JOURNAL OF BACTERIOLOGY, July 1986, p. 350–355
0021-9193/86/070350-06$02.00/0

Vol. 167, No. 1

Characterization of *iucA* and *iucC* Genes of the Aerobactin System of Plasmid ColV-K30 in *Escherichia coli*

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Received 13 January 1986/Accepted 15 April 1986

A cloned 8.3-kilobase-pair DNA fragment carrying all the genes (*iucABCD iutA*) of the aerobactin iron transport system of plasmid pColV-K30 was subjected to in vitro mutagenesis to afford mutant genes *iucA*, *iucC*, and *iucA iucC*. Complementation analyses and identification of aerobactin precursors accumulated by *Escherichia coli* cells harboring the different constructions allowed assignment of the *iucA* and *iucC* genes to discrete steps in biosynthesis of the siderophore from N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine and citrate. Plasmid pVLN10, a derivative carrying a DNA fragment complementing an *iucC* mutation, expressed in a minicell system a single 62,000-dalton protein as the product of this gene.

Under conditions of iron stress many microorganisms induce a variety of high-affinity iron transport systems to overcome the unavailability of this essential element. These systems have in common the activation of a number of genes which determine the synthesis of iron-chelating, low-molecular-weight molecules (siderophores) and outer membrane receptors for the transport of the ferrisiderophore complexes or both (18). Many virulent *Escherichia coli* strains have a particular system for iron transport mediated by the hydroxamate siderophore aerobactin. Although this system can be either plasmid- or chromosome borne, it was first described (22, 23) and cloned (3) from the large plasmid pColV-K30. Several approaches have been used by different investigators (6, 8, 10, 11) to ascertain the organization and regulation of the aerobactin gene cluster of pColV-K30. Data from our (8) and other (6, 10) laboratories indicate that the entire system is determined by an 8.3-kilobase-pair (kb) DNA fragment which carries an operon with five genes in the order *iucABCD iutA* (*iuc*, iron uptake chelate; *iut*, iron uptake transport; see Fig. 1). Of these, one (*iutA*) determines a 74-kilodalton (kDa) outer membrane protein which acts as receptor for the ferriaerobactin complex (20), whereas the others (*iucABCD*) are involved in the biosynthesis of the siderophore. In particular, *iucD* mediates the N^{ϵ}-hydroxylation of L-lysine, and *iucB* mediates the acetylation of N^{ϵ}-hydroxylysine. The *iucC* gene was shown to be related to the synthetase reaction, whereas the function of *iucA* remained in our previous work unassigned (8). The product of gene *iucB* has been positively identified as a 33-kDa protein (8; M. Coy, B. Paw, A. Bindereif, and J. B. Neilands, Biochemistry, in press), and the products of *iucA* and *iucD* have only been detected in minicells as 63- and 53-kDa proteins, respectively. No polypeptide has been assigned definitively to the *iucC* gene, although different minicell preparations have been reported to generate a band variously reported as 27 kDa (5), 32 kDa (16), or 45 kDa (6) and most recently designated as undefined polypeptide S (8). The expression of all the proteins is transcriptionally regulated by iron (4), most likely through a negative regulation by the product of the *fur* gene (12).

