Supplementary Materials

2 Fig. S1 In-house program used for processing of raw pyrosequencing data and

3 analysis of the pyrosequencing data at species level.



5

1

Fig. S2 PCR-DGGE profiles of duplicated chemostat experiments conducted 6 under the same operation conditions with two inocula collected from the same 7 8 donor at ten months intermittence. A. DGGE profile of V3 region of 16S rRNA gene^a; B. Phylogenetic tree based on UPGMA method analyzed by Quantity One. 9 10 ^aHU08 and HU09 represent the different fecal inocula of the same donor collected in 11 2008 and 2009. HF represents the chemostat samples inoculated with human feces. 12 08VI, 09VI, 08VL and 09VL represent the chemostat samples cultured with VI and VL media in 2008 and 2009. M represents markers used for DGGE profiles. 13



14



¹⁷ ^aSamples were collected from duplicated chemostat experiments conducted under the ¹⁸ same operation conditions with two human fecal inocula taken from the same donor at ¹⁹ ten months intermittence. HU08 and HU09 represent the different fecal inocula of the ²⁰ same donor collected in 2008 and 2009. HF08VI, HF09VI, HF08VL and HF09VL ²¹ represent the chemostats samples with VI and VL media conducted in 2008 and 2009.





Fig. S4 The effect of sequencing reads on the patterns of human fecal microbiota
compositions^a.

^aPearson correlation between samples was analyzed by SPSS software based on the percentage of each bacterial classification unit at genus levels. Un represents

29 unclassified.





| Target bacterial group | Primer name | Primer sequence (5'-3') | Amplicon size (bp) | Annealing temp (\mathcal{C}) | Reference |
|-----------------------------|----------------|---------------------------|-----------------------|----------------------------------|-----------|
| Total bastaria | Bac1114F | CGGCAACGAGCGCAACCC | 161 | 66 | (5) |
| Total Dacteria | Bac1275R | CCATTGTAGCACGTGTGTAGCC | 101 | 00 | (3) |
| Destausidas anous | Bfra-F | ATAGCCTTTCGAAAGRAAGAT | 405 | 50 | (8) |
| Bacterolaes group | Bfra-R | CCAGTATCAACTGCAATTTTA | 495 | 50 | (8) |
| Clostridium | CcocF | CGGTACCTGACTAAGAAGC | 420 | 55 | (11) |
| coccoides-Eubacterium group | CcocR | AGTTTYATTCTTGCGAACG | 429 | 55 | (11) |
| Frederick | Eco1457F | CATTGACGTTACCCGCAGAAGAAGC | 105 | (2) | (1) |
| Enterobacteriaceae | Eco1652R | CTCTACGAGACTCAAGCTTGC | 195 | 03 | (1) |
| | Bif164F | GGGTGGTAATGCCGGATG | 427 | 50 | (1) |
| Bifidobacterium genus | Bif601R | TAAGCCATGGACTTTCACACC | 437 | 59 | (1) |
| Lactobacillus group | LactoF | TGGAAACAGRTGCTAATACCG | 222 | (\mathbf{c}) | |
| | LactoR | GTCCATTGTGGAAGATTCCC | 233 | 62 | (0) |

Table S1 PCR primers used for qPCR assay.

| Subjects ^b | HU | HFVI | HFVL | СН | CFVI | CFVL |
|-------------------------|--------|--------|--------|--------|--------|--------|
| No of sequences | 1751 | 1456 | 1043 | 1684 | 1643 | 1082 |
| No of OTUs (98% cutoff) | 141 | 143 | 96 | 308 | 69 | 91 |
| ACE | 280.58 | 221.04 | 247.65 | 864.66 | 173.71 | 218.92 |
| Shannon | 3.38 | 3.38 | 2.35 | 4.75 | 1.46 | 2.7 |
| Chao | 200.04 | 204.11 | 172.56 | 568.55 | 128.5 | 164.93 |
| Evenness ^c | 0.45 | 0.46 | 0.34 | 0.64 | 0.20 | 0.39 |
| Good's coverage (%) | 96.69 | 95.95 | 95.21 | 90.68 | 97.87 | 95.75 |

33 Table S2 Basic information for biodiversity analysis of pyrosequencing^a.

^aBasic information calculated using Mothur (version 1.11.0).

