

Culture of previously uncultured members of the human gut microbiota by culturomics

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Supplementary Tables 1 to 15

Supplementary Table 1: Main characteristics of the samples tested in the culturomics studies

	Category	N° samples	Sample Name	Origin	Clinical status	Age	Sex	BMI	N° colonies tested
Previous studies									
A	First study	3	Data previously described						32500
B	Previously published	352	Data previously described						47453
Total		355							79953
Current study									
C	70 conditions	12	Raiatea	French Polynesia	Healthy	39	M	24	12735
			Inde	India	Healthy	33	M	21	14482
			Polynésie8	French Polynesia	Obese	49	M	27	10674
			Amaz1	Amazonia	Healthy	10	F	A	17338
			Amaz2	Amazonia	Healthy	16	F	A	34700
			VIH 1	France	AIDS-infected patient	32	M	23	10290
			VIH3	France	AIDS-infected patient	46	M	21	16090
			HIV11	France	AIDS-infected patient	36	M	25	10630
			HIV12	France	AIDS-infected patient	21	M	19	9326
			HIV13	France	AIDS-infected patient	31	M	20	10136
			HIV4	France	AIDS-infected patient	72	F	22	8064
			HIV8	France	AIDS-infected patient	32	M	24	5800
D	18 conditions	40	Gab3	Gabon	Healthy	27	M	22	14868
			Gab5	Gabon	Healthy	16	M	19	14532
			SAOB1	Saudi Arabia	Obese individual	25	M	51	8538
			SA OB3	Saudi Arabia	Obese individual	24	M	52	23476
			SA OB6	Saudi Arabia	Obese individual	25	M	46	8896
			SAN1	Saudi Arabia	Healthy	25	M	20	16584
			SAN7	Saudi Arabia	Healthy	27	M	23	9732
			Touareg 8	Niger	Healthy	na	M	na	7600
			Marasme 03		Malnutrition				5700
			Marasme 06		Malnutrition				6800
			Marasme 9	Niger	Malnutrition	na	M	na	12753
			BD8	Saudi Arabia	Healthy	29	M	51,6	10254
			BD2	Saudi Arabia	Healthy	19	M	14	9200
			BD4	Saudi Arabia	Healthy	34	M	18.4	7200
			BD10	Saudi Arabia	Healthy	19	M	24.4	12564
			BD11	Saudi Arabia	Healthy	31	M	25.5	9897
			BD9	Saudi Arabia	Healthy	42	F	20.8	13024
			BD3	Saudi Arabia	Healthy	50	F	25.7	8200
			Control N6	Niger	Healthy	7 m 13	F	17	11474
			Control N2	Niger	Healthy	m	F	15	11352
			Control N12		Healthy				11686
			Control S07	Senegal	Healthy	3	F	15	11750
			Control S05	Senegal	Healthy	3	M	14	12540

		Control S500		Healthy				11008
		Kwashiorkor 5004	Senegal	Malnutrition	7 m	F	na	11402
		Kwashiorkor 1 (Niger)	Niger	Malnutrition	na	na	na	4895
		Kwashiorkor 01 (Senegal)	Senegal	Malnutrition	8 m	F	15	12422
		Kwashiorkor 02	Senegal	Malnutrition	4	F	15	17210
		Kwashiorkor 4	Senegal	Malnutrition	4 m	F	11	9312
		Kwashiorkor 5 (Senegal)	Senegal	Malnutrition	1	M	12	8402
		Kwashiorkor 6	Senegal	Malnutrition	6 m	M	12	8358
		Kwashiorkor 8 (Niger)	Niger	Malnutrition	na	na	na	9587
		Kwashiorkor 10 (Senegal)	Senegal	Malnutrition	2 m	M	13	9820
		Kwashiorkor 12	Niger	Malnutrition	na	na	na	7854
		Kwashiorkor 14	Niger	Malnutrition	na	na	na	8654
		Amaz10	Amazonia	Healthy				1020
		Amaz15	Amazonia	Healthy	10	M	na	1020
		Amaz14	Amazonia	Healthy				15693
		Amaz20	Amazonia	Healthy				15700
		Pygmies 1	Congo	Healthy	35	F	na	6500
E	Cohortes							
	Pilgrims	254	Nd	nd	nd	nd	nd	3000
	Bariatric surgery	30	Nd	nd	nd	nd	nd	33650
	Preterm	30	Nd	nd	nd	nd	nd	3000
	Acidophiles	16	Nd	nd	nd	nd	nd	12968
F	Fresh stools	28	Nd	France	Healthy	nd	nd	59688
G	Proteobacteria	110	Nd	nd	nd	nd	nd	18036
H	Microaerophilie	198	Nd	nd	nd	nd	nd	41392
I	Microcolonies	10	Nd	France	Healthy	nd	nd	9620
J	Halophiles	215	Nd	nd	nd	nd	nd	91220
K	Small bowel and Colonic	19	Nd	nd	nd	nd	nd	31048
Total		973						901,364

nd= not detailed ; na : not available, m= months

Supplementary Table 2A: The 70 most effective culture conditions selected after the pioneer study.

