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Genetic and Physical Maps of *Klebsiella aerogenes* Genes for Histidine Utilization (*hut*)

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Summary. Deletion derivatives of the *hut*-containing plasmid pCB101 were tested against point mutants defective in individual genes of the histidine utilization (*hut*) operons using a complementation/recombination assay. Location of the genes of the right operon, *hutU* and *hutH*, was confirmed by direct assay of the gene products, urocanase and histidase; location of the repressor gene was identified by measuring the ability of the plasmid-carried genes to repress the formation of histidase from a chromosomal location. The analysis of eight deletion plasmids unambiguously confirms the map order of the *hut* genes as *hutI-G-C-U-H*, and demonstrates that, in *Klebsiella aerogenes*, the *hutU* and *hutH* genes are transcribed from their own promoter. In addition, the genetic map of *hut* can be aligned with the restriction map of the *hut* DNA in plasmid pCB101. One of the deletion plasmids studied apparently encodes a defective histidase subunit that is trans-dominant to active histidase. Another deletion, which completely removes the left operon, *hutIG*, allows high level expression of the *hutUH* operon and thus overproduction of a toxic intermediate.

Introduction

The amino acid histidine can be degraded by *Klebsiella aerogenes* in a sequence of four enzymatic reactions (reviewed by Magasanik 1978). Histidine is first deaminated by histidase (the product of the *hutH* gene) to urocanic acid, the physiological inducer of the entire degradative system. Urocanase (encoded by *hutU*) then converts urocanic acid to imidazolone propionate, a toxic intermediate. Imidazolone propionate hydrolase (encoded by *hutI*) then cleaves the ring to yield formimino glutamic acid, which is further cleaved by formiminoglutamate hydrolase (encoded by *hutG*) to give glutamic acid, a potentially useable carbon and nitrogen source, and formamide, which is excreted. The histidine utilization (*hut*) genes are clustered on the *K. aerogenes* chromosome in a region between *gal* and *bio* (Goldberg and Magasanik 1975). The *hut* genes are absent from *Escherichia coli* but are found in the *gal-bio* region in *Salmonella typhimurium* where they are arranged as two adjacent operons.

The *hut* operons of *K. aerogenes* provide an attractive model system to study regulation of gene expression in bacteria (Magasanik 1978). Expression of *hut* genes is controlled at many levels. (1) The *hutC* gene product is a regulated repressor whose natural inducer, urocanate, is produced by the first enzyme of the pathway, histidase, and is destroyed by the second enzyme, urocanase, (Schlesinger et al. 1965; Prival and Magasanik 1971) (2) *hut* expression requires a positive activator, either CAP+cAMP in response to carbon limited growth, or an unknown factor(s) in response to nitrogen-limited growth (Neidhardt and Magasanik 1957; Prival and Magasanik 1971), and (3) *hut* expression further requires the presence of an exogenous electron acceptor (Goldberg and Hanau 1980). All these effects are at the transcriptional level and their targets seem to lie in the operator-promoter regions.

In the preceding paper (Boylan et al. 1983), we described the restriction endonuclease cleavage map of the *hut* region from *K. aerogenes* and the isolation of a set of deletions removing various segments of this DNA. In the present paper, we use these deletions to determine the map order of the structural genes, confirming the order deduced from transductional analysis (Goldberg and Magasanik 1975). We also show that the *K. aerogenes hut* cluster, like that in *S. typhimurium*, is transcribed in the direction from *hutI* to *hutH*, and is arranged into at least two independent transcription units with *hutUH* expressed even in the absence of the entire *hutIG* operon. The results presented here and in the accompanying paper (Boylan et al. 1983) allow us to identify the location of *hutP*, the promoter region of the *hutUH* operon, for our studies of regulated *in vitro* transcription using components purified from *K. aerogenes*.

