

1                    **Updating the repertoire of cultured bacteria from the human being**

2                    Ami Diakite<sup>1,2</sup>, Grégory Dubourg<sup>1,2</sup>, Didier Raoult<sup>1,2\*</sup>

3

4    <sup>1</sup>*Aix Marseille Université, IRD, AP-HM, MEPHI, Marseille, France*

5    <sup>2</sup>*IHU Méditerranée Infection, Marseille, France*

6    **\*Corresponding author** : Didier Raoult<sup>1</sup>, Aix Marseille Université, IHU Méditerranée

7    Infection, Marseille, France, E-mail : [didier.raoult@gmail.com](mailto:didier.raoult@gmail.com)

8

9    Phone number: +33 4 13 73 24 01

10   Fax number: +33 4 13 73 24 02

11

12

13

14

15

16

17

18

19

20 **Abstract**

21 The recent renewal of cultural approach has substantially enriched knowledge of the human  
22 microbiota, notably through the discovery of new taxa from various anatomical sites. As an  
23 increasing number of these recent species are currently considered beneficial or harmful for  
24 human health, a constant updating of the repertoire of bacteria and archaea isolated from  
25 humans by culture is essential. Herein, we show that the number of cultured bacterial species  
26 associated with human beings increased, from 2776 in 2018, to 3253 in 2020, representing a  
27 17% increase in 2 years by adding 477 species, of which 64% are new species (N=307). A  
28 wide majority of the species added (i.e., 63%) were isolated using the culturomics approach,  
29 while 16% were cultured as part of clinical microbiology laboratories. Human microbiota  
30 studies would benefit from the completeness of the repertoire of bacteria associated with  
31 human beings, which would require continued efforts to culture microbes from human  
32 specimens.

33

34 **Keywords: Human repertoire; Bacteria: Culturomics**

35

36

37

38

39

40

41

## 42        **1. Introduction**

43        The Human microbiota is defined as all the bacteria, parasites, viruses, fungi and archaea  
44        found in the human body. In terms of biomass, the number of bacteria colonizing the human  
45        body has been estimated to be equal to the number of human cells [1]. However, the bacterial  
46        diversity associated with the human host remains so far unknown. Indeed, the tools dedicated  
47        to the elucidation of the human microbiota suffers from various biases that were extensively  
48        discussed [2]. There is however a growing evidence of the influence of the human microbiota  
49        in a variety of diseases, including obesity, diabetes and cancer [3–6].

50        In addition, the advent of bacteriotherapy and the growing use of the fecal microbiota  
51        transplantation (FMT) demonstrate the clinical importance of these microorganisms [7,8].  
52        While most of this data is derived from high-throughput sequencing methods by screening for  
53        taxa of potential medical interest, biological objects remain essential. Unattributed sequences  
54        of potential anticancer prokaryotes have been shown to have already been isolated [9].  
55        Various cultivation approaches have indeed recently contributed to the renewal of culture.  
56        Among these, culturomics is defined as a high-throughput culture technique combining  
57        variations in culture conditions and rapid identification of microorganisms using mass  
58        spectrometry type Matrix Assisted Laser Desorption Ionization - Time of Flight (MALDI-  
59        TOF MS) [10]. But there are many other high-throughput cultural approaches currently  
60        developed to reduce the metagenomics dark matter [11,12] resulting in an increasing number  
61        of new bacterial species discovered from human being. Reference databases used for  
62        taxonomic assignments currently do not include the entirety of sequences derived from these  
63        novel taxa. We therefore believe that updating the previous repertoire is crucial to improve the  
64        identification of sequences generated by high-throughput sequencing approaches. We have  
65        already provided such repertoires in 2015 and 2018 and spontaneously propose herein to  
66        update the repertoire of human bacteria isolated at least once in humans [13,14].

