A NEW MAP LOCATION FOR THE *ilvB* LOCUS OF *ESCHERICHIA COLI*

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ABSTRACT

We isolated, in *E. coli* K12, new alleles of the *ilvB* locus, the structural gene for acetolactate synthase isoenzyme I, and showed them to map at or near the *ilvB619* site. The map position of the *ilvB* locus was redetermined because plasmids containing the *ilvC-cya* portion of the chromosome did not complement mutations at the *ilvB locus*. Furthermore, diploids for the *ilvEDAC* genes formed with these plasmids in an *ilvHI* background facilitated the isolation of the new *ilvB* alleles. The *ilvB* locus was remapped and found to be located at 81.5 minutes, between the *uhp* and *dnaA loci*. This location was determined by two- and three-point transductional crosses, deletion mapping and complementation with newly isolated plasmids. One of the new alleles of the *ilvB* gene is a *mu-1* insertion. When present in the donor strain, this allele interferes with the linkage of genes flanking the *mu-1* insertion, as well as the linkage of genes to either side of the *mu-1* insertion.

IN *Escherichia coli* K12, the biosynthesis of the branched chain amino acids requires a carbon-to-carbon condensation step catalyzed by an acetolactate synthase activity (AHAS–EC 4.1.3.18; IACCARINO *et al.* 1978). This metabolic step is complex because it is the first step in valine biosynthesis and the second step in isoleucine biosynthesis (see Figure 1).

Our present understanding of the genetics and physiology of the acetolactate synthase reaction is mainly the result of a series of investigations in which it was shown that *E. coli* K12 contains three structural genes for three isoenzymes, AHAS I, AHAS II and AHAS III, each of which can catalyze the synthesis of both acetolactate and acetohydroxybutyrate (GUARDIOLA, DE FELICE and IACCARINO 1974; DEFELICE *et al.* 1974a, b; FAVRE *et al.* 1976 GUARDIOLA *et al.* 1977). Prior to these studies, the assumption that acetolactate synthase activity was obligatory for branched chain amino acid synthesis was inferred from the inability to find another enzyme to synthesize acetolactate and acetohydroxybutyrate and from the observation that this AHAS activity was inhibited by valine and coordinately controlled (under certain conditions) with other *ilv* gene products (IACCARINO *et al.* 1978). The aforementioned studies established that a strain containing mutations in the *ilvG* gene and the *ilvI* gene retained an Ilv$^+$ phenotype because this strain remained *ilvB*$^+$. However, following

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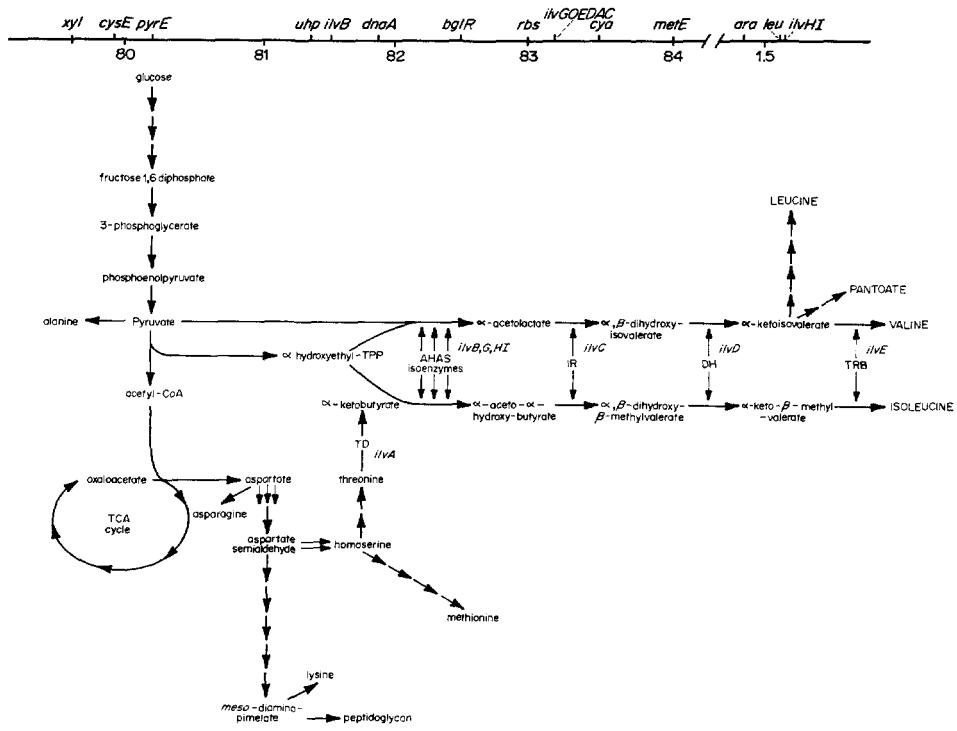

FIGURE 1.—Branched chain amino acids: gene-enzyme relationships. The top of the figure shows a portion of the standard genetic map of *Escherichia coli* K12 after BACHMANN, Low and TAYLOR 1976. The *ilvA* gene product is L-threonine hydro-lyase (deaminating), EC 4.2.1.16; trivial name is theonine deaminase or TD. The *ilvB* gene product is acetolactate pyruvate-lyase (carboxylating) EC 4.1.3.18; trivial name is acetolactate synthase I or AHAS I. This latter gene product is one of three isoenzymes with the same EC number. The other two gene products are: AHAS II, the *ilvG* gene product and AHAS III, the *ilvHI* gene product. The *ilvC* gene product is 3-alkyl-2,3-dihydroxyacid: NADP+ oxidoreductase (isomerizing) EC 4.1.3.18; trivial name is isomeroreductase or IR. The *ilvD* gene product is 2,3 dihydroxy acid-lyase, EC 4.2.1.9; trivial name is dihydroxy acid dehydrase or DH. The *ilvE* gene product is transaminase B or TRB; this enzyme does not have a formal name. A claim was made for a separate valine transaminase (GUARDIOLA 1977), but recent evidence shows that one protein catalyzes both transamination reactions (LEE-PENG, HERMODSON and KOHLHAW 1979; ADAMS, LAWTHER and HATFIELD 1979). The *ilvO* gene product is unknown, but mutations at this locus increase the level of the *ilvEDA* gene products and allow for *ilvG* gene expression. This locus was recently re-mapped by COHEN and JONES 1976. The map position of the *ilvG* gene with respect to the *ilvO* gene was determined by SMITH *et al.* (1979).