35 ^bHU and CH represent inocula of human feces and chicken cecal contents. HFVI,

36 HFVL, CFVI and CFVL represent chemostat products of human fecal and chicken

37 cecal microbiota grown in VI and VL media.

³⁸ ^cThe Shannon index of evenness was calculated with the formula E = H/ln(S), where

39 H is the Shannon diversity index and S is the total number of sequences in that group.

| Genus | $\mathbf{HU}^{\mathbf{b}}$ | | CH ^b | |
|------------------------------|------------------------------|--------|------------------------------|--------|
| Faecalibacterium | Faecalibacterium prausnitzii | 23.47% | Faecalibacterium prausnitzii | 7.66% |
| Bacteroides | Bacteroides vulgatus | 31.88% | Bacteroides plebeius | 6.77% |
| | | | Bacteroides uniformis | 1.43% |
| | | | Bacteroides clarus | 1.71% |
| | | | Bacteroides faecis | 0.95% |
| | | | Bacteroides barnesiae | 0.51% |
| | | 31.88% | | 11.37% |
| Unclassified Lachnospiraceae | Eubacterium eligens | 1.00% | Eubacterium xylanophilum | 3.38% |
| | Eubacterium fissicatena | 0.86% | Eubacterium fissicatena | 0.83% |
| | Eubacteriaceae bacterium | 1.14% | Ruminococcus gnavus | 0.48% |
| | Butyrate-producing bacterium | 0.69% | Coprococcus comes | 0.48% |
| | Eggerthella lenta | 1.88% | | |
| | Lachnospira pectinoschiza | 5.54% | | |
| | Lachnospiraceae bacterium | 1.58% | Clostridium methylpentosum | 0.47% |
| | Clostridium saccharolyticum | 0.99% | Clostridium hathewayi | 0.36% |
| | Clostridium populeti | 0.29% | Clostridium populeti | 1.66% |
| | | 13.97% | | 7.66% |
| Parabacteroides | Parabacteroides merdae | 0.34% | Parabacteroides merdae | 11.46% |
| | Parabacteroides distasonis | 0.18% | Parabacteroides johnsonii | 2.08% |
| | | 0.52% | | 13.54% |
| Roseburia | Roseburia intestinalis | 15.63% | Roseburia faecis | 3.21% |

40 Table S3 Comparative composition of major genera at species level between human fecal and chicken cecal microbiota^a

- 41 ^a Sequences with 98% similarity are regarded as the same OTU.
- 42 ^bHU, human fecal microbiota; CH, chicken cecal microbiota.