Materials and Methods

Bacteria and Growth Media. The strains used in this study are listed in Table 1. Minimal medium contained W salts (Prival and Magasanik 1971), carbon sources at 0.4%, and nitrogen sources at 0.2%. When histidine or urocanate was sole carbon and nitrogen source, it was added to 0.4%. A solution of glutamine (Calbiochem grade A) was freshly prepared before addition to medium. Inducer (histidine or urocanate) was present at 0.2%. Plasmid-containing strains were grown in the presence of ampicillin at 25 μg/ml (*E. coli*) or 500 μg/ml (*K. aerogenes*).

Transformations. Competent cells were prepared and transformed with plasmid DNA as described by Lederberg and Cohen (1974) and transformants were selected on LB plates

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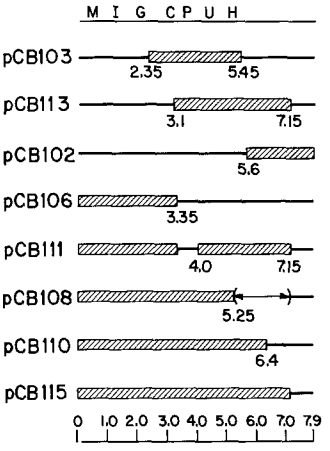
Table 1. Bacterial strains employed

Strain	Genotype	Origin
Escherichia coli		
EB649	*hsr, lac, gal, rpsL594* (pBR322)	transformation of EG47
EB841	*hss-1, thi-1, lacY1, tonA21, supE44* (pCB106)	transformation of RH202
EB890	*hss-1, thi-1, lacY1, tonA21, supE44* (*pCB102*)	transformation of RH202
EB896	*hss-1, thi-1, lacY1, tonA21, supE44*, (pCB101)	transformation of RH202
EG47	*hsr lac gal rpsL594*	(Goldberg et al. 1974)
RH202	*hss-1, thi-1, lacY1, tonA21, supE44*	(Adams et al. 1979)
Klebsiella aerogenes		
KB897	*hutC516, hutI519*, (pCB102)	transformation of KG2005
KB899	*hutC516, hutI519*, (pCB113)	transformation of KG2005
KB936	*hutC516, hutI519*, (pCB101)	transformation of KG2005
KB937	*hutC516, hutI519*, (pCB103)	transformation of KG2005
KB965	*hutC516, hutI519*, (pBR322)	transformation of KG2005
KG2000	*hut*$^+$	(Goldberg and Magasanik 1975)
KG2003	*hutC516, hutH518*	(Goldberg and Magasanik 1975)
KG2005	*hutC516, hutI519*	(Goldberg and Magasanik 1975)
KG2007	*hutC517, hutU520*	(Goldberg and Magasanik 1975)
KG2010	*hutG504*	(Goldberg and Magasanik 1975)

supplemented with ampicillin. Transformation of *K. aerogenes* has not been described previously and the frequency of transformants seen with *K. aerogenes* strain was quite similar to that seen with *E. coli*. To test for complementation/recombination, five colonies from the LB ampicillin selection plate were picked with sterile toothpicks and patched onto minimal medium plates with histidine or urocanate as sole carbon source. A solid patch for each of the colonies indicated complementation, growth of 5–35 colonies in each of the five patches indicated recombination; no growth whatsoever in each of the five patches indicated neither complementation nor recombination. No other phenotype was seen.

Enzyme Assays. Histidase and urocanase were assayed as described by Brill and Magasanik (1969) except that whole, undisrupted cells were used and CTAB (hexadecyltrimethylammonium bromide) was added to the reaction mixtures at 100 μg/ml to render the cells permeable (Bender et al. 1977). One unit of activity is the amount of enzyme required to produce 1 nmol of product or degrade 1 nmol of substrate per min at 37° C. Whole cells were assayed for protein by the method of Lowry et al. (1951) using crystaline bovine serum albumin (Sigma) as standard

