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pH Regulates Genes for Flagellar Motility, Catabolism, and Oxidative Stress in *Escherichia coli* K-12†

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Gene expression profiles of *Escherichia coli* K-12 W3110 were compared as a function of steady-state external pH. Cultures were grown to an optical density at 600 nm of 0.3 in potassium-modified Luria-Bertani medium buffered at pH 5.0, 7.0, and 8.7. For each of the three pH conditions, cDNA from RNA of five independent cultures was hybridized to Affymetrix *E. coli* arrays. Analysis of variance with an α level of 0.001 resulted in 98% power to detect genes showing a twofold difference in expression. Normalized expression indices were calculated for each gene and intergenic region (IG). Differential expression among the three pH classes was observed for 763 genes and 353 IGs. Hierarchical clustering yielded six well-defined clusters of pH profiles, designated Acid High (highest expression at pH 5.0), Acid Low (lowest expression at pH 5.0), Base High (highest at pH 8.7), Base Low (lowest at pH 8.7), Neutral High (highest at pH 7.0, lower in acid or base), and Neutral Low (lowest at pH 7.0, higher at both pH extremes). Flagellar and chemotaxis genes were repressed at pH 8.7 (Base Low cluster), where the cell's transmembrane proton potential is diminished by the maintenance of an inverted pH gradient. High pH also repressed the proton pumps cytochrome o (*cyo*) and NADH dehydrogenases I and II. By contrast, the proton-importing ATP synthase F_1F_o and the microaerophilic cytochrome *d* (*cyd*), which minimizes proton export, were induced at pH 8.7. These observations are consistent with a model in which high pH represses synthesis of flagella, which expend proton motive force, while stepping up electron transport and ATPase components that keep protons inside the cell. Acid-induced genes, on the other hand, were coinduced by conditions associated with increased metabolic rate, such as oxidative stress. All six pH-dependent clusters included envelope and periplasmic proteins, which directly experience external pH. Overall, this study showed that (i) low pH accelerates acid consumption and proton export, while coinducing oxidative stress and heat shock regulons; (ii) high pH accelerates proton import, while repressing the energy-expensive flagellar and chemotaxis regulons; and (iii) pH differentially regulates a large number of periplasmic and envelope proteins.

Escherichia coli and related enteric bacteria respond to a wide range of pH stresses by regulating gene expression (for reviews see references 21 and 68) and protein profiles (73, 82). Enteric bacteria encounter a wide range of external pHs in their natural habitat, the human digestive tract (17). Colonization of the intestine requires transient survival through the stomach at pH 1 to 2 (fasting) or 2 to 7 (transiently, during feeding) (18), as well as exposure to pancreatic secretions at pH 10 (25) followed by growth and persistence at a range of external pHs of 5 to 8 (20). Growth at a pH substantially higher or lower than the cytoplasmic pH 7.6 induces protective responses with two fundamental aims: to maintain internal pH homeostasis and to prepare the cell to survive future exposure to more extreme pH conditions (below pH 5 or above pH 9) that no longer permit growth (11, 41, 70).

The effects of pH on enteric bacteria contribute to disease. Low pH enhances expression of numerous virulence factors, such as the ToxR-ToxT virulence regulon in *Vibrio cholerae* (7), the *phoP-phoQ* regulon of *Salmonella enterica* (6), and the pH 6 antigen of *Yersinia pestis* (50). Acid stress contributes to food preservation; many food preservatives are membrane-permeant acids whose uptake is enhanced by acid (60), and acid interacts in complex ways with both temperature and organic food preservatives (65).

While growth in acid challenges pH homeostasis, the pH difference across the inner cell membrane (ΔpH) nevertheless contributes cell energy in the form of proton potential or proton motive force (Δp). The proton potential powers motility, ATP synthesis, and catabolite transport (for a review see reference 29). But low pH also amplifies the uptake of membrane-permeant acids that dissipate the proton potential (59). Thus, we expect low pH to induce a combination of positive and negative responses.

Much of bacterial catabolism affects pH, and in *E. coli* a growing number of catabolic enzymes and catabolite transporters are known to be regulated by pH (21, 73). Sugar fermentation initially generates short-chain acids that are excreted but accumulate and reenter the cytoplasm, causing acidification. Thus, it is not surprising that sugar transporters such as OmpF and the maltose regulon are down-regulated at low pH (13). Consumption of acids by the tricarboxylic acid (TCA) cycle causes alkalinization, a common result of growth to stationary phase in tryptone-based media (66, 73). Catabolism of amino acids by decarboxylases generates alkaline amines, which help the cell counteract external acidification, for example, the lysine and arginine decarboxylases (4, 27, 45, 47, 71). High pH,

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however, induces deaminases that generate acids, such as tryptophan deaminase (*tnaAB*) and serine deaminase (*sda*) (9, 73, 82).

A complicated case is that of the glutamic acid decarboxylase genes *gadA* and *gadBC* (12, 44). The *gad* system enables cells to survive extreme acid (77), but its expression is induced mainly at high pH, or in Luria-Bertani medium grown to stationary phase, where pH naturally increases (73, 82). An alternative role of *gad*, particularly under anaerobiosis, may be to channel its product γ-aminobutyric acid into fermentation acids.

Even mild acid (pH 6 to 7) greatly amplifies the uptake of membrane-permeant weak acids such as acetate. Permeant acids pass through the bacterial membrane and dissociate in the cytoplasm, causing accumulation of anions and depression of internal pH (34, 56). Acetate concentrations rise as cell density increases, and acetate induces a large number of genes and proteins (3, 35). Growth inhibition occurs as a result of both lower internal pH and the differential ability of anions to inhibit metabolism (60). The effect of permeant acids is critical in the human colon, where the concentration of short-chain fatty acids totals approximately 100 mM (15).

While numerous responses to pH stress are known, the mechanisms by which *E. coli* maintains its internal pH at 7.6 remain poorly understood. The electron transport chain pumps protons outside the cell, and the H^+-ATPase either exports or imports protons, but mutants in these components maintain pH homeostasis. There is evidence that potassium exchange contributes to pH homeostasis in external acid (5, 10, 52, 80), but the precise mechanisms remain unclear. At high pH, the electrical potential ($\Delta\psi$) is diminished in order to compensate for the inverted ΔpH. The sodium-proton antiporter NhaA contributes to internal pH maintenance under sodium stress (24, 75). High pH also induces major stress systems such as heat shock response (1, 28, 74), the SOS regulon (63), and the CpxP envelope stress response (16).

At more extreme pH values, well below the growth range (as low as pH 1.5 for clinical isolates) *E. coli* can retain viability for many hours, a phenomenon termed acid survival or acid resistance. Acid resistance is enhanced by many genes induced during growth at the acid end of the pH range (pH 5) or growth to stationary phase. Acid-induced acid resistance factors include periplasmic chaperones such as the *hdeA* product (23), envelope proteins such as OsmY, and redox modulators such as Tpx (73, 78). A complex acid resistance regulon including the *gad* system is regulated by transcription factors GadX-GadW and EvgA-YdeO, as well as by RpoS, H-NS, and cyclic AMP (11, 12, 44, 79). *E. coli* also exhibits base resistance, the ability to survive at or above pH 10 (58, 70). Base resistance requires *rpoS* and components of the *gad* system (30).

Finally, pH may affect flagellar motility, although the present picture is unclear. According to one report, growth in acid represses flagellar genes and eliminates motility (72), whereas another group finds motility enhanced by acetate and propionate, which cause acid stress (53).

To investigate acid and base response, we used microarrays to compare *E. coli* gene expression at low, neutral, or high external pH. Past microarray studies of pH response have been limited by their absence of pH conditions above pH 7 (44, 78); their use of glucose minimal medium (78), in which many catabolic genes are repressed; and their focus on only a single acid resistance regulon (44). Our experimental design included both acid and base conditions, as well as pH 7.0. For each growth condition, five independent cultures were hybridized separately, a number of replicates that ensured detection of virtually all expression ratios of at least twofold. The coregulation of numerous genes within operons confirmed the biological relevance of our expression ratios. Our study revealed unexpected patterns of pH response and clarified the overlap of pH stress with other stress responses.

MATERIALS AND METHODS

Growth conditions. *E. coli* K-12 strain W3110 (R. VanBogelen and F. Neidhardt) was grown overnight in unbuffered potassium-modified Luria broth (LBK) (10 g of tryptone/liter, 5 g of yeast extract/liter, 7.45 g of KCl/liter). For pH-controlled growth, media were buffered with 100 mM homopiperazine-*N*,*N*′-bis-2-(ethanesulfonic acid) (HOMOPIPES) (pK_a, 4.55 and 8.12). The pH of the media were adjusted to 5.0, 7.0, or 8.7 with KOH solution to avoid extra sodium ions, which stress cells at high pH (24). To maximize aeration and maintain logarithmic growth, the overnight culture was diluted 1,000-fold into 12 ml of buffered medium in a 125-ml baffled flask and rotated at 240 rpm. Cultures were grown at 37°C to an optical density at 600 nm of 0.3. For all cultures, the pH was tested after growth to ensure that the values were maintained at ±0.2 pH unit of the pH of the original uninoculated medium.

To observe motility, we used *E. coli* K-12 strain RP437 and *S. enterica* serovar Typhimurium SJW1103 from a laboratory in which strains are maintained for motility (M. Macnab). Culture was spotted on tryptone-KCl soft-agar plates (0.35% Bacto Agar) and incubated at 37°C until cells swam out. Culture was picked from the leading edge of the swimming cells and inoculated into LBK for overnight growth. For quantitative assay of motility, 5 μl of culture was spotted in triplicate on plates containing tryptone-KCl with 100 mM sulfonate buffer of appropriate pK_a (73). After growth for 8 h, the diameter of motile cell growth was measured.

RNA isolation. Bacterial RNA was isolated using the Qiagen RNeasy kit with on-column DNA digestion (Qiagen), with additional DNA removal with Ambion DNase. To perform this additional DNase digestion, RNA was precipitated and redissolved in 85 μl of nuclease-free water. We then added 10 μl of 10× DNase I buffer and 5 μl of (1-U/μl) DNase I (Ambion). The DNase reaction mixture was incubated at 37°C for 30 min and then chilled on ice. A second RNeasy column purification was performed.

cDNA preparation and array hybridization. For microarrays, standard methods were used for cDNA synthesis, fragmentation, and end-terminus biotin labeling, based on Affymetrix protocols. Labeled cDNA was hybridized to *E. coli* Affymetrix Antisense Genome Arrays. Hybridized arrays were stained with streptavidin-phycoerythrin with the use of the Affymetrix Fluidic Station. After staining, arrays were scanned with a GC2500 scanner.

Statistical analysis of gene expression. The experiment was designed so as to minimize both false-positive and false-negative results for expressed genes. Five full replicates (with respect to *E. coli* growth, RNA isolation, sample preparation, and array hybridization) were performed for each pH condition.

The median within-group variance in expression for all genes in the data set was 0.031 (or standard deviation, 0.175). To test for significant differences in expression between the pH classes, one-way analysis of variance (ANOVA) was performed at a significance level of 0.001; thus, of every thousand genes tested, only one false positive would be expected. For a gene with average within-group variability, our sample size provided statistical power of 98% to detect a twofold difference in gene expression among pH groups. That is, only 2% of genes that show a twofold difference in expression between any two pH groups would be missed (false negatives).

