

Supplementary Information for:

Construction and characterization of stable multi-species biofilm formed by nine core gut bacteria on wheat fiber

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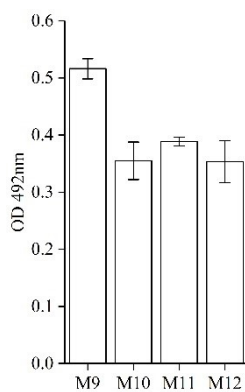


Fig. S1. The biofilm formation ability detected by XTT reduction assay at the absorbance value at 492 nm. M9: *B. ovatus*, *P. distasonis*, *B. uniformis*, *B. cellulosilyticus*, *E. coli*, *B. fragilis*, *P. dorei*, *B. stercoris*, and *B. longum*; M10: *B. ovatus*, *P. distasonis*, *B. uniformis*, *B. cellulosilyticus*, *E. coli*, *B. fragilis*, *P. dorei*, *B. stercoris*, *B. longum*, and *B. xylanisolvans*; M11: *B. ovatus*, *P. distasonis*, *B. uniformis*, *B. cellulosilyticus*, *E. coli*, *B. fragilis*, *P. dorei*, *B. stercoris*, *B. longum*, *B. xylanisolvans*, and *P. merdae*; M12: *B. ovatus*, *P. distasonis*, *B. uniformis*, *B. cellulosilyticus*, *E. coli*, *B. fragilis*, *P. dorei*, *B. stercoris*, *B. longum*, *B. xylanisolvans*, *R. torques*, and *F. prausnitzii*.

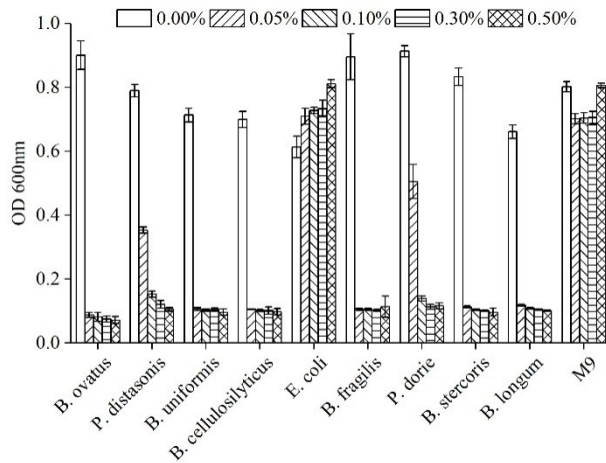


Fig. S2. The absorbance values at OD 600nm of mono and multi-species biofilms with different bile salt concentrations (0.00%, 0.05%, 0.1%, 0.3%, and 0.5%).

Table S1. The information of 18 species core gut bacteria.

Strain name	Abbreviation	Source	Characteristic
<i>Bacteroides ovatus</i> CCFM1342	B. ovatus	Culture Collection of Food Microorganisms (CCFM), Jiangnan University	hemicellulose degradation
<i>Parabacteroides distasonis</i> CCFM1377	P. distasonis	CCFM, Jiangnan University	hemicellulose degradation
<i>Bacteroides uniformis</i> CCFM1358	B. uniformis	CCFM, Jiangnan University	hemicellulose degradation
<i>Bacteroides cellulosilyticus</i> FSDTAELIBH15	B. cellulosilyticus	CCFM, Jiangnan University	cellulose degradation
<i>Bacteroides thetaiotaomicron</i> FTJS1K7	B. thetaiotaomicron	CCFM, Jiangnan University	hemicellulose degradation
<i>Bacteroides vulgatus</i> FSDTAHCMXY14	B. vulgatus	CCFM, Jiangnan University	hemicellulose degradation
<i>Escherichia coli</i> CCFM1378	E. coli	CCFM, Jiangnan University	-
<i>Bacteroides fragilis</i> FSDTAHCK_B8	B. fragilis	CCFM, Jiangnan University	-
<i>Phocaeicola dorei</i> FSDLZ62K4	P. dorei	CCFM, Jiangnan University	-
<i>Bacteroides stercoris</i> FFJLY21K3	B. stercoris	CCFM, Jiangnan University	-
<i>Bifidobacterium longum</i> FBJCY2M11	B. longum	CCFM, Jiangnan University	-
<i>Bacteroides caccae</i> FNMHLBE15K4	B. caccae	CCFM, Jiangnan University	-
<i>Bacteroides xylanisolvens</i> FGDLZ48K3	B. xylanisolvens	CCFM, Jiangnan University	-
<i>Streptococcus salivarius</i> K12	S. salivarius	CCFM, Jiangnan University	-
<i>Parabacteroides merdae</i> FSDTAELIBH14	P. merdae	CCFM, Jiangnan University	-
<i>Alistipes onderdonkii</i> DSM 19147	A. onderdonkii	DSMZ	-
<i>Ruminococcus torques</i> ATCC 27756	R. torques	ATCC	-
<i>Faecalibacterium prausnitzii</i> A2-165	F. prausnitzii	DSMZ	-

Table S2. Details of modified YCFA medium (1L)

Formulation	Content	Formulation	Content
Casein peptone	10 g	Cellobiose	2g
Tryptone	10 g	Maltose	2 g
Yeast extract	5 g	Biotin	0.01 mg
Sodium acetate	5 g	Vitamin B12	0.01 mg
Lactose-H ₂ O	5 g	p-aminobenzoic acid	0.03 mg
NaHCO ₃	4 g	Folic acid	0.05 mg
(NH ₄) ₂ SO ₄	1.8 g	Pyridoxine-HCl	0.15 mg
KH ₂ PO ₄	0.45 g	Vitamin B1	0.05 mg
NaCl	0.9 g	Riboflavin	0.05 mg
MgSO ₄ × 7 H ₂ O	0.045 g	D-Ca-pantothenate	5 mg
CaCl ₂	0.09 g	Hemin chloride	1%
L-Cysteine-HCl	1.445 g	Clarify bovine rumen fluid	0.5%

Glucose	2g		
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Table S3. Details of strain-specific primers.