Table 2. Complementation of *hut* deletion plasmids



Strain	KG2005	KG2010	KG2007	KG2003
Defect	I$^-$	G$^-$	U$^-$	H$^-$
pCB103 his	+	c	−	c
pCB103 uro	+	−	−	
pCB113 his	+	+	−	−
pCB113 uro	+	+	−	
pCB102 his	c	c	c	−
pCB102 uro	+	+	+	
pCB106 his	−	−	c	c
pCB106 uro	−	−	c	
pCB111 his	−	−	−	−
pCB111 uro	−	−	−	
pCB108 his	−	−	−	c
pCB108 uro	−	−	−	
pCB110 his	−	−	−	−
pCB110 uro	−	−	−	
pCB115 his	−	−	−	−
pCB115 uro	−	−	−	

Deletions are indicated by a cross-hatch and are placed with respect to the *Hin*dIII sites of the cloned *hut* DNA. Only the *K. aerogenes* DNA from the plasmids is shown here. *Klebsiella aerogenes* strains KG2005 (*hutI*), KG2010 (*hutG*), KG2007 (*hutU*) and KG2003 (*hutH*) were transformed according to the procedure described in Materials and Methods. Transformant colonies appearing on LB plates supplemented with ampicillin were patched directly onto both histidine and urocanate minimal media plates and scored for growth after 26 h at 37° C. Strain KG2003 does not require any additional genes for growth on urocanate so the growth on urocanate is not reported for this strain. + = solid growth in streak, indicating complementation, − = no growth in streak, C = 5–35 colonies in streak area indicating recombinations

Results

Complementation of hut Mutants with hut Deletion Plasmids

Plasmid pCB101 is a derivative of pBR322 carrying the *hut* genes of *K. aerogenes*. Plasmids deleted for varying amounts of *hut* DNA (Boylan et al. 1983) were used to transform *K. aerogenes* strains defective in one of the four *hut* structural genes (Goldberg and Magasanik 1975), and the transformants were tested for the ability to utilize histidine or urocanate as sole carbon source (Table 2).

Plasmid pCB103 allowed the *hutI* strain KG2005 to grow as a solid patch on plates containing either histidine or urocanate as sole carbon and nitrogen source, demonstrating that pCB103 complements the *hutI* mutation. No such complementation was seen when pCB103 was tested in either a *hutG* or *hutH* strain, but several colonies grew in the streaked area indicating that recombination had occurred. Thus pCB103 carries an active *hutI*$^+$ gene and at least part of the *hutG* and *hutH* genes. The failure of pCB103 to show either complementation or recombination in a *hutU* strain showed that at least the portion of the *hutU* gene identified by the *hutU* allele used here is removed by the deletion in pCB103. Plasmid pCB113, whose deletion maps further to the right of that in pCB103, complemented not only the *hutI* mutation, but also the *hutG*. However, it could neither complement nor recombine with the *hutH* mutation to provide a *hutH*$^+$ gene (Table 2). Therefore the *hutIG* region occupies the left end of the cloned *hut* fragment as depicted in Table 2. This orientation is confirmed

by the complementation data for plasmid pCB102. The mutations in *hutI*, *hutG*, and *hutU* are complemented for growth of the transformant on urocanate by pCB102 (Table 2). The failure of pCB102 to complement these same mutations for growth on histidine will be discussed later. The *hutH* transformant did not grow at all on histidine plates nor did it yield colonies in the patch, indicating that the pCB102 deletion removes the region of *hutH* defined by the *hutH518* allele. Plasmid pCB110 whose deletion enters the *hutH* region from the left, was also unable to give $hutH^+$ recombinants with the *hutH518* allele. Thus the *hutH518* mutation must lie between map positions 5.6 and 6.4, the region missing from both the deletion in pCB102 and that in pCB110. Other plasmids which contain this region, even in an inverted orientation (see pCB108), did recombine with *hutH518*.

