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Phage Types and Genotypes of Shiga Toxin-Producing *Escherichia coli* O157 in Finland

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This study examined Shiga toxin-producing *Escherichia coli* (STEC) O157, using phage typing, pulsed-field gel electrophoresis, and typing of Shiga toxin variant genes by PCR with restriction fragment length polymorphism in an epidemiological survey of STEC O157 isolated from humans in Finland between 1990 and 1999.

Human infections caused by Shiga toxin-producing *Escherichia coli* (STEC) O157 have been reported from over 30 countries on six continents (12). In Finland, with a population of 5.1 million, the incidence rate of STEC O157 infections rose from 0.06 in 1990 (9) to 1.0 in 1997 and then decreased to 0.3 in 1999 (13). The only recorded STEC O157 outbreak in Finland occurred in 1997 (14). Pulsed-field gel electrophoresis (PFGE) has been widely used for subtyping of STEC O157 (6). However, an internationally standardized library of PFGE fingerprints has not yet been created, in contrast to phage typing for STEC O157 (1, 10). In this study, 105 STEC O157:H7 and O157:H$^-$ strains isolated from cultures sent from Finnish hospital laboratories to the Laboratory of Enteric Pathogens, National Public Health Institute (9), during 1990 to 1999 were phage typed, genotyped by PFGE, and investigated for Shiga toxin variant genes by PCR with restriction fragment length polymorphism. In addition, the relevant patient data, including recent foreign travel, were collected on a special form accompanying the isolate.

For phage typing, the bacteria were grown in double-strength nutrient broth (Difco Laboratories, Detroit, Mich.) shaken for 1.5 h at 37°C, flooded onto double-strength nutrient agar plates (Difco), and phage typed (1, 10). The results were interpreted according to a phage type list of 66 confirmed and 14 provisional phage types. A result of RDNC (for reacts but does not conform) was confirmed by phage typing five additional colonies of the strain.

In PFGE, bacterial growth on nutrient agar was suspended in TEN buffer (0.1 M Tris-HCl, 0.15 M NaCl, 0.1 M EDTA, pH 7.5) to an A_{600} of 0.280 to 0.310. The suspension was embedded equally in 1.8% InCert agarose (FMC BioProducts, Rockland, Maine) and digested overnight with 0.15 mg of proteinase K (Boehringer Mannheim, Indianapolis, Ind.) per ml at 50°C in 3 ml of ES buffer (0.5 M EDTA, 1% *N*-lauroylsarcosine). The plugs were washed as described previously (9). The PFGE method and the library of PFGE types were standardized nationally (L. Rantala, M. Saari, S. Hallanvuo, A. Siitonen, and T. Honkanen-Buzalski, Abstr. Nordic PFGE meeting, 25–26, 2000). The PFGE types were coded from 1.1 to 1.59.

PCR-RFLP was executed as described previously (4, 11) with minor modifications. A loopful of bacteria on sorbitol-MacConkey plates was suspended in 500 μl of sterile water, boiled for 5 min, and centrifuged briefly, and 1 μl was used as a template. In the PCR, the final elongation was for 10 min at 72°C. The amplified DNA was restricted with 20 U of *Hin*cII and *Acc*I enzymes (New England Biolabs) (4).

Of the 105 strains studied, 98 (93%) belonged to nine confirmed phage types (PTs): PT2, −4, −8, −14, −21/28, −34, −49, −50 and −88 (Table 1). The most common PT was PT2, representing 56% of all isolates, followed by PT4 (11%), PT49 (10%), and PT8 (6%). Seven strains (7%) did not conform to any published phage type and were designated RDNC, which formed three distinct reaction patterns. Of all strains, 93 iso-

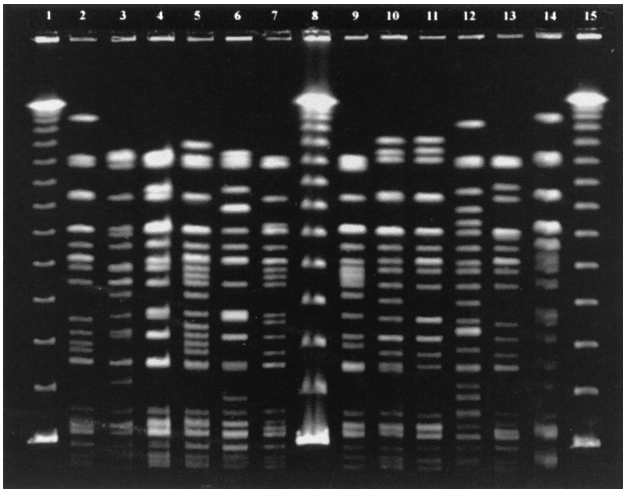

FIG. 1. PFGE fingerprint patterns of the STEC O157 isolates of foreign and of domestic origin in Finland. Lanes 1, 8, and 15, bacteriophage lambda ladder PFG marker 340 (New England Biolabs Inc., Beverly, Mass.); lane 2, type PT8/1.30 (Czech Republic); lane 3, type RDNC 3/1.49 (Tunisia); lane 4, type RDNC 3/1.11 (Spain); lane 5, type PT8/1.52 (Spain); lane 6, type PT8/1.9 (Turkey); lane 7, type PT8/1.29 (Turkey); lane 9, type RDNC 3/1.59 (Greece); lane 10, type PT2/1.5 (Finland and Russia); lane 11, type PT4/1.47 (Finland and Sweden); lane 12, type RDNC 3/1.48 (Greece); lane 13, type PT8/1.32 (Finland); lane 14, O157:H7 control strain (no. G5244) from the Centers for Disease Control and Prevention.

