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Use of Deoxyribose by Intestinal and Extraintestinal Pathogenic *Escherichia coli* Strains: a Metabolic Adaptation Involved in Competitiveness

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We showed that the *deoK* operon, which confers the ability to use deoxyribose as a carbon source, is more common among pathogenic than commensal *Escherichia coli* strains. The expression of the *deoK* operon increases the competitiveness of clinical isolates, suggesting that this biochemical characteristic plays a role in host infectivity.

Both pathogenic and nonpathogenic strains of *Escherichia coli* exist. The nonpathogenic strains are found in the normal intestinal flora of humans and animals, and the pathogenic strains are a leading cause of death and morbidity worldwide, particularly in developing countries. Pathogenicity islands (PAIs) carrying virulence genes have been characterized. However, most sequences within PAIs are still of unknown function. PAI I_{AL862} from the human blood *E. coli* isolate AL862 was previously described (18). The *afa-8* operon is the only region encoding a virulence factor that has been identified in this new PAI (18). Here, we demonstrate the presence of the *deoK* operon in this PAI. This operon codes for the use of deoxyribose, a sugar that is not fermented by *E. coli* K-12.

We observed that partially sequenced regions of PAI I_{AL862} (18) showed similarities with the *deoK* operon from *Salmonella enterica* serovar Typhi (2, 30). Using primers deduced from

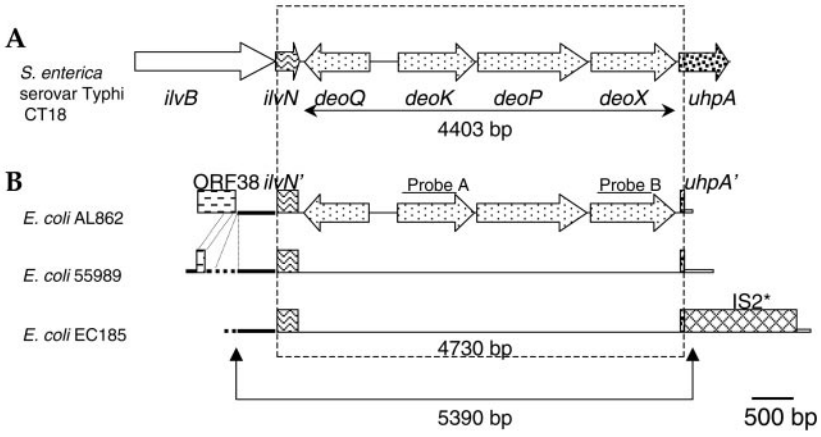

FIG. 1. Genetic organization of the *deoK* region in *E. coli*. (A) Genetic organization of the *deoK* operon in *S. enterica* serovar Typhi strain CT18. The *deoK* operon spans 4,403 bp. (B) Schematic diagram of the genomic regions carrying the *deoK* operon from three *E. coli* strains. The regions from AL862 used as probes A and B are shown. The size of the common region carrying the *deoK* operon in the three *E. coli* strains is indicated (5,390 bp). The 4,730-bp region conserved in the *E. coli* and *S. enterica* strains is indicated by dashed lines. Boxes indicate the coding sequences, showing their orientations and positions. Noncoding regions are represented by lines. Identical symbols on boxes and lines indicate regions with similarities.

