Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Information

Non-lethal growth inhibition by arresting the starch utilization system of clinically relevant human isolates of *Bacteroides dorei*

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1. General Experiment Details

1.1 TYG Media

Tryptone, yeast extract, and glucose (TYG) agar and broth were prepared following the procedure described in the Anaerobe Laboratory Manual.[1]

1.2 TYG Media Components

Media components for TYG were obtained from the following sources and used without further purification.

Table S1. TYG media components

Compound	Supplier	Catalog #
Ammonium Sulfate	Alfa Aesar	A11682
Calcium Chloride	BDH	BDH0224
Ferrous Sulfate Heptahydrate	Amresco	0387
Glucose	BDH	BDH8005
Hematin Porcine	Sigma Aldrich	H3281
L-Cysteine	Amresco	J994
L-Histidine	Amresco	1B1164
Monobasic Potassium Phosphate	BDH	BDH9268
Sodium Chloride	Alfa Aesar	12314
Tryptone	Amresco	J859
Vitamin B_{12}	Acros	405925000
Vitamin K_3	Spectrum Chemical	ME105
Yeast Extract	Amresco	J850

1.3 Ruminococcus (RUM) and modified Ruminococcus (MRUM) Media (100 mL)

RUM medium was adapted from the recipe reported by Flint, et. al.[2] and prepared as follows:

40 mL of deionized H_2O was added to 1 g tryptone, 0.25 g yeast extract, 0.4 g $NaHCO_3$, 0.09 g ammonium sulfate, 0.1 mg calcium (D)-pantothenate, 0.1 mg nicotinamide, 0.01 mg folinic acid calcium salt hydrate, 5 mL K^+/Na^+ salts (4.5 g K_2HPO_4, 4.5 g KH_2PO_4, 9 g NaCl, 500 mL deionized H_2O), 1 mL of RUM vitamin mix (1 mg biotin, 1 mg cobalamin, 3 mg *p*-amino benzoic acid, 5 mg folic acid, 15 mg pyridoxamine, 5 mg thiamine, 5 mg riboflavin, 100 mL deionized H_2O), 800 μL 1.9 mM hematin/ 0.2 M (L)-histidine solution, 500 μL 200X $MgSO_4$ solution (0.88 g in 100 mL deionized H_2O), 500 μL 200X $CaCl_2$ solution (1.8 g in 100 mL deionized H_2O), 183 μL acetic acid, 67 μL propionic acid, 26.4 μL isobutyric acid, 10.8 μL valeric acid, and 10.8 μL isovaleric acid. The resulting solution was brought to a pH of 6.8 and 2 mL of 100x (L)-cysteine (50 mg/mL 10mM HCl) was added. The solution was brought to a final volume of 100 mL, 0.5 g of the appropriate carbohydrate source was added, and the resulting medium was filter sterilized.

MRUM was prepared in the same manner, but only 0.25 g tryptone and 0.0625 g of yeast extract were added in order to limit the amount of additional carbohydrate sources present.

1.4 Ruminococcus (RUM) and MRUM Media Components

Table S2. RUM and MRUM media components

Compound	Supplier	Catalog #
Acetic Acid	VWR	97065-042
Ammonium Sulfate	Alfa Aesar	A11682
Biotin	Alfa Aesar	A14207
Calcium Chloride	BDH	BDH0224
D-Calcium Pantothenate	Acros	243300050
D-(+)-Maltose Monohydrate	Alfa Aesar	A16266
Dibasic Potassium Phosphate	G - Biosciences	RC-081
Folic Acid	Fisher Scientific	BP25195
Folinic Acid	Alfa Aesar	H60181
Glucose	BDH	BDH8005
Hematin Porcine	Sigma Aldrich	H3281
Isobutyric Acid	Acros	122520250
Isovaleric Acid	Acros	156690100
L-Cysteine	Amresco	J994
L-Histidine	Amresco	1B1164
Magnesium Sulfate	BDH	BDH0246
Monobasic Potassium Phosphate	BDH	BDH9268
Nicotinamide	Acros	128271000
p–Aminobenzoic Acid	Alfa Aesar	A12673
Propionic Acid	Acros	149300010
Pullulan	TCI	P0978
Pyridoxamine	Alfa Aesar	J62679
Riboflavin	Acros	132350250
Starch From Potato	Alfa Aesar	A11961
Sodium Bicarbonate	BDH	BDH8011
Sodium Chloride	Alfa Aesar	12314
Thiamine	Fisher Scientific	BP892
Tryptone	Amresco	J859
Valeric Acid	Acros	149571000
Vitamin B_{12} (Cyanocobalamin)	Acros	405925000
Yeast Extract	Amresco	J850