The present study was undertaken to ascertain the role of the *iucA* gene in the biosynthesis of aerobactin and to determine whether the synthetase reaction proceeds via a single- or double-step mechanism. The analyses of aerobactin precursors accumulated by *E. coli* strains harboring plasmids with *iucA*, *iucC*, and *iucA* plus *iucC* mutations in the operon show that gene *iucA* is involved in the synthetase reaction and that this occurs through two discrete steps mediated by the products of genes *iucA* and *iucC*. The product of gene *iucC* was identified as a 62-kDa polypeptide.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* HB101 (15) was the recipient of all the recombinant plasmids. An *ent fep* derivative of this strain carrying the plasmid pABN6 (constructed by Albrecht Bindereif) was used as an indicator of aerobactin production. This plasmid (Fig. 1) carries a 6.3-kb DNA insert from pColV-K30 in pBR322 coding for the outer membrane receptor for ferriaerobactin. Therefore, strain HB101 *ent fep*(pABN6) relies on exogenous aerobactin for growth under low iron conditions (see below). *E. coli* X1488 was used for the preparation of minicells (17). Plasmids employed are depicted in Fig. 1 and 3. Plasmids pVLN8 ($iucA^+B^+C^+D^+$ $iutA^+$), pABN15 ($iucA^+$), pABN5 ($iucA^+B^+C^+D^+$), and pABN11 ($iucA^+B^+C^-D^+$) have been described previously (8). Plasmid pVLN10 carrying the $iucC^+$ gene was constructed by cloning a ca. 2.6-kb *Ava*I-*Kpn*I fragment of pVLN8 in the *Xma*I-*Bam*HI sites of pUC8 (21) to place expression of the *iucC* gene product under the control of the *lac* promoter. Plasmid pVLN17 was obtained by cloning the 6.1-kb *Sal*I-*Eco*RI fragment of pABN5 in the corresponding sites of pUC9 (21). All procedures for the construction of the remaining plasmids have also been described previously (15).

To obtain an *iucA* mutation in pVLN8 without affecting the expression of the other genes of the operon (8), we linearized the plasmid with *Sal*I restriction nuclease and subjected the digestion products to further digestion with an excess of S1 nuclease (15), followed by religation of the resulting blunt ends. This treatment was assumed to lead to loss of a few base pairs around the *Sal*I point of the operon within the *iucA* gene beyond the removal of the protruding single-stranded ends. *E. coli* HB101 was transformed with the ligation products, and chloramphenicol-resistant clones failing to produce aerobactin were screened. The polypeptide profiles produced by plasmids derived from such clones were examined in *E. coli* X1488 minicells. We chose a clone carrying a plasmid, pVLN15, which gave in minicell analysis

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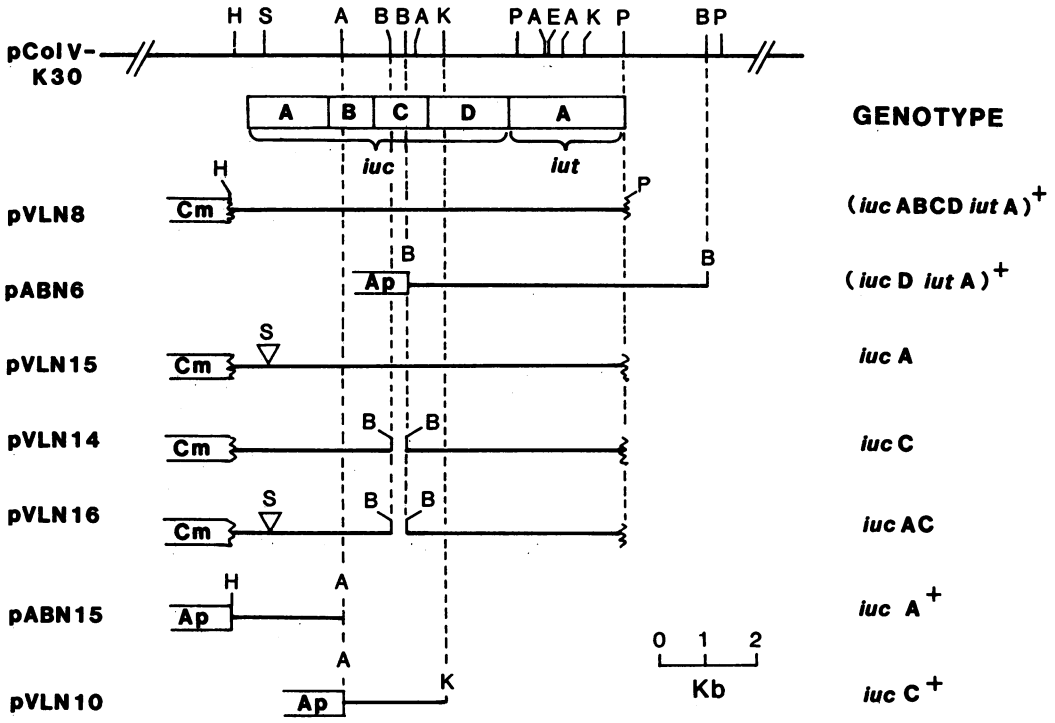

