Sample preparation for sulfonate measurements

Solvents/Chemicals:

Acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents for mass spectrometry were of analytical grade purity. Water (resistivity 18.2 MΩcm) was purified using a Milli-Q-System (Millipore, Milford, MA, USA). Formic acid was purchased from Honeywell Fluka (Muskrgon, MI, USA).Cysteic acid and isethionic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). 2,3-dihydroxypropane-1-sulfonate and sulfoquinovose were purchased from MCAT GmbH (Donaueschingen, Germany). Taurine was purchased from Roth GmbH(Karlruhe, Germany). Ti(III) nitrilotriacetate (Sigma-Aldrich, Germany)

Anoxic phosphate-buffered saline (PBS): 8.5 g / l NaCl, 0.3 g / l KH2PO4, Na2HPO4 0.6 g / l, 0.1 g / l Bacteriological Peptone, 1 mg / l Resazurin, 40 mM Sodium DL-lactate , 40 mM Sodium formate pH 7.0, N2/CO2 (80/20, v / v) as gas phase, autoclaved at 121°C for 15 min. all compounds from Fluka ,Muskrgon, MI, USA.

DS-medium: 19 mM NH4Cl, 17 mM NaCl, 2 mM MgCl2 x 6 H2O, 7 mM KCl, 0.3 mM CaCl2 x 2 H2O, 1 mM K2HPO4, 40 mM sodium DL-lactate, 40 mM sodium formate, 3.5 mg/l yeast extract, 1 ml/l selenite-tungstate solution 1 ml/l trace element solution (see Table 5), 1.2 µM 1,4-naphthoquinone and 2 µM resazurin. The medium was adjusted to pH 7.4, gas flashed with N2/CO2. all compounds from Fluka ,Muskrgon, MI, USA.

Solution	Components with concentration
Trace-element solution	10 ml/l HCl 1.5 g/l $FeCl_2$ x 4 H_2O 70 mg/l $ZnCl_2$ 100 mg/l $MnCl_2$ x 4 H_2O 6 mg/l H_3BO_3 190 mg/l $CoCl_2$ x 6 H_2O 2 mg/l $CuCl_2$ x 2 H_2O 24 mg/l $NiCl_2$ x 6 H_2O 36 mg/l Na_2MoO_4 x 2 H_2O
Selenite-tungstate solution	500 mg/l NaOH 3 mg/l Na_2SeO_3 x 5 H_2O 4 mg/l Na_2WO_4 x 2 H_2O

Seven-vitamin solution	100 mg/l vitamin B_{12} 80 mg/l p-amino benzoic acid 20 mg/l D (+)-biotin 200 mg/l nicotinic acid 100 mg/l calcium pantothenate 300 mg/l pyridoxine hydrochloride 200 mg/l thiamine hydrochloride x 2 H_2O
Ti(III) nitrilotriacetate solution	19.2 g/l nitrilotriacetic acid diluted in anoxic distilled water, pH of 9 adjusted with NaOH 19.2 ml 20% $TiCl_3$ (Acros) pH of 7adjusted with Na_2CO_3 (80 g/l)

Work steps calibration samples

One g of the human fecal sample was homogenized in anoxic phosphate-buffered saline (PBS, pH 7.0, Table 4) by vortexing with glass beads (c. 3 mm; Roth, Germany) to yield a 10% fecal suspension. The fecal suspension was further diluted to 1% in a Hungate tube containing anoxic PBS supplemented with 3.18 mM of sterile filtered Ti(III) nitrilotriacetate (Sigma-Aldrich, Germany) as reductant. Subsequently, fecal slurry aliquotes were centrifuged (14.000 x g, 4°C, 5 min) and the supernatant frozen until further processing. Fecal slurries were , fecal supernatants were serially diluted using 50% acetonitrile and 0.1% formic acid in water to obtain final dilution of 1:10,000 and spiked with cysteate, sulfoquinovose (SQ), 2,3-dihydroxypropane-1-sulfonate (DHPS), taurine and isethionate for calibration. 500 µl of each sample were placed in glass vials (Wicom, Heppenheim, Germany) and stored at - 80 °C until LC-MS/MS analysis.

Work steps for application pilot study

For each 10 ml incubation, DS medium supplemented with either taurine (20 mM) or sulfoquinovose (SQ, 4 mM) was inoculated with the SIHUMI bacteria or the SIHUMI consortium and B. wadsworthia. Control incubations of medium containing only sulfonates or bacteria were included. The incubations were conducted under anoxic conditions at 37 °C in duplicates. Samples (600 µl) were withdrawn after 0, 3, 24 and 48 h for quantification of sulfoquinovose (SQ), 2,3-dihydroxypropane-1-sulfonate (DHPS), taurine and isethionate. For the analysis of sulfonates by LC-MS/MS-MRM, aliquots of 250 µl for each sample were centrifuged (14 000 x g, 4 °C, 5 min) and 50 µl of the supernatant stored at – 20 °C until further processing. Samples were thawed, centrifuged at 18 000 x g at RT for 2 min and the supernatant was diluted 1:10 000 in 50% aqueous acetonitrile. Subsequently, 500 µl of each sample were placed in glass vials (Wicom, Heppenheim, Germany) and stored at - 80 °C until LC-MS/MS analysis.