Model-based expression analysis with dChip software (40) was performed on the probe-level data from Affymetrix's DAT files. The model relates target RNA levels to the probe signals by a linear function that weights the significance of all oligonucleotide probes for each gene. The analysis includes normalization, which rescales data from different arrays so that comparisons can be made among arrays. Each array was normalized to a baseline array from a pH 7 culture, by using local regression on an invariant set of probes (62). Model-based expression indices were calculated for each gene on each array by using only the perfect match probes (61), and outlier detection was performed (39). Only probe sets that received an Affymetrix call of "present" on greater than 50% of the arrays were used in subsequent analyses. "Present" or "absent" calls use information

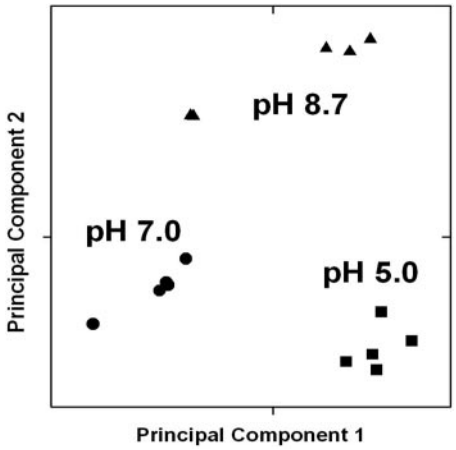

FIG. 1. Principal component analysis. The gene expression profiles of the arrays were visualized in two-dimensional Euclidian space, by using BRB ArrayTools software as described under Materials and Methods. The first and second principal components are shown. pH 5.0, squares; pH 7.0, circles; pH 8.7, triangles.

from paired perfect-match and single-base-mismatch probes. Four thousand six hundred fifty probe sets passed this criterion.

For genes whose probe sets passed the 50% screen, one-way ANOVA was performed on the $\log_2$-transformed model-based expression indices, on a gene-by-gene basis. For each gene that displayed significant differences in expression among the classes, pairwise comparisons of pH classes were determined using Tukey's multiple comparisons procedure to control the familywise error rate for the *t* test.

Additional analyses were performed to explore categories of differential gene expression. Global relationships among arrays were visualized by performing a principal component analysis (81) on the expression data and plotting arrays in two-dimensional space corresponding to the first two principal components. The gene expression profiles of the arrays were visualized in two-dimensional Euclidian space, by using BRB ArrayTools software. In addition, categories of differential expression profiles across the pH classes were generated by a hierarchical cluster analysis of differentially expressed genes, based on the average linkage method (19) with BRB ArrayTools.

RESULTS

Growth range of pH. To study the full range of pH response, we selected the widest pH range (pH 5.0 to 8.7) in which cultures maintained reasonable doubling times and approximately constant pH throughout growth. Culture media were adjusted to pH 5.0, 7.0, and 8.7. The doubling time for *E. coli* cultured at pH 5.0 and 8.7 was approximately 25 min and at pH 7.0 was 18 min. All cultures were grown to an optical density of 0.3 in order to facilitate at least five complete replications. The final pH of growth cultures was found to be within ±0.2 of the initial pH. The internal pH of the cytoplasm is approximately 7.6 (69); thus, growth at external pH 7.0 might induce some acid response.

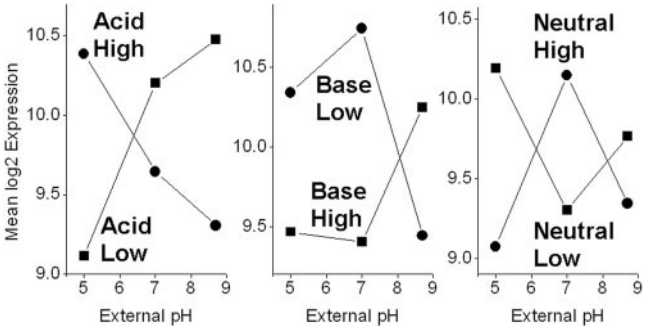

FIG. 3. Cluster mean expression profiles. The mean expression profiles over pH are plotted for the six clusters defined in Fig. 2.

Probe hybridization. To determine differential gene expression, the $\log_2$ transforms of normalized model-based expression values of genes were compared. Of the 7,231 genes and intergenic regions (IGs) on the array, 4,650 loci were detected on more than half (eight or more) of the 15 arrays. These loci, constituting about 70% of the total array, were taken for further analysis.

Principal component analysis. Global relationships among arrays were visualized by performing a principal component analysis (81) on the expression data (Fig. 1). Before dimensional reduction, each array existed in 4,650-dimensional space (one dimension for each of the 4,650 intensity values). The array comparisons were plotted in two-dimensional space, corresponding to the first and second principal components of variation. The first principal component for each array is the weighted linear combination of intensity values that shows maximum variation, whereas the second principal component is a weighted linear combination orthogonal to the first component that has maximum variance.

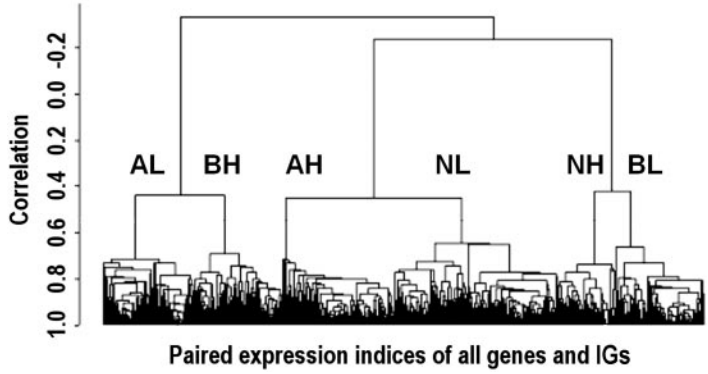

FIG. 2. Cluster analysis of differentially expressed genes. The dendrogram was generated based on the average linkage method (19) with BRB ArrayTools. At a correlation of 0.6, six clusters of related gene expression were designated Acid High (AH), Acid Low (AL), Base High (BH), Base Low (BL), Neutral High (NH), and Neutral Low (NL).

The principal component analysis indicated that the microarrays from each of the three pH conditions appeared in distinct groups (Fig. 1). Within-class variability was small relative to variability among pH levels. The pH 8.7 arrays showed the greatest degree of separation, clustering into two groups based on the date on which the arrays were hybridized, but this difference was small compared to the differences between pH classes.

ANOVA for significance of expression profiles. We compared gene expression among the three pH groups on a gene-by-gene basis using one-way ANOVA at a significance level of 0.001. The significance level indicates the probability of a false positive, and we therefore expect $0.001 \times 4{,}650 = 4.65$ false-positive genes (i.e., genes that are not truly differentially expressed but that appear in our differentially expressed list) in our full analysis. Of the 4,650 loci with eight or more "present" calls on arrays, 761 genes and 353 IGs showed a significant F value for differential expression among the three pH classes. Thus, about 17% of *E. coli* genes showed significant modulation of expression as a function of pH.

Cluster analysis. As a first attempt at categorizing differentially expressed genes, we performed a hierarchical cluster analysis (19) of differentially expressed genes (Fig. 2). We used average linkage and one minus the centered Pearson correlation as the distance metric. At a correlation value of approximately 0.6, the dendrogram generated six clusters of gene expression profiles.

Within each of the six clusters, the average profiles were determined for all the gene expression indices ($\log_2$ intensity values) across the three pH conditions (Fig. 3). The clusters were defined by their mean expression profiles across the three pH conditions. The Acid High cluster showed highest expression at pH 5.0, declining at pH 7.0 and 8.7. It included 160 genes and 49 IGs. Acid Low (113 genes, 57 IGs) showed approximately the reverse profile, with its lowest expression at pH 5.0, rising at pH 7.0 and 8.7. Base High (93 genes, 70 IGs) showed low expression at pH 5.0 and 7.0 and higher expression at pH 8.7, whereas Base Low (123 genes, 40 IGs) showed the reverse, higher expression at pH 5.0 and 7.0 than at pH 8.7. The Neutral High cluster (93 genes, 14 IGs) showed highest expression at pH 7.0 and lower expression at both pH extremes. The Neutral Low cluster (181 genes 123 IGs) showed the lowest expression at pH 7.0 and higher expression at both pH extremes, although the mean expression was substantially greater at pH 5.0 than at pH 8.7; a number of acid-induced genes fell in this category.

Table 1 lists the genes that fell into each cluster; details of description and Blattner open reading frame (ORF) numbers are available online in Table S1 in the supplemental material. In many cases, all or most of the ORFs in a given operon were induced in the same cluster; see, for example, the *atp* operon (Base High cluster) and the *flg* and *fli* operons (Base Low cluster).

Known acid-induced genes and acid resistance genes such as *sucBC* and *hdeA* (73) generally fell under Acid High, Base Low, or Neutral Low, a cluster whose mean expression indices were actually twofold higher in acid than in base (Fig. 3). These results are generally consistent with the cluster pH profiles and with the structure of the cluster dendrogram, in which the Acid High profile correlates most closely with the Neutral Low profile. Most known base-induced genes, such as *alx* (*ygjT*) (8, 73) and *tnaA* (9), fell under Base High or Acid Low.

For IGs, the cluster assignment and expression ratios are presented online in Table S2 in the supplemental material. Expression of an IG may result from a small regulatory RNA that lies between protein-encoding genes (2, 43), or it may indicate the tail end of mRNA containing pH-regulated genes. For example, the IGs upstream of *tnaC* (*tnaA* leader peptide) and downstream of *tnaB* both were repressed in acid, as are *tnaA* and *tnaB*.

Individual gene expression ratios. For genes whose overall expression profile yielded a significant F value (one-way ANOVA), we used the Tukey procedure to determine ratios of average model-based expression indices from cultures at pH 5.0 versus pH 7.0, at pH 8.7 versus pH 7.0, and at pH 8.7 versus pH 5.0. The full list of individual $\log_2$ expression ratios for all analyzed genes is presented in Table S1 in the supplemental material and for IGs is presented in Table S2 in the supplemental material; for genes of particular interest grouped in functional categories, the data are presented in Tables 3 through 7. Expression ratios that are significant at $\alpha = 0.001$ are shown in boldface.

The genes most strongly regulated by pH are summarized in Table 2. These genes each showed an expression ratio of at least fourfold ($\log_2 = 2$) between two of the pH classes. Note that the two genes most strongly induced in acid are ORFs with no known function, *yhcN* and *yagU*. Other acid-induced genes include those for catabolic enzymes in pathways that consume acids, such as *sdhCD* (succinate dehydrogenase). Genes repressed at high pH include several members of the flagellar regulon, including the main flagellar subunit *fliC* (for a review see reference 42).

The genes most strongly induced at high pH included *tnaC*, encoding the tryptophanase leader peptide (26), as well as *tnaA* (tryptophanase) and the Trp transporter gene *tnaB*, with its leader peptide gene *tnaC*. Previously in proteomic gels, we found tryptophanase to be the most highly expressed protein observed at high pH (9). The alkali-inducible protease gene *cpxP* (16) was also strongly induced. Members of the maltose transport regulon (*malEKM*) were strongly repressed by acid, consistent with previous reports (31, 73). But proteins strongly induced by base also included those from genes of unknown function, such as *yifO* and *ymgD*.

