Supporting Information

Chromosomal Instability in Enterohaemorrhagic *Escherichia coli* O157:H7: Impact on Adherence, Tellurite Resistance and Colony Phenotype

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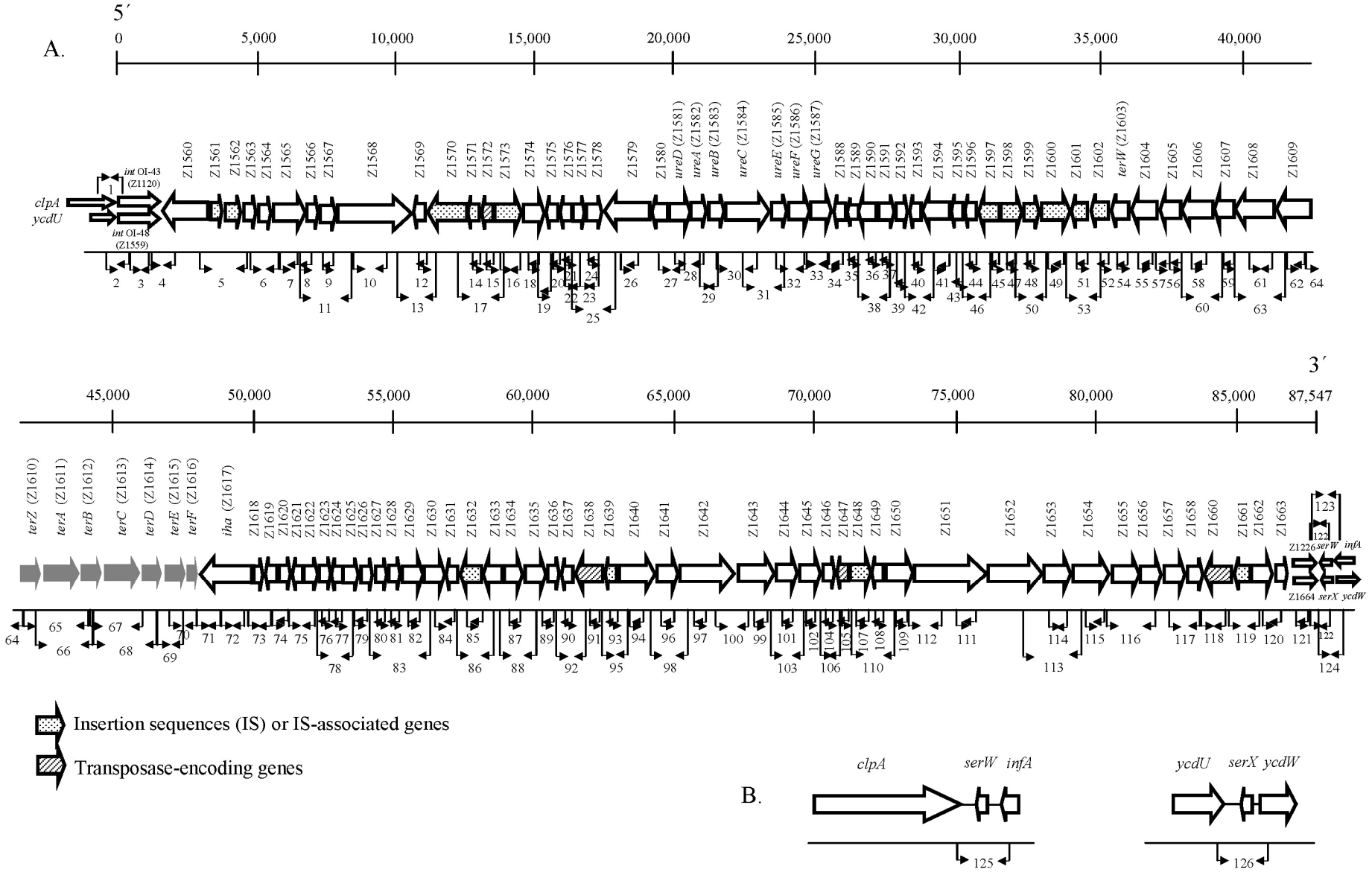
A.
5′
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87,547
3′
clpA
ycdU
int OI-43 (Z1120)
int OI-48 (Z1559)
ureD (Z1581)
ureA (Z1582)
ureB (Z1583)
ureC (Z1584)
ureE (Z1585)
ureF (Z1586)
ureG (Z1587)
terW (Z1603)
terZ (Z1610)
terA (Z1611)
terB (Z1612)
terC (Z1613)
terD (Z1614)
terE (Z1615)
terF (Z1616)
iha (Z1617)
serW
infA
serX
ycdW
Insertion sequences (IS) or IS-associated genes
Transposase-encoding genes
B.
clpA
serW
infA
ycdU
serX
ycdW

Fig. S1. PCR strategy to characterize tellurite resistance (Tel^R)-encoding islands and their integration sites in L and S colonies of *E. coli* O157:H7 strains.

A. Physical map of O island (OI) 48 and its flanks in *E. coli* O157:H7 strain EDL933 and positions of primers used to map Tel^R-encoding islands. Large arrows indicate ORFs of OI 48 (designations above arrows). Striped arrows denote putative transposases. Dotted arrows indicate insertion sequences (IS) and IS-associated genes. *ter* genes are grey. The duplicated arrows at the 5′ and 3′ends of the island indicate respective ORFs at these positions in OI 48 (Z1559 [putative P4-family integrase of OI 48] and Z1664, respectively; lower arrows) and in OI 43 (Z1120 [putative P4-family integrase of OI 43] and Z1226, respectively; upper arrows). Moreover, genes upstream of the first ORF of OI 48 (*ycdU*) and OI 43 (*clpA*) and downstream of the last ORF of OI 48 (*serX*, *ycdW*) and OI 43 (*serW*, *infA*) are indicated by double arrows. The scale (in bp) is above the graph. Small arrows indicate positions of PCR primers; the numbers below the small arrows indicate PCR numbers in Table S1, which describes PCR conditions.

B. Strategy to investigate occupancy of *serW* and *serX*, the respective integration sites for OI 43 and OI 48 in EDL933.

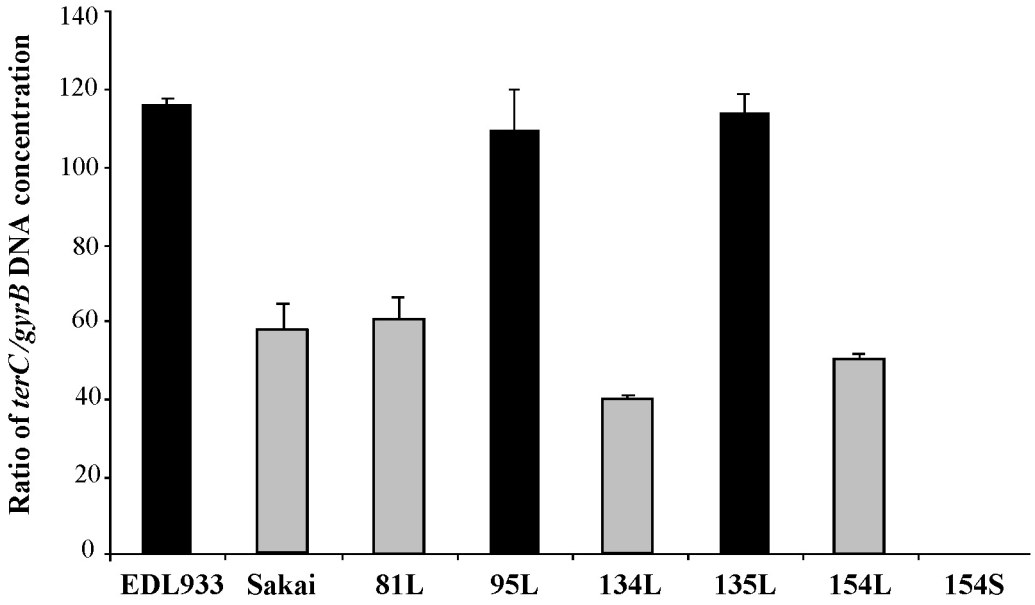

Fig. S2. Number of copies of Tel^{R}-encoding island in L strains. The *terC* DNA was quantified using light-cycler PCR and normalized to *gyrB* DNA. The *terC*/*gyrB* DNA ratio for each strain represents a mean ± standard deviation from three independent experiments. Strains with two copies or one copy of *terC* are depicted by black or grey bars, respectively. Strains EDL933 (GenBank accession no. AE005174; 2 copies of the *ter* cluster), Sakai RIMD 0509952 (GenBank accession no. NC_002695; 1 copy of the *ter* cluster), and 154S (lacking the *ter* cluster) were used as controls.