## 67        2. Bibliographical methods

68        A manual query was conducted on three major databases, including Google Scholar, PubMed  
69        and Web of science. We started by defining terms and text words referring to Bacteria (Q1),  
70        Archaea (Q2), Man (Q3) and Culture Technology (Q4). Then, we combined these terms in  
71        order to search the different databases. We build different requests for bacteria and archaea  
72        (*Supplementary Data 1*). Considering the period covered by the preceding repertoire update,  
73        we focused our literature search on data published from 01/01/2018 to 25/02/2020. Using  
74        request for bacteria, we retrieved 5950, 27472 and 44562 papers on Google Scholar, PubMed  
75        and Web of science databases, respectively. Using request for archaea, we retrieved 92, 642  
76        and 2,368 papers on Google Scholar, PubMed and Web of science databases, respectively.  
77        These data were exported to the EndNote version X9 platform (<https://endnote.com>) in order  
78        to sort and analyze the publications. Following removal of duplicates, we obtained 66,622  
79        publications from bacterial request and 2,731 publications from archaeal request (*Figure 1A,*  
80        *1B*). After analysis of the titles and abstracts, we excluded 56590 studies that did not involve  
81        humans, did not use culture-dependent methods and that did not focus on bacteria. Then, we  
82        analyzed the full paper and supplementary data of 10,032 publications and excluded those that  
83        did not add more bacterial species when compared to the dataset from Bilen et al. in 2018  
84        [14]. We checked the name of each species, filled in their taxonomic information, the origin  
85        of their first isolation, whether they were found in pathological situations and whether they  
86        were isolated for diagnostic or research purposes. These information were collected from the  
87        websites [www.ncbi.nlm.nih.gov/taxonomy](http://www.ncbi.nlm.nih.gov/taxonomy), [www.bacdivi.dsmz.de](http://www.bacdivi.dsmz.de) and the original papers  
88        relating to these bacteria. The oxygen tolerance of bacterial species was determined using the  
89        database [https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/list-of-](https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/list-of-prokaryotes-according-to-their-aerotolerant-or-obligate-anaerobic-metabolism/)  
90        [prokaryotes-according-to-their-aerotolerant-or-obligate-anaerobic-metabolism/](https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/list-of-prokaryotes-according-to-their-aerotolerant-or-obligate-anaerobic-metabolism/)  
91        [www.mediterranee-infection.com](http://www.mediterranee-infection.com).

92 **3. Number of species added and description of their characteristics.**

93 Based on our bibliographical research, we were able to add 475 bacterial species and 2  
94 archaea to the last update of the human bacterial repertoire in 2018 by Bilen *et al* [14]. This  
95 represents a 17% increase of the repertoire in 2 years (*Figure 2, Supplementary Table 1*). The  
96 477 species added to this repertoire represent 9 phyla with a majority of *Firmicutes* (N=238;  
97 50%), followed by *Actinobacteria* (N=109; 23%), *Proteobacteria* (N=73; 15%) and  
98 *Bacteroidetes* (N=46; 10%). Phyla such as *Euryarchaeota* (N=2), *Fusobacteria* (N=2),  
99 *Synergistetes* (N=1), *Deferribacteres* (N=1) and *Chlamydiae* (N=1) each represented less than  
100 1% of the total (*Supplementary Figure 1A*). The unique bacterial species isolated in each of  
101 these three phyla are *Anaerobaculum hydrogeniformans* (isolated from gut), *Simkania*  
102 *negevensis* (isolated from oral cavity) and *Mucispirillum schaedleri* (isolated from breast  
103 milk), respectively. For this latter, it represents the second member of the *Deferribacteres*  
104 phylum ever isolated from the human being. This distribution of phylum is consistent with  
105 what was demonstrated in the previous human repertoire by Bilen *et al.* in 2018[14]. The 9  
106 phyla are divided into 240 bacterial genera, the majority of which belong to the *Clostridium*  
107 (N=25), *Bifidobacterium* (N=20) and *Bacillus* (N=18) genera (*Supplementary Figure 1B*).  
108 Anaerobic species constitute half of the species added (N=239; 50%). Majority of the species  
109 added, 64% (N=307), are new species discovered from humans samples. The remaining 36%  
110 (N=170) species are bacterial species first isolated from the environment (N=75; 16%),  
111 animals (N=72; 15%) and food (N=23; 5%) (*Supplementary Figure 2*).

