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# NOTES

# Nucleotide Sequence of the Gene Encoding the Repressor for the Histidine Utilization Genes of *Klebsiella aerogenes*

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**The *hutC* gene of *Klebsiella aerogenes* encodes a repressor that regulates expression of the histidine utilization (*hut*) operons. The DNA sequence of a region known to contain *hutC* was determined and shown to contain two long rightward-reading open reading frames (ORFs). One of these ORFs was identified as the 3′ portion of the *hutG* gene. The other ORF was the *hutC* gene. The repressor predicted from the *hutC* sequence contained a helix-turn-helix motif strongly similar to that seen in other DNA-binding proteins, such as *lac* repressor and the catabolite gene activator protein. This motif was located in the N-terminal portion of the protein, and this portion of the protein seemed to be sufficient to allow repression of the *hutUH* operon but insufficient to allow interaction with the inducer. The presence of a promoterlike sequence and a ribosome-binding site immediately upstream of the *hutC* gene explained the earlier observation that *hutC* can be transcribed independently of the other *hut* operon genes. The predicted amino acid sequence of *hut* repressor strongly resembled that of the corresponding protein from *Pseudomonas putida* (S. L. Allison and A. T. Phillips, J. Bacteriol. 172:5470–5476, 1990). An unexpected, leftward-reading ORF extending from about the middle of *hutC* into the preceding (*hutG*) gene was also detected. The deduced amino acid sequence of this leftward ORF was quite distinct from that of an unexpected ORF of similar size found immediately downstream of the *P. putida hutC* gene. The nonstandard codon usage of this leftward ORF and the expression of repressor activity from plasmids with deletions in this region made it unlikely that this ORF was necessary for repressor activity.**

The enteric bacterium *Klebsiella aerogenes* has the ability to use the amino acid histidine as its sole source of carbon, nitrogen, or both (12). The histidine utilization (*hut*) genes encoding this catabolic potential lie clustered on the *K. aerogenes* chromosome between *gal* and *bio* (7), in roughly the site occupied by *att*$^{\lambda}$ in the related species *Escherichia coli* (which lacks *hut* genes). The genes are arranged in the order *hut(M)IGC(P)UH*, where *hutH*, *hutU*, *hutI*, and *hutG* encode the four enzymes that catabolize histidine to ammonia, glutamate, and formamide; *hut(M)* is the region needed to control expression from *hutIp*; and *hut(P)* is the region needed to control expression from *hutUp* and from an oppositely directed promoter, Pc, of unknown function (15). The *hut*-specific repressor, encoded by *hutC*, blocks expression of all the *hut* genes in the absence of inducer (12).

The *hutU* and *hutH* genes are transcribed as an operon (A. Schwacha, J. A. Cohen, K. B. Gehring, and R. A. Bender, submitted for publication). The histidase encoded by *hutH* and urocanase encoded by *hutU* are responsible for the synthesis and degradation of the physiological inducer (urocanate) from exogenously supplied histidine (12). Although urocanate is normally generated by metabolism of histidine, *K. aerogenes* also has an inducible urocanate permease, allowing direct induction by urocanate (12). The capacity of this permease is sufficient to allow growth with urocanate as the sole carbon or nitrogen source, but such growth is slower than that seen with histidine, suggesting that the activity of urocanate permease is rather modest (12). The products of the *hutI* and *hutG* genes of *K. aerogenes*, imidazolone proprionate hydrolase and formiminoglutamate hydrolase, respectively, convert the toxic intermediate imidazolone proprionate (produced by urocanase) to glutamate and formamide (12).

In *Salmonella typhimurium*, *hutI*, *hutG*, and *hutC* form a single *hutIGC* operon (12). In *K. aerogenes*, *hutC* is transcribed independently of *hutI* and *hutG* (Schwacha et al., submitted). Nevertheless, there is considerable similarity between the *hut* genes of *K. aerogenes* and those of *S. typhimurium*; a continuous heteroduplex can be formed across the entire length of *hut*, with one strand encoding the *S. typhimurium hut* and the other strand encoding the *K. aerogenes hut* (3). Thus, the independent transcription of the *K. aerogenes hutC* gene was unexpected (Schwacha et al., submitted) and required further investigation.