The 70 most effective culture conditions	
1.	5% sheep blood agar 0,45µm, Aerobic 37°C
2.	5% sheep blood agar Anaerobic, 37°C (Anaerobic cabinet)
3.	5% sheep blood agar, Aerobic 28°C
4.	5% sheep blood agar, Aerobic 37°C
5.	5% sheep blood agar, Anaerobic 28°C
6.	5% sheep blood agar, Anaerobic 37°C
7.	5% sheep blood agar, microaerophilic, 37°C
8.	BCP, Aerobic 37°C
9.	BCYE, Aerobe 37°C, 2,5% CO ₂
10.	Bordetella, Aerobic 37°C 2,5% CO ₂
11.	Brain Heart Infusion + 5% 0.2µm filtered stool, Aerobic 37°C
12.	Brain Heart Infusion + NaCl 15g/l, 37°C Aerobic
13.	Brain Heart Infusion + NaCl 1g/l, Aerobic 37°C
14.	Brain Heart Infusion + NaCl 3g/l, Aerobic 37°C
15.	Brain Heart Infusion + sheep blood 5%, Aerobic 37°C
16.	Brain Heart Infusion + sheep blood 5%, Anaerobic, 37°C
17.	Brain Heart Infusion + Vanco µg/l, Aerobic 37°C
18.	Brain Heart Infusion 3% NaCl, Anaerobic 37°C
19.	Brain Heart Infusion, 57°C Ae
20.	Brain Heart Infusion, Anaerobic 55°C
21.	Brucella, Aerobic 37°C
22.	CaCO ₃ , aerobe 37°C
23.	Co culture amoeba
24.	EMB, Aerobic 37°C
25.	Filtration 5% sheep blood agar 5µm Anaerobic , 37°C
26.	Filtration Brain Heart Infusion 0,45 µm, Aerobic 37°C
27.	Filtration Brain Heart Infusion 0.8 µm, Aerobic 37°C
28.	Filtration Brain Heart Infusion 0.8µm, Anaerobic 37°C
29.	Filtration Brain Heart Infusion 5µm, Aerobic 37°C
30.	Filtration Brain Heart Infusion 5µm, Anaerobic 37°C
31.	Glu Asp Aerobic 37°C
32.	HTM Aerobic 37°C, 2,5% CO ₂
33.	M17, Aerobic 37°C
34.	Marine medium, Aerobic 28°C
35.	MOD-2, Aerobic 37°C
36.	MRS, Anaerobic 37°C
37.	Mueller Hinton vancomycine 50µg/l, Aerobic
38.	Mueller Hinton Ae 37°C
39.	Orange Ae 37°C
40.	Passive filtration with BSKH, 5% sheep blood agar, Aerobic 37°C
41.	Passive filtration with Leptospira broth, 5% sheep blood agar, aerobic 37°C

42. Passive filtration with Leptospira broth, 5% sheep blood agar, microaerophilic 37°C
 43. Phage T1 + T4, then 5% sheep blood agar, aerobe 37°C
 44. Preincubation in blood culture bottle 11 days with rumen, 5% sheep blood agar, Anaerobic 37°C
 45. Preincubation in blood culture bottle 2 days with rumen + filtered stool, 5% sheep blood agar, Anaerobic 37°C
 46. Preincubation in blood culture bottle 26 days with rumen and sheep blood, 5% sheep blood agar Anaerobic 37°C
 47. Preincubation in blood culture bottle 3 days with 5 ml sheep blood, 5% sheep blood agar Anaerobic 37°C
 48. Preincubation in blood culture bottle 3 days with rumen and sheep blood, 5% sheep blood agar, Anaerobic 37°C
 49. Preincubation in blood culture bottle 4 days after thermic shock, 5% sheep blood agar, Aerobic 45°C
 50. Preincubation in blood culture bottle Anaerobic 10 days, Brain Heart Infusion, Anaerobic 37°C
 51. Preincubation in blood culture bottle Anaerobic 10 days, Schaedler Kana-Vanco, Anaerobic 37°C
 52. Preincubation in blood culture bottle Anaerobic 14 days with 8ml rumen fluid, 5% sheep blood agar, Anaerobic 37°C
 53. Preincubation in blood culture bottle Anaerobic 3 days with 8ml rumen fluid, 5% sheep blood agar, Anaerobic 37°C
 54. Preincubation in blood culture bottle Anaerobic 5 days, 5% sheep blood agar Anaerobic 37°C
 55. Preincubation in blood culture bottle Anaerobic 5 days, Schaedler kana vanco 37°C
 56. Preincubation in blood culture bottle with thioglycolate, 4 days, 5% sheep blood agar Anaerobic 37°C
 57. Preincubation in in blood culture bottle aerobic 3 days with 8ml rumen fluid, 5% sheep blood agar, Aerobic 37°C
 58. Preincubation in in blood culture bottle Anaerobic 30 days with 8ml rumen fluid, 5% sheep blood agar, Anaerobic 37°C
 59. PVX Ae 37°C 5% CO₂
 60. R2A, Aerobic 37°C
 61. Sabouraud 37°C
 62. Schaedler Kana Vanco, Anaerobic 37°C
 63. Schaedler, Anaerobic 37°C
 64. Thermic shock 65°C 5% sheep blood agar Ae 37°C
 65. Thermic shock, Preincubation in blood culture Anaerobic 15 days, 5% sheep blood agar, Anaerobic 37°C
 66. Thermic shock, Preincubation in blood culture Anaerobic 40 days, 5% sheep blood agar, Anaerobic 37°C
 67. Thermic shock, Preincubation in blood culture Anaerobic 50days, 5% sheep blood agar, Anaerobic 37°C
 68. Thioglycolate + agar + 5% human blood, microaerophilic 37°C
 69. TSA, Aerobic 37°C
 70. Wilkins Chalgren, Aerobic 37°C
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Supplementary Table 2B: The 18 standardized liquid culture conditions ranked in ascending number of species isolated.

