

Microbial culturomics: a general perspective

Only 45 bacterial species obtained in culture in the present work were common to all three analysed stool samples and could be named 'the culturomics core microbiome'. The majority of the isolated species (63.6%) were cultured from only one stool sample, indicative of large inter-individual diversity of the human gut microbiome (Fig. S4). Thus, microbial culturomics allowed the detection of numerous new bacteria from each tested sample (Tables 1 and 2).

Although we used a total of 212 different culture conditions for the three stool samples, 100% of the species grew under only 70 culture conditions, and 73% of the species were identified with only 20 conditions (Fig. S5; Table S3). These results provide guidance for future culturomics studies, which will benefit from using the set of conditions shown to be efficient in this study before

developing new culture approaches. Although a limited number of culture conditions allowed us to grow the majority of the common bacterial species, a more thorough approach that used many 'exotic' culture conditions substantially expanded the repertoire by allowing the isolation of less abundant bacteria.

'Giant' bacteria and giant virus

The typical diameter of the isolated bacteria ranged from 0.5 to 1.5 μm [21]. However, the largest isolated bacterium, *Microvirga massiliensis*, reached 2.28 μm according to transmission electron microscopy, and was also shown to possess the largest genome (9.35 Mb) of any bacterium previously obtained from a human sample (Tables 1 and 2; Fig. 1). The giant (194 nm in diameter) Senegalvirus isolated by amoebal co-culture is the first giant virus ever isolated from the human gut. The genome of Senegalvirus (Genbank JF909596–JF909602) is closely related to those of Marseillevirus (96% identity) and Lausannevirus [22] (Fig. S3), suggesting that this is a new strain of Marseillevirus. Preliminary work indicates the presence of antibodies against this virus in the serum and stool of the subject.

Genome sequencing of new bacteria

The genomes of all 31 new bacterial species and genera (Tables 1 and 2; Fig. 2) isolated from the two African stool samples and the French stool sample were sequenced, generating a total of 110.4 Mb of unique sequence, and are freely available in the EMBL database (http://www.ebi.ac.uk/embl/Submission/index.html). The genome sizes of the new bacteria ranged from 1.7 to 9.35 Mb. Among the predicted gene products of the new genomes, 2.9–24.1% had no readily detectable homologues, i.e. they represented ORFans. Altogether, the present study yielded *c.* 10 000 previously unknown genes.

Comparison with 16S rRNA sequencing

The pyrosequencing that was performed as part of this study identified 126, 138 and 157 phylotypes corresponding to known species from the three stools, respectively (Tables S4, S5 and S6). For the three stools taken together, the microbial culturomics approach yielded 340 bacterial species from seven phyla and 117 genera, whereas pyrosequencing identified 282 species from six phyla and 91 genera. However, a dramatic difference was observed with culturomics: only 51 phylotypes were common between the two approaches (15% of the culturomics set) (Fig. 3a). Among the 'culturomics core microbiome' of 45 cultured species, only 12 (26%) were detected by pyrosequencing. Similarly, only 44 genera (38% of the culturomics set) were shared between the two approaches (Fig. 3b). Altogether, 416 phylotypes of previously uncultured bacteria were identified. Notably, the sequence from a new genus cultured here (*Senegalemassilia anaerobia*) had been previously identified as an uncultured bacterium by metagenomics, demonstrating the capacity of culturomics to grow such supposedly 'unculturable' microorganisms [23–27]. Finally, the molecular techniques did not identify pathogenic bacteria, such as *Salmonella*, that were detected by culturomics (Fig. 4).

