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NOTES

Uropathogenic *Escherichia coli* Strains Generally Lack Functional Trg and Tap Chemoreceptors Found in the Majority of *E. coli* Strains Strictly Residing in the Gut

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The prevalence and function of four chemoreceptors, Tsr, Tar, Trg, and Tap, were determined for a collection of uropathogenic, fecal-commensal, and diarrheagenic *Escherichia coli* strains. *tar* and *tsr* were present or functional in nearly all isolates. However, *trg* and *tap* were significantly less prevalent or functional among the uropathogenic *E. coli* strains (both in 6% of strains) than among fecal-commensal strains (both in ≥50% of strains) or diarrheal strains (both in ≥75% of strains) ($P < 0.02$).

Escherichia coli, the predominant facultative anaerobe of the diverse microflora found in the gastrointestinal tract in humans and animals (19), causes both diarrheal and intestinal diseases as well as extraintestinal disease, including urinary tract infection (UTI) (12). Uropathogenic *E. coli* (UPEC) strains, particularly strains belonging to phylogenetic group B2 (20), are the cause of more than 80% of uncomplicated UTIs among otherwise healthy individuals (28). The intestinal tract is considered the primary reservoir for urinary tract infection, where bacteria, including UPEC, from the anus gain access to the periurethral area and establish infection in an ascending manner.

The gastrointestinal tract and the urinary tract represent two different nutritional niches for UPEC. The function of the gastrointestinal tract of humans and other animals is to break down ingested food into simpler nutrients and expel the remaining, unused food as waste. In the large intestine, approximately 10^{12} bacteria per gram of contents have been recovered; diverse genera, including *Escherichia coli*, are represented in these bacteria (19, 23). UPEC must compete with this array of bacteria in the large intestine for the remaining nutrients that have not been absorbed by the small intestine. Conversely, the urinary tract is primarily a sterile environment. Metabolic by-products are excreted from the kidneys and combine with water to form urine. Trace amounts of protein (<10 mg/dl) and amino acids (0.4 to 1.1 g/day) can be detected in the urine by urinalysis or chromatography; however, glucose and other simple carbohydrates are generally not detectable in the urine of healthy individuals (4, 7, 8, 24).

Bacteria utilize multiple mechanisms to detect changes in the chemical composition of the surrounding environment (3). The most extensively studied mechanism is flagellum-mediated chemotaxis, where transmembrane chemoreceptors relay signals to the flagellar motor to direct movement toward a more favorable environment (27). In the urinary tract, motility and chemotaxis offer a selective advantage (14). Four transmembrane chemoreceptors, or methyl-accepting chemotaxis proteins (MCPs), that generally mediate chemotaxis to amino acids (Tsr to serine and Tar to aspartate), saccharides (Trg to ribose and galactose), and dipeptides (Tap) have been identified for *E. coli* (26). Additionally, the Tar receptor has been shown to mediate chemotaxis to maltose (22). Tsr, Tar, Trg, and Tap are composed of four domains: a periplasmic ligand-binding domain (proximal to the N terminus), two transmembrane domains, and a cytoplasmic signaling domain (proximal to the C terminus) (6, 13).

To explore potential uropathogen-specific traits, our laboratory has also examined the chemotaxis of UPEC and other intestinal *E. coli* strains toward additional simple chemical attractants that have been functionally and genetically linked to the four well-characterized MCP receptors. Our initial observations revealed that representative UPEC pyelonephritis and cystitis strains, CFT073 and F11, respectively, did not possess functional Trg and Tap chemotactic responses; this was supported by inspection of both genomes. To assess whether this was a uropathogen-specific trend, we determined the prevalence of each of the four MCP receptor genes for 21 uropathogenic and 13 fecal-commensal isolates by Southern blotting or PCR analysis. From these analyses, four different genotypes were observed: tsr^+ tar^+ trg^+ tap^+ (11 strains), tsr^+ tar^+ trg^+ (5 strains), tsr^+ tar^+ (17 strains), and negative for all four chemoreceptors (1 nonmotile strain). Six uropathogenic and four fecal-commensal strains, representing different receptor profiles, were tested for their ability to respond chemotactically to known chemoattractants for the four MCP receptors with a modified capillary chemotaxis assay (1, 21). Addition-

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TABLE 1. Prevalence and function of MCPs among different *E. coli* strains

