**Supplementary material**

1. **Materials and Methods**
   1. **Reagents/chemicals used in this study**

* 1-propanol (Sigma, St. Louis, USA);
* 1,3-propanediol (Sigma, St. Louis, USA);
* 2-propanol (Honeywell Riedel-de Haen, Munich, Germany);
* Acetic acid glacial (Sigma, St. Louis, USA);
* Ammonium Carbonate- (NH4)2CO3 (Merck KGaA, Darmstadt, Germany);
* Bile bovine (Sigma, St. Louis, USA);
* Bile salts (Sigma, St. Louis, USA);
* Bile acid assay kit (Sigma, St. Louis, USA);
* Butyric acid (Sigma, St. Louis, USA);
* Calcium chloride dihydrate - CaCl2(H2O)2
* Calcium chloride hexahydrate - CaCl2(H2O)6 (Sigma, St. Louis, USA);
* Defibrinated sheep blood (Oxoid Limited, Basingstoke, UK);
* Dipotassium hydrogen phosphate- K2HPO4 (Honeywell Fluka, Seelze, Germany);
* DL-lactic acid (Sigma, St. Louis, USA);
* Fructooligosaccharides from chicory root (FOS) (Megazyme, Bray, Ireland);
* Glycerol – analytical grade (Fisher Scientific, Loughborough, UK);
* Hemin (Sigma, St. Louis, USA);
* Hemoglobin (Sigma, St. Louis, USA);
* Hydrochloric acid- HCl (Honeywell Fluka, Seelze, Germany);
* L-cysteine HCl (Sigma-Aldrich, St. Louis, MO, USA);
* Magnesium chloride hexahydrate- MgCl2(H2O)6 (Panreac, Barcelona, Spain);
* Magnesium sulfate heptahydrate- MgSO4(H2O)7 (Sigma, St. Louis, USA);
* Molico skim milk powder- SKM (Nestlé S.A., Vevey, Switzerland);
* Na-p- tosyl-L-arginine methyl esther hydrochloride - TAME (Sigma, St. Louis, USA);
* Pancreatin from porcine pancreas (Sigma, St. Louis, USA);
* Pepsin from porcine gastric mucose powder (Sigma, St. Louis, USA);
* Peptone from animal tissue (Sigma, St. Louis, USA);
* Phosphate buffered saline (Dulbecco A) (Oxoid Limited, Basingstoke, UK);
* Potassium chloride- KCl (Honeywell Fluka, Seelze, Germany);
* Potassium dihydrogen phosphate- KH2PO4 (Merck KGaA, Darmstadt, Germany);
* Propionic acid (Sigma, St. Louis, USA);
* Resazurin sodium salt (Sigma, St. Louis, USA);
* Sodium chloride- NaCl (Honeywell Fluka, Seelze, Germany);
* Sodium hydrogen carbonate- NaHCO3 (Panreac, Barcelona, Spain);
* Sodium hydroxide – NaOH (LabChem, Zelienople, USA);
* Sulfuric acid - H2SO4 (Honeywell Fluka, Seelze, Germany);
* Trichloroacetic acid – TCA (Sigma, St. Louis, USA);
* Tris(hydroxymethyl)aminomethane hydrochloride (Merck KGaA , Darmstadt, Germany)
* Tween 80 (Sigma, St. Louis, USA);
* Vitamin K1 (Sigma, St. Louis, USA);
* Yeast extract (Sigma, St. Louis, USA).
  1. **Culture media**

**•** Bifidus selective medium agar- BSMA ( Sigma, St. Louis, USA);

• Columbia agar base- CBA (Liofilchem, Roseto degli Abruzzi, Italy);

• de Man, Rogosa and Sharpe agar- MRSA (Biokar Diagnostics, Allonne, France);

• MacConkey agar- MCA (Biolife, Milan, Italy);

• Violet red bile glucose agar – VRBG (Biokar diagnostics, Allonne, France).