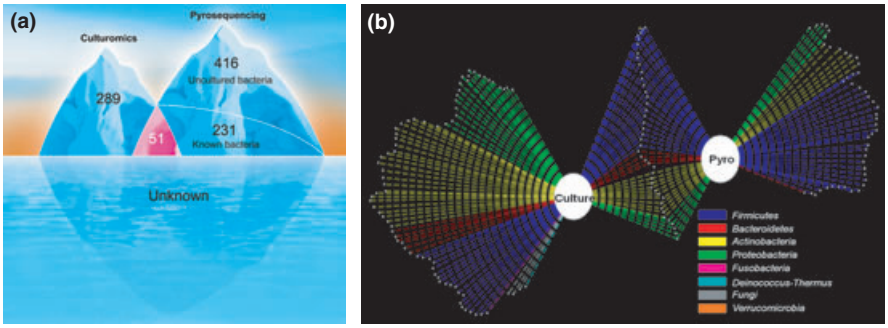

FIG. 3. Identification of bacteria in the human gut by culturomics and metagenomics. (a) The two 'icebergs' represent the 340 cultured bacterial species and the 698 phylotypes identified by pyrosequencing. The overlap between the two sets of species, i.e. the 51 species detected by both approaches, is shown in purple. Below the 'sea level' is the projected unknown part of the human gut microbiome. (b) Taxonomic distribution of organisms identified by culturomics and pyrosequencing. The ovoid shape denoted 'Culture' indicates all of the bacterial and fungal genera identified by culturomics from the three stool samples. The ovoid shape denoted 'Pyro' indicates genera identified by 16S amplicon pyrosequencing of the three stool samples. The dashed coloured lines show the phylum membership of the respective genus node. The two shapes with dashed lines at left and right represent the bacterial and fungal genera identified by only one technique (pyrosequencing or culturomics), whereas the shape in the middle represents the genera identified by both culturomics and pyrosequencing.

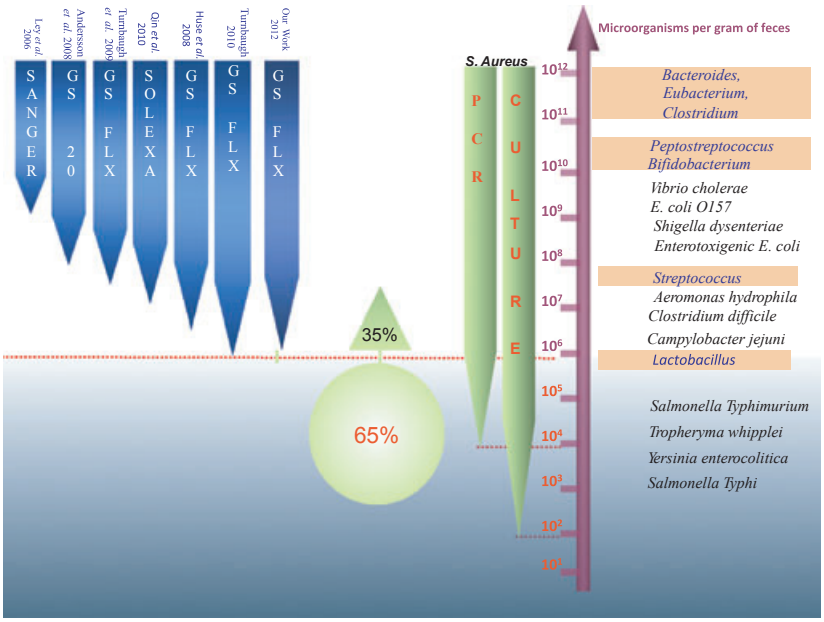

FIG. 4. The detection thresholds of metagenomic and culturomic approaches. The detection threshold of metagenomic methods correlates with the concentration of bacteria in the investigated sample divided by the number of generated sequences. The blue pointed shapes show the detection depth of different published metagenomic analyses of the human gut microbiome. The upper dotted red line shows the detection threshold of the most powerful available metagenomic methods, the middle line shows the detection threshold of PCR, and the lower line shows the detection threshold of culturomics. The latter two thresholds were determined by detection of *Staphylococcus aureus* that was added to the samples in varying concentrations (indicated by green pointed shapes). Among the 340 cultivated bacterial species, 29 were identified only after several days of incubation in an anaerobic blood culture bottle, so their concentrations in the original samples could not be estimated. Among the remaining 311 bacteria, 203 (65%) were found at concentrations of $<10^6$ CFU/g of stool, i.e. below the detection threshold of metagenomic methods.

Discussion

Metagenomics is currently thought of as the mainstream of microbiome studies, in particular as applied to the human gut. Unexpectedly, however, in a direct comparison, we described more known bacterial species by systematically applying a large sample of culture conditions (the approach we denoted culturomics) than by pyrosequencing (Fig. 3a). Moreover, we found a dramatic divergence between the sets of bacteria identified by the two approaches at the level of both species and genera. The detection by culturomics of numerous bacteria that go undetected in genomic and metagenomic studies is far from being trivial, even if most of these microorganisms are of low abundance. Undoubtedly, a minority population, as in the famous short story [28], can have a substantial effect on the ecology of the gut microbiota and on human health. Indeed, *c.* 65% of bacterial species from the three samples were detected at concentrations between 10^3 and 10^6 CFU/mL, which are below the detection thresholds of large-scale molecular studies, demonstrating the major 'depth bias' that is characteristic of the metagenomic approaches (Fig. 4). In support of this conclusion, culture methods allowed the detection of *Staphylococcus aureus* that was added to the stools at a concentration that was 100 times lower than the concentration detectable by molecular tools (Fig. 4).