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Reference or source	Deoxyribose[a]
Nonpathogenic *E. coli* strains			
K-12 MG1655	Nonpathogenic reference strain (genome sequenced)	5	−
MG1655 Rif	Spontaneous rifampicin-resistant derivative of MG1655	This study	−
MG1655 Nal	Spontaneous nalidixic acid-resistant derivative of MG1655	This study	−
EC185	Isolate from feces of a healthy volunteer	15	+
ExPEC strains			
AL862	Human blood isolate	18	+
CFT073	Isolate from blood of a patient with symptomatic pyelonephritis (genome sequenced)	31	+
CFT073*deoK*	CFT073 isogenic mutant with *deoK* gene deleted	This study	−
CFT073 Nal	Spontaneous nalidixic acid-resistant derivative of CFT073	This study	+
InPEC strains			
55989	Enteroaggregative strain producing AAF-III fimbriae (tetracycline resistant)	3	+
55989*deoK*	55989 isogenic mutant with of *deoK* gene deleted	This study	−
AL866	55989*deoK* carrying pILL1314	This study	+
AL868	55989*deoK* carrying pUC18	This study	−
55989 Nal	Spontaneous nalidixic acid-resistant derivative of 55989	This study	+
AL867	55989 Nal carrying pUC18	This study	+
55989 Rif	Spontaneous rifampicin-resistant derivative of 55989	This study	+
Plasmids			
pILL1272	45-kb Sau3A fragment containing the *deoK* operon of AL862 cloned into pHC79 (carbenicillin resistant)	18	+
pILL1307	pILL1272 containing a *deoK* gene mutated by partial deletion and insertion of the kanamycin resistance cassette (carbenicillin and kanamycin resistant)	This study	−
pILL1287	45-kb Sau3A fragment containing the *deoK* operon of 55989 cloned into pHC79 (carbenicillin resistant)	3	+
pUC18	*E. coli* cloning vector (carbenicillin resistant)	32	−
pILL1314	BamHI-KpnI PCR product corresponding to the *deoK* gene from 55989 inserted into pUC18 (carbenicillin resistant)	This study	−

[a] Growth on minimal medium supplemented with 0.1% deoxyribose and hybridized with probe A.

these sequences, we amplified and sequenced a 5,840-bp segment from pILL1272, a cosmid from the AL862 library (Table 1). A 4,375-bp region was 78% identical to the *deoK* operon from *S. enterica*, which is composed of four genes (*deoQ*, *deoK*, *deoP*, and *deoM*) (Fig. 1). Few bacteria are able to catabolize deoxyribose. Deoxyribokinase (product of the *deoK* gene), which catalyzes the ATP-dependent phosphorylation of 2-D-deoxyribose, has only been identified in *Lactobacillus planta-*

TABLE 2. Use of deoxyribose by nonpathogenic and pathogenic *E. coli* strains

Strain type (no. of isolates)	Reference(s) or source	No. (%) of isolates with deoxyribose as a carbon source[c]
Nonpathogenic *E. coli*[a] (61)		14 (22.9)
ECOR collection (25)	24	6 (24)
French collection (36)	15	8 (22.2)
Pathogenic *E. coli* (293)		133 (45.4)*
ExPEC (202)		87 (43.1)*
Archetypal (CFT073, J96, 536, RS218)[b] (4)	6, 8, 28, 31	2 (50)
Pyelonephritis isolates[b] (88)	1	42 (47.7)
Sepsis isolates (110)	15	43 (39)
InPEC (91)		46 (50.5)**
Enterotoxigenic (5)	29 and this study	3 (60)
Enterohemorrhagic (9)	7, 25, 27, 33 and C. Martin	1 (11)
Enteropathogenic (11)	20 and E. Oswald and A. Aidara-Kane	3 (27)
Diffusely adherent enteropathogenic (11)	17	11 (100)
Diffusely adherent (20)	4, 19	9 (45)
Enteroaggregative (27)	3, 22, 23	15 (55.5)
Adherent invasive (8)	21	4 (50)

[a] Isolates from feces of healthy individuals.
[b] Similar results were obtained by hybridization and growth assays.
[c] The data marked with asterisks were compared by chi-square analysis. The prevalences were significantly different from that in nonpathogenic strains (*, $P < 0.01$; **, $P < 0.001$).