E. coli subtype[a]	Strain (characteristic)[b]	Presence of gene or chemotactic function[c]							
		tsr		*tar*		*trg*		*tap*	
		Gene	Phenotype[d]	Gene	Phenotype[e]	Gene	Phenotype[f]	Gene	Phenotype[g]
Uropathogenic	CFT001	+		+		−		−	
	CFT026	+		+		−		−	
	CFT049	+		+		−		−	
	CFT073	+	+	+	+	−*	−	−	−
	CFT108	+	+	+	+	+	−	+	−
	CFT142	+	+	+	+	+	−	−	−
	CFT180	+		+		−		−	
	CFT195	+		+		−		−	
	CFT204 (NC)	+		+		−*		−	
	CFT269 (NC)	+		+		−		−	
	CFT323	+		+		+		−	
	CFT325 (NC)	+		+		+		−	
	F3	+	+	+	+	−	−	−	−
	F8 (NC)	+		+		+		+	
	F11	+	+	+	+	+§	−	−	−
	F12	+		+		−		−	
	F15 (NC)	+		+		+		+	
	F24	+		+		−		−	
	F39	+	+	+	+	+	−	+	+
	F54	+		+		−		−	
	F63	+		+		−		−	
	UTI89	+		+		−		−	
Fecal-commensal	EFC1	+		+		+		+	
	EFC2	+	+	+	+	−	−	−	−
	EFC3 (NC)	+		+		+		−	
	EFC4 (NC)	+		+		+		+	
	EFC5	+		+		−		−	
	EFC6 (NC)	−		−		−		−	
	EFC7	+		+		+		+	
	EFC8 (NC)	+		+		+		+	
	EFC9	+	+	+	+	+	+	+	+
	EFC10	+		+		−		−	
	EFC25	+	+	+	+	+	−	+	+
	EFC26	+		+		−		−	
	HS	+		+		+		+	
	K-12	+	+	+	+	+	+	+	+
Diarrheagenic	**EAEC 042**	+	+	+	+	+	+	+	−
	EIEC 53638	+		+		+		+§	
	EPEC B171 (NM)	+		+		−		+	
	EPEC E110019	+§		+		+		+	
	EPEC E22	+	+	+	+	+	+	+	+
	EPEC E2348/69	+	+	+	+	−	−	−	+
	EHEC EDL933	+	+	+	+	+	+	+	+
	ETEC B7A	+		+		+		+	
	ETEC E24377A	+		+		+		+	

[a] Eleven UPEC strains (CFT) were randomly sampled from a collection of 67 isolates cultured from urine and blood samples from patients (43 women and 24 men) with acute pyelonephritis (18). Eight UPEC strains (F) were randomly sampled from a collection of 38 isolates from urine samples from women with cystitis but not pyelonephritis (9, 25). Twelve fecal-commensal *E. coli* strains (EFC) were randomly sampled from a previous collection of 28 control strains of *E. coli* from fecal samples from healthy women (20 to 50 years old) who had not had symptomatic UTI or known bacteriuria within 6 months prior to the collection and had not experienced diarrhea or received antibiotics within 1 month prior to collection (18). Four diarrheagenic *E. coli* strains, used for both genomic and chemotaxis studies, were described previously by our laboratory as part of a collection provided by J. Nataro (University of Maryland, Baltimore, MD) (10).

[b] NM, nonmotile; NC, nonchemotactic; strains in boldface indicate that genome sequences are available at NCBI; EHEC, enterohemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; ETEC, enterotoxigenic *E. coli*.

[c] +, presence; −, absence; *, truncation in DNA sequence; §, premature stop in protein sequence.

[d] Positive chemotaxis to either L-serine, L-alanine, or L-glycine.

[e] Positive chemotaxis to either L-aspartate, L-glutamate, or maltose.

[f] Positive chemotaxis to either L-ribose, L-galactose, or L-glucose.

[g] Positive chemotaxis to L-Gly-Leu.

ally, MCP sequence analysis was conducted with a genomic sequence available for nine diarrheagenic *E. coli* strains; of these strains, four were chosen to determine MCP functionality. In 49 of 56 cases, the presence of the intact gene correlated with the function of that particular receptor. After the genomic and functional results were combined, Trg and Tap were found to be significantly less prevalent or functional among UPEC strains than among fecal-commensal or diarrheagenic strains ($P < 0.02$). Overall, data from these studies suggest that UPEC strains have evolved under selective pressures that are different

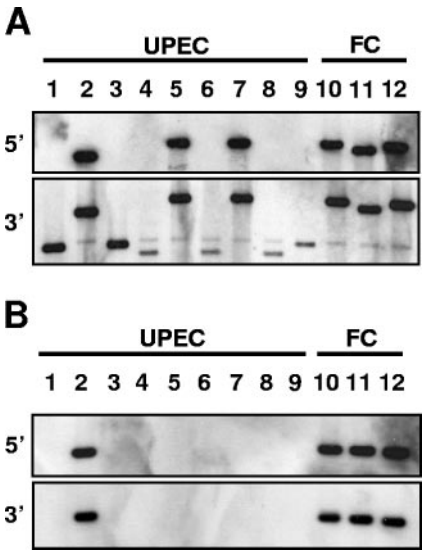

FIG. 1. Presence of *trg* and *tap* gene sequences among uropathogenic and fecal-commensal strains as determined by Southern blot analysis. BglII-digested genomic DNA from each of nine UPEC and three fecal-commensal (FC) strains was electrophoresed on an 0.8% agarose gel and subjected to Southern blot analysis with digoxigenin-labeled 5′ and 3′ fragments of the *trg* (A) and *tap* (B) genes. Lane designations are the same for both blots. Lanes: 1, CFT073; 2, CFT108; 3, CFT204; 4, CFT269; 5, CFT325; 6, F3; 7, F11; 8, F24; 9, F54; 10, EFC4; 11, EFC9; 12, K-12.

from other intestinal *E. coli* strains, reflective of the urinary tract and gut environments, respectively.