Culturomics standardization by 18 culture conditions	
1.	Preincubation in aerobic blood culture bottle with stool filtered at 5µm, then 5% sheep blood agar, Aerobic condition, 37°C
2.	Preincubation in anaerobic blood culture bottle after thermic shock at 80°C during 20min, then 5% sheep blood agar, Anaerobic condition, 37°C
3.	Preincubation in anaerobic condition in marine broth, then 5% sheep blood agar, Anaerobic condition, 37°C
4.	Preincubation in aerobic blood culture bottle with 5ml sheep blood, then 5% sheep blood agar, Aerobic condition, 37°C
5.	Preincubation in aerobic marine broth, then 5% sheep blood agar, Aerobic condition 37°C
6.	Preincubation in aerobic blood culture bottle with rumen fluid and sheep blood, then 5% sheep blood agar, Aerobic condition, 37°C
7.	Preincubation in aerobic condition in trypticase soy broth, then 5% sheep blood agar, Aerobic condition, 37°C
8.	Preincubation in aerobic condition in 5% sheep blood broth, then 5% sheep blood agar, Aerobic condition, 37°C
9.	Preincubation in anaerobic blood culture bottle with stool filtered at 5µm, then 5% sheep blood agar, Anaerobic condition, 37°C
10.	Preincubation in aerobic condition in Brain Heart Infusion broth with 5% sheep blood, then 5% sheep blood agar, Aerobic condition, 37°C
11.	Preincubation in anaerobic condition in 5% sheep blood broth, then 5% sheep blood agar , Anaerobic condition, 28°C
12.	Preincubation in aerobic blood culture bottle with rumen fluid, then 5% sheep blood agar , Aerobic condition, 37°C
13.	Preincubation in aerobic condition in 5% sheep blood broth, then 5% sheep blood agar, Aerobic condition, 28°C
14.	Preincubation in anaerobic blood culture bottle with 5ml sheep blood, then 5% sheep blood agar , Anaerobic condition, 37°C
15.	Preincubation in anaerobic blood culture bottle, then 5% sheep blood agar , Anaerobic condition, 37°C
16.	Preincubation in anaerobic blood culture bottle with rumen fluid and sheep blood, then 5% sheep blood agar, Anaerobic condition, 37°C
17.	Preincubation in anaerobic condition in 5% sheep blood broth, then 5% sheep blood agar, Anaerobic condition, 37°C
18.	Preincubation in anaerobic blood culture bottle with rumen fluid, then 5% sheep blood agar , Anaerobic condition, 37°C

Supplementary Table 2C: Culture conditions used during the cohort that included 15 preterm neonates with necrotizing enterocolitis and the 15 controls.

54 culture conditions performed for the NEC cohort	
1.	5% Columbia sheep blood agar, aerobe, 37°C
2.	5% Columbia sheep blood agar, anaerobe, 37°C
3.	Chocolat Polyvitex, aerobe, 37°C
4.	Chocolat Polyvitex, anaerobe, 37°C
5.	Mueller Hinton, aerobe, 37°C
6.	Mueller Hinton, anaerobe, 37°C
7.	Schaedler Kana-Vanco + 5% sheep blood, aerobe, 37°C
8.	Schaedler Kana-Vanco + 5% sheep blood, anaerobe, 37°C
9.	Brain Heart Infusion Agar, aerobe, 37°C
10.	Brain Heart Infusion Agar, anaerobe, 37°C
11.	Brain Heart Infusion + sheep blood 5% + vancomycin 10 µg/ml, aerobe, 37°C
12.	Brain Heart Infusion + sheep blood 5% + vancomycin 10 µg/ml, anaerobe, 37°C
13.	Orange aerobe, aerobe, 37°C
14.	Bromocresol Purple, 37°C
15.	Inoculation blood culture bottle day 2 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, aerobe, 37°C
16.	Inoculation blood culture bottle day 2 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, anaerobe, 37°C
17.	Inoculation blood culture bottle day 3 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, aerobe, 37°C
18.	Inoculation blood culture bottle day 3 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, anaerobe, 37°C
19.	Inoculation blood culture bottle day 7 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, aerobe, 37°C
20.	Inoculation blood culture bottle day 7 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, anaerobe, 37°C
21.	Inoculation blood culture bottle day 14 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, aerobe, 37°C
22.	Inoculation blood culture bottle day 14 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, anaerobe, 37°C
23.	Inoculation blood culture bottle day 30 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, aerobe, 37°C
24.	Inoculation blood culture bottle day 30 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, anaerobe, 37°C
25.	Inoculation blood culture bottle day 2 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, aerobe, 37°C
26.	Inoculation blood culture bottle day 2 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, anaerobe, 37°C
27.	Inoculation blood culture bottle day 3 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, aerobe, 37°C
28.	Inoculation blood culture bottle day 3 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, anaerobe, 37°C
29.	Inoculation blood culture bottle day 7 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, aerobe, 37°C
30.	Inoculation blood culture bottle day 7 with 5 ml sheep blood, 10 mL Rumen, then