Flagellar and chemotaxis regulons. Motility in *E. coli* is governed by the flagellar chemotaxis regulon including 50 components in 19 operons, governed by the major regulators FlhC and FlhD (42, 76). The expression of the regulatory operon *flhCD* is controlled by numerous environmental response systems, such as adenylate cyclase (37), RcsCDB (22), and ClpXP (76).

Nearly all the genes of the flagellar regulons (47 genes) were repressed at high pH (Table 2). Forty-one genes fell in the Base Low cluster, which means that the bulk of significant expression difference occurred between pH 7.0 and 8.7. (The other six genes were Acid High.) These genes were among the most strongly base-repressed genes in the arrays (Table 2); for instance, *fliC*, encoding the flagellin monomer, had the lowest pH 8.7/pH 7.0 ratio observed, down-regulated about 20-fold (Table 3). Some of the *che* and *mot* genes showed a relatively

TABLE 1. Clusters of pH-dependent genes

Cluster	Genes							
Acid High	*aceK*	*dapB*	*gatA*	*iap*	*murE*	*sirA* (*yhhP*)	*ybjN*	*ygdQ*
	acnA	*dcyD* (*yedO*)	*gatB*	*icdA*	*murF*	*sprT*	*ycdB*	*yggA*
	acpD	*dhaH*[e] (*ycgC*)	*gatC*	*idi*	*nfnB*	*sthA* (*udhA*)	*ycdN*	*yggJ*
	add	*dhaK*[e] (*ycgT*)	*gatD*	*ispF* (*ygbB*)	*nfsA* (*mdaA*)	*sucA*	*ycdO*[a]	*ygiW*
	ahpF	*dhaL*[e] (*ycgS*)	*gatY*[a,c]	*kgtP*	*nuoC*	*sucB*[a]	*ycfP*	*yhcN*
	alaT	*edd*	*gatZ*	*lipA*	*nuoN*	*sucC*[a]	*ycfR*	*yheN*
	alaU	*eno*	*glcC*	*lldD*	*pdhR*	*sucD*	*ydgD*	*yheO*
	apbA	*fdoG*	*gltA*	*lpxD*	*pdxY*	*tas*	*ydhM*	*yieE*
	aroA	*fimA*	*gltB*	*lysC*	*proP*	*tehA*	*ydiH*	*yieF*
	aroH	*fimC*	*gltD*	*lysU*[a]	*rbsA*	*thrC*	*yeaS*	*yigI*
	aroP	*fimI*	*gpmA*	*map*	*rbsC*	*trpB*	*yecD*	*yiiS*[d]
	b0725	*flgH*	*grxA*	*marA*[a]	*rcsA*	*uspD* (*yiiT*)	*yecS*	*yjeM*
	b1364	*flhA*	*gshA*	*marB*	*rimI*	*xseA*	*yejG*	*yjgK*
	bcsE (*yhjS*)	*flhE*	*gshB*	*marR*	*rpoE*	*yabN*	*yfcA*	*yjjU*
	cadA[a,b]	*fliP*	*hdeA*[c,d]	*mdaB*	*rseA*	*yagU*	*yfcD*	*ykgA*
	cfa[c]	*fliQ*	*hdeB*[c,d]	*mdtG* (*yceE*)	*rseB*	*ybaK*	*yfcE*	*ylaC*
	cyaA	*fliR*	*hemB*	*menF*	*sdhA*	*ybfD*	*yfiD*[a,c]	*yncD*
	cyoB	*fliY*[c]	*hsdR*	*metN* (*abc*)	*sdhB*	*ybgF*	*yfjF*	*yodC*
	dadA	*fumA*	*hsdS*	*mltC*	*serV*	*ybiC*	*yfjG*	*yohN*
	dadX	*gapC*	*hslJ*	*mqo* (*yojH*)	*sfcA*	*ybjC*	*ygbE*	*yraQ*
Acid Low	*acrR*	*dcuS* (*yjdH*)	*gcvT*	*maa* (*ylaD*)	*ppiA*	*tatD* (*yigW*)	*yccA*	*yhaL*
	alx[e] (*ygjT*)	*dksA*[c,e]	*glgS*	*manX*	*pppA*	*tgt*	*yccK*	*yhiI*
	atpH	*dniR*	*glpA*	*mhpC*	*proV*	*tnaA*[e]	*ycfS*	*yjgF*[e]
	b3913	*dppC*	*hflB*	*miaA*	*purD*	*tnaB*	*ydhF*	*yjiA*
	bax	*dusA* (*yjbN*)	*hflC*	*mutL*	*purL*	*tnaC*	*yfiA*	*yjiX*
	bioB	*eco*	*hflK*	*nadA*	*purN*	*treB*	*ygaH*	*yjiY*
	borD (*ybcU*)	*fdhD*	*hflX*	*nmpC*	*putA*	*treC*	*ygaU*	*ykfF*
	btuB	*fhuD*	*htpX*	*nrdD*	*recR*	*trs5*	*ygaZ*	*ylaB*
	cirA	*folP*	*ilvB*	*nrdG*	*rpoH*	*ubiB* (*yigR*)	*ygdR*	*ylbF*
	cpxA	*frdA*	*ilvG*	*nudH* (*ygdP*)	*rzpD* (*ybcT*)	*yagE*	*yggH*	*yncE*
	cpxP[f]	*frdC*	*ilvN*	*ompF*[e]	*sdaB*	*yahA*	*yghG*	*yqjA*
	cpxR	*ftnB* (*yecI*)	*ivbL*	*phnA* (*yjdM*)	*sdaC*	*yajC*	*yghJ*	*yqjC*
	dadX	*fucI*	*katG*	*pitA*	*secD*	*ybaL*	*yghJ*	*yqjD*
	dcuR (*yjdG*)	*gcvH*	*kdtA*	*pntA*	*ssb*[e]	*ybfA*	*ygiB*	*ytfJ*
Base High	*artI*[e]	*b1171*	*dedA*	*glpX*	*osmB*	*recA*	*uvrY*	*yieG*
	artM	*b1172*	*dinI*	*glyA*	*potD*	*rnk*	*yceI*[e]	*yihA*
	asnA	*b3837*	*dinJ*	*gpmB*	*prsA*	*rpiA*[e]	*yciB*	*yijD*
	aspA	*carA*	*dsbA*[e]	*himD*	*purA*	*sdaA*[e]	*yciC*	*yjfO*
	atpA	*coaA*	*emrR*	*hisC*[e]	*purC*	*serU*	*yciI*	*yjgD*
	atpB	*codB*	*eptB* (*yhjW*)	*hisF*	*purH*	*slt*	*ydcG*	*ynfD*
	atpC	*cspD*	*fadL*	*hisH*	*purK*	*speA*	*ydeH*	*yqaE*
	atpD	*cvpA*	*folD*	*hisI*	*purM*	*speD*	*yebE*	*yqgB*
	atpE	*cydA*	*fucR*	*hisJ*	*pyrB*	*spy*	*yeeI*	*yqjB*
	atpF	*cydB*	*glmU*	*ispE* (*ychB*)	*pyrC*	*tatA*	*yehU*	
	atpG	*cydC*	*glpB*	*malT*	*pyrL*	*tatB*	*yfiQ*	
	atpI	*cydD*	*glpC*	*mdoB*	*rdoA* (*yihE*)	*ubiE* (*menG*)	*ygiC*	
Base Low	*adhE*[c]	*dnaJ*	*flgM*	*fliS*	*ibpB*[d]	*speG*	*ybeZ*	*yhhQ*
	aer	*dnaK* (*ycgT*)	*flgN*	*fliT*	*lipB*	*srlA*	*ybgL*	*yhiN*[d]
	cca	*dsbC*	*fliA*	*fliZ*	*lon*	*srlB*	*ybiX*	*yhjH*
	cdd	*fdoH*	*fliC*	*galM*	*modB*	*srlD*	*ybjX*	*yi81*
	cheA	*flaG*	*fliD*	*gapA*[e]	*motA*	*srlE*	*ycgR*	*yiaD*
	cheB	*flaX*	*fliE*	*gdhA*	*motB*	*srlR*	*ycjX*	*yjdA*
	cheR	*flgB*	*fliF*	*gntX* (*yhgH*)	*nhaB*	*tap*	*yeaD*	*ykfB*
	cheW	*flgC*	*fliG*	*groL* (*mopA*)	*nlpA*	*tar*	*yebW*	*ymdA*
	cheY	*flgD*	*fliH*	*groS* (*mopB*)	*nupG*	*tsr*	*yecR*	*yneE*
	cheZ	*flgE*	*fliI*	*grpE*	*ompT*	*tsx*	*yedM*	*ynfB*
	clpB	*flgF*	*fliJ*	*hlpA*	*pdxK*[d]	*udp*	*yeeR*	*zntR* (*yhdM*)
	clpX	*flgG*	*fliK*	*hslO* (*yrfI*)	*prlC*	*yafE*	*yffB*	
	deoA	*flgI*	*fliL*	*hslU*	*rbsB*	*yafY*	*ygaZ*	
	deoB	*flgJ*	*fliM*	*hslV*	*rbsD*	*ybbN*	*ygbF*	
	deoC	*flgK*	*fliN*	*htpG*	*rbsK*	*ybeX*	*ygiS*	
	deoD	*flgL*	*fliO*	*htrG* (*ygiM*)	*sfa* (*ymcE*)	*ybeY*	*yheL*	