Prevalence of MCP genes among different *E. coli* strains. Twelve pyelonephritis isolates (isolates with CFT prefix), nine cystitis isolates (isolates with F prefix), and thirteen fecal-commensal strains (isolates with EFC prefix and K-12) were examined by PCR for the prevalences of the four MCP genes. Two PCRs were designed for each MCP gene (*trg*, *tap*, *tar*, and *tsr*) to amplify both the 5′ end and the 3′ end of each gene. For all amplifications, no discrepancy between the 5′- and 3′-end PCR results was observed; both reactions were either positive or negative. The prevalence rates were as follows: *trg* was identified in 4 of 12 (33%) pyelonephritis isolates, 4 of 9 (44%) cystitis isolates, and 8 of 13 (62%) fecal-commensal isolates; *tap* was identified in only 1 of 12 (8%) pyelonephritis isolates, 3 of 9 (33%) cystitis isolates, and 7 of 13 (54%) fecal-commensal isolates; *tar* was identified in 12 of 12 (100%) pyelonephritis isolates, 9 of 9 (100%) cystitis isolates, and 12 of 13 (92%) fecal-commensal isolates; and *tsr* was identified in 12 of 12 (100%) pyelonephritis isolates, 9 of 9 (100%) cystitis isolates, and 12 of 13 (92%) fecal-commensal isolates (Table 1). Only the fecal-commensal strain EFC6 lacked all four MCP genes; this strain was also nonchemotactic in 0.25% tryptone broth agar.

To confirm the PCR findings, the 5′- and 3′-end PCRs were repeated to generate probes for Southern blotting, and the prevalences of the *trg*, *tap*, *tar*, and *tsr* genes were determined for 12 *E. coli* strains. Of the strains tested by PCR, five pyelonephritis isolates, four cystitis isolates, and three fecal-commensal strains were selected for Southern analysis. The 5′- and 3′-end Southern blots for the *trg* gene are shown in Fig. 1A. Six of the strains were positive for *trg*: two pyelonephritis (Fig. 1A, lanes 2 and 5), one cystitis (Fig. 1A, lane 7), and three fecal-commensal (Fig. 1A, lanes 10 to 12) strains. The band visible in lanes 1 and 3 of the 3′ *trg* Southern blot (Fig. 1A) corresponds to a truncated version of the *trg* gene; also, Fig. 1B shows the 5′- and 3′-end Southern blots for the *tap* gene. Four of the strains were positive for *tap*: one pyelonephritis (Fig. 1B, lane 2) and three fecal-commensal (Fig. 1B, lanes 10 to 12) strains. None of the cystitis strains tested by Southern blotting contained the *tap* gene. Additionally, as predicted by PCR, all strains examined by Southern blot analysis were positive for *tsr* and *tar* (data not shown). Positive amplification or hybridization resulting in the expected size of the *tsr*, *tar*, *trg*, or *tap* gene is evidence that the gene is present in that particular strain. All *tsr*, *tar*, *trg*, and *tap* genotypic data, as determined by Southern blotting and PCR, are included in Table 1. (Primers used for the Southern blot and PCR studies are available upon request.) The prevalences of *tsr*, *tar*, *trg*, and *tap* were also determined for one additional UPEC strain (UTI89), one fecal-commensal strain (HS), and nine diarrheagenic strains using the genome sequence data available at NCBI (Table 1).

Chemotaxis to human urine and validation of the capillary chemotaxis assay. To determine whether our *E. coli* isolates possessed an ability to respond chemotactically to human urine, similar to that observed by Herrmann and Burman (11), one pyelonephritis strain (CFT073), one cystitis strain (F11), three fecal-commensal strains (K-12, EFC5, and EFC9), and motility and chemotaxis mutants of UPEC strain CFT073 (Δ*fliC* and Δ*cheY* mutants, respectively) were subjected to a modified capillary chemotaxis assay (1, 21). The complementable *fliC* and *cheY* mutants (14) were used as negative controls for motility and chemotaxis, respectively, as nonmotile mutants should also be nonchemotactic in this assay. Strains were cultured from motility agar and grown in tryptone broth to mid-exponential phase (optical density at 600 nm of 0.3 to 0.4), harvested by centrifugation to remove all spent medium, resuspended in chemotaxis buffer (10 mM potassium phosphate buffer [pH 7.0], 0.1 mM potassium EDTA [pH 8.0]), and