FIG. 1. Restriction nuclease maps of pVLN8 and related plasmids. The inserts are lined up with respect to the original segment of pColV-K30 and to the locations of the five genes. The vectors carrying the different inserts are represented by open boxes in which the selective markers (Cm, chloramphenicol resistance; Ap, ampicillin resistance) are designated. The zigzag lines at the vector-insert junctions indicate that the original restriction sites were lost during the cloning procedures. Transcription occurs in all cases from left to right through either the main aerobactin promoter (4) in pVLN8, pVLN15, pVLN14, pVLN16, and pABN15 or the *lac* promoter of vector pUC8 in pVLN10. The insert of pABN6 is also transcribed left to right, although the precise start point is unknown. The inverted triangle at the *Sal*I site of pVLN15 and pVLN16 gives the position of a small in-frame deletion (see text for details). Restriction sites: H, *Hin*dIII; S, *Sal*I; A, *Ava*I; B, *Bam*HI; K, *Kpn*I; P, *Pvu*II; E, *Eco*RI.

a protein pattern apparently identical to that of pVLN8. The in vitro treatment is believed to have resulted in a small in-frame deletion in the *iucA* gene that led to synthesis of an inactive product (see Discussion). Although the precise extent of the deletion was not determined, a mutational event in *iucA* was confirmed by restriction analysis, complementation with pABN15 (*iucA*$^+$), and the polypeptide profile of minicells carrying pVLN15 (see Results). An *iucC* mutation in pVLN8, pVLN15, and pVLN17 was constructed by deleting the 0.3-kb *Bam*HI fragment, affording, respectively, pVLN14, pVLN16, and pVLN18 (Fig. 1; see Fig. 3).

Hydroxamate analyses. To analyze for the presence of aerobactin or its precursors in the culture supernatant fluids of *E. coli* carrying the different plasmids, we adopted the following procedure. Flasks with 10 ml of Tris succinate medium (22) supplemented with 10% LB broth (15) and the corresponding growth factors and antibiotics were inoculated with 0.1 ml of an overnight culture of the appropriate strain in LB broth (15). After 30 h of incubation at 37°C with vigorous shaking, the cells were removed by centrifugation, and 1 to 60 μl of the supernatant fluids was submitted to analyses by either thin-layer chromatography (TLC) on cellulose plates (Sigmacell type 100) with a pyridine-ethyl acetate-acetic acid-water (5:5:1:3, vol/vol) solvent system or paper electrophoresis in pyridine-acetate (19) (pH 5.6) for 1 h at 25 V/cm. Iron perchlorate stain (2), 1% ferric chloride in ethanol, or an ultrasensitive chrome azurol S stain devised by Bernhard Schwyn (submitted for publication) was used to develop the hydroxamate spots. Purified aerobactin, N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine, and N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine (8) were used as markers (see Fig. 4 for structures). In addition, production of aerobactin was detected by growth of *E. coli* HB101 *ent fep*(pABN6) in iron-deficient NBD plates (10). About 10^7 cells of this strain per plate were spread with top agar, and the strains to be tested for aerobactin production were spot inoculated on the surface. After 48 h of incubation at 37°C, aerobactin presence was indicated by a halo of growth of the aerobactin-dependent indicator strain around the colony.

Minicell analyses. The polypeptides encoded by the different plasmid constructions were examined in a minicell system as described previously (17). Minicell-producing *E. coli* X1488 was transformed with the appropriate plasmids, and minicells were isolated through two consecutive sucrose density gradients and labeled with [^{35}S]methionine as specified in reference 8. Labeled polypeptides were analyzed by 10% or 10 to 15% gradient acrylamide gel electrophoresis (14) followed by enhancement (Autofluor enhancer; National Diagnostics) and fluorography.

