

Supplementary materials: for the article:

Zafar H. et al. Evaluation of a *Salmonella* Strain Isolated from Honeybee Gut as a Potential Live Oral Vaccine Against Lethal Infection of *Salmonella* Typhimurium

Pol J Microbiol. 2019. Article in Press.

Table SI

The details of the safety evaluation test.

Group SR1

Antigen	Post vaccination Hours	Results based on the average of readings of five rabbits					
		Physical abnormalities	Average body temperature	Daily food intake	Water intake	Weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	102.9	Normal	Normal	Normal	1×10^3
	48	-	102.5	Dropped	Dropped	Normal	1×10^4
	72	-	103.1	Normal	Normal	Normal	1×10^4
	96	-	102.5	Normal	Normal	Normal	1×10^5
	120	-	103.1	Normal	Normal	Normal	1×10^5
	144	-	101.6	Normal	Normal	Normal	1×10^4
	168	-	102.2	Normal	Normal	Normal	1×10^4

Group SR2

Antigen	Post vaccination hours	Results based on the average of readings of five rabbits					
		Physical abnormalities	Average body Temperature	Food intake	Water Intake	Changes in weight	Recovery of HB1 antigen CFU/ml (Average)
HB1 live antigen	24	None	103.2	Normal	Normal	Normal	1×10^5
	48	-	102.9	Normal	Normal	Normal	1×10^8
	72	-	102.5	Normal	Normal	Normal	1×10^8
	96	-	102.7	Normal	Normal	Normal	1×10^7
	120	-	102.1	Normal	Normal	Normal	1×10^7
	144	-	101.9	Normal	Normal	Normal	1×10^6
	168	-	102.5	Normal	Normal	Normal	1×10^7

Group SR3 (control)

Antigen	Post vaccination Hours	Results based on the average of readings obtained from of the five rabbits					
		Physical abnormalities	Average body Temperature	Food intake	Water Intake	Changes in weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	100.7	Normal	Normal	Normal	-
	48	-	102.0	Normal	Normal	Normal	-
	72	-	102.1	Normal	Normal	Normal	-
	96	-	102.5	Normal	Normal	Normal	-
	120	-	102.3	Normal	Normal	Normal	-
	144	-	102.4	Normal	Normal	Normal	-
	168	-	102.3	Normal	Normal	Normal	-

Table SII
Table for determination of LD50 of *S. Typhimurium* (ATCC 14028).

Groups	Dilution of bacteria	Dose cells	Observed values		Accumulative values		Rates	
			Dead	Alive	Total dead	Total alive	Fractional ratio	Percent ratio
1	10 ⁻²	1×10 ⁶	6/6	0/6	15	0	15/15	100%
2	10 ⁻³	1×10 ⁵	4/6	2/6	9	2	9/11	80%
3	10 ⁻⁴	1×10 ⁴	3/6	3/6	5	5	5/10	50%
4	10 ⁻⁵	1×10 ³	1/6	5/6	2	10	2/12	16%
5	10 ⁻⁶	1×10 ²	1/6	5/6	1	15	1/16	6%
6	10 ⁻⁷	1×10 ¹	0/6	6/6	0	21	0/21	0
7	PBS		0/6	6/6	0	27	0/27	0

No of rabbits in each group was 6, while total rabbits were 42.

The percentage of mortality was calculated according to the method of Reed and Munch, (1938). Proportional distance = % mortality above 50% - 50% / % mortality above 50% - mortality below 50% . The LD50 of *S. Typhimurium* (ATCC14028) was considered to be 1×10⁴ cfu/ml.

Table SIII
Results of Challenge/Protection test against *S. Typhimurium* applied at 70 days post immunization.

Vaccinated groups	No. of animals challenged	Morbidity	Survival	Percentage protection
R1	10	3	7/10	70
R2	10	0	10/10	100
R3	10	2	2/10	80
R4	10	0	10/10	10

Appendix A

The recovery percentage of isolates from the human, animal and insect enteric sources.

Type of sample	Species	No. of samples	Growth in TTB	<i>Salmonella</i> colonies on SSA/BSA	Total <i>Salmonella</i> isolates
Stool/ feces/ dropping/ insect gut contents	Human TY(1-15)	15	4(26.6%)	3(20%)	3(20%) TY1, TY2 TY3
	Cat CT (1-15)	15	3(20%)	1(6.6%)	1(6.6%) CT7
	Sheep SP (1-15)	15	3(20%)	1(6.6%)	1(6.6%) SP6
	Honey bee HB (1-15)	15	5(33.3%)	2(13.3%)	2(13.3%) HB1, HB11
	Poultry birds PB (1-15)	15	4(26.6%)	2(13.3%)	2(13.3%) PB2, PB9
	Horse HE (1-15)	15	1(6.6%)	1(6.6%)	1(6.6%) HE13
Total		90	20(22.2%)	10(11.1%)	10(11.1%)

TTB = Tetrathionate broth,
SSA = *Salmonella-Shigella* agar
BSA =Bismuth-Sulphite agar

Appendix B

Colony morphology of *Salmonella* isolates.