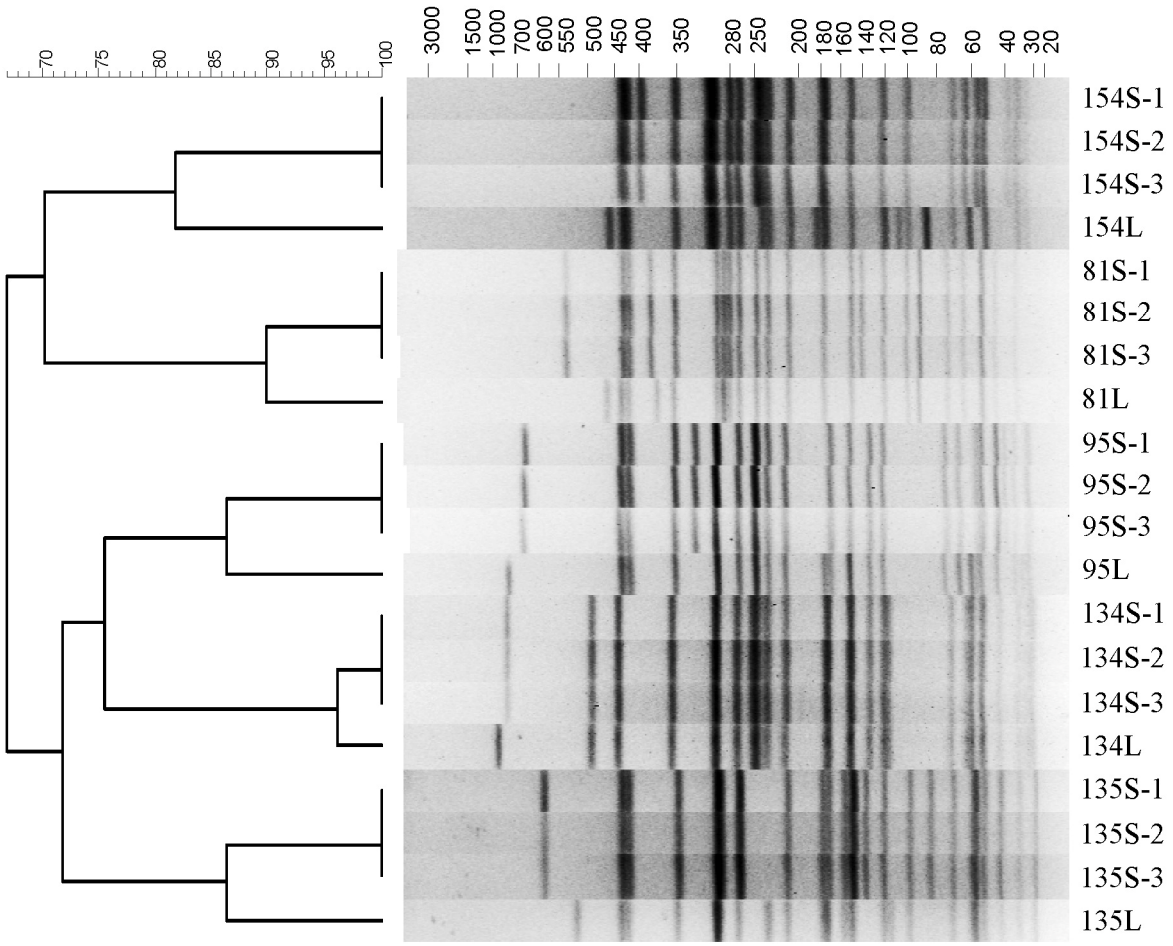

Fig. S3. PFGE of *Xba*I-digested genomic DNA from L and S strains. Chromosomal DNA from strains 81L, 95L, 134L, 135L, and 154 L and from three independent S colonies derived from each L strain was digested with *Xba*I and subjected to PFGE as described in Experimental procedures. The cluster analysis was performed using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) in BioNumerics software 5.1. Strain numbers are shown to the right from the gel (three independent S colonies derived from each L strain are designated by strain number and the end numbers 1 to 3). Molecular weight scale (based on *Xba*I-digested DNA of *Salmonella enterica* serovar Braenderup strain H9812) is given above the gel.

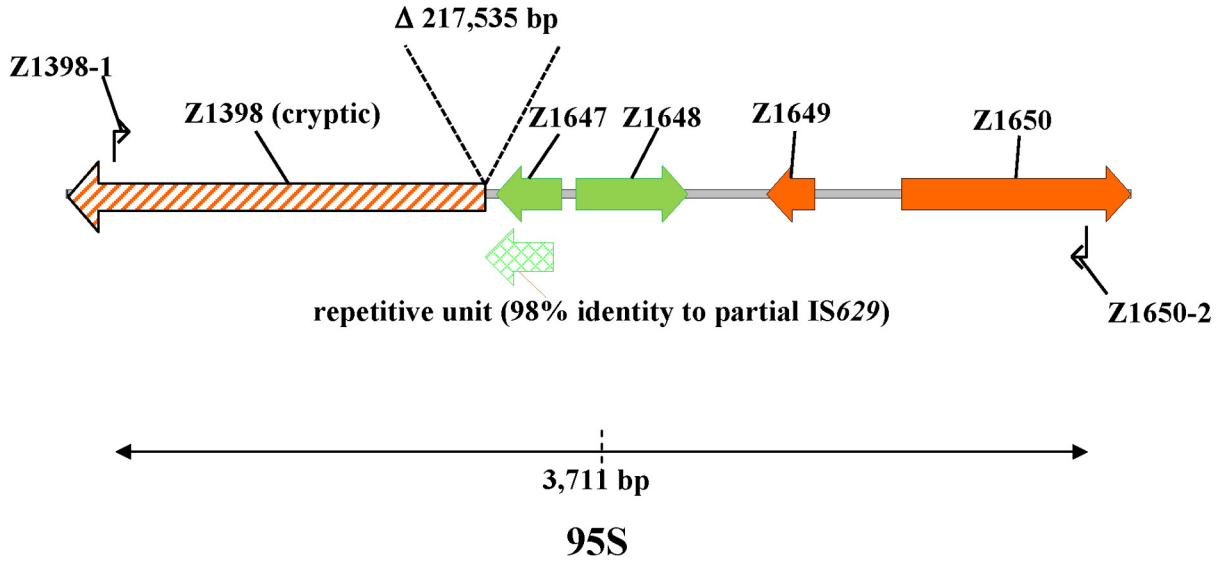

Fig. S4. Deletion of OI 48 and the upstream core genome region in strain 95S. The size and genetic organisation of the connecting fragment amplified with primers Z1398-1 and Z1650-2 (small arrows) that spans the internal deletion of OI 48 plus deleted parts of the core chromosome. Position and size of the deleted DNA region (217,535 bp) is indicated above the graph by dotted lines. ORFs with similarity to mobile genetic elements (putative transposases and insertion sequences) are highlighted in green.

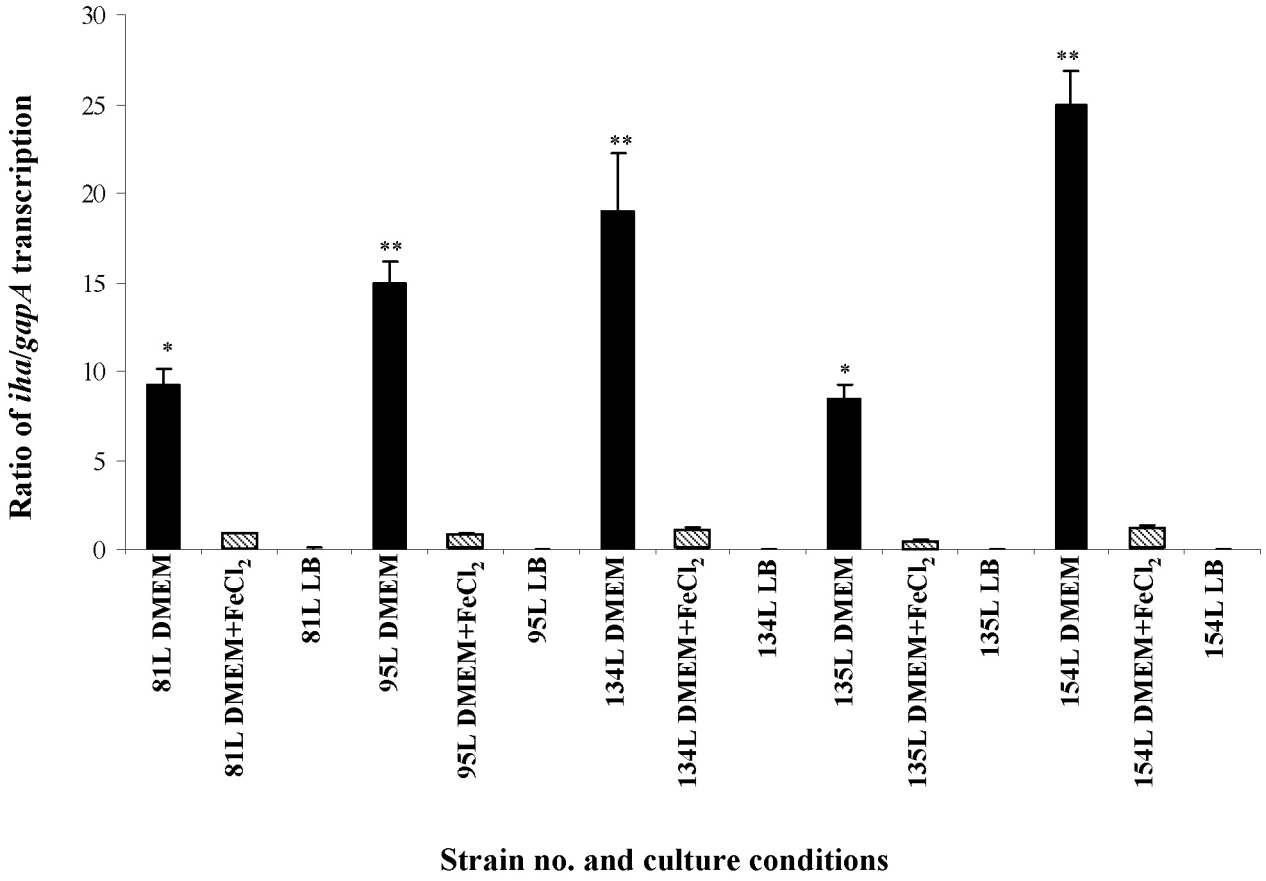

Fig. S5. *iha* transcription in L strains is upregulated in iron-limited conditions. The *iha*-positive L strains were cultured in DMEM (iron content < 0.05 μg ml^{-1}), DMEM with 10 μM $FeCl_2$ (iron content 0.50 μg ml^{-1}) or LB broth (iron content 0.59 μg ml^{-1}) and *iha* transcription was determined using quantitative real-time reverse transcription PCR and normalized to *gapA* transcription. The ratio of *iha*/*gapA* transcription was 8.4-24.9, ≤ 1.0, and ≤ 0.04, respectively. Data are presented as means ± standard deviations from three experiments performed with three independently isolated RNA preparations. Differences between relative transcription of *iha* in DMEM and in each of the other two media are indicated * ($p < 0.05$) and ** ($p < 0.001$) (unpaired Student´s *t* test).

