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Escherichia coli B/r *leuK* Mutant Lacking Pseudouridine Synthase I Activity

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***Escherichia coli* B/r strain EB146 containing mutation *leuK16* has elevated levels of enzymes involved in the synthesis of leucine, valine, isoleucine, histidine, and tryptophan (Brown et al., J. Bacteriol. 135:542–550, 1978). We show here that strain EB146 (*leuK16*) has properties that are similar to those of *E. coli* and *Salmonella typhimurium hisT* strains. In $tRNA_1^{Leu}$ from both *hisT* and *leuK* strains, positions 39 and 41 are uridine residues rather than pseudouridine residues. Furthermore, in $tRNA_3^{Leu}$ and $tRNA_4^{Leu}$ from a *leuK* strain, uridine residues at positions 39 and 40, respectively, are unmodified. Pseudouridine synthase I activity is missing in extracts of strain EB146 (*leuK16*), and extracts of strain EB146 (*leuK16*) and of a *hisT* strain do not complement one another in vitro. Four phenotypes of strain EB146 (*leuK16*), leucine excretion, wrinkled colony morphology, and elevated levels of *leu* and *his* enzymes, are complemented by a plasmid having a 1.65-kilobase DNA fragment containing the *E. coli* K-12 *hisT* locus. These results indicate that either *leuK* codes for pseudouridine synthase I (and is thus a *hisT* locus in reality) or, less likely, it codes for a product that affects the synthesis or activity of pseudouridine synthase I.**

Escherichia coli B/r strain EB146 containing mutation *leuK16* has elevated levels of enzymes involved in the synthesis of leucine, valine, isoleucine, histidine, and tryptophan (5). Genetic experiments suggested that *leuK16* mapped near *gal* and that it was dominant to the wild allele. Taken together, these results defined a new locus, *leuK*, that in some way interacted with diverse operons involved in amino acid biosynthesis. More recent unpublished experiments by E. Kline indicate that previous conclusions (5) regarding the map location and dominance characteristic of *leuK* are invalid. The experiments described below indicate that strain EB146 (*leuK16*) has a number of properties in common with *hisT* strains. The simplest interpretation of our results is that *leuK* is, in fact, a *hisT* locus.

tRNA was isolated from parent strain EB145 and mutant strain EB146. The profile of charged $tRNA^{Leu}$ species emerging from Sepharose 4B, eluted with a reverse $(NH_4)_2SO_4$ gradient (19), is shown in Fig. 1. In our hands, $tRNA^{Leu}$ isoaccepting species emerged in three peaks, the first containing $tRNA_2^{Leu}$ and $tRNA_5^{Leu}$, the second containing $tRNA_3^{Leu}$ and $tRNA_1^{Leu}$, and the third containing $tRNA_4^{Leu}$ (see Table 1 for definitions of $tRNA^{Leu}$ isoaccepting species). In the profiles published by Hatfield (19), which are of higher resolution, $tRNA_2^{Leu}$ elutes with $tRNA_5^{Leu}$ and $tRNA_3^{Leu}$ elutes before $tRNA_1^{Leu}$. Figure 1 shows the results of a double-label experiment employing [^{14}C]leucine and [^{3}H]leucine charged to tRNA from the parent (EB145) and mutant (EB146) strains, respectively. For tRNA from strain EB146, the first and last peaks were shifted relative to the parent, indicating that $tRNA_2^{Leu}$ or $tRNA_5^{Leu}$ plus $tRNA_4^{Leu}$ or all three, are altered in the mutant. On the other hand, there were no differences in the elution profiles of $tRNA^{Val}$ or $tRNA^{Ile}$ species between the parent and mutant strains (J. Jones, unpublished data).

Isoaccepting species $tRNA_1^{Leu}$, $tRNA_3^{Leu}$, and $tRNA_4^{Leu}$ were purified by a combination of the derivatization procedure of Gillam et al. (16) and two-dimensional polyacrylamide gel electrophoresis (14). No differences in mobility were observed between the tRNAs of the mutant and wild-type strains on two-dimensional gels. Purified $tRNA_4^{Leu}$ species from both mutant and parent strains were sequenced by the chemical method of Peattie (29). The sequence of wild-type $tRNA_4^{Leu}$ shows gaps at positions 33, 40, and 66, corresponding to known positions of pseudouridine (ψ) (35). In the sequence of $tRNA_4^{Leu}$ from the mutant, there is a band in the uridine (U) track at position 40 (Fig. 2) but not at position 33 or 66 (data not shown). No differences other than the one noted above were observed between the two sequences. These results indicate that the modification of $tRNA_4^{Leu}$ at position 40 is affected by the *leuK* mutation but that modifications of positions 33 and 66 are not.

