Characterization of Mixed-Species Biofilms Formed by Four Gut Microbiota

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Table S1. Details of different strains used in this study.

Species	Number	label
Bifidobacterium longum subsp. Infantis	FJND16M4	A
Bifidobacterium longum subsp. Infantis	FBJCP1M11	B
Enterococcus faecalis	E1	C
Enterococcus faecalis	JNGMMB7	D
Bacteroides ovatus	FTJS5K9	E
Bacteroides ovatus	FNXYCHL6K1	F
Lactobacillus gasseri	FHNFQ11L7	G
Lactobacillus gasseri	FHNFQ14L5	H

Table S2. Details of medium (1L) used in this study.

Formulation	Content	
Casein peptone	10.00	g
tryptone	10.00	g
Yeast extract	2.50	g
Sodium acetate	5.00	g
Lactose × H_2O	5.00	g
Glucose	5.00	g
$MgSO_4$ × 7 H_20	45.00	mg
$CaCl_2$ × 2 H_20	90.00	mg
K_2HPO_4	0.45	g
KH_2PO_4	0.45	g
NaCl	0.90	g
Resazurin	1.00	mg
Distilled water	1000.00	mL
$NaHCO_3$	4.00	g
L-Cysteine-HCl	1.00	g
Hemin	10.00	mg

Cellobiose	2.00	g
$(NH_4)_2SO_4$	0.90	g
Biotin	0.01	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Vitamin B_1	0.05	mg
Riboflavin	0.05	mg
D-Ca-pantothenate	5.00	mg
Lipoic acid	5.00	mg
Nicotinic acid	2.00	mg
Acetic acid	1.50	mL
Propionic acid	0.70	mL
Iso-Butyric acid	90.00	μL
n-Valeric acid	100	μL
Iso-Valeric acid	100.00	μL
Clarified rumen fluid	10	mL
PH	6.2 – 6.4	

Table S3. Primer sequences used in qPCR for species.

Species	Primer name	Sequence (5′-3′)	Amplicon size (bp)	Reference
Bifidobacterium longum subsp. Infantis	*B. longum*_F *B. longum*_R	ATCCGTCCGACCCAGACC CTCGACATCCTCACGGCC	123	[1]
Enterococcus faecalis	*E. faecalis*_F *E. faecalis*_R	CGCTTCTTTCCTCCCGAGT GCCATGCGGCATAAACTG	143	[2]
Bacteroides ovatus	*B. ovatus*_F *B. ovatus*_R	AAGACAACGATGGATAGGG GTT TTCACGCTACTTGGCTGGT	136	[3]
Lactobacillus gasseri	*L. gasseri*_F *L. gasseri*_R	AGCGAGCTTGCCTAGATGAA TTTG TCTTTTAAACTCTAGACATGC GTC	171	[4]

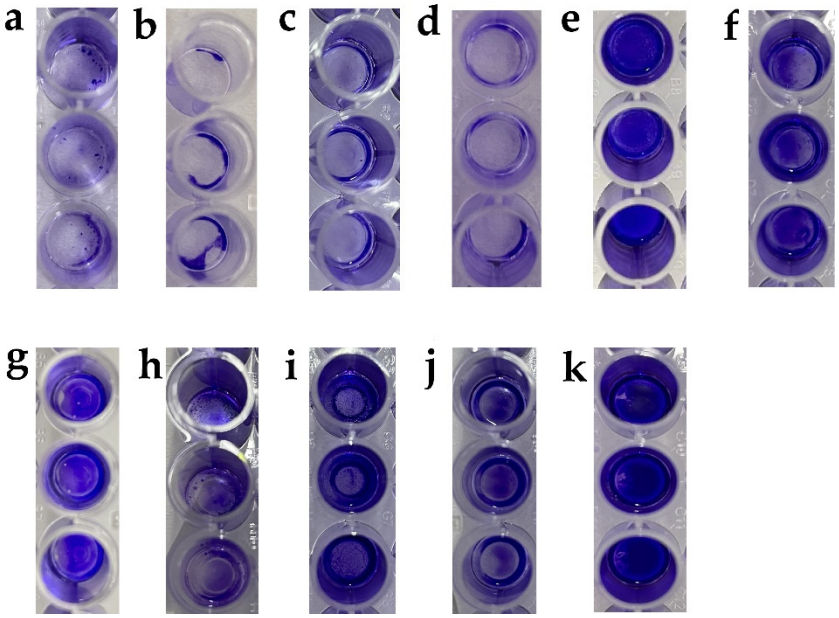

Figure S1. Images of the crystal violet staining of different combinations. (a) *B. longum*; (b) *E. faecalis*; (c) *B. ovatus*; (d) *L. gasseri*; (e) *B. longum* + *E. faecalis*; (f) *B. longum* + *B. ovatus*; (g) *B. longum* + *L. gasseri*; (h) *E. faecalis* + *B. ovatus*; (i) *E. faecalis* + *L. gasseri*; (j) *B. ovatus* + *L. gasseri*; (k) *B. longum* + *E. faecalis* + *B. ovatus* + *L. gasseri*.

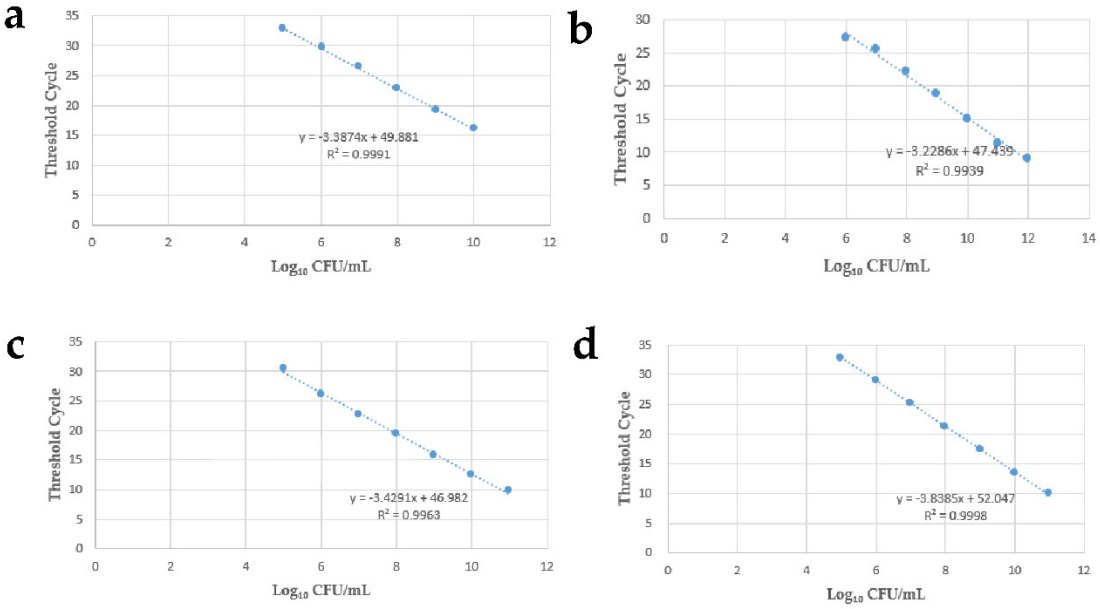

Figure S2. Standard curves of qPCR. (a) *B. longum*. (b) *E. faecalis*. (c) *B. ovatus*. (d) *L. gasseri.*

Reference

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