* 1. **Apparatus used in this study**
* 1 kDa molecular weight cut-off regenerated cellulose dialysis tubing Spectra/Por® 6 (Spectrum, New Brunswick, USA);
* Agilent 1260 II series HPLC (Agilent, Santa Clara, Califórnia, USA);
* Alpha 2-4 LSC plus model (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany)
* Anaerobic cabinet, Whitley A35 workstation (Don Whitley Scientific, Bingley, UK);
* FerMac 260 pH controller (Electrolab Biotech Ltd., Gloucestershire, UK);
* Ion-exclusion Aminex HPX-87H column (Biorad, Hercules, California, USA);
* Heraeus™ Megafuge™ 16R Centrifuge (Thermo Fischer Scientific, Waltham, USA)
* Mixwel® laboratory blender (Alliance Bio Expertise Guipry, France);
* MR Hei-Tec magnetic stirrer ( Heidolph Instruments GmbH & CO. KG, Schwabach, Germany)
* MST magnetic stirrer (Velp Scientifica, Usmate Velate, Italy)
* OxoidTM AnaeroGenTM 2.5 L sachet (Thermo Fischer Scientific, Waltham, USA);
* OxoidTM AnaeroJarTM 2.5 L (Thermo Fischer Scientific, Waltham, USA);
* Reax top vortex ( Heidolph Instruments GmbH & CO. KG, Schwabach, Germany)
* SevenCompact pH meter (Mettler Toledo, Urdorf, Switzerland);
* Synergy H1 Hybrid Multi-Mode Reader (BioTek, Winooski, USA);
* UV-1900 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan);
* Tamper proof specimen 1-L containers (Sigma, St. Louis, USA).
  1. **Human fecal sample collection protocol**

The fecal samples were collected into a clean tamper proof specimen 1 L-container. The containers with the feces were placed in the OxoidTM AnaeroJarTM 2.5 L with an OxoidTM AnaeroGenTM 2.5 L sachet, closed until opened up inside of the anaerobic cabinet, Whitley A35 workstation and used within 2 h of collection. Under the anaerobic cabinet atmosphere (nitrogen 80%, carbon dioxide 10%, hydrogen 10%), fecal content of each donor was sampled in equal amounts, placed into an empty pre-weight tamper proof specimen 1 L-container and weighed to obtain a pooled fecal inoculum.

**Table S1-** Bacterial viable cell counts (log CFU/mL, mean ± SD) in different culture media of the fresh fecal individual and pooled inoculums. Different letters mark statically significant (p<0.05) differences between conditions at each culture media.

|  |  |  |
| --- | --- | --- |
| **Inoculum** | **Culture media** | **Bacterial concentration**  **(log CFU/mL)** |
| **D1** | CBA | 9.34 ± 0.04b,c |
| VRBGA | 6.36 ± 0.09d |
| MCA | 5.51 ± 0.01e |
| MRSA | 6.92 ± 0.05c |
| BSMA | 8.29 ± 0.02d |
| **D2** | CBA | 9.54 ± 0.01a |
| VRBGA | 6.67 ± 0.03c |
| MCA | 6.68 ± 0.05c |
| MRSA | 8.31 ± 0.09b |
| BSMA | 8.92 ± 0.02a |
| **D3** | CBA | 9.51 ± 0.04a,b |
| VRBGA | 6.29 ± 0.03d |
| MCA | 6.27 ± 0.08d |
| MRSA | 8.61 ± 0.03a |
| BSMA | 8.75 ± 0.01b |
| **D4** | CBA | 9.05 ± 0.09d |
| VRBGA | 7.95 ± 0.05a |
| MCA | 7.91 ± 0.03a |
| MRSA | 5.35 ± 0.03d |
| BSMA | 8.44 ± 0.02c |
| **D5** | CBA | 8.95 ± 0.10d |
| VRBGA | 6.49 ± 0.31c,d |
| MCA | 6.60 ± 0.19c |
| MRSA | 8.18 ± 0.08b |
| BSMA | 6.38 ± 0.02e |
| **P fresh** | CBA | 9.23 ± 0.01c |
| VRBGA | 7.11 ± 0.15b |
| MCA | 7.10 ± 0.15b |
| MRSA | 8.19 ± 0.08b |
| BSMA | 8.68 ± 0.04b |

**Table S2**- Bacterial viable cell counts (log CFU/mL, mean ± SD) in different culture media of different storage conditions of human pooled fecal inoculum. Different letters mark statically significant (p<0.05) differences between each conditions at each culture media.

|  |  |  |
| --- | --- | --- |
| **Inoculum** | **Culture media** | **Bacterial concentration (log CFU/mL)** |
| **I)** | CBA | 9.10 ± 0.08a |
| MCA | 7.30 ± 0.09a |
| MRSA + 0.1 % (w/v) cysteine | 8.67 ± 0.03a |
| **II)** | CBA | 7.14 ± 0.12d |
| MCA | 6.10 ± 0.06c |
| MRSA + 0.1 % (w/v) cysteine | 6.31 ± 0.05d |
| **III)** | CBA | 8.25 ± 0.09c |
| MCA | 6.13 ± 0.01c |
| MRSA + 0.1 % (w/v) cysteine | 7.21 ± 0.10c |
| **IV) (15 days)** | CBA | 9.05 ± 0.03a |
| MCA | 6.84 ± 0.02b |
| MRSA + 0.1 % (w/v) cysteine | 8.41 ± 0.14b |
| **IV) (90 days)** | CBA | 8.52 ± 0.02b |
| MCA | 6.56 ± 0.07c |
| MRSA + 0.1 % (w/v) cysteine | 8.39 ± 0.04b |