- Chocolat Polyvitex agar, anaerobe, 37°C
31. Inoculation blood culture bottle day 14 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, aerobic, 37°C
 32. Inoculation blood culture bottle day 14 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, anaerobe, 37°C
 33. Inoculation blood culture bottle day 30 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, aerobic, 37°C
 34. Inoculation blood culture bottle day 30 with 5 ml sheep blood, 10 mL Rumen, then Chocolat Polyvitex agar, anaerobe, 37°C
 35. Inoculation blood culture bottle day 2 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, aerobic, 37°C
 36. Inoculation blood culture bottle day 2 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, anaerobe, 37°C
 37. Inoculation blood culture bottle day 3 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, aerobic, 37°C
 38. Inoculation blood culture bottle day 3 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, anaerobe, 37°C
 39. Inoculation blood culture bottle day 7 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, aerobic, 37°C
 40. Inoculation blood culture bottle day 7 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, anaerobe, 37°C
 41. Inoculation blood culture bottle day 14 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, aerobic, 37°C
 42. Inoculation blood culture bottle day 14 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, anaerobe, 37°C
 43. Inoculation blood culture bottle day 30 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, aerobic, 37°C
 44. Inoculation blood culture bottle day 30 with 5 ml sheep blood, 10 mL Rumen, then Schaedler Kana-Vanco + 5% sheep blood agar, anaerobe, 37°C
 45. Inoculation blood culture bottle day 2 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, aerobic, 37°C
 46. Inoculation blood culture bottle day 2 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, anaerobe, 37°C
 47. Inoculation blood culture bottle day 3 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, aerobic, 37°C
 48. Inoculation blood culture bottle day 3 with 5 ml sheep blood, 10 mL Rumen, then 5% Columbia sheep blood agar, anaerobe, 37°C
 49. Inoculation blood culture bottle day 7 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, aerobic, 37°C
 50. Inoculation blood culture bottle day 7 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, anaerobe, 37°C
 51. Inoculation blood culture bottle day 14 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, aerobic, 37°C
 52. Inoculation blood culture bottle day 14 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, anaerobe, 37°C
 53. Inoculation blood culture bottle day 30 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, aerobic, 37°C
 54. Inoculation blood culture bottle day 30 with 5 ml sheep blood, 10 mL Rumen, then Brain Heart Infusion agar, anaerobe, 37°C
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Supplementary Table 3: List of the 477 species isolated from gut by other laboratories but not by the previously published works of culturomics, classified by phyla and by preferred atmosphere of growth.

Supplementary Table 4A: List of the 1,057 bacterial species isolated in this project; new species are highlighted in bold. We used the main categories than in Fig. 1 and in main manuscript. C= 70 culture conditions; D= 18 culture conditions; E= cohorts; F= Fresh stools; G= Proteobacteria project; H= Microaerophilic project; I= Halophilic bacteria project; J= Microcolonies detection; K= Small bowel and colonic samples. H (GUT) = previously known from human gut; NH : 1st isolation in human; H = 1st isolation in human gut but previously known from human; newspe= new species. X= species isolated in culturomics for the first time in this project.

Supplementary Table 4B: List of the 1,170 bacterial species isolated by culturomics including previously published studies (columns A and B). A= First culturomics study; B= other previously published studies; C= 70 culture conditions; D= 18 culture conditions; E= cohorts; F= Fresh stools; G= Proteobacteria project; H= Microaerophilic project; I= Halophilic bacteria project; J= Microcolonies detection; K= Small intestine and colonic samples. H(GUT) = previously known from human gut; NH : 1st isolation in human; H = 1st isolation in human gut but previously known from human; newspe= new species. X= species isolated in culturomics for the first time in this project.

Supplementary Table 5: List of the 247 new bacterial species isolated by culturomics (197 in the present study plus 50 previously reported species) with CSUR accession number, DSMZ accession number, and GenBank accession number of the 16SrRNA. For the bacterial species already described in the literature, the PMID is available, as well as the list number of the species already officially recognized by the International Committee of Taxonomy. Bacteria that we failed to subculture are highlighted by a star (*). New bacterial species published as ‘new species announcement’ are highlighted by a pound (#). The Archaea species are highlighted by two stars (**). Finally, the 50 new bacterial species are underlined in grey. *subculture failure; # archaea; NA : not available

Supplementary Table 6: Reads recovered by metagenomics from the HMP data. The 247 16S rRNA of new bacterial species are reported in the table. *subculture failure, # archaea

Supplementary Table7: Main characteristics of the genomes of the new bacterial species isolated by culturomics (genome size, ORFans number and percentage). *subculture failure, # archaea

Supplementary Table 8: Number of ORFans identified from our new species and retrieved from the assembly of whole metagenomic studies by HMP.

Species	ORFans finding
<i>Ihuella massiliensis</i>	136
<i>Butyricimonas phoceensis</i>	132
<i>Intestinimonas massiliensis</i>	112
<i>Clostridium phoceense</i>	99
<i>Bacteroides timonense</i>	95
<i>Alistipes ihumii</i>	95
<i>Blautia phoceensis</i>	83
<i>Clostridium saudii</i>	76
<i>Clostridium bouchedurhonense</i>	63
<i>Gabonia massiliensis</i>	55
<i>Polynesia massiliensis</i>	43
<i>Alistipes jeddahensis</i>	39
<i>Neglecta timonensis</i>	36
<i>Alistipes obesihominis</i>	35
<i>Blautia massiliensis</i>	25
<i>Sanguibacteroides massiliense</i>	22
<i>Megasphaera massiliensis</i>	21
<i>Guyana massiliensis*</i>	19
<i>Alistipes provencensis</i>	14
<i>Bacteroides neonati</i>	11
<i>Dakarella massiliensis</i>	9
<i>Ruminococcus phoceensis</i>	9
<i>Fournierella massiliensis</i>	8
<i>Alistipes senegalensis</i>	7
<i>Drancourtella massiliensis</i>	7
<i>Emergencia timonensis</i>	6
<i>Holdemania massiliensis</i>	6
<i>Alistipes timonensis</i>	6
<i>Stoquefichus massiliensis</i>	6
<i>Clostridium dakarene</i>	5
<i>Collinsella massilioamazoniensis</i>	5
<i>Intestinimonas gabonensis</i>	5
<i>Gorbachella massiliensis</i>	5
<i>Stoquefichus jeddahensis</i>	3
<i>Senegalimassilia anaerobia</i>	3
<i>Christensenella massiliensis</i>	2
<i>Ruminococcus massiliensis*</i>	2
<i>Eubacterium massiliense</i>	2
<i>Gorillibacterium timonense</i>	2
<i>Clostridium massiliosenegalense</i>	2
<i>Prevotella phoceensis</i>	2