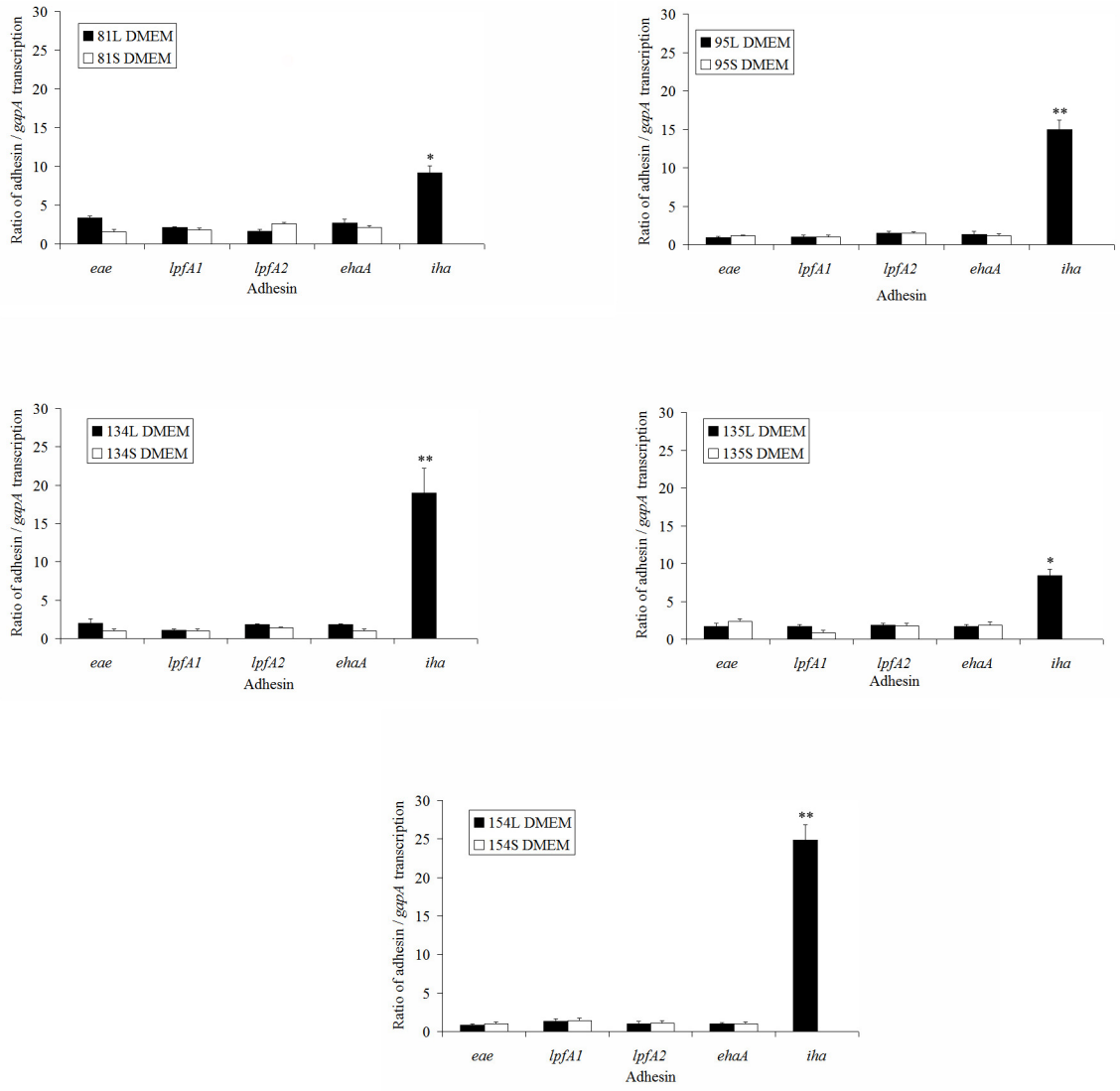

Fig. S6. Transcription analysis of *iha* and non-*iha* adhesin genes (*eae*, *lpfA1*, *lpfA2*, *ehaA*) in L and S strains grown under iron-limited conditions. The strains were cultured in DMEM (iron content < 0.05 µg ml^{-1}) and transcription of each gene was determined using quantitative real-time reverse transcription PCR and normalized to *gapA* transcription. Data are presented as means ± standard deviations from three experiments performed with three independently isolated RNA preparations. Differences between relative transcription of *iha* and each of the non-*iha* adhesin genes are indicated * ($p < 0.05$) and ** ($p < 0.001$) (unpaired Student´s *t* test).

Table S1. PCR primers and conditions used for mapping and analysis of deletions of Tel^R-encoding islands.

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
1	clpA-f int-4	CTGTTTGGTTCGCTGGTGGAC CCATTTCCCCCGCCCCTTTAC	*clpA*-integrase OI 43 (left junction of OI 43 and the core genome)	94°C, 30 s	56°C, 60 s	72°C, 60 s	756	2161-2181[d] 280-260
2	serX-F OI 48 CI-R	TGTCGATTCCTCTGGCATAA AAGTTGCTCTCGCATTATCC	*ycdU*-integrase OI 48 (left junction of OI 48 and the core genome)	95°C, 45 s	58°C, 45 s	72°C, 90 s	678	913-932[e] 397-378
3	int-1 int-2	AATCTGAGAAAGTCGCTATCC CACTGACGGCTTTCTTTTTAC	integrase OI 43/OI 48	94°C, 30 s	54°C, 60 s	72°C, 60 s	708	435-455 1142-1122
4	Z1559-1 Z1560-2	GAATGAAAGCCAGGATAC ATAAGCCTGTTTGATGGA	Z1559-Z1560	94°C, 30 s	50°C, 60 s	72°C, 60 s	767	972-989 1738-1721
5	Z1560-1 Z1563-2	TAGCCAGAACACCAAAAC AGTTCAGGCATCCGTAAG	Z1560-Z1563	94°C, 30 s	51°C, 60 s	72°C, 90 s	1,228	3310-3327 4537-4520
6	Z1563-1 Z1565-4	CAGCAGTCAAAGGTGTTA CGGATAAGGATTTTCTGG	Z1563-Z1565	94°C, 30 s	49°C, 60 s	72°C, 60 s	743	4708-4725 5450-5433
7	Z1565-1 Z1565-2	AACGCATCTGAATAAACC AGACAACCGCACATACTC	Z1565	94°C, 30 s	51°C, 60 s	72°C, 60 s	688	5849-5866 6536-6518
8	Z1565-3 Z1566-2	TGTCGCACCTAACTTTGA ATTTTTTCATACCTATAA	Z1565-1566	94°C, 30 s	48°C, 60 s	72°C, 60 s	381	6779-6796 7159-7142

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
9	Z1567-1 Z1567-2	ATTCATTTTATCGGCTAC TACGCCTCAGAATAATAC	Z1567	94°C, 30 s	49°C, 60 s	72°C, 60 s	256	7885-7902 8140-8123
10	Z1568-1 Z1568-4	ATCAGTGGGCTTTTCTTG ATCGCAGAAAATGAAACC	Z1568	94°C, 30 s	53°C, 60 s	72°C, 60 s	862	9120-9137 9981-9964
11	Z1565-3 Z1568-2	TGTCGCACCTAACTTTGA GAGACCAGACGGGCATTT	Z1565-Z1568	94°C, 30 s	51°C, 60 s	72°C, 120 s	1,628	6779-6796 8406-8389
12	Z1569-1 Z1569-2	TTTGCCATAATCGCTTCT ATCGGTAATCTTGAGGAA	Z1569	94°C, 30 s	50°C, 60 s	72°C, 60 s	212	10907-10924 11118-11101
13	Z1568-3 Z1570-2	CGTATCCTGTGCTGCTCA TCCTGTTCTTCGGCTCTG	Z1568-Z1570	94°C, 60 s	54°C, 60 s	72°C, 90 s	1,042	10605-10622 11646-11629
14	Z1571-1 Z1571-2	TACCTTCCAGCAGCATCG GTTACCTTTCGGGACCAA	Z1571	94°C, 30 s	56°C, 60 s	72°C, 60 s	290	13097-13114 13386-13369
15	Z1572-1 Z1572-2	GCTTACGATTACGCTGAA TATGGAAGAACGGAACAG	Z1572	94°C, 30 s	52°C, 60 s	72°C, 60 s	255	13858-13875 14112-14095
16	Z1573-1 Z1573-4	GTATCCAGGAGCGGTTAT GAGATATTCCCCAGACGA	Z1573	94°C, 60 s	54°C, 60 s	72°C, 60 s	758	14316-14332 15073-15056
17	Z1570-1 Z1573-2	CAGCAGGAAGATGTCGTC CCGCTCATCACTTTGTTT	Z1570-Z1573	94°C, 60 s	54°C, 60 s	72°C, 120 s	1,423	12962-12979 14384-14367
18	Z1574-3 Z1574-4	CAAAGCAGACAAAATGGA GGTATTTATGCTGGTTGC	Z1574	94°C, 60 s	53°C, 60 s	72°C, 60 s	332	15333-15350 15664-15647