Continued on following page

TABLE 1—*Continued*

Cluster	Genes							
Neutral High	*allA* (*ybbT*)	*frwB*	*idnT*	*menA*	*pmrD*	*uxaC*	*yhdT*	*yjhD*
	cdh	*ftsA*	*lamB*[e]	*mltB*	*psiF*	*yagF*	*yhfS*	*ykgF*
	dnaA	*ftsQ*	*lnt*	*modC*	*pstA*	*ybaV*	*yhfU*	*ymdB*
	dnaN	*fucK*	*lpxB*	*murG*	*pstB*	*ybcS*	*yhgE*	*yoaE*
	entE	*galK*	*malE*[c,e]	*napC*	*pstC*	*ybjG*	*yhiQ*	*yohK*
	fecA	*gntT*	*malF*	*narY*	*pstS*	*ycdZ*	*yicG*	*yqfA*
	fecB	*gpmM* (*yibO*)	*malG*	*nrdF*	*rne*	*ycjF*	*yihF*	*yqjH*
	fecC	*gpsA*	*malK*	*pflC*	*tdcB*	*ycjO*	*yjaB*	*yrfF*
	fecD	*hrpB*	*malM*	*pheA*	*tdcC*	*yddB*	*yjeJ*	*yrfG*
	fecE	*hslR* (*yrfH*)	*malP*	*phoB*	*trkA*	*ydhF*	*yjfL*	
	fepA	*hupA*	*malQ*	*phoU*	*ulaE* (*sgaU*)	*yghD*	*yjgM*	
	fhuC	*hybA*	*mdlB*	*pinO* (*pioO*)	*ulaR* (*yjfQ*)	*yhaM*	*yjgW*	
Neutral Low	*aceE*	*emrA*	*hpt*	*nadE*	*pfkB*[c]	*rho* (*sun*)	*ubiB*	*yeeZ*
	aceF[a]	*fabI*	*iadA*	*ndh*	*pflA*	*ribA*	*ubiH*	*yehS*
	acnB	*fadR*	*insA1*	*ndk*	*pflB*[c]	*rmf*	*xerC*	*yehT*
	adk	*fdx*	*insA2*	*nemA*	*pgi*	*rnb*	*yadG*	*yeiG*
	ahpC	*fnr*	*kdsA*	*nuoG*	*pheM*	*rnt*	*yadH*	*yfaE*
	aldA	*folE*	*kdsB*	*nuoH*	*pncB*	*rsd* (*yjaE*)	*yaiA*	*yfbQ*
	apaG	*fpr*	*lgt*	*nuoI*	*pps* (*ppsA*)	*rsuA*	*ybgC*	*yfcM*
	argS	*galF*	*lldP*	*nuoJ*	*ppx*	*sdhC*	*ybhB*	*yfcZ*
	aspC	*gapC*	*lpdA*[a]	*nuoK*	*proC*	*sdhD*	*ycaR*	*yfdG*
	avtA	*ghrA* (*ycdW*)	*luxS*[c] (*ygaG*)	*nuoL*	*pta*[c,e]	*serC*	*ycbK*	*yfdI*
	b0100	*glf*	*lysP*	*ompR*	*ptsG*	*sfsB* (*nlp*)	*ycbL*	*yfhB*
	can (*yadF*)	*gloA*	*mdtJ*	*ompX*[a,e]	*ptsH*	*sodB*[a]	*ycdX*	*yfjW*
	cld (*wzzB*)	*gltX*	*mdtL*	*oppA*[c,e]	*ptsI*	*sppA*	*ycdY*	*ygdI*
	cyoA	*gmhA*	*menB*	*oppB*	*ptsO*	*sra* (*rpsV*)	*ychH*	*yhbJ*
	cyoC	*gnd*	*mepA*	*oppC*	*purU*	*surA*	*ychJ*	*yheM*
	cyoD	*gppA*	*metG*	*oppD*	*pyrG*	*tatE* (*ybeC*)	*ydfG*	*yhjR*
	cyoE	*gpt*	*metK*	*oppF*	*rcsB*	*tehB*	*ydiH*	*yieP*
	cysK[e]	*grxB*[c]	*miaB* (*yleA*)	*pal*	*relA*	*tolB*	*ydjN*	*yjbQ*
	cysZ	*guaA*	*mipA* (*yeaF*)	*pdxH*	*rfbC*	*tolQ*	*yeaC*	*yjeQ*
	dld	*guaB*[a]	*mreB*	*pepB*	*rfbD*	*tpiA*	*yeaK*	*yjeS*
	dps[c]	*hemM*	*mrp*	*pepN*	*rfe*	*tpx*[a,e]	*yeaQ*[c]	*yjgP*
	dsbB	*hha*	*mtn* (*pfs*)	*pepP*	*rhlB*	*typA* (*yihK*)	*yeeN*	

[a] Acid induced (68, 73, 82).
[b] Data from five arrays from pH 5.0; no significant expression at pH 7.0 and 8.7.
[c] Acetate induced (3, 35).
[d] Extreme acid resistance (44, 78, 79).
[e] Base induced (68, 73, 82).
[f] Base induced (16).

small degree of repression in acid compared to that at pH 7.0 but overall were repressed at high pH.

The major regulator operon *flhCD*, however, showed no effect of pH. Thus, either the *flhCD* probes failed to show up in our arrays or pH may affect expression posttranscriptionally.

Motility assays. The effect of pH on motility was tested by spotting motile cultures of *E. coli* K-12 RP437 and *S. enterica* serovar Typhimurium SJW1103 on motility agar buffered at a range of pH values (Fig. 4). Both species showed a steady decline of motility as pH increased. The decline was particularly steep between pH 7.5 and 8.7.

Catabolism and proton transport. Several enzymes for catabolism of sugars and amino acids show a pH dependence that minimizes acid production at low external pH or maximizes acids at high pH (68, 73). Our microarrays revealed many new components, showing the broad scope of pH regulation of catabolism (Table 4).

Many operons encoding processes of glycolysis and the TCA cycle, such as *aceEF* (pyruvate dehydrogenase), *dhaKL* (dihydroxyacetone kinase), *pta* (phosphotransacetylase), and *pts* (glucose phosphotransferase), showed elevated expression in acid. Others, however, were elevated at high pH. Operons elevated at high pH tended to be those induced by anaerobiosis, such as *glpABC* (anaerobic glycerol-3-phosphate dehydrogenase), *pflBA* (anaerobic pyruvate formate lyase), and *dcu* (anaerobic fumarate respiration). The *mal* system, however, is strongly repressed by acid (13, 31) and showed up as such in our arrays.

Membrane-bound systems for proton and electron transport were regulated by acid or base along lines largely consistent with their relative degree of export or import of H^+. An example is the *atp* operon encoding F_1F_o ATP synthase (32), which imports H^+ during oxidative respiration. Most of the *atp* genes were strongly upregulated at high pH, whereas *ndh* and *nuo* (the NADH dehydrogenases I and II), which export H^+, were down-regulated. The *sdh* gene (succinate dehydrogenase), which contributes electrons for proton export, is also down-regulated at high pH. On the other hand, cytochrome *d* oxidase (*cyd*) is expressed in preference to cytochrome *o* oxidase (*cyo*) at high pH, presumably because it exports half as many H^+ per electron (14).

Enzymes for degradation of amino acids showed pH regulation as expected, with high pH favoring deaminase operons such as *tna* (tryptophan deaminase), *sda* (serine deaminase), and *tdcB* (threonine dehydratase). Acid induced only one of the decarboxylase operons, *cad* (lysine decarboxylase). Several

TABLE 2. Strongest pH-dependent expression ratios (fourfold or higher)

pH dependence and pH ratio	Gene	Log_2 ratio	pH dependence and pH ratio	Gene	Log_2 ratio
Acid induced			Base induced		
5.0/7.0	*yagU*	3.220	8.7/7.0	*yifO*	2.769
	yhcN	3.064		*ymgD*	2.221
	sdhC	2.728			
	lysP	2.662	8.7/5.0	*tnaC*	5.517
	sdhD	2.349		*cpxP*	4.234
	cfa	2.075		*tnaA*	4.028
	nemA	2.060		*nmpC*	3.961
				treB	3.895
				yjiY	3.665
5.0/8.7	*yhcN*	4.199		*treC*	3.233
	yagU	3.962		*b3913*	3.176
	fliC	3.396		*yifO*	3.095
	fimA	2.579		*borD*	3.088
	cfa	2.555		*tnaB*	2.993
	gltB	2.271		*ycfS*	2.820
	ydiY	2.193		*yghJ*	2.762
	ycdN	2.147		*ymgD*	2.433
	yncD	2.117		*yccA*	2.378
	mqo	2.075		*yfiA*	2.364
	dhaH	2.074		*yebE*	2.343
	cheA	2.074		*yjiX*	2.292
	motB	1.997		*nrdD*	2.182
				dniR	2.081
				alx	2.009
7.0/8.7	*fliC*	4.561		*mutL*	2.000
	malM	3.780			
	malK	3.748	7.0/5.0	*tnaC*	5.026
	lamB	3.735		*lamB*	4.881
	malP	3.373		*malK*	4.790
	motB	3.238		*malM*	4.643
	cheA	3.199		*yjiY*	4.359
	cheZ	3.199		*nmpC*	4.012
	flxA	3.007		*malP*	4.000
	malE	2.933		*tnaA*	3.805
	malQ	2.883		*malE*	3.425
	cheW	2.785		*borD*	3.378
	ibpB	2.618		*malQ*	3.322
	yhjH	2.522		*cpxP*	3.232
	htpG	2.439		*treB*	3.156
	deoC	2.267		*yghJ*	3.081
	pstS	2.262		*yjiX*	2.959
	dnaK	2.249		*fecB*	2.842
	tar	2.213		*ompF*	2.834
	yjdA	2.208		*treC*	2.613
	dnaJ	2.155		*fecA*	2.608
	yrfG	2.055		*pstS*	2.479
	yheL	2.031		*b3913*	2.177
	deoA	2.003		*fecE*	2.098

decarboxylases are known to be induced by acid, but their induction is repressed by oxygen (4, 30), which may explain their absence in our highly aerobic cultures.

Oxidative stress and salicylate stress. Several acid stress genes are known to overlap with oxidative stress, for example, the alkyl hydroperoxide reductase *ahpC* (9, 84), and certain permeant acids such as salicylate are considered oxidative stress agents (54). We surveyed our pH-regulated genes for overlap with response to H_2O_2, paraquat, and salicylate, as reported in references 54 and 84 (Table 5).

Of the 73 pH-dependent genes known to be induced by H_2O_2, paraquat, or salicylate, virtually all were induced by acid or repressed by base. This finding confirms our hypothesis of a strong connection between acid stress and oxidative stress. It may be that low pH amplifies the toxicity of oxygen radicals. Genes repressed by paraquat or salicylate were repressed in acid or induced at high pH, such as the base-inducible membrane protein gene *alx*, the histidine cyclase gene *hisF*, and outer membrane protein gene *ompF*. An exception to these generalizations was the maltose regulon (*lamB*, *malE*, and *malK*), which was repressed by acid but induced by paraquat.