The lower part of the figure depicts the metabolic interrelationships between valine and other members of the pyruvate family (alanine, leucine and the pantoate portion of coenzyme A) and isoleucine, a member of the aspartate family (meso-diaminopimelate, lysine, methionine, threonine and asparagine). The thinner arrows stand for isoenzymes, while the thicker arrows represent other biosynthetic steps. Although the isoleucine pathway and the valine pathway are linked by their consumption of α-hydroxyethyl groups and a series of four common enzymes, the major part of their carbon skeletons are made from different sources, which themselves (pyruvate and oxalacetate) have a precursor-product relationship.

mutagenesis, the *ilvB619* allele was introduced into strain MI261, producing the first reported Ilv⁻ *E. coli* strain lacking acetolactate synthase activity (GUARDIOLA, DE FELICE and IACCARINO 1974). The *ilvB619* mutation was subsequently shown to be in the structural gene for *ilvB* by isolating a temperature-sensitive revertant whose phenotype was conditionally Ilv⁻ and whose AHAS I enzyme activity was also temperature sensitive as compared to the wild type (GUARDIOLA *et al.* 1977).

AHAS I and AHAS III activities are inhibited by valine; AHAS II is resistant to valine inhibition (FAVRE *et al.* 1976). However, AHAS II is not expressed in the wild-type strain, even though the *ilvG* gene is structurally intact, because of a mechanism of expression of the *ilvEDA* operon that excludes *ilvG* expression (DE FELICE *et al.* 1977). As a consequence, addition of valine to minimal media inhibits AHAS activity, causing a starvation for isoleucine and a concomitant growth inhibition (DE FELICE *et al.* 1979).

The map location of the *ilvHI* genes between *leu* and *azi* and their identification as the structural genes for AHAS III were unambiguously obtained by three-point testcrosses, deletion mapping and the isolation of temperature-sensitive mutations for both an Ilv⁺ phenotype and enzyme activity (DE FELICE *et al.* 1974a, b; DE FELICE, NEWMAN and LEVINTHAL 1978a). The *ilvB619* allele was shown to be 54%-linked to the *bgl* locus by transduction, and preliminarily assigned to a location between the endpoint of the *ilvDAC115* deletion and the *cya* gene (GUARDIOLA, DE FELICE and IACCARINO 1974). This location coincided with the one previously reported for the *ilvB* locus (RAMAKRISHNAN and ADELBERG 1965).

We chose the analog D,L-5′5′5′ trifluoroleucine (TFL) because it was used in Salmonella to isolate regulatory mutations (CALVO, FREUNDLICH and UMBARGER 1969) and because *E. coli* K12 is not sensitive to it (RENNERT and ANKER 1963). In this study, we report that one class of mutations selected for sensitivity to branched chain amino acid analogs are structural gene mutations at the *ilvB* locus. Such a result is consistent both with the previous observations of GUARDIOLA *et al.* (1977) that strains containing only single AHAS isoenzymes show altered responses to such analogs and our previous report of other classes of mutations with similar phenotypes that affect the amount, rather than the structure, of the AHAS isoenzymes (NEWMAN, T. and M. LEVINTHAL 1975, *Abst. H26 Annual Meetings Am. Soc. Microbiol.* p. 100).

MATERIALS AND METHODS

Bacterial strains: All of the bacterial strains used in this study are derivatives of *Escherichia coli* K12. Their genotypes and origins are listed in Table 1. The genetic nomenclature used conforms to that of BACHMANN, LOW and TAYLOR (1976).

Growth Media: The minimal medium used was Vogel-Bonner Citrate (VBC; VOGEL and BONNER 1956). Carbon sources were added to a final concentration of 0.2% (w/v). L-amino acids and nucleosides, when required, were supplemented at 20 μg/ml unless otherwise noted. Exceptions are L-valine 40μg/ml, L-L-diaminopimelic acid (DAP) 100 μg/ml and thymine 50 μg/ml. All vitamin requirements were supplied at 2 μg/ml.

TABLE 1

Bacterial strains

Strain	Sex	Partial genotype	Source
AB313	Hfr	*thr-1 leuB6 rpsL*	A. L. TAYLOR *via* CGSC
AB1206	F'14	*thi-1 his-4 proA2 lacY1 galK2 rpsL8 tfr-3 supE44*	J. PITTARD *via* CGSC
AT2243-11	HfrC	*pyrE41 uhp-2 relA1 metB1 tonA2*	H. KORNBERG *via* CGSC
AT2538	F⁻	*thi-1 pyrE60 argE3 his-4 proA2 thr-1 leuB6 mtl-1 xyl-5 ara-14 galK2 lacY1 rpsL31 supE44*	A. L. TAYLOR *via* CGSC
CSH61	HfrC	*thi-1 trpR*	MILLER 1972
CU12	F⁻	*galT12 rbs-115*	H. E. UMBARGER
CU44	F⁻	*ilvC462 rbs-115*	H. E. UMBARGER
CU97	F⁻	*galT12 ilvD530*	FAVRE, IACCARINO and LEVINTHAL 1974
E177	F⁻	*thr-1 leuB6 thi-1 thyA6 deoC1 dnaA177 lacY1 rpsL67 tonA2 supE44*	J. WECHSLER *via* CGSC
JM15	F⁻	*cysE50 tfr-8*	M. JONES MORTIMER *via* CGSC
MI261	HfrH	*ilv-603 ilvG605 ilvH612 ilvI614 thi-1 leuB6 relA1* λ⁻	DE FELICE *et al.* 1977
MI316	HfrH	*ilvB619 ilvH612 ilvI614 thi-1 glyA ara bgl*	DE FELICE *et al.* 1977
PS277	F⁻	*ilvB807 galT12*	This paper
PS372	HfrC	*ilvB800::mu-1 thi-1 trpR*	This paper
PS519	F⁻	*cya-283 gal*	BRICKMAN, SOLL and BECKWITH 1973
PS607	F⁻	*ilvA454 rbs-13 gal*	DES Rbs⁻ from *ilvA454 gal*
PS1079	F⁻	*ara-42 rbs-115 xyl-7 lacY1*	(P1) CU12 × ara-42 *cya* derivative KL983 select Cya+
PS1087	F⁻	*ilvA454 ara-42 xyl-7 lacY1 mglP1*	P1 (*ilvA454*) × PS1079 select Rbs+
PS1199	HfrC	Δ*ara leu ilvHI863 trpR thi-1*	DE FELICE, NEWMAN and LEVINTHAL 1978a
PS1282	HfrC	*car-53 ilvB800::mu-1 rbs-115 thi-1 trpR*	P1 (M1162a) × PS1160 select Leu+
PS1283	HfrC	Δ*ara leu ilvHI863 ilvB800::mu-1 rbs-115 thi-1 trpR*	P1 (PS1199) × PS1282 select Car+
PS1418	F⁻	*bgl-30 PS1079*	Spont. arbutin utilizer of PS1079
PS1431	HfrC	*bgl-32 PS1283*	Spont. arbutin utilizer of PS1283
PS1432	HfrC	Δ*ara leu ilvHI863 rbs-115 trpR thi-1 bgl-32*	P1 (CSH61) × PS1431 select IV+
PS1434	HfrC	*bgl-32*+ PS1432	as PS1432
PS1466	F⁻	φ80λdilv+/Δ*ara leu ilvHI863 xyl-7 lacY1 mglP1*	This paper
PS1480	F⁻	*ilvB801* PS1466	This paper
PS1481	F⁻	*ilvB805* PS1466	This paper
PS1482	F⁻	*ilvB806* PS1466	This paper
PS1498	HfrC	*nalA* PS1431	Spont. Nal^r PS1431
PS1506	HfrC	*ilvB801* Δ*(ara-ilvHI863)*	This paper
PS1507	HfrC	*ilvB805* Δ*(ara-ilvHI863)*	This paper

