

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 (Karlsruhe, 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 ₂ O
	70 mg/l ZnCl ₂
	100 mg/l MnCl ₂ x 4 H ₂ O
	6 mg/l H ₃ BO ₃
	190 mg/l CoCl ₂ x 6 H ₂ O
	2 mg/l CuCl ₂ x 2 H ₂ O
	24 mg/l NiCl ₂ x 6 H ₂ O
Selenite-tungstate solution	36 mg/l Na ₂ MoO ₄ x 2 H ₂ O
	500 mg/l NaOH
	3 mg/l Na ₂ SeO ₃ x 5 H ₂ O
	4 mg/l Na ₂ WO ₄ x 2 H ₂ O

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 ₂ O
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 7 adjusted with Na ₂ CO 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.