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Alkaline Induction of a Novel Gene Locus, *alx*, in *Escherichia coli*

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A novel pH-regulated locus inducible over 100-fold in alkaline media was identified in *Escherichia coli* through screening of 93,000 Mu dI1734 (*lacZ* Km^r) operon fusions at pH 6.5 and pH 8.5. Four *lacZ* fusions that showed expression only at the higher pH were mapped at 67.5 min by P1 transduction crosses. The locus was designated *alx*.

Escherichia coli can grow over a wide range of external pHs (pHs 5 to 9) while maintaining an internal pH within the narrow range of pHs 7.4 to 7.8 (16, 22, 24). The essential mechanisms of pH homeostasis in *E. coli* remain unclear (for reviews, see references 2 and 15); a sodium-proton antiporter may be required for homeostasis in alkaline media (10). Acid conditions elicit a repellent chemotactic response, whereas basic conditions elicit an attractant response (11, 17).

The mechanism of adjustment to a pH change might involve differential gene expression, as in the bacterial response systems for heat shock, osmotic shock, and nutrient starvation (5, 13). So far, however, there are few reports of pH-regulated gene expression. We reported the isolation of *lacZ* fusions to a locus induced several hundredfold at a low pH (*exa*, since mapped to *cadA*) as well as fusions induced by membrane-permeable weak acids but not by external acidity (*ina*) (21). External acid induction was also reported for fusions to the arginine decarboxylase gene (*adi*) (1a). The transcription of *ompF* is depressed at a low external pH, showing pH dependence over the range of pHs 5.0 to 7.5 (7). Heat shock genes show transient induction at a high external pH (23).

We have used *lacZ* fusions to identify a novel pH-dependent locus, designated *alx*, which maps at 67.5 min. To our knowledge, this is the first report of a gene in *E. coli* showing induction in the extreme alkaline range of growth.

Isolation of *alx*::*lacZ* fusions. All strains are listed in Table 1. Mu dI1734 *lacZ* operon fusions (3) in host strain M8820 were grown in Luria broth containing appropriate pH buffers at 100 mM. The methods for the generation, screening, and characterization of the *lacZ* operon fusions were as described previously (19, 21), with the modifications noted below. During the fusion procedure, all media were buffered at pH 6.5 with 100 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], a condition under which alkaline-inducible genes would show low transcription and, therefore, Mu insertion should not be inhibited (6). The indicator for β-galactosidase was 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal).

Approximately 93,000 fusion colonies were screened for alkaline inducibility by replica plating onto media buffered at pH 8.5 with 100 mM TAPS [tris(hydroxymethyl)methylaminopropanesulfonic acid]. Four isolates produced blue colonies at pH 8.5 and white ones at pH 6.5. These were designated *alx-1* through *alx-4*.

Characterization of the expression of *alx* fusions. The level of β-galactosidase expression for the *alx-1* fusion increased 10-fold over the range of pH 5 to pH 8 (Fig. 1). With a small additional increase in alkalinity, to pH 8.7, expression increased sharply, up to 18-fold over the level at pH 8 (an increase of 180-fold over the level at pH 5). The results for *alx-2*, *alx-3*, and *alx-4* were similar (data not shown). The pH dependence of *alx-1* over the acid-to-neutral range was similar to the pH dependence reported for *ompF*; however, a sharp increase in the alkaline range has not been shown for *ompF* (7).

The time course of induction of *alx-1* was observed in a shift from neutrality to a high pH by dilution into a concentrated buffer (Fig. 2). The buffer and salt concentrations throughout the pH shift were designed so as to minimize the osmotic change while maximizing the net change in pH; the final pH of the alkaline-shifted culture after growth was 8.45. Parallel cultures were grown at pH 7.0 or at pH 8.5 without a shift in pH. Doubling times for all three cultures were virtually the same (data not shown). In the alkaline-shifted culture, the expression of *alx-1* increased immediately and reached the steady-state level after about 80 min. This induction pattern contrasted with the reported alkaline induction of heat shock genes, which show transient peaks at 5 to 10 min (23).