**DNA sequence of *hutC*.** P1-mediated generalized transduction had shown that *hutC* lies within the *hut* gene cluster between *hutG* and *hutU* (7). Deletion analysis of the cloned *hut* genes had established that *hutC* lies between map units 2.4 and 3.5 on the physical map of *hut* (4). Finally, insertion mutagenesis with the transposon Tn*1000* (γδ) had established that *hutC* lies between 2.6 and 3.5 map units (Schwacha et al., submitted), where map units are measured in kilobases (kb) from the left end [corresponding to the *hut(M)* region] of the *hut* DNA, cloned as a *Hin*dIII fragment in plasmid pCB101 (5). A set of subclones derived directly or indirectly from this region of pCB101 was obtained to facilitate analysis of the *hutC* region. The fragment of *hut* DNA present in each clone is shown in Fig. 1.

Plasmids pJAC1, pJAC2, pAS3, and pAS4 were constructed by ligation of *hutC*-containing fragments from pCB101 (4) in either orientation into the *Pst*I site of pUC8

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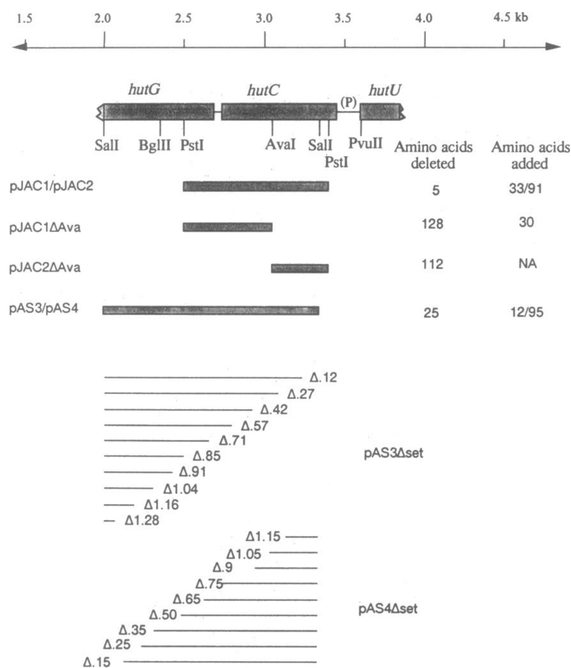


FIG. 1. *hutC* region of *K. aerogenes hut* DNA. The top line represents the map coordinates (in kb measured from the *Hin*dIII site at the left end of the operon) of the standard restriction map of *hut* (5). The second line shows a partial restriction map of the region, with sites relevant to this discussion aligned with the location of the structural genes and the *hut(P)* region (15). The shaded bars in the four lines beneath the restriction map illustrate the DNA cloned in each of the plasmids listed at the left. Where two entries are separated by a slash, the fragment was cloned in both orientations. The amino acids deleted from the C-terminal end of *hut* repressor are indicated, and the number of amino acids fused to the C-terminal end as a result of the cloning and deletion are also shown. (NA, not applicable; the deletion removes not only five amino acids at the C-terminus but also 112 amino acids at the N-terminus and all the expression signals for *hutC* expression.) The lower two sets of bars indicate the DNA remaining in the deletion plasmids used for determining the DNA sequence.

(for pJAC1 and pJAC2) or the *Sal*I site of pUC18 (for pAS3 and pAS4). The pUC18 vector used here carried the frameshift mutation in the *lacZ* alpha peptide (11). Deletions of pJAC1 and pJAC2 were made by cleavage with *Ava*I and religation under dilute conditions. Unidirectional deletions in pAS3 and pAS4 were made by the method of Hennikoff (9) with the unique *Xba*I (5′ overhang, susceptible to exonuclease III digestion) and *Sac*I (3′ overhang, resistant to exonuclease III) sites in the polylinker as entry sites for exonuclease III. The complete DNA sequence of both strands was determined by the dideoxy method with either reverse transcriptase (2) or modified T7 DNA polymerase (18) and double-stranded supercoiled DNA as the template.

The sequence of the 1,380-base-pair (bp) *Sal*I fragment and the adjacent *Sal*I-*Pvu*II fragment (previously reported in reference 14) gave a total size of 1,625 bp and spanned the region from map positions 2.0 to 3.6. The *Sal*I site at position 2.0 is known to lie within *hutG* (4), and the *Pvu*II site at position 3.6 is known to lie within the *hutU* gene (Schwacha et al., submitted), thus bracketing the *hutC* gene completely. The region contained two extensive, rightward-reading open reading frames (ORFs). One began at the left end of the fragment (i.e., within *hutG*) and extended to bp 666. This ORF presumably encodes the 3′ portion of the *hutG* gene. The other ORF extended from bp 731 to 1453, immediately adjacent to the *hut(P)* region. This second ORF was tentatively assigned to *hutC*.