FIG. 2. Chemotaxis of uropathogenic, fecal-commensal, and diarrheagenic *E. coli* strains to human urine, amino acids, saccharides, and dipeptide. Numerous pyelonephritis (blue), cystitis (red), fecal-commensal (green), and diarrheagenic (magenta) *E. coli* strains were assayed for their ability to respond chemotactically to human urine (A), L-serine (B), L-aspartate (C), D-ribose (D), D-galactose (E), and L-Gly-Leu (F). Strains were cultured from motility agar and grown to mid-exponential phase (optical density at 600 nm of 0.3 to 0.4) in tryptone broth, pelleted, and resuspended in chemotaxis buffer to starve at 30°C for 1 h. Starved bacterial suspensions (500 μl of ~10^{10} total CFU) were added to wells of chemotaxis chambers along with microcapillaries filled with attractant (undiluted or diluted in chemotaxis buffer) or chemotaxis buffer alone as a negative control. Chemotaxis chambers were incubated at 30°C for 90 min before the capillaries were removed and the contents were diluted and plated. Data are represented in a box-and-whiskers format, with the box extending from the 25th percentile to the 75th percentile, with a line at the median or 50th percentile. Whiskers extend from the box to include the highest and lowest data points. Filled boxes indicate capillaries filled with attractant, while open boxes indicate capillaries filled with chemotaxis buffer only. All strain names are indicated along the *y* axis. *, $P < 0.05$.