The doubling time for the growth of the *alx-1* fusion strain was no different from that of parent strain M8820 at a neutral or high pH (data not shown). Thus, the fusion does not interrupt an "essential" gene, given the growth media tested. The steady-state level of *alx-1* expression showed no sensitivity to increased osmolarity (up to 500 mM NaCl or KCl) or anaerobiosis (over the range of pHs 6.5 to 8.7). Agents which perturb the internal pH, such as the membrane-permeable weak acid benzoate (10 mM at pH 6.5) and the weak base diethanolamine (40 mM at pH 8.0), had no effect on *alx-1* expression. Also, added iron (to 10 μM ferric sulfate) had no effect. Thus, the induction of *alx-1* appears to depend specifically on the external pH.

Alkaline induction of *alx* was also observed in a modified minimal medium (7) which contained 40 mM KCl, 21 mM NH_4Cl, 100 μM $CaCl_2$, 1 mM $MgCl_2$, 0.7 μM $FeCl_3$, 25 μM Na_2SO_4, 1 mM KH_2PO_4, 50 mM PIPES, 50 mM TAPS, 3 μM thiamine, 0.02% Casamino Acids, and either 4% glucose or 4% glycerol. The observation of pH dependence of expression in a medium containing both PIPES and TAPS ruled out the effect of the particular buffers used.

Mapping of *alx*. Mapping of fusion loci was performed by standard methods of Hfr mating and P1 transduction (12, 14). Crosses showed that the four *alx* fusions are linked to *zgh-5*::Tn*10* (at 67.2 min) and *zgh-6*::Tn*10* (at 68 min) (Table 2). The data support a map location of about 67.5 min for *alx*.

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TABLE 1. Strains of *E. coli* K-12 used in this study

Strain	Description or genotype	Source or reference
M8820	F⁻ *araD139 (ara-leu)7697 (proAB-argF-lacIPOZYA)XIII rpsL*	M. Casadaban
POI1734	M8820 Mu dI1734 (Km^r *lacZYA*) *ara*::Mu *cts3*	M. Casadaban
JLS8838	M8820 *alx-1*::Mu dI1734	This study
CS1562	*tolC6*::Tn*10* λ⁻	B. Bachmann
CAG18164	*zgh-3075*::Tn*10*	20
TH5	*zgh-5*::Tn*10* Hfr *metB1 relA1*	18
TH6	*zgh-6*::Tn*10* Hfr *metB1 relA1*	18

The nearest known locus is *uxaAC*, at 67.6 min (18), which does not require alkaline conditions for high levels of expression (9). Another likely candidate for an alkaline-regulated gene, *tdcABC* (threonine deaminase), maps at 68 min (18). It is known that *E. coli* produces deaminases at a high pH (4). However, threonine deaminase does not require alkaline conditions, and it does show anaerobic induction (8).

In summary, we identified a novel locus, *alx*, whose expression shows pH dependence throughout the viable range of external pHs for *E. coli*, with a steep increase in expression above pH 8. The induction of *alx* at a high external pH is comparable to the induction of *exa* at a low external pH (21). On the other hand, the pH dependence of *alx* over the range of pHs 5 to 7.5 contrasts with the flat level of expression of the acid-inducible locus over the neutral-to-alkaline range (21); in this respect, the pH dependence of *alx* resembles that of *ompF* (7).