Isolate	SSA		BSA	
	Morphology	Diameter range	Morphology	Diameter range
TY1	Straw colored with blackish center	0.9-1.5 mm	Black colored	0.8-1.5 mm
TY2	Straw colored with blackish center	0.8-1.5mm	Black colored	0.75- 1mm
TY3	Straw colored with blackish center	0.75-1 mm	Black colored	0.75-1.5 mm
CT 7	Straw colored with blackish center	0.5-0.75 mm	Black colored	0.5-1mm
SP 6	Straw colored with blackish center	0.9-1 mm	Circular Straw color	0.5-1.5 mm
HB 1	Straw colored with blackish center	0.8-1mm	Circular Straw colored	0.75-1.5 mm
HB 11	Straw colored with blackish center	0.7- 1mm	Circular Straw colored	0.9-1 mm
PB 2	Straw colored with blackish center	0.8-1 mm	Circular Straw colored	0.8- 1.5mm
PB 9	Straw colored with blackish center	0.6-0.75 mm	Circular Straw colored	0.5-1 mm
HE 13	Straw colored with blackish center	Up to 1mm	Circular Straw colored	0.9-1.5 mm

Appendix C

Fasta sequence of TY1 (Accession# MH985341) alongwith top 4 hits from BLAST.

>TY1

```
CTCGAGCTACCATGCACTCAACGGTAACAGGAAGCACTTGCTGCTTTGCT
GACGAGTGGCGGACGGGTGAGTAGTGTCTGGGAAACTGCCTGATGGAGGG
GGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAA
AGAGGGGGACCTTCGGGCCTCTTGCCATCATATGTGCCCATATGGGATTA
TCTTGTTGGTGAGGTAACGGCTCACCTGGGCGACGATCCCTATCTGGTCT
GAGAGGATGACCACCCACACTGGAAGTGGAGACACGGTCCACACTCCTACG
GGAGGCAGCAGTGGGGAATATTGCACAGTGGGCGCAAGCCTGATGCACCC
ATGCCGCGTGTATGAAAAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGG
GAAGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAAAA
GAAGCACCGGCTAACTCCGTGCCAGCACCCGCGGTAATACGGAGGGTGCA
AGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCACGCGGTCTGTCAA
GTCAGATGTGATATCCCCGGTCTCAACCTGAGAAGTGCATTCGAAACTGG
CAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCGGGTGTAGCGGTGATA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAGCGCGGCCCTGCACAAG
ACTGACGCTCATGTGCGAAGCGTGGAGAGCAACAGGATTAGATCCTCTGA
TACTCACGCCGTACACGATGTCTACGTGAGTTGTGCCGTGACGCGTGCTC
AGAGCTAACGCGTAAGTACACCCGCCTGGAGAAGCTACGGGCCCGAC
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Descriptions

Sequences producing significant alignments:

Select: All None Selected: 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	1236	1236	98%	0.0	93%	NR_074799.1
<input type="checkbox"/> Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA gene, partial sequence	1230	1230	98%	0.0	93%	NR_104709.1
<input type="checkbox"/> Salmonella enterica subsp. salamae strain DSM 9220 16S ribosomal RNA gene, partial sequence	1227	1227	98%	0.0	93%	NR_044372.1
<input type="checkbox"/> Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA, partial sequence	1225	1225	98%	0.0	93%	NR_074910.1

FASTA sequence of TY2 (Accession# MH985355) alongwith top 4 hits from BLAST.

>TY2

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CTCGAGCTACCATGCACTCAACGGTAACAGGAAGCACTTGCTGCTTTGCT
GACGAGTGGCGGACGGGTGAGTAGTGTCTGGGAAACTGCCTGATGGAGGG
GGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAA
AGAGGGGGACCTTCGGGCCTCTTGCCATCATATGTGCCCATATGGGATTA
TCTTGTTGGTGAGGTAACGGCTCACAGGGCGACGATCCCTATCTGGTCT
GAGAGGATGACCACCCACACTGGAAGTGGAGACACGGTCCACACTCCTACG
GGAGGCAGCAGTGGGGAATATTGCACAGTGGGCGCAAGCCTGATGCACCC
ATGCCGCGTGTATGAAAAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGG
GAAGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAAAA
GAAGCACCGGCTAACTCCGTGCCAGCACCCGCGGTAATACGGAGGGTGCA
AGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCACGCGGTCTGTCAA
GTCAGATGTGATATCCCCGGTCTCAACCTGAGAAGTGCATTCGAAACTGG
CAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCGGGTGTAGCGGTGATA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAGCGCGGCCCTGCACAAG
ACTGACGCTCATGTGCGAAGCGTGGAGAGCAACAGGATTAGATCCTCTGA
TACTCACGCCGTACACGATGTCTACGTGAGTTGTGCCGTGACGCGTGCTC
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AGAGCTAACGCGTAAGTACACCCGCCTGGAGAAGCTACGGGCCCGAC

Sequences producing significant alignments:

Select: All None Selected: 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Salmonella typhi strain RG-07/06 16S ribosomal RNA gene, partial sequence	1197	1197	100%	0.0	95%	EF195174.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Typhi strain S.typhi Hk.4 16S ribosomal RNA gene, partial sequence	782	782	72%	0.0	92%	KX946133.1
<input type="checkbox"/> Pseudomonas fluorescens strain GB6 16S ribosomal RNA gene, partial sequence	588	588	74%	1e-163	86%	KR058828.1
<input type="checkbox"/> Serratia marcescens subsp. sakuensis strain LO7 16S ribosomal RNA gene, partial sequence	568	568	74%	1e-157	85%	MG198695.1

FASTA sequence of TY3 (Accession# MH989533) alongwith top 4 hits from BLAST.

>TY3