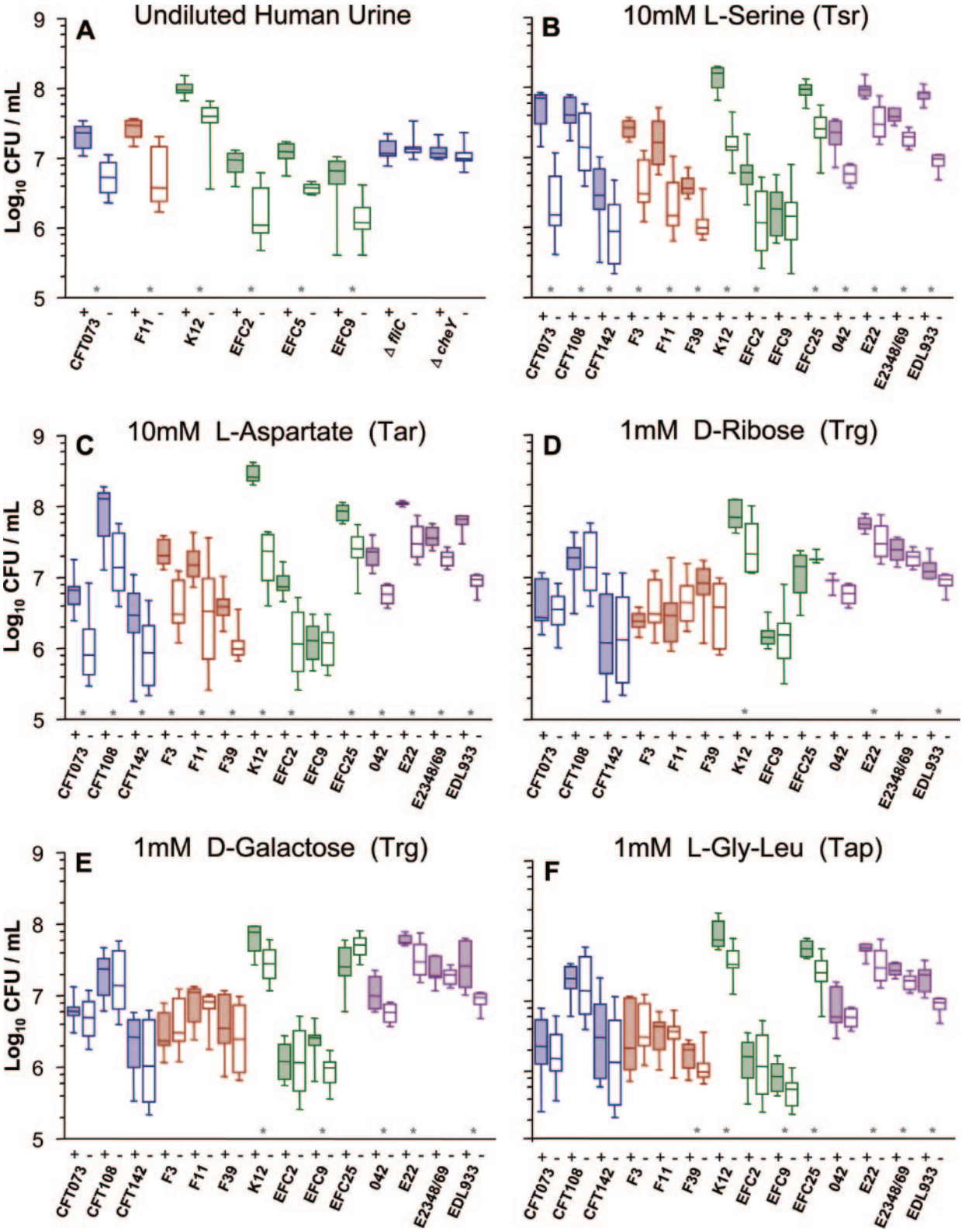

FIG. 2

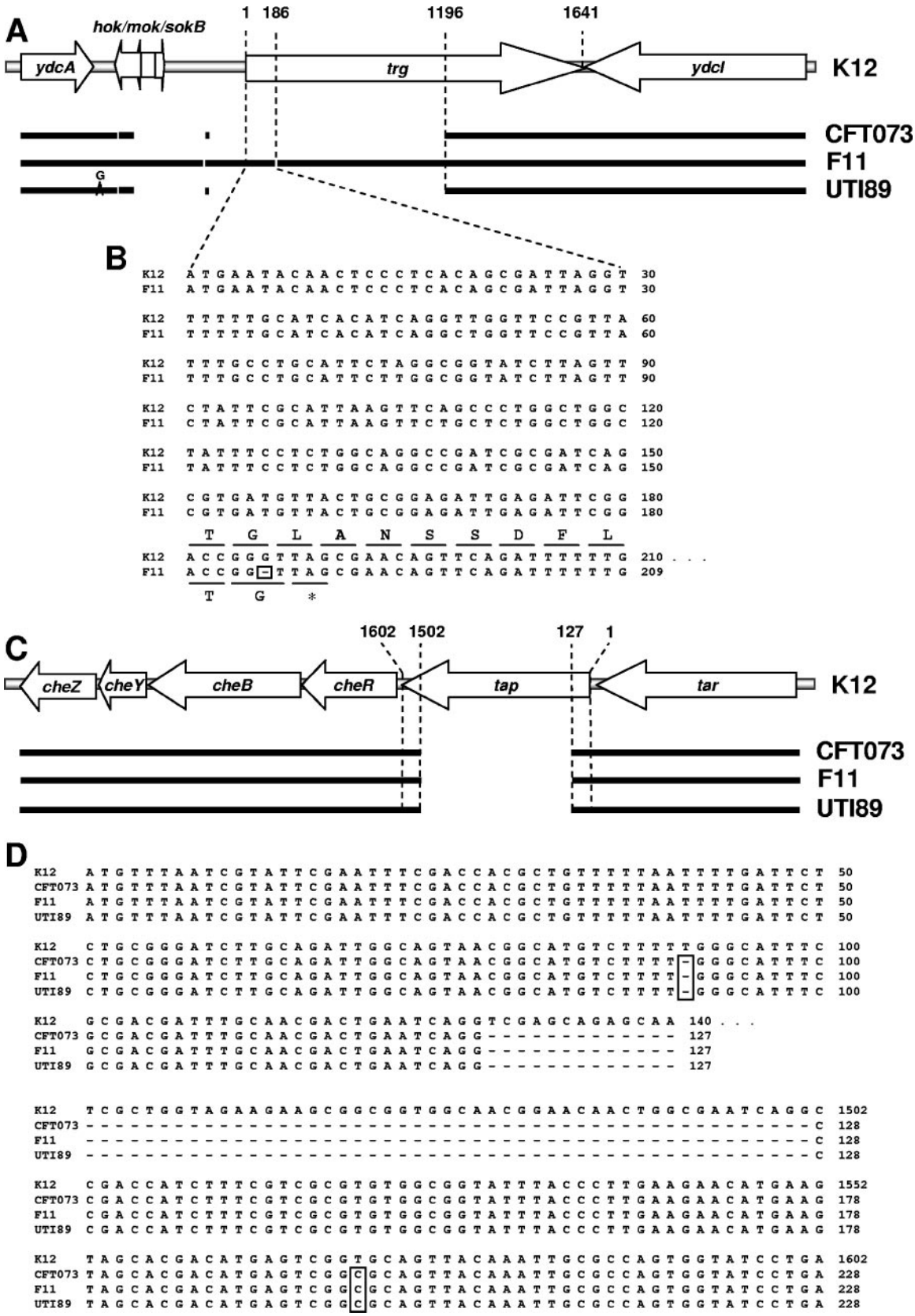

FIG. 3. Deletion and nonsense mutations of *trg* and clonal deletion of *tap* from the *tar-tap-cheRBYZ* operon of different UPEC strains compared to full-length *trg* and *tap* in K-12. (A) The *trg* gene region from K-12 was aligned with homologous regions from UPEC strains CFT073, F11, and UTI89 by using ClustalW software (MegAlign, Lasergene 7). Bold lines indicate regions of homology of the *trg* genes of CFT073, F11, and UTI89