**Table S3**- Bacterial viable cell counts (log CFU/mL, mean ± SD) in different culture media of inoculum A (fresh) and B (frozen). Different letters mark statically significant (p<0.05) differences between each conditions at each culture media.

|  |  |  |
| --- | --- | --- |
| **Inoculum** | **Media** | **Bacterial concentration (log CFU/mL)** |
| **A** | CBA | 9.10 ± 0.08a |
| MCA | 7.30 ± 0.09a |
| MRSA + 0.1 % (w/v) cysteine | 8.67 ± 0.03a |
| **B** | CBA | 8.18 ± 0.11b |
| MCA | 6.24 ± 0.11b |
| MRSA + 0.1 % (w/v) cysteine | 8.20 ± 0.05b |

**Table S4-** Concentration (mM, means ± SD) of organic acids produced along fermentation time in inoculum A and B. Nd- not detected. Different letters mark statically significant (p<0.05) differences between the same conditions at each sampling time in each inoculum.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Conditions** | | **Time (h)** | **Lactate** | **Acetate** | **Propionate** | **Butyrate** |
| **Inoculum A** | **IC** | 0 | Nd | Nd | Nd | Nd |
| 6 | Ndb | 6.71 ± 0.30a | 1.76 ± 0.04a | 0.96 ± 0.03a |
| 24 | Nd | 21.72 ± 0.51a | 3.31 ± 0.00a | 2.99 ± 0.06a |
| 30 | Nd | 22.23 ± 0.28a | 3.33 ± 0.05a | 2.96 ± 0.04a |
| 48 | Nd | 23.48 ± 1.61a | 3.63 ± 0.09a | 2.93 ± 0.02a |
| **SKM** | 0 | Nd | Nd | Nd | Nd |
| 6 | 17.03 ± 0.57a | 32.70 ± 1.91a | 6.71 ± 0.14a | 1.52 ± 0.42a |
| 24 | Nd | 55.44 ± 3.35a | 14.64 ± 1.96a | 6.10 ± 1.55a |
| 30 | Nd | 57.20 ± 1.47a | 15.22 ± 1.84a | 6.58 ± 1.03a |
| 48 | Nd | 58.98 ± 1.12a | 15.41 ± 1.71a | 6.27 ± 1.02a |
| **SKM + 0.1% (w/w) FOS** | 0 | Nd | Nd | Nd | Nd |
| 6 | 16.65 ± 2.14a | 31.91 ± 1.86 a | 6.65 ± 0.26a | 1.81 ± 0.71a |
| 24 | Nd | 53.66 ± 0.29a | 13.17 ± 0.74a | 6.85 ± 0.16a |
| 30 | Nd | 55.26 ± 0.46a | 13.45 ± 0.90a | 6.68 ± 0.28a |
| 48 | Nd | 56.32 ± 0.84a | 14.14 ± 0.38a | 6.88 ± 0.37a |
| **Inoculum B** | **IC** | 0 | Nd | Nd | Nd | Nd |
| 6 | 0.69 ± 0.02a | 1.20 ± 0.09b | 0.98 ± 0.00b | Ndb |
| 24 | Nd | 4.17 ± 1.03b | 1.82 ± 0.34b | Ndb |
| 30 | Nd | 8.35 ± 2.03b | 1.85 ± 0.11b | 1.38 ± 0.00b |
| 48 | Nd | 14.72 ± 0.97b | 2.45 ± 0.21b | 1.36 ± 0.58a |
| **SKM** | 0 | Nd | Nd | Nd | Nd |
| 6 | 15.71 ± 1.65a | 25.01 ± 3.73a | 1.36 ± 0.34b | Ndb |
| 24 | Nd | 48.54 ± 6.92a | 12.73 ± 0.05a | 3.55 ± 1.46a |
| 30 | Nd | 52.63 ± 3.48a | 13.24 ± 0.55a | 4.51 ± 0.62a |
| 48 | Nd | 55.78 ± 4.35a | 16.05 ± 0.18a | 5.17 ± 0.70a |
| **SKM + 0.1% (w/w) FOS** | 0 | Nd | Nd | Nd | Nd |
| 6 | 11.78 ± 0.02a | 13.54 ± 2.16b | 0.34 ± 0.06b | 0.44 ± 0.29a |
| 24 | Nd | 44.35 ± 7.12a | 11.64 ± 1.15a | 3.80 ± 0.59b |
| 30 | Nd | 43.72 ± 5.43a | 12.37 ± 0.91a | 3.77 ± 0.61b |
| 48 | Nd | 49.72 ± 7.03a | 15.86 ± 2.31a | 4.91 ± 1.44a |