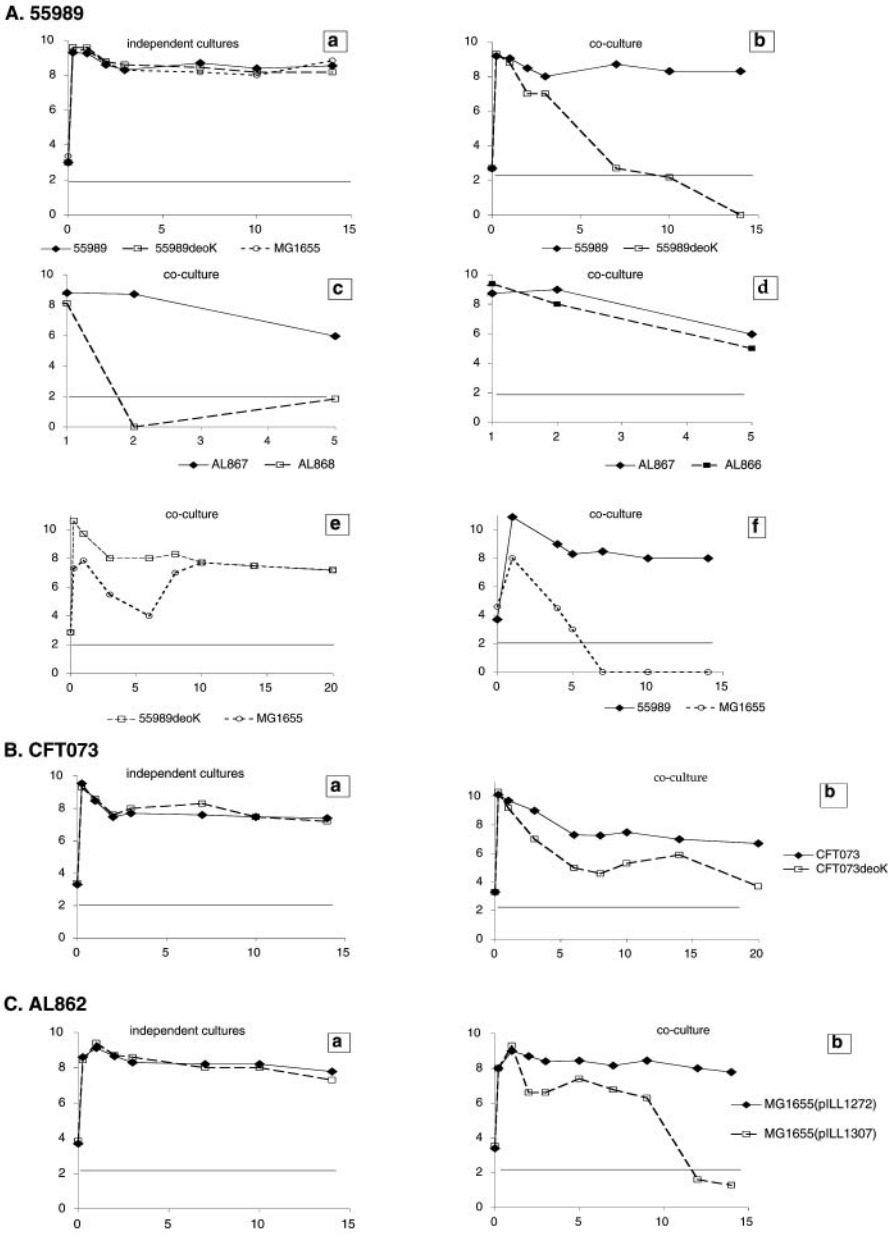

FIG. 2. Experiments conducted with isolates 55989 (A) and CFT073 (B) and with isolate MG1655 carrying the *deoK* operon from AL862 (C). Bacteria were grown separately or in coculture for 2 to 3 weeks. Samples were taken periodically, and viable counts were determined by plating out serial dilutions on Luria-Bertani agar supplemented with antibiotic or not, as appropriate. Each assay was performed at least twice. The detection limit of the titration method is <100 CFU/ml. Wild-type strains were cocultured with their respective *deoK* mutants (Ab, Bb, and Cb). Similar results were obtained in coculture experiments with either the parental 55989 isolate or its Nal derivative. The 55989*deoK* mutant was transcomplemented with the cloned *deoK* gene (AL866) and cocultured with AL867 (Ad; compare to Ac as a negative control). Strain 55989 carrying the *deoK* operon or without it was also cocultured with the commensal strain MG1655 (Af or Ae, respectively). Cocultures were performed with the parental 55989 and MG1655 isolates, the 55989 Nal and MG1655 Rif derivatives, or the 55989 Rif and MG1655 Nal derivatives. Similar results were also obtained when 55989 Nal was cocultured with MG1655 Rif at a 1:100 ratio.

rum, *Selenomonas ruminantium*, and *S. enterica* (12, 16, 26). We showed that *E. coli* strains AL862 and MG1655(pILL1272) were able to grow on K5 minimal medium (11) containing 0.1% 2-D-deoxyribose ribose (vol/vol) as the sole carbon source for 24 to 48 h at 37°C, meaning that they express the *deoK* operon.