Purified $tRNA_3^{Leu}$ from the parent and mutant were analyzed by procedures that give both the nucleotide sequence and the identity of modified bases (17, 18). In $tRNA_3^{Leu}$ from the mutant but not the parent, the U at position 39 is unmodified (Fig. 3). In all other respects, the sequences from the mutant and parent were identical, corresponding to the sequence derived by the Sanger procedure (4). Purified $tRNA_1^{Leu}$ from the parent and mutant were analyzed in a similar fashion. In $tRNA_1^{Leu}$ from the mutant, neither of the Us at positions 39 and 41 was modified to ψ as they were in the parent, but the U at position 66 was normally modified (data not shown).

To summarize, for three different $tRNA^{Leu}$ isoaccepting species analyzed, tRNAs from the mutant lacked ψ residues within the 3′ side of the anticodon loop (at positions 39, 40, or 41) but contained ψ residues within the TψC loop and, for $tRNA_4^{Leu}$, within the 5′ side of the anticodon loop. Some preliminary experiments indicate that the *leuK* mutation affects ψ modification within $tRNA_5^{Leu}$ in the same way as for $tRNA_4^{Leu}$ (L. Searles, unpublished data).

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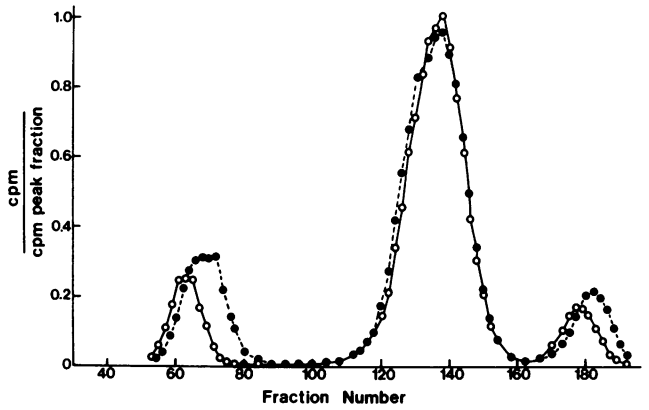

FIG. 1. Sepharose 4B chromatography of tRNAs from strains EB145 (○) and EB146 (*leuK*) (●). Strains were grown and tRNA was isolated as described by Zubay (37). Conditions for tRNA charging are described by Blank and Söll (3). An extract of an *araB9 gal-205 S. typhimurium* strain containing tRNA synthetases was prepared as described by Muench and Berg (27). Samples of 500 μg of tRNA were charged with 1 μCi of [^{3}H]leucine (400 mCi/mmol) (*leuK* tRNA) or 2 μCi of [^{14}C]leucine (200 mCi/mmol) (parent tRNA). The two samples were mixed, applied to the column, and eluted as described by Hatfield (19).

The pleiotropic effects of the *leuK* mutation upon amino acid biosynthetic operons (5) and the effect of the *leuK* mutation upon the U→ψ modification in tRNA^{Leu} species invite comparison with *hisT* mutations in *Salmonella typhimurium* and *E. coli*. Mutations in *hisT* result in elevated expression not only of the *his* operon (32), but also of several other amino acid biosynthetic operons, including the *leu* operon (10, 23, 30). *hisT* is the structural gene for pseudouridine synthase I, an enzyme that converts U to ψ within the anticodon loop of several tRNAs, including tRNA$_1^{Leu}$ (10, 32). In fact, the ψ modification pattern of tRNA$_1^{Leu}$ from a *hisT* strain (39 and 41 unmodified; 66 modified) is just that reported here for tRNA$_1^{Leu}$ from a *leuK* strain.