<i>Christensenella timonensis</i>	1
<i>Clostridium nigeriense</i>	1
<i>Dielma fastidiosa</i>	1
<i>Peptoniphilus grossensis</i>	1
<i>Peptoniphilus obesihominis</i>	1
<i>Clostridium amazonitimonense</i>	1
<i>Bacillus massiliosenegalensis</i>	1
<i>Clostridium senegalense</i>	1
<i>Enterobacter massiliensis</i>	1
<i>Enorma massiliensis</i>	1
<i>Niameyia massiliensis</i>	1
<i>Anaerococcus rubiinfantis</i>	1
<i>Anaerotruncus rubiinfantis</i>	1
Total	1,326
*subculture failure	

Supplementary Table 9: List of the new species identified in the 396 and 239 human gut microbiome samples from Nielsen et al. and Browne et al., respectively^{15,16}. *subculture failure

Supplementary Table 10: List of the 84 metagenomes used for the study exploring the dark matter. We used the main categories than in Fig. 1 and in main manuscript : B= other previously published studies; C= 70 culture conditions; D= 18 culture conditions; E= cohorts; F= Fresh stools; I= Halophilic bacteria project;

Metagenomic code	Stool specimen	Category of project
A315	Amazonie 2	C
M1034	BLA bariatric (before)	E
M1030	MC bariatric (before)	E
M1015	BMI bariatric (after)	E
A309	Amazonie 20	D
M1019	GMT bariatric	E
A31	VIH8	C
A150	SAN-7	D
M933	LL bariatric (after)	E
A420	Kw 1	D
KW01	KW01 Senegal	D
M1046	CCH bariatric (before)	E
M631	BD4	D
M1037	DN bariatric (after)	E
M407	Senegalese Stool NDIOP 6	I
M932	LL bariatric (before)	E
M406	Senegalese Stool NDIOP 5	I
M638	BD11 Stool human bédouin	D
M1018	GMT bariatric (before)	E
M927	Senegalese Stool NDIOP 4	I
KW04	KW04	D
M405	Senegalese Stool NDIOP 4	I
M400	VIH 12_Protocole 5	C
M394	VIH 3_Protocole 5	C
M1016	MS bariatric (before)	E
M1017	MS bariatric (after)	E
A430	Kw 14	D
A144	SAN-1	D
KW06	KW 06	D
M404	Senegalese Stool NDIOP 3	J
M926	CC bariatric (before)	E
M1033	HA bariatric (after)	E
M901	N12 control Niger	D
M401	VIH 13_Protocole 5	C
A312	Amazonie 15	D
M1035	BLA bariatric (after)	E
S07	Control S07 Sénégal	D
M893	N2 control Niger	D

A428	Kw 12	D
M1032	HA bariatric (before)	E
M1047	CCH bariatric (after)	E
A310	Amazonie 1	C
M629	BD2 Stool human bédouin	D
A8	Touareg	D
A140	OB-6	D
GD2	Fresh stool 2	F
M1036	DN bariatric (before)	E
M1029	FE bariatric (after)	E
M1021	BC bariatric (after)	E
KW02	KW02	D
A307	Amazonie 14	D
M935	MM bariatric (after)	E
A418	MR9	D
M636	BD9 Stool human bédouin	D
A2	Réa 2	B
A39	Polynésie 8	C
A9	VIH1	C
A425	Kwashiorkor 8	D
M930	BM bariatric (before)	E
M1020	BC bariatric (before)	E
KW10	Kw 10	D
M1014	BMI bariatric (before)	E
A302	Amazonie 10	D
M637	BD10 Stool human bédouin	D
M897	N6 control Niger	E
M1031	MC bariatric (after)	E
A10	Réa 1	B
MR09	MR09	D
M929	ACZ bariatric (after)	E
A21	Inde	C
S05	Control S05 Sénégal	D
M931	BM bariatric (after)	E
A3	Raiatea	C
M928	ACZ bariatric (before)	E
A17	VIH4	C
A137	OB-3	D
M402	Senegalese Stool NDIOP 1	I
M635	BD8	D
M1028	FE bariatric (before)	E
M403	Senegalese Stool NDIOP 2	I
M934	MM bariatric (before)	E
A294	Touareg 8	D
A46	VIH11	C
A135	OB1	D

Supplementary Table 11: Identification of Operational Taxonomic Units (OTUs) and assigned species from the gut metagenomes: The information concerning these samples is available in Supplementary Table 10.