**Features of the DNA sequence.** At the 5′ end of *hutC* there was a sequence with strong similarity to known promoter sequences (bp 657 to 685, marked by overlining in Fig. 2). According to Mulligan et al. (13), this would probably be a strong promoter, since its homology score was 52, similar to that of the promoter of the *bla* gene (encoding β-lactamase) in pBR322. About 25 bp downstream of the putative start of transcription, there was a sequence strongly homologous to the Shine-Dalgarno sequence, followed closely by an ATG codon. The presence of a putative promoter just upstream of the putative translation initiation signal suggests that the *hutC* gene is transcribed from its own promoter.

At the 3′ end of *hutC*, 10 bp downstream from the terminator TGA codon, there was a potential stem-loop structure with 10 bases in each half of the putative stem and a 7-base loop (bp 1466 to 1492). This structure was followed by an A+T-rich region. The stem-loop structure was initially detected because of anomalous mobility of single-stranded DNA in sequencing gels (14) in the presence of high concentrations of urea and is thus assumed to be significant. This structure might represent the signal to terminate *hutC* transcription.

The *hutC* nucleotide sequence predicts a protein of 241 amino acids with a molecular weight of 27,218. Codon usage analysis (8) showed that *hutC* and the 3′ region of *hutG* had codon usage typical for *E. coli* over the predicted coding regions, and the frequency of unusual codons increased as one entered a predicted noncoding area. The deduced amino acid composition of *hut* repressor was also quite similar to that of an average *E. coli* protein with few exceptions. The predicted *hutC* product had about threefold less asparagine and lysine and about threefold more histidine than average, with the lower lysine content matched by a slightly higher arginine content. Although these deviations from a statistical mean may not be significant in themselves, it is interesting to note two unusual, nitrogen-rich sequences: Arg-His-His-Gln-His-Arg near the middle of the protein (bp 1022 to 1039), and Arg-Arg-Arg near the C-terminus (bp 1361 to 1369). The unusual Arg-Arg-Arg sequence lies in the middle of a stretch of 11 amino acids exactly conserved between *P. putida* (1) and *K. aerogenes* (Pro-Cys-Leu-Leu-Ile-Arg-Arg-Arg-Thr-Trp-Ser). It is tempting to speculate that this sequence may be important either for urocanate binding or for subunit interactions, but we have no data bearing on these questions. The local hydrophobicity analysis, determined by the method of Kyte and Doolittle (10), shows no evidence of membrane association. A Chou-Fasman secondary-structure analysis (6) predicts about eight regions of α-helix, composing 43% of the sequence.

Two adjacent helices near the amino terminus showed strong similarity to the helix-turn-helix motif found in many DNA-binding proteins (16). Figure 3 shows this region and the analogous regions of two well-characterized DNA-binding proteins from *E. coli* and the *hutC* gene product from *P. putida* (1). There was very strong similarity between the *hutC* protein sequences from *K. aerogenes* and *P. putida* in this region and complete identity of the second helix in the helix-turn-helix motif, suggesting that these two proteins may bind rather similar DNA sites. The promoter region of the *K. aerogenes hutUH* operon (tightly regulated by *hutC* products) contained the sequence CTTGTATAGACAAG between the −10 and −35 regions of the promoter (bp 1540