TABLE 1—Continued

Strain	Sex	Partial genotype	Source
PS1508	HfrC	*ilvB806 Δ(ara-ilvHI863)*	This paper
PS1514	F-	*leu+ ara+* AT2538	P1 (CSH61) × AT2538
PS1516	F-	*thr+ Δara leu ilvHI863* PS1514	Mating PS1199 × PS1514 select Thr+
PS1518	F-	*pyrE+* PS1516	P1 (PS1431) × PS1516 select Pyr+
PS1519	F-	*ilvB800::mu-1 pyrE+* PS1516	P1 (PS1431) × PS1516 select Pyr+
PS1521	F-	*ilvB801 pyrE+* PS1516	P1 (PS1506) × PS1516 select Pyr+
PS1523	F-	*ilvB805 pyrE+* PS1516	P1 (PS1507) × PS1516 select Pyr+
PS1525	F-	*ilvB806 pyrE+* PS1516	P1 (PS1508) × PS1516 select Pyr+
PS1526	F-	*ilvB807 pyrE+* PS1516	P1 (PS277) × PS1516 select Pyr+
PS1551	F-	*ilvB619 pyr+* PS1516	P1 (M1316) × PS1516 select Pyr+
PS1556	F-	*Δara leu ilvHI+* PS1518	P1 (CSH61) × PS1518 select Leu+
PS1557	F-	*Δara leu ilvHI+* PS1519	P1 (CSH61) × PS1518 select Leu+
PS1558	F-	*Δara leu ilvHI+* PS1523	P1 (CSH61) × PS1518 select Leu+
PS1559	F-	*Δara leu ilvHI+* PS1523	P1 (CSH61) × PS1523 select Leu+
PS1560	F-	*Δara leu ilvHI+* PS1525	P1 (CSH61) × PS1525 select Leu+
PS1561	F-	*Δara leu ilvHI+* PS1526	P1 (CSH61) × PS1526 select Leu+
PS1562	F-	*Δara leu ilvHI+* PS1551	P1 (CSH61) × PS1551 select Leu+
PS1567	F-	*rbs-115 bgl-30* PS1519	P1 (PS1418) × PS1519 select Bgl+
PS1581	HfrC	*ilvB800::mu-1* AT2243–11	P1 (PS1431) × AT2243–11 select Pyr+
PS1596	F-	*ilvB800::mu-1 bgl-32 rbs-115 thi-1 argE3 rpsL31 Δara leu ilvHI863 mtl-1 xyl-5 galK2 lacY1 recA1*	This paper
PS1598	F-	*cya-283 rbs+* PS1567	P1 (PS519) × PS1567 select Rbs+ (+cAMP)
PS1650		*nalA* PS1518	Spont. Nal^r PS1518
RM309	F-	*tna cysB*	R. SOMERVILLE
SH1002	F'	F'shF8/*ilvD145 metE46 trp pro thi mtl malA ara gal lac recA rpsL tonA bfe tsx*	S. HIRAGA
SH1026	F'	F'shF8–7/*ilvD145 metE46 trp pro thi mtl malA ara gal lac recA rpsL tonA bfe tsx*	S. HIRAGA

Solid media were prepared by the addition of 1.5% agar to the appropriate medium before autoclaving. A special solid medium was used for the selection of *ara* mutations. It consisted of VBC supplemented with 0.2% glycerol, 1.4% L-arabinose and 2.5% ribitol (SCHLEIF 1972). Nal^r (*nalA*) mutants were isolated, selected for and scored in VBC containing glucose and 10 μg/ml nalidixic acid (MILLER 1972).

Rich media were Luria broth (LB) Difco (prepared according to the instructions on the package) and Superbroth (HOWE 1973). Indicator media used to test sugar fermentation consisted of MacConkey base agar with a 1% carbon source. D-maltose was used to score for the *cya* locus. Arbutin was used to score the *bg1R* locus; in this case, 0.1% bromthymol blue was added to the MacConkey base agar.

Mutagenesis: 2-aminopurine mutagenesis was performed as described by MILLER (1972). Mu-1 mutagenesis was accomplished by infecting cells at an MOI of 1 and incubating the cells at 37° for one hr to promote adsorption. Mu-1 phage lysates were prepared according to the procedures of HOWE (1973). Penicillin selections for Ilv^- mutants were performed in VBC plus 0.2% glucose by incubating 10^8 cells in the presence of 333 units of Penicillin-G/ml for 12 to 18 hr at 37° or for 24 to 36 hr when incubated at 30°. The analog-sensitive mutants were enriched for by adding 100 μg/ml TFL to the above medium. This procedure was found to enrich for mutants better than using high concentrations of penicillin for shorter times. Following incubation, cells were plated for growth. Screening for mutants was carried out by picking colonies onto a grid and replica plating to the appropriate media to identify the mutants.

Transduction: Generalized transduction was performed using the P1 clm^r *clr-100* transducing phage using the techniques of ROSNER (1972). The φ80λ CI857t68 $dilv^+$ phage of AVITABLE *et al.* (1972) was used for specialized transduction according to the procedures of PRESS *et al.* (1971).

Matings: Bacterial matings were conducted according to the methods of MILLER (1972), using a donor-to-recipient ratio of 1:20. Liquid matings for the transfer of F′ factors were carried out for 30 min at 37°. Cells were plated after centrifugation and washing with an 0.8% saline solution. Counterselection of the donor was usually done with 100 μg?ml streptomycin since the recipient usually contained an *rpsL* mutation.