112 **4. Contribution of Culturomics to the expansion of the human bacterial repertoire.**

113 Culturomics is defined as a high-throughput culture method that has so far led to the  
114 discovery of hundreds of new taxa associated with humans [10]. In this work, we qualified as  
115 culturomics all studies using a high-throughput culture method with or without reference to

116 the term culturomics and evaluated whether the species included in this study were isolated by  
117 this approach or not [11,12]. As a result, 301 bacterial species were isolated as a part of  
118 culturomics studies, representing a contribution of 63% of the total number of species added  
119 by this work (N=301/477) (*Figure 2, Supplementary Table 1*). Most of these species are  
120 strictly anaerobic (64%; N=194/301) and are new taxa (73%; N=220/301). Gut is the most  
121 represented anatomical site as it is the source of 73% (N=160/220) of these new taxa,  
122 followed by vagina (9%; N=19/220), skin (5%; N=11/220) and less than 5% in other sites  
123 such as oral cavity (N=9), urine (N=7), respiratory tract (N=6), breast milk (N=4), blood  
124 (N=3) and nasopharyngeal swabs (N=1). These new species are mostly anaerobes  
125 (N=150/220; 68%). The remaining species (27%; N=81/301) isolated from humans through  
126 culturomics are species of non-human origin known previously. They have been found at 99%  
127 (N=80/81) in the gut and 1% (N=1/81) in breast milk with an anaerobic proportion estimated  
128 at 54% (N=44/81).

## 129 **5. The role of clinical microbiology in updating the repertoire of human microbes**

130 Microbial culture in the diagnostic laboratory has also been the subject of several innovations  
131 that make it faster and more efficient. The introduction of mass spectrometry type Matrix  
132 Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF MS) in the identification  
133 of bacterial species in the routine laboratory has revolutionized bacterial identification by  
134 progressively abandoning morphological and biochemical methods [15]. MALDI-TOF MS  
135 therefore saved a considerable amount of time and increased the colony identification rate  
136 because the database used for spectrum comparison is versatile. It currently represents a  
137 powerful screening tool. Indeed, it has probably led microbiologists to test an increasing  
138 number of colonies cultivated from clinical specimens that have contributed to expand the  
139 human bacterial repertoire. In this work, we tried to evaluate the percentage of species added  
140 using the microbial culture method in the diagnostic laboratory. As a result, 16% (N=76/477)

141 of the species added to the repertoire were isolated from clinical specimen (*Figure 2*,  
142 *Supplementary Table 1*). Most of these species are tolerant to oxygen (i.e., 88%, 67/76) and  
143 are new taxa (64/76; 84%). The clinical specimens were respiratory samples (25%; N=16/64),  
144 blood cultures (23%; N=15/64), wounds (11%, N=7/64) and other sites with low percentages.  
145 The species that are not new (N=12/76; 16%) are species already known from the  
146 environment, animals and food.

## 147 **6. Updating the human archaeal repertoire**

148 Archaea are prokaryotic organisms that were first discovered in extreme environments and  
149 were gradually isolated from humans, in particular from the intestinal microbiota and, more  
150 recently, from the breast milk [16,17]. Importantly, archaeal species were isolated from  
151 clinical specimen, including urine [18]. The most important discovery was the detection of  
152 *Methanobrevibacter smithii* in the blood of febrile patients, including subjects with  
153 endocarditis. These findings were screened by PCR and confirmed by various assays,  
154 including culture. Regarding the repertoire, only 10 of 715 species of archaea known  
155 according to NCBI taxonomy have so far been found by culture in humans in 2018[14]. The  
156 present study enables to add two species of archaea, including "*Candidatus*  
157 *Methanomethylophilus Alvus*" and "*Candidatus Methanomassiliicoccus intestinalis*",  
158 representing a new order of methanogens related to *Thermoplasmata*. Both species were  
159 isolated from the human intestinal microbiota, thus increasing the number of archaea isolated  
160 by culture from humans to a total of 12 species [19,20].