```
TCTCGAGCTCCATGCACTCAACGGTAACAGGAAGCACTTGCTGCTTTGCT
GACGAGTGGCGGACGGGTGAGTAGTGTCTGGGAATCTGCCTGATGGAGGG
GGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAA
AGAGGGGGACCTTCGGGCCTCTTGCCCTCATATGTGCCCATATGGGATTA
TCTTGTGTTGGTGAAGTAACGGCTCACCAGGGCGACTATCCCTATCTGGTCT
GAGAGGATGACCACCACTCTGGAAGTGAAGACACGGTCCACACTCCTACG
GGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCACCC
ATGCCGCGTGTATGAAAAGGCCTTCGGGTTGTAAAGTACTTTCCGCGGG
GAGGAAGGTGTTGTGTTTAATAACCGCAGCATTGACGTTACCCGCAAAA
AAAACACCCGCTATCTCCGTGCCAGCACCCGCGGAAATACAGAGGGTGCA
AGCGTTTATCAGAATTTCTGGGGCGTAAAGCGCACGCACGCGCTCTGTCA
AGTCAGATGTGAAATCCCCCGGGCCTCCCCCTGGGGAAGTGCATTCTAAA
CTGGCAGGCTTGAGTCTTGTAAGGGGGGGTAAAAATTCCATGTGTAGCG
GTGAAATGCGTAGAGATCTGGAAGAATACCGGTGGCGAAAGCGGCCCCCT
GCGACAAAGACTGACGCTCCTGTGCGAAAGCGTGAGAGCACACAAGTATA
TATACCTCCTGAGTGCTCCACGCGTAAAGATGTACTACATTAGAGTTGT
GCCCTTAGAGGCGTGTCTTCCGGATCATAACGCTTTTATATACACCCCGC
TCTTGGGAGTGT
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Sequences producing significant alignments:

Select: All None Selected: 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Salmonella typhi strain AK-1 16S ribosomal RNA gene, partial sequence	1286	1286	100%	0.0	95%	DQ480723.1
<input type="checkbox"/> Uncultured Citrobacter sp. clone bsc55 16S ribosomal RNA gene, partial sequence	1284	1284	99%	0.0	95%	KC011154.1
<input type="checkbox"/> Citrobacter freundii strain 58 16S ribosomal RNA gene, partial sequence	1279	1279	99%	0.0	95%	MH399241.1
<input type="checkbox"/> Citrobacter freundii strain B9-C2 chromosome	1279	10135	99%	0.0	95%	CP027849.1

Fasta sequence of HB1 (Accession #MH985334) alongwith top 4 hits from BLAST.