Enzyme assays and chemicals: The growth of cells, the preparation of extracts and the enzyme assay for L-threonine deaminase (EC 4.2.1.16) and acetohydroxy acid synthase (EC 4.1.3.18) have been previously described (DE FELICE and LEVINTHAL 1977).

Chemicals used were of the highest grade commercially available and purchased from either Calbiochem or Sigma. All amino acids were of the L form except for D,L-5′-5′-5′ trifluoroleucine (Fairfield Chemical Co.)

RESULTS

The isolation and characterization of analog-sensitive mutants: We performed both chemical mutagenesis and mutagenesis with the mu-1 phage, followed by a penicillin selection as described above in MATERIALS AND METHODS. This procedure yielded two mutations (*ilvB800*::mu-1 and *ilvB807*) that upon subsequent analysis proved to be alleles of the *ilvB* locus. Ilv^+ strains containing mutations at the *ilvB* locus possess reduced amounts of total AHAS activity (GUARDIOLA *et al.* 1977), and the remaining activity has unique kinetic parameters including a rate less dependent upon added FAD than AHAS I (DE FELICE, SQUIRES and LEVINTHAL 1978b). The data presented in Table 2 represent steady-state measurements of threonine deaminase (EC 4.2.16) and total AHAS-specific activities in extracts derived from presumptive *ilvB* mutants (the origins of the *ilvB801*, *805*, and *806* mutations are described below). These strains may be compared to isogenic $ilvB^+$ PS1556. We assayed enzyme activities under two growth conditions, namely, minimal glucose and minimal glucose plus the three

TABLE 2

Enzyme activities of strains containing a presumptive ilvB *mutation*

Strain	Growth medium	TD	AHAS	% AHAS S.A.* − FAD
PS1556	excess ILV	25	32	16
(*ilvB+*)	minimal	38	78	19
PS1557	excess ILV	18	15	77
(*ilvB800::mu-1*)	minimal	27	16	78
PS1558	excess ILV	18	14	80
(*ilvB801*)	minimal	48	20	82
PS1559	excess ILV	17	14	83
(*ilvB805*)	minimal	22	16	77
PS1560	excess ILV	23	15	80
(*ilvB806*)	minimal	79	19	74
PS1561	excess ILV	16	16	77
(*ilvB807*)	minimal	26	19	72
PS1562	excess ILV	17	11	76
(*ilvB619*)	minimal	36	13	74

* This value refers to the amount of activity in the extracts independent of the cofactor FAD in the assay. The specific activities are nmoles product/min/mg protein. Details of extract preparation and enzyme assay may be found in DE FELICE and LEVINTHAL (1977). TD stands for L-threonine deaminase and AHAS stands for acetolactate synthase.

branched-chain amino acids (excess ILV). Table 2 reveals that all strains have reduced amounts of AHAS activity in both growth conditions, and the AHAS levels are largely unresponsive to the absence of isoleucine, valine and leucine in the growth medium. These latter two observations are consistent with an *ilvB ilvHI*$^+$ genotype for strains PS1577–1562. A further confirmation is the observation that the AHAS activity is more independent of FAD addition to the extracts than is the control. The lowered amounts of threonine deaminase activity in the mutant strains is seen only during growth in repressing medium (excess ILV), and even then the differences are less than 50% when compared to the *ilvB*$^+$ control, but the differences may be due to the pleiotropic phenotype of *ilvB* mutations (DE FELICE *et al.* 1977).

We introduced an *ilvHI* mutation into an *ilvB800::mu-1*-containing strain by transduction. The resulting phenotype was Ilv$^-$, confirming that strain PS372 contains an *ilvB* allele or a mutation preventing *ilvB* gene expression.

We performed a reciprocal genetic test of *ilvB* function with the *ilvB807* allele. In this case, we introduced the *ilvB807* allele into an *ilvHI*-containing strain. The phenotype was Ilv$^-$, confirming that an Ilv$^-$ phenotype caused by a lack of AHAS activity requires two mutations, one at the *ilvB* locus and one at the *ilvI* locus.

Plasmids containing an ilvB$^+$ *allele:* Figure 2 shows the genotypes of some F′ plasmids that were used to ascertain the map location of the *ilvB* locus. In the first test, the recipient strain was PS1598 (Ilv$^-$ Cya$^-$), which contains the *cya-283* mutation and the *ilvB800::mu-1* allele. The donor strain was AB1206, the ancestral F′14-containing strain (PITTARD, LOUTIT and ADELBERG 1963). We

selected Cya$^+$ transconjugants and, following single colony isolation, demonstrated their diploidy by showing segregation for the ability to ferment maltose. When tested for the unselected marker *ilvB*$^+$, all eighteen of the diploids required isoleucine and valine. This surprising result suggested that the *ilvB* locus was located outside the map interval covered by the F'14 plasmid. However, we had confirmed the previous result (see below) that *ilvB619* was 54%-linked (by co-transduction) to the *bglR* locus; thus, we tested the shF8 plasmid and some of its derivatives kindly provided by S. HIRAGA.

The plasmid shF8 was selected from HfrKL25 by its ability to complement an *ilvD145* mutation in a *recA1* background and was further screened for its ability to replicate in an Hfr strain (Poh$^+$). The Poh$^+$ phenotype was correlated with the inclusion of the *bglR-dnaA* region of the chromosome in the plasmid. Plasmids line shF8–7 exhibit a Poh$^-$ phenotype and contain deletions (see Figure 2, WADA, YURA and HIRAGA 1977).

The plasmid shF8 complements the *ilvB* mutations in strains PS1498, 1506, 1507, 1508, as well as an *ilvA454* mutation and other loci in the *bgl-dnaA* region. However, the shF8–7 plasmid containing a deletion in this region did not complement any of the *ilvB* mutations, but did complement *ilvA454*. In general, we find that *ilvB* mutations (in an *ilvHI* background) are complemented by a Poh$^+$ plasmid, but not by a Poh$^-$ plasmid (see Figure 2). Therefore, the *ilvB* locus maps between *uhp* and *rbs*.

A new map location for the ilvB *locus:* We determined the co-transduction frequency between the *ilvB* and the *rbs* gene clusters (see Figure 3; Table 7), by measuring the frequency of inheritance of various unselected donor markers when Rbs$^+$ transductants were selected. We found a 4.3% co-transduction frequency between *rbs* and the *ilvB619* gene in the donor (231 transductants tested) and a 3.5% co-transduction frequency between the same *rbs* allele and an *ilvB*$^+$ gene repairing the *ilvB800::mu-1* mutation (280 transductants). In these crosses, we also found *rbs* linked 0.4% to the *uhp-2* allele, 78%-linked to the *ilvC462* mutation, and 54%-linked to the *bgl* region.