43 Table S4 Comparative microbiota compositions of human fecal and chicken cecal

44 inocula and their corresponded chemostat products grown in VI and VL media

45 at genus level^a

| | % in total sequences | | | | | | |
|----------------------------------|----------------------|-------|-------|-------|-------|-------|--|
| Genus | HU^b | HFVI | HFVL | СН | CFVI | CFVL | |
| Parabacteroides | 0.51 | 2.95 | 0.38 | 11.76 | 0 | 0.46 | |
| Bacteroides | 31.81 | 50.62 | 3.84 | 9.98 | 0.12 | 12.85 | |
| unclassified Lachnospiraceae | 13.94 | 21.43 | 70.76 | 6.83 | 0 | 4.07 | |
| Faecalibacterium | 23.42 | 0 | 0 | 6.65 | 0 | 0.19 | |
| Naxibacter | 0 | 0 | 0 | 5.17 | 0 | 0 | |
| unclassified Ruminococcaceae | 0.34 | 2.20 | 0 | 3.27 | 0 | 8.97 | |
| Olsenella | 0 | 0 | 0 | 3.09 | 0 | 0 | |
| unclassified Oxalobacteraceae | 0 | 0 | 0 | 3.09 | 0 | 0 | |
| Roseburia | 15.59 | 4.46 | 0 | 2.79 | 0 | 0.09 | |
| Subdoligranulum | 0 | 0 | 0 | 2.32 | 0 | 3.51 | |
| Paraprevotella | 0 | 0 | 0 | 2.26 | 0 | 0.000 | |
| Escherichia/Shigella | 0 | 0.07 | 0.10 | 2.14 | 0.06 | 0.09 | |
| Oscillibacter | 0.29 | 0.76 | 0 | 2.08 | 0.06 | 9.98 | |
| unclassified Flavobacteriaceae | 0 | 0.07 | 0.10 | 2.02 | 0 | 0 | |
| unclassified Prevotellaceae | 0 | 0 | 0 | 1.96 | 0 | 0.19 | |
| Prevotella | 0.06 | 0 | 0 | 1.84 | 0 | 4.71 | |
| Megamonas | 0 | 0 | 0 | 1.54 | 0 | 0 | |
| Succiniclasticum | 0 | 0 | 0 | 1.49 | 0 | 0 | |
| unclassified Erysipelotrichaceae | 0.34 | 0.07 | 0.86 | 1.37 | 0 | 0.09 | |
| Enterococcus | 0 | 0 | 0.48 | 1.37 | 0 | 0 | |
| Streptococcus | 0 | 0 | 0.10 | 1.37 | 0 | 0 | |
| Slackia | 0 | 0 | 0 | 1.37 | 0 | 0 | |
| Fastidiosipila | 0 | 0 | 0 | 1.31 | 0 | 15.62 | |
| unclassified Porphyromonadaceae | 0 | 0 | 0 | 0.95 | 0 | 4.62 | |
| Cloacibacillus | 0 | 0.28 | 0 | 0.71 | 0 | 0 | |
| Janthinobacterium | 0 | 0 | 0.19 | 0.65 | 0 | 0.19 | |
| Alistipes | 0.40 | 3.30 | 0. | 0.59 | 0 | 0 | |
| Bifidobacterium | 0 | 0 | 0 | 0.53 | 79.37 | 0 | |
| unclassified Comamonadaceae | 0 | 0 | 0 | 0.53 | 0 | 0 | |
| unclassified Coriobacteriaceae | 0 | 0 | 0 | 0.48 | 0.06 | 0 | |
| Massilia | 0 | 0 | 0 | 0.48 | 0 | 0 | |
| Coprococcus | 2.34 | 0.69 | 0.19 | 0.36 | 0 | 2.96 | |
| Eubacterium | 0 | 0 | 0 | 0.36 | 0 | 0 | |
| Pseudomonas | 0.06 | 0 | 0.10 | 0.30 | 0.24 | 0 | |
| Hespellia | 0.06 | 0 | 0 | 0.30 | 0 | 0 | |
| Collinsella | 0 | 0 | 0 | 0.30 | 0 | 0 | |