RESULTS

Mutations in genes *iucA*, *iucC*, and *iucA* plus *iucC* of pVLN8. Plasmid pVLN8 consists of an 8.3-kb insertion in vector pACYC184 and carries the complete aerobactin operon (8). The position of the different genes with respect to the restriction map of the DNA insert was previously reported (8). We took advantage of convenient restriction sites

TABLE 1. Hydroxamates excreted by *E. coli* cells carrying plasmid pVLN8 and derivatives

Plasmids	Genotype	Hydroxamates produced[a]		
		Aerobactin	N^{ϵ}-Acetyl-N^{ϵ}-hydroxylysine	N^{α}-Citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine
Single plasmids				
pVLN8	*iucA*$^+$*B*$^+$*C*$^+$*D*$^+$ *iutA*$^+$	+	+	ND
pVLN15	*iucA*	−	⊕	−
pVLN14	*iucC*	−	+	⊕
pVLN16	*iucA iucC*	−	+	−
Complementation				
pVLN15 plus pABN15	*iucA* + *iucA*$^+$	+	+	ND
pVLN14 plus pVLN10	*iucC* + *iucC*$^+$	+	+	ND
pVLN16 plus pABN15	*iucA iucC* + *iucA*$^+$	−	+	⊕
pVLN16 plus pVLN10	*iucA iucC* + *iucC*$^+$	−	+	−

[a] Cells harboring single plasmids were grown in Tris succinate medium, and the hydroxamates were assayed as described in the text. For strains carrying two plasmids, *E. coli* HB101 cells were transformed in sequence with the constructions indicated and propagated in the above-mentioned iron-deficient medium. The appropriate antibiotics (chloramphenicol and chloramphenicol plus ampicillin) were used to ensure retention of the plasmids. The hydroxamates were identified by comigration with reference standards of aerobactin, N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine, and N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine. Aerobactin, which was also detected by bioassay, was always accompanied by a certain level of N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine. Hydroxamates critical for functional assignment of *iucA* are circled. ND, Not determined.

to generate selective in vitro mutations in *iucA* or *iucC* or both genes, obtaining plasmids pVLN15 (*iucA*), pVLN14 (*iucC*), and pVLN16 (*iucA iucC*) (Fig. 1). Cells carrying these plasmids produced hydroxamate-positive material (2) but failed to synthesize aerobactin, as shown by bioassays. Mutations in *iucA* and *iucC* were complemented by pABN15 and pVLN10, respectively (see below), indicating in each case that the rest of the genes were still functional.

Aerobactin precursors accumulated by *iucA* and *iucC* mutants. To ascertain the role of the products of *iucA* and *iucC* genes in aerobactin synthesis, the hydroxamates accumulated in the culture medium by cells harboring plasmids with the corresponding mutations were analyzed qualitatively in paper electrophoresis and TLC systems. These two procedures allowed the easy identification of the hydroxamates produced. On electrophoretic analysis, N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine behaved as a neutral compound (8), whereas N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine moved as an anionic species which migrated toward the anode faster than aerobactin. Furthermore, in TLC the hydroxamates gave R_fs of 0.34 (aerobactin), 0.45 (N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine), and 0.24 (N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine).

Table 1 summarizes the results obtained. *E. coli* HB101 (pVLN16, *iucA iucC*) and HB101 (pVLN15, *iucA*) excreted into the medium a single neutral hydroxamate which comigrated with N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine in the TLC and paper electrophoresis systems, whereas *E. coli* HB101 (pVLN14, *iucC*) excreted two hydroxamates which comigrated with N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine and N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine. Plasmid pABN15, carrying the intact *iucA* gene (8), restored the synthesis of N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine in cells harboring pVLN16 (*iucA iucC*), whereas pVLN10, carrying the intact *iucC* gene, did not change the hydroxamate production pattern of the same cells. Finally, as indicated in Table 1, aerobactin production by cells carrying pVLN15 (*iucA*) was restored by pABN15 (*iucA*$^+$) as were cells carrying pVLN14 (*iucC*) by pVLN10 (*iucC*$^+$). Production of aerobactin was confirmed by bioassay with *E. coli* HB101 *ent fep*(pABN6). No hydroxamate spot besides those which comigrated with either N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine or N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine was detected in any of the systems used.