>HB1

```
CCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCATGTCGAAC
GGTAACAGGAAGCAGCTTGCCTCTTCGCTGACGAGTGGCGGACGGGTGAG
TAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTA
GCTAATACAGCATAATGTTCGCAGGACCAAGAGGGGGACCTTCGGGCCTC
TTGCCATCAGATGTGCCTAGATGGGATTAGCTTGTGTTGGTGAAGTAAACGGC
TCACCAAGGCGACGATCCTTAGCTGGTCTGAGATGATGACCAGCCACACT
GGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGCCCAATAT
TCTACTTATTTTTCAACCCTCATCCAGGGATGCAGCGTGTATGAAGAAGG
CCTTCGGGTCGGATTGTACTGGCAGCGCCGTGGTTGGGGATTTGGCTTAT
```

AACCTTGTTTCATTGACGATGGGCGCAGAAGAAGCACCCGGCTAACTCAGTG
 CCAGCAGCCGCGGTAATACGGAAGGTGCAAGCGTTAATCGGAATTACTGG
 GCGTAAAGCGCACGCCGGCGGTCTGTCAAGTTCGGATGTGAAATCCCCGGG
 CTCAACCTGGGAACTGCATTCGAAACTGGCGGGCTGGAGTCTTGTAGAGG
 GGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAAT
 ACCGGTGGCGAAGGCGGCCCTGGACTTAGACTGACGCTCAGGTGCGAA
 AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
 GATGTCTACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCCAACG
 CGTTAAGTAGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACCTCAAATG
 AATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAAATTTCGATGC
 AACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAAGTTTGCAGAGAT
 GCGAATGTGCCTTCGGGAACCTGTGAGACAGGTGCTGCATGGCTGTCGTCA
 GCTCGTGTGTGAAATGTTGGGTTAAGTCGGGCAACGAGCGCAACCCTTA
 TCCTTTGTTGCCAGCGGTCCCCCGGGGACACGGGGATGACGGCCTGACT
 TAAAGGGGATTAAGGCGGGGATTAGATCTTGTATCATCCCCTTTACGAC
 CAGAGCTACACACGTGCGACAATGGCGCATACAAAGAGAAGCGACCTCGC
 GAGAGCAAGCGGACCTCATAAAGTGCCTCGTAGTCTGGACTGGAGTCTGC
 AACTCGACTCCATGAAGTCGGAATCGCCCGGAATTGTGCATCAGGGTGCC
 AGGGAGATTAGGAACCGGGGCCTTGTAGACAGGTCCTGTCACAGGATGGG
 AGTGGGTGCAAAAGAAGTCGGTAGCTTAACCTTCATAGCCCAAAAAGGG
 CTTTTTGAACATGTCTTGGGTGTTGTGGAAACCCCTAGG

Descriptions

Sequences producing significant alignments:

Select: All None Selected: 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Salmonella enterica subsp. arizonae strain ATCC 13314 16S ribosomal RNA gene, partial sequence	2089	2089	96%	0.0	93%	NR_041696.1
Salmonella enterica subsp. arizonae strain NCTC7307 genome assembly, chromosome_1	2063	14221	96%	0.0	93%	LS483466.1
Salmonella enterica subsp. enterica strain SA20100345, complete genome	2063	14022	96%	0.0	93%	CP022504.1
Salmonella enterica subsp. arizonae serovar 62-z36-str. RKS2983, complete genome	2063	14197	96%	0.0	93%	CP006693.1

Fasta sequence of HB11 (Accession# MH985335) alongwith top 4 hits from BLAST.

>HB11

TGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTG
 CTGCTTCGCAGACGAGTCCCGGACGGGTGANTAATGTCTGGGAACTGCC
 TGATGGAGGGGGATAACTACTGGAAACGGAGGCTGGTACCGCATAACGTC
 GCAAGACCAAGAGGGGGACCTTCGGGCCTCTTGGGATCAGTTGTGCCCT
 GATGGGGTTAGCTGGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCC
 TAGCTGGTCTGAGAGGATGACCAGCCACACTGGAAGTGGAGACACGGTGGA
 GACACCTACGGGAGGCAGCAGTGGGGAAATGTTGCACAATGGGCGCAAGCC
 TGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAACTAA
 GGGCAACAGAGAGGAAGGTGTTGTGGTTAATTTCCGCAGCAATTGACGTT
 ACCCGCAGAAGTTGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
 GGGGGGTGCAAGCGTTAATCGGGGGTACTGGGCGTAAAGCGCACGCAGGC
 GGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCAT
 TCGAAACTGGCAGGCTTGAAGTCTTGTGGAGGGGGGTAGAATTCCAGGTGT
 AGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGGG
 CCCTGGACAAAGACTGACGCTCAGGTGCGTTTTCGTGGGGAGCAAACAGG
 ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCTACTTGGAGGCTG
 TGCCCTTGGAGCCGTGGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCTGG

GGAGTACGGCCGCAAGGTAAAACTCGGGTGGGTTGACGGCCCCCGCAG
 GGGCGGTGGAGCATGTGGTTTAAATTCGATGCAACGCGAAGAACCTTACCT
 GGTCTTGACATCCACGGAAGGTCTCAGAGATGAGACTGTGCCTTCGGGAG
 CCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTT
 GGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTC
 CGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGG
 GATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTA
 CAATGGCGCATAAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCAT
 AAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTC
 GGAATCGCTAGTAATCGTGGATCAGTTTGG

Descriptions

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Salmonella enterica subsp. houtenae strain DSM 9221 16S ribosomal RNA gene, partial sequence	2172	2172	99%	0.0	96%	NR_044371.1
Salmonella enterica subsp. indica strain DSM 14848 16S ribosomal RNA gene, partial sequence	2097	2097	99%	0.0	95%	NR_044370.1
Salmonella enterica subsp. salamae strain DSM 9220 16S ribosomal RNA gene, partial sequence	2089	2089	99%	0.0	95%	NR_044372.1
Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	2084	2084	99%	0.0	95%	NR_074799.1

FASTA sequence of CT7 (Accession# MH985332) alongwith top 4 hits from BLAST.

> CT7

GGCCTCTTGCCTTCAAATGTGCCAGATGGGATTAGCTAGTTGGTGTGGTAACGG
 CTCCCCAAGGCGACG
 ATCCCTAGCTGGTCTGAGAGGATGACCAGCCTCTCTGGAAGTCTGAGACACGGTCCA
 GACTCCTACGGGAGG
 CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCAGCGT
 GTATGAAGAAGGCCTT
 CGGGTTGTAAAGTACTTTCAGTGGGGTGGAAAGGTGTTGTGGTTAATAACAGCAGC
 AATTGACGTTACCAG
 CAGAAGAAGCACCGGCTAACTCCGTGCCTGCAGCCGCGGTAATACGGAGGGTGC
 AAGCGTTAATCGGAAT
 TACTGGGCGTAAAGCGCCCGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGG
 GCTCAACCTGGGAAC
 TGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGT
 AGCGGTGAAATGCGT
 AAAGATCTGGAGGAATACCGGTGGTGAAGGCGGCCCCCTGGACAAAACTGACG
 CTCAGGTGCGAAAGCG
 TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCCCGCCGTAAACGATGTCTAC
 TTGGAGGTTGTGCC
 TTGAGGTGTGGCTTCCGGAGCTAACGTGTTAAGTAGACCGCCTGGGGAGTACGGC
 CGCAAGGTTAAAACT
 CAAATGAATTGACGGGGCCCGCCAAGTGGTGGAGCATGTGGTTTAAATTCGATG
 CAACGCGAAGAACCT
 TACCTGGTCTTGACATCCACAGAACTTCCAGAGATGGATTGGTGCCTTCGGGAA
 CTGTGAGACAGGTGC
 TGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAG
 CGCAACCCTTATCCT
 TTGTTGCCTGTGATTAGGTCGGGAACTCAAAGGAACTGCCAGTGATAAACTGGA
 GGAAGGTGGGGATGA

CGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCA
TACAAAAAGAAGCGA
CCTCGTATGAGCAAGTGGACCTCATAAAGTGTGTCGTAGTCCGGATTGGAGTCTG
CAACTCGACTCCTTG
AAGTCGGAATCGCTAGTAATCGTGGATCAAATGCCACGGTGAATACGTTCCCGG
GCCTTGTACACACCG
CCCGTCTCCCCTTGGGAGTGGGTTGCAAAGAAGTAGGTAGCTTAACCTTCGGGA
GGGGGCTTACCCCTTTG

Descriptions

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Typhimurium strain ATCC 13311 16S ribosomal RNA gene, partial sequence	2145	2145	100%	0.0	97%	NR_116126.1
<input type="checkbox"/> Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA, partial sequence	2134	2134	100%	0.0	97%	NR_074910.1
<input type="checkbox"/> Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA gene, partial sequence	2124	2124	100%	0.0	97%	NR_104709.1
<input type="checkbox"/> Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	2111	2111	100%	0.0	97%	NR_074799.1

FASTA sequence of SP6 (Accession #MH985331) alongwith top 4 hits from BLAST.