N° of sample	Sample Name	Total OTUs number	OTU's without identified species	Total of bacterial species	Non-Human bacteria	Non-gut bacteria bacteria	Human bacteria	New species	Gut bacteria not found by culturomics	Non-gut bacteria not found by culturomics
1	A315	800	751	49	2	1	17	12	13	4
2	M1034	450	379	71	3	6	37	9	13	3
3	M1030	499	445	54	4	4	20	12	14	0
4	M1015	432	350	82	4	6	49	9	8	6
5	A309	656	600	56	2	3	21	12	13	5
6	M1019	358	304	54	1	4	32	11	6	0
7	A31	853	801	52	3	3	17	13	14	2
8	A150	64	21	43	1	1	22	11	8	0
9	M933	1311	1237	74	3	5	33	14	12	7
10	A420	431	389	42	2	3	22	6	6	3
11	KW01	484	443	41	1	4	28	1	4	3
12	M1046	362	316	46	1	2	23	7	10	3
13	M631	541	483	58	4	4	24	11	13	2
14	M1037	238	194	44	1	3	24	7	6	3
15	M407	349	270	79	6	6	31	14	15	7
16	M932	1140	1087	53	1	5	26	7	12	2
17	M406	296	249	47	4	2	23	5	10	3
18	M638	554	495	59	3	2	31	6	13	4
19	M1018	342	305	37	1	0	20	8	8	0
20	M927	997	934	63	1	3	35	9	12	3
21	KW04	200	157	43	2	6	24	4	3	4
22	M405	374	309	65	11	1	18	14	14	7
23	M400	287	261	26	0	0	21	2	1	2
24	M394	430	389	41	1	2	18	11	7	2
25	M1016	301	265	36	2	0	15	7	11	1
26	M1017	454	399	55	4	0	26	8	16	1
27	A430	540	491	49	6	7	22	6	3	5
28	A144	83	20	63	3	4	28	12	9	7
29	KW06	125	92	33	0	5	21	3	3	1
30	M404	185	123	62	11	5	17	10	14	5
31	M926	951	876	75	0	5	43	10	9	8
32	M1033	380	326	54	1	3	32	6	9	3
33	M901	956	888	68	4	3	36	12	9	4
34	M401	286	261	25	1	1	12	5	5	1
35	A312	462	406	56	4	1	19	12	18	2
36	M1035	424	352	72	4	4	40	14	7	3
37	S07	807	750	57	5	6	30	6	5	5
38	M893	728	670	58	2	1	29	12	12	2
39	A428	527	486	41	2	4	29	2	2	2
40	M1032	426	368	58	1	3	30	13	11	0
41	M1047	459	399	60	6	3	26	8	16	1
42	A310	596	539	57	4	1	22	10	14	6
43	M629	692	589	103	4	8	48	13	13	17
44	A8	439	366	73	6	1	31	25	10	0
45	A140	94	39	55	1	2	24	10	16	2
46	GD2	119	105	14	0	0	7	6	0	1
47	M1036	274	236	38	0	2	20	8	7	1
48	M1029	302	249	53	1	3	25	14	10	0
49	M1021	275	236	39	1	1	20	7	10	0
50	KW02	1084	1021	63	3	1	43	7	4	5
51	A307	638	590	48	3	0	16	11	14	4
52	M935	1217	1126	91	4	9	42	18	14	4
53	A418	248	210	38	2	5	25	4	1	1
54	M636	815	759	56	3	2	27	8	14	2
55	A2	135	96	39	0	1	21	13	2	2
56	A39	276	234	42	2	0	22	9	8	1
57	A9	625	567	58	0	1	32	15	9	1
58	A425	189	143	46	2	3	24	8	7	2
59	M930	1202	1161	41	2	1	18	8	11	1
60	M1020	313	275	38	2	0	22	6	6	2
61	KW10	1429	1354	75	2	2	45	17	8	1
62	M1014	416	364	52	0	1	27	12	6	6
63	A302	616	570	46	2	0	18	10	14	2
64	M637	611	559	52	2	1	27	9	11	2
65	M897	1320	1231	89	5	0	45	18	16	5
66	M1031	605	544	61	3	2	29	13	13	1

67	A10	478	435	43	2	4	26	8	2	1
68	MR09	484	457	27	0	2	17	3	3	2
69	M929	1445	1345	100	2	6	51	20	19	2
70	A21	407	371	36	0	1	18	7	10	0
71	S05	1925	1843	82	2	1	38	16	18	7
72	M931	844	767	77	5	4	33	15	10	10
73	A3	131	110	21	0	0	17	2	1	1
74	M928	1242	1167	75	5	6	39	7	6	12
75	A17	812	743	69	3	2	36	13	15	0
76	A137	91	12	79	4	5	39	10	13	8
77	M402	318	275	43	9	0	12	7	11	4
78	M635	522	467	55	3	1	20	11	14	6
79	M1028	378	326	52	2	7	28	6	8	1
80	M403	275	220	55	7	0	15	10	17	6
81	M934	723	641	82	5	10	37	14	13	3
82	A294	868	802	66	5	0	23	14	20	4
83	A46	430	384	46	1	1	22	12	8	2
84	A135	73	15	58	3	2	28	12	11	2
Total unique		4158	3602	556	47	61	210	102	50	86
Culturomics (420)								Not Culturomics (136)		