Envelope and periplasmic stress. A large part of *E. coli* function takes place in the outer membrane and envelope (48) and the periplasm (49), compartments essentially exposed to "extracellular" pH. Thus, it is not surprising that several envelope and periplasmic components show pH-dependent expression (16, 23, 73, 82). Our microarrays revealed an even greater number of such responses (Table 6). Both acid and base induction were observed. Acid-induced periplasmic proteins in-

TABLE 3. Flagellar and chemotaxis genes

Gene	Function	Log_2 pH ratio[a]			Class[b]
		5/7	8.7/7	8.7/5	
cheA	Chemotaxis sensor kinase	**−1.125**	**−3.199**	**−2.0474**	BL
cheB	Protein methylesterase	**−1.050**	**−1.578**	**−0.528**	BL
cheR	Chemotaxis MCP[c] methyltransferase	**−0.564**	**−1.013**	−0.448	BL
cheW	Chemotaxis signal transducer	**−1.336**	**−2.785**	**−1.449**	BL
cheY	Response regulator for chemotactic signal	**−1.089**	**−1.310**	−0.221	BL
cheZ	CheY-P phosphatase	**−1.505**	**−3.199**	**−1.694**	BL
flgA	Flagellar synthesis	−0.261	**−1.056**	**−0.795**	BL
flgB	Basal body rod subunit	−0.192	**−1.120**	**−0.928**	BL
flgC	Basal body rod subunit	−0.257	**−1.241**	**−0.984**	BL
flgD	Basal body rod modification	0.133	**−1.241**	**−1.107**	BL
flgE	Hook subunit	0.272	**−0.856**	**−1.128**	BL
flgF	Basal body rod subunit	0.109	**−1.239**	**−1.348**	BL
flgG	Basal body rod major subunit	0.220	**−1.116**	**−1.335**	BL
flgI	Basal body P-ring	−0.011	**−1.218**	**−1.207**	BL
flgJ	Flagellum-specific muramidase	0.030	**−0.857**	**−0.886**	BL
flgK	Flagellar synthesis	−0.211	**−1.875**	**−1.664**	BL
flgL	Flagellar synthesis	0.111	**−1.165**	**−1.276**	BL
flgM	Anti-sigma 28 (FliA); regulates FlhD	−0.292	**−1.424**	**−1.132**	BL
flgN	Flagellar synthesis	−0.295	**−1.567**	**−1.272**	BL
flhA	Flagellar export pore protein	0.333	−0.528	**−0.861**	AH
flhE	Function unknown	0.492	−0.454	**−0.946**	AH
fliA	Sigma 28; regulates class III flagellar genes	−0.150	**−1.127**	**−0.976**	BL
fliC	Flagellin subunit, H-antigen	−1.165	**−4.561**	**−3.396**	BL
fliD	Hook-associated protein	−0.311	**−1.953**	**−1.641**	BL
fliE	Flagellar synthesis; basal body component	−0.478	**−1.861**	**−1.203**	BL
fliF	Flagellar basal body M-ring	−0.159	**−1.216**	**−1.057**	BL
fliG	Motor switching and energizing	0.182	**−1.313**	**−1.495**	BL
fliH	Negative regulator of FliI; flagellar assembly and export	0.389	**−1.245**	**−1.634**	BL
fliI	Membrane ATPase, flagellar, axial subunit export	0.270	**−1.238**	**1.508**	BL
fliJ	Flagellar biosynthesis	−0.068	**−1.404**	**−1.336**	BL
fliK	Hook filament junction	0.278	**−1.186**	**−1.464**	BL
fliL	Rotational direction of flagella	−0.102	**−1.083**	**−0.981**	BL
fliM	Flagellar synthesis, motor switching and energizing	0.029	**−1.053**	**−1.082**	BL
fliN	Flagellar switch	0.273	**−0.875**	**−1.148**	BL
fliO	Flagellar synthesis	0.191	**−0.921**	**−1.112**	BL
fliP	Flagellar synthesis	0.419	−0.669	**−1.087**	AH
fliQ	Flagellar synthesis	0.463	−0.980	**−1.444**	AH
fliR	Flagellar synthesis	0.924	−0.899	**−1.822**	AH
fliS	Cytosolic chaperone inhibits premature FliC assembly	−0.174	**−1.947**	**−1.772**	BL
fliT	Flagellar synthesis	−0.323	**−1.021**	**−0.698**	BL
fliY	Cystine-binding protein, periplasmic; may regulate FliA (sigma 28)	0.233	−0.252	**−0.484**	AH
fliZ	Not required for motility; may regulate FliA (sigma 28)	0.144	**−1.165**	**−1.309**	BL
motA	Flagellar rotation	**−0.682**	**−1.847**	**−1.166**	BL
motB	Flagellar rotation	**−1.241**	**−3.238**	**−1.997**	BL
tap	Dipeptide chemoreceptor	−0.622	**−1.089**	−0.466	BL
tar	Aspartate, maltose chemoreceptor	**−0.519**	**−2.213**	**−1.694**	BL
tsr	Serine chemoreceptor	**−1.061**	**−1.826**	**−0.765**	BL
ycgR	Suppresses *hns* motility defect	−0.531	**−0.783**	−0.252	BL
yhjH	Suppresses *hns* motility defect	**−0.972**	**−2.522**	**−1.550**	BL

[a] Boldface for ratios indicates significance ($\alpha = 0.001$).
[b] BL, Base Low; AH, Acid High.
[c] MCP, methyl-accepting chemotaxis protein.

cluded the well-known acid chaperone from *hdeAB* (23), as well as the newly observed TolA-binding protein (*ybgF*) and the lipoprotein from *pal*. High pH induced the ferric transporters from *fecAB* and *fhuD*, possibly due to low iron solubility at high pH. At high pH, various transport proteins and redox modulators such as that from *dsbA* are known to be induced. In addition, several additional base-induced periplasmic and envelope proteins appeared, including the vitamin B_{12} transporter from *btuB*, the outer membrane protein from *nmpC*, and the peptidylprolyl-*cis-trans*-isomerase from *ppiA*.

Universal stress and heat shock. Various heat shock and universal stress proteins are inducible by the permeant acid benzoate, such as the products of *clpB*, *htpG*, *dnaK*, *groS*, and *uspA* (38). Some of these showed pH response in our microarrays (Table 7). The DNA damage response gene *uspD* was acid induced, as was *dps*, encoding the DNA-binding protein involved in stationary phase and acid resistance. Acid induced

rseAB, the antisigma regulators of the *rpoE* envelope heat stress system (1). High pH induced the *rpoH* heat shock sigma 32 gene (28) as well as heat shock proteasome genes *hslUV* and regulators *hslOR*.

DISCUSSION

Overall, our work revealed a large number of genes not previously known to be regulated by pH. Furthermore, many of these genes had no previously known function or response, such as *yhcN* and *yagU* (induced by acid) and *yifO* and *ymcG* (induced by base).

An important question is to assess the biological relevance of the expression ratios reported (36, 51). Most of the ratios we reported as significant (boldface in Tables 3 through 7) are greater than twofold ($\log_2 = 1$). In many cases, all or most members of an operon fell in the same cluster and show similar expression profiles; the flagellar regulon was particularly consistent (Table 3). The gene probes are synthesized on the array independently of their operon map; thus, parallel expression profiles within operons do not reflect array position. Note that even genes with significant expression ratios of less than 2 ($\log_2 = 1$) tend to group with their operons. In previous studies, comparison with quantitative reverse transcriptase real-time PCR shows that microarray ratios, while quantitatively consistent, generally underestimate the actual differences in mRNA levels between the biological systems compared (83).

Flagellar biosynthesis and motility. The effects of pH in flagellar biosynthesis and motility remain poorly understood. It has long been known that low external pH (thus, large ΔpH) contributes to the proton motive force that drives flagellar rotation (33). The cytoplasmic pH, however, must remain high; permeant acids such as acetate and benzoate, which depress internal pH and decrease proton motive force, are chemotactic repellents (67) and impair rotation of the flagellar motor (46). Low pH elicits negative chemotaxis (55, 67), whereas a pH increase up to 8.3 elicits a positive response (55).

In recent reports acid stress is associated with low motility (72), yet acetate has been reported to induce the flagellar regulon and enhance motility (53). We believe that the previous reports are limited in several ways. Reference 72 does not compare pH conditions directly but notes repression of flagellar genes in an *hns* mutant in which acid resistance is increased. The motility assay is not clearly described, and the acid dependence of *flhDC-cat* expression was observed on plasmids, not in the genome. Reference 53 reports induction of chromosomal *flhDC-lacZ* fusions by acetate. Those authors' assays of motility, however, show relatively small differences between pH conditions.

Our microarrays showed strong evidence for suppression of motility and chemotaxis at high pH. This evidence was supported by the decrease in motility at high pH, observed for both *E. coli* and *S. enterica* serovar Typhimurium, which swims twice as fast as *E. coli*. We also found weaker evidence for repression of *che* and *mot* genes at pH 5, but the flagellar synthesis genes were strongly induced at low pH. Overall, our data point to alkaline suppression of flagellar motility. Work in progress shows that, at high pH, the number of flagella per cell is decreased to one to three per cell (about 20% of normal) (S. Aizawa and J. Slonczewski, unpublished data).

No pH dependence was observed for the flagellar regulators *flhD* and *flhC*. On the other hand, in a microarray study of anaerobic cultures, *flhD* and *flhC* are induced by acid (E. Hayes and J. L. Slonczewski, unpublished data). Acid induction of these regulators would be consistent with the report of their induction by acetate (53). We did see acid induction of two known activators of *flhDC*: adenylate cyclase *cyaA* (37) (Acid High) and *dnaK-dnaJ-grpE* (64) (Base Low). We saw no acid induction of other flagellar activators such as *crp* (37), nor did we see alkaline induction of the negative flagellar regulator *rcsCDB* (22).

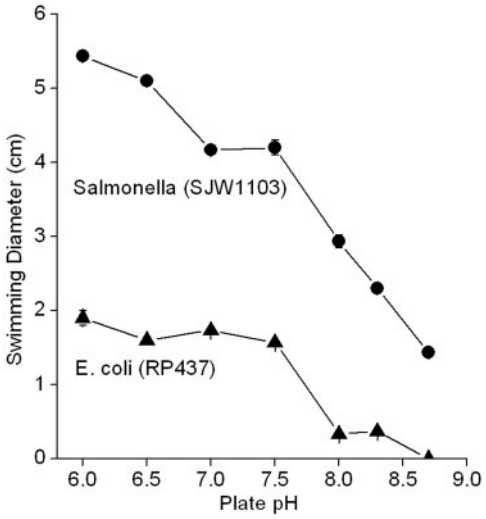

FIG. 4. Swimming distance as a function of pH. *E. coli* K-12 RP437 and *S. enterica* serovar Typhimurium SJW1103 were spotted on soft-agar plates as described under Materials and Methods. Error bars represent standard errors of the means ($n = 3$); in most cases their size was smaller than the symbol.

An alternative model is that pH regulation of the flagellar regulon is mediated by proteolysis, as in the case of ClpXP proteolysis of FlhD and FlhC (76). We find that ClpX is downregulated at high pH (Base Low cluster), but a different protease could be involved.

Catabolism. The picture of catabolism is more complicated, but in general our expression ratios confirm our present hypotheses of pH regulation while extending our knowledge to many more components. Systems that consume acids are enhanced at low pH. On the other hand, initial import and breakdown of some sugars, such as maltose, are favored at high pH, where they may quickly generate a large burst of fermentation acids.

With respect to proton export, *E. coli* appears to prefer components such as ATP synthase that import protons at high pH (counteracting the alkaline stress on cytoplasmic pH) and prefers to minimize proton export associated with the terminal oxidase *cyd* in preference to *cyo*. This observation is consistent with the previous report that *cyd* expression is higher at pH 7.5 than at pH 5.0 in an *fnr* mutant (14), although in those experiments *cyo* expression also increased with pH. It is likely that our broader range of pH classes (up to pH 8.7) provided a clearer picture of pH regulation of *cyo* and *cyd*.