Identification of the product of the *iucC* gene. To identify the polypeptide product of the *iucC* gene, we transformed *E.*

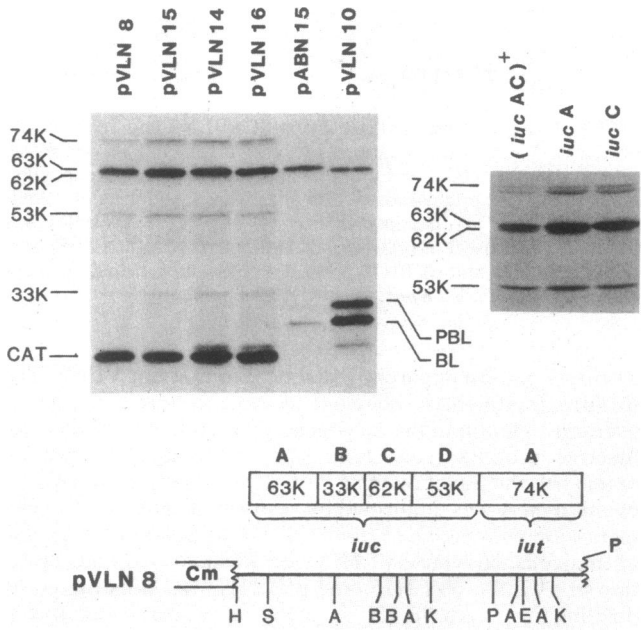

FIG. 2. Expression in *E. coli* minicells of polypeptides encoded by different plasmids. *E. coli* X1488 was transformed with the plasmids indicated above the lanes. Purified minicells were incubated with [^{35}S]methionine, and the labeled polypeptides were analyzed by electrophoresis on a 10% polyacrylamide gel followed by autoradiography. On the right side of the figure, peptides of the high-molecular-weight zone of the three first lanes are shown resolved in a 10 to 15% acrylamide gel. Polypeptides encoded by the vectors are: CAT, chloramphenicol acetyltransferase; PBL, pre-beta-lactamase; and BL, beta-lactamase. Another variably expressed polypeptide of ca. 25 kDa (most likely a decomposition product) in the last two lanes is also vector encoded. Note in the lanes of pVLN14 (*iucC*) and pVLN16 (*iucA iucC*) the absence of the 62-kDa protein and the concomitant appearance of an apparently truncated product located just above the CAT band. Minicells carrying pABN15 overexpressed the 63-kDa protein. On the bottom of the figure may be found the complete assignment of the genes of the cluster. The 63-, 33-, 53-, and 74-kDa proteins were previously mapped (8). The restriction sites are designated as in Fig. 1; Cm, chloramphenicol resistance. K, Kilodalton.

