SUPPLEMENTAL DATA

Table S1. Primers used in this study

	Primers name	Gene (name)	Primer sequence 5' → 3'	References
Autotransporter	*ehaA*$^{\alpha}$-F	z0402 (*ehaA*)	ATATCGGCTAAAGTGGAACAGGTCC	(1)
	ehaA$^{\alpha}$-R		CCTAACGCTAACTCAGAGTTGGTGC	This study
	ehaA$^{\alpha}$-F		GACGGGTGAGCAGAAACAAACC	(1)
	ehaA$^{\alpha}$-R		ATCCAGGTAACCTGTGCTTGCG	(1)
	ehaB$^{\alpha}$-F	z0469 (*ehaB*)	AGTGCATGACTACTGATTGTGCTG	(1)
	ehaB$^{\alpha}$-R		CACATTAAACAAACCGCTCTGG	(1)
	ehaB$^{\beta}$-F		CCGTTGTTCTGCAACAGATGG	(1)
	ehaB$^{\beta}$-R		TCACCGTTCACATCGTTATCG	(1)
	ehaC$^{\alpha}$-F	yfaL (*ehaC*)	ATGAATGTCGGCGA(T/C)ACGC	(1)
	ehaC$^{\alpha}$-R		AGCGTACCGGA(G/A)GCGATTTG	(1)
	ehaC$^{\beta}$-F		ATGGTCAGACGCTGAATTTACG	(1)
	ehaC$^{\beta}$-R		TCCCTTTCTGCCACGCCACG	(1)
	ehaD$^{\alpha}$-F	ypjA (*ehaD*)	CACAACCATAAATGGTGGTCG	(1)
	ehaD$^{\alpha}$-R		TCTTTCTCAACAACCTGCCG	(1)
	ehaD$^{\beta}$-F		ATTCTCTGGGCGGCTATGCC	(1)
	ehaD$^{\beta}$-R		TTCCACGGGGATTCCACACC	(1)
	ehaG$^{\alpha}$-F	z5029 (*ehaG*)	GTGGAATGTTGTCGTCGTTTGGG	This study
	ehaG$^{\alpha}$-R		CTCTCGACTGGCTACCATTA	This study
	ehaG$^{\beta}$-F		AATACCCAGAGCATTACTAACCTG	(2)
	ehaG$^{\beta}$-R		TTGGGTCTACAAATTACAAGGT	(2)
	saa-F	LH0174 (saa)	CGTGATGAACAGGCTATTGC	(3)
	saa-R		ATGGACATGCCTGTGGCAAC	(3)
	espP-F	L7020 (e*spP*)	AAACAGCAGGCACTTGAACG	(4)
	espP-R		GGAGTCGTCAGTCAGTAGAT	(4)
	yejO-F	z3449 (*yejO*)	CTAACACTCCACAATCCGTC	This study
	yejO-R		CGCAAATTACCCCTTCAACC	This study

	Primers name	Gene (name)	Primer sequence 5' → 3'	References
Fimbriae	*loc1-F*	z0024	CTGGTCTCATTCTGGTCA	This study
	loc1-R		GGATGTCGATCAAGGAAC	This study
	loc2-F	z0152 (*yadN O157)*	AAAAGCACTTCTCGCAGC	This study
	loc2-R		GGCTGTTCGGTGCTTTAA	This study
	loc2b-F	ECO26_0144 (yadN nonO157)	TTGCTGTTTGGTCTGCGA	This study
	loc2b-R		CAGTGGTCTGGTTGTTGA	This study
	loc3-F	*z0686* (*sfmA*)	GCGGTACAATTCACTTTGAAGG	(5)
	loc3-R		CATTTGCTTGCCCTGCTGATGC	(5)
	loc4-F	*z0872* (*ybgD*)	TGTTCCATCCAGGCAAAG	This study
	loc4-R		GTGAAGTTATTGAAGCACC	This study
	loc5-F	*z1286* (*ycbQ*)	CTGTGGTATGTGCAACGTCC	(5)
	loc5-R		CCCCGTAGCGATATAATCAAC	(5)
	loc6-F	*z1538*	CCTACAGTCACTTTTCAGGG	(5)
	Loc6-R		GATTAATTAGAGGTAGCTCAGG	(5)
	loc7-F	*z2206*	CCAGTATTTTTCCATTACGC	This study
	loc7-R		CCGGTGGAGTTGTCATAA	This study
	loc8-F	*z1676* to *z1677* (*csgA-CsgC*)	CAGGTGTTGTTCCTCAGT	This study
	loc8-R		AAAGTGCCGCAAGGAGTAA	This study
	loc9-F	*z3277* (*yehB*)	TACCGAAGGTGATTGTGC	This study
	loc9-R		GATGCTTATGTCAATGGGC	This study
	Loc10-F	*z3601*	CTGCTTCGCTTTGCCTTT	This study
	Loc10-R		CTGGAAGTTGATATGGGTG	This study
	Loc11-F	*z4498* (*yraH*)	CTTTTCGCAGGTAATGCCG	(5)
	Loc11-R		TTAACGACAGCGTCTGCTTC	This study
	loc12-F	*z4968*	GTAACCRACGCGGGTATCAA	This study
	loc12-R		GCAGCAGGATTACTGGAA	This study
	loc13-F	*z5222* to *z5224*	GAGGATAAAAGCAAASCGCC	This study
	loc13-R		TCATCGGTMTGCCAGTAT	This study
	loc14-F	*z5913* (*fimI*)	GTCGTTGCTGCCAATGTTTGC	(5)
	loc14-R		GAAATGTAGCGAAGTAGAGCC	(5)