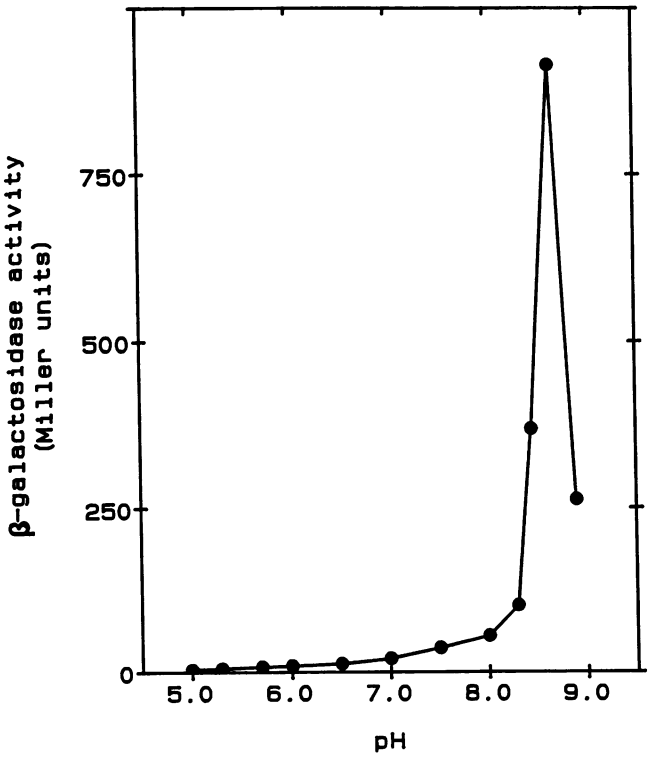

FIG. 1. External pH dependence of β-galactosidase expressed by the *alx-1*::*lacZ* fusion. Cultures of JLS8838 were grown overnight in buffered Luria broth, diluted 1:100, and grown at 30°C to an optical density at 600 nm of 0.2. All growth media included 100 mM buffers appropriate for the pHs as described previously (21); TAPS was used at pH 8.0 to 9.0. The pH was retested after the growth of bacteria.

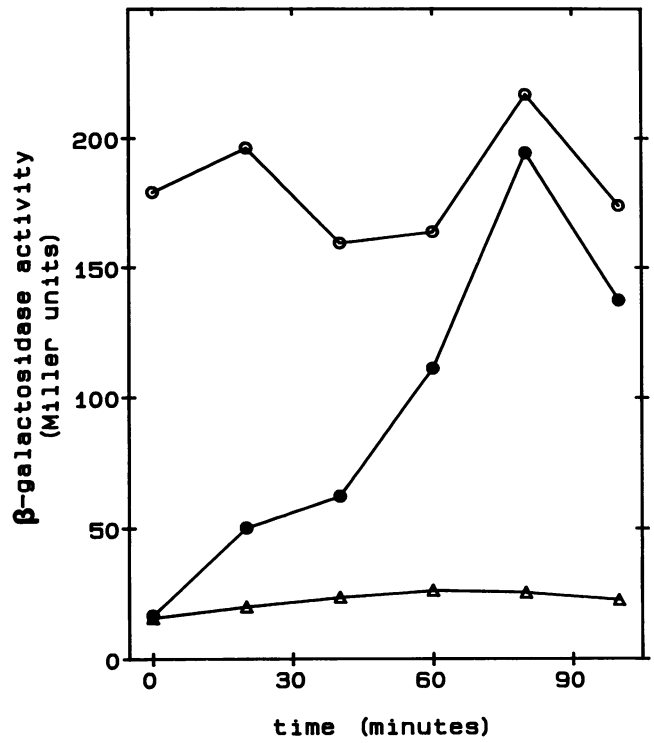

FIG. 2. Time course of induction of *alx-1* at a high external pH. Cultures were grown at 30°C to an optical density at 600 nm of 0.4 to 0.5 in buffered Luria broth and diluted fivefold at time zero in prewarmed buffered Luria broth. The buffers were 10 mM PIPES (pH 7.0)–40 mM NaCl diluted into 50 mM TAPS (pH 8.7) (●), 50 mM TAPS (pH 8.7) diluted into 50 mM TAPS (pH 8.7) (○), and 10 mM PIPES (pH 7.0)–40 mM NaCl diluted into 50 mM PIPES (pH 7.0) (△). At 60 min, each culture was rediluted fivefold to maintain logarithmic growth. The final pHs of each culture measured at the end of the time course were 8.45 (●), 8.5 (○), and 7.0 (△).

Although all of our isolates mapped to a single locus, other alkaline-inducible loci could exist. The indicator used, X-Gal, only permits the detection of loci with near-zero levels of *lacZ* expression under the uninduced condition.

Our findings add to the growing picture of pH-regulated gene expression, confirming our prediction (21) that distinct gene loci will emerge showing induction at a high external pH and at a low external pH. We now are attempting to isolate regulator genes comparable to the *ear* locus in *Salmonella* spp., which negatively regulates the acid-inducible locus *aniG* (1). We also are cloning the *alx* fusions and hope to identify alkaline-specific control sequences.

TABLE 2. Cotransduction of *alx*::*lacZ* fusions with Tn*10* markers[a]

Tn*10* marker	Map position (min)	% Cotransduction of:			
		alx-1	*alx-2*	*alx-3*	*alx-4*
tolC::Tn*10*	66.5	8	ND	ND	ND
zgh-3075::Tn*10*	67.0	49	ND	ND	ND
zgh-5::Tn*10*	67.2	52	63	63	64
zgh-6::Tn*10*	68.0	38	43	44	42

[a] The numbers of tetracycline-resistant transductants screened per cross were 300 for *alx-1* and 200 for *alx-2* through *alx-4*. ND, Not done.

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