This comparison prompted us to measure pseudouridine synthase I in parent, *hisT*, and *leuK* strains by using the ^{3}H release assay developed by Cortese et al. (10). The basis of this assay is that in the conversion of U to ψ, a hydrogen is released from the carbon atom at position 5 of the pyrimidine ring. tRNA from a *leuK* strain acted as a substrate for a pseudouridine synthase activity that was present in crude extracts of wild-type *S. typhimurium*, *E. coli* K-12, and *E. coli* B/r (Table 2; experiment 1). Extracts prepared from *hisT* strains of *S. typhimurium* and *E. coli* did not catalyze release of ^{3}H from this substrate (Table 2, experiment 2), suggesting that the activity observed in experiment 1 was due to pseudouridine synthase I activity. An extract from mutant strain EB146 (*leuK16*) behaved like extracts from *hisT* strains, i.e., it did not catalyze ^{3}H release from the tRNA substrate (Table 2, experiment 2). The lack of activity in extracts prepared from strain EB146 (*leuK16*) was not the result of a diffusible inhibitor because such extracts did not inhibit the activity present in wild-type extracts (Table 2, experiment 4). Extracts prepared from *leuK* and *hisT* strains did not complement one another in vitro (Table 2, experiment 3).

The foregoing analysis points up the similarities between *leuK* and *hisT* mutations. The two loci differ in their map position: *hisT* is about 40% linked to *purF* by P1 transduction (7, 31), whereas *leuK* is reportedly linked to *gal* (5). Upon reexamining the linkage of *leuK* to *gal*, E. Kline (personal communication) observed the following. Almost all Gal$^+$ transductants from a cross between phage P1 grown on EB146 (*gal*$^+$ *leuK*) and a *gal* recipient indeed excreted leucine (a phenotype associated with *leuK*). However, the same result was obtained from a control cross between phage P1 grown on EB145 (*gal*$^+$ *leuK*$^+$) and a *gal* recipient, indicating that the leucine excretion observed in these crosses is not due to cotransduction of *leuK* and *gal* (E. Kline, personal communication).

To determine whether the *leuK16* mutation is linked to *purF*, the following crosses were performed. *E. coli* K-12 strain CV875 (*purF*) was transduced to prototrophy with P1 phage grown on *E. coli* B strain EB146 (*purF*$^+$ *leuK16*). Transductants were scored for leucine excretion by an auxanographic test (9). None of 170 transductants analyzed excreted leucine (Table 3). Another cross was performed with recipient *E. coli* B strain CV878 (*purF*::Tn*10*) and phage grown on *E. coli* B strain EB146 (*leuK16*); prototrophic transductants were scored for both leucine excretion and

TABLE 1. Identity of tRNA^{Leu} isoaccepting species

tRNA^{Leu} species[a]	Elution from Sepharose 4B[b]	Anticodon (3'-5')[c]	Codon(s)
1	4	GAC	CUG
2	1 or 2	GAU	CUA, CUG
3	3	GAG	CUC, CUU
4	5	AAU	UUA, UUG
5	1 or 2	AAC	UUG

[a] Species designation refers to order of elution from an RPC-5 column.
[b] Figure 1 and reference 19.
[c] References 3, 4, 11, 13, 20, 28, and 35; L. Searles, unpublished data.

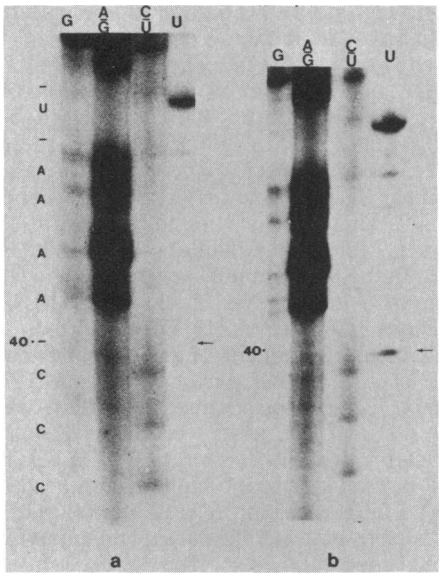

FIG. 2. Partial nucleotide sequence of tRNA$_4^{Leu}$ from (a) strain EB145 (parent) and (b) strain EB146 (*leuK16*). Purified species were end-labeled with pCp using RNA ligase (6) and sequenced by the chemical method of Peattie (29). Samples were applied to a 20% acrylamide–0.67% bisacrylamide–8 M urea gel.