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
19	Z1574-1 Z1575-2	CTCTCGGTGTTGGACTGG CAAACCGAACGCTGAAGA	Z1574-Z1575	94°C, 30 s	53°C, 60 s	72°C, 60 s	462	15458-15475 15919-15902
20	Z1575-1 Z1575-4	TATTATTTTCCCTGTCAC AGGAGGTTCTGTTATGGA	Z1575	94°C, 60 s	48°C, 60 s	72°C, 60 s	244	15837-15854 16080-16063
21	Z1576-3 Z1576-4	CGGAGACGCCATTATTTT TTTGAACTCCTGATGTCG	Z1576	94°C, 30 s	48°C, 60 s	72°C, 60 s	202	16141-16158 16342-16325
22	Z1576-3 Z1577-2	CGGAGACGCCATTATTTT ATTGATGACCTCTTTGCT	Z1576-Z1577	94°C, 30 s	50°C, 60 s	72°C, 60 s	439	16141-16158 16579-16562
23	Z1577-1 Z1578-2	AGGTCATCAATCTGGTAT GCAACTAAAATCGTAATC	Z1577-Z1578	94°C, 30 s	48°C, 60 s	72°C, 60 s	824	16569-16586 17392-17375
24	Z1578-3 Z1578-2	GAGTGCATTCTGGTTCTG GCAACTAAAATCGTAATC	Z1578	94°C, 30 s	47°C, 60 s	72°C, 60 s	224	17169-17186 17392-17375
25	Z1576-1 Z1579-4	CGGCATCATTCATACTGG TCGTTACTGGGGTTATGG	Z1576-Z1579	94°C, 60 s	51°C, 60 s	72°C, 120 s	1,243	16351-16368 17593-17576
26	Z1579-1 Z1579-2	GGATTCGCTTACATACAC TGTGGTTTTTATTTACGG	Z1579	94°C, 30 s	48°C, 60 s	72°C, 60 s	630	18145-18162 18774-18757
27	Z1580-1 UreD-r	TGTCGCTTCCCCAAGAAAAGG GCGTGGCTCCGGCGTAGTTTT	Z1580-*ureD* (Z1581)	94°C, 30 s	58°C, 60 s	72°C, 90 s	1,081	19578-19598 20658-20638
28	UreD-f UreA-r	CGTCATCATGTCGGTCTGCTCA CAGATTATCGGATTATGGACGGTA	*ureD-ureA* (Z1581-Z1582)	94°C, 30 s	58°C, 60 s	72°C, 90 s	1,040	20070-20091 21109-21086

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
29	UreA-f UreB-r	GACTCCAAGAGAAAAAGACAAACTA ACCCATTATATCTCCGCGGAAACCG	*ureA-ureB* (Z1582-Z1583)	94°C, 30 s	56°C, 60 s	72°C, 60 s	600	20816-20840 21415-21391
30	UreB-f UreC-r	GCTACCTGCAGTATTATCGTTGAA GAGGAAGGCAGAATATTGGG	*ureB-ureC* (Z1583-Z1584)	94°C, 30 s	57°C, 60 s	72°C, 90 s	1,146	21176-21199 22321-22302
31	UreC-f UreE-r	TCTAACGCCACAACCTGTAC CGTGATTATGGGCGTGCGACTT	*ureC-ureE* (Z1584-Z1585)	94°C, 30 s	56°C, 60 s	72°C, 120 s	1,666	21924-21943 23589-23568
32	UreE-f UreF-r	GAGACCCCGGCTCAGACAACT ACGGAATAATCGGGAATACTGGG	*ureE-ureF* (Z1585-Z1586)	94°C, 30 s	60°C, 60 s	72°C, 90 s	1,110	23169-23189 24278-24256
33	UreF-f UreG-r	ACTGGAGTGGGCAGTGGAAGC GATGTTTTGCAGACCTTCACCAC	*ureF-ureG* (Z1586-Z1587)	94°C, 30 s	59°C, 60 s	72°C, 90 s	1,182	23693-23713 24874-24852
34	UreG-f Z1588-2	GGTCCGGTCGGCTCAGGTAAA GTTTGAAAAATCGGCATCTGG	*ureG* (Z1587)-Z1588	94°C, 30 s	57°C, 60 s	72°C, 60 s	699	24335-24355 25033-25013
35	Z1589-3 Z1589-2	AACTGATAACCGATACCG AGGCACTTTAGGACAATG	Z1589	94°C, 30 s	51°C, 60 s	72°C, 60 s	226	25172-25189 25397-25380
36	Z1590-1 Z1590-2	CATCTGGGTTTTTACTTC AAAAGTTATGATGATTGC	Z1590	94°C, 30 s	47°C, 60 s	72°C, 40 s	106	25584-25601 25689-25672
37	Z1591-1 Z1591-4	ATTGAACTGGATGGTGTA CCTTTTTTCGCATCAACA	Z1591	94°C, 30 s	48°C, 60 s	72°C, 40 s	155	26227-26244 26381-26364
38	Z1589-1 Z1591-2	GAGTACCGAGCCCTTTTC AAACGGATGCGACTTAGA	Z1589-Z1591	94°C, 30 s	52°C, 60 s	72°C, 90 s	945	25348-25365 26292-26275

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
39	Z1592-3 Z1592-4	GCCTGACCATGTTGCTGA GCAAGAACGCCGCAATTT	Z1592	94°C, 30 s	52°C, 60 s	72°C, 40 s	93	26724-26741 26816-26799
40	Z1593-1 Z1593-2	ACTTTATTGATTTACTGC ATAGTTTTAGCAATACCT	Z1593	94°C, 30 s	45°C, 60 s	72°C, 40 s	135	27259-27276 27393-27376
41	Z1594-1 Z1594-4	ATAGTGGATTTCGTTTCA CCCAGAAACACGATAAAG	Z1594	94°C, 30 s	47°C, 60 s	72°C, 60 s	622	27779-27796 28400-28383
42	Z1592-1 Z1594-2	TGTAAACCGTAAGAAACC AATAATGAAACGAAATCC	Z1592-Z1594	94°C, 30 s	49°C, 60 s	72°C, 90 s	983	26819-26836 27801-27784
43	Z1595-3 Z1595-4	GCAGACTGGCATAAAAAC ACACGCAAGCGACCAAAT	Z1595	94°C, 30 s	48°C, 60 s	72°C, 40 s	121	28839-28856 28959-28942
44	Z1596-1 Z1596-2	TTTCGGGCGCATCATCAG GCCGGAACAATACCTCAG	Z1596	94°C, 30 s	54°C, 60 s	72°C, 40 s	131	29429-29446 29559-29542
45	Z1597-1 Z1597-4	CTCAAGGGGCAGCAACTC ATACGGCAATGGCGACAA	Z1597	94°C, 30 s	57°C, 60 s	72°C, 60 s	396	29928-29945 30323-30306
46	Z1595-1 Z1597-2	CGCTTCTTATGACTGTGC GAAGACCGACTGGAAGAG	Z1595-Z1597	94°C, 30 s	54°C, 60 s	72°C, 90 s	1,059	28902-28919 29960-29943
47	Z1598-3 Z1598-4	TAAACTGGCTTCACAACC GGCGGAACTCAACTTTAC	Z1598	94°C, 30 s	52°C, 60 s	72°C, 60 s	278	30780-30797 31057-31040
48	Z1599-1 Z1599-2	TATGCGTAAATCCTTCAA TGGATACCTTACCGTCAC	Z1599	94°C, 30 s	52°C, 60 s	72°C, 60 s	209	31187-31204 31395-31378

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
49	Z1600-3 Z1600-4	CGGGCATTACTCTCCAAC CGCTGCCAAAGAACATAA	Z1600	94°C, 30 s	56°C, 60 s	72°C, 60 s	745	32214-32231 32958-32941
50	Z1598-1 Z1600-2	TCCGCTTTTATCCACTCC TATTTCCCGCTCCTCCTC	Z1598-Z1600	94°C, 30 s	55°C, 60 s	72°C, 60 s	696	30993-31010 31688-31671
51	Z1601-1 Z1601-2	ATGTGCTGCTGGCGATAC TGGAAGAGCAGATGAAGC	Z1601	94°C, 30 s	55°C, 60 s	72°C, 60 s	496	33376-33393 33871-33854
52	Z1602-1 Z1602-4	ATGCCCTTTCACCTGTTG ATGCTGACGCCTGATAAC	Z1602	94°C, 30 s	54°C, 60 s	72°C, 60 s	315	34098-34115 34412-34395
53	Z1600-1 Z1602-2	AGGCGGATAATAATGCTG TTCACTGGCTGGTCTTTG	Z1600-Z1602	94°C, 30 s	55°C, 60 s	72°C, 120 s	1,264	32881-32898 34144-34127
54	TerW-1 TerW-2	TTCTCTACCGCTTCACTT TCAAATACAGCAAGGCAG	*terW* (Z1603)	94°C, 60 s	55°C, 60 s	72°C, 60 s	250	35047-35064 35296-35279
55	Z1604-3 Z1604-4	GTGGTGGGCATAGTTTTC CGAGAGCGAAGGCATTAG	Z1604	94°C, 30 s	54°C, 60 s	72°C, 60 s	490	35753-35770 36242-36225
56	Z1605-3 Z1605-4	GCGGTCAGGGCAAAATCA ATCCCCGTCGCCCTCAAA	Z1605	94°C, 30 s	58°C, 60 s	72°C, 60 s	476	37024-37041 37499-37482
57	Z1604-1 Z1605-2	GGTATTCTCACGCTCCAC GGGACCTATGCCAATCTG	Z1604-Z1605	94°C, 30 s	55°C, 60 s	72°C, 60 s	444	36119-36136 36562-36545
58	Z1606-1 Z1606-2	ACGGTATCGGCAAGCATC TGACATGGGGAAAACCTC	Z1606	94°C, 30 s	56°C, 60 s	72°C, 60 s	402	37986-38003 38387-38370