| Butyricicoccus | 0.11 | 0.14 | 0.29 | 0.24 | 0 | 0.92 |
|----------------------------------|------|------|------|------|------|------|
| Blautia | 0.57 | 0 | 0.38 | 0.24 | 0 | 0 |
| Methylobacterium | 0.06 | 0 | 0 | 0.24 | 0 | 0 |
| Peptococcus | 0 | 0 | 0 | 0.24 | 0 | 0 |
| Marvinbryantia | 0 | 0 | 0 | 0.24 | 0 | 0 |
| Pseudoramibacter | 0 | 0 | 0 | 0.24 | 0 | 0 |
| Butyrivibrio | 0.57 | 0.41 | 2.01 | 0.18 | 0 | 0.28 |
| Desulfonatronum | 0 | 0 | 0 | 0.18 | 0 | 0.19 |
| Coprobacillus | 1.26 | 0 | 0 | 0.18 | 0 | 0 |
| Catenibacterium | 0 | 0 | 0.10 | 0.18 | 0 | 0 |
| Hallella | 0 | 0 | 0 | 0.18 | 0 | 0 |
| inclassified Veillonellaceae | 0 | 0 | 0 | 0.18 | 0 | 0 |
| Acetanaerobacterium | 0 | 0.34 | 0 | 0.12 | 0 | 2.31 |
| Moryella | 0 | 0 | 0 | 0.12 | 0 | 0 |
| Lactococcus | 0 | 0 | 0.77 | 0.06 | 0 | 0.09 |
| Dorea | 0.46 | 0 | 0.77 | 0.06 | 0 | 0 |
| Butyricimonas | 0.29 | 0 | 0 | 0.06 | 0 | 0 |
| Barnesiella | 0 | 0 | 0.38 | 0.06 | 0 | 0 |
| Anaerofilum | 0 | 0 | 0 | 0.06 | 0 | 0 |
| ΓM7 | 0 | 0 | 0 | 0.06 | 0 | 0 |
| Megasphaera | 0 | 0 | 0 | 0.06 | 0 | 0 |
| unclassified Actinosynnemataceae | 0 | 0 | 0 | 0.06 | 0 | 0 |
| unclassified Alcaligenaceae | 0 | 0 | 0 | 0.06 | 0 | 0 |
| Weeksella | 0.17 | 0.55 | 0.10 | 0 | 0 | 9.34 |
| Desulfovibrio | 0 | 0 | 0 | 0 | 0 | 0.65 |
| Hydrogenoanaerobacterium | 0 | 0 | 0 | 0 | 0 | 0.37 |
| Ethanoligenens | 0 | 0.07 | 0 | 0 | 0 | 0.19 |
| Clostridium | 0 | 0 | 0.67 | 0 | 0.18 | 0.09 |
| unclassified Clostridiaceae 1 | 0 | 0 | 0 | 0 | 0 | 0.09 |
| Veillonella | 0.06 | 0 | 0.10 | 0 | 8.34 | 0 |
| Lactobacillus | 0 | 0 | 0 | 0 | 7.30 | 0 |
| unclassified Bifidobacteriaceae | 0 | 0 | 0 | 0 | 3.53 | 0 |
| Alloscardovia | 0 | 0 | 0 | 0 | 0.43 | 0 |
| Eggerthella | 0 | 0 | 0 | 0 | 0.24 | 0 |
| Fusobacterium | 3.54 | 1.10 | 3.26 | 0 | 0 | 0 |
| Sutterella | 1.83 | 2.20 | 0.77 | 0 | 0 | 0 |
| Phascolarctobacterium | 0.34 | 0.07 | 0.19 | 0 | 0 | 0 |
| Parasporobacterium | 0.29 | 0 | 0 | 0 | 0 | 0 |
| Catonella | 0.29 | 0 | 0 | 0 | 0 | 0 |
| Odoribacter | 0.23 | 0 | 0 | 0 | 0 | 0 |
| Acinetobacter | 0.11 | 0 | 0 | 0 | 0 | 0 |
| Anaerobacter | 0.11 | 0 | 0 | 0 | 0 | 0 |
| Haemophilus | 0.11 | 0 | 0 | 0 | 0 | 0 |
| Sporacetigenium | 0.11 | 0 | 0 | 0 | 0 | 0 |

| Sporobacterium | 0.06 | 0.34 | 0 | 0 | 0 | 0 |
|---------------------------------|------|------|------|---|---|---|
| Turicibacter | 0.06 | 0 | 0 | 0 | 0 | 0 |
| Anaerosporobacter | 0 | 1.79 | 8.44 | 0 | 0 | 0 |
| Pyramidobacter | 0 | 0.55 | 1.34 | 0 | 0 | 0 |
| Pantoea | 0 | 0 | 0.96 | 0 | 0 | 0 |
| Anaerostipes | 0 | 0 | 0.67 | 0 | 0 | 0 |
| Dialister | 0 | 0 | 0.58 | 0 | 0 | 0 |
| unclassified Enterobacteriaceae | 0 | 0 | 0.29 | 0 | 0 | 0 |
| Enterobacter | 0 | 0 | 0.29 | 0 | 0 | 0 |
| Solobacterium | 0 | 0 | 0.19 | 0 | 0 | 0 |
| Providencia | 0 | 0.28 | 0.10 | 0 | 0 | 0 |
| Cronobacter | 0 | 0 | 0.10 | 0 | 0 | 0 |
| Anaerovorax | 0 | 1.92 | 0 | 0 | 0 | 0 |
| Ruminococcus | 0 | 1.03 | 0 | 0 | 0 | 0 |
| Lawsonia | 0 | 0.14 | 0 | 0 | 0 | 0 |
| Erysipelothrix | 0 | 0.14 | 0 | 0 | 0 | 0 |
| Holdemania | 0 | 0.14 | 0 | 0 | 0 | 0 |
| Akkermansia | 0 | 0.07 | 0 | 0 | 0 | 0 |
| Anaerotruncus | 0 | 0.07 | 0 | 0 | 0 | 0 |
| Pseudobutyrivibrio | 0 | 0.07 | 0 | 0 | 0 | 0 |