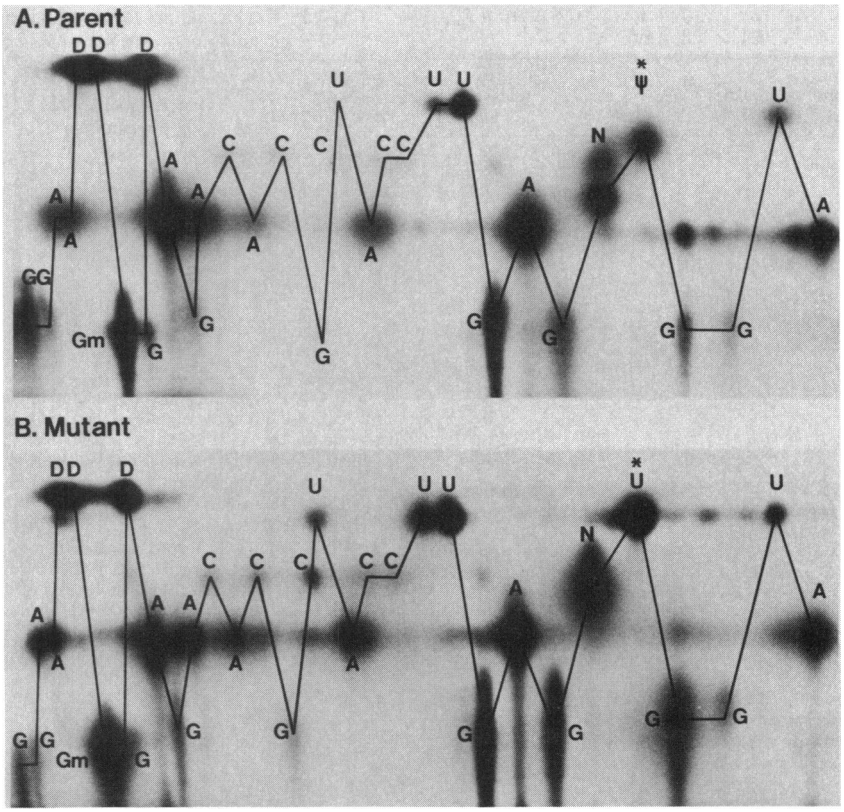

FIG. 3. Partial nucleotide sequence of $tRNA_3^{Leu}$ from strains EB145 (A) and EB146 (*leuK*) (B). Purified samples were analyzed by the rapid read-out sequencing method of Gupta and Randerath (17) as modified by Gupta et al. (18). The asterisk marks the difference in sequence between the parent and mutant tRNAs.

wrinkled colony morphology. We observed that strain EB146 (*leuK16*), like *hisT*-containing strains of *S. typhimurium*, had a wrinkled colony morphology when grown on plates containing 2% glucose and 2% gluconate. Again, no linkage of *purF* to *leuK16* was found among 300 transductants tested. In addition, we did not observe linkage of *purF* to *leuK16* in a transduction involving phage grown on a *purF*::Tn*10* donor and a *leuK16* recipient when selection was made for tetracycline resistance associated with Tn*10* (Table 3).

To summarize, the assignment of *leuK* to a location near *gal* (5) is incorrect. Our transduction experiments do not define the location of *leuK*. If *leuK* is a *hisT* locus, then our inability to detect linkage to *purF* suggests that these loci are not closely linked in *E. coli* B. However, the possibility remains that the two loci are linked, but that we did not observe linkage because of factors relating to interspecies crosses.

To determine whether phenotypes associated with the *leuK16* mutation were complemented by a wild-type *hisT* locus, *E. coli* B strain EB146 (*leuK16*) was transformed separately with plasmids ψ300 and pNU61, containing the *hisT* locus from *E. coli* K-12 on 2.3- and 1.65-kilobase fragments, respectively (25). The resulting transformants had normal colony morphology, did not excrete leucine, and had normal (low) levels of β-isopropylmalate dehydrogenase (*leuB* product) and histidinol phosphatase (*hisB* product) (Table 4). These results strongly suggest that *hisT* complements the *leuK16* mutation.

The simplest interpretation of the biochemical and genetic complementation experiments described here is that *leuK16* is a *hisT* mutation. Other more complicated possibilities can be imagined. For example, the wild-type *leuK* gene may code for a product that regulates the synthesis or activity of pseudouridine synthase I. The fact that an *E. coli* B *leuK* mutation is complemented by an *E. coli* K-12 *hisT* gene may reflect a difference in regulation between the two organisms or may be a result of high plasmid copy number.