1.5 Bacteroides dorei Overnight Cultures

B. dorei (JR01, JR02, JR03, and JR04) from freezer stocks was grown on TYG agar for at least 48 hours in a GasPak™ Anaerobic Jar with a BD GasPak ™ EZ anaerobe container system (cat. number 260678) and incubated at 37 ˚C. TYG overnight cultures were prepared from these plates following the procedure described in our previous communication.[3] RUM overnight

cultures were prepared from 100 μL of the TYG overnight using the protocol substituting RUM for TYG. Culture tubes were incubated for 18 – 24 h. at 37 ˚C.

1.6 Bacteroides dorei 96 Well Plate Assay

In an anaerobic chamber with a hydrogen level maintained at 2.0% using a gas mix of 85% nitrogen/10% carbon dioxide/5% hydrogen and a balance of nitrogen, 500 μL of the RUM Glucose overnight was centrifuged at 8,000 rpm using a Chemglass MLX-108-CLS Mini Centrifuge for 1 min until a pellet formed and the resulting supernatant was removed. The pellet was suspended in 500 μL of 2x MRUM medium with no carbohydrate source. This suspension was used to inoculate 25 mL of 2x MRUM medium. 100 μL of the inoculated medium was aliquoted in to the wells of a 96 well plate, followed by 100 μL of the corresponding carbohydrate source at a 2x concentration or deionized H_2O as a control. If acarbose (Alfa Aesar, J61737) was being tested, it was added to the 2x carbohydrate source also at a 2x concentration. Plates were then inserted into a BioTek Epoch 2 Microplate Spectrophotometer where they were incubated at 37 ˚C for 48 h with a 2 ˚C gradient. During incubation, the optical density (OD) at 600 nm was measured every half hour. Prior to each measurement, there was a 15 s shake. The resulting ODs were used to generate growth curves for each of the *B. dorei* strains in the presence or absence of acarbose.