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TABLE 1. Characteristics of STEC O157:H7 and O157:H$^-$ isolates in Finland from 1990 to 2000

Strain	Origin	Isolation yr/mo	Serotype	Sorbitol fermentation	PT	PFGE type	*stx* variant gene(s)
IH 40962	Turkey	1990/5	O157:H7	−	8	1.9	stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 40986	Finland	1990/7	O157:H7	−	34	1.10	stx_1 or stx_{1v}
IH 41039	Spain	1990/10	O157:H7	−	8	1.52	stx_1 or stx_{1v} + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 41285	Finland	1992/10	O157:H7	−	49	1.8	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53393	Spain	1996/7	O157:H7	−	RDNC3	1.11	stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53401	Finland	1996/12	O157:H7	−	49	1.38	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53404	Finland	1997/1	O157:H7	−	RDNC2	1.3	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53407	Finland	1997/3	O157:H7	−	4	1.15	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53409	Finland	1997/5	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53410	Finland	1997/5	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53414	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53415	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53427	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53429	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53416	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53430	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53432	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53417	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53433	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53435	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53423	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53419	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53421	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53440	Finland	1997/7	O157:H$^-$	+	RDNC1	1.4	stx_2
IH 53425	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53467	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53438	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53441	Finland	1997/7	O157:H7	−	2	1.5	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53442	Russia	1997/7	O157:H7	−	2	1.5	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53436	Finland	1997/7	O157:H7	−	49	1.8	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53464	Finland	1997/7	O157:H7	−	49	1.21	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53443	Finland	1997/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53461	Finland	1997/8	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53459	Finland	1997/8	O157:H7	−	49	1.21	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53474	Finland	1997/8	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53476	Finland	1997/9	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53475	Cruise, Finland and Sweden	1997/9	O157:H7	−	4	1.47	stx_2
IH 53478	Cruise, Finland and Sweden	1997/9	O157:H7	−	4	1.47	stx_2
IH 53480	Cruise, Finland and Sweden	1997/9	O157:H7	−	4	1.47	stx_2
IH 53489	Finland	1997/9	O157:H7	−	2	1.55	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53490	Finland	1997/9	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53491	Finland	1997/9	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53492	Finland	1997/9	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53493	Finland	1997/9	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53494	Finland	1997/9	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53495	Finland	1997/9	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53496	Finland	1997/9	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53500	Finland	1997/9	O157:H7	−	2	1.55	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53497	Finland	1997/9	O157:H7	−	2	1.55	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 53498	Finland	1997/9	O157:H7	−	2	1.3	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56726	Finland	1997/10	O157:H7	−	2	1.3	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56728	Finland	1997/10	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56730	Finland	1997/11	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56738	Finland	1997/12	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56740	Finland	1997/12	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56741	Finland	1997/12	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56742	Finland	1997/12	O157:H7	−	49	1.21	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56743	Finland	1997/12	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56744	Finland	1998/1	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56745	Finland	1998/1	O157:H7	−	49	1.12	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56746	Finland	1998/1	O157:H7	−	21/28	1.56	stx_1 or stx_{1v} + stx_2
IH 56747	Finland	1998/1	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56748	Finland	1998/1	O157:H7	−	49	1.12	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56767	Finland	1998/1	O157:H7	−	49	1.12	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56768	Finland	1998/1	O157:H7	−	49	1.12	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56769	Finland	1998/1	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56770	Finland	1998/1	O157:H7	−		1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$