⁴⁶ ^aResults are expressed as the percentage of specified sequence in total bacterial

47 sequences. The phylogenetic assignment was based on RDP Classifier.

⁴⁸ ^b HU and CH represent inocula of human feces and chicken cecal contents. HFVI,

49 HFVL, CFVI and CFVL represent chemostat products of human fecal and chicken

50 cecal microbiota grown in VI and VL media.

| Samples | Dry m | atter | Crude pr | otein | Star | ch | $\mathbf{NSP}^{	imes}$ | | Sugars | |
|----------------------------------------|------------------------|--------|---------------------------------------------|-------|-----------|-------|------------------------|-------|-----------|-------|
| Samples | Chicken | Human | Chicken | Human | Chicken | Human | Chicken | Human | Chicken | Human |
| Terminal ileal (g d ⁻¹) | 20.3±0.13 [§] | 101.30 | 2.51±0.05 | 17.20 | 1.37±0.06 | 1.30 | 0.17±0.01 | 23.30 | 4.02±0.04 | 20.8 |
| Feces (g d^{-1}) | 17.8±0.15 | 34.10 | 0.30 ± 0.04 $(1.92\pm0.09)^{\text{Y}}$ | 9.80 | 0.88±0.08 | 0.30 | 0.09±0.01 | 8.30 | 0.48±0.05 | 0.4 |
| Post-ileal digestibility (%) | 12.32 | 66.34 | 88.05 (23.51) [¥] | 43.02 | 35.77 | 76.92 | 47.06 | 64.38 | 88.06 | 98.08 |

51 Table S5 Comparison of post-ileal digestibility of major nutrients from chicken and human samples^{*}.

⁸All data for chicken are shown as mean and SD, n=18. The post-ileal digestibility was calculated as the difference between the terminal ileal

53 output $(g d^{-1})$ and feces $(g d^{-1})$ relative to the terminal ileal output $(g d^{-1})$. All data for humans were cited by Coles *et al.* (3).

⁵⁴ [§]The daily ileal output in dry matter for adult chickens is predicted with the daily total dry matter in excreta plus 2.5 gram based on a previous

55 study on intact and cecectomized adult chickens (10).

 $^{\text{Y}}$ The data outside of the brackets represent true protein, excluding non-protein nitrogen (uric acid). The data inside the brackets represent the crude protein, including both protein and non-protein nitrogen.

⁵⁸ [×]Non-starch polysaccharides.

59 Table S6 Molar ratios of VFA in chemostats, SHIME[§] or batch fermentation inoculated with human fecal microbiota studied by different

60 countries.

| Carbohydrates (%) | Techniques | Inocula source | Acetate | Propionate | Butyrate | Reference |
|------------------------------------------------------------------------------------------------------|--------------------|----------------|---------|------------|----------|--------------------------------|
| starch 0.2; pectin 0.05; inulin 0.05; xylan 0.05; | | | 70 | 17 | 13 | |
| arabinogalactan 0.05; guar gum 0.05 | chemostats | British | 83 | 9 | 8 | (7) |
| starch 0.3, arabinogalactan 0.1, pectin 0.2, xylan 0.1, glucose 0.04 | SHIME [§] | American | 51 | 15 | 22 | (4) |
| starch 0.2; pectin 0.05; Raftiline LS 0.05; xylan 0.05; arabinogalactan 0.05; guar gum 0.05 | chemostats | British | 61 | 24 | 15 | (2) |
| Konjac glucomannan (KGM) 0.2% | Batch fermentation | Japanese | 50 | 30 | 13 | (9) |
| carrageenan oligosaccharides (KCO) 0.8% | chemostats | Chinese | 44 | 40 | 16 | Unpublished data in our lab |
| carrageenan oligosaccharides (KCO) 0.8% | Batch fermentation | Chinese | 53 | 29 | 18 | Unpublished data in our lab |