Table S2. Number of STEC colonies positive for Congo red or calcofluor staining classified by seropathotypes.

Seropathotype[b]	M9-Congo Red[a]					M9-Calcofluor[a]				
	30°C		37°C		All	30°C		37°C		All
	24h	48h	24h	48h	conditions	24h	48h	24h	48h	conditions
A (15)	0	9	7	15	15	0	2	0	1	3
B (21)	5	12	10	19	19	2	9	7	8	11
C (3)	0	3	1	2	3	0	1	0	0	1
Total (39)	5	24	18	36	37	2	12	7	9	15

[a] Results are average for 3 independent biological replicates.

[b] Number in parentheses indicates number of isolates.

Table S3. Serotype distribution of autotransporter and fimbrial genes among STEC strains.

Serotype	No of strains	No. of strains positive by PCR for autotranpsorters genes:												
		ehaA $^{\alpha}$	*ehaA* $^{\beta}$	*ehaB* $^{\alpha}$	*ehaB* $^{\beta}$	*ehaC* $^{\alpha}$	*ehaC* $^{\beta}$	*ehaD* $^{\alpha}$	*ehaD* $^{\beta}$	*ehaG* $^{\alpha}$	*ehaG* $^{\beta}$	*saa*	*espP*	*yejO*
O157:H7 (A)	14	14	14	14	14	14	14	14	14	14	14	0	14	14
O157:NM (A)	1	1	1	1	1	1	1	0	1	0	1	0	1	1
O157:H26 (C)	1	0	0	0	1	1	1	1	1	1	0	0	0	1
O26:H11 (B)	4	4	4	0	4	4	4	0	0	4	4	0	4	4
O45:H2 (B)	4	4	4	0	4	4	4	0	0	4	4	0	0	4
O103:H2 (B)	1	1	1	0	1	1	1	1	1	1	1	0	1	1
O111:H8 (B)	1	1	1	0	0	1	1	0	0	1	1	0	0	1
O111:NM (B)	4	4	4	0	4	4	4	0	0	4	4	0	0	4
O121:H19 (B)	4	4	4	4	4	4	4	0	0	4	4	0	3	3
O145:H25 (C)	1	1	1	0	1	1	1	1	0	1	1	0	1	1
O145:NM (B)	3	3	3	0	3	3	3	1	0	3	3	0	2	2
O113:H21 (C)	1	1	1	0	1	1	1	0	0	1	1	1	1	0
Total	**39**	38	38	19	38	39	39	15	17	38	38	1	27	36

		No. of strains positive by PCR for fimbrial genes:													
		z0024	*yadN*	*sfmA*	*ybgD*	*ycbQ*	*z1538*	*z2206*	*sgA-csgC*	*yehB*	*z3601*	*yraH*	*z4968*	*z22-z52*	*fim*
O157:H7 (A)	14	14	14	14	14	14	14	13	14	14	14	14	14	13	14
O157:NM (A)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
O157:H26 (C)	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1
O26:H11 (B)	4	0	4	4	4	4	0	3	4	4	4	4	4	4	4
O45:H2 (B)	4	0	4	4	4	4	0	4	4	2	4	4	4	0	4
O103:H2 (B)	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1
O111:H8 (B)	1	0	1	1	1	1	0	1	1	0	1	1	1	1	1
O111:NM (B)	4	0	4	4	4	4	0	4	4	4	4	4	4	4	4
O121:H19 (B)	4	0	4	4	4	4	0	4	4	0	4	4	4	4	4
O145:H25 (C)	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1
O145:NM (B)	3	2	3	3	3	2	0	3	3	3	3	2	2	1	3
O113:H21 (C)	1	0	1	1	1	1	0	1	1	0	1	1	0	1	1
Total	**39**	18	39	39	39	38	15	37	39	31	39	38	37	29	39