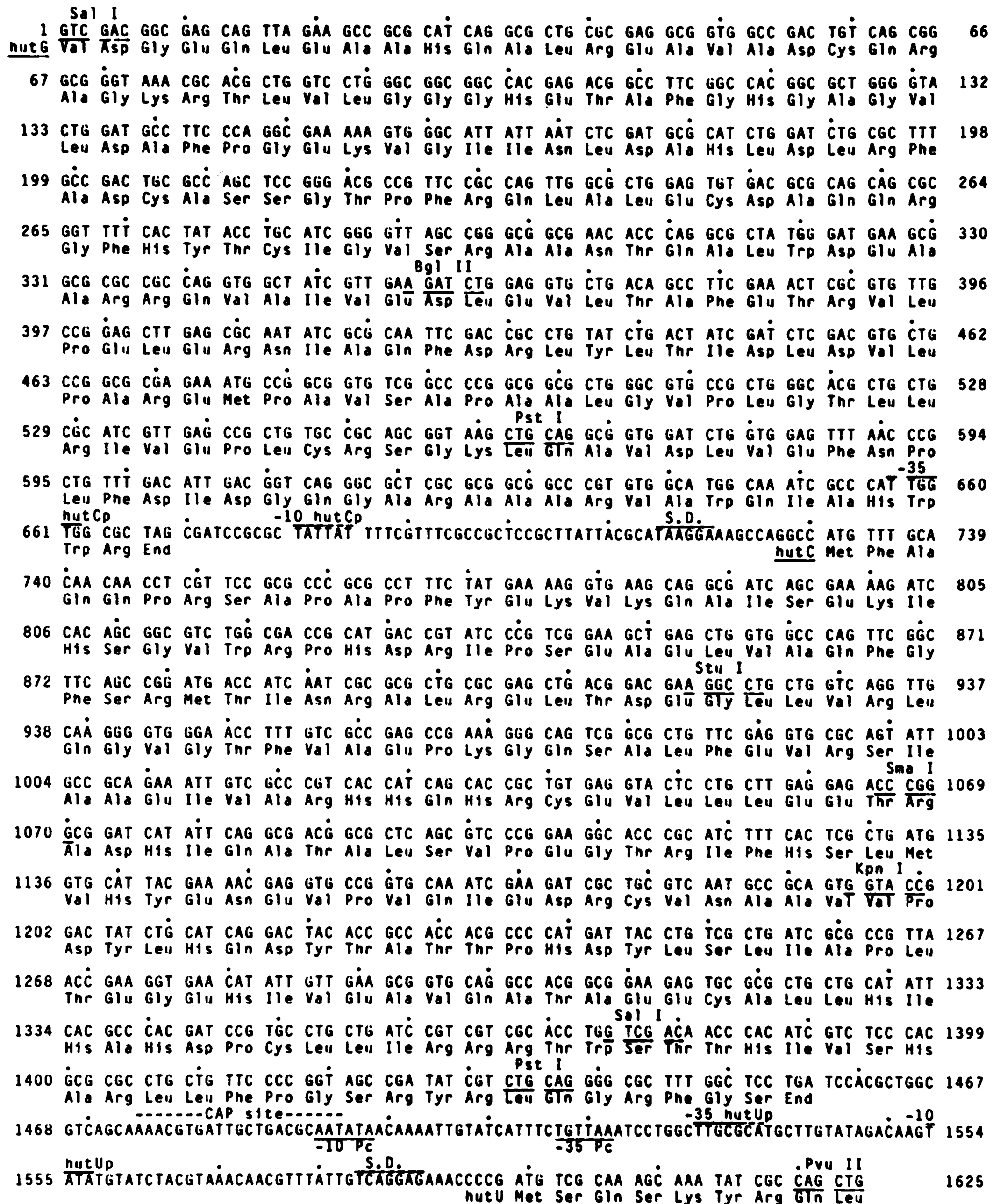

FIG. 2. DNA sequence of the *hutC* region from *K. aerogenes* (GenBank accession number M34604). The DNA sequence of a fragment from the *Sal*I site at map position 2.0 to the *Pvu*II site at position 3.6 was determined. Two long ORFs are translated, and their predicted amino acid sequences are shown. The first, encoded by bases 1 to 666, corresponds to the C-terminal portion of the *hutG* product. The *hutC* product (repressor) is encoded by bases 731 to 1453. The coding sequence for the *hutU* product (urocanase) begins at base 1599. Transcriptional and translational control signals are overlined. Restriction sites are underlined. The region from bases 1375 to 1625 was presented previously (14). S.D., Shine-Dalgarno sequence. CAP, Catabolite gene activation protein. Pc, A leftward-oriented promoter of unknown function (15).

to 1553 in Fig. 2). This sequence may be the *K. aerogenes hut* operator, since it was virtually identical to the sequence CTTGTACATACAAG, known to bind *P. putida* repressor (1). Expression of the *P. putida hutC* gene may be autogenously regulated, since an apparent operator site overlaps the putative promoter of *P. putida hutC* (1). A reasonable match to the left half of this sequence (CTTATTA) at bp 704 to 710 lay at +13 to +19 relative to the putative start site of transcription of the *K. aerogenes hutC* gene. The significance of this sequence is unknown, but it may represent a further similarity to the *P. putida hutC* gene.