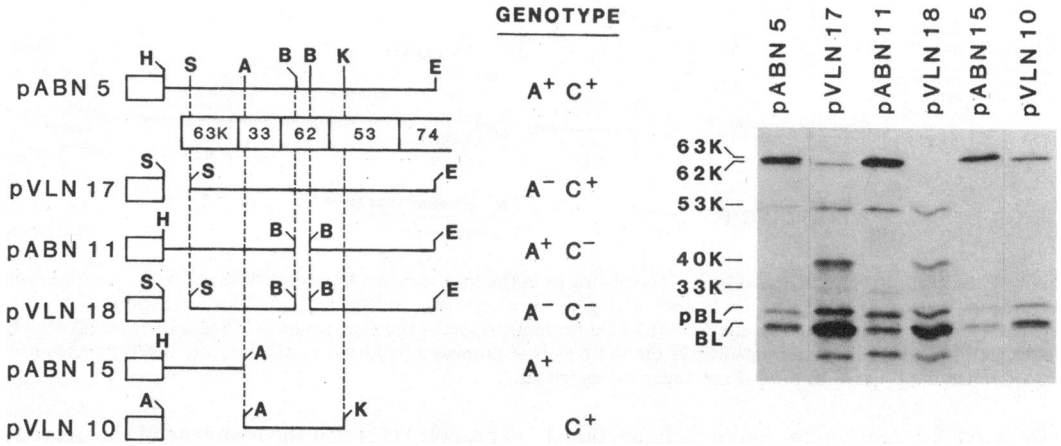

FIG. 3. Identification of the product of gene *iucC*. The labeled polypeptides produced by minicells carrying the various plasmids are shown on the right side of the figure. All plasmids are shown aligned with the restriction sites in pABN5. The genotype indicated refers to *iucA* or *iucC*. Transcription is from left to right via the promoter of the aerobactin system (pABN5, pABN11, pABN15) or the *lac* promoter of pUC9 (pVLN17, pVLN18) and pUC8 (pVLN10). Abbreviations are the same as those used in Fig. 2. Note the absence of the 62-kDa band in the lane corresponding to pVLN18.

coli X1488 with pVLN10 (*iucC*$^+$), pVLN14 (*iucC*), and pVLN16 (*iucA iucC*). As controls, transformants with pABN15 and pVLN8 were also obtained. Minicells derived from the corresponding transformants were labeled, and the polypeptides were analyzed in acrylamide gels. The pattern of labeled polypeptides of these strains is shown in Fig. 2. Minicells carrying pVLN10 (*iucC*$^+$) expressed, in addition to vector-encoded proteins, a 62-kDa polypeptide. This band appears below, but very close to, the 63-kDa protein, the product of the gene *iucA*. The 62-kDa band was apparently absent in the *iucC* mutants. These data suggested assignment of the 62-kDa polypeptide as the product of *iucC*.

Confirmation of this assignment was obtained by minicell analysis of strains carrying plasmids pVLN17 and pVLN18. The insert of pVLN17 carries the intact sequences of genes *iucBCD* of the aerobactin operon but lacks the main promoter as well as about 230 base pairs of the *iucA* gene (4) owing to a *Hin*dIII-*Sal*I deletion of the insert of pABN5. Plasmid pVLN17 also lacks *iutA* owing to a large deletion in this gene (8). However, the other genes are still transcribed in the proper orientation with the *lac* promoter of pUC9. Plasmid pVLN18 is an *iucC* derivative of pVLN17.

Minicell analyses of the peptides encoded by these plasmids are shown in Fig. 3. As controls, minicells from pABN5, pABN11, pABN15, and pVLN10 were examined in the same gel. In pVLN17, the deletion in *iucA* led to production of a truncated polypeptide of about 40 kDa, whereas the products of the remaining genes *iucB* (33-kDa protein), *iucC* (62 kDa), and *iucD* (53 kDa) were produced. In this case the 62-kDa protein from *iucC* is perfectly visible without the interference of the 63-kDa *iucA* product. Furthermore, as expected, the 62-kDa protein is absent in pVLN18, an *iucC* derivative. This absence is more difficult to detect if *iucA* is intact, as in the case of pABN11 (which is also an *iucC* mutant).

It should be mentioned that in the gel shown in Fig. 3 the lanes corresponding to pVLN17 and pVLN18 had to be somewhat overexposed with respect to the others for a clear identification of the peptides produced. This indicates that the deletion in *iucA* in those plasmids has a substantial polar effect on the rest of the genes, which are expressed at a relatively low level. As expected, cells carrying pVLN17 and pVLN18 excreted into the medium low levels of a single hydroxamate identified as N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine (data not shown), about 10-fold below the amounts found with pVLN15 (*iucA*) and pVLN16 (*iucA iucC*).