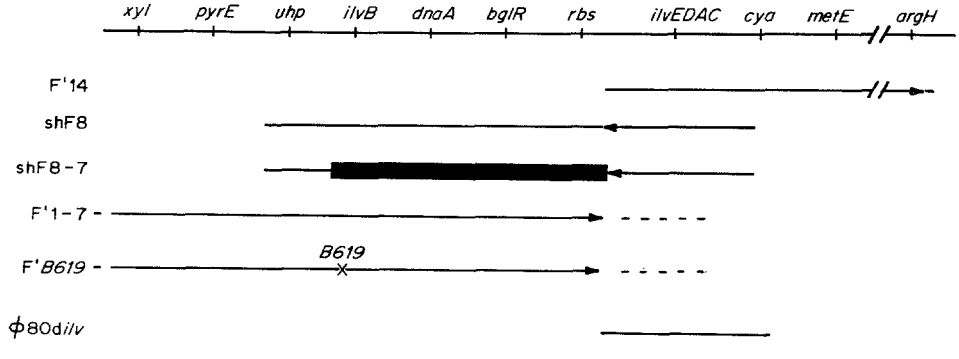

FIGURE 2.—Plasmids carrying regions of the genome from *xyl*-argH. A solid line indicates the genes known to be carried on the plasmid by complementation tests in a *recA* host. The short dashes indicate that exact endpoints are not known. Arrows denote the origin and orientation of the fertility factor. A bar indicates regions that have been deleted.

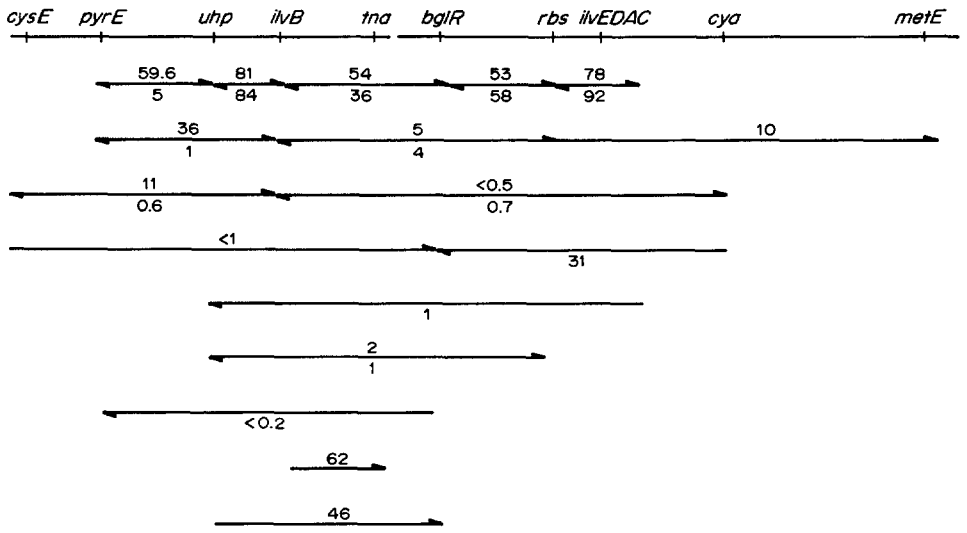

FIGURE 3.—Reciprocal co-transduction frequencies of markers near *ilvB*. The arrowheads point towards the unselected marker. Thus, when *pyrE*+ transductants are selected and the frequency of occurrence of the donor *uhp* allele is scored, the two markers show co-transduction in 59.6% of the cases. However, if *uhp*+ transductants are selected and the donor *pyrE* allele is scored, a co-transduction frequency of 5% is obtained. This asymmetry of results, first observed by MASTERS 1977, is discussed in the text.

TABLE 3

The linkage of uhp *to the individual* ilvB *alleles*

Recipient	Number of Ilv+ transductants analyzed	% Donor marker	
		uhp	*Δara leu ilvHI*+
PS1519 (*ilvB800::mu-1*)	231	78	8.2
PS1551 (*ilvB619*)	230	73	0.8
PS1521 (*ilvB801*)	230	79	2.0
PS1523 (*ilvB805*)	231	84	7.7
PS1525 (*ilvB806*)	276	86	7.6
PS1526 (*ilvB807*)	231	86	7.3

The donor is a P1 lysate grown on AT2243-11 (*uhp-2*). The *uhp-2* allele was scored as growth on 0.2% glucose-6-phosphate as the sole carbon source with the appropriate supplements. Repair of the *Δara leu ilvHI863* deletion was scored as Leu+.

We tested the linkage of the *uhp* gene to the *ilvB* locus by crossing a P1 phage lysate grown on a strain containing a *uhp* mutation with various *ilvB ilvHI* mutant strains and selecting for Ilv$^+$ transductants (Table 3). When we select for Ilv$^+$, we obtain either *ilvHI*$^+$ or *ilvB*$^+$ recombinants. However, the *ilvHI*$^+$ recombinants can be recognized when a strain carrying the Δ(*ara-ilvHI863*) deletion is used, as this class will be Ara$^+$ and Leu$^+$. Table 3 shows that all *ilvB* alleles are closely linked to the *uhp-2* allele of the donor.

Three-point testcrosses to locate the ilvB *locus:* We performed three-point testcrosses with strains containing the *ilvB800*::*mu-1* mutation. These crosses are presented in Table 4, where in each case we select Ilv$^+$ transductants and score Bgl, Rbs, or Uhp. In the first cross, both the donor and the recipient contain the *ara leu ilvHI863* deletion, so that all Ilv$^+$ recombinants are *ilvB*$^+$. In crosses two and three, the Ilv$^+$ transductants were scored for Leu$^+$, and this class was excluded from the three-point data. Cross number one confirms the order *ilvB–bgl–rbs*: if *ilvB* were located between *bgl* and *rbs*, we would not expect such a high proportion of *bgl*$^+$ *rbs*$^+$ transductants. In cross number two, we reverse the *bgl* and *rbs* genotype as compared to cross one. The data of Table 4 reveal that now the *bgl*$^-$ *rbs*$^-$ recombinant class becomes significant, as expected