## 161 **7. Description of the current human bacterial repertoire**

162 The aim of this work was to update the latest repertoire of bacteria and archaea cultured at  
163 least once in humans carried out in 2018 by Bilen. et al [14]. Species identified in this work  
164 were isolated from humans according to our data between 1982 and 2020 with a peak in 2019

165 (N=239; 50%) (*Supplementary Figure 3*). A significant number of species (N=113; 24%)  
166 were reported in 2018, the year of the last update where data collection ceased in April 2018.  
167 The year 2020 represents 9% (N=41) of the added species, while 18% of the species added  
168 (N=84) were found between the years 1980 and 2017, which are periods normally covered by  
169 the previous repertoires. This underlines the exhaustiveness of the methodology used herein,  
170 that was mostly performed and verified manually. As a result, 477 were added, an increase of  
171 17% in two years. The number of bacteria and archaea isolated from humans thereby  
172 increases from N=2776 to N=3253, with the proportion of new species increasing from 19%  
173 to 26% (N=831) (*Figure 3, Supplementary Table 2*). Most species are *Firmicutes* 37%,  
174 *Proteobacteria* 25%, *Actinobacteria* 25% and *Bacteroidetes* (*Figure 4*). A total of 711  
175 bacterial genera compose the human repertoire to date with the addition of 108 genera yet  
176 unknown in humans. The genera *Mycobacterium* (N=163), *Clostridium* (N=133), *Bacillus*  
177 (N=108), *Corynebacterium* (N=89) and *Streptococcus* (N=78) are the most represented in  
178 terms of number of species. Despite the diversification of human anatomical sites subjected to  
179 high-throughput culture analysis, the intestinal tract remains the site where the most species  
180 are isolated. Indeed, 52% (N=247) of the total species added by this study (N=477) came  
181 from the gut of which 67% (N=166) are new taxa. IHU Méditerranée-Infection contributed up  
182 to 20% (N= 639/3253) of the total human bacteria and archaea repertoire, mostly through the  
183 discovery of new species (*Figure 5*). The proportion of anaerobic species increased globally  
184 from 24% of the previous repertoire (N=662) to 28% in this new repertoire (N=901) with the  
185 addition of the 239 anaerobic species, most of which are new species. These findings show  
186 that the anaerobes constitute a substantial fraction of the microbial dark matter associated with  
187 humans.

## 188 **8. Conclusion**



189 This present study makes it possible to significantly increase the repertoire of human-  
190 associated bacteria in a very short period of time. We are currently witnessing the revival of  
191 culture, by culturomics, but not only, as many works have reported the isolation of new taxa  
192 through innovative cultural strategies [11,12,21,22]. Update of such repertoire is thus crucial  
193 as the taxa recently discovered are implied in human health and disease[9,23]. Infectious  
194 diseases are also concerned as new species discovered thanks to culturomics were further  
195 detected in clinical specimen [24]. We believe that such repertoire could also help in the  
196 interpretation of datasets from high sequencing approaches. Indeed, with the current increase  
197 of the sequencing depth, the number of sequences amplified is exponentially growing. It can  
198 therefore be tricky to determine the relevance of the identification of certain taxa as  
199 part of human microbiome studies. Some sequences could be due to the presence of  
200 contaminants, or transient microbes that are not commensal to the studied anatomical site.  
201 This is also supported by the fact that not all microbes detected are viable in a human  
202 specimen [25]. This repertoire, along with other repertoires dedicated to specific human  
203 ecological niches [13,26–29], may at least partly help to resolve these interpretation issues.  
204 Improving culture approaches to isolate of microbes that are considered "unculturable" is  
205 therefore crucial, as shown by Kaboré et al for the culture of *Gemmata* genus. Known for  
206 being tedious to cultivate, it was isolated using a culture medium including the skeleton of  
207 marine sponges and the aqueous heat filtrate of the skeleton sponge [30]. Belkacemi et al., for  
208 the same purpose, developed a successful culture technique based on passive filtration for the  
209 culture of *Treponema* species [31]. Thanks to metagenomic studies, we know that the number  
210 of species of archaea in humans is much higher compared to the number cultivated [32]. To  
211 date, we have listed several 12 species of archaea cultivated at least once in humans. It is  
212 therefore imperative to develop culture techniques to be able to isolate them and explore their  
213 role as they are often found to be associated with several bacterial infections, such as