starved at 30°C for 1 h. Starved bacterial suspensions (500 μl containing ~10^8 CFU/ml) were added to the wells of chemotaxis chambers, along with microcapillaries filled with human urine or chemotaxis buffer alone. Capillaries filled with chemotaxis buffer alone allow for the measurement of random motility of bacteria into the capillary, caused either by the bacteria or by the mechanical movement of the capillary into the chambers. The chemotaxis chambers were incubated at 30°C for 90 min before the capillaries were removed, and the contents were diluted and plated. Strains were considered to be chemotactic to urine if, after a 90-min incubation at 30°C, the mean number of bacteria was significantly greater within the capillaries filled with urine than in the capillaries filled with chemotaxis buffer alone, as determined using a Student's *t* test (InStat; GraphPad Software). As observed by Herrmann and Burman (11), all UPEC and fecal-commensal *E. coli* strains, with the exception of the nonmotile and nonchemotactic mutants, showed significant chemotaxis to undiluted human urine (Fig. 2A). As expected, both the *fliC* and *cheY* mutants were found to be nonchemotactic and were used as a validation of this assay (Fig. 2A). Additionally, UPEC strain CFT073 and its flagellar mutants are known to grow in urine and not in chemotaxis buffer; however, we observed no growth in urine during the 90-min incubation at 30°C, as evidenced by the fact that we did not observe any significantly higher numbers of bacteria in the capillaries filled with urine than in the capillaries filled with chemotaxis buffer. Given that all *E. coli* strains tested for chemotaxis to urine possess the *tsr* and *tar* genes (encoding the amino acid receptors) (Table 1) and not all strains possess the *trg* or *tap* genes (encoding the saccharide and dipeptide receptors) (Table 1), chemotaxis to urine is most likely attributable to the presence of amino acids in the urine. The use of human urine was approved by the Institutional Review Board of the University of Michigan Medical School.

Determination of MCP function using the capillary chemotaxis assay. To explore potential uropathogen-specific traits, six uropathogenic strains, three or four fecal-commensal strains, and four diarrheagenic *E. coli* strains were tested for their ability to respond chemotactically to L-serine, L-aspartate, D-ribose, D-galactose, and L-Gly-Leu, in addition to other known attractants for the Tsr, Tar, Trg, and Tap MCPs, by a capillary chemotaxis assay. For all chemotaxis assays, optimal attractant concentrations were selected based on previous work using *E. coli* strain K-12 (2, 16, 17). For these studies, K-12 was used as the positive control and as a fecal-commensal strain.

All uropathogenic strains, three of four fecal-commensal strains (including the positive control K-12), and all diarrheagenic *E. coli* strains responded chemotactically to L-serine (Fig. 2B), indicating that each of these strains possesses functional Tsr receptors (Table 1). Although the fecal-commensal strain EFC9 did not respond chemotactically toward L-serine, it did so to another attractant, L-glycine, which also indicates Tsr function (Table 1). Fecal-commensal strains K-12, EFC2, and EFC25 were also shown to respond chemotactically to L-glycine (Table 1). UPEC strains CFT073 and F11 also responded chemotactically to L-alanine and L-glycine (Table 1). Overall, the results were consistent, even though fecal-commensal strain EFC9 preferentially responded to L-glycine over L-serine. Taken together, these data indicate that all uropathogenic, fecal-commensal, and diarrheagenic *E. coli* strains tested possess a functional Tsr MCP (Table 1). Interestingly, UPEC strains CFT073 and F11 and fecal-commensal strains K-12 and EFC9 followed a pattern of chemotaxis similar to that of the D-serine enantiomer (data not shown).

The functionality of the Tar receptor was assessed by measuring the chemotaxis of various uropathogenic, fecal-commensal, and diarrheagenic *E. coli* strains to L-aspartate, L-glutamate, and maltose. Interestingly, all strains except the fecal-commensal strain EFC9 can respond chemotactically to L-aspartate (Fig. 2C). As with the functionality of the Tsr receptor, EFC9 can respond chemotactically to a minor Tar attractant, maltose (Table 1). UPEC strain CFT073 and fecal-commensal strain K-12 were also shown to respond chemotactically to maltose, whereas UPEC strain F11 was not (Table 1). Moreover, only UPEC strain F11, not CFT073 or fecal-commensal strain EFC9, was shown to respond chemotactically to L-glutamate (Table 1). Despite the preference for the attractant, these data provide evidence that Tar is functional in all of the uropathogenic, fecal-commensal, and diarrheagenic *E. coli* strains tested (Table 1).

To examine the function of the Trg MCP receptor in UPEC and other *E. coli* strains, chemotactic responses to D-ribose, D-galactose, and D-glucose were assessed using a standard capillary chemotaxis assay as described above. Fecal-commensal strain K-12, enteropathogenic (EPEC) strain E22, and enterohemorrhagic *E. coli* (EHEC) strain EDL933 responded chemotactically to both D-ribose and D-galactose (Fig. 2D and E). Fecal-commensal strain EFC9 and enteroaggregative *E. coli* (EAEC) strain 042 were observed to respond chemotactically only to D-galactose (Fig. 3E). Additionally, fecal-commensal strains K-12 and EFC9 were found to respond chemotactically to D-glucose (data not shown). None of the UPEC strains tested responded chemotactically to D-ribose, D-galactose, or D-glucose (Fig. 2D and E and data not shown). Overall, the Trg MCP was determined to be functional in 0% of the uropatho-