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
59	Z1607-3 Z1607-4	GACTGCCAGCCCTTTCTC ATCGCACCCTGAATCCAC	Z1607	94°C, 30 s	56°C, 60 s	72°C, 60 s	500	38898-38915 39397-39380
60	Z1605-1 Z1607-2	CTCCAGGCAAATCACCAG GCTGGCTGCTTGAAAAAC	Z1605-Z1607	94°C, 30 s	56°C, 60 s	72°C, 120 s	1,376	37521-37538 38896-38879
61	Z1608-1 Z1608-2	CCCCTTGTGGACGGAGAC TACTCGGCAGGCACATTC	Z1608	94°C, 30 s	57°C, 60 s	72°C, 60 s	668	39817-39834 40484-40467
62	Z1609-3 Z1609-4	GCTTCCTTCGTTTACCAG AACCATTCAGACCTACGG	Z1609	94°C, 30 s	54°C, 60 s	72°C, 60 s	550	40954-40971 41503-41486
63	Z1607-1 Z1609-2	AAAGGAAATGGCGGACTC CCTTCCTGGGTTATTTGC	Z1607-Z1609	94°C, 30 s	56°C, 60 s	72°C, 90 s	1,135	39664-39681 40798-40781
64	Z1609-1 Z1610-2	GTGGATGCCGTAGGTCTG ACCGAGACCGAAATGAAG	Z1609-Z1610	94°C, 30 s	50°C, 60 s	72°C, 60 s	631	41479-41496 42109-42092
65	TerZ1 TerA2	GACGGTATCACTCAGCAAAGAATC TGGCGGGTCAGTTCGTCAC	*terZ-terB* (Z1610-Z1612)	94°C, 30 s	58°C, 60 s	72°C, 150 s	1,800	42052-42075 43851-43833
66	TerA1 TerB2	TATCGTTTCAGCGGTTATTC TCGCAACGGCAATACCAACACG	*terZ-terB* (Z1610-Z1612)	94°C, 30 s	56°C, 60 s	72°C, 120 s	1,568	42586-42605 44153-44132
67	TerB1 TerC2	AGGCCGTGACGAACTGACC GAAACACTCATAAAATAACCTCTT	*terB-terD* (Z1612-Z1614)	94°C, 30 s	55°C, 60 s	72°C, 120 s	1,540	43828-43846 45367-45344
68	TerC1 TerD2	TCCTGGCGCTGAAAGAT CCGAACAGCATGGCAGTCT	*terB-terD* (Z1612-Z1614)	94°C, 30 s	56°C, 60 s	72°C, 150 s	1,742	44088-44104 45829-45811

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
69	TerD1 TerE2	AGTAAAGCAGCTCCGTCAAT CCGTCCCGTTGTCGTTGTTGTAA	*terD-terE* (Z1614-Z1615)	94°C, 30 s	55°C, 60 s	72°C, 90 s	1,023	45396-45415 46418-46396
70	TerE1 TerF2	TAAAAGGCGGCAACGTATCTCTGA CAATGACAACGGTGATCG	*terE-terF* (Z1615-Z1616)	94°C, 30 s	56°C, 60 s	72°C, 120 s	1,267	46020-46043 47286-47269
71	Z1616-1 Z1617-2	GCATTGAGATAGCACTGG GTGAATGCGAAACTGAAC	Z1616-Z1617	94°C, 30 s	53°C, 60 s	72°C, 90 s	1,058	47221-47238 48278-48261
72	Iha-I Iha-II	CAGTTCAGTTTCGCATTCACC GTATGGCTCTGATGCGATG	*iha* (Z1617)	94°C, 30 s	56°C, 60 s	72°C, 90 s	1,305	48259-48279 49563-49545
73	Z1617-1 Z1619-2	GACAGGGAATGACTACGG TGTAAAGCGGGGAGTAAC	Z1617-Z1619	94°C, 30 s	51°C, 60 s	72°C, 60 s	785	49952-49969 50736-50719
74	Z1619-1 Z1620-2	AAAACTCTTGACGCATAC TAGCCATACGACTTTACC	Z1619-Z1620	94°C, 30 s	48°C, 60 s	72°C, 60 s	764	50840-50857 51603-51586
75	Z1620-3 Z1622-2	AAAGTCGTATGGCTATGG ACATTCCGTCCAACAGAG	Z1620-Z1622	94°C, 30 s	49°C, 60 s	72°C, 60 s	841	51589-51606 52429-52412
76	Z1623-1 Z1623-2	AGATGGATTTCGCTGGAC ATGACATTGTAATAAGGC	Z1623	94°C, 30 s	46°C, 60 s	72°C, 40 s	112	53166-53183 53277-53260
77	Z1624-1 Z1624-2	AGTGGAAAATAGGTTAGG AAAATAAAAAGGCTGGAG	Z1624	94°C, 30 s	48°C, 60 s	72°C, 40 s	104	53491-53508 53594-53577
78	Z1622-1 Z1625-2	ACACAGGAGCCAGAATAC TAAATCAGGCAAAACCAG	Z1622-Z1625	94°C, 30 s	51°C, 60 s	72°C, 120 s	1,438	52528-52545 53965-53948

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
79	Z1625-1 Z1626-2	AGACTCTCGTTGCTGGTG CGGGATACCAGGGATTAC	Z1625-Z1626	94°C, 30 s	53°C, 60 s	72°C, 60 s	639	54043-54060 54681-54664
80	Z1627-1 Z1627-2	ACAACAAGCCACGACAGC TGCTCCACTTCACTTTTG	Z1627	94°C, 30 s	51°C, 60 s	72°C, 40 s	179	54773-54790 54951-54934
81	Z1628-1 Z1628-2	GCGCTGGTATTGTGGAAC TCAGGAAGGGATAACAAC	Z1628	94°C, 30 s	51°C, 60 s	72°C, 40 s	105	55060-55077 55164-55147
82	Z1629-1 Z1629-2	TACCCATTGATTTCTCTG TATATTTAGCAGCAACCA	Z1629	94°C, 30 s	49°C, 60 s	72°C, 60 s	313	55739-55756 56051-56034
83	Z1626-1 Z1630-2	ATCCCTGGTATCCCGTAA TTTCCTTTACAGCACCTC	Z1626-Z1630	94°C, 30 s	51°C, 60 s	72°C, 120 s	1,612	54667-54684 56278-56261
84	Z1630-1 Z1631-2	GATTGTGTTTGGCGAAGG CGCCTCCGAAAGATGTAA	Z1630-Z1631	94°C, 30 s	52°C, 60 s	72°C, 60 s	603	56574-56591 57176-57159
85	Z1632-1 Z1632-2	AGATAATGCCCGATGACC CGGGCAGTGATGTGATTG	Z1632	94°C, 30 s	55°C, 60 s	72°C, 60 s	385	57255-57272 57639-57622
86	Z1631-1 Z1633-2	TAAAGAACCGACTCCAAG TTGTTTTCCTCATTGTTC	Z1631-Z1633	94°C, 30 s	51°C, 60 s	72°C, 90 s	1,094	57133-57150 58226-58209
87	Z1634-1 Z1634-2	GTTGGCATTTCACTTCAC CTGCGACATAAAGGACTG	Z1634	94°C, 30 s	53°C, 60 s	72°C, 60 s	403	59167-59184 59569-59552
88	Z1633-1 Z1635-2	AAGGTGGAAGTGCTGAAA TCGTTCCGTTCTGTAAAA	Z1633-Z1635	94°C, 30 s	51°C, 60 s	72°C, 90 s	1,166	58572-58589 59737-59720

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
89	Z1635-1 Z1636-2	TGTTCAGGTTTGGCAGTC ACAAGCCCAGAAAACCAC	Z1635-Z1636	94°C, 30 s	54°C, 60 s	72°C, 60 s	450	60166-60183 60615-60598
90	Z1637-1 Z1637-2	TTTATTCTGTTTCTTCCC TTACCGAAAGTCAATCAG	Z1637	94°C, 30 s	50°C, 60 s	72°C, 40 s	175	60686-60703 60860-60843
91	Z1638-3 Z1638-4	AAAATCAGCCACCCACAG GCAACAGCGACATCATCC	Z1638	94°C, 30 s	56°C, 60 s	72°C, 60 s	316	61952-61969 62267-62250
92	Z1636-1 Z1638-2	GTTTTCTGGGCTTGTCTC GGCAGAAGCAGAAAAAGC	Z1636-Z1638	94°C, 30 s	51°C, 60 s	72°C, 90 s	929	60601-60618 61529-61512
93	Z1639-1 Z1639-2	AACTCCGCCTTCGCAAAA CGTTTTTCCCCCGAGGTC	Z1639	94°C, 30 s	57°C, 60 s	72°C, 60 s	290	62381-62398 62670-62653
94	Z1640-1 Z1640-2	GCTGATGCGTTACCACAC ACGATTTGACAGGCTTTG	Z1640	94°C, 30 s	54°C, 60 s	72°C, 60 s	504	63088-63105 63591-63574
95	Z1638-1 Z1640-4	ATGATGTCGCTGTTGCTG TGCTTCATCTCCGCTACG	Z1638-Z1640	94°C, 30 s	55°C, 60 s	72°C, 60 s	680	62252-62269 62931-62914
96	Z1641-1 Z1641-2	GAAATGACGACAGAGAGG CGTTTTTACTCTGTCCTG	Z1641	94°C, 30 s	50°C, 60 s	72°C, 60 s	429	64220-64237 64648-64631
97	Z1642-3 Z1642-4	GAAAAGATGGACAAACAG TGTTTCTGAATCCAATCC	Z1642	94°C, 30 s	49°C, 60 s	72°C, 60 s	370	64863-64880 65232-65215
98	Z1640-3 Z1642-2	GTTTGTGGCTGGTGTCTG TGTGTTTTACCTGTTCCA	Z1640-Z1642	94°C, 30 s	51°C, 60 s	72°C, 90 s	986	63786-63803 64771-64754