>SP6

TACTCGAGCTCCATGCAATCAACGGTAAGTGGAAAGCACTTGCTGCTTTGC
TGACGAGTGGCGGACGGGTGAGTAGTGTCTGGGAAACTGCCTGATGGAGG
GGGATAACTACTGGAACGGTGGCTAATACCGCATAACGTCGCAAGACCA
AAGAGGGGGACCTTCGGGCCTCTTGCCATCATATGTGCCCATATGGGATT
ATCTTGTTGGTGAAGTAACGGCTCACCAGGGCGACGATCCCTATCTGGTC
TGAGAGGATGACCACCCACACTGGAAGTACACACGGTCCACACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAGTGGGCGCAAGCCTGATGCACC
CATGCCGCGTGTATGAAAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGG
GGAAGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAA
AAAAGCACCGGCTAACTCCGTGCCAGCACCCGCGGTAATACAGAGGGTGC
AAGCGTTAATCGGAATTACTGGGCGTAGAGCGCACGCACGCGGTCTGTCA
AGTCAGATGTGAAATCCCCGGGCTAACCTGAGAACTGCATTCGAAACTG
GCTGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCGGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAAGAATACCGGTGGCGAGCGCGGCCCCCTGCAC
AAGACTGACGCTCATGTGCGAAGCGTGGAGAGCAACGGGATTAGATACCC
TGATACTCCACGCCGTAACGATGTCTACTGAGTGTGCCGTGAAGGCGTC
CTCGGAGCTACGCGTTAAGTACACCCGTCTGAGAGTACGGACCGCG

Descriptions

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Dublin strain DSPV 595T 16S ribosomal RNA gene, partial sequence	1240	1240	99%	0.0	93%	FJ997268.1
<input type="checkbox"/> Uncultured bacterium clone 16saw/25-2d10 p1k 16S ribosomal RNA gene, partial sequence	1240	1240	98%	0.0	93%	EF605231.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Gaminara str. SA20063285 chromosome, complete genome	1236	8644	98%	0.0	93%	CP030288.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Milwaukee str. SA19950795 chromosome, complete genome	1236	8554	98%	0.0	93%	CP030175.1

Fasta sequence of PB2 (Accession# MH985338) alongwith top 4 hits from BLAST.

>PB2

AAATTGAAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTA
ACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACG
AGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGAT

AACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAG
GGGGACCTTCCCGCCACAAGCCAGCACATCTCGGCTGATCCCATTACCAA
GTTGGTGAGGTAACGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGTCTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATTGGGGGAAAAATGATGCAGGGATGG
GGCGTGTATGAAGAAGGCCCTTCGGGTTGTAAGTACTTTCAGCGGGGAGG
AAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAGAAGAAG
CACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGGTGCAAGCG
TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCG
GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTCGAAACTGGCAGG
CTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGGGCGAAGGCGGCCCCCTGGACAAAG
ACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT
GGTAGTCCACGCCGTAACGATGTCTACTTGGAGGTTGTGCCCTTGAGAG
CGTGGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCATGACAGTACGGCC
GCAAGGTTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAG
CATGTGATCCGATTTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACAT
CCACGGAAGTTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAG
TGCTGCATGGCTGTTCGTCAGCTCGTGTGTGAAATGTTGGGTAAAGTTC
GCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACT
CAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGAATCGCAGGTA
C

Description	Max score	Total score	Query cover	E value	Ident	Accession
Salmonella.gallinarum 16S ribosomal RNA gene, partial sequence	2004	2004	99%	0.0	97%	AF057360.1
Salmonella enterica subsp. enterica serovar Anatum strain R16.0676 chromosome, complete genome	1958	13318	99%	0.0	96%	CP029800.1
Salmonella enterica subsp. enterica serovar Anatum str. USDA-ARS-USMARC-1765, complete genome	1958	13377	99%	0.0	96%	CP014659.2
Salmonella enterica subsp. enterica serovar Anatum str. USDA-ARS-USMARC-1781, complete genome	1958	13294	99%	0.0	96%	CP014666.2

Fasta sequence of PB9 (Accession# MH985340) alongwith top 4 hits from BLAST.
>PB9

AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTA
ACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACG
AGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGACGCGGAT
AACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAG
GGGGACCTTCCGGCCTCTTGCCATCAGATGTGCCAGATGGGATTAGCTT
GTTGGTGAGGTAACGGCTCACCAAGGCGACGATCCCTTCCTGGTCTGAGA
GGATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC
CGCGTGTATGAAGAAGGCCCTTCGGGTTGTAAGTACTTTCAGCGGGGAGG
AAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAGAAGAAG
CACCGGCAAACGCCGTGCCTGCAGCCGCGGTAATACGGAGGGGTGCAAGCG
TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCG
GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTCGAAACTGGCAGG
CTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGAC
TGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAACGATGTCTACTTGGAGGTTGTGCCCTTGAGGCGT
GGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCTGGGGAGTACGGCCGCA

AGGTTAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
 GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCA
 CGGAAGTTTTTCAGAGATGAGAATGGTGCCTTCGGGAACCGTGAGACAGGT
 GCTGCATGGCTGTCGTCAGCTCGTGTGTTGTGGGGTGTTCCTTAAGTGGGG
 CTTCGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTC
 AAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGT
 CATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATAAC
 AAAGAGAAGCGCCACGCGTGTGCGAGGGGAGGTCATGGGGTACGACGCA
 GGCCGGAAAGGGGTCGGCAACTCGACTCCAGGAAGTCGGAATCGCTAGTA
 ATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACAC
 CGCCCGTCACACCATGGGAGTGGGTTGCAAAGAAGTAGGTAGCTTAACC
 TTCGGGAGGGCGCTTACCACCTTTGTGATTCATGACTGGGGTGAAGTCGTA
 ACAAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTTACCTT

Descriptions

Sequences producing significant alignments:

Select: All None Selected: 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Salmonella paratyphi strain A3 16S ribosomal RNA gene, partial sequence	2647	2647	100%	0.0	98%	EU118078.1
<input type="checkbox"/> Salmonella paratyphi strain A5 16S ribosomal RNA gene, partial sequence	2641	2641	100%	0.0	97%	EU118080.1
<input type="checkbox"/> Salmonella enterica subsp. enterica serovar Gaminara str. SA20063285 chromosome, complete genome	2639	18317	100%	0.0	97%	CP030288.1
<input type="checkbox"/> Salmonella enterica strain SA20041606 chromosome	2639	18151	100%	0.0	97%	CP030236.1

Fasta sequence of HE13 (Accession# MH985333) alongiwth top 4 hits from BLAST.

>HE13

ATTGAACGCTGGTGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGA
 AACAGCTTGCTGCTTCGCTGACGAGTGGCGACCGGGTGAGTAATGTCTGG
 GAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTGGCTAATACAG
 CATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAG
 ATGTGCCAGATGGGATTAGCTTGTGGTGAGGTAACGGCTCACCAAGGC
 GACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAAGTGA
 CACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG
 GCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTT
 GTAAAGTACTTTCAGCGGGGAGCAAGGTGTTGTGGTTAATAACCGCAGCA
 ATTGAAGTTACCCGCAGAAGAAGCACC GGCTAACTCCGTGCCAACACAG
 CGGTAATAAGGAGGGTGCAATTGTTAATCGGAATTACTGGGCGTAAAGCG
 CACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGG
 GAACTGCATTTCGAAACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAAT
 TCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG
 AAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA
 GCAAACAGGATTAGATACCCTGGTAGTCCAAGCCGTAACGATGTCTACT
 TGCAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTAG
 ACCGCTGGGCAGTACGGCAGCAAGGTTAAAACTCAAATGAATTGACGGG
 GGCCAGCACAATGGTGCAGCATGTGGTTTAATTCGATGCAAAGCGAAGA
 ACCTTACCTGGTCTTGACATCCACAGAANNNTCCAGAGATGAATTNGTGC
 CTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAACTCGTGTG
 TGAAATGTTGGGTTAAGTCCAACAAAGAGCGCAACCCTTATCCTTTGTTG
 CCAACGATTAGGTGGGGAAGTCAAAGGAGACTGCCAGTGATAAACTGCAG
 CAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAAGACCAGGGCTACA
 CAAGTGCTACAATGGTGCATACAAAGAGAATTGACCTCGCGAGAGCAAGC

GCACCTCATAAAGTGCGTCGTAGTCCGCATTGGAGTCTGCAACTCGACTC
 CATGAAGTAGGAATCGCTAGTAATCGTGCATCAGGGTGCCAAGGTGAATA
 AGTTGGGGGGCCTTGTACACACCGCCCGTCACACCATGGCAGTGGGTTGC
 AAAAGAAGTAGGTAGCTTAACCTTGGGCAGGGCGCTTACCACTTTGTGAT
 TCATGACTGGGGTGAAG

Descriptions

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA, partial sequence	2418	2418	100%	0.0	97%	NR_104709.1
<input type="checkbox"/>	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA, partial sequence	2401	2401	100%	0.0	96%	NR_074910.1
<input type="checkbox"/>	Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	2385	2385	100%	0.0	96%	NR_074799.1
<input type="checkbox"/>	Salmonella enterica subsp. enterica strain ATCC 13311 16S ribosomal RNA, partial sequence	2375	2375	100%	0.0	96%	NR_119108.1

Appendix E

Results of safety test for HB1 antigen (0.5 ml) in Group SR1, 7 days post oral immunization.