There was also considerable similarity between the helix-turn-helix region of the *hutC* product from *K. aerogenes* and the DNA-binding domains of the *crp* and *lacI* gene products from *E. coli*, especially in the right (i.e., more C-terminal) helices of the three proteins, the helix thought to be most

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ka | *hutC* | 34 | asp | arg | ile | pro | ser | glu | ala | glu | leu | val | ala | gln | phe | gly | phe | ser | arg | met | thr | ile | asn | arg | ala | leu | arg | glu | leu | thr |
| Pp | *hutC* | 41 | his | arg | val | pro | ser | glu | ser | glu | leu | val | asn | glu | leu | gly | phe | ser | arg | met | thr | ile | asn | arg | ala | leu | arg | glu | leu | thr |
| Ec | *lacI* | 4 | --- | pro | val | --- | thr | leu | tyr | asp | val | ala | glu | tyr | ala | gly | val | ser | tyr | gln | thr | val | ser | arg | val | val | asn | gln | ala | ser |
| Ec | *crp* | 165 | ile | lys | ile | --- | thr | arg | gln | glu | ile | gly | gln | ile | val | gly | cys | ser | arg | glu | thr | val | gly | arg | ile | leu | lys | met | leu | glu |

FIG. 3. Deduced amino acid sequence of the helix-turn-helix region of *hut* repressor. The amino acid sequence of the *K. aerogenes* (Ka) *hut* repressor helix-turn-helix region was aligned with the known DNA-binding domains of the *lac* repressor protein (*lacI* product) and the catabolite gene activator protein (*crp* gene product) from *E. coli* (Ec) as well as the predicted amino acid sequence of the *P. putida* (Pp) *hut* repressor (*hutC* product). Dark boxes surrounding amino acids indicate identities; light boxes indicate conservative substitutions. The numbers at the left indicate the residue number of the first amino acid shown.

involved in DNA sequence recognition. Since many bacterial operators share a common core sequence (R. Osuna and R. A. Bender, unpublished data), the similarities among *hutC*, *lacI*, and *crp* (as well as *trpR* [1]) may not be surprising.

A protein homology search of the Protein Identification Resource (PIR) database produced only one sequence of significant homology to *hutC*, the *E. coli* protein A sequence (accession no. A30263). This gene (whose function in unknown) is located upstream of the genes for pyruvate dehydrogenase and encodes a protein similar in size to *hutC* (17). The predicted amino acid sequences of the N-terminal regions of both proteins (amino acids 13 to 77 in *hutC* and amino acids 15 to 79 in protein A) have an identity of 38% and a similarity of 53% if conservative changes of amino acids are counted. This region corresponds to the helix-turn-helix region of *hutC*, suggesting that protein A might be a DNA-binding protein.

**Repressor activity and *hutC* deletions.** Previous data have shown that the *hutC* gene, encoding repressor, is located entirely within a DNA segment bounded by *Bgl*II restriction sites at map positions 2.35 and 5.45 (4). Moreover, when this *Bgl*II fragment, cloned in pBR322, was tested for complementation against the *hutC515* allele, the fragment was fully capable of providing repressor (Schwacha et al., submitted). We therefore tested the ability of smaller DNA fragments from the *hutC* region to complement a *hutC* mutation (Table 1). Plasmids pJAC1 and pJAC2 contain the entire *hutC* ORF except for the last five amino acids at the C-terminus. In pJAC1, these are replaced with 33 amino acids encoded by the cloning vector (leftward through the *lac* promoter and beyond). In pJAC2, the five deleted amino acids are replaced with 91 amino acids from the alpha-peptide (Fig. 1). Although both pJAC1 and pJAC2 encoded an activity capable of repressing the chromosomal copy of *hut*, neither allowed induction by histidine. The failure of induction may result from loss of the inducer-binding site in these deleted repressors or from overproduction of a fully active repressor at a level where the formation of the physiological inducer (urocanic acid) from histidine is insufficient to inactivate all the accumulated repressor.