Under amino acid catabolism, relatively few new components of pH response were observed. This makes sense, because most amino acid decarboxylases are repressed by oxygen

TABLE 4. Catabolism and respiration

Group	Gene	Function	Log$_2$ pH ratio[a]			Class[b]
			5/7	8.7/7	8.7/5	
Sugar catabolism and TCA cycle	*aceE*	Pyruvate dehydrogenase	**1.928**	0.527	**−1.401**	NL
	aceF	Pyruvate dehydrogenase dihydrolipoamide acetyltransferase	**1.802**	0.402	**1.401**	NL
	aceK	Isocitrate dehydrogenase kinase/phosphatase	**0.667**	0.038	**−0.629**	AH
	acnA	Aconitase A, stationary phase induced	**0.769**	−0.143	**−0.912**	AH
	acnB	Aconitase B; 2-methylaconitate hydratase	**1.036**	−0.041	**−1.077**	NL
	acrR	Regulator for *acrA* and *acrB*	**−0.873**	0.164	**1.037**	AL
	dcuR (*yjdG*)	Fumurate respiration regulator (anaerobic)	−0.220	0.055	**0.275**	AL
	dcuS (*yjdH*)	Fumurate respiration regulator (anaerobic)	**−0.324**	0.065	**0.390**	AL
	dhaK	Dihydroxyacetone kinase, subunit I	**1.041**	−0.379	**−1.420**	AH
	dhaL	Dihydroxyacetone kinase, subunit II	**0.742**	−0.385	**−1.127**	AH
	dld	D-Lactate dehydrogenase	**0.919**	0.566	−0.353	NL
	eno	Enolase; RNA degradosome	**0.299**	0.052	−0.247	AH
	fucI	L-Fucose isomerase	**−0.693**	**−0.173**	0.520	AL
	fucK	L-Fuculose kinase	**−1.259**	**−0.519**	0.740	NH
	fucR	Positive regulator, *fuc* operon	−0.046	**0.495**	**0.541**	BH
	galF	Putative regulator of *galU*				NL
	galK	Galactokinase	**−0.616**	**−0.562**	0.053	BL
	galM	Galactose mutarotase; aldose-1-epimerase	**−0.619**	**−0.647**	−0.028	
	gapA	Glyceraldehyde 3-P dehydrogenase A	0.093	**−0.752**	**−0.844**	BL
	gatA	Galactitol-specific enzyme IIA of PTS[d]	**0.929**	−0.626	**−1.555**	AH
	gatB	Galactitol-specific enzyme IIB of PTS	0.632	−0.895	**−1.528**	AH
	gatC	Galactitol-specific enzyme IIC of PTS	**0.973**	**−0.949**	**−1.922**	AH
	gatD	Galactitol-1-phosphate dehydrogenase	**1.037**	−0.893	**−1.930**	AH
	gatY	D-Tagatose-1,6-bisphosphate aldolase, class II	**0.846**	−0.483	**−1.329**	AH
	gatZ	Enhances GatY activity	**0.774**	−0.605	**−1.380**	AH
	glpA	Glycerol-3-phosphate dehydrogenase large subunit (anaerobic)	−0.200	0.558	**0.758**	AL
	glpB	Glycerol-3-phosphate membrane anchor (anaerobic)	−0.124	0.437	**0.562**	BH
	glpC	Glycerol-3-phosphate dehydrogenase (anaerobic) small subunit	−0.169	0.469	**0.638**	BH
	glpX	Fructose 1,6-bisphosphatase	−0.162	0.375	**0.537**	BH
	gltA	Citrate synthase	0.288	−0.559	**−0.846**	AH
	gnd	Gluconate-6-phosphate dehydrogenase	**0.895**	0.351	**−0.544**	NL
	gntT	High-affinity gluconate transport	**−0.613**	−0.362	0.251	NH
	gpsA	Glycerol-3-phosphate dehydrogenase	**−0.492**	**−0.534**	−0.041	NH
	gpmA	Phosphoglycerate mutase I	0.303	−0.118	**−0.421**	AH
	icdA	Isocitrate dehydrogenase	**1.211**	0.061	**−1.272**	AH
	lldD	L-Lactate dehydrogenase	0.613	−0.554	**−1.167**	AH
	lldP	L-Lactate permease; glycolate uptake	**1.527**	0.219	**−1.307**	NL
	lpdA	Lipoamide dehydrogenase; E3 component of pyruvate and 2-oxoglutarate dehydrogenase complexes	**1.507**	0.281	**−1.226**	NL
	malE	Maltose-binding protein, periplasmic	**−3.425**	**−2.933**	0.491	NH
	malF	Maltose transport, inner membrane	**−1.016**	**−0.935**	0.082	NH
	malG	Maltose transport, inner membrane subunit	**−1.577**	**−1.502**	0.075	NH
	malK	Maltose transport, ATP-binding subunit	**−4.790**	−3.748	**1.041**	NH
	malM	Periplasmic protein, *mal* regulon	**−4.643**	**−3.780**	**0.863**	NH
	malP	Maltodextrin phosphorylase	**−4.000**	**−3.373**	0.627	NH
	malQ	Amylomaltase	**−3.322**	**−2.883**	0.439	NH
	malT	*mal* positive regulator	−0.103	**0.617**	**0.720**	BH
	pdhR	Pyruvate dehydrogenase operon repressor	**0.755**	−0.109	**−0.863**	AH
	pfkB	6-Phosphofructokinase-2	**0.363**	0.095	**−0.268**	NL
	pflA	Pyruvate formate lyase I activase	**0.964**	0.667	−0.297	NL
	pflB	Pyruvate formate lyase I (anaerobic)	**0.812**	0.578	−0.234	NL
	pgi	Glucose phosphate isomerase	**0.654**	0.177	**−0.477**	NL
	pta	Phosphotransacetylase	**1.167**	0.639	−0.528	NL
	ptsG	Glucose PTS enzyme IIBC	**0.841**	**0.544**	−0.287	NL
	ptsH	PTS system histidine phosphocarrier protein Hpr	**0.369**	**0.564**	0.195	NL
	ptsI	PTS system enzyme I	0.241	**0.339**	0.098	NL
	ptsO	NPr, N-regulated HPr-like protein	**0.639**	0.168	**−0.470**	NL
	rpiA	Ribose-5-phosphate isomerase A	0.180	**0.364**	0.184	BH
	srlA	Sorbitol-specific enzyme II of PTS	**−1.156**	**−1.720**	−0.564	BL
	srlB	Sorbitol-specific enzyme III of PTS	**−0.908**	**−1.140**	−0.232	BL
	srlD	Sorbitol-6-phosphate dehydrogenase	**−0.660**	**−1.334**	**−0.674**	BL
	srlE	srl operon protein	**−1.013**	**−1.613**	**−0.599**	BL
	srlR	srl regulator	−0.151	**0.804**	**−0.654**	BL
	sucA	2-Oxoglutarate dehydrogenase, E1 component	**0.850**	**−0.851**	**−1.701**	AH
	sucB	Dihydrolipoamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex (E2)	**0.566**	−1.073	**−1.638**	AH
	sucC	Succinyl-CoA[c] synthase beta subunit	0.494	−1.019	**−1.513**	AH
	sucD	Succinyl-CoA synthase alpha subunit	0.526	−1.225	**−1.751**	AH
	tpiA	Triosephosphate isomerase	**0.772**	0.245	−0.526	NL

Continued on following page

TABLE 4—*Continued*

Group	Gene	Function	Log_2 pH ratio[a]			Class[b]
			5/7	8.7/7	8.7/5	
	treB	Trehalose-specific PTS enzyme II	**−3.156**	**0.739**	**3.895**	AL
	treC	Trehalose-6-phosphate hydrolase	**−2.613**	**0.620**	**3.233**	AL
Proton transport and electron transport chain	*atpA*	ATP synthase subunit alpha, F_1	0.111	**0.460**	**0.349**	BH
	atpB	ATP synthase subunit a, F_0	0.125	**0.484**	**0.609**	BH
	atpC	ATP synthase subunit epsilon, F_1	0.266	**1.005**	**0.739**	BH
	atpD	ATP synthase subunit beta, F_1	0.542	**1.172**	0.630	BH
	atpE	ATP synthase subunit c, F_0	0.182	**0.503**	0.321	BH
	atpF	ATP synthase subunit b, F_0	−0.055	0.321	**0.377**	BH
	atpG	ATP synthase subunit gamma, F_1	0.372	**0.632**	**0.260**	BH
	atpH	ATP synthase subunit delta, F_1	−0.172	0.186	**0.358**	AL
	atpI	ATP synthase subunit, F_1F_0-type proton-ATPase	0.017	**0.499**	**0.482**	BH
	cydA	Cytochrome *d* (bd-I) terminal oxidase subunit I (microaerobic)	−0.268	**0.872**	**1.140**	BH
	cydB	Cytochrome *d* (bd-I) terminal oxidase subunit II (microaerobic)	−0.097	**0.777**	**0.874**	BH
	cydC	Cysteine exporter to periplasm required for Cyd assembly	0.135	**0.490**	**0.355**	BH
	cydD	Cysteine exporter to periplasm required for cytochrome assembly	0.232	**0.574**	0.342	BH
	cyoA	Cytochrome *o* oxidase subunit II	**1.160**	0.217	**−0.943**	NL
	cyoB	Cytochrome *o* oxidase subunit I	**0.812**	−0.204	**−1.016**	NL
	cyoC	Cytochrome *o* oxidase subunit III	**1.026**	0.019	**−1.007**	NL
	cyoD	Cytochrome *o* oxidase subunit IV	**0.829**	−0.192	**−1.021**	NL
	cyoE	Cytochrome *o* oxidase subunit protoheme IX farnesyltransferase	**1.094**	0.225	**−0.869**	NL
	fdoG	Formate dehydrogenase-O, major selenopeptide subunit	0.353	−0.138	**−0.491**	AH
	fdoH	Formate dehydrogenase-O Fe-S subunit	0.003	**−0.414**	**−0.416**	BL
	frdA	Fumarate reductase flavoprotein subunit	−0.305	0.046	0.351	AL
	frdC	Fumarate reductase membrane anchor polypeptide	**−0.378**	−0.008	**0.370**	AL
	fumA	Fumarase A	0.595	**−0.647**	**−1.242**	AH
	nfsA (*mdaA*)	Nitroreductase A	**0.561**	−0.419	**−0.980**	AH
	mdaB	Probable nitroreductase or quinone reductase	**0.444**	−0.223	−0.667	AH
	napC	Cytochrome electron source for NapAB, membrane bound	**−0.797**	−0.382	0.416	NH
	ndh	Respiratory NADH dehydrogenase II; NADH:ubiquinone oxidoreductase II	**0.789**	0.161	**−0.628**	NL
	nuoC	NADH:ubiquinone oxidoreductase subunit C	0.440	−0.084	**−0.524**	AH
	nuoG	NADH:ubiquinone oxidoreductase subunit G; NADH dehydrogenase I	**0.525**	0.103	−0.442	NL
	nuoH	NADH:ubiquinone oxidoreductase subunit H; NADH dehydrogenase I	**0.564**	0.185	**−0.379**	NL
	nuoI	NADH:ubiquinone oxidoreductase subunit I; NADH dehydrogenase I	**0.792**	0.310	−0.481	NL
	nuoJ	NADH:ubiquinone oxidoreductase subunit J; NADH dehydrogenase I	**0.468**	0.234	−0.234	NL
	nuoK	NADH:ubiquinone oxidoreductase subunit K; NADH dehydrogenase I	**0.766**	**0.460**	−0.306	NL
	nuoL	NADH:ubiquinone oxidoreductase subunit L; NADH dehydrogenase I	**0.311**	0.078	−0.233	NL
	nuoN	NADH:ubiquinone oxidoreductase subunit N; NADH dehydrogenase I	0.126	−0.210	**−0.336**	AH
	sdhA	Succinate dehydrogenase flavoprotein subunit	**1.438**	−0.018	**−1.458**	AH
	sdhB	Succinate dehydrogenase iron-sulfur protein	**1.833**	0.329	**−1.505**	AH
	sdhC	Succinate dehydrogenase membrane anchor subunit, cytochrome b_{556}	**2.728**	0.890	**−1.838**	NL
	sdhD	Succinate dehydrogenase hydrophobic subunit	**2.349**	0.595	**−1.754**	NL
Amino acid catabolism and transport	*artI*	Arginine periplasmic binding protein	0.286	**0.655**	0.369	BH
	artM	Arginine periplasmic binding protein	−0.115	0.293	**0.407**	BH
	cadA	Lysine decarboxylase, degradative	**1.024**	0.137	**−0.887**	AH
	cysK	*O*-Acetylserine sulfhydrylase A (cysteine synthase)	**1.204**	**1.351**	0.147	NL
	dadA	D-Amino acid dehydrogenase	**1.273**	−0.229	**−1.501**	AH
	dadX	D-Amino acid dehydrogenase	**0.683**	−0.393	**−1.076**	AH
	dppC	Dipeptide permease system	**−0.346**	−0.032	0.315	AL
	gdhA	Glutamate dehydrogenase	−0.080	**−0.496**	−0.416	BL
	hisC	Histidinol-phosphate aminotransferase	0.253	**0.984**	**0.731**	BH
	hisF	Imidazole glycerol phosphate synthase (cyclase)	0.150	**0.703**	**0.553**	BH
	hisH	Amidotransferase of imidazole glycerol phosphate synthase	0.034	**0.393**	**0.359**	BH
	hisI	PR-ATP pyrophosphatase and PR-AMP cyclohydrolase	0.234	**0.641**	**0.407**	BH
	hisJ	Histidine-binding protein	−0.149	**0.400**	**0.549**	BH
	lysC	Aspartokinase III	**1.464**	−0.226	**−1.690**	AH
	lysP	Lysine permease	**2.662**	0.963	**−1.698**	NL
	lysU	Lysine-tRNA ligase	**0.544**	−0.077	**−0.621**	AH
	potD	Putrescine-ornithine transporter	0.053	**0.509**	**0.456**	BH
	sdaA	L-Serine deaminase, degradative	−0.409	**0.638**	**1.048**	BH
	sdaB	L-Serine deaminase	**−1.111**	−0.434	**0.678**	AL
	sdaC	H+/serine symporter; regulator of serine deaminase	**−1.205**	−0.410	**0.794**	AL
	tnaA	Tryptophan deaminase, degradative; also deaminases serine and cysteine	**−3.805**	0.223	**4.028**	AL
	tnaB	Tryptophan transporter	**−1.840**	1.153	**2.993**	AL
	tnaC	*tnaA* leader peptide	**−5.026**	0.490	**5.517**	AL
	tdcB	Threonine dehydratase, degradative	**−0.849**	−0.296	0.553	NH
	ydfG	L-*allo*-Threonine, L-serine, D-serine dehydrogenase	**0.407**	0.287	−0.120	NL