to the K-12 *trg* gene and surrounding DNA sequence. Gaps within the lines indicate that regions of *trg* and flanking sequence have been deleted from the UPEC strains. Both UPEC strains CFT073 and UTI89 appear to have acquired the same (or clonal) deletion (from the exact position upstream to position 1196 of *trg*). F11 has acquired a couple of smaller deletions, including that at position 186 of *trg* which results in a frame shift and premature stop in Trg translation. (B) An alignment of the 5′ region of both *trg* sequences from K-12 and F11 is shown along with the deletion at position 186 in F11 and the predicted effect on translation of both Trg sequences. An asterisk indicates the stop codon. (C) The *tar-tap-cheRBYZ* operon from K-12 was aligned with the same operon from UPEC strains CFT073, F11, and UTI89 using ClustalV software (MegAlign, Lasergene 7). Bold lines indicate regions of homology of CFT073, F11, and UTI89 to the K-12 *tar-tap-cheRBYZ* operon. Gaps within the lines indicate regions of the *tap* gene that have been deleted from the UPEC strains. All UPEC strains appear to have acquired the same (or clonal) deletion (from positions 109 to 1484 of *tap*). (D) An alignment of the 5′ and 3′ regions of *tap* from K-12, CFT073, F11, and UTI89 is shown and highlights the clonality of the *tap* deletion in the UPEC strains.

genic ($n = 6$), 50% of the fecal-commensal ($n = 4$), and 75% of the diarrheagenic ($n = 4$) *E. coli* strains tested (Fig. 2D and E).

To examine Tap function, six uropathogenic, four fecal-commensal, and four diarrheagenic *E. coli* strains were tested for their ability to respond chemotactically to the dipeptide L-Gly-Leu. Uropathogenic strain F39, fecal-commensal strains K-12, EFC9, and EFC25, diarrheagenic strains EPEC E22 and E2348/69, and EHEC strain EDL933 were found to be significantly chemotactic in response to L-Gly-Leu (Fig. 2F). Subsequently, Tap was determined to be functional in 17% of the uropathogenic ($n = 6$), 75% of the fecal-commensal ($n = 4$), and 75% of the diarrheagenic ($n = 4$) *E. coli* strains tested (Fig. 2F).

Determining trends based on genetic and functional data. In 49 of 56 cases, the function of the receptor correlated with the presence of that particular MCP gene. For 6 of 56 cases, a positive genotype was observed for a strain with a negative phenotype (as with CFT108 *trg* and *tap*, CFT142 *trg*, F39 *trg*, EFC25 *trg*, and EAEC 042 *tap*). To resolve this difference, the *trg* and *tap* gene sequences obtained either from NCBI (EAEC 042) or from amplification and sequencing (CFT108, CFT142, F39, and EFC25) were compared to the *trg* and *tap* gene sequences from the positive control strain K-12. Upon comparison of the gene sequences, each of the *trg* and *tap* genes appeared to be intact (data not shown). Therefore, the loss of phenotype could be explained by the differences in optimal attractant concentrations for different strains, the preference for an atypical attractant, or the inherent variation in the data diminishing the sensitivity of the chemotaxis assay. Additionally, since only the open reading frames were examined, our analysis cannot exclude upstream mutations that could adversely affect MCP gene expression. In only 1 of 56 cases was a negative genotype observed for a strain with a positive phenotype (as with EPEC E2348/69 *tap*). EPEC strain E2348/69 was determined by genomic analysis to lack *tap*; however, this strain showed a significant chemotactic response to L-Gly-Leu, which is indicative of Tap function. Although the causes for this anomaly are not clear, the disparity could be due to a difference in receptor preference that allows EPEC E2348/69 Tsr or Tar to include sensing of dipeptides. Since there were so few exceptions where the genotypic data did not match the functional data, we were able to combine the functional data with the genome-predicted functions to analyze larger sample sizes and determine the significance of the distribution and function of the four MCPs among the different *E. coli* subtypes. We predicted that if other anomalies existed, they would not affect the overall significance of the trends observed.

During this analysis, we excluded any strains that were known to be nonmotile (such as EPEC strain B171) or shown to be nonchemotactic in 0.25% tryptone broth agar. In particular, 10 of the 45 original *E. coli* strains were determined not to be chemotactic in 0.25% tryptone broth agar (Table 1). Although it is possible that these strains could be motile in a different soft agar medium, it is difficult to determine whether this defect is in motility and not just chemotaxis. Since we are ultimately interested in determining the function of Tsr, Tar, Trg, and Tap among different motile *E. coli* subtypes, these nonchemotactic strains were omitted from further analysis.

