

Supplementary Information

Isolation, potential beneficial properties, and storage stability assessment of direct-fed microbial consortium from wild-type chickens' (*Gallus gallus*) intestines

Probiotics and Antimicrobial Proteins

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Table S1 Primer sequence for specific antibiotic resistance genes

Antibiotic	Primer name	Sequence (5'- 3')	Annealing temp (°C)
Ampicillin (AM)	502 F	AATGAAGCCATACCAAACGAC	54.7
	632 R	CGGATAAAGTTGCAGGACCA	
Chloramphenicol (CL)	387 F	CGTTTATGCCAACCGTCCTG	57.3
	525 R	GGCTATACTGGCGATGCT	
Erythromycin (E)	300 F	GATATTTGGAAGTATACCCT	47.7
	521 R	GTGTCTTAATAAACTTACCCG	
Vancomycin A (VA-A)	382 F	GGGAAAACGACAATTGC	52
	615 R	GTACAATGTGGCCGTTA	
Vancomycin B (VA-B)	420 F	ATGGGAAGCCGATAGTC	48
	612 R	GATTCGTTCTCGACC	
Aminoglycosides (AMG)	194 F	TAATGCCTACTCAGACCA	58.7
	409 R	AAGTATCTCGCTAATTCACC	
Tetracycline M (TE-M)	408 F	GTGGACAAAGGTACAACGAG	64
	R	CGGTAAAGTTCGTCACACAC	
Tetracycline W (TE-W)	F	GAGAGCCTGCTATATGCCAGC	64
	R	GGGCGTATCCACAATGTTAAC	

Table S2 Primer sequence for *Lactobacillus* at the species level

Target	Primer name	Sequence (5'- 3')	Annealing temp (°C)	Amplicon size (bp)
<i>Lactobacillus acidophilus</i>	496 F 692 R	CAACCAGAAAGTCACGGCTA ATCTACGCATTCCACCGCTA	57.0	302
<i>Lactobacillus casei</i>	606 F 781 R	AGTCTGATGTGAAAGCCCTC ATGGGTAGCGAACAGGATTAG	56.6	175
<i>Lactobacillus helveticos</i>	611 F 786 R	ATGCTGATGTGAAAGCCCTC CTAATCCTGTTTCGCTACCCAT	56.2	175
<i>Lactobacillus rhamnosus</i>	995 F 1298 R	CTTCCGGTACGAACAACCTCT AGTCCGGTGTTTGGTTTAGC	57.0	176
<i>Lactobacillus cremoris</i>	1091 F 1278 R	CCCCTATTGTTAGTTGCCAT TTGCAGCCTACAATCCGAAC	54.8	207

Table S3 Specific primer design for proportion bacteria identification by qPCR

Target	Primer name	Sequence (5'- 3')	Annealing temp (°C)	Reference
Total Bacteria (universal primers)	bact1369F	CGGTGAATACGTTTCYCGG	56	Suzuki et al. [1]
	Prok1492R	GGWTACCTTGTTACGACTT		
Total <i>Lactobacillus</i>	F	AGCAGTAGGGAATCTTCCA	56	Rinttilä et al. [2]
	R	CACCGCTACACATGGAG		
Total <i>Enterobacteria</i>	F	ATGTTACAACCAAAGCGTAC A	56	Takahashi et al. [3]
	R	TTACCYTGACGCTTAAGTGC		

Table S4 Summary of 16S rRNA gene sequencing reads for chicken gastrointestinal tract bacteria across samples

Samples	Sample type	Input Raw reads	Filtered	Denois ed Forward	Denois ed Reverse	Merged	Non chimeras	Merged (%)
BC10C	Cecum	28463	11053	10835	10895	10122	7690	76.0
BC1C	Cecum	37729	14083	13936	14001	13510	12029	89.0
BC2I	Ileum	24180	9838	9451	9702	8963	6962	77.7
BC3C	Cecum	17216	6749	6535	6559	6187	4940	79.8
BC4I	Ileum	29339	13621	13454	13516	13083	7520	57.5
BC5I	Ileum	29489	11446	11298	11317	10803	8754	81.0
BC6C	Cecum	24108	9390	9341	9382	9173	8229	89.7
BC7I	Ileum	25633	10237	10178	10149	9974	8573	86.0
Total		216157	86417	85028	85521	81815	64697	79.1

Table S5 Bacterial relative abundance (%) at phyla level (top 10) of ileum and cecum chicken intestine

Phyla	Samples							
	Cecum				Ileum			
	BC10C	BC1C	BC3C	BC6C	BC2I	BC4I	BC5I	BC7I
Firmicutes	44.486346	17.1335938	16.82186	13.52534	38.063775	100	41.29541	8.596757
Fusobacteriota	2.873862	0.9643362	7.08502	0	3.389831	0	0	0
Proteobacteria	52.639792	81.90207	76.09312	86.47466	58.546395	0	58.70459	91.403243

Table S6 Bacterial relative abundance (%) at the order level (top 10) of ileum and cecum chicken intestine

Order	Samples							
	Cecum				Ileum			
	BC10C	BC1C	BC3C	BC6C	BC2I	BC4I	BC5I	BC7I
Lactobacillales	2.87	13.45	11.88	13.28	15.38	10.17	30.10	5.027
Clostridiales	39.57	0	3.64	0.24	9.55	85.80	5.837	0.875
Lachnospirales	0	0	0	0	0	0.29	0	0
Oscillospirales	1.743	0.91	1.30	0	2.03	0	0	0
Peptostreptococcales-Tissierellales	0.30	2.77	0	0	11.10	3.74	5.36	2.70
Fusobacteriales	2.87	0.96	7.09	0	3.39	0	0	0
Enterobacterales	52.64	81.90	76.09	86.48	58.55	0	58.71	91.40

References

1. Suzuki MT, Taylor LT, DeLong EF (2000) Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Appl Environ Microbiol* 66:4605–4614. <https://doi.org/10.1128/AEM.66.11.4605-4614.2000>
2. Rinttilä T, Kassinen A, Malinen E, et al (2004) Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J Appl Microbiol* 97:1166–1177. <https://doi.org/10.1111/j.1365-2672.2004.02409.x>
3. Takahashi H, Saito R, Miya S, et al (2017) Development of quantitative real-time PCR for detection and enumeration of Enterobacteriaceae. *Int J Food Microbiol* 246:92–97. <https://doi.org/10.1016/j.ijfoodmicro.2016.12.015>