TABLE 4

Three-point testcrosses locating ilvB800::mu-1 *between* uhp *and* bgl

Cross no.	Strain	Smallest class as double crossovers			Number of unselected transductants			
					bgl$^-$*rbs*$^-$	*bgl*$^-$*rbs*$^+$	*bgl*$^+$*rbs*$^-$	*bgl*$^+$*rbs*$^+$
	PS1432 (*bgl-32 rbs-115*)	+	−	−				
1.		*ilvB800*	*bgl*	*rbs*	6	90	0	84
	PS1519 (*ilvB800::mu-1*)	−	+	+				
	CSH61 (*ilvB*+)	+	+	+				
2.		*ilvB800*	*bgl*	*rbs*	117	4	133	12
	PS1431 (*ilvB800::mu-1 bgl-32 rbs-115*)	−	−	−				
					bgl$^-$*uhp*$^-$	*bgl*$^-$*uhp*$^+$	*bgl*$^+$*uhp*$^-$	*bgl*$^+$*uhp*$^+$
	AT2243-11 (*uhp-2*)	−	+	+				
3.		*uhp*	*ilvB800*	*bgl*	59	17	106	30
	PS1567 (*ilvB800::mu-1 bgl-32*)	+	−	−				

All recipients contain the Δ*(ara-ilvHI863)* deletion. For complete genotypes for all strains, see Table 1. All data are corrected for repairs of the deletion so that the data represent transductional events at *ilvB* only. In all three crosses, Ilv+ transductants were selected on glucose-minimal plates, and the unselected classes scored following replica plating to the appropriate medium.

from the map order. We do not consider the order *bgl–rbs–ilvB* because of the low co-transduction between *ilvB* and *rbs* as compared to the linkage between *ilvC* and *rbs* and because of the results of cross number three. This cross locates *ilvB* between *uhp* and *bgl*. If the order were *uhp–bgl–ilvB*, we would not expect so many *bgl*$^-$ *uhp*$^-$ transductants. The data favor the order *uhp–ilvB–bgl* over *ilvB–uhp–bgl* (17 *bgl*$^-$ *uhp*$^+$ transductants *vs.* 30 *bgl*$^+$ *uhp*$^+$ transductants).

The order of ilvB *and* dnaA: We were particularly interested in the map order of *ilvB* and *dnaA* because *dnaA* is located near *oriC* (BACHMANN, LOW and TAYLOR 1976). Accordingly, P1 phage were grown on strain E177 (*dnaA177*) and crossed to PS1567 (Table 5). Both Ilv$^+$ and Rbs$^+$ transductants were selected from the same transducing mixture. We incubated the cross at 30° because *dnaA177* is a thermosensitive lesion; its phenotype is growth sensitive at 40° on complex medium. As shown in Table 9, *dnaA177* was not inherited by any of the transductants. However, the frequency of inheritance of the donor *bgl* allele in both selections is reduced when the *dnaA177*-containing strain is the donor as compared to the *dnaA*$^+$ donor. These results could be explained if *dnaA177* were lethal in strain PS1567. We know that the *dnaA177* locus is present in our lysates of E177 because it can be transferred as an unselected marker to strain PS1581 by co-transduction (5/27) with *uhp*. The 2% inheritance of the donor's *bgl* marker when *ilvB* is selected would represent the quadruple crossover class, whereas the less severe linkage reduction between *bgl* and *rbs*, when *Rbs*$^+$ transductants are selected, suggests the order *dnaA–bgl–rbs*. This technique of mapping lethals by looking for missing classes was first proposed by MORGAN (1912). On this basis, we assign the map order *ilvB–dnaA–bgl–rbs*.

Additional tests of the new map location for the ilvB *gene:* We performed a number of additional experiments to test certain predictions suggested by the new map location for the *ilvB* locus. First, we confirmed by transduction that the *ilvB619* allele and the *ilvB800::mu-1* allele were closely linked. We conducted a linkage test by using a single recipient, MI316, and P1 phage lysates. Donor phage were grown on two isogenic strains (PS1283 and PS1434) that differed only at the *ilvB800::mu-1* locus. We selected *glyA*$^+$ transductants in order to monitor the number of transducing particles; both lysates had equal

TABLE 5

Disruption of linkage between ilvB *and* bgl *by* dnaA177

Donor	Selection	Number scored	% Transductants inheriting donor's unselected markers			
			ilvB	*dnaA*	*bgl*	*rbs*
E177 (*dnaA177*)	IlvB+	311	100	0	2.3	1.0
	Rbs+	539	0.2	0	35	100
CSH61 (*dnaA*+)	IlvB+	1598	100	—	58	4.4
	Rbs+	1073	2.4	—	57	100

The recipient in all crosses was PS1431 (*ilvB800::mu-1 bgl-32 rbs-115* Δ*ara leu ilvHI863*). All selections for IlvB have been determined by monitoring repairs of the deletion by scoring Leu+. Scoring of the *dnaA177* allele was done at 42° on nutrient agar.

transducing ability by this criterion. The linkage between the two mutations may then be determined by comparing the observed numbers of Ilv$^+$ transductants between the P1 lysate grown on PS1283 (*ilvB800::mu-1*) with the lysate derived from PS1434 (isogenic *ilvB*$^+$). We observed a 50-fold reduction in the appearance of *Ilv*$^+$ transductants when the donor strain was PS1283, showing that *ilvB619* and *ilvB800::mu-1* are closely linked.

Another prediction tested is that the *ilvB*$^+$ allele should not be included on the ϕ80*dilv*$^+$ transducing phage isolated from an F'16 plasmid (LoSchiavo *et al.* 1975). The diploid strain PS1466 (*ilvB*$^+$ Δ(*ara-ilvHI863*)/ϕ80λCI857 dilv*EDAC*$^+$) was subjected to 2-aminopurine mutagenesis, followed by penicillin selection, and plated on minimal glucose leucine plates containing suboptimal amounts of isoleucine and valine (2 μg/ml). Three independent mutants requiring isoleucine and valine (arising from different mutagenesis tubes) were isolated from a total of 300 colonies picked after penicillin selection. All three colonies were shown to contain the ϕ80 *dilv*$^+$ transducing phage.

The presumptive *ilvB* mutants (PS1480,1481,1482) were used as recipients in a P1-mediated transduction. The donor strain was *leu*$^+$ *ilvHI*$^+$ *rbs bgl*, and Ilv$^+$ transductants were selected on minimal glucose containing leucine. As can be seen in Table 6, Ilv$^+$ transductants arose either by a recombination event in the *bgl* region or by the replacement of the *ara leu ilvHI* deletion. Strain MI316, containing the *ilvB619* mutation in an *ilvH612 I614* background, shows comparable inheritance of the donor *bgl* and *rbs* alleles when Ilv$^+$ transductants are selected. Strain PS1431 contains a mu insertion at or near the *ilvB619* locus. When strain PS1431 is used as a recipient in this type of cross, it gives similar results to both the *ilvB619*-containing strain (PS878) and the newly isolated *ilvB* mutations.