2. Growth curves of *B. dorei* JR01, JR02, JR03, and JR04 in MRUM with no additional carbohydrate

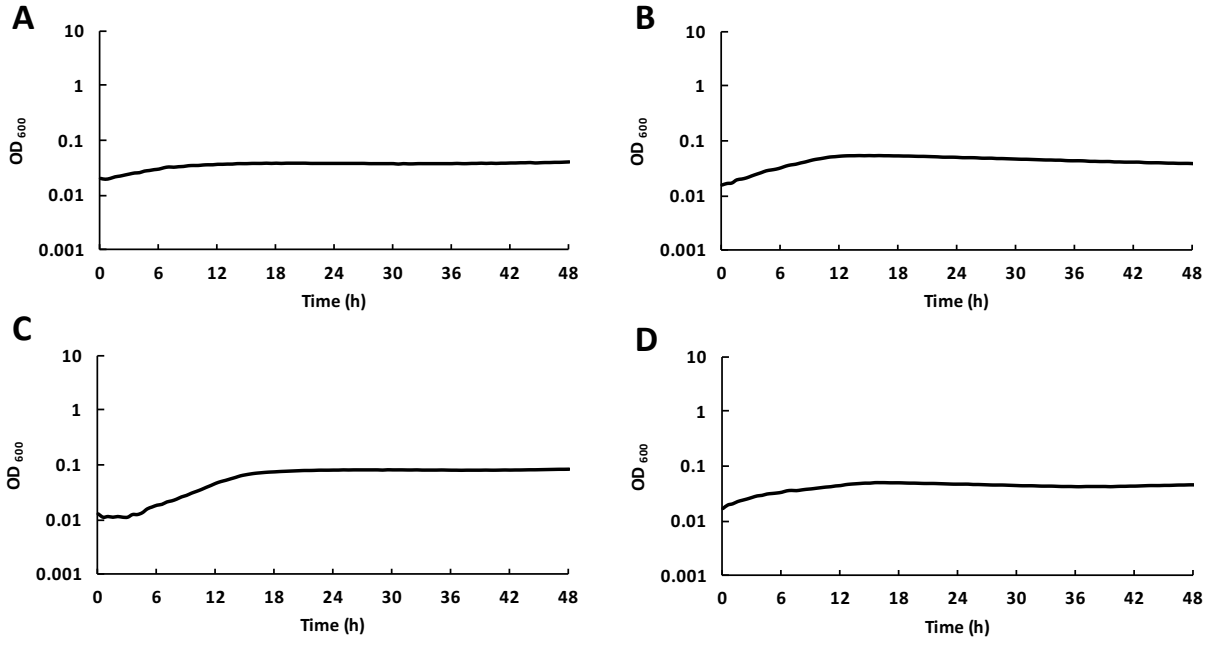

Figure S1. Growth curves of all four *B. dorei* strains in MRUM with no additional carbohydrate source. **A)** *B. dorei* JR01 **B)** *B. dorei* JR02 **C)** *B. dorei* JR03 **D)** *B. dorei* JR04

As seen in **Figure S1**, MRUM with no additional carbohydrate source allows for limited growth, permitting us to use this medium as a control to investigate *B. dorei* carbohydrate utilization.

3. Growth curves of *B. dorei* JR01, JR02, and JR04 on glucose based carbohydrates

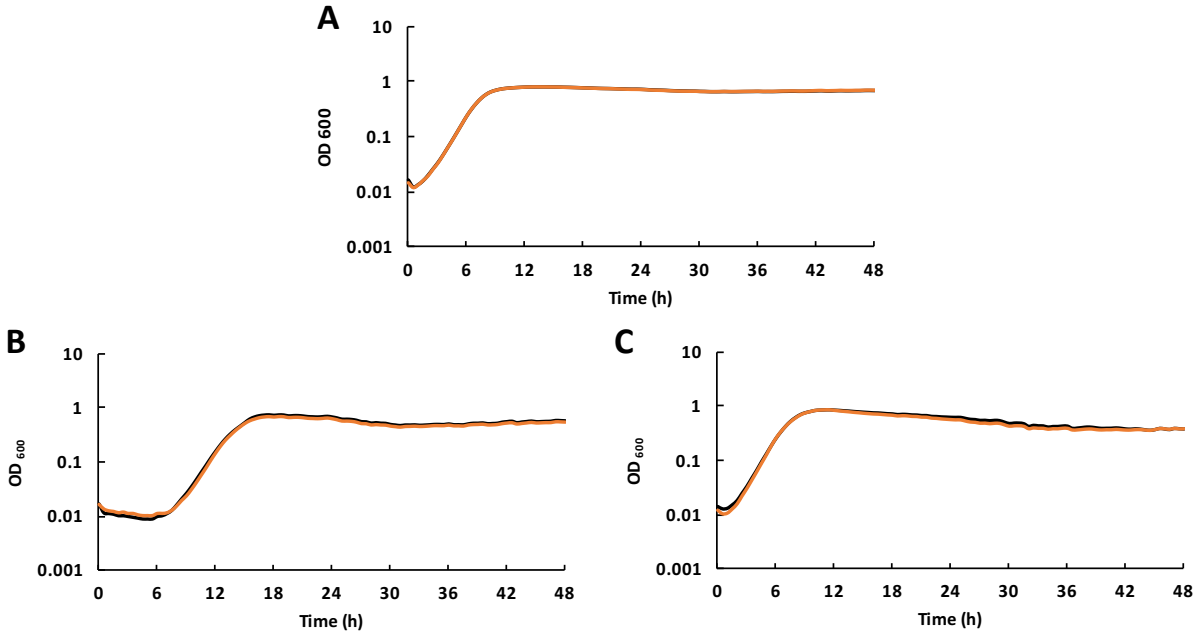

Figure S2. Growth curves of various *B. dorei* strains in glucose, with (orange line) and without (black line) 100 μM acarbose. **A)** *B. dorei* JR01 **B)** *B. dorei* JR02 **C)** *B. dorei* JR04