214 periodontitis, chronic sinusitis, muscular or cerebral abscesses. *Methanobrevibacter smithii*  
215 was detected in blood cultures from febrile patients, thus suggesting that these microbes  
216 should not be longer neglected. Guindo et al. had, for example, developed a technique for  
217 cultivating methanogens archaea through the production of hydrogen necessary for their  
218 growth by a chemical technique based on the use of iron filings and acetic acid [33]. In  
219 parallel, we observed that most of the newly isolated species were anaerobic species, hence  
220 the need to develop anaerobic culture strategies. The fact that most of the new taxa isolated  
221 from clinical specimen are, on the contrary, tolerant to oxygen, reflects the gradual  
222 abandonment of anaerobic culture in clinical microbiology. Yet, Diakit  et al., in analyzing  
223 the cost-effectiveness of the culture conditions used in culturomic studies, showed that  
224 anaerobic culture conditions allow the isolation of 5 times more bacteria than aerobic culture  
225 conditions [34]. Efforts must continue to cultivate bacterial species that do not tolerate  
226 oxygen, including extremely oxygen-sensitive bacteria (EOS). To this end, special attention  
227 should be paid to maintaining anaerobiosis from specimen collection to incubation [35]. The  
228 important contribution of culturomics technique initiated 8 years ago (2012) to the human  
229 repertoire demonstrates its effectiveness in highlighting and understanding the diversity of the  
230 human microbiota. These innovative approaches to culture techniques are to be encouraged in  
231 order to continue isolating bacteria and filling the dark matter of high-throughput sequencing  
232 approaches.

## 233 **References**

- 234 [1] R. Sender, S. Fuchs, R. Milo, Revised Estimates for the Number of Human and  
235 Bacteria Cells in the Body, PLoS Biol. 14 (2016) e1002533.  
236 <https://doi.org/10.1371/journal.pbio.1002533>.
- 237 [2] J.-C. Lagier, G. Dubourg, M. Million, F. Cadoret, M. Bilen, F. Fenollar, A. Levasseur,  
238 J.-M. Rolain, P.-E. Fournier, D. Raoult, Culturing the human microbiota and culturomics,  
239 Nat. Rev. Microbiol. 16 (2018) 540–550. <https://doi.org/10.1038/s41579-018-0041-0>.

- 240 [3] I. Sekirov, S.L. Russell, L.C.M. Antunes, B.B. Finlay, Gut Microbiota in Health and  
241 Disease, *Physiological Reviews*. 90 (2010) 859–904.  
242 <https://doi.org/10.1152/physrev.00045.2009>.
- 243 [4] L. Zhao, The gut microbiota and obesity: from correlation to causality, *Nature*  
244 *Reviews Microbiology*. 11 (2013) 639–647. <https://doi.org/10.1038/nrmicro3089>.
- 245 [5] N. Larsen, F.K. Vogensen, F.W.J. van den Berg, D.S. Nielsen, A.S. Andreasen, B.K.  
246 Pedersen, W.A. Al-Soud, S.J. Sørensen, L.H. Hansen, M. Jakobsen, Gut microbiota in human  
247 adults with type 2 diabetes differs from non-diabetic adults, *PLoS ONE*. 5 (2010) e9085.  
248 <https://doi.org/10.1371/journal.pone.0009085>.
- 249 [6] L. Zitvogel, L. Galluzzi, S. Viaud, M. Vétizou, R. Daillère, M. Merad, G. Kroemer,  
250 Cancer and the gut microbiota: An unexpected link, *Science Translational Medicine*. 7 (2015)  
251 271ps1-271ps1. <https://doi.org/10.1126/scitranslmed.3010473>.
- 252 [7] N. Kobyliak, C. Conte, G. Cammarota, A.P. Haley, I. Styriak, L. Gaspar, J. Fusek, L.  
253 Rodrigo, P. Kruzliak, Probiotics in prevention and treatment of obesity: a critical view, *Nutr*  
254 *Metab (Lond)*. 13 (2016) 14. <https://doi.org/10.1186/s12986-016-0067-0>.
- 255 [8] M. Hocquart, J.-C. Lagier, N. Cassir, N. Saidani, C. Eldin, J. Kerbaj, M. Delord, C.  
256 Valles, P. Brouqui, D. Raoult, M. Million, Early Fecal Microbiota Transplantation Improves  
257 Survival in Severe *Clostridium difficile* Infections, *Clin Infect Dis*. 66 (2018) 645–650.  
258 <https://doi.org/10.1093/cid/cix762>.
- 259 [9] J.-C. Lagier, M. Million, A.H. Togo, S. Khelaifia, D. Raoult, Culturomics provides  
260 critical prokaryote strains for anti-*Listeria* and anti-cancer probiotics, *Int. J. Antimicrob.*  
261 *Agents*. 54 (2019) 407–409. <https://doi.org/10.1016/j.ijantimicag.2019.05.017>.
- 262 [10] J.-C. Lagier, P. Hugon, S. Khelaifia, P.-E. Fournier, B.L. Scola, D. Raoult, The  
263 Rebirth of Culture in Microbiology through the Example of Culturomics To Study Human  
264 Gut Microbiota, *Clinical Microbiology Reviews*. 28 (2015) 237–264.  
265 <https://doi.org/10.1128/CMR.00014-14>.
- 266 [11] Y. Zou, W. Xue, G. Luo, Z. Deng, P. Qin, R. Guo, H. Sun, Y. Xia, S. Liang, Y. Dai,  
267 D. Wan, R. Jiang, L. Su, Q. Feng, Z. Jie, T. Guo, Z. Xia, C. Liu, J. Yu, Y. Lin, S. Tang, G.  
268 Huo, X. Xu, Y. Hou, X. Liu, J. Wang, H. Yang, K. Kristiansen, J. Li, H. Jia, L. Xiao, 1,520  
269 reference genomes from cultivated human gut bacteria enable functional microbiome  
270 analyses, *Nat. Biotechnol*. 37 (2019) 179–185. <https://doi.org/10.1038/s41587-018-0008-8>.
- 271 [12] H.P. Browne, S.C. Forster, B.O. Anonye, N. Kumar, B.A. Neville, M.D. Stares, D.  
272 Goulding, T.D. Lawley, Culturing of “unculturable” human microbiota reveals novel taxa and  
273 extensive sporulation, *Nature*. 533 (2016) 543–546. <https://doi.org/10.1038/nature17645>.
- 274 [13] P. Hugon, J.-C. Lagier, P. Colson, F. Bittar, D. Raoult, Repertoire of human gut  
275 microbes, *Microb. Pathog*. 106 (2017) 103–112.  
276 <https://doi.org/10.1016/j.micpath.2016.06.020>.