TABLE 6

Linkage of Ilv+ transductants to leu *and* bgl *regions of the chromosomes*

Donor	Recipient	No. Ilv+ transductants having the unselected donor marker*		
		rbs	*bgl*	*leu*
PS1418(rbs-115 bgl-30)	PS1480 (*ilvB801 Δara leu ilvHI863*)	2	125	3
PS1418	PS1481 (*ilvB805 Δara leu ilvHI863*)	6	123	20
PS1418	PS1482 (*ilvB806 Δara leu ilvHI863*)	1	131	13
PS1434(rbs-115)	MI316 (*ilvB619 bgl ilvH612 ilvI614*)	7	81	nd
CSH61(*rbs*+ *bgl*+ *leu*+)	PS1431 (*ilvB800::mu-1 bgl-32 Δara leu ilvHI863*)	10	92	28

* In each case, 180 Ilv$^+$ transductants were scored for their *Rbs*, *Leu* and *Bgl* phenotypes, as described in MATERIALS AND METHODS. The abbreviation "nd" means not determined, since both donor and recipient are *ilvHI*$^-$.

We expect to see Ilv$^+$ transductants occurring at two loci if the induced mutation is at the *ilvB* locus, since either gene specifying a Val^s AHAS can be repaired to give prototrophy. In crosses 1 through 3 of Table 6, we performed a separate selection for Leu$^+$ (from the same transduction tube) under conditions where the Ilv phenotype was unselected. In all three cases, we found that 100% (45 of 45 tested in each case) of the Leu$^+$ transductants were also Ilv$^+$. The data shown in Table 2 demonstrate that the enzyme activity profile for the new *ilvB* mutant strains matches that of *ilvB800::mu-1* and *ilvB619*. We conclude that *ilvB* mutations in a strain diploid for the *ilvEDAC* region have been isolated.

We further tested whether these newly isolated *ilvB* mutations were located in the same region of the chromosome as the *ilvB800::mu-1* allele by isolating a series of episomes containing the *ilvB* alleles and showing that the episome failed to complement an *ilvB800::mu-1*-containing strain in a *recA1* background (PS1596). We found that mating Hfr AB313 with PS1596 and selecting for Xyl$^+$ produced episomes that made PS1596 Ilv$^+$, presumably by the introduction of the *ilvB*$^+$ gene. These diploids are all valine sensitive and thus do not reflect the expression of the *ilvG* gene. We introduced the *ilvB619*, *B801*, *B805*, *B806* and *B807* alleles into AB313 and repeated the cross. In each case, all of the Xyl$^+$ transconjugants remained Ilv$^-$, indicating that the episomes carried an *ilvB*$^-$ allele, or they did not carry the *ilvB* gene at all. To insure that a mutant *ilvB* allele was on the episome, the diploids of PS1596 were crossed to a recA$^+$ Δ(ara-ilvHI863)-containing strain (PS1650). In each case, some of the Xyl$^+$ exconjugants were Ilv$^+$, which can occur only by recombination between the *ilvB*$^-$ allele on the episome and the recipient chromosome. Therefore, each of these mutations is allelic to *ilvB800::mu-1*, and they can be considered as structural mutations in the *ilvB* gene.

In Table 7, we have pooled all of our two-point transduction data. We grew P1 phage on a variety of mutant strains and crossed them to recipients containing either one of the *ilvB* alleles, or the *pyrE60* allele or the *rbs-115* mutation, or an *ilvD530* or an *ilvC462* mutation. All the *ilvB* alleles are closely linked to *uhp* and *tna* (the structural gene for tryptophanase). However, in our hands, scoring *tna* as an unselected marker was tedious and unreliable. Notice that *pyrE* and *ilvB* appear to have a co-transduction frequency of 0.5% when an *ilvB* mutation is the recipient and a *pyrE*-containing strain is the donor (line 1, column 3); whereas, when the donor and recipient are reversed, the apparent co-transduction frequency is 35% (line 3, column 4). This result can be contrasted with the *rbs-ilvB* co-transduction frequency, which is the same in reciprocal crosses (line 1, column 7 and line 4, column 4). Asymmetry of co-transduction values when the donor and recipient are reversed has been observed in this region when the markers being tested are separated by the origin of replicaton (MASTERS 1977).

The effect of mu-1 phage lysogeny on co-transduction frequencies: Our mapping data for the location of *ilvB* are internally consistent with the data published for the markers used in this study (BACHMANN, LOW and TAYLOR 1976, WECHSLER and ZDZIENICKA 1975). A comparison of the co-transduction values

TABLE 7

Co-transduction frequencies in the pyrE-metE *region*

Recipients	Donors									
	cysE	*pyrE*	*uhp*	*ilvB*	*tna*	*bgl*	*rbs*	*ilvD,C*	*cya*	*metE*
ilvB	0.011	0.005	0.813	—	0.70	0.53	0.052	—	0.005	—
	(168)	(2234)	(1559)		(183)	(2525)	(1879)		(373)	
pyrE	—	—	0.59	0.35	—	0.10	0.003	0.007	—	—
			(230)	(715)		(851)	(273)	(133)		
rbs	—	<0.001	0.004	0.037	—	0.58	—	0.78	—	0.10
		(623)	(539)	(999)		(858)		(231)		(100)
ilvD or *ilvC*	—	—	—	—	0.56	—	0.95	—	0.71	0.21
					(135)		(180)		(154)	(576)

"( )" indicates the number of transductants scored for the linkage computation. This experiment was conducted by growing P1 phage on a strain containing a mutation in the gene indicated. The recipient had a mutation in the gene indicated, and repair of this mutation was selected. The *cysE50* mutation was unselected on minimal cystine, the *pyrE60* allele was unselected on minimal plus uracil and cytosine, the *metE70* allele was unselected on minimal methionine and the *tna* allele was unselected on minimal medium, as were all the fermentation markers. The other alleles used were *rbs-115*, *uhp-6*, *bgl-30*, *ilvD530*, *ilvC462*, *cya-283*. For the *ilvB* data, *ilvB801*, *ilvB804*, *ilvB805*, *ilvB806*, *ilvB807* and *ilvB619* were used both as donor and recipients; *ilvB800::mu-1* was used only as a recipient.

found by MASTERS (1977) with those in Figure 3 show essentially identical frequencies.

