

Supplemental material

1 Supplemental Methods:

1.1 Isolation of Peyer's patch (PP) -specific *Lactobacilli*.

The PPs were aseptically removed, and tissue specimens were placed in Betadine antiseptic solution (Seton Healthcare Group plc, Oldham, UK) for 3 min to disinfect the surface. Subsequently, tissues were vortexed in multiple 500-mL aliquots of phosphate-buffered saline to encourage the removal of any bacteria on the tissue surface. Final washes were retained and analyzed by both culture-dependent and culture-independent techniques to determine whether surface decontamination was successful. After homogenization, 1 g of sample was suspended in 10 ml of 0.85% NaCl and diluted by 10^3 , then 1 ml of each dilution was inoculated into 5 mL of Mann Rogosa Sharp (MRS) broth and incubated in a CO₂ saturated anaerobic chamber at 30° C for 24 h. In order to increase specificity, cultures obtained from the more diluted samples were spread onto *Lactobacillus* anaerobic MRS plates with vancomycin and bromocresol green agar (LAMVAB) to isolate *Lactobacillus*. Single colonies randomly taken from the LABVAB were purified by streaking out twice on MRS broths under anaerobic conditions at 37° C. Purity of the isolates was confirmed by repeated streaking and sub-culturing in fresh MRS agar, followed by microscopic examination.

1.2 Amplified fragment length polymorphism (AFLP) typing.

For discrimination of the strains, the AFLP analysis method was performed using

was performed to construct a tree plot using the unweighted pair-group method with arithmetic averages (UPGMA) in the SAHN program of the NTSYS-pc software.

1.3 Isolation of the epithelium from the crypts.

Crypt epithelia were isolated from jejunum according to the method described by Flint ² with minor modification. Briefly, intestines pieces were washed in HBSS with 0.5 mM DTT for 5 min at 4 °C with constant stirring at 200 rpm for 30 min. Detached tissues pellets were transferred to 115 mL of chelating buffer, incubated at 4 °C for 20 min with constant stirring at 100 rpm. Suspension were re-suspended in 25 mL of fresh chelating buffer in a 50 mL centrifuge tube for washing by inverting the tube for 60 times at 2~8 °C. Detached pieces were collected in a new tube. Fresh chelating buffer (20 mL) was added to the wash tube and washed for three times. Detached pieces were collected every time. The purity of crypts was examined by microscopy. Samples were harvested by centrifugation under 1000 rpm for 5 min and stored at –80 °C after addition of 1 mL of Trizol.

REFERENCE

- 1 K. Watanabe, J. Fujimoto, Y. Tomii, M. Sasamoto, H. Makino, Y. Kudo and S. Okada, *Int. J. Syst. Evol. Microbiol.*, 2009, **59**, 754–760.
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2 Supplemental Tables:

Table S1. Adapter and primer oligonucleotides used in AFLP analyses

Oligonucleotide	Sequence (5'-3')
AFLP adapters	
EcoRI adapter	CTCGTAGACTGCGTACC CATCTGACGCATGGTTAA
MseI adapter	GACGATGAGTCCTGAG CTACTCAGGACTCAT
Core sequences of AFLP primers without selective bases	
EcoRI core	GACTGCGTACCAATTC
MseI core	GATGAGTCCTGAGTAA
Selective amplification	
EcoRI-G	GACTGCGTACCAATTCTG
MseI-CC	GATGAGTCCTGAGTAACC

TableS2. Primer sequence used for Real-time reverse transcription-PCR for gene expression of crypts

Gene name	Sequence (5'-3')
Tlr2	F:AAAATGTCGTTCAAGGAG R:TTGCTGAAGAGGACTGTT
Tlr4	F:GGAACAAACAGCCTGAGACAC R:CAAGGGATAAGAACGCTGAGAA
Tlr9	F:GGTGTGGAACATCATTCT R:ATACGGTTGGAGATCAAG
Nod2	F:ACAGCACGTCAGGGAAGTACCAG R:CAGGCAAAGATTCTCCGACCC
Myd88	F:TGGCATGCCTCCATCATAGTTAACC R:GTCAGAAACAACCACCACCATGC
Nfkb	F:AGGCTTCTGGGCCTTATGTG R:TGCTTCTCTCGCCAGGAATAC
CRS1C	F:TGCTCTTCAAGATGTAGCCCAACG R:TGGAGCTTGGGTGGTGATTGCA
CRS4C	F:GCATGGAATCTGGGTCAAGATAAC R:AGAAGGAAGAGCAATCAAGGCTAAG
RegIII γ	F:TTCCTGTCCTCCATGATCAAAA R:CATCCACCTCTGTTGGGTTC
defcr-rs10	F:ATCATCCAGGTGATTCCCAGCCAT R:TTCCGGGTCTCCAAAGGAAACAGA
β -actin	F:GGGTCAGAAGGACTCCTATG R:GTAACAATGCCATGTTCAAT