Plasmid pCB102 Encodes a Trans-dominant Negative Histidase

As noted above, pCB102 fails to complement *hutI*, *hutG*, or *hutU* mutants for growth on histidine despite the presence of active *hutI*, *hutG*, and *hutU* genes on the plasmid (Table 2). The presence of an active *hutU* gene was confirmed by direct assay for urocanase in strain EB890, an *E. coli* strain carrying pCB102. Urocanase expression was indistinguishable both in quantity and in inducibility from that in strain EB896 carrying a complete *hut* operon on pCB101. Furthermore, no histidase was produced from pCB102, in agreement with the complementation data showing the absence of an active *hutH* gene. A similar analysis of *K. aerogenes* strain KB897 carrying a wild type *hut* operon on the chromosome as well as pCB102 again suggested that urocanase was synthesized from the pCB102 gene and that this synthesis was induced by urocanate. Urocanase synthesis in KB897 was not induced by histidine, however, perhaps reflecting the increased gene dosage for the inducer-degrading enzyme, urocanase, relative to the inducer-forming enzyme, histidase.

Since pCB102 does not provide an active *hutH* gene, the histidase formed in strain KB897 must have been chromosomally encoded. Nevertheless, plasmid pCB102 interfered with the normal regulation of this chromosomally encoded *hutH* product. Whereas urocanase showed the normal ten-fold induction by urocanate, histidase levels rose less than three-fold upon induction (Table 3). Furthermore, the maximal histidase level seen in the presence of pCB102 was only 20% of that seen in the absence of pCB102 (strains KB897 and KB965). Thus, induction of the *hutUH* operon on pCB102 blocked the expression of induced levels of histidase for the chromosomal *hutH* gene in trans, recalling the *trans*-dominant *hutH* mutation described previously (Hagan et al. 1974).

Histidine Toxicity and pCB106

Strains carrying pCB106 express high levels of *hutU* and *hutH* product (Table 3, line 4), yet pCB106 does not complement *hutU* or *hutH* mutations (Table 2). Even hut^+ strains transformed with pCB106 are severely defective for growth on histidine as sole carbon source (not shown). The product of the *hutU* gene product, 4-imidazolone-5-propionate (IP), is toxic to *K. aerogenes* unless further metabolized by the enzymes encoded by *hutI* and *hutG* (Schlesinger and Magasanik 1965). Since pCB106 lacks *hutI* and *hutG* genes to balance the high number of copies of *hutH* and *hutU*, transformants with pCB106 might accumulate high levels of IP when provided with histidine or urocanate. This could explain why even a hut^+ strain grew poorly on histidine or urocanate as sole carbon source when carrying pCB106. When an *E. coli* strain transformed with pCB106 (EB841) was grown in a derepressing medium and transferred from histidine-free to histidine-supplemented medium, the cells died rapidly (Fig. 1). In contrast, *E. coli* strain EB649 containing only pBR322 grew very slowly with histidine as sole nitrogen source (probably via transaminations) while strain EB896 containing pCB101 (with a complete *hut* gene cluster) grew well (Fig. 1).

Table 3. Regulation of histidase and urocanase formation in plasmid-bearing strains

Strain	Plasmid	Inducer	Specific activity (μ/mg)	
			Histidase[a]	Urocanase
EB649	pBR322	none	≦1.0	≦0.3
		uro	≦1.0	≦0.3
EB896	pCB101	none	17.6	1.3
		uro	52	4.3
EB890	pCB102	none	≦1.0	1.1
		uro	≦1.0	6.0
EB841	pCB106	none	182	14.4
		uro	125	12.4
KG2000	none	none	4.3	0.61
		his	52.8	5.6
		uro	45.1	5.4
KB965	pBR322	none	47.3	4
		his	53	6.1
		uro	56.4	5.6
KB936	pCB101	none	17.9	1.5
		his	172	14.8
		uro	117	10.6
KB937	pCB103	none	52.4	ND[b]
		his	48.7	ND
		uro	46	ND
KB899	pCB113	none	43.5	ND
		his	51.6	ND
		uro	44.2	ND
KB897	pCB102	none	3.75	1
		his	5.1	1.1
		uro	9.4	9.4

Cultures were grown and enzyme assays were carried out as in Methods Growth medium consisted of 0.4% glucose and 0.2% ammonium sulfate in minimal salts. Strain KG2000 has an active repressor gene ($hutC^+$) expressed from the chromosome; all other *K. aerogenes* strains carry the *hutC516* allele on the chromosome abolishing production of active repressor from the chromosome. *E. coli* strains lack *hut* genes (including repressor). All *K. aerogenes* strains designations begin with K while *E. coli* designations begin with E