Continued on following page

TABLE 1—*Continued*

Strain	Origin	Isolation yr/mo	Serotype	Sorbitol fermentation	PT	PFGE type	*stx* variant gene(s)
IH 56771	Finland	1998/1	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56775	Finland	1998/1	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56776	Finland	1998/1	O157:H7	+	88	1.13	Not determined
IH 56777	Finland	1998/1	O157:H7	−	4	1.15	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56778	Finland	1998/1	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56779	Finland	1998/2	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56827	Finland	1998/4	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56833	Greece	1998/6	O157:H7	−	RDNC3	1.59	stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56834	Finland	1998/6	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56835	Finland	1998/6	O157:H7	−	4	1.15	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56836	Finland	1998/6	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56839	Finland	1998/7	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56842	Turkey	1998/8	O157:H7	−	8	1.29	stx_1 or stx_{1v} + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56843	Finland	1998/8	O157:H7	−	2	1.1	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56844	Czech Republic	1998/8	O157:H$^-$	−	8	1.30	stx_1 or stx_{1v} + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56845	Finland	1998/8	O157:H7	−	14	1.1	stx_2
IH 56858	Finland	1998/10	O157:H7	−	4	1.57	stx_2
IH 56863	Finland	1998/10	O157:H7	−	2	1.31	stx_2
IH 56868	Finland	1998/10	O157:H7	−	4	1.58	stx_2
IH 56869	Finland	1998/10	O157:H7	−	4	1.57	stx_2
IH 56870	Finland	1998/10	O157:H7	−	4	1.57	stx_2
IH 56893	Finland	1999/2	O157:H7	−	50	1.3	stx_2
IH 56894	Finland	1999/2	O157:H7	−	50	1.3	stx_2
IH 56909	Finland	1999/3	O157:H$^-$	+	88	1.53	stx_2
IH 56926	Finland	1999/3	O157:H$^-$	+	88	1.53	stx_2
IH 56927	Finland	1999/3	O157:H$^-$	+	88	1.53	stx_2
IH 56929	Finland	1999/3	O157:H$^-$	+	88	1.54	stx_2
IH 56958	Finland	1999/4	O157:H7	−	4	1.47	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56966	Finland	1999/4	O157:H7	−	4	1.37	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 56969	Finland	1999/4	O157:H−	−	88	1.50	stx_2
IH 56970	Finland	1999/5	O157:H7	−	2	1.28	stx_2
IH 56975	Finland	1999/5	O157:H7	−	2	1.28	stx_2
IH 57034	Finland	1999/6	O157:H7	−	8	1.32	stx_1 or stx_{1v} + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 57044	Finland	1999/7	O157:H7	−	2	1.28	stx_2
IH 57046	Finland	1999/7	O157:H7	−	8	1.32	stx_1 or stx_{1v} + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 57065	Greece	1999/10	O157:H7	−	RDNC3	1.48	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 57068	Tunisia	1999/11	O157:H7	−	RDNC3	1.49	stx_2 + stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$
IH 57086	Finland	1999/12	O157:H$^-$	+	RDNC1	1.51	stx_2

lates (89%) were of domestic origin. Among these, the predominant PT was PT2 (62%; 58 isolates), followed by PT49 (11%; 10 isolates) and PT4 (10%; 9 isolates). Three strains (3%) were designated RDNC. In PFGE, the isolates of domestic origin were distributed in 24 types. The main types were 1.1 (53%), 1.3 (5%), and 1.12 (4%). Twelve isolates were associated with recent travel abroad. Among these isolates, the PT distribution was PT2 (8%), PT4 (25%), PT8 (33%), and RDNC (33%). By PFGE, 10 types were detected, of which two types (1.5 and 1.47) were also found indigenously (Table 1, Fig. 1). Among all isolates, the most common PT-PFGE combinations were PT2/1.1 (46%), PT49/1.12 (4%), and PT4/1.47 (4%). In PCR-RFLP, the majority of the strains (70%; 74 strains) were positive for stx_2 and either stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$. These genes associated mainly with PT2/1.1 (48 strains), PT4 (5 strains), and RDNC (3 strains). The strains carrying stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$ only were all of foreign origin; they were from Turkey (PT8/1.9), Spain (RDNC3/1.11), and Greece (RDNC3/1.59). The five strains carrying stx_1 and stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$ all belonged to PT8; three of them were associated with recent travel to Spain (PT8/1.52), Turkey (PT8/1.29), and the Czech Republic (PT8/1.30).

The distribution of PTs in Finland was similar to that found in several other countries, especially in Europe (2, 3, 7, 8, 15). While the range of PT and PFGE types isolated from human infections in Finland indicates several domestic sources for STEC O157 infections, only one sporadic infection could be linked to a known food item, unpasteurized milk (16). However, various food items or environmental sources have caused STEC O157 infections worldwide (12). Based on these data, further studies of the prevalence of STEC O157 bacteria in Finland will be needed. In this study, 70% of the strains of the most common PT (PT2) possessed stx_2 and stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$. These strains were isolated mainly during the STEC outbreak in Finland in July 1997 (14), although this subtype was seen for the first time in May 1997 (9). The reason for the emergence of these strains can only be speculated on; it took place about 2 years after Finland joined the European Union (EU). In fact, the swift emergence and similarity of the strains in Finland and other European countries even speak for the effect of EU on this issue. For example, in Great Britain, PT2 strains possessing stx_2 and stx_{2c} have caused several outbreaks (18). The other common domestic types, PT4 and PT49, have also been common in Great Britain (17). Also, sorbitol-positive

O157:H$^-$ strains of PT88 carrying the stx_2 gene were found in our study. Similar strains have caused a large outbreak in Germany (2) and have been isolated in the Czech Republic (5). In infections of foreign origin, strains of PT8 carrying stx_1 and stx_{2c}, stx_{2vha}, or $stx_{2vOX393}$ have predominated in Finland. Interestingly, PT8 strains possessing the stx_1 and stx_2 genes have also been found in The Netherlands and Great Britain (7, 17).

This study used STEC O157 phage typing for the first time as an extensive epidemiological surveillance method in Finland. The information gained on the PT distribution in Finland may help to trace O157 infections at the EU level and help to assess the need to standardize new PTs indicated by RDNC.

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