The *ilvB800* mu-1 allele can be used in mapping by observing the alteration of co-transduction frequencies. When mu-1 inserts between two genes, the co-transduction of those two genes is greatly reduced when the mu-1 lysogen is used as a donor in P1 transduction (BUKHARI and TAYLOR 1971). We show that the *ilvB800* mu-1 allele when used as a donor disrupts the linkage between both *pyrE* and *bglR* and *uhp* and *bglR* (Table 8). Both of these observations are consistent with the co-transduction data and the three-point testcross analysis of these genes. The most interesting observation from Table 8 is that the presence of mu-1 in the donor can actually increase the co-transduction frequencies of adjacent markers. Without mu-1 in the donor, we observe a co-transduction frequency of 5.2% between *uhp* and *pyrE* when we select *Uhp*$^+$ and score *pyrE*. If we perform the identical cross with a donor containing mu-1 (inserted in the *ilvB* gene) adjacent to the *uhp* gene, the apparent co-transduction frequency increases to 12.8%. However, the co-transduction frequency in this cross depends in large part upon the number of transducing fragments containing both the *uhp*$^+$ and *pyrE* alleles. We expect an equal number of transducing fragments containing the *uhp*$^+$ gene and genes clockwise to it (not containing the *pyrE* gene) and transducing fragments containing the *uhp*$^+$ gene and the genes counter-clockwise to it (containing the pyrE$^+$ gene). If the mu phage insertion reduced the latter class of transducing fragments, while leaving the frequency of the former class unaffected, we would see the observed apparent increase of co-transduction frequency when it is measured as we have in Table 8. However, if the absolute number of Uhp$^+$ transductants is measured, we should be able to see a reduction in Uhp$^+$ transductants due to the reduction of transducing fragments that must carry the integrated mu-1 phage. This prediction is verified in Table 9. When the relative transducing abilities of lysates are compared, we not only see a decrease in the number of Uhp$^+$ transductants, but also the expected decrease in Uhp$^+$ Pyr$^+$ transductants.

TABLE 8

Linkage distortion when ilvB800::mu-1 *is used as a donor in P1 mediated transduction*

Selected phenotype	Unselected phenotype	% Donor phenotype PS1432 —	mu-1	PS1431 +
PyrE+	Uhp	59.6		12.1
PyrE+	IlvB	24.0		0.9
PyrE+	BglR	9.6		0.3
Uhp+	Pyr	5.2		12.8
Uhp+	Bgl	45.9		0.4
Uhp+	Rbs	1.7		0.4

In this cross, the donor is PS1506 (ilvB801) and the recipient is PS1516 (*pyrE41 Δara leu ilvHI863*). The other recipient strain is AT2243–11 (*pyrE60 uhp-2*). The number of transductants in each cross analyzed was 231.

TABLE 9

Reduction of P1 transducing ability due to mu-1 prophage in the donor

Selected phenotype	Relative transducing potential* PS1432 donor —	mu-1	PS1431 +
Met+	1.0		1.0
Uhp+	9.1		0.12
Uhp+ PyrE+	0.1		0.01

* Equal aliquots of transduction mixture were plated from the same tube for each cross. AT2243-11 (*metB1 uhp-2 pyrE41*) was the recipient. Duplicate plate counts determined the number of transductants that were then standardized to the number of Met+ transductants obtained per 0.1 ml of transduction mixture plated.

DISCUSSION

We feel that our evidence for the map location of the *ilvB* locus is conclusive. The mutations that we describe all lower the total activity of AHAS; they all have an Ilv⁻ phenotype when combined with an *ilvHI* mutation, and they are closely linked to *ilvB619*. We have shown that *ilvB* mutations can be isolated in a strain diploid for the *ilvEDAC* gene cluster. We believe that this result rules out the published map location for *ilvB*. We have presented data for a large number of two-point crosses that locate the *ilvB* alleles between the *uhp* and *dnaA* genes. We have noted the phenomenon, first described by MASTERS (1977), that reciprocal transduction data between two markers separated by the replication origin are not equivalent, but depend on which marker is selected. It is possible that this or a related phenomenon caused the discrepancy between our results and those published previously (GUARDIOLA, DE FELICE and IACCARINO 1974). In the reported cross, *Ilv*+ was selected and *cya* was an unselected marker. MASTERS (1977) reported that although the *uhp* and *ilv* genes are co-transducible with a frequency of less than 1%, a large proportion (up to 40%) of the transductant clones contain a few cells expressing the unselected marker. We have found a similar phenomenon in crosses with *ilvB* and *cya*. An additional problem not anticipated in the prior mapping studies was the interaction between the *ilvB* gene and the *cya* gene. A recent study by FREUNDLICH (1977) suggested that cAMP is required for maximal expression of the *ilvB* gene. MAXINE LEVINTHAL (personal communication) has observed that an *ilvB*+ *ilvHI cya*+ strain grows faster in glucose minimal medium than an *ilvB*+ *ilvHI cya* strain. Therefore, we can suppose that in a mixed clone of *cya*+ and *cya*⁻ transductants, the former genotype would be favored over the latter.

LOSCHIAVO *et al.* (1975) showed that a ϕ80λd*ilv*+ transducing phage would complement an *ilvB ilvHI* double-mutant strain. However, the Ilv+ transductants were Val^r (M. IACCARINO, personal communication). We have confirmed this result with both the F'14 episome and the ϕ80d*ilv*+ transducing phage. In the latter case, we find that the number of transducing particles for AHAS function is 1000-fold lower than the number for threonine deaminase or dehydrase function. We suggest that the unexpressed *ilvG* gene, which is known to be on both

of these plasmids (FAVRE *et al.* 1976), is expressed in a small fraction of the plasmids when an *Ilv*$^+$ phenotype is selected in an *ilvB ilvHI* double mutant. Using the F'14 plasmid, we find that if *cya*$^+$ is selected in an Ilv$^-$ (AHAS$^-$) mutant strain, then all of the diploids (19 of 19 tested and verified by segregation analysis) remain Ilv$^-$; whereas, if an identical test is performed with an *ilvD* mutant strain, all of the diploids are *Ilv*$^+$ (T. NEWMAN and M. LEVINTHAL, unpublished). We believe that the above information explains our observation that chromosomal *ilvB* mutations could be isolated from a strain lysogenized with the ϕ80λd*ilvEDAC*.

Finally, our results with mapping studies on the *mu-1* insertion requires some comment. The use of linkage disruption between two markers flanking a mu insertion is a useful mapping technique, provided the proper controls are also done. We would recommend determining the transducing particle frequency for the regions at and near the transduction site. Furthermore, the flanking marker co-transduction frequency should be compared to the reduction in linkage caused by the mu insertion when it is *both* clockwise and counterclockwise to the co-transduced markers.

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