[a] Inducers were His (0.2% histidine) urocanate (Uro) (0.2% urocanate)

[b] Not determined

Location of the Repressor Gene (hutC)

Both pCB102 and pCB106 promoted urocanase synthesis in *E. coli*. The expression of urocanase from pCB102 re-

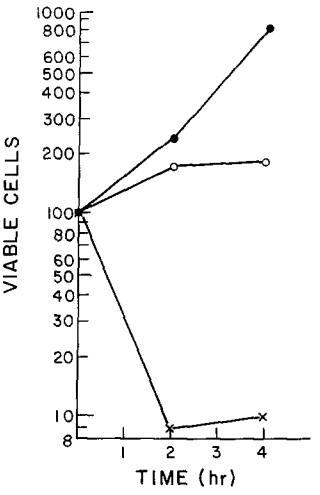


Fig. 1. The effects of histidine on the viability of a pCB106 transformant. Strains were grown overnight at 30° C in a medium containing 0.4% glucose, 0.2% glutamine and 0.2% urocanate. The next morning, cultures were diluted with fresh medium and their growth was followed by Klett units and viable counts. The growing cultures were then washed twice with W salts (See Materials and Methods) and brought to a Klett value of 10 in a 0.4% glucose and 0.4% histidine medium. Five ml cultures were grown at 37° C in 125 ml Klett flasks. Viable counts were determined by diluting a sample of the culture and plating on LB amp plates. The value at $t=0$ was set at 100 and subsequent titres are expressed relative to this value. o, strain EB649, which carries pBR322; •, strain EB896, which carries pCB101; ×, strain EB841, which carries pCB106

quired inducer, whereas that from pCB106 did not (Table 3, strains EB890 and EB841). Thus the repressor gene (*hutC*) was expressed from pCB102 but not from pCB106. The location of the *hutC* gene was further delimited by testing the ability of two plasmids that do not express *hutH* to repress *hutH* expression from a chromosomal *hut* which lacks an active *hutC* product of its own. Neither pCB103 nor pCB113 provided active repressor for the regulation of chromosomally encoded histidase or urocanase expression (Table 3, strains KB937 and KB899). Thus *hutC* lies at least partially to the right of the endpoint of the deletion in pCB113 (at position 3.15) and at least partially to the left of the endpoint of the deletion in pCB106 (at position 3.35).

Discussion

The data presented here yield an unambiguous map order of the *hut* genes based on deletions, confirming the order deduced from transductional analysis. The deletion plasmid pCB103 carries *hutI* and at least part of *hutG* and *hutH* since pCB103 recombines with *hutG* and *hutH* mutations and complements a *hutI* mutation. Plasmid pCB113 carries DNA corresponding to the left end of the deletion in pCB103 and expresses *hutG* but not *hutC*, *hutU* or *hutH* activity in complementation tests, establishing the order *hutI*, *hutG*, (*hutCUH*). Plasmid pCB102, whose deletion enters *hut* from the right, expresses all the *hut* genes except *hutH*. Thus the *hutH* gene is rightmost and the order is *hutI*, *hutG*, (*hutCU*), *hutH*. Finally, plasmid pCB106, whose deletion enters *hut* from the left, lacks *hutI*, *hutG*, and *hutC* activities, but expresses both *hutU* and *hutH*. Therefore *hutC* is to the left of *hutU* and the final map order must be *hutI*, *hutG*, *hutC*, *hutU*, *hutH*.