- 277 [14] M. Bilen, J.-C. Dufour, J.-C. Lagier, F. Cadoret, Z. Daoud, G. Dubourg, D. Raoult,  
278 The contribution of culturomics to the repertoire of isolated human bacterial and archaeal  
279 species, *Microbiome*. 6 (2018) 94. <https://doi.org/10.1186/s40168-018-0485-5>.
- 280 [15] P. Seng, M. Drancourt, F. Gouriet, B. La Scola, P.-E. Fournier, J.M. Rolain, D. Raoult,  
281 Ongoing Revolution in Bacteriology: Routine Identification of Bacteria by Matrix-Assisted  
282 Laser Desorption Ionization Time-of-Flight Mass Spectrometry, *Clin Infect Dis*. 49 (2009)  
283 543–551. <https://doi.org/10.1086/600885>.
- 284 [16] N. Gaci, G. Borrel, W. Tottey, P.W. O’Toole, J.-F. Brugère, Archaea and the human  
285 gut: New beginning of an old story, *World J Gastroenterol*. 20 (2014) 16062–16078.  
286 <https://doi.org/10.3748/wjg.v20.i43.16062>.
- 287 [17] A.H. Togo, G. Grine, S. Khelaifia, C. des Robert, V. Brevaut, A. Caputo, E. Baptiste,  
288 M. Bonnet, A. Levasseur, M. Drancourt, M. Million, D. Raoult, Culture of Methanogenic  
289 Archaea from Human Colostrum and Milk, *Scientific Reports*. 9 (2019) 18653.  
290 <https://doi.org/10.1038/s41598-019-54759-x>.
- 291 [18] G. Grine, R. Lotte, D. Chirio, A. Chevalier, D. Raoult, M. Drancourt, R. Ruimy, Co-  
292 culture of *Methanobrevibacter smithii* with enterobacteria during urinary infection,  
293 *EBioMedicine*. 43 (2019) 333–337. <https://doi.org/10.1016/j.ebiom.2019.04.037>.
- 294 [19] G. Borrel, H.M.B. Harris, W. Tottey, A. Mihajlovski, N. Parisot, E. Peyretailade, P.  
295 Peyret, S. Gribaldo, P.W. O’Toole, J.-F. Brugère, Genome sequence of “*Candidatus*  
296 *Methanomethylophilus alvus*” Mx1201, a methanogenic archaeon from the human gut  
297 belonging to a seventh order of methanogens, *J. Bacteriol*. 194 (2012) 6944–6945.  
298 <https://doi.org/10.1128/JB.01867-12>.
- 299 [20] G. Borrel, H.M.B. Harris, N. Parisot, N. Gaci, W. Tottey, A. Mihajlovski, J. Deane, S.  
300 Gribaldo, O. Bardot, E. Peyretailade, P. Peyret, P.W. O’Toole, J.-F. Brugère, Genome  
301 Sequence of “*Candidatus Methanomassiliicoccus intestinalis*” Issoire-Mx1, a Third  
302 Thermoplasmatales-Related Methanogenic Archaeon from Human Feces, *Genome Announc*.  
303 1 (2013). <https://doi.org/10.1128/genomeA.00453-13>.
- 304 [21] S. Ghimire, C. Roy, S. Wongkuna, L. Antony, A. Maji, M.C. Keena, A. Foley, J.  
305 Scaria, Identification of *Clostridioides difficile*-Inhibiting Gut Commensals Using  
306 Culturomics, Phenotyping, and Combinatorial Community Assembly, *mSystems*. 5 (2020).  
307 <https://doi.org/10.1128/mSystems.00620-19>.
- 308 [22] Y. Chang, F. Hou, Z. Pan, Z. Huang, N. Han, L. Bin, H. Deng, Z. Li, L. Ding, H. Gao,  
309 F. Zhi, R. Yang, Y. Bi, Optimization of Culturomics Strategy in Human Fecal Samples, *Front*  
310 *Microbiol*. 10 (2019) 2891. <https://doi.org/10.3389/fmicb.2019.02891>.
- 311 [23] C. Gómez-Gallego, S. Pohl, S. Salminen, W.M. De Vos, W. Kneifel, *Akkermansia*  
312 *muciniphila*: a novel functional microbe with probiotic properties, *Benef Microbes*. 7 (2016)  
313 571–584. <https://doi.org/10.3920/BM2016.0009>.