TABLE 1. Complementation of the *hutC515* mutation by cloned DNA fragments from the *hutC* region[a]

| Chromosome | Plasmid | Histidase sp act (U/mg) | |
|---|---|---|---|
| | | No inducer | With inducer |
| *hutC*$^+$ | None | 3 | 48 |
| *hutC515* | None | 38 | 58 |
| | pJAC1 | ≤5 | ≤4 |
| | pJAC2 | ≤3 | ≤3 |
| | pJAC1ΔAva | 12 | 25 |
| | pJAC2ΔAva | 54 | 64 |
| | pAS4 | 23 | 25 |

[a] The *hutC515* mutant strains carrying the plasmids indicated were grown as described before (4). Inducibility of histidase formation was monitored as an indication of *hut* repressor activity. The *hutC515* and *hutC*$^+$ strains both carry the *recA3011* allele and are otherwise isogenic. Inducer (0.2% [wt/vol] histidine) was added as indicated.

Plasmids pAS4 and pJAC1ΔAva had more material removed from the C-terminus (25 and 128 amino acids, respectively) and replaced with 95 and 30 amino acids, respectively, from vector sequences. Both plasmids conferred little if any *hutC* activity despite their high copy number and little or no response to inducer. Plasmid pJAC2ΔAva lacks the entire N-terminal half (112 amino acids) of *hutC* and was entirely lacking in complementation activity. These deletions are consistent with the idea that the DNA-binding domain of the *hut* repressor lies in the N-terminal portion, which includes the helix-turn-helix motif.

Several lines of evidence argue that the *hutC* ORF does indeed encode the *hut* repressor. (i) The predicted protein product bears a striking similarity to the *hutC* gene product from the distantly related bacterium *P. putida* (1), with about 60% of the deduced protein sequence being identical. (ii) All subclones containing at least the 5′ region (encoding the first 112 amino acids form *hutC*) are able to encode a product that represses *hutUH* to a greater or lesser extent. (iii) An in-frame translational fusion (pJAC2) between *hutC* and the *lacZ* alpha-peptide of pUC8 (replacing the last five amino acids of *hutC* with *lacZ* alpha peptide, beginning at its 15th codon) results in expression of alpha-complementation activity (data not shown). This fusion demonstrates that there is rightward transcription and translation coming from *hutC* in vivo. Note that this fusion protein retained both alpha-complementation activity and *hut* repression activity but lost inducibility (Table 1).

It is clear that *hutC* can be expressed independently of the *hutIGC* cluster (Schwacha et al., submitted). The presence of sequences that resemble a good transcription initiation site and a good translation initiation site just upstream of *hutC* is thus not surprising. The question of whether *hutC* is also part of a *hutIGC* operon is more difficult to answer. Sixty-one base pairs separate the stop codon of *hutG* from the presumed initiation codon of *hutC*, rather long for an intergenic region in a standard operon. Moreover, the presence of a potential stem-loop structure (bp 667 to 681) followed by a T-rich run may indicate the presence of a transcriptional terminator (or attenuator) immediately following the coding sequence of *hutG*. Thus, it is possible that *hutC* is not a member of a *hutIGC* operon, as is found in the related organism *S. typhimurium*. Alternatively, the *K. aerogenes hutC* gene may be expressed both independently and as a member of a *hutIGC* operon, perhaps with an attenuation between *hutIG* and *hutC*.

Close inspection of this sequence reveals four ORFs

greater than 100 amino acids in length in the *hutC* region extending in the opposite direction. From codon usage predictions, none of these ORFs would be predicted to encode a protein. Examination of the 5′ areas preceding each ORF reveals extremely weak or nonexistent promoters and Shine-Dalgarno homologies. The largest ORF (ORFB, encoded by nucleotides 1240 to 590) lies about 250 bp away from the nearest promoter sequence, Pc at bp 1522 to 1493 (15). The DNA fragments in plasmids pJAC1 and pJAC2 would have left this ORF intact but removed the promoter. The observation that these plasmids still expressed repression activity but an activity that could not respond to inducer is inconsistent with ORFBs encoding *hut* repressor. Although it is tempting to dismiss this ORF, it must be noted that the *P. putida hutC* region also contains an extra ORF (1). In *P. putida*, the ORF is immediately downstream of *hutC* (1) and is of about the same size as ORFB. The predicted amino acid sequences deduced for the two ORFs are quite different. We have no evidence for a product from ORFB and no phenotype that can be associated with its presence or absence.

In summary, a region known to contain *hutC* contains a sequence sufficient to encode a 27,218-dalton protein that shows similarity to other bacterial DNA-binding proteins. Deletions removing various portions of this sequence suggest that the amino-terminal portion of the repressor may be sufficient for at least some DNA binding. The sequence of *hutC* from *K. aerogenes* is remarkably similar to that of *hutC* from *P. putida* even to the maintenance of a high G+C content.

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