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
99	Z1643-1 Z1643-2	CTTTATGGGGCTGGTGTC GCTCCCCAGGTCATTCTC	Z1643	94°C, 30 s	55°C, 60 s	72°C, 60 s	544	67869-67886 68412-68395
100	Z1642-1 Z1643-4	GGTTATTGAAGTGGATGG CACAAGATGGAAAAGCAC	Z1642-Z1643	94°C, 30 s	50°C, 60 s	72°C, 60 s	821	66665-66682 67485-67468
101	Z1644-1 Z1644-2	GGTGAGAAGAAACTGCTG CCGCTAATCTGTTTCAAT	Z1644	94°C, 30 s	53°C, 60 s	72°C, 60 s	368	68766-68783 69133-69116
102	Z1645-1 Z1645-2	GTCATCCATCAACCAGTG TGTTATTCGCTGTTTCTG	Z1645	94°C, 30 s	50°C, 60 s	72°C, 60 s	371	69702-69719 70072-70055
103	Z1643-3 Z1645-4	GCGTATGAAAGAAAGGAG TTCATCGCATAAAAGTTG	Z1643-Z1645	94°C, 30 s	53°C, 60 s	72°C, 90 s	1,091	68526-68543 69616-69599
104	Z1646-1 Z1646-2	GATGAAAGTGAATGGTGT TTGACAAGACGACGTTAG	Z1646	94°C, 30 s	48°C, 60 s	72°C, 40 s	151	70248-70265 70398-70381
105	Z1647-3 Z1647-4	CCAGCCTTTCCAGCAATC TACGGCGGTCAGTTTACG	Z1647	94°C, 30 s	51°C, 60 s	72°C, 60 s	169	70970-70987 71138-71121
106	Z1645-3 Z1647-2	ATTTTATGAGCCCGATGC CGGCAGAAGCAGAAAAAG	Z1645-Z1647	94°C, 30 s	53°C, 60 s	72°C, 60 s	880	70078-70095 70957-70940
107	Z1648-1 Z1648-2	TACAGGACTGGCGAAAAG TCTGAGTGGAAGTGGATA	Z1648	94°C, 30 s	51°C, 60 s	72°C, 60 s	300	71208-71225 71507-71490
108	Z1649-1 Z1649-2	GCCGCCTCATCATTATCC ACGATGAAAACTATGACG	Z1649	94°C, 30 s	47°C, 60 s	72°C, 40 s	94	71966-71983 72059-72042

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
109	Z1650-1 Z1650-2	TCGCTTTCCCGTCACATC TGCCCCCAGACAGATTCC	Z1650	94°C, 30 s	58°C, 60 s	72°C, 60 s	665	72494-72511 73158-73141
110	Z1647-1 Z1650-4	TAAACTGACCGCCGTATG GTAAGGCAGGGAGGAGAG	Z1647-Z1650	94°C, 30 s	52°C, 60 s	72°C, 120 s	1,371	71123-71140 72493-72476
111	Z1651-1 Z1651-2	ACCACCACGAACCTCACC GACGGACGAAGCCATAGC	Z1651 (AIDA-I)	94°C, 30 s	57°C, 60 s	72°C, 60 s	532	75211-75228 75742-75725
112	Z1650-3 Z1651-4	AAACAGATGCGGTATTCC ACAGCAGCAAGAGACAGC	Z1650-Z1651	94°C, 30 s	55°C, 60 s	72°C, 60 s	837	72978-72995 73814-73797
113	Z1652-1 Z1654-4	TGGGAAAACTATGACGAG AATGCTGGTCCCTGAAAC	Z1652-Z1654	94°C, 30 s	54°C, 60 s	72°C, 120 s	1,369	78684-78701 80052-80035
114	Z1653-1 Z1653-2	CTGGCTGACGGAGATGAC GATGCTGCTCTTCACTGC	Z1653	94°C, 30 s	55°C, 60 s	72°C, 60 s	545	79261-79278 79805-79788
115	Z1654-1 Z1654-2	CGTGCTGAACTGGGTGTC GCAATACCGCCTCTGACC	Z1654	94°C, 30 s	57°C, 60 s	72°C, 60 s	487	80440-80457 80926-80909
116	Z1654-3 Z1656-2	CGGAAGAGGCAGATTTGG GAAGACACGGGGCTCCAG	Z1654-Z1656	94°C, 30 s	56°C, 60 s	72°C, 120 s	1,472	80945-80961 82416-82399
117	Z1657-2 Z1658-2	ACACCCAGTAAGGCAGAC GTGCTGGTGTCGGATAAA	Z1657-Z1658	94°C, 30 s	54°C, 60 s	72°C, 60 s	939	83086-83103 84024-84007
118	Z1658-1 Z1660-2	GCCTTTCCGCTGCTGATG CGACGATTGCTGGGAAGG	Z1658-Z1660	94°C, 30 s	57°C, 60 s	72°C, 60 s	578	83859-83876 84436-84419

PCR No.	Primer[a]	Sequence (5´ - 3´)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
119	Z1660-1 Z1662-2	ATGATGTCGCTGTTGCTG TAGCCGTTATCGCAGACC	Z1660-Z1662	94°C, 30 s	55°C, 60 s	72°C, 60 s	779	85124-85141 85902-85885
120	Z1662-1 Z1663-2	GCTACTCCCTTCGTGTTG CGATACAGGGCAACAGTG	Z1662-Z1663	94°C, 30 s	54°C, 60 s	72°C, 60 s	512	85899-85916 86410-86393
121	Z1664-1 Z1664-2	CTGGCTGAAATCTCGGTGCTG GATCACCCCTCGCTCAAACAC	Z1226, Z1664[f]	94°C, 30 s	57°C, 60 s	72°C, 60 s	327	86689-86709 87015-86995
122	LG-1 SerWX-2	AAATCAGCGAAGCGAACA TGTCCGAGTGGCTGAAGG	Z1226/Z1664 - *serW/serX*	94°C, 30 s	54°C, 60 s	72°C, 60 s	694	86936-86953 25-8[g]
123	LG43 InfA-r	GTAAACCAATCCCCGACAACC CCTGACGGGCGACAAAGTGAC	Z1226-*infA* (right junction of OI 43 and the core genome)	94°C, 30 s	55°C, 60 s	72°C, 60 s	781	87188-87208 79-59[h]
124	OI 48 CI-F serX-R	TTTGCCAGTATGCTCAAAGA GGTATCGAACGTTGGGTGAT	Z1664 downstr-*ycdW* (right junction of OI 48 and the core genome)	95°C, 45 s	58°C, 45 s	72°C, 90 s	505	87420-87401 75-56[i]
125	SerW-3 SerW-4	CGCTGGATAAAGAGAAAAATG GCAAAAACTACATCCGCATCC	*serW* intact	94°C, 30 s	54°C, 60 s	72°C, 90 s	859	2201-2221[d] 98-78[h]
126	serX-F serX-R	TGTCGATTCCTCTGGCATAA GGTATCGAACGTTGGGTGAT	*serX* intact	95°C, 45 s	60°C, 45 s	72°C, 90 s	624	913-932[e] 75-56[i]

PCR No.	Primer[a]	Sequence (5´ - 3´)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
127	Z1565F Z1638R	TGAAAGTCTGGCGATGAGTG GTACTACCGTCAGCCGGAAA	internal deletion of OI 48 in strain 134S	95°C, 45 s	60°C, 30 s	68°C, 240 s	2,017	6257-6276 62046-62027
128	Z1567F Z1648R	ATGCTGGCGGGATATTACTG GGATTTTCCAGCGTCATGTT	internal deletion of OI 48 in strains 81S/154S	95°C, 45 s	60°C, 30 s	68°C, 240 s	4,825/ 2,379	7831-7850 71571-71552
129	134S-F2 134S-R2	CAGAGTTGTACCGGTTATTGAGG CCATTTCGTAATTGGGATTAGC	walking primer for sequencing of PCR product 127	- -	59°C 59°C	- -	- -	n.a.[j] n.a.
130	81S-F2 81S-F3 81S-F4 81S-R2 81S-R3 81S-R4 154S-R2	ACGTTAGTGTGGCCAGTTCC ATGATGTCGCTGTTGCTGAC GCCCTAATTATCCTCCCGAAT ACCGGCCAGTCTGCTATG TGCGGTCATCACGAACATAC ATACGGCGGGAGACTTTTTC GTTCCATTGCCCCAAAGATT	walking primer for sequencing of PCR products 128	- - - - - - -	59°C 59°C 59°C 59°C 59°C 59°C 59°C	- - - - - - -	- - - - - - -	n.a. n.a. n.a. n.a. n.a. n.a. n.a.
131	RecAF RecAR	CCGGTAAAACCACGCTGAC CCTGACCGATCTTCTCACCT	RecA	95°C, 30 s	54°C, 45 s	72°C, 60 s	693	212-230[k] 904-885[k]
132	TerE1 TerE2	TAAAAGGCGGCAACGTATCTCTGA CCGTCCCGTTGTCGTTGTTGTAA	TerE	95°C, 30 s	58°C, 45 s	72°C, 60 s	399	46020-46043 46418-46396

PCR No.	Primer[a]	Sequence (5´ - 3´)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
133	Z1567F	ATGCTGGCGGGATATTACTG	5' junction of internal	94°C, 30 s	53°C, 30 s	68°C, 5 m	3,464	7831-7850
	Z1568-4	ATCGCAGAAAATGAAACC	deletion in strain 81L					9981-9964
						-		
	81S-F2	ACGTTAGTGTGGCCAGTTCC	walking primer for	-	59°C	-	-	n.a.
	81S-F3	ATGATGTCGCTGTTGCTGAC	sequencing	-	59°C	-	-	n.a.
	Z1568-R1	ACCTGAGGTAATTGCGCTTT		-	59°C	-	-	9360-9341
	Z1568-R2	CCAGCGCAGCAGGTACAC		-	59°C		-	8846-8829
134	Z1637F	CGCTGATTGACTTTCGGTAAT	3' junction of internal	94°C, 30 s	61°C, 30 s	68°C, 10 m	~6,200	60841-60861
	Z1648R	GGATTTTCCAGCGTCATGTT	deletion in strain 81L					71571-71552
	Z1637/38F	ATTCTGGTACCGGTTGATGC	walking primer for	-	59°C	-	-	61342-61361
	81S-R2	ACCGGCCAGTCTGCTATG	sequencing[l]	-	59°C	-	-	n.a.
	81S-R3	TGCGGTCATCACGAACATAC		-	59°C	-	-	n.a.
	81S-R4	ATACGGCGGGAGACTTTTTC		-	59°C	-	-	n.a.
	81S-R5	CGGAACGTTACCTCACAGC		-	59°C	-	-	n.a.