- 314 [24] G. Dubourg, S. Baron, F. Cadoret, C. Couderc, P.-E. Fournier, J.-C. Lagier, D. Raoult,  
315 From Culturomics to Clinical Microbiology and Forward, *Emerging Infect. Dis.* 24 (2018)  
316 1683–1690. <https://doi.org/10.3201/eid2409.170995>.
- 317 [25] S. Bellali, J.-C. Lagier, D. Raoult, J. Bou Khalil, Among Live and Dead Bacteria, the  
318 Optimization of Sample Collection and Processing Remains Essential in Recovering Gut  
319 Microbiota Components., *Front Microbiol.* 10 (2019) 1606.  
320 <https://doi.org/10.3389/fmicb.2019.01606>.
- 321 [26] K. Diop, J.-C. Dufour, A. Levasseur, F. Fenollar, Exhaustive repertoire of human  
322 vaginal microbiota, *Human Microbiome Journal.* (2019).
- 323 [27] A. Morand, F. Cornu, J.-C. Dufour, M. Tsimaratos, J.-C. Lagier, D. Raoult, Human  
324 Bacterial Repertoire of the Urinary Tract: a Potential Paradigm Shift., *J Clin Microbiol.* 57  
325 (2019). <https://doi.org/10.1128/JCM.00675-18>.
- 326 [28] A. Togo, J.-C. Dufour, J.-C. Lagier, G. Dubourg, D. Raoult, M. Million, Repertoire of  
327 human breast and milk microbiota: a systematic review., *Future Microbiol.* 14 (2019) 623–  
328 641. <https://doi.org/10.2217/fmb-2018-0317>.
- 329 [29] M.D. Fonkou, J.-C. Dufour, G. Dubourg, D. Raoult, Repertoire of bacterial species  
330 cultured from the human oral cavity and respiratory tract. *Future Microbiol.* 13 (2018) 1611–  
331 1624. <https://doi.org/10.2217/fmb-2018-0181>.
- 332 [30] O.D. Kaboré, S. Godreuil, M. Drancourt, Improved culture of fastidious Gemmata  
333 spp. bacteria using marine sponge skeletons, *Sci Rep.* 9 (2019) 11707.  
334 <https://doi.org/10.1038/s41598-019-48293-z>.
- 335 [31] S. Belkacemi, J. Bou Khalil, Y. Ominami, A. Hisada, A. Fontanini, A. Caputo, A.  
336 Levasseur, J.-C. Lagier, S. Khelaifia, D. Raoult, Passive Filtration, Rapid Scanning Electron  
337 Microscopy, and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass  
338 Spectrometry for *Treponema* Culture and Identification from the Oral Cavity, *J. Clin.*  
339 *Microbiol.* 57 (2019). <https://doi.org/10.1128/JCM.00517-19>.
- 340 [32] H.T.T. Huynh, M. Pignoly, V.D. Nkamga, M. Drancourt, G. Aboudharam, The  
341 repertoire of archaea cultivated from severe periodontitis, *PLoS ONE.* 10 (2015) e0121565.  
342 <https://doi.org/10.1371/journal.pone.0121565>.
- 343 [33] C.O. Guindo, E. Terrer, E. Chabrière, G. Aboudharam, M. Drancourt, G. Grine,  
344 Culture of salivary methanogens assisted by chemically produced hydrogen, *Anaerobe.* 61  
345 (2020) 102128. <https://doi.org/10.1016/j.anaerobe.2019.102128>.
- 346 [34] A. Diakite, G. Dubourg, N. Dione, P. Afouda, S. Bellali, I.I. Ngom, C. Valles, M.L.  
347 Tall, J.-C. Lagier, D. Raoult, Optimization and standardization of the culturomics technique  
348 for human microbiome exploration, *Sci Rep.* 10 (2020) 9674. [https://doi.org/10.1038/s41598-](https://doi.org/10.1038/s41598-020-66738-8)  
349 [020-66738-8](https://doi.org/10.1038/s41598-020-66738-8).