[a] Values in boldface are significant ($\alpha = 0.001$).
[b] NL, Neutral Low; AH, Acid High; AL, Acid Low; BH, Base High; BL, Base Low; NH, Neutral High.
[c] CoA, coenzyme A.
[d] PTS, phosphotransferase.

TABLE 5. pH-regulated oxidative stress response[a]

Gene	Function	Log$_2$ pH ratio[b]			PQ, Sal, or H_2O_2	Class[c]
		5/7	8.7/7	8.7/5		
acnA	Aconitase A	**0.769**	−0.143	**−0.912**	Sal	AH
adhE	Acetaldehyde-coenzyme A dehydrogenase	0.043	−0.380	−0.422	Sal	BL
ahpC	Alkyl hydroperoxide reductase small subunit	**1.003**	0.436	**−0.568**	PQ, H_2O_2	NL
ahpF	Alkyl hydroperoxide reductase large subunit	**0.777**	−0.222	**−0.999**	H_2O_2	AH
aldA	Aldehyde dehydrogenase, NAD linked	**0.773**	**0.764**	−0.009	PQ	NL
alx (*ygjT*)	Membrane protein, alkali induced	**−1.317**	**0.692**	**2.009**	PQ−	AL
artI	Periplasmic arginine binding protein	0.286	**0.655**	0.369	PQ	BH
aspA	Aspartate ammonia-lyase (aspartase)	−0.592	0.770	**1.362**	PQ−	BH
carA	Carbamoylphosphate synthase small subunit	0.029	**1.059**	1.030	PQ−	BH
cfa	Cyclopropane fatty acid synthase	**2.075**	−0.480	**−2.555**	Sal	AH
cyaA	Adenylate cyclase	0.691	−0.156	**−0.846**	Sal	AH
cyoD	Cytochrome *o* oxidase subunit IV	**0.829**	−0.192	**−1.021**	PQ	NL
cysK	Cysteine synthase	**1.204**	**1.351**	0.147	PQ, Sal, H_2O_2	NL
dadX	Alanine racemase	**0.683**	−0.393	**−1.076**	PQ	AH
deoA	Thymidine phosphorylase	**−1.031**	**−2.003**	**−0.972**	Sal	BL
deoB	Deoxyribouratase, phosphopentomutase	**−0.731**	**−1.502**	**−0.771**	PQ, Sal	BL
dhaH	Dihydroxyacetone phosphoryl donor	**1.547**	−0.527	**−2.074**	Sal	AH
dhaK	Dihydroxyacetone kinase	**1.041**	−0.379	**−1.420**	Sal	AH
dnaK	HSP-70-type molecular chaperone	−0.894	**−2.249**	−1.356	Sal	BL
dps	Stress response DNA-binding protein	**1.130**	0.105	**−1.025**	PQ, Sal, H_2O_2	NL
fliS	Flagellar synthesis; flagellar regulon member	−0.174	**−1.947**	**−1.772**	PQ−	BL
fpr	Ferredoxin $NADP^+$ reductase; anaerobic	**0.565**	0.275	−0.289	PQ, H_2O_2	NL
gapA	GAPDH[d] A	0.093	**−0.752**	**−0.844**	Sal	BL
gatA	Galactitol-specific enzyme IIA of PTS[e]	**0.929**	−0.626	**−1.555**	PQ, Sal	AH
gatB	Galactitol-specific enzyme IIB of PTS	0.632	−0.895	**−1.528**	PQ, Sal	AH
gatC	Galactitol-specific enzyme IIC of PTS	**0.973**	**−0.949**	**−1.922**	Sal	AH
gatD	Galactitol-1-phosphate dehydrogenase	**1.037**	**−0.893**	**−1.930**	PQ, Sal	AH
gatZ	Tagatose 6-phosphate aldolase 2	**0.774**	−0.605	**−1.380**	Sal	AH
gltA	Citrate synthase	0.288	−0.559	**−0.846**	PQ, Sal	AH
gltB	Glutamate synthase, large subunit	**0.846**	**−1.425**	**−2.271**	Sal	AH
grxA	Glutaredoxin 1	**1.666**	−0.106	**−1.772**	H_2O_2	AH
gshB	Glutathione synthetase	**0.768**	−0.081	**−0.848**	Sal	AH
hdeA	Periplasmic acid chaperone	0.841	−0.326	**−1.167**	Sal	AH
hdeB	Periplasmic acid chaperone	0.782	−0.622	**−1.404**	Sal	AH
hisF	Cyclase component of IGP synthase	0.150	**0.703**	**0.553**	PQ−	BH
ibpB	Chaperone, HSP20 family	−1.691	**−2.618**	−0.928	H_2O_2	BL
katG	Catalase hydrogen peroxidase 1	**−0.578**	−0.313	0.265	H_2O_2	AL
lamB	Maltose high-affinity uptake	**−4.881**	**−3.735**	**1.146**	PQ	NH
lldP	L-Lactate permease	**1.527**	0.219	**−1.307**	Sal	NL
lysU	Lysyl tRNA synthetase, inducible	**0.544**	−0.077	**−0.621**	Sal	AH
malE	Maltose-binding protein, periplasmic	**−3.425**	**−2.933**	**0.491**	PQ	NH
malK	Maltose transport complex, ATP-binding subunit	**−4.790**	**−3.748**	**1.041**	PQ	NH
manX	PTS family, mannose-specific enzyme IIA component	0.000	0.517	0.517	Sal	AL
map	Methionine aminopeptidase	**0.510**	**0.056**	**−0.454**	PQ	AH
marA	Multiple antibiotic resistance	**1.321**	**−0.516**	**−1.836**	PQ, Sal	AH
marB	Regulator for *mar*	**0.894**	−0.172	**−1.066**	Sal	AH
marR	Repressor of *mar*	0.352	−0.437	**−0.789**	Sal	AH
mdaB	Drug activity modulator	**0.444**	−0.223	−0.667	Sal	AH
murF	D-Alanyl:D-alanine adding to cell wall	0.216	−0.170	**−0.386**	PQ	AH
nfnB	Nitrofurantoin resistance; nitroreductase	**0.818**	−0.204	**−1.022**	PQ, Sal	AH
nuoI	NADH dehydrogenase I subunit	**0.792**	0.310	−0.481	PQ	NL
nuoK	NADH dehydrogenase I subunit	**0.766**	**0.460**	−0.306	PQ	NL
ompF	Outer membrane porin	**−2.834**	−0.892	**1.942**	PQ−	AL
ompT	Outer membrane protease VII	−0.815	**−1.204**	−0.389	Sal−	BL
pdhR	Repressor of *pdh*	**0.755**	−0.109	**−0.863**	PQ	AH
pepN	Aminopeptidase N	**0.671**	0.118	**−0.553**	Sal	NL
pflB	Pyruvate formate lyase I (anaerobic)	**0.812**	0.578	−0.234	Sal	NL
pgi	Glucose phosphate isomerase	**0.654**	0.177	**−0.477**	PQ	NL
ptsG	PTS family IIC, glucose specific	**0.841**	**0.554**	−0.287	PQ	NL
putA	Proline dehydrogenase	**−1.062**	−0.353	0.708	Sal	AL
pyrB	Aspartate transcarbamylase	0.133	0.608	0.475	PQ−, Sal−	BH
sdhB	Succinate dehydrogenase	**1.833**	0.329	**−1.505**	PQ	AH
tnaA	Tryptophanase	**−3.805**	0.223	**4.028**	H_2O_2	AL
treB	Tre-specific PTS enzyme II	**−3.156**	**0.739**	**3.895**	Sal−	AL
yahA	Putative repressor	**−0.667**	0.422	**1.089**	PQ−	AL
yaiA	Function unknown	**1.154**	0.416	**−0.738**	H_2O_2	NL
ybjC	Function unknown	0.463	−0.447	**−0.910**	PQ, Sal	AH
ycfR	Function unknown	0.537	−0.819	**−1.356**	H_2O_2	AH
yfiA	Stabilizes ribosome against dissociation	**−1.782**	0.581	**2.364**	PQ−, Sal, H_2O_2	AL
yggJ	Function unknown	**0.576**	−0.178	**−0.755**	Sal	AH
yqjD	Function unknown	**−0.347**	**0.378**	**0.725**	Sal	AL
yncE	Function unknown	**−0.476**	0.331	**0.808**	PQ, Sal	AL

[a] Oxidative response is based on data in references 54 and 84. Induction was by H_2O_2, paraquat (PQ), or sodium salicylate (Sal). Repression is indicated by minus sign (Sal−, PQ−).

