

Supplementary Materials

Evaluation of curcumin and copper acetate against *Salmonella* Typhimurium infection, intestinal permeability, and cecal microbiota composition in broiler chickens

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Identification of genus *Salmonella* in microbiota analysis

In this study, we orally gavaged all day-of-hatch chicks in all groups except for NC with *S. Typhimurium* PHL-2020 strain. When we analyzed MiSeq sequence reads of 16S rRNA genes from cecal samples using DADA2 algorithm in QIIME2, we detected only one amplicon sequence variant (ASV) that matched to genus *Salmonella*. The sequence of this ASV (373 nucleotides long) is shown below:

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TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAAT
CCCCGGGCTCAACCTGGGAACTGCATTTCGAAACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTA
GCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGC
GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCTACTTGGAGGTTGTGCC
TTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCTGGGGAGTACGGCCGCAAGGTTA
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The frequency of this AVS reads was total 695 in 7 samples as shown in the table below.

Index	Treatment	Before rarefaction		After rarefaction	
		genus <i>Salmonella</i>	Total read counts	genus <i>Salmonella</i>	Total read counts
ANA-1-1	NC	0	94583	0	69566
ANA-1-2	NC	0	75139	0	69566
ANA-1-3	NC	0	94729	0	69566
ANA-1-4	NC	0	121233	0	69566
ANA-1-5	NC	0	109204	0	69566
ANA-2-1	PC	47	83388	43	69566
ANA-2-2	PC	63	100502	41	69566
ANA-2-3	PC	165	96924	123	69566
ANA-2-4	PC	159	102306	110	69566
ANA-2-5	PC	236	111518	138	69566
ANA-3-1	CA	0	104855	0	69566
ANA-3-2	CA	0	78143	0	69566
ANA-3-3	CA	0	69566	0	69566
ANA-3-4	CA	0	113760	0	69566
ANA-3-5	CA	0	81440	0	69566
ANA-4-1	CR	0	118198	0	69566
ANA-4-2	CR	0	144899	0	69566
ANA-4-3	CR	0	112376	0	69566
ANA-4-4	CR	0	132693	0	69566
ANA-4-5	CR	0	126829	0	69566
ANA-5-1	CA-CR	0	150582	0	69566
ANA-5-2	CA-CR	25	114467	14	69566
ANA-5-3	CA-CR	0	155679	0	69566
ANA-5-4	CA-CR	0	148901	0	69566
ANA-5-5	CA-CR	0	127595	0	69566
Total		695		469	

To assess the accuracy of the taxonomic assignment for this ASV, we performed BLAST analysis of this ASV sequence against the major genera in family Enterobacteriaceae, *Escherichia*, *Salmonella* and *Shigella* in Nucleotide collection (nr/nt) using default settings. The BLAST results below showed that this ASV has 100% identity (373/373) with genus *Salmonella*, while it was lower with genus *Escherichia* (98.93-99.20%) and *Shigella* (97.32-99.20%). The result strongly supports that the taxonomic assignment of this ASV to genus *Salmonella* in this study was accurate.

Top 10 hits in genus *Escherichia*

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/> Escherichia coli isolate AV41 16S ribosomal RNA gene, partial sequence	700	700	100%	0.0	99.20%	MH040909.1
<input type="checkbox"/> Escherichia coli strain E21 16S ribosomal RNA gene, partial sequence	700	700	100%	0.0	99.20%	KY780356.1
<input type="checkbox"/> Escherichia coli strain E152 16S ribosomal RNA gene, partial sequence	700	700	100%	0.0	99.20%	KY765056.1
<input type="checkbox"/> Escherichia coli strain E114 16S ribosomal RNA gene, partial sequence	700	700	100%	0.0	99.20%	KY765048.1
<input type="checkbox"/> Escherichia coli plasmid pV266-a DNA, contig: V266-a_scaffold_6, strain: V266	700	700	100%	0.0	99.20%	LC056477.1
<input type="checkbox"/> Escherichia coli strain 30 16S ribosomal RNA gene, partial sequence	700	700	100%	0.0	99.20%	KU052648.1
<input type="checkbox"/> Escherichia coli strain SUS9EC 16S ribosomal RNA gene, partial sequence	700	700	100%	0.0	99.20%	KF991482.1
<input type="checkbox"/> Escherichia coli strain 5.4 16S ribosomal RNA gene, partial sequence	700	700	100%	0.0	99.20%	AY319394.1
<input type="checkbox"/> Escherichia coli strain BGS18 16S ribosomal RNA gene, partial sequence	694	694	100%	0.0	98.93%	MN209843.1
<input type="checkbox"/> Escherichia coli strain Cp15 16S ribosomal RNA gene, partial sequence	694	694	100%	0.0	98.93%	MH295806.1