Supplementary Table 12: List of the 120 species isolated by Browne et al.¹⁵

Phylum	closest 16S BLAST match to named bacterium-99 or 100% match unless otherwise stated	Novelty	Type of species
1 Bacteroidetes	<i>Alistipes finegoldii</i>	characterised	H(Gut)
2 Firmicutes	<i>Anaerostipes hadrus</i>	characterised	H(Gut)
3 Firmicutes	<i>Anaerostipes hadrus</i> _98%	novel species	NS
4 Firmicutes	<i>Anaerotruncus colihominis</i>	characterised	H(Gut)
5 Firmicutes	<i>Anaerotruncus colihominis</i> _91%	novel genus	NS
6 Bacteroidetes	<i>Bacteroides caccae</i>	characterised	H(Gut)
7 Bacteroidetes	<i>Bacteroides coprocola</i> _94%	novel species	NS
8 Bacteroidetes	<i>Bacteroides finegoldii</i>	characterised	H(Gut)
9 Bacteroidetes	<i>Bacteroides finegoldii</i> _98%	novel species	NS
10 Bacteroidetes	<i>Bacteroides intestinalis</i> _98%	novel species	NS
11 Bacteroidetes	<i>Bacteroides ovatus</i>	characterised	H(Gut)
12 Bacteroidetes	<i>Bacteroides plebius</i> _95%	novel species	NS
13 Bacteroidetes	<i>Bacteroides salyersiae</i>	characterised	H(Gut)
14 Bacteroidetes	<i>Bacteroides thetaiotaomicron</i>	characterised	H(Gut)
15 Bacteroidetes	<i>Bacteroides uniformis</i>	characterised	H(Gut)
16 Bacteroidetes	<i>Bacteroides vulgatus</i>	characterised	H(Gut)
17 Bacteroidetes	<i>Bacteroides xylanisolvens</i>	characterised	H(Gut)
18 Firmicutes	<i>Balutia luti</i> _96%	novel species	NS
19 Actinobacteria	<i>Bifidobacterium adolescentis</i>	characterised	H(Gut)
20 Actinobacteria	<i>Bifidobacterium bifidum</i>	characterised	H(Gut)
21 Actinobacteria	<i>Bifidobacterium pseudocatenulatum</i>	characterised	H(Gut)
22 Firmicutes	<i>Blautia hydrogenotrophica</i>	characterised	H(Gut)
23 Firmicutes	<i>Blautia hydrogenotrophica</i> _96%	novel genus	NS
24 Firmicutes	<i>Blautia luti</i> _95%	novel species	NS
25 Firmicutes	<i>Blautia luti</i> _96%	novel species	NS
26 Firmicutes	<i>Blautia luti</i> _98%	novel species	NS
27 Firmicutes	<i>Blautia obesum</i>	characterised	H(Gut)
28 Firmicutes	<i>Blautia producta</i> _94%	novel species	NS
29 Firmicutes	<i>Blautia wexlerae</i>	characterised	H(Gut)
30 Firmicutes	<i>Butyrivibrio pullicaecorum</i> _94%	novel species	NS
31 Firmicutes	<i>Butyrivibrio pullicaecorum</i> _94%	novel species	NS
32 Firmicutes	<i>Catenibacterium mitsuokai</i>	characterised	H(Gut)
33 Firmicutes	<i>Clostridium baratti</i>	characterised	H(Gut)
34 Firmicutes	<i>Clostridium bartlettii</i>	characterised	H(Gut)
35 Firmicutes	<i>Clostridium boltae</i> _94%	novel species	NS
36 Firmicutes	<i>Clostridium celerecrescens</i> _93%	novel genus	NS
37 Firmicutes	<i>Clostridium celerecrescens</i> _93%	novel genus	NS
38 Firmicutes	<i>Clostridium clostridioforme</i>	characterised	H(Gut)
39 Firmicutes	<i>Clostridium clostridioforme</i> _93%	novel genus	NS
40 Firmicutes	<i>Clostridium clostridioforme</i> _98%	novel species	NS
41 Firmicutes	<i>Clostridium cocleatum</i> _93%	novel species	NS
42 Firmicutes	<i>Clostridium disporicum</i>	characterised	H(Gut)
43 Firmicutes	<i>Clostridium disporicum</i> _98%	novel species	NS

44	Firmicutes	<i>Clostridium ghonii</i> _98%	novel species	NS
45	Firmicutes	<i>Clostridium hathewayi</i>	characterised	H(Gut)
46	Firmicutes	<i>Clostridium hathewayi</i> _92%	novel genus	NS
47	Firmicutes	<i>Clostridium innocuum</i>	characterised	H(Gut)
48	Firmicutes	<i>Clostridium innocuum</i> _95%	novel species	NS
49	Firmicutes	<i>Clostridium lituseburense</i> _98%	novel species	NS
50	Firmicutes	<i>Clostridium methylpentosum</i> _92%	novel species	NS
51	Firmicutes	<i>Clostridium nexile</i> _94%	novel species	NS
52	Firmicutes	<i>Clostridium oroticum</i> _95%	novel genus	NS
53	Firmicutes	<i>Clostridium oroticum</i> _95%	novel genus	NS
54	Firmicutes	<i>Clostridium oroticum</i> _96%	novel species	NS
55	Firmicutes	<i>Clostridium paraputrificum</i>	characterised	H(Gut)
56	Firmicutes	<i>Clostridium perfringens</i>	characterised	H(Gut)
57	Firmicutes	<i>Clostridium saccharogumia</i> _93%	novel species	NS
58	Firmicutes	<i>Clostridium saccharolyticum</i> _94%	novel species	NS
59	Firmicutes	<i>Clostridium thermocellum</i> _86%	novel family 2	NS
60	Firmicutes	<i>Clostridium thermocellum</i> _87%	novel genus	NS
61	Firmicutes	<i>Clostridium xylanolyticum</i> _95%	novel genus	NS
62	Firmicutes	<i>Clostridium xylanolyticum</i> _96%	novel species	NS
63	Actinobacteria	<i>Collinsella aerofaciens</i>	characterised	H(Gut)
64	Actinobacteria	<i>Collinsella aerofaciens</i> _92%	novel species	NS
65	Firmicutes	<i>Coprococcus comes</i>	characterised	H(Gut)
66	Firmicutes	<i>Coprococcus eutactus</i>	characterised	H(Gut)
67	Firmicutes	<i>Coprococcus eutactus</i> _97%	novel species	NS
68	Firmicutes	<i>Dorea formicigenerans</i> _98%	novel species	NS
69	Firmicutes	<i>Dorea longicatena</i>	characterised	H(Gut)
70	Firmicutes	<i>Eubacterium contortum</i>	characterised	H
71	Firmicutes	<i>Eubacterium contortum</i> _97%	novel genus	NS
72	Firmicutes	<i>Eubacterium eligens</i>	characterised	H(Gut)
73	Firmicutes	<i>Eubacterium fissicatens</i> _95%	novel species	NS
74	Firmicutes	<i>Eubacterium hallii</i>	characterised	H(Gut)
75	Firmicutes	<i>Eubacterium hallii</i> _97%	novel species	NS
76	Firmicutes	<i>Eubacterium infirmum</i> _91%	novel family 1	NS
77	Firmicutes	<i>Eubacterium ramulus</i>	characterised	H(Gut)
78	Firmicutes	<i>Eubacterium rectale</i>	characterised	H(Gut)
79	Firmicutes	<i>Eubacterium siraeum</i>	characterised	H(Gut)
80	Firmicutes	<i>Faecalibacterium prausnitzii</i>	characterised	H(Gut)
81	Firmicutes	<i>Faecalibacterium prausnitzii</i> _98%	novel species	NS
82	Firmicutes	<i>Flavonifractor plautii</i>	characterised	H(Gut)
83	Firmicutes	<i>Flavonifractor plautii</i> _94%	novel species	NS
84	Firmicutes	<i>Flavonifractor plautii</i> _95%	novel species	NS
85	Firmicutes	<i>Flavonifractor plautii</i> _95%	novel genus	NS
86	Firmicutes	<i>Flavonifractor plautii</i> _95%	novel species	NS
87	Firmicutes	<i>Flavonifractor plautii</i> _96%	novel genus	NS
88	Firmicutes	<i>Flavonifractor plautii</i> _97%	novel genus	NS
89	Firmicutes	<i>Fusicatenibacter saccharivorans</i>	novel genus	NS
90	Firmicutes	<i>Fusicatenibacter saccharivorans</i> _93%	novel genus	NS
91	Firmicutes	<i>Lachnospira pectinoschiza</i>	characterised	NH