[b] Values in boldface indicate significance ($\alpha = 0.001$).

[c] AH, Acid High; AL, Acid Low; BH, Base High; BL, Base Low; NH, Neutral High; NL, Neutral Low.

[d] GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

[e] PTS, phosphotransferase.

TABLE 6. Envelope and periplasmic genes

Gene	Function	Log_2 pH ratio[a]			Class[b]
		5/7	8.7/7	8.7/5	
artI	Periplasmic binding protein of Arg transport system	0.286	**0.655**	0.369	BH
artM	Arginine periplasmic binding protein	−0.115	0.293	**0.407**	BH
btuB	B-12 transporter, outer membrane receptor	**−0.667**	0.037	**0.704**	AL
cirA	Colicin I receptor production	**−0.885**	0.260	**1.145**	AL
clpX	ATPase subunit of ClpXP protease	−0.361	**−0.625**	−0.264	BL
cpxA	Periplasmic stress sensor (CpxAR)	**−0.461**	**0.230**	**0.691**	AL
cpxP	CpxAR-regulated periplasmic stress protein	**−3.232**	**1.002**	**4.234**	AL
cpxR	Periplasmic stress response regulator (CpxAR)	**−0.662**	**0.590**	**1.251**	AL
dsbA	Thiol:disulfide interchange, periplasmic	0.086	**1.117**	**1.031**	BH
dsbC	Disulfide bond isomerase, periplasmic chaperone	−0.288	**−0.534**	−0.246	BL
fadL	Fatty acid transport, outer membrane	−0.861	**1.069**	**1.931**	BH
fecA	Outer membrane ferric citrate receptor	**−2.608**	**−1.128**	**1.480**	NH
fecB	Periplasmic ferric citrate-binding protein	**−2.842**	**−1.538**	**1.304**	NH
fepA	Ferrienterobactin outer membrane receptor	**−0.966**	−0.289	**0.676**	NH
fhuD	Ferric hydroxamate binding protein; hydroxamate-dependent iron uptake	**−0.767**	−0.180	**0.587**	AL
fliY	Cystine-binding protein, periplasmic	0.233	−0.252	**−0.484**	AH
hdeA	Acid periplasmic chaperone	0.841	−0.326	**−1.167**	AH
hdeB	Acid periplasmic protein	0.782	−0.622	**−1.404**	AH
hisJ	High-affinity histidine-binding protein	−0.149	**0.400**	**0.549**	BH
hlpA	Periplasmic chaperone for OMPs[c]	0.099	**−0.661**	**−0.759**	BL
lamB	Maltoporin, maltose high-affinity uptake; phage lambda receptor	**−4.881**	**−3.735**	**1.146**	NH
lon	DNA-binding, ATP-dependent protease	−0.600	**−1.666**	**−1.065**	BL
malE	Maltose-binding protein, periplasmic	**−3.425**	**−2.933**	0.491	NH
malM	Maltose operon periplasmic protein	**−4.643**	**−3.780**	**0.863**	NH
mltB	Membrane-bound murein hydrolase	**−0.739**	−0.419	0.320	NH
nmpC	Outer membrane	**−4.012**	−0.051	**3.961**	AL
ompF	Outer membrane porin protein 1a	**−2.834**	−0.892	**1.942**	AL
ompT	Outer membrane protease VII	−0.815	**−1.204**	−0.389	BL
ompX	OMP, induces RNAP-sigma E	**1.523**	0.439	**−1.083**	NL
oppA	Periplasmic oligopeptide binding protein	**1.358**	0.406	**−0.953**	NL
pal	Lipoprotein associated with peptidoglycan	**0.532**	0.379	−0.153	NL
potD	Spermidine-binding membrane protein; regulates *pot*	0.053	**0.509**	**0.456**	BH
ppiA	Rotamase; peptidylprolyl-*cis-trans*-isomerase A	**−0.526**	**0.231**	**0.757**	AL
pstS	High-affinity, periplasmic phosphate binding protein	**−2.479**	**−2.262**	0.217	NH
rbsB	D-Ribose binding protein, periplasmic	−0.749	−1.150	−0.401	BL
rseB	Periplasmic, binds RseA; enhances RpoE-RseA cytoplasmic complex formation	**0.631**	−0.105	**−0.736**	AH
secD	SecDF-YajC inner membrane secretion complex	−0.165	0.287	**0.452**	AL
surA	Periplasmic outer membrane porin chaperone, stationary phase	**0.310**	0.209	−0.101	NL
tatA	Twin arginine translocation	0.043	**0.679**	**−0.636**	BH
tatB	Twin arginine translocation	0.075	0.446	0.372	BH
tolB	Group A colicin uptake and tolerance	0.498	0.421	−0.077	NL
tpX	Thiol peroxidase, antioxidant	0.637	0.178	**−0.459**	NL
tsx	Phage T6, colicin K resistance; nucleoside channel	**−0.707**	**−0.904**	−0.196	BL
ybgF	TolA-binding periplasmic protein	**0.312**	−0.007	**−0.318**	AH
yceI	Function unknown; periplasmic protein	−0.036	**1.038**	**1.074**	BH
yhcN	Periplasmic protein	**3.064**	**−1.136**	**−4.199**	AH

[a] Values in boldface indicate significance ($\alpha = 0.001$).
[b] AH, Acid High; AL, Acid Low; BH, Base High; BL, Base Low; NH, Neutral High; NL, Neutral Low.
[c] OMPs, outer membrane proteins.
[d] RNAP, RNA polymerase.

(4, 68), as are deaminases such as *sdaA* (82). In preliminary experiments, we have repeated our microarray study on cultures grown anaerobically. Under anaerobiosis, several amino acid decarboxylases and deaminases show pH-dependent expression (Hayes and Slonczewski, unpublished).

Stress responses. Several stress responses are known to interact with pH stress and pH resistance, including oxidative stress, heat shock, and envelope stress (for reviews see references 21 and 68). The overlap with salicylate stress could be explained in part by salicylate's effect as a permeant acid, stressing internal pH (60). The *mar* drug resistance operon is known to be coinduced by aromatic permeant acids and low pH (69) under regulation by MarR as well as by the superoxide regulator SoxRA (57).

Beyond salicylate, however, a large number of oxidative stress genes inducible by H_2O_2 or by paraquat showed significant pH-dependent expression, nearly all induced by acid or repressed by base. This finding confirms our hypothesis of a strong connection between acid stress and oxidative stress. Since so much of aerobic respiration is stepped up at pH 5, including cytochrome *o* oxidase, it is likely that acid conditions accelerate the production of oxygen radicals, thus inducing a partial oxidative stress response.

Various envelope and periplasmic stress responses are induced by acid, contributing to acid resistance; the best characterized in terms of mechanism is the acid-induced periplasmic chaperone HdeA (23). Extracellular acid induces a dimer-to-monomer transition in HdeA, which then suppresses aggrega-

TABLE 7. Universal stress and heat shock response genes

Gene	Function	Log_2 pH ratio[a]			Class[b]
		5/7	8.7/7	8.7/5	
ahpC	Alkyl hydroperoxide reductase	**1.003**	0.436	**−0.568**	NL
ahpF	NAD(P)H:peroxiredoxin oxidoreductase	**0.777**	−0.222	**−0.999**	AH
cfa	Cyclopropane fatty acid synthase; acid resistance in stationary phase	**2.075**	−0.480	**−2.555**	AH
clpB	ClpB protease, ATP-dependent chaperone	−0.219	**−1.963**	**−1.744**	BL
cysK	Cysteine synthase, *o*-acetylserine sulfhydrylase A	**1.204**	**1.351**	0.147	NL
cysZ	Unknown function	**0.440**	0.212	−0.228	NL
dinI	Inhibits RecA coprotease	0.132	**0.568**	0.436	BH
dinJ	Induced by DNA damage	0.683	**1.192**	0.508	BH
dnaJ	DnaK cochaperone	−0.908	**−2.155**	**−1.247**	BL
dnaK	HSP-70-type molecular chaperone	−0.894	**−2.249**	−1.356	BL
dps	Stress response DNA-binding protein	**1.130**	0.105	**−1.025**	NL
grpE	Nucleotide exchange factor for DnaKJ	−0.491	**−1.141**	**−0.650**	BL
grxA	Glutaredoxin 1	**1.666**	−0.106	**−1.772**	AH
hdeA	Acid periplasmic chaperone	0.841	−0.326	**−1.167**	AH
hdeB	Acid periplasmic chaperone	0.782	−0.622	**−1.404**	AH
hslJ	Heat-inducible novobiocin resistance	0.368	−0.676	**−1.044**	AH
hslU	Heat-inducible ATP-dependent protease	−0.745	**−1.688**	−0.946	BL
hslV	Heat-inducible ATP-dependent protease	−1.125	**−1.913**	−0.788	BL
ibpB	Heat-inducible chaperone, HSP20 family	−1.691	**−2.618**	−0.928	BL
hslO	Hsp33, cytoplasmic heat shock chaperone activated by disulfide bond formation	**−1.453**	**−1.737**	−0.284	BL
hslR (*yrfH*)	Hsp15, heat shock, binds RNA and DNA	**−1.773**	**−1.940**	−0.168	NH
katG	Catalase-hydrogen peroxidase I	**−0.578**	−0.313	0.265	AL
rpoE	RNAP[c] sigma E, envelope heat stress	0.310	−0.427	**−0.738**	AH
rpoH	RNAP sigma 32, heat shock regulons	−0.378	0.084	**0.462**	AL
rseA	Anti-RpoE sigma factor, spans inner membrane	**0.419**	−0.159	**−0.578**	AH
rseB	Binds periplasmic domain of anti-RpoE sigma RseA	**0.631**	−0.105	**−0.736**	AH
sodB	Superoxide dismutase, Fe; acid inducible	**0.752**	0.280	−0.472	NL
ycdB	Function unknown, peroxidase homolog	**0.654**	−0.329	**−0.983**	AH
ycdO	Acid inducible, function unknown	**0.842**	**−0.625**	**−1.467**	AH
uspD (*yiiT*)	UV resistance	**1.333**	−0.497	**−1.830**	AH

[a] Values in boldface indicate significance ($\alpha = 0.001$).
[b] AH, Acid High; AL, Acid Low; BH, Base High; BL, Base Low; NH, Neutral High; NL, Neutral Low.
[c] RNAP, RNA polymerase.

tion by acid-denatured proteins. Our study reveals additional potential contributors to acid resistance and base resistance, including genes of unknown function such as *yhcN*, induced by acid, and *yceI*, induced by base.

Our study presents the most comprehensive picture to date of acid and base response by *E. coli* grown aerobically in complex medium. Overall, low pH accelerates acid consumption and proton export, while coinducing oxidative stress, possibly through increased production of oxygen radicals. High pH accelerates proton import while repressing the energy-expensive systems of flagellar biosynthesis and chemotaxis. Finally, pH differentially regulates a large number of periplasmic and envelope stress systems, as well as transporters, chaperones, and redox regulators.

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