The remaining discussion, relating to the expression of *hisT* mutations in different organisms, assumes that *leuK16* is a *hisT* mutation. In *S. typhimurium*, a *hisT* mutation causes marked elevation of *his* operon expression (32) and modest elevation in the expression of the *leu* and *ilv* operons (10, 30). In *E. coli* K-12, a *hisT* mutation has a substantial effect upon the *his* operon but only a small effect upon *leu* and *ilv* operons (22). These results may be compared with those for *E. coli* B/r containing *leuK16*: the *leu* operon is most highly derepressed, with the *his*, *ilv*, and *trp* operons being substantially elevated (5). Note that both column chromatographic profiles of charged tRNAs and enzyme assays suggest that pseudouridine synthase I activity is totally absent in each of the three strains compared (10, 22). Another phenotype relevant to this discussion is growth rate. *S. typhimurium* (23) and *E. coli* K-12 (7) *hisT* strains grow more slowly than the parent, but this is not the case for *E. coli* B/r strain EB146 (5).

The differences described above may be due to one or a combination of the following possibilities.

(i) The *his*, *leu*, *ilv*, and *trp* operons of *E. coli* K-12 and *S. typhimurium* are controlled by transcription attenuation (21).

TABLE 2. Pseudouridine synthase I assays using tRNA from *E. coli* EB146 (*leuK*) as substrate

Expt[a]	Source of enzyme[b]	^3H released[c] (cpm)
1	LT2	624
	K-12	621
	B/r	836
2	LT2 *hisT*	90
	K-12 *hisT*	80
	B/r *leuK*	97
3	B/r *leuK* + LT2 *hisT*	95
	B/r *leuK* + K-12 *hisT*	81
4	B/r *leuK*	0
	LT2	552
	K-12	660
	B/r	748
	B/r *leuK* + B/r	731
	B/r *leuK* + K-12	671
	B/r *leuK* + LT2	550

[a] Experiments 1, 2, and 3 were carried out at one time. A count of 20 cpm, representing the result obtained for a sample lacking extract, was subtracted from each value. For experiment 4, a background of 143 cpm was subtracted.

[b] An extract was prepared as described by Cortese et al. (10). Strains: LT2, *S. typhimurium* LT2; K-12, *E. coli* K-12; B/r, *E. coli* B/r strain EB145; B/r (*leuK*), *E. coli* B/r strain EB146 (*leuK*).

[c] The ^3H-labeled tRNA substrate was prepared by isolating tRNA (37) from strain EB146 (*leuK16*) grown in Vogel-Bonner medium (33) with 0.2% glucose containing [^3H]uridine (42 Ci/mmol). The specific activity of the tRNA was about 2,500 cpm/pmol. The assay was carried out as described by Cortese et al. (10). Each assay contained 100 μg of protein and tRNA containing 2×10^5 cpm. In cases where two extracts were mixed, 100 μg of protein from each strain was used. Assays were performed for 30 min at 37°C. Under these conditions, ^3H release had reached a maximum.

TABLE 4. Complementation of several *leuK* phenotypes by plasmids carrying the *E. coli* K-12 *hisT* gene

E. coli B strain	Containing plasmid[a]	Sp act	
		Histidinol phosphatase[b]	β-IPM dehydrogenase[c]
B145 (*leuK*$^+$)	None	0.44	4.9
	ψ300	0.26	4.7
	pNU61	0.26	3.3
	pBR322	0.44	3.7
EB146 (*leuK16*)	None	6.9	14.2
	ψ300	0.38	4.2
	pNU61	0.44	3.8
	pBR322	6.0	26.5

[a] *hisT* is part of a multigene operon containing *usg* (upstream of *hisT*; codes for a 36, 364-dalton polypeptide of unknown origin) and a putative third gene upstream of *usg* (1,25). Plasmid ψ300 has this operon on a 2.3-kilobase fragment inserted into plasmid pBR322. Plasmid pNU61, a derivative of ψ300, has a 650-base-pair deletion within the *usg* gene but still codes for normal levels of pseudouridine synthase I.

[b] Histidinol phosphatase (E.C. 3.1.3.15) was measured by a toluenized cell assay (24). Specific activity is change in absorbancy at 820 nm per ml of suspension having 1 absorbancy unit at 650 nm, per 15 min at 37°C.