92	Firmicutes	<i>Lachnospira pectinoschiza</i> 91%	novel genus	NS
93	Firmicutes	<i>Megasphaera elsdenii</i> 95%	novel species	NS
94	Firmicutes	<i>Mitsuokella jalaludinii</i>	characterised	H(Gut)
95	Firmicutes	<i>Oscillibacter valericigenes</i> 96%	novel species	NS
96	Bacteroidetes	<i>Parabacteroides distasonis</i>	characterised	H(Gut)
97	Bacteroidetes	<i>Parabacteroides merdae</i>	characterised	H(Gut)
98	Bacteroidetes	<i>Prevotella copri</i>	characterised	H(Gut)
99	Firmicutes	<i>Roseburia faecis</i>	characterised	H(Gut)
100	Firmicutes	<i>Roseburia faecis</i> 95%	novel genus	NS
101	Firmicutes	<i>Roseburia hominis</i>	characterised	H(Gut)
102	Firmicutes	<i>Roseburia intestinalis</i>	characterised	H(Gut)
103	Firmicutes	<i>Roseburia inulinivorans</i> 94%	novel species	NS
104	Firmicutes	<i>Roseburia inulinivorans</i>	characterised	H(Gut)
105	Firmicutes	<i>Ruminococcus albus</i> 95%	novel species	NS
106	Firmicutes	<i>Ruminococcus albus</i> 98%	novel species	NS
107	Firmicutes	<i>Ruminococcus bromii</i>	characterised	H(Gut)
108	Firmicutes	<i>Ruminococcus bromii</i> 93%	novel species	NS
109	Firmicutes	<i>Ruminococcus bromii</i> 94%	novel species	NS
110	Firmicutes	<i>Ruminococcus flavefaciens</i> 93%	novel genus	NS
111	Firmicutes	<i>Ruminococcus flavefaciens</i> 95%	novel species	NS
112	Firmicutes	<i>Ruminococcus gnavus</i>	characterised	H(Gut)
113	Firmicutes	<i>Ruminococcus gnavus</i> 98%	novel species	NS
114	Firmicutes	<i>Ruminococcus obeum</i>	characterised	H(Gut)
115	Firmicutes	<i>Ruminococcus obeum</i> 96%	novel species	NS
116	Firmicutes	<i>Ruminococcus obeum</i> 98%	novel species	NS
117	Firmicutes	<i>Ruminococcus torques</i>	characterised	H(Gut)
118	Firmicutes	<i>Ruminococcus torques</i> 97%	novel genus	NS
119	Firmicutes	<i>Sarcina ventriculi</i>	characterised	H(Gut)
120	Firmicutes	<i>Turicibacter sanguinis</i>	characterised	H(Gut)

Supplementary Table 13: List of the bacterial species formerly isolated by culturomics and then identified in our clinical microbiology laboratory in La Timone, Marseille, France.

Strain	CSUR accession number	Type of samples	N° of time isolated
<i>Actinomyces grossensis</i>	CSURP242	Puncture fluid	4
		Abcesses	1
<i>Actinomyces ihumii</i>	CSURP2006	Abcesses	6
<i>Anaerococcus senegalensis</i>	CSURP156	Puncture fluid	1
<i>Anaerosalibacter massiliensis</i>	CSURP762	Diarrhea	1
<i>Bacteroides timonense</i>	CSURP194	Blood culture	3
<i>Butyrivibrio phoceensis</i>	CSURP2478	Diarrhea	1
<i>Clostridium jedahtimonense</i>	CSURP1230	Diarrhea	7
<i>Clostridium massilioamazoniensis</i>	CSURP1360	Diarrhea	1
<i>Clostridium saudii</i>	CSURP697	Diarrhea	12
<i>Peptoniphilus grossensis</i>	CSURP184	Abcesses	5
		Biopsies	6
		Material	1
		Puncture fluid	5
		Peritoneal fluid	1
<i>Polynesia massiliensis</i>	CSURP1280	Urine	1
<i>Pseudomonas massiliensis</i>	CSURP1334	Skin	1
Total			57

Supplementary Table 14: Bacterial species isolated by culturomics, unidentified by MALDI-TOF and requiring 16SrRNA amplification and sequencing for identification.

Supplementary Table 15: Genome of the bacterial species isolated from the gastrointestinal tract and referenced by the Human Microbiome Project (available 29 March 2016). The bacterial species officially recognized by the International Committee on Systematic Bacteriology are highlighted in green.