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
135	Z1565F	TGAAAGTCTGGCGATGAGTG	5’ junction of internal	95°C, 30 s	45°C, 30 s	68°C, 5 m	3197	6257-6276
	Z1567-2	TACGCCTCAGAATAATAC	deletion in strain 134L					8140-8123
	134S-F2	CAGAGTTGTACCGGTTATTGAGG	walking primer for	-	59°C	-	-	n.a.
	134L-F1	GTGCTGCTGGTTACCTCGTT	sequencing	-	59°C	-	-	n.a.
	ECs5244-2	CCAGCCTTCCCAGCAATCGTC		-	59°C	-	-	n.a.
	134L-R1	CTGGAAAGTCAGGGCGAATA		-	59°C	-	-	n.a.
	ECs5244-1	TCAGCAACAGCGACATCATCC		-	59°C	-	-	n.a.
	134S-R2	CCATTTCGTAATTGGGATTAGC		-	59°C	-	-	n.a.
136	Z1636-1	GTTTTCTGGGCTTGTCTC	3’ junction of internal	95°C, 30 s	53°C, 30 s	68°C, 5 m	1,446	60601-60618
	Z1638R	GGATTTTCCAGCGTCATGTT	deletion in strain 134L					31082-31063
	Z1638-2	GGCAGAAGCAGAAAAAGC	walking primer for sequencing	-	52°C	-	-	61529-61512

PCR No.	Primer[a]	Sequence (5′ - 3′)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
137	Z1567F	ATGCTGGCGGGATATTACTG	5’ junction of internal	94°C, 30 s	61°C, 30 s	68°C, 10 m	3,213	7831-7850
	Z1574R	CGACTGCTGCACCTTCATAA	deletion in strain 154L					15403-15384
	ECs5244-2	CCAGCCTTCCCAGCAATCGTC	walking primer for	-	59°C	-	-	n.a.
	81S-F3	ATGATGTCGCTGTTGCTGAC	sequencing	-	59°C	-	-	n.a.
	Z1573R	ACAGGGCTTTACCTGTCAGC		-	59°C	-	-	14772-14753
	Z1573-2	CCGCTCATCACTTTGTTT		-	50°C	-	-	14384-14367
	ECs5243-1	GCGATTCGTATGGTTCTGGA		-	59°C	-	-	n.a.
	ECs5244-1	TCAGCAACAGCGACATCATCC		-	59°C	-	-	n.a

PCR No.	Primer[a]	Sequence (5´ - 3´)	Target	PCR conditions[b]			PCR product (bp)	Position of the primer[c]
				Denaturing	Annealing	Extension		
138	Z1640F	CTGCTGGATTTACGGGACAT	3' junction of internal	94°C, 30 s	60°C, 30 s	68°C, 10 m	6,424	63370-63389
	Z1648R	GGATTTTCCAGCGTCATGTT	deletion in strain 154L					71571-71552
	Z1640-3	GTTTGTGGCTGGTGTCTG	walking primer for	-	54°C	-	-	63786-63803
	Z1641-1	GAAATGACGACAGAGAGG	sequencing	-	50°C	-	-	64220-64237
	Z1642-3	GAAAAGATGGACAAACAG		-	49°C	-	-	64863-64880
	Z1642F	GAGAAGGCTGGCGCTATTTA		-	59°C	-	-	65291-65310
	Z1642F2	TCAGGAACATGGTGCCAGTA		-	59°C	-	-	65800-65819
	ECs5244-2	CCAGCCTTCCCAGCAATCGTC		-	59°C	-	-	n.a.
	81S-F3	ATGATGTCGCTGTTGCTGAC		-	59°C	-	-	n.a.
	ECs5243-1	GCGATTCGTATGGTTCTGGA		-	59°C	-	-	n.a.
	ECs5244-1	TCAGCAACAGCGACATCATCC		-	59°C	-	-	n.a.
	Z1647R	AAACCGTGCAGAAGTGGAAC		-	59°C	-	-	71046-71027
	Z1642/43R	CACACCTGGAAAATCCATGC		-	59°C	-	-	67171-67152
	Z1642R	AATGGAGTCTTCCCGACCTT		-	59°C	-	-	66661-66642

a. Primers were derived from the sequence of O island (OI) 48 of *E. coli* O157:H7 strain EDL933 (GenBank accession number AE005174; genomic position 1454242 to 1541789), which is identical in its nucleotide sequence to OI 43 of EDL933, and from the genes flanking OI 48 (*ycdU*, *serX*, *ycdW*) and OI 43 (*clpA*, *serW*, *infA*) in the genome of strain EDL933.

b. All PCRs included 30 cycles as indicated, preceded by denaturation (94°C, 5 min) and followed by a final extension (72°C, 5 min).

c. Positions of PCR primers within the sequence of OI 48 of *E. coli* O157:H7 strain EDL933 are indicated unless specified otherwise; position of the first nucleotide of OI 48 (1454242) is considered as position 1.

d. Position of the primer within *clpA* (Z1119), which is located directly upstream of the integrase gene of OI 43 in strain EDL933 (Fig. S1A).

e. Position of the primer within *ycdU* (Z1558), which is located directly upstream of the integrase gene of OI 48 in strain EDL933 (Fig. S1A).

f. Last genes of OI 43 and OI 48, respectively.

g. Positions of the primer within *serW* and *serX* genes (which are 100% identical).

h. Position of the primer within *infA* (Z1228), which is located downstream of OI 43 (Fig. S1A).

i. Position of the primer within *ycdW* (Z1666) which is located downstream of OI 48 (Fig. S1A).

j. n.a., not applicable

k. Position of the primer within *recA*(Z4002).

l. Full sequencing of the PCR product was not possible due to the presence of an additional IS*629* besides Z1638/Z1339 in the PCR fragment. However, the existence of two IS elements has been verified by restriction digestion with *Hind*III, a single cutting enzyme in IS*629*, which resulted in three restriction fragments of the expected size (data not shown). According to sequencing data additional *Hind*III restriction sites in flanking regions were excluded.

Table S2. PCR primers for analysis of the core genome deletions in strain 95S.

Primer	Sequence (5´- 3´)	Target	Annealing temperature	PCR product (bp)
ycdU-1 ycdU-2	GTGGGATTCTGGCCCTGGTGC GCGTACCGCCTGGAGAAGCG	Z1558 (*ycdU*)	59°C	538
Z1549-1 Z1549-2	GCACTGAGCCCATCTATC ATTCGGCACATAGTCCAG	Z1549	55°C	554
Z1539-1 Z1539-2	AGCCATCACTATCACAAGCAA CCTTCAGGGATTAAGTGCAAA	Z1539	58°C	494
Z1528-1 Z1528-2	TGATTGCTAACGGAGCATTG GCAGCGCGGATAAAAACTAC	Z1528	59°C	712
Z1522-1 Z1522-2	GCATTTTCAACCTCATCC TCCACCTTCCCTATTTCC	Z1522	54°C	674
Z1513-1 Z1513-2	CCAGGCGAATCATAAGAC TCGCTGAACCGCATTATC	Z1513	55°C	607
Z1505-1 Z1505-2	AATCAGCCCGAACACAAC CTGGATGGCGGTAATGAC	Z1505	55°C	574
Z1495-1 Z1495-2	GGCAACTGTTACGCACTC CGAATGCCTCACGAATAC	Z1495	55°C	1224
Z1486-1 Z1486-2	CCGAATGATGATGAAACC ACATCCTGCTTACCATCC	Z1486	54°C	791
Z1477-1 Z1477-2	CGTGAAGCCTGGTTTGTC GCCCCTGAATAACCTCTG	Z1477	55°C	784
LP43 LP44	ATCCTATTCCCGGGAGTTTACG GCGTCATCGTATACACAGGAGC	Z1464	57°C	584
wrbA1 wrbA2	ATGGCTAAAGTTCTGGTG CTCCTGTTGAAGATTAGC	Z1423	53°C	600
Z1409-1 Z1409-2	TGCGTAACCAATCACCGTAA CTGAATTAATCGGCGCTCTC	Z1409	59°C	605
Z1408-1 Z1408-2	GGCTCCCTTGTGGAGCCTTTTT CGCCAGTAAGGTGTTGGTTCCCG	Z1408	57°C	155

Primer	Sequence (5´- 3´)	Target	Annealing temperature	PCR product (bp)
Z1406-1 Z1406-2	TCCTGATGATGGCAGCAAAGACG GGGCCACGTTGACCCTGCTC	Z1406	57°C	120
Z1405-1 Z1405-2	CGCTGTTGGTCCTCGCAGGC ACCTTTGATCGCAAAAGCGGCA	Z1405	58°C	162
Z1404-1 Z1404-2	GCGCGTAGTGGACGTGGTGG GACGCCAGCTGCGTTTGCTG	Z1404	59°C	259
Z1403-1 Z1403-2	ACACAGCACATCGCGACCCG TCAGCAGATCCAGGCGTTGCC	Z1403	59°C	364
Z1402-1 Z1402-2	CACAGGGCAACAGGGGCGAC TTCCGTTGGCCCCGTGGAGA	Z1402	60°C	504
Z1401-1 Z1401-2	TTGCGCAGGCTGGCTACGTC TGGCGCTGTTCCCATGGCTG	Z1401	59°C	946
Z1400-1 Z1400-2	AGCGTTACCCAGCGCTTCCG CGCAGGCTAACCCGGAGCTG	Z1400	59°C	647
Z1399-1 Z1399-2	GACCTCGGGAGCGATTGCGG CATTGGCGAGCGCTTGCTGC	Z1399	59°C	241
Z1398-1 Z1398-2	GCAGGCTGGTGCCGACAGAG GAAGTGCGCAGCGAGCTGGA	Z1398	60°C	1174
Z1397-1 Z1397-2	TCGTCATGGTGTGCGTGCCC ATCCTTCCCCACCCCGGCTC	Z1397	59°C	666
Z1396-1 Z1396-2	TAGCGTTGGTGCGCACTGGG CAGCGTGATGCCTGCCGTGA	Z1396	59°C	775
Z1395-1 Z1395-2	CCTGCTGGTGACGTGGCTGG ACGGCAAACTCCAGAGCGCC	Z1395	59°C	917
Z1394-1 Z1394-2	ACAGCCCGCAGGTGATTG TCAATGGGCGGCGGTAAC	Z1349	58°C	352
Z1376-1 Z1376-2	TCCACGGGGCTGAAAATC GGAGAGCGTTTTCGTCAC	Z1376	56°C	375
Z1349-1 Z1349-2	GTGAGGGCGTTTATTTTG GCATTCAGTCCCAGTGTC	Z1349	56°C	844