Type of antigen	Post immunization Hours	Results based on the average of readings of 5 rabbits					
		Physical abnormalities	Average Body temperature	Daily food intake	Water intake	Weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	102.9	Normal	Normal	Normal	1×10^3
	48	-	102.5	Dropped	Dropped	Normal	1×10^4
	72	-	103.1	Normal	Normal	Normal	1×10^4
	96	-	102.5	Normal	Normal	Normal	1×10^5
	120	-	103.1	Normal	Normal	Normal	1×10^5
	144	-	101.6	Normal	Normal	Normal	1×10^4
	168	-	102.2	Normal	Normal	Normal	1×10^4

Results of Safety test for HB1 antigen (1ml) in Group SR2 7 days post oral immunization.

Type of antigen	Post immunization hours	Results based on the average of readings of 5 rabbits					
		Physical abnormalities	Average Body Temperature	Food intake	Water Intake	Changes in weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	103.2	Normal	Normal	Normal	1×10^6
	48	-	102.9	Normal	Normal	Normal	1×10^8
	72	-	102.5	Normal	Normal	Normal	1×10^8
	96	-	102.7	Normal	Normal	Normal	1×10^7
	120	-	102.1	Normal	Normal	Normal	1×10^7
	144	-	101.9	Normal	Normal	Normal	1×10^6
	168	-	102.5	Normal	Normal	Normal	1×10^7

Results of Safety test for control group of rabbits given normal saline 0.5ml.

Type of antigen	Post vaccination Hours	Results based on the average of readings obtained from of the 5 rabbits					
		Physical abnormalities	Average Body Temperature	Food intake	Water Intake	Changes in weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	100.7	Normal	Normal	Normal	-
	48	-	102.0	Normal	Normal	Normal	-
	72	-	102.1	Normal	Normal	Normal	-
	96	-	102.5	Normal	Normal	Normal	-
	120	-	102.3	Normal	Normal	Normal	-
	144	-	102.4	Normal	Normal	Normal	-
	168	-	102.3	Normal	Normal	Normal	-

Appendix F

Results of IHA antibody titers in the four groups of immunized rabbits.

Sampling post vaccination days	Indirect hemagglutination titer in the four group of rabbits			
	R1	R2	R3	R4
Day 10	1:16	1:8	1:8	-
	1:4	1:16	1:16	-
	1:8	1:16	1:16	-
	1:8	1:32	1:32	-
	1:16	1:8	1:8	-
	1:16	1:8	1:8	-
	1:8	1:8	1:8	-
	1:8	1:8	1:8	-
	1:4	1:16	1:16	-
	1:16	1:16	1:16	-
Day 20	1:32	1:64	1:32	-
	1:16	1:32	1:32	-
	1:8	1:32	1:32	-
	1:16	1:32	1:32	-
	1:16	1:64	1:16	-
	1:32	1:32	1:32	-
	1:32	1:32	1:32	-
	1:16	1:16	1:32	-
	1:8	1:128	1:16	-
	1:16	1:64	1:32	-
Day 30	1:64	1:128	1:16	-
	1:64	1:64	1:32	-
	1:16	1:64	1:64	-
	1:8	1:64	1:64	-
	1:32	1:256	1:32	-
	1:64	1:128	1:32	-
	1:32	1:64	1:64	-
	1:32	1:64	1:64	-
	1:32	1:128	1:64	-
	1:64	1:64	1:64	-
Day 40	1:128	1:512	1:64	-
	1:64	1:256	1:128	-
	1:32	1:64	1:256	-
	1:32	1:64	1:128	-
	1:64	1:256	1:32	-
	1:64	1:64	1:64	-
	1:128	1:64	1:128	-
	1:64	1:64	1:256	-
	1:32	1:256	1:256	-
	1:128	1:64	1:128	-
Day 50	1:256	1:64	1:128	-
	1:128	1:64	1:64	-
	1:64	1:32	1:128	-
	1:32	1:32	1:64	-
	1:64	1:64	1:64	-
	1:32	1:32	1:64	-
	1:128	1:64	1:128	-
	1:64	1:32	1:128	-

	1:32	1:128	1:128	-
	1:128	1:32	1:16	-
Day 60	1:64	1:32	1:8	-
	1:32	1:64	1:4	-
	1:16	1:16	1:8	-
	1:32	1:64	1:16	-
	1:8	1:32	1:16	-
	1:16	1:16	1:4	-
	1:64	1:16	1:4	-
	1:16	1:8	1:16	-
	1:32	1:64	1:32	-
	1:128	1:32	1:8	-
Day 70	1:4	1:16	1:4	-
	1:4	1:4	1:2	-
	1:16	1:8	1:4	-
	1:8	1:4	1:4	-
	1:2	1:8	1:8	-
	1:4	1:2	-	-
	1:8	-	1:4	-
	-	1:4	1:8	-
	1:4	1:4	-	-
	1:32	1:32	1:16	-