DISCUSSION

Plasmid pVLN8 has some features which make it particularly convenient for the study of aerobactin synthesis. It carries an 8.3-kb insert derived from pColV-K30 and encodes the entire aerobactin system, i.e., genes for both production and transport of the siderophore (8). Its vector, pACYC184 (7), is compatible with ColE1-derived plasmids, thus facilitating complementation analyses of pVLN8-derived mutants with defined DNA fragments cloned in pBR322 derivatives. Finally, its relatively low copy number relieves in part the stress undergone by cells harboring the iron-regulated aerobactin system in a multicopy plasmid under iron-limiting conditions.

A previous study (8) from our laboratory and independent reports from others (6, 10) had established a general overview of the regulation of the aerobactin operon (4) and the organization and function of its genes. However, the gene product of *iucC* was not identified, and the components of the synthetase reaction were not completely characterized. We used different plasmid derivatives of pVLN8 to address these two issues.

In vitro-generated mutants in the *iucA*, *iucC*, or *iucA* plus *iucC* locations of pVLN8 were constructed, and the hydroxamate products of *E. coli* cells harboring the corresponding plasmids were analyzed. Previous *iucC* mutants isolated in our laboratory did not show a decrease in expression of the downstream genes, as detected by minicells (8). However, a *Sal*I frameshift mutation in *iucA* was reported to have a severe polar effect on the rest of the genes of the operon (8). To avoid this effect, we constructed an *iucA* mutant whose gene product was inactive by virtue of a small deletion around its *Sal*I point but in which the reading frame of the sequence was apparently retained, as shown by the fact that no difference in expression of the derived polypeptide products of pVLN15 (*iucA*) and the parental pVLN8 was found in a minicell system.

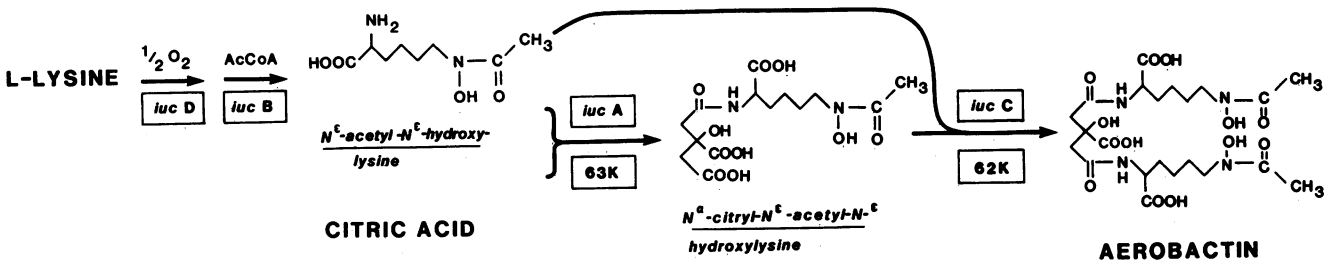

FIG. 4. Proposed aerobactin synthetase reaction. The substrates in the first step are N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine, previously formed from lysine and acetyl coenzyme A (AcCoA) by *iucD* and *iucB* gene products (8), and citric acid. The formation of N^{ϵ}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine is catalyzed by the product of gene *iucA* (63-kDa protein), whereas the product of *iucC* (62-kDa protein) attaches a second side chain to yield aerobactin. The biosynthetic pathway is the same as that proposed by Gross et al. (11), and discrepancies in the size reported for the products of genes *iucA*, *iucB*, and *iucD* are regarded as trivial.

Either mutation (*iucA* or *iucC*) in the operon left functional the other two genes involved in the biosynthesis: *iucB*, coding for the N^{ϵ}-hydroxylysine acetylase (8; Coy et al., in press), and *iucD*, required for the N^{ϵ}-oxygenation of L-lysine (8). Therefore, the only step in aerobactin synthesis in which genes *iucA* and *iucC* may be involved is the reaction in which two side chains of N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine are attached to a citric acid backbone. The prochirality of the two distal carboxyl groups of the citrate molecule requires that this reaction take place in two steps. Indeed, we previously reported (8) that an *iucC* mutation in the aerobactin operon leads to the accumulation of a hydroxamic acid consisting of a single N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine residue attached to one of the carboxyl residues of citric acid. We confirmed this with *E. coli* cells carrying the pVLN14 (*iucC*) plasmid. Furthermore, the hydroxamate production pattern of mutants *iucA* (pVLN15) and *iucA iucC* (pVLN16) as well as the accumulation of N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine obtained in cells carrying pVLN16 (*iucA iucC*) plus pABN15 ($iucA^{+}$) can only be explained by a function of the gene *iucA* product in the attachment of a first N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine to a carboxylic group of citric acid followed by the reaction of the resulting N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine with a second N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine, mediated by the product of gene *iucC*, to produce aerobactin (Fig. 4).