For the remaining strains, where the functions of Tsr, Tar, Trg, and Tap were not examined, the function of these four MCP receptors was predicted based on the data generated by Southern blotting and PCR or by analyzing genome sequence data publicly available at NCBI. Every strain was predicted to have a functional Tar receptor, whereas the only strain predicted to have a nonfunctional Tsr receptor was EPEC strain E110019 (Table 1). After comparing the *tsr* genes from E110019 and K-12, it was evident that a single nucleotide transition (C1120T) that resulted in a nonsense mutation in *tsr* (data not shown) occurred. After the functional data were combined with the genome-predicted functions of those strains for which no functional data were available, it was determined that Tar was functional among 100% of the motile uropathogenic, fecal-commensal, and diarrheagenic *E. coli* strains ($n = 35$) and that Tsr was functional among 100% of the uropathogenic ($n = 17$), 100% of motile fecal-commensal ($n = 10$), and 88% of the motile diarrheagenic ($n = 8$) *E. coli* strains (Table 1). Trg and Tap were found to be functional or prevalent in 1 of 17 and 1 of 17 motile uropathogenic, 5 of 10 and 6 of 10 motile fecal-commensal, and 7 of 8 and 6 of 8 motile diarrheagenic *E. coli* strains, respectively (Table 1). Moreover, Trg and Tap were found to be significantly more prevalent or functional among fecal-commensal ($P = 0.0152$ and 0.0042, respectively) and diarrheagenic ($P = 0.0001$ and 0.0010, respectively) *E. coli* strains than among UPEC strains, as determined by a Fisher's exact contingency table (InStat; GraphPad software).

Analysis of *trg* and *tap* DNA sequences. To determine the nature of Trg and Tap dysfunction, we aligned homologous *trg* and *tap* regions from sequenced UPEC strains CFT073, F11, and UTI89 to *trg* and *tap* regions from K-12 by use of ClustalW and ClustalV software, respectively (MegAlign; Lasergene 7). Alignments of the *trg* and *tap* genetic regions are shown schematically in Fig. 3. The deletion of *trg* in UPEC strains CFT073 and UTI89 appears to be clonal (Fig. 3A), ending at position 1196 of *trg*. Interestingly, F11 has acquired only two small deletions; however, one deletion in particular (position 186 of *trg*) predicts a frame shift and premature stop in Trg translation (Fig. 3B). After inspection of the *tar-tap-cheRBYZ* operon multiple alignment, it was determined that *tap* was deleted from positions 109 to 1484 in each of the UPEC strains (Fig. 3C). The multiple sequence alignments of the 5′ and 3′ regions of *tap* are shown in Fig. 3D to highlight the clonality of this deletion. This is intriguing because the clonal deletion of *tap* does not appear to cause polar effects on *cheRBYZ* transcription or translation, since CFT073, F11, and UTI89 all undergo chemotaxis normally. For other UPEC strains, such as CFT108, CFT142, and F39, the loss of Trg or Tap (or both) functions appears to have occurred via other unknown mechanisms as the coding sequences appear to be intact (data not shown). Altogether, data from these studies demonstrate a general lack of Trg and Tap function among UPEC strains, and not other intestinal *E. coli* strains, and provide different mechanisms (clonal deletion, frame-shift mutations, etc.) by which UPEC strains have lost Trg and Tap function.

Conclusions. Since all *E. coli* strains were shown to respond chemotactically to human urine, this study does not supply proof that UPEC has an advantage in gaining access to the urinary tract; instead, it provides evidence of a lack of selective pressure on UPEC strains that is different from other intestinal *E. coli* strains. What sets uropathogenic *E. coli* strains apart from other intestinal *E. coli* strains is the fact that UPEC

strains are able to colonize the urinary tract and persist in this environment. Otherwise, the life cycles of these *E. coli* subtypes are likely similar; both uropathogenic and strictly intestinal *E. coli* strains spend part of their life cycles outside the animal host and can be transmitted to a new animal host via fecal-oral contamination, where the bacteria subsequently reside in the large intestine or colon. Mechanical access to the urinary tract from the anus is possible for both strictly intestinal and uropathogenic *E. coli* strains, but the ability to survive within the urinary tract is unique to UPEC. Our laboratory has recently shown that although flagellum-mediated motility and chemotaxis are not required for colonization, they are significant aids in UPEC's efficient colonization of the murine urinary tract and increase the fitness of the bacterium (14). As for the importance of motility and chemotaxis during gut colonization, the fact that it is generally accepted that most wild-type *E. coli* strains are motile and chemotactic would suggest that motility and chemotaxis are involved to some extent in the colonization of the gastrointestinal tract (11, 15). If chemotaxis is important for UPEC during gut colonization, we hypothesize that the general inability of UPEC to respond chemotactically to saccharides and dipeptides would not be deleterious to UPEC's ability to colonize and compete for nutrients since most UPEC strains are able to respond chemotactically to amino acids present in the gut. Additionally, that the majority of fecal-commensal and diarrheagenic *E. coli* strains in this study were shown to maintain all four MCP receptors suggests that chemotaxis to amino acids, saccharides, and dipeptides is important during some stage of the organism's life cycle.

Our knowledge of the life cycles of UPEC and the strictly intestinal *E. coli* strains together with the findings presented in this report suggest that there are different selective pressures on both subtypes of *E. coli*. Assuming that UPEC and other intestinal *E. coli* strains are under the same pressures in the gut and the environment outside of the host, then the differences in MCP prevalence and function are likely to have evolved within the urinary tract due to a lack of selective pressure to retain *trg* and *tap*. Therefore, these data suggest that the retention of the two amino acid MCP receptors, Tsr and Tar, emphasizes the likely importance of chemotaxis to amino acids rather than saccharides and dipeptides by uropathogenic *E. coli* in the urinary tract.

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