The paradigm shift in microbiome study that seems to be brought about by culturomics became possible thanks to the breakthrough in the application of MALDI-TOF MS [10]. In comparison with the most rapid conventional phenotypic identification method for bacteria (Vitek System; Biomerieux, Marcy l'Etoile, France), MALDI-TOF MS reduces by *c.* 55-fold the time to bacterial identification and reduces the costs by at least a factor of 5 [10]. Therefore, MALDI-TOF MS is currently the most time-effective and cost-effective identification method available for culture-based microbiota studies.

In the present culturomics study, the actual analysis of microbial cultures involved only three students (JCL,MM,PH) that performed the experience on the three stools during 2 years in a single laboratory. Nevertheless, this limited effort yielded 174 bacterial species that have not been previously reported from the human gut microbiota. Genome sequencing of these bacteria would increase by *c.* 18% the number of

sequenced bacterial genomes from the human gut (975) that have been identified by several laboratories within the human microbiome project [29].

The present limited culturomics study shows that microbial biodiversity in the human gut is substantially broader than predicted on the basis of genomic and metagenomic analyses [27,30]. Interestingly, culturomics also 'broke the records' for the largest bacterium and virus isolated from humans so far. By using different atmospheres, temperatures, pH, nutrients, minerals, antibiotics or phages, 'microbial culturomics' provides comprehensive culture conditions simulating, reproducing or mimicking the entirety of selective constraints that have shaped the gut microbiota for millions of years. In fact, each isolated microorganism is one among the possible viable solutions to the evolutionary equation whose constants are the selective constraints of the environment, corresponding here to the human gut. This is why microbial culturomics is the best way to capture the functional and viable gut microbiota biodiversity of each human individual through large-scale isolation, and to capture the deepest informational genetic gut biodiversity by sequencing the complete genomes of the previously isolated microorganisms. In the future, the use of the most effective conditions and automatic colony picking will further deepen this field of research.

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Author Contributions

Conception and and design of the experiments: D. Raoult. Performance of the experiments: J. C. Lagier, M. Million, P. Hugon, I. Pagnier, C. Robert, and B. La Scola. Analysis of the data: J. C. Lagier, F. Armougom, P. Hugon, I. Pagnier, F. Bittar, G. Fournous, G. Gimenez, E. V. Koonin, B. La Scola, and D. Raoult. Contribution of reagents/material/analysis tools: J. C. Lagier, F. Armougom, P. Hugon, I. Pagnier, F. Bittar, G. Fournous, G. Gimenez, M. Million, B. La Scola, and D. Raoult. Writing of the paper: J. C. Lagier, F. Armougom, E. V. Koonin, and D. Raoult.

Transparency Declaration

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. A comparison of the species identified from the cultures of the different samples.

Figure S2. The protocol used for the rumen fluid preparation.

Figure S3. A genomic comparison between Marseillevirus, Senegalvirus and Lausannevirus.

Figure S4. A Venn diagram representing the number of species cultivated from each of the stool samples.

Figure S5. The number of species that were cultivated in 10–70 conditions out of the 212 tested culture conditions.

Figure S6. The percentage of new species and of the total number of species cultured that grew in only one culture condition or in multiple culture conditions.

Table S1. Culture conditions for microbial culturomics characterization from the stool samples of the N'Diop and Dielmo individuals and the obese French individual.

Table S2. The 345 bacterial and fungal species cultured from the N'Diop, Dielmo and obese patient stool samples.

Table S3. The 20 best culture conditions, which facilitated the identification of 73% of the bacterial species.

Table S4. Cultivated species identified in a Senegalese stool sample from Dielmo village.

Table S5. Cultivated phylotypes identified in the stool sample from an obese patient.

Table S6. Cultivated phylotypes identified in a Senegalese stool sample from N'Diop village.

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