Minicell analyses of pVLN10, in comparison with different *iucC* mutants, enabled assignment of a ca. 62-kDa polypeptide as the product of this gene. The failure in previous work (6, 8, 11) to identify this protein can be explained by its almost complete overlapping in polyacrylamide gels with the 63-kDa protein, the product of gene *iucA*.

Some data are available (1) on the aerobactin synthetase of *Aerobacter aerogenes* 62-I, the microorganism in which aerobactin was first described (9). Although this enzyme has not been purified, gel filtration data indicate a molecular size over 100 kDa for the protein. Assuming a similarity between this enzyme and the aerobactin synthetase from pColV-K30, these results suggest that the enzyme may be a complex with at least two subunits.

Elucidation of the restriction map of the aerobactin region of pColV-K30 (3) has facilitated investigation of the corresponding gene cluster in closely related plasmids. Subsequent to the submission of this paper, the recent work of Gross et al. (11) with pColV-K311 came to our attention. These findings are in complete agreement with our report (8) on the presence of four biosynthetic and one transport gene in the aerobactin operon. As in our case (8), Gross et al. (11) failed to locate the product of the third gene of the operon, which is the major contribution of our present paper. The proposal (11) that the first gene of the operon is required for the primary step in the synthetase reaction was based mainly on the production of N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine by cells carrying a plasmid similar to our pVLN17. We now provide further evidence on the function of the 63-kDa protein (product of *iucA*) by showing that the intact $iucA^{+}$ gene restores the formation of N^{α}-citryl-N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine in an *iucA iucC* double mutant (Table 1). The composition and organization of the aerobactin genes of ColV plasmids K30 and K311 appear to be identical despite minor differences in estimates of the size of the gene products. An important contribution of both papers (8, 11) is the demonstration via different approaches that the suggested (13) role of the 53-kDa protein in aerobactin transport is clearly incorrect.

Regarding the nomenclature of the genes in the aerobactin operon, we prefer to retain the original designation introduced by Williams and co-workers (22, 23), namely, *iuc* and *iut* for biosynthetic and transport genes, respectively. We assign to the biosynthetic genes the sequence *iucABCD*, which corresponds to their genetic arrangement. The same genes have been designated by the Braun group (11) as *aerDBCA*, apparently as a means of specifying the order of participation of their products in aerobactin synthesis. However, both previous studies (8, 11), confirmed in the present investigation, indicate that the 63-kDa protein performs the first step in the synthetase reaction. Thus, to be consistent the designation of genes *aerD* and *aerC* would have to be exchanged. Pending a consensus on this point the following correlations should be noted: *iucA* = *aerD*, *iucB* = *aerB*, *iucC* = *aerC*, and *iucD* = *aerA*.

The present work completes the first entire gene-product-function analysis of any siderophore gene complex from any microbial species. Important questions regarding the enzymology and regulation of the system remain. In particular, the mechanism of the enzyme which oxygenates L-lysine and the possible presence of additional regulatory sites apart from the main promoter (4) immediately upstream from gene *iucA* must await further studies.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants AM17146 and AI04156 from the National Institutes of Health and by National Science Foundation grant PCM 78-12198. V.d.L. was supported by National Institutes of Health Fogarty International Fellowship 1FO5TW03577-01-BI-5.

Albrecht Bindereif and Bernhard Schwyn provided plasmid pABN6 and an unpublished method for siderophore detection, respectively. The assistance of Paul Gill and Jeff Buyer in devising the TLC systems is also acknowledged.

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