350 [35] E. Guilhot, S. Khelaifia, B. La Scola, D. Raoult, G. Dubourg, Methods for culturing  
351 anaerobes from human specimen, *Future Microbiol.* 13 (2018) 369–381.  
352 <https://doi.org/10.2217/fmb-2017-0170>.

353

354

355

356 **Fig. 1:** Flow chart of bibliographical bacterial (A) and archaea (B) request.

357 **Fig. 2:** All 477 added species isolated at least once from humans by culture. Using the online  
358 tool wordle ([www.wordle.net](http://www.wordle.net)), the size of the name of each species is proportional to the  
359 number of times it occurs in the repertoire. The circles realized thanks to the cytoscape  
360 software ([www.cytoscape.org](http://www.cytoscape.org)) represent the partition of the culturomics technique, clinical  
361 microbiology and other culture techniques in this update of the repertoire. Red edges  
362 represent the contribution of IHU Méditerranée infection and green edges represent that of  
363 other laboratories. Orange dots at species level represent new taxa and the grey dots represent  
364 already known species other than humans.

365 **Fig. 3:** Evolution of the number of known bacterial and archaea species in humans through  
366 culture in 2015, 2018 and 2020. Percentage added in relation to the previous repertoire is  
367 represented by arrow. Orange proportion represents evolution of new species isolated from  
368 2015 to nowadays.

369 **Fig. 4:** Distribution in terms of phylum and genera of the 3253 species of the current human  
370 bacterial and archaea repertoire. In blue the distribution of phylum from the last update of the  
371 repertoire in 2018 by Bilen et al, and in orange the distribution of phylum added by this study.

372 **Fig. 5:** All 3253 species of the current human bacterial isolated at least once from humans  
373 using culture. Using the online tool wordart (<https://wordart.com/>), the size of the name of  
374 each species is proportional to the number of times it occurs in the database. Circle in dark

- 375 blue represent IHU Méditerranée infection contribution in the total repertoire and circle in
- 376 light blue represent species firstly isolated by IHU Méditerranée infection in environment.