[c] β-Isopropylmalate (β-IPM) dehydrogenase (E.C. 1.1.1.85) was assayed by a modification of the procedure of Burns et al. (8). Cells were permeabilized by treatment for 5 min with $CHCl_3$. Specific activity is change in absorbancy at 540 nm per ml of suspension having 1 absorbancy unit at 550 nm, per 15 min at 37°C.

The structure of the control regions of the relevant operons may be different in different organisms. This is not true for the *his* operons of *E. coli* K-12 and *S. typhimurium* (which differ by two nucleotides) (2, 12), but it is certainly the case for the *leu* operons of these two organisms (18 of 84 nucleotides within the leader regions differ) (34). For *E. coli* B/r, there is no relevant nucleotide sequence information. Conceivably, the number, position, or identity of control codons may be different in *E. coli* B/r.

(ii) There may be strain-related differences in the structures of some tRNA species. Available information (15) suggests that both sequence and modification differences exist but are few.

(iii) Regulation by transcription attenuation is known to be affected by factors influencing both transcription and translation. For example, mutations affecting the structure of RNA polymerase cause altered expression of the *trp* operon (36). It is not unlikely that some component(s) of the transcription or translation machinery in these strains differ. Certainly, such differences could explain the growth rate and regulatory patterns observed.

TABLE 3. Transduction crosses performed to determine the map position of *leuK*[a]

Phage P1 *vir* grown on *E. coli* B strain[b]	Recipient[b]			Selected marker	% of transductants that[c]:	
	Organism	Strain	Relevant genotype		Excrete leucine	Have wrinkled phenotype
EB146 (*leuK16*)	*E. coli* K-12	CV875	*purF*::Tn*10*	*purF*$^+$	0 (170)	0 (170)
EB145	*E. coli* K-12	CV875	*purF*::Tn*10*	*purF*$^+$	0 (40)	0 (40)
EB146 (*leuK16*)	*E. coli* B/r	CV878	*purF*::Tn*10*	*purF*$^+$	0 (232)	0 (232)
EB145	*E. coli* B/r	CV878	*purF*::Tn*10*	*purF*$^+$	0 (54)	0 (54)
CV877 (*purF*::Tn*10*)	*E. coli* B/r	EB146	*leuK16*	*tet*	100 (43)	100 (43)
CV877 (*purF*::Tn*10*)	*E. coli* B/r	EB145	*leuK*$^+$	*tet*	0 (35)	0 (35)

[a] Transductions were carried out by procedures described by Miller (26) in a minimal medium (9) containing 0.2% glucose. Selection for tetracycline resistance was on L-plates (26) containing 25 μg of tetracycline per ml. Problems of restriction in transductions between strains B/r and K-12 were reduced by incubating the recipient at 55°C for 30 min before performing the transduction or by using strains lacking restriction systems.

[b] Genotypes of strains were as follows. Strain EB146: *dau-5 rpsL* (*mal*$^+$ λ^s from *E. coli* K-12) *leuK16*. Strain CV875: F$^-$ *rpsL lac gal-1,2* T1^r T7^r *hsdR hsdM purF77*::Tn*10*. Strain CV877: *hsdR11 met-100* (*mal*$^+$ λ^s from *E. coli* K-12) *gal-151 purF77*:Tn*10*. Strain CV878: *purF77*::Tn*10 dau-5 rpsL*(*mal*$^+$ λ^s from *E. coli* K-12). Strain CV875 was constructed by transducing *E. coli* K-12 strain EG47 (F$^-$ *hsdR hsdM rpsL lac gal-1,2* T1^r T7^r) with phage P1 *vir* grown on *E. coli* K-12 strain NK6035 [Δ(*gpt-lac*)*5 purF77*::Tn*10 relA1 spoT1* thi-1 λ$^-$] and selecting for tetracycline resistance. Strain CV877 was derived from *E. coli* B strain WA837 [*hsdR11 gal-151 met-100* (*mal*$^+$ λ^s from *E. coli* K-12)] by transduction with phage P1 *vir* grown on *E. coli* K-12 strain NK6035 and selection for tetracycline resistance. Strain CV878 is a *purF*::Tn*10* derivative of strain EB145 obtained by transduction with phage P1 *vir* grown on strain CV877.

[c] *leuK16* causes overproduction and excretion of leucine, a phenotype that was scored by an auxanographic test described by Calvo et al. (9). Plates for measuring the wrinkled colony morphology contained in addition to 2% glucose, 2% gluconate which accentuated the phenotype. The number in parentheses is the total number of colonies analyzed.

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