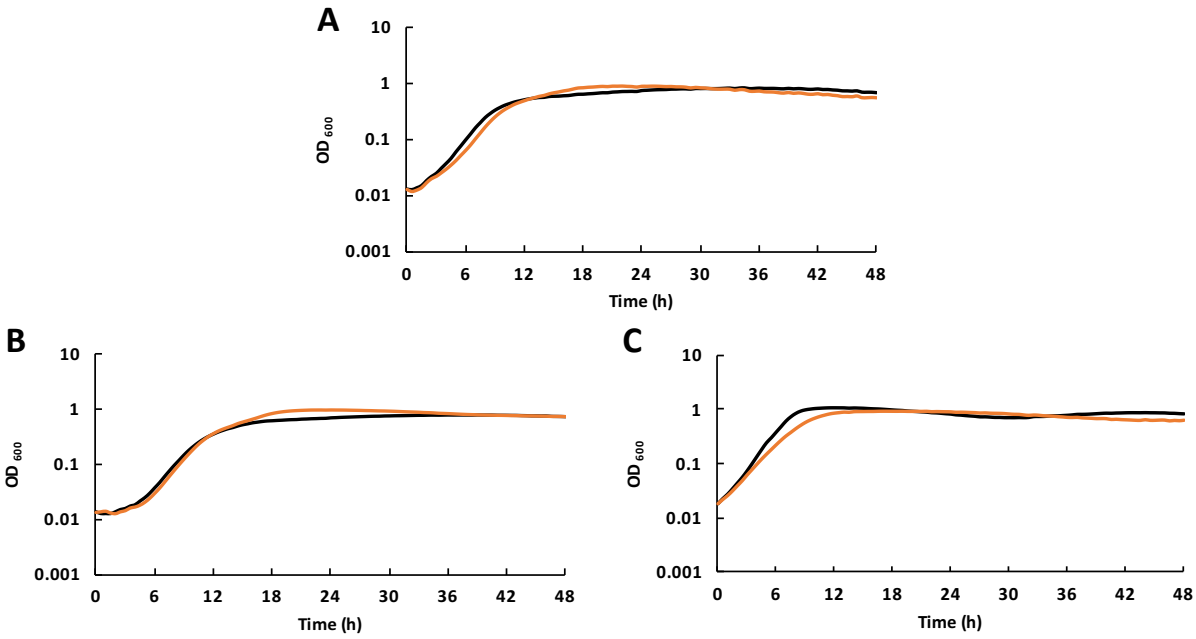

Figure S3. Growth curves of various *B. dorei* strains in maltose, with (orange line) and without (black line) 100 μM acarbose. **A)** *B. dorei* JR01 **B)** *B. dorei* JR02 **C)** *B. dorei* JR04

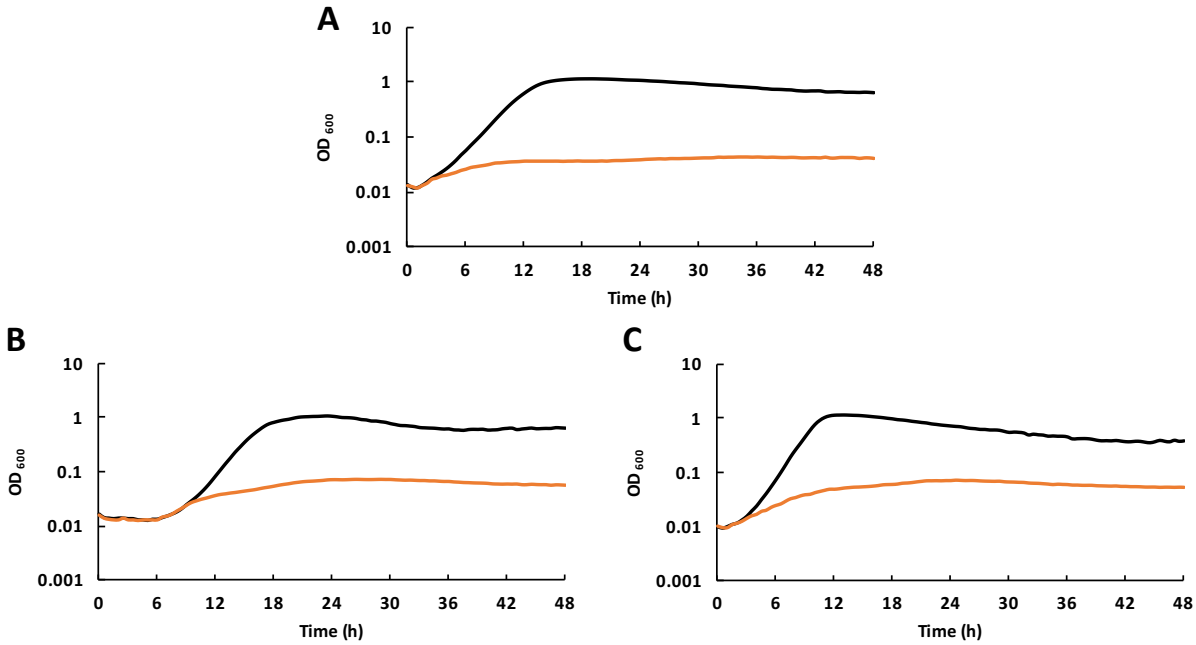

Figure S4. Growth curves of various *B. dorei* strains in pullulan, with (orange line) and without (black line) 100 μM acarbose. **A)** *B. dorei* JR01 **B)** *B. dorei* JR02 **C)** *B. dorei* JR04