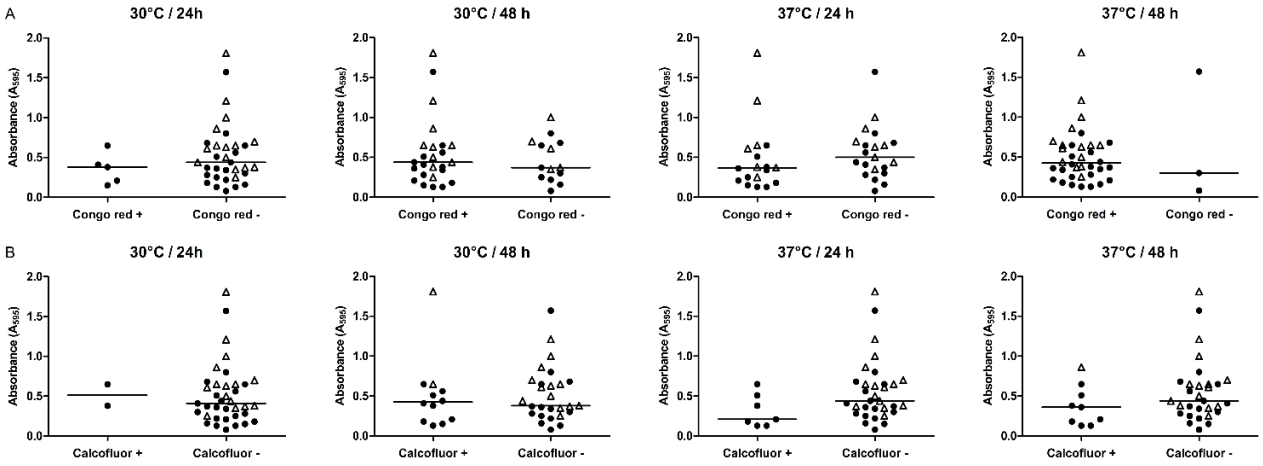

Figure S1. Biofilm formation and Congo red (A) or calcofluor white staining (B). The ability to bind CR on M9-CR agar (A) or CF on M9-CF agar (B) after 24 h or 48 h of incubation at 30°C or 37°C was not correlated neither with the 24 h biofilm formed in M9 plus glucose (0.4% wt/vol) in microtiter plates at 30°C, nor with seropathotypes. Open triangles represents seropathotype A isolates and filled dots represents seropathotypes B–C isolates. Results are the average for 3 independent biological replicates. The horizontal bars represent the median. Statistical analysis was performed by using a Mann Whitney test with two-tailed distribution.

REFERENCES

1. **Wells TJ, Sherlock O, Rivas L, Mahajan A, Beatson SA, Torpdahl M, Webb RI, Allsopp LP, Gobius KS, Gally DL, Schembri MA.** 2008. EhaA is a novel autotransporter protein of enterohemorrhagic *Escherichia coli* O157:H7 that contributes to adhesion and biofilm formation. Environ Microbiol **10:**589–604. http://dx.doi.org/10.1111/j.1462-2920.2007.01479.x.
2. **Totsika M, Wells TJ, Beloin C, Valle J, Allsopp LP, King NP, Ghigo JM, Schembri MA.** 2012. Molecular characterization of the EhaG and UpaG trimeric autotransporter proteins from pathogenic *Escherichia coli*. Appl Environ Microbiol **78:**2179–2189. http://dx.doi.org/10.1128/AEM.06680-11.
3. **Paton AW, Srimanote P, Woodrow MC, Paton JC.** 2001. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. Infect Immun **69:**6999–7009. http://dx.doi.org/10.1128/IAI.69.11.6999-7009.2001.
4. **Brunder W, Schmidt H, Frosch M, Karch H.** 1999. The large plasmids of Shiga toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. Microbiology **145 (Pt 5):**1005–1014. http://dx.doi.org/10.1099/13500872-145-5-1005.
5. **Low AS, Holden N, Rosser T, Roe AJ, Constantinidou C, Hobman JL, Smith DG, Low JC, Gally DL.** 2006. Analysis of fimbrial gene clusters and their expression in enterohaemorrhagic *Escherichia coli* O157:H7. Environ Microbiol **8:**1033–1047. http://dx.doi.org/10.1111/j.1462-2920.2006.00995.x.