Supplemental Online Materials & Methods

Culture conditions

All strains were cultivated on MTC-6 defined medium, and were transferred through defined medium three times prior to their use as inoculum (2%vol transfers), in order to remove traces of complex media. Strains were frozen at -80C when in late exponential phase.

Strains LL1025 (WT) and LL1144 (*ΔnfnAB*::Kan^r) were grown in 125ml glass serum bottles, with 5ml transferred to a sterile, purged glass Balch tube (post inoculation). The culture volumes are as follows:

-LL1025: 50ml MTC-6 + 0.05ml culture. 5ml transferred to Balch tube after mixing well.

-LL1144: 50ml MTC-6 + 2.5ml culture. 5ml transferred to Balch tube after mixing well.

The 5ml Balch tubes were used to monitor the OD600 of each bottle using a Thermo Scientific Genesys 10 VIS spectrophotometer. On occasion throughout the growth of the cultures, the volume of the Balch tubes was quickly re-mixed with the contents of the serum bottle, and replaced back (using sterile needles and within the anaerobic chamber), in order to confirm OD600 readings were accurate. At the time of harvest, these 5ml tubes were added to each respective 50ml conical for preparation of cell pellets. Bottles were allowed to rest on the bench to cool to less than 30C, before centrifuging at 7197g for 15 minutes in an Eppendorf 5430 centrifuge equipped with a F-35-6-30 rotor. The resulting supernatant was separated by decantation, prior to freezing at -80C.

Medium composition and preparation

Media was filter sterilized immediately after preparation into sterilized serum bottles, and purged of oxygen using twenty 45 second cycles of UHP N^2 gas (Airgas) and vacuum. Bottles were released of residual pressure inside an anaerobic chamber (Coy) using a sterile filter and needle, prior to inoculation. All media used for the purpose of this study was inoculated within two hours of preparation. All fermentations were incubated in an orbital shaking incubator (Innova 4080 Incubator Shaker, New Brunswick Scientific) held at a temperature of 55C and a 180 rpm.

MTC-6 medium was prepared using anaerobic stock solutions of filter sterilized media components. All stock solutions were prepared in advance and used within six weeks, save the vitamin and trace element formulations. Solution D and E were stored in darkness to prevent photodegradation of media components, and solution E was also stored at 4C for vitamin preservation. All solutions were purged of oxygen with twenty 45 second cycles of UHP N_2 gas and vacuum prior to storage. The stock solution compositions used in this study are noted below.

All media for reference fermentations was prepared using fresh cellobiose (Sigma) and MOPS buffer (3-(N-morpholino)propanesulfonic acid, sodium salt). After completing dissolving granular cellobiose and MOPS in

deionized water (MilliQ, ~60% of final medium volume) with gentle stirring, required volumes of stock solutions were drawn aseptically via syringe. Extra volume was drawn in order to avoid volumetric inaccuracies due to syringe dead volumes, and added slowly to the stirring medium. The medium was then brought up to ~97-99% of the final volume in a graduated cylinder, and brought to a final pH of 6.2 in a beaker with a stir bar, using 10% H_2SO_4. The medium was returned to the cylinder to complete the final volume with deionized water.

Serum bottles were sterilized by 30 minutes of autoclave. All reference fermentations utilized a new, unpierced butyl rubber stopper (20mm, Chemglass, #21) for consistency, with aluminum tear-off crimps. Bottles were fitted with a 23g needle and 0.2um filter for pressure equalization during media addition. 52ml of medium was drawn to 60ml syringes (BD) and removed of any air bubbles prior to fitting an aseptically prepared 0.2um filter with a 21g 1-1/2 needle. Excess volume after filter absorption was released under the sterile field of a Bunsen burner, and both needle and butyl stopper were flame sterilized prior to injection. Vacuum lines were attached to the outgas filters to draw in the medium. The same outgas filters were then used for N_2/vacuum purging (20 cycles of 45 seconds).

Stock Solutions:

Solution B (200ml, 40ml/L MTC): Potassium citrate monohydrate (C6H7K3O8), 10.0000g; Citric acid monohydrate (C6H8O7 . H2O), 6.2500g; Sodium Sulfate (Na2SO4), 5.0000g; Potassium phosphate monobasic (KH2PO4), 5.0000g; Sodium Bicarbonate (NaHCO3), 12.5000g.

Solution C (400ml, 20ml/L MTC): Ammonium chloride (NH4Cl), 30.0000g.

Solution D (400ml, 20ml/L MTC): Magnesium chloride (MgCl2 * 6H2O), 20.0000g; Calcium chloride (CaCl2 * H2O), 4.0000g; Iron (II) chloride (FeCl2 * 6H2O), 2.0000g; L-cysteine HCl monohydrate, 20.0000g; Trace mineral solution F, 20ml.

Solution E (200ml, 20ml/L MTC): Pyridoxamine HCl, 0.2000g; P-aminobenzoic acid, 0.0400g; d-biotin, 0.0200g; Vitamin B12, 0.0200g; Thiamine, 0.0040g.

Solution F (500ml): MnCl2*4H2O, 0.2500g; CoCl2*6H2O, 0.2500g; ZnCl2, 0.1000g; CuCl2*2H2O, 0.0250g; H3BO3, 0.0250g; Na2MoO4*2H2O, 0.0250g; NiCl2*6H2O, 0.0250g.

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MTC-6, per liter:	Final Concentration (g/L)
Cellobiose	5
MOPS sodium salt	9.3
Potassium citrate monohydrate	2
Citric acid monohydrate	1.25
Sodium sulfate, Na2SO4	1
Potassium phosphate, KH2PO4	1
Sodium bicarbonate, NaHCO3	2.5
Ammonium chloride, NH4Cl	1.5
Magnesium chloride, MgCl2 * 6H2O	1
Calcium chloride, CaCl2 * H2O	0.2
Iron (II) chloride, FeCl2 * 6H2O	0.1
L-cysteine HCl monohydrate	1
Pyridoxamine HCl	0.02
p-aminobenzoic acid	0.004
d-biotin	0.002
Vitamin B12	0.002
Thiamine	0.004
MnCl2*4H2O	0.0005
CoCl2*6H2O	0.0005
ZnCl2	0.0002
H3BO3	0.00005
Na2MoO4*2H2O	0.00005
NiCl2*6H2O	0.00005

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