Database searches showed that the *deoK* operon from AL862 is highly conserved in the uropathogenic *E. coli* isolate CFT073 (99% identity over 4,378 bp) (31). However, the genomes of the commensal strain MG1655 of *E. coli* K-12 (5) and the enterohemorrhagic *E. coli* O157:H7 strains EDL933 and Sakai do not contain this operon (14, 25). We investigated 354 pathogenic and commensal isolates from various collections (Table 2). Colony hybridization assays (performed as described in reference 13) and growth assays on deoxyribose minimal medium with 130 clinical isolates showed that the presence of

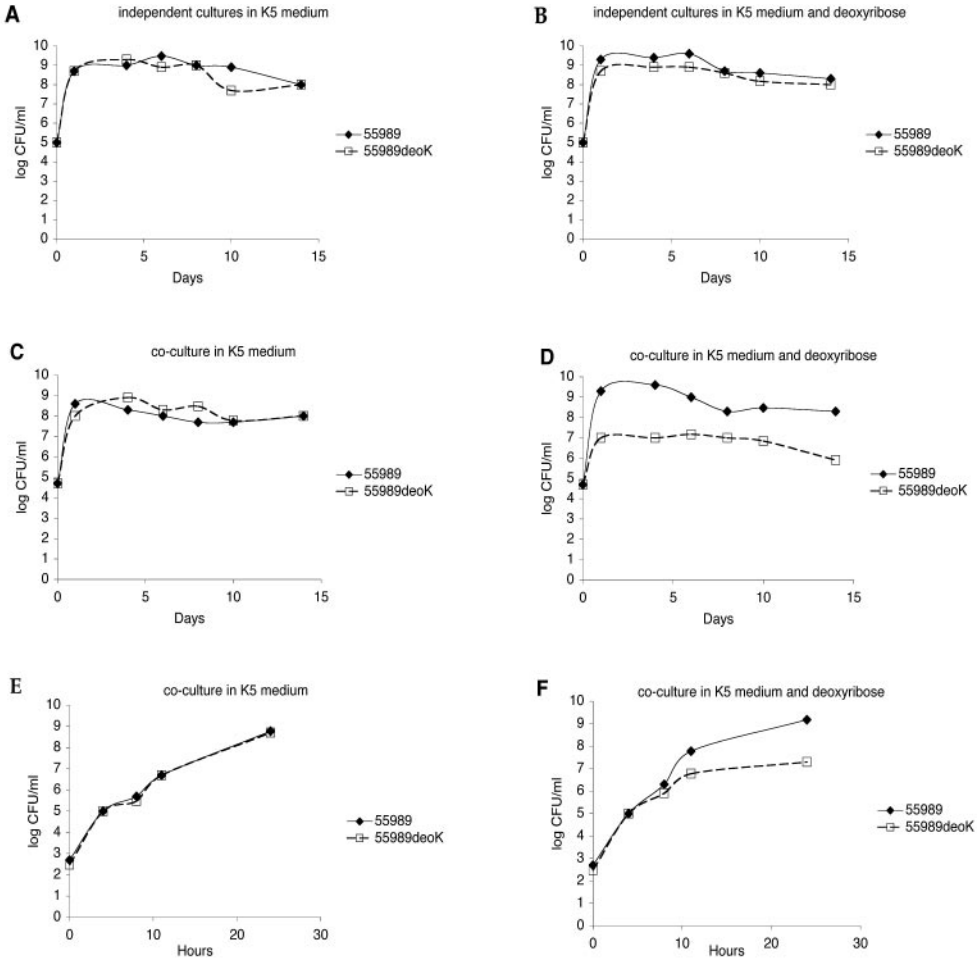

FIG. 3. Survival of 55989 derivatives in coculture experiments in K5 minimal medium containing deoxyribose or without it. Strains 55989 and 55989*deoK* were grown separately for two weeks (A and B), in coculture for two weeks (C and D), or in coculture for 24 h (E and F).