Since the deletions used in this study were derived from restriction endonuclease digests, we can align the genetic map deduced above with the restriction map presented in the accompanying paper (Boylan et al. 1983). For example, the *hutG* extends rightward beyond the *Bgl*II site at map position 2.35 but ends before the *Kpn*I site at 3.1 since the *Bgl*II-generated deletion in pCB103 abolished *hutG* expression but the *Kpn*I-generated deletion in pCB113 did not. Similarly, at least part of *hutC* must lie to the right of 3.1 since the *Kpn*I-generated deletion in pCB113 abolished *hutC* expression. Since *hutU* and *hutH* are expressed at normal levels from pCB106, the promoter region of the *hutUH* operon, here called *hutP*, must lie to the right of the *Sal*I site at map position 3.35.

The data in Table 3 show clearly that the deletion in pCB102 interrupts the *hutH* gene, but the ability of pCB102 expression to interfere with chromosomal *hutH*-encoded histidase activity strongly resembles the *trans*-dominant *hutH* mutation described by Hagen et al. (1974). This would predict that a portion of *hutH* remains and that the polypeptide produced by this fusion of *hutH* sequences with "foreign" DNA sufficiently resembles the histidase polypeptide to co-assemble with normal histidase subunits resulting in an inactive histidase multimer. The high copy number of the plasmid would tend to accentuate this effect. If mixing of normal and defective subunits is in fact the explanation for the trans-dominant efect of pCB102 on chromosomal histidase expression (Table 3), then although the pCB102 deletion enters the *hutH* gene from the right, it probably leaves a substantial portion of the *hutH* gene intact. Therefore, *hutH* probably extends only a short distance to the right of the *Bam*HI site at map position 5.45.

The trans-dominant, histidase-negative phenotype exhibited by pCB102 would explain the inability of pCB102 to complement any of the *hut* mutants for growth on histidine since little or no active histidase would be produced in such strains. Since histidase activity is not required for metabolizing urocanate, the complementation pattern for growth on urocanate is that which would be expected of a plasmid carrying and expressing *hutI*, *hutG*, and *hutU*. This interference of pCB102 with normal histidase expression also explains the failure of histidine to act as inducer in strain KB897 (Table 3). Urocanase, the enzyme which degrades the inducer, is expressed both from the chromosome and from the plasmid, whereas histidase, the enzyme which synthesizes inducer from histidine, is very poorly expressed so inducer degradation exceeds synthesis. The failure of pCB106, which expresses *hutU* and *hutH*, (Table 3) to complement a *hutU* or *hutH* mutation can also be explained on physiological grounds. The high copy number of the *hutUH* operon leads to the histidine-dependent (or urocanate-dependent) production of the toxic intermediate imidazolone propionate (IP). If IP is not further metabolized in *hutI* strains or is metabolized slowly by a single-copy chromosomal gene, IP accumulates and kills the cell. Thus, while pCB106 carries the information to correct the *hutU* and *hutH* mutations by recombination, growth on histidine or urocanate as sole source of carbon and/or nitrogen is suicidal (Fig. 1).

A map showing the physical locations of the *hut* genes is shown in Table 2. About 1 Kb has been allowed for the four structural genes and a little over 0.5 Kb for the *hutC*

gene. This is consistent with the size of the products of the *hutG*, U and H genes from other organisms (Hagen et al. 1974; Magasanik et al. 1971). Our *K. aerogenes* map is similar to the map of *hut* from *S. typhimurium*. Blumenberg and Magasanik (1981) found that *hut* C, P, U and H end before 3.15, 3.7, 4.85 and 6.75 respectively. By comparing the *K. aerogenes* and *S. typhimurium hut* restriction maps, the region of low homology between the two *hut* operons identified by heteroduplex analysis (Blumenberg and Magasanik 1979) contains the *hutC* region and not *hutP* as previously reported.

The placement of the *hut* genes with respect to the restriction sites which have been mapped on the cloned *hut* DNA (Boylan et al. 1983) facilitates the studies of in vitro transcription from *hutP* now being pursued by our laboratory (Nieuwkoop, Boylan, and Bender, manuscript in preparation). We can now locate *hutP* at map position 3.5. Plasmid pCB111, which contains *hut* DNA only from the 3.35–4.0 region, carries *hutP* and would be an excellent choice for further molecular characterization of the regulation of *hutUH* transcription.

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