Primer	Sequence (5´- 3´)	Target	Annealing temperature	PCR product (bp)
Z1329-1 Z1329-3	GCAGTTCCGGTAATCCTGTG AATCCAGGAAATTGCAGCAC	Z1329	60°C	331
Z1309-1 Z1309-2	ACCGATCATTGTTTGGCAGT GAAACTCCTGTTTCGGCGTA	Z1309	60°C	543
Z1297-1 Z1297-2	GGCTGACGGTGGTGAAAATAC GTTTTGCTGATTTCGCCACAC	Z1297	56°C	405
Z1280-1 Z1280-2	AAATCCTGACGCTGCCTTCTG CAGGTCGGTAAGCATTTCCAC	Z1280	58°C	602
Z1270-1 Z1270-2	ATCCCTCGTTCCGTCTTGTCG TGGCTGATTCTCTTCGGTTTC	Z1270	57°C	438
Z1256-1 Z1256-2	TGGCACTAGCGGCATTACATC CCGAGGAGGCGTCAAGGAAAA	Z1256	57°C	327
Z1243-1 Z1243-2	CAGGGCGAGCAATGTAAGGTG ATGATGCTGCCGTTTTGCTTG	Z1243	55°C	225
Z1232-1 Z1232-2	TTTGATGTAGCCGTTTTCCAG CCGTGGAGTTTCTGCTTGTGC	Z1232	56°C	406
clpA-1 clpA-2	GAATCAGCTTGCGCGCGTGG TTCGTCCAGCAGCAGCACCG	Z1119 (*clpA*)	59°C	1168
Z1118-1 Z1118-2	TTCGCGACGCGCTAAAACCG TGGTTTCTGCAACCTCGGCGG	Z1118	59°C	198
Z1117-1 Z1117-2	TGCCTTTTGGCCCCTGGTGG CATCTGCCCTGAAGGCGGCG	Z1117	59°C	125
Z1116-1 Z1116-2	TGCTTCGCGCCCAGGAGTTG ACCTGCGCACGACCGTTCAG	Z1116	59°C	838
Z1115-1 Z1115-2	TGCTGGCGACCGGAAAGCTG CCGGATCGCCTAACGCCGAC	Z1115	59°C	804
Z1112-1 Z1112-2	CGCCGACGGTATTCAGCCCG ATCGCCCTTACCTTGCGGCG	Z1112	59°C	649
Z1110-1 Z1110-2	CTGGCGTCTGGGGCCAAGTG CAGCCACAGCACGCGTGAGA	Z1110	59°C	595

Primer	Sequence (5´- 3´)	Target	Annealing temperature	PCR product (bp)
Z1109-1 Z1109-2	CAGCCGCCCTGGAAGATGGC ATTGCCCAGGTTGTCGGCGG	Z1109	59°C	344
Z1108-1 Z1108-2	CGCGCTCCCGATTACCGGAC CGCTTCGCCAACAAGCTGCG	Z1108	59°C	617
Z1107-1 Z1107-2	AGCATTTCGCCGTGGGTGTAG GCAGGGGATGTGTGGTAAAAC	Z1107	57°C	762
Z1097-1 Z1097-2	CGCGGAAAATATTCCAACAG TCATCAGCAAATCCAAGCAC	Z1097	59°C	573
Z1087-1 Z1087-2	TGGTTTGCTGGTGGGTATTGG CGGCATCTTTGGTAACTTTGG	Z1087	55°C	500
Z1074-1 Z1074-2	TCGGCATCTCAATCTGGCTAC CATTGGCGTTTTAGTCGTATC	Z1074	56°C	908
Z1063-1 Z1063-2	ATTCCGAAGTTGGCGATGATG GCCGTAGTTTTTGCCTTTTTG	Z1063	56°C	443
Z1054-1 Z1054-2	TTTGCTTTCCCCATTCCTTAC CTCTTTTTGCCCTTTACCTTC	Z1054	56°C	480
Z1045-1 Z1045-2	TTACCCACCGAGCCAATGACC TCCACTCAGCCTAACCTCAGC	Z1045	58°C	998
Z1035-1 Z1035-2	TAGCAGGAACCACAGACCAAG TGGCGTCTATTCAAGGTGGAG	Z1035	57°C	347
Z1026-1 Z1026-2	ACCCATAATCGCCGTCATCAG GGATACTACCCGCACGATGAC	Z1026	58°C	826
Z1017-1 Z1017-2	CCACGGTAATAAATCGCAAGG TGATTTCGCACCACCTTCTCC	Z1017	58°C	436

All primers were designed in this study except for LP43 and LP44 (Friedrich *et al.,* 2002) and wrbA1 and wrbA2 (Bielaszewska *et al*., 2006b).

Table S3. Quantitative real time RT-PCR assays to determine transcription of *iha* and non-*iha* adhesins in L and S strains.

Primer	Sequence (5´ - 3´)	Target	Annealing temperature	PCR product (bp)	Reference
GapA_for GapA_rev	GTTGTCGCTGAAGCAACTGG AGCGTTGGAAACGATGTCCT	*gapA*	53°C - 60°C	171	Blumer *et al*., 2005
K14924 K14925	TTACCAGCGATACCAAGAGC CAACATGACCGATGACAAGG	*eae*	59°C	126	Chen *et al.*, 2007
CMD306 CMD307	GGCTGAATCTGCAGGAAAGCAACA TGCAGGCTGACAGAATCATCCACA	*iha*	60°C	95	Léveillé *et al.,* 2006
ehaA-F ehaA-R1	AGCGTTAGGGGCACAGGAAG CCTCGCTGCCACTGACAATA	*ehaA*	54°C	161	This study[a]
LPFA1-CF LPFA1-CR1	GGTTGGTGACAAATCCCCG CGTCTGGCCTTTACTCAGA	*lpfA*1 (O157/OI-141)	53°C	244	Torres *et al.,* 2009
LPFA2-F1 LPFA2-CR	GCAGGTCACCTACAGGCGGC GCTAATACCAGCGGCAGCATCGT	*lpfA*2 (O157/OI-154)	57°C	306	This study[b] Torres *et al.,* 2009

a. The primers were derived from a 851-bp fragment of *ehaA* passenger domain sequenced in our laboratory, which was 100% identical in all L and S strains and also to the respective sequences in EHEC O157:H7 strains EDL933 and Sakai (data not shown).

b. The primer was derived from the sequence of *lpfA2* of strain EDL933 (GenBank accession no. AE005174).

Table S4. Plasmids and *E. coli* constructs used in this study.

Plasmid or construct	Description	Source or reference
Plasmids		
pWKS30	*ori* pSC101, *bla*	Wang and Kushner, 1991
pWKS30*iha*	pWKS30 with a 2,916-bp fragment containing *iha* from EHEC O157:H7 Sakai (RIMD 0509952)	This study
pKD3	*bla* FRT *cat ori*Rγ	Datsenko and Wanner, 2000
pKD46	*bla araC*-P*araB*-γ-β-exo *ori*R101 *repA*101 (ts)	Datsenko and Wanner, 2000
pKD4	*kan* FRT *cat ori*Rγ	Datsenko and Wanner, 2000
pKD4*iha*	pKD4 with a 2,916-bp fragment (*BstB*I/*Hind*III) containing *iha* from EHEC O157:H7 Sakai (RIMD 0509952)	This study
pCP20	*bla cat flp* (ts)	Cherepanov and Wackernagel, 1995
pGEM-T easy	*bla lacZ*α	Promega
pTerE	pGEM-T Easy containing a 399-bp internal *terE* fragment	This study
Constructs		
154LΔ*iha*	154LΔ*iha*	This study
154LΔ*iha*/pWKS30*iha*	154LΔ*iha* containing pWKS30*iha*, Ap^R	This study
154LΔ*iha*/pWKS30	154LΔ*iha* containing pWKS30, Ap^R	This study
154S *glmS*::*iha*	154S chromosomally complemented (downstream of *glmS*) with *iha* from EHEC O157:H7 Sakai (RIMD 0509952), Km^R	This study
SakaiΔ*iha*	EHEC O157:H7 Sakai (RIMD 0509952)Δ*iha*	This study
SakaiΔ*iha glmS*::*iha*	EHEC O157:H7 SakaiΔ*iha* chromosomally complemented (downstream of *glmS*) with *iha* from EHEC O157:H7 Sakai (RIMD 0509952), Km^R	This study

Table S5. Primers used for construction of *iha* deletion and complementation mutants.

Primer[a]	Sequence (5′ - 3′)
del_iha_for	GTATTTGTGTATTGTCTTGCCGGTTAACATGATCGGAGATTAGTAATATGGTGTAGGCTGGAGCTGCTT
del_iha_rev	TGCAGTGGCAACGTATTCTACCGTCAGTGATAGCGTTTTGTTATTATCACATATGAATATCCTCCTTAGTTCCTA
iha_for2	GGTTTCGAACAATGTGGGCTGACATGA
iha_rev	CGGTAGCTGCAAAACTTAACG
iha-pKD4_for	CCTTTTTGCGTGGCCAGTGCCAAGCTTGCATGCAGATTGCAGCATTACACAACAATGTGGGCTGACATGA
iha-pKD4_rev	AAAGTATAGGAACTTCGAAGCAGCTCCAGCCTACACAATCGCTCAAGACCGGTAGCTGCAAAACTTAACG
iha-int_for	AACTCCATTAATTTGATATTATTCCTGTGTGTTATCCATTTATTAAATTTAACAATGTGGGCTGACATGA
iha-int_rev	ACTTACCAGCATTAATAATCTAACCTCATTCCATATAATTAACTTCAGCTACGGCTGACATGGGAATTAG

a. All primers were designed in this study.