the *deoK* operon was always correlated with the use of this sugar. Probes A and B (Fig. 1) were amplified from AL862 DNA with previously described primers (18). Although we found that both pathogenic and commensal strains harbored the *deoK* operon, our results strongly suggested that this operon is associated with the pathogenicity of the strain (45.4% of pathogenic strains versus 22.9% of commensal strains harbored *deoK*; $P < 0.01$). Approximately half of the *deoK*-positive commensal isolates studied carried sequences encoding at least one virulence factor produced by extraintestinal pathogenic *E. coli* (ExPEC) (data not shown) and, consequently, could be considered potential ExPEC strains resident in the bowel.

We created nonpolar mutations by replacing the *deoK* gene with a PCR product containing a kanamycin resistance cassette as described previously (9). PCR analysis was performed to confirm the replacement of the gene. The deletion of the *deoK* gene did not result in any apparent growth defects. A study of parental strains, mutants, and transcomplemented mutants showed that the *deoK* gene is involved in use of the sugar (Table 1). These data were confirmed by determination of the deoxyribokinase specific activity and by comparison of the proteomes of *deoK*-positive [AL862, 55989, CFT073, and MG1655(pILL1287)] and *deoK*-negative (55989*deoK* and MG1655) strains, as previously described (10, 30). Deoxyribokinase activity (0.005 to 0.014 U/mg) and deoxyribokinase (identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry [data not shown]) could only be detected in soluble extracts from *deoK*-positive isolates that had been grown in minimal medium in the presence of deoxyribose.

Sequencing and comparison of the *deoK* operons from three pathogenic (AL862, 55989, and CFT073) and one commensal (EC185) *E. coli* strain showed that this operon is conserved (98% identity), as is an ~1-kb DNA region surrounding it (750 bp on the left side and 280 bp on the right side) in all the strains tested (Fig. 1). A few base pairs (242 bp upstream and 115 bp downstream) directly flanking the operon corresponded to partial sequences of the *ilvN* and *uhpA* genes, between which the *deoK* operon is inserted in *S. enterica* (30). The sequences following these two truncated genes did not share similarity with sequences in the databases. These data suggested that *E. coli* acquired the *deoK* operon by horizontal transfer, possibly from *S. enterica*.

Deoxyribose is a sugar that is exclusively derived from DNA degradation, and the human diet leads to a high concentration

of DNA in the intestine. We therefore hypothesized that deoxyribose catabolism plays a role in the colonization of the intestine by both ExPEC (where they are resident) and intestinal pathogenic *E. coli* (InPEC) strains, by conferring the ability to use a limiting nutrient. In agreement with this hypothesis, we demonstrated that *deoK*-positive strains were able to ferment deoxyribose. As the intestine is a complex organ that contains various and variable limiting nutrients, we carried out coculture experiments (with equal numbers of wild-type and *deoK* mutant colonies) in rich and minimal medium (K5 broth containing pyruvate as a constant carbon source) supplemented with or without deoxyribose. Although the growth rates were similar in independent cultures, the *deoK* mutants were less competitive than the corresponding wild-type strains in the coculture experiments. This effect clearly depends on the presence of both an active *deoK* gene and deoxyribose in the medium (Fig. 2 and 3). The 55989*deoK* mutant was totally eliminated after several days in rich medium but only suffered from a loss of fitness in minimal medium. Levels of enzyme activity after the growth of strain 55989 in the different media (data not shown) suggested that competitive differences are probably related to differences in deoxyribokinase activities. Although the MG1655 strain and the 55989*deoK* mutant showed similar fitness patterns in our coculture experiments, the parental 55989 isolate totally outcompeted the commensal strain after 6 days. Thus, the acquisition of the *deoK* operon by *E. coli* strains might confer an evolutionary fitness advantage, especially for pathogenic strains. In conclusion, our results agree with other reports, suggesting that metabolic functions specific for pathogenic strains play a role in host infectivity.

Nucleotide sequence accession numbers. The GenBank accession numbers for the 5,840-bp region of pILL1272, the 6,105-bp region of pILL1287, and the 6,921-bp region of strain EC185 reported in this paper are AY299335, AY298765, and AY299336, respectively.

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