61

62 [§]SHIME was represent for Simulator of the Human Intestinal Microbial Ecosystem.

| 63 | | Appendix | | | | |
|----------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|--|--|
| 64 | The se | quence of the phylotype of unclassified Lachnospiraceae coming from human | | | | |
| 65 | microbiota which was enriched in the VL medium in vitro chemostat system. | | | | | |
| 66 | >the pl | hylotype | | | | |
| 67 | CCTA | CGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGAT | | | | |
| 68 | CCAG | CGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCA | | | | |
| 69 | GCAG | GGAAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGT | | | | |
| 70 | GCCA | GCAGCCGCGGTAAT | | | | |
| 71 | | | | | | |
| 72 | Supp | lementary references | | | | |
| 73 74 75 76 | 1. | Bartosch, S., A. Fite, G. T. Macfarlane, and M. E. McMurdo. 2004. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. | | | | |
| 77 78 79 80 | 2. | Child, M. W., A. Kennedy, A. W. Walker, B. Bahrami, S. Macfarlane, and G. T. Macfarlane. 2006. Studies on the effect of system retention time on bacterial populations colonizing a three-stage continuous culture model of the human large gut using FISH techniques. FEMS Microbiol. Ecol. 55 :299-310. | | | | |
| 81 82 83 | 3. | Coles, L. T., P. J. Moughan, A. Awati, A. J. Darragh, and M. L. Zou. 2010. Predicted apparent digestion of energy-yielding nutrients differs between the upper and lower digestive tracts in rats and humans. J. Nutr. 140:469-476 | | | | |
| 84 85 86 | 4. | De Boever, P., B. Deplancke, and W. Verstraete. 2000. Fermentation by gut microbiota cultured in a simulator of the human intestinal microbial ecosystem is improved by supplementing a sovgerm powder I. Nutr 130 :2599-2606 | | | | |
| 87 88 89 | 5. | Denman, S. E., and C. S. McSweeney. 2006. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. FEMS Microbiol Ecol. 58 :572-582 | | | | |
| 90 91 92 | 6. | Frank, J. A., C. I. Reich, S. Sharma, J. S. Weisbaum, B. A. Wilson, and G. J. Olsen. 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA | | | | |
| 92 93 94 95 | 7. | Macfarlane, S., E. J. Woodmansey, and G. T. Macfarlane. 2005. Colonization of mucin by human intestinal bacteria and establishment of biofilm communities in a two-stage continuous culture system. Appl. Environ. Microbiol. 71 :7482-7402 | | | | |
| 95 96 | 8. | Matsuki, T., K. Watanabe, J. Fujimoto, Y. Miyamoto, T. Takada, K. Matsumoto, H. | | | | |

97 Oyaizu, and R. Tanaka. 2002. Development of 16S rRNA-gene-targeted group-specific 98 primers for the detection and identification of predominant bacteria in human feces. Appl. 99 Environ. Microbiol. 68:5445-5451. 100 9. Matsuura, Y. 1998. Degradation of konjac glucomannan by enzymes in human feces and 101 formation of short-chain fatty acids by intestinal anaerobic bacteria. J. Nutr. Sci. Vitaminol. 102 (Tokyo) 44:423-436. 103 10. Rezvani, M. R. 2007. Standardisation of precaecal and total tract amino acid digestibility 104 measurement in laying hens Martin Luther University, Halle Wittenberg. 105 Rinttila, T., A. Kassinen, E. Malinen, L. Krogius, and A. Palva. 2004. Development of an 11. 106 extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous 107 bacteria in faecal samples by real-time PCR. J. Appl. Microbiol. 97:1166-1177. 108