Table S3 Group-Specific Primers Based on 16S rRNA Sequences Used for qRT- PCR

Target bacterial group	primer	Sequence (5'-3')	Annealing T (°C)	reference
All bacteria	F-Eub 338	ACTCCTACGGGAGGCAGCAG	60	3
	R-Eub 518	ATTACCGCGGCTGCTGG		
Firmicutes	Firm934F	GGAGYATGTGGTTTAATTCGAAGC	60	4
		A		
	Firm1060R	AGCTGACGACAACCATGCAC		
Bacteroidetes	Bact934F	GGARCATGTGGTTTAATTCGATGAT	60	4
	Bact1060R	AGCTGACGACAACCATGCAG		
Bacteroides	-	CGATGGATAGGGGTTCTGAGAGGA	60	5
	-	GCTGGCACGGAGTTAGCCGA		
Lactobacillus	Lac1	AGCAGTAGGGAATCTTCCA	55	6
	Lab-0677r	CACCGCTACACATGGAG		

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- 1 K. Watanabe, J. Fujimoto, Y. Tomii, M. Sasamoto, H. Makino, Y. Kudo and S. Okada, *Int. J. Syst. Evol. Microbiol.*, 2009, **59**, 754–760.
- 2 N. Flint, F. L. Cove and G. S. Evans, *Biochem. J.*, 1991, **280** (Pt 2, 331–334.
- 3 J. Walter, G. W. Tannock, A. Tilsalatisjarvi, S

Table S4. Primers with different amplification regions and its reaction solution.

primer	Sequence(5'-3')	Target	Procedure	Reference
27F 534R	AGAGTTTGATCCTGGCTCAG ATTACCGCGGCTGCTGG	v1-v3	96°C, 1min; 55 °C, 1min; 72 °C, 1min	7
27F 907R	AGAGTTTGATCCTGGCTCAG CCGTCAATTCCTTTGAGTTT	v1-v5	94°C, 1min; 55 °C, 1min; 72 °C, 2min	8
27F 1492R	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	v1-v9	96°C, 1min; 55 °C, 1min; 72 °C, 1min	9
334F 939R	CCAGACTCCTACGGGAGGCAGC CTTGTGCGGGCCCCCGTCAATTC	v3-v5	94°C, 1min; 69 °C, 1min; 72°C, 1.5min	10
968F 1401R	AACGCGAAGAACCTTAC CGGTGTGTACAAGACCC	v6-v8	94 °C, 20s; 54 °C, 20s; 68 °C, 40s	8
530F 1492R	GTGCCAGCMGCCGCGG GGTTACCTTGTTACGACTT	v4-v9	94 °C, 1min; 55 °C, 1min; 72 °C, 2min	8

REFERENCE

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- 2 N. Flint, F. L

Table S5: The fragment profiles with virtual digestion online.

Enzyme	Forward fragment	Reverse fragment
AflIII	119, 134, 348, 363, 474, 507, 511, 524	74, 130, 132, 259, 473, 488, 489,
DdeI	57, 124, 131, 135, 138, 159, 204, 259, 260, 261, 267, 291, 317, 372, 373, 387, 398, 412, 415, 419, 420, 421, 422, 423	89, 90, 91, 97, 114, 179, 182, 183, 185, 229, 446
EcoRI	288, 312, 316, 333, 338, 339, 340	260, 261, 264, 265, 266, 267
DpnII	90	104, 105, 235, 237, 455, 492, 515, 516, 517, 518, 519, 520, 521
HinfI	29	92, 286, 314
Sau96I	207, 208, 212, 281, 375, 392, 395, 397, 398, 399, 400	103, 104, 202, 204, 205, 206, 207, 209, 398, 399, 400, 401
AluI	61, 100, 156, 244, 282, 284	65, 78, 79, 451, 454, 500, 503, 504, 507
MaeII	119, 121, 125, 136, 148, 149	74, 257, 403, 404, 405, 406, 407, 408, 409, 412, 456, 457, 458, 461

3 Supplemental Figure.

Figure S1

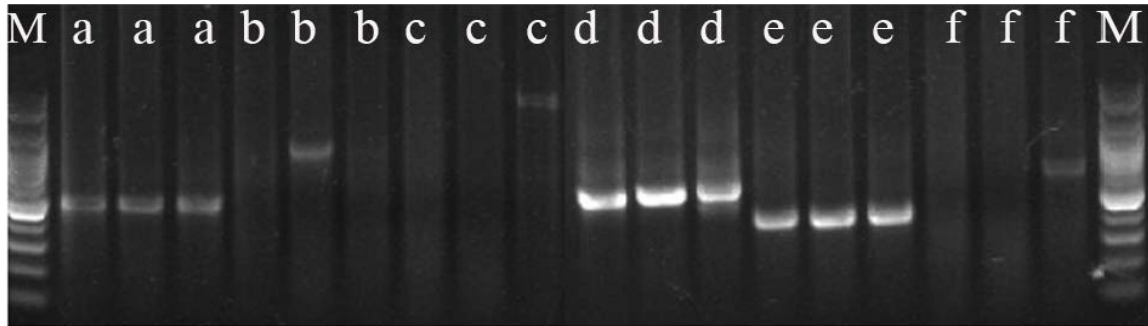


Figure S1 Gel electrophoresis of PCR products with primers a-f for eDNA amplification. a, 27F/534R; b, 27F/907R; c, 27F/1492R; d, 334F/939R; e, 968F/1401R; f, 530F/1492R.