Species	Primer name	Sequence (5'-3')	Amplicon size (bp)
<i>B. ovatus</i>	B. ovatus-F	GGGCCTATTATCGCAACCGA	114
	B. ovatus-R	AGGTGCATACGTAGACGGAC	
<i>P. distasonis</i>	P. distasonis-F	TGCCTATCAGAGGGGGATAAC	78
	P. distasonis-R	GCAAATATTCCCATGCGGGAT	
<i>B. uniformis</i>	B. uniformis-F	GGAATTCCCGAAAAGACCTTCCG	99
	B. uniformis-R	GTAGGCATTGCTGTCTTTGCACAT	
<i>B. cellulosilyticus</i>	B. cellulosilyticus-F	AGCAGGCGGAATTCGATAAG	96
	B. cellulosilyticus-R	GTGTACAGTGCCAGGCATAA	
<i>E. coli</i>	E. coli-F	CAACGAACTGAACTGGCAGA	121
	E. coli-R	CATTACGCTGCGATGGAT	
<i>B. fragilis</i>	B. fragilis-F	TCAGGAAGAAAGCTTGCT	163
	B. fragilis-R	CATCCTTTACCGAATCCT	
<i>P. dorei</i>	P. dorei-F	AAGCGGCTTCAAGAAACAGG	100
	P. dorei-R	GTGCCCTTTACCTTGGGAAC	
<i>B. stercoris</i>	B. stercoris-F	TGGGACAGGCATCCATCAAC	211
	B. stercoris-R	CCGAGCGACAGTACCATAACC	
<i>B. longum</i>	B. longum-F	ATCCGTCCGACCCAGACC	123
	B. longum-R	CTCGACATCCTCACGGCC	

Table S4. The group information of mono-bacterium and mixed strains.

No. group	Mono and multi-species	Label
1	<i>B. ovatus</i>	A
2	<i>P. distasonis</i>	B
3	<i>B. uniformis</i>	C
4	<i>B. cellulosilyticus</i>	D
5	<i>B. thetaiotaomicron</i>	E
6	<i>B. vulgatus</i>	F
7	<i>B. ovatus</i> + <i>P. distasonis</i>	A+B
8	<i>B. ovatus</i> + <i>B. uniformis</i>	A+C
9	<i>B. ovatus</i> + <i>B. cellulosilyticus</i>	A+D
10	<i>B. ovatus</i> + <i>B. thetaiotaomicron</i>	A+E
11	<i>B. ovatus</i> + <i>B. vulgatus</i>	A+F
12	<i>P. distasonis</i> + <i>B. uniformis</i>	B+C
13	<i>P. distasonis</i> + <i>B. cellulosilyticus</i>	B+D
14	<i>P. distasonis</i> + <i>B. thetaiotaomicron</i>	B+E
15	<i>P. distasonis</i> + <i>B. vulgatus</i>	B+F
16	<i>B. uniformis</i> + <i>B. cellulosilyticus</i>	C+D
17	<i>B. uniformis</i> + <i>B. thetaiotaomicron</i>	C+E
18	<i>B. uniformis</i> + <i>B. vulgatus</i>	C+F
19	<i>B. cellulosilyticus</i> + <i>B. thetaiotaomicron</i>	D+E
20	<i>B. cellulosilyticus</i> + <i>B. vulgatus</i>	D+F
21	<i>B. thetaiotaomicron</i> + <i>B. vulgatus</i>	E+F
22	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. uniformis</i>	A+B+C
23	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. cellulosilyticus</i>	A+B+D
24	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. thetaiotaomicron</i>	A+B+E
25	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. uniformis</i> + <i>B. cellulosilyticus</i>	A+B+C+D
26	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. uniformis</i> + <i>B. thetaiotaomicron</i>	A+B+C+E
27	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. thetaiotaomicron</i> + <i>B. cellulosilyticus</i>	A+B+D+E
28	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. uniformis</i> + <i>B. cellulosilyticus</i> + <i>B. thetaiotaomicron</i>	A+B+C+D+E
29	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. uniformis</i> + <i>B. cellulosilyticus</i> + <i>B. vulgatus</i>	A+B+C+D+F
30	<i>B. ovatus</i> + <i>P. distasonis</i> + <i>B. uniformis</i> + <i>B. cellulosilyticus</i> + <i>B. thetaiotaomicron</i> + <i>B. vulgatus</i>	A+B+C+D+E+F

Table S5. The qPCR standard curves of the 9 bacteria.

Strains	Standard curves	R ²
<i>B. ovatus</i>	y = -3.2165x + 46.878	R ² = 0.9996
<i>P. distasonis</i>	y = -3.1102x + 52.574	R ² = 0.9997
<i>B. uniformis</i>	y = -3.1237x + 48.786	R ² = 0.9994

<i>B. cellulosilyticus</i>	$y = -3.3114x + 50.962$	$R^2 = 0.9998$
<i>E. coli</i>	$y = -3.132x + 53.105$	$R^2 = 0.9985$
<i>B. fragilis</i>	$y = -3.3471x + 51.198$	$R^2 = 0.9996$
<i>P. dorie</i>	$y = -3.1504x + 46.351$	$R^2 = 0.9996$
<i>B. stercoris</i>	$y = -3.3236x + 45.784$	$R^2 = 0.9993$
<i>B. longum</i>	$y = -2.8275x + 54.028$	$R^2 = 0.9962$