Top 10 hits in genus *Salmonella*

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Typhimurium strain KKP3079 16S ribosomal RNA gene, partial sequence	717	717	100%	0.0	100.00%	MW033548.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Typhi strain PU4 16S ribosomal RNA gene, partial sequence	717	717	100%	0.0	100.00%	MW029937.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Typhimurium isolate S.Tm LT2p22_assembled_genome_assembly_chromosome:1_Linear	717	4846	100%	0.0	100.00%	LR881463.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Typhi str. TY2 16S ribosomal RNA gene, partial sequence	717	717	100%	0.0	100.00%	MT975685.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar London strain HA1-SP5, complete sequence	717	5024	100%	0.0	100.00%	CP060134.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar London strain HA3-IN1, complete sequence	717	4994	100%	0.0	100.00%	CP060132.1
<input type="checkbox"/> Salmonella enterica strain K_SA184 chromosome, complete genome	717	5024	100%	0.0	100.00%	CP061159.1
<input type="checkbox"/> Uncultured Salmonella sp. clone asv_67_2323 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence	717	717	100%	0.0	100.00%	MT967568.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Albany strain R17_5974 chromosome, complete genome	717	5024	100%	0.0	100.00%	CP060730.1
<input type="checkbox"/> Salmonella enterica strain SLR1_8250 chromosome, complete genome	717	5024	100%	0.0	100.00%	CP060522.1

Top 10 hits in genus *Shigella*

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/> Uncultured Shigella sp. clone COG-15 16S ribosomal RNA gene, partial sequence	700	700	100%	0.0	99.20%	KJ910361.1
<input type="checkbox"/> Shigella dysenteriae strain ISO2 16S ribosomal RNA gene, partial sequence	689	689	100%	0.0	98.66%	KY971287.1
<input type="checkbox"/> Shigella sonnei strain ALL-7 16S ribosomal RNA gene, partial sequence	677	677	100%	0.0	98.12%	KJ639000.1
<input type="checkbox"/> Shigella sonnei strain 08BF03TD 16S ribosomal RNA gene, partial sequence	671	671	100%	0.0	97.86%	KX146471.1
<input type="checkbox"/> Shigella sonnei strain MG1 16S ribosomal RNA gene, partial sequence	667	667	100%	0.0	98.40%	KT824796.1
<input type="checkbox"/> Shigella sp. 86.3 16S ribosomal RNA gene, partial sequence	665	665	100%	0.0	97.59%	KU362663.1
<input type="checkbox"/> Shigella sp. R-52921 16S ribosomal RNA gene, partial sequence	665	665	100%	0.0	97.59%	KT185161.1
<input type="checkbox"/> Shigella sp. R-52920 16S ribosomal RNA gene, partial sequence	665	665	100%	0.0	97.59%	KT185160.1
<input type="checkbox"/> Shigella sonnei strain HZMJW 1-3 16S ribosomal RNA gene, partial sequence	660	660	100%	0.0	97.32%	MT605407.1
<input type="checkbox"/> Uncultured Shigella sp. clone 4_166 16S ribosomal RNA gene, partial sequence	660	660	100%	0.0	97.32%	GU271883.1