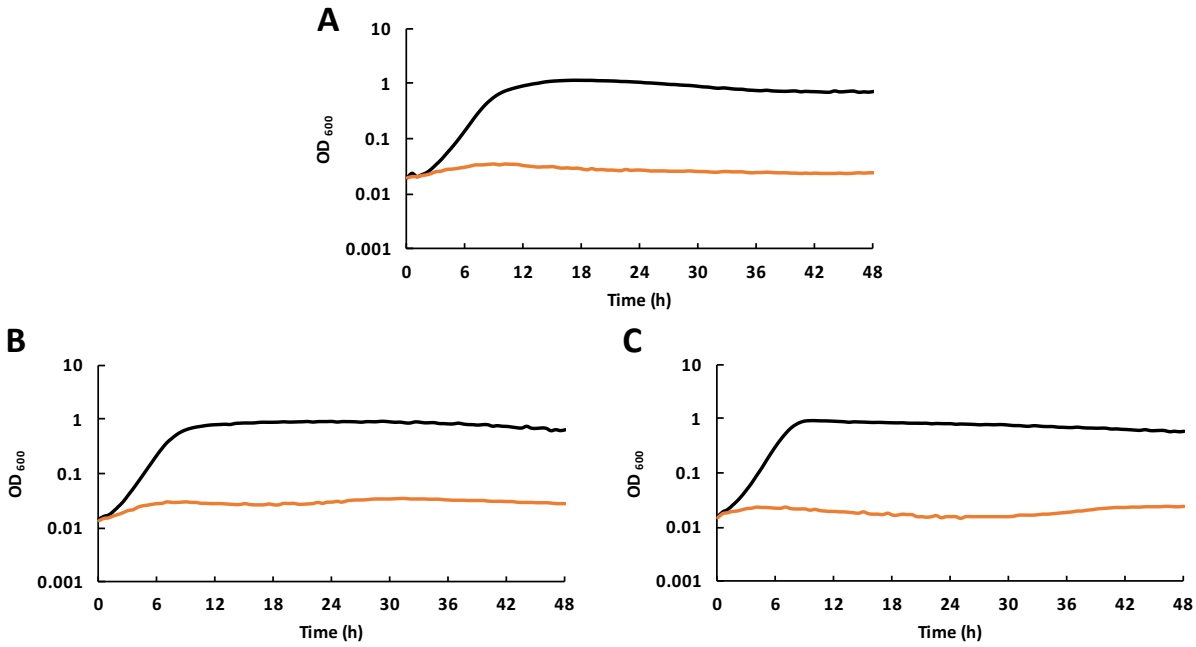

Figure S5. Growth curves of various *B. dorei* strains in potato starch, with (orange line) and without (black line) 100 μM acarbose. **A)** *B. dorei* JR01 **B)** *B. dorei* JR02 **C)** *B. dorei* JR04

Figures S2 through **S5** depict the growth of *B. dorei* JR01, JR02, and JR04 in the various glucose-based carbohydrate sources. All three strains grow in a similar manner to JR03 as can be seen in **Figure 2** and **Table 1**. No acarbose based inhibition is seen with glucose and maltose, but all four strains show about a 90% growth inhibition (about 10% growth compared to non-treated cultures) in the glucose-based polysaccharides pullulan and potato starch. This again reinforces our premise that treatment with acarbose is both non-lethal and interrupts the starch utilization mechanisms belonging to the bacteria.

Supplementary References:

[1] *Anaerobe Laboratory Manual* (Eds.: L. V. Holdeman, E. D. Cato, W. E. C. Moore) Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, **1977**.
[2] Ze, X.; David, Y. B.; Laverde-Gomez, J. A.; Dassa, B.; Sheridan, P. O.; Duncan, S. H.; Louis, P.; Henrissat, B.; Juge, N.; Koropatkin, N. M.; Bayer, E. A.; Flint, H. J., *mBio.* **2015**, *6,* e01058-15.
[3] A.D. Santilli, E. W. Dawson, K. J. Whitehead, D. C. Whithead, *ACS Chem. Biol.*, **2018**, *13*, 1165-1172.