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# Mutant of *Escherichia coli* K-12 Missing Acetolactate Synthase Activity

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A mutant requiring isoleucine and valine for growth, because of the absence of acetolactate synthase activity, has been isolated. At least one of three different genes (*ilvG*, *ilvB*, *ilvI*) is required for the expression of acetolactate synthase activity, thus suggesting the presence of three different acetolactate synthase isoenzymes.

The assumption that acetohydroxy acid synthase (acetolactate pyruvate-lyase[carboxylating], EC 4.1.3.18; called acetolactate synthase) activity is obligatory for isoleucine and valine biosynthesis in *Escherichia coli* K-12 and other *Enterobacteriaceae* is based principally on the fact that no other enzyme has been described that forms acetohydroxybutyrate and acetolactate, known precursors of isoleucine and valine, respectively. Furthermore, this enzyme activity is inhibited by valine and is repressed or derepressed together with other *ilv* gene products (18).

Isoleucine- and valine-requiring ($Ilv^-$) mutants, lacking the acetolactate synthase, have never been isolated from *E. coli* K-12, *E. coli* B, or *Salmonella typhimurium*. This failure could be due to the presence of more than one acetolactate synthase activity. The presence of two acetolactate synthase activities, apparently involved in isoleucine and valine biosynthesis, one sensitive ($Val^s$) and one resistant ($Val^r$) to valine inhibition, has been reported for *S. typhimurium*, *E. coli* B (1, 9, 10), and *Aerobacter aerogenes* (8). Evidence for the occurrence of two acetolactate synthases in *E. coli* K-12 has been reported (12, 14). Working with the same strain, we were able to detect a $Val^r$ acetolactate synthetic activity only when the *ilv0603* mutation was in the strain. This activity was abolished by the *ilvG605* (amber) mutation (M. Iaccarino, R. Favre, J. P. O'Neill, and M. Freundlich, manuscript in preparation). We have also found mutations in another gene, *ilvI*, located close to *leu*, which abolish the presence of another acetolactate synthase (M. De Felice, J. Guardiola, B. Esposito, and M. Iaccarino, manuscript in preparation). When a strain containing mutations in both the *ilvG* and the *ilvI* genes is prepared by transduction (strain MI261), the double mutant is still $Ilv^+$ and extracts of this strain still contain acetolactate synthase activity. Since a gene has been described, (*ilvB*; 14, 15) that has been implicated in the expression of acetolactate synthase activity, it is possible that among the $Ilv^-$ mutants isolated from strain MI261 (*ilv0603*, *ilvG605*, *ilvI614*) there are those with lesions in the *ilvB* gene that lack acetolactate synthase activity. Mutants were isolated from strain MI261 by ultraviolet mutagenesis (with a dose that kills approximately 90% of the bacteria) and penicillin counterselection (4). Nineteen $Ilv^-$ mutants were obtained and purified by single colony isolation, and then acetolactate synthase activity was measured qualitatively as follows. Logarithmic phase cultures of these strains were grown in minimal medium to a concentration of $8 \times 10^8$ cells per ml. To a 1-ml portion of each culture, 2.4 ml of a solution containing 4.25 mmol of NaOH, 2.5 mg of creatine, and 25 mg of $\alpha$-naphtol (freshly prepared) was added, and the mixture was incubated at 37 C for 60 min. Although an $Ilv^+$ strain developed a red color owing to the presence of small amounts of acetoin or diacetyl (16), 3 of the 19 $Ilv^-$ strains gave no color, thus indicating the absence of acetolactate synthase activity. These three strains (no. 1, 10, and 12 in Table 2), the parental strain MI261, and two strains (no. 5 and 8) randomly chosen from the remaining 16 strains, were assayed for acetolactate synthase activity (16). Strains 1, 10, and 12 showed very low activity, whereas strains 5 and 8 showed a level of acetolactate synthase activity comparable to that of the parental strain (Table 2). Strain 1 was retained for further study and was called MI262. The mutation causing the $Ilv^-$ phenotype was named *ilvB619*. Strain MI262 still contained a low level (~0.3 nmol per min

TABLE 1. *Bacterial strains*[a]

| Strain | Genotype | Origin |
|---|---|---|
| Ca85 | *thi, his, lac*$_{am}$, HfrH | J. Beckwith |
| MI148a | *thi, metE, proC, purE, trp, lysA, ara, xyl, lacZ, azi, str, tonA, tsx;* F$^-$ | (7) |
| MI261 | *thi-1, leu-8, ilv0603, ilvG605, ilvH612*[b], ilvI614; HfrH | This laboratory (DeFelice et al., manuscript in preparation) |
| MI262 | *thi-1, leu-8, ilv0603, ilvG605, ilvH612, ilvI614, ilvB619;* HfrH | This paper |
| MI262b | *thi-1, glyA, ilv0603, ilvG605, ilvH612, ilvI614, ilvB619;* HfrH | *glyA* derivative of a Leu$^+$ Ara$^-$ transductant of strain MI262 |
| MI262g | *thi-1, glyA, bgl*[c]*, cya, ilv0603, ilvG605, ilvH612, ilvI614, ilvB619, ara;* HfrH | *bgl, cya* (17) derivative of strain MI262b |
| MI262H | *thi-1, glyA, ilvDAC115, ilvH612, ilvI614, ara;* HfrH | Cross of MI262b and AB3590 (J. Guardiola, M. De Felice and M. Iaccarino, manuscript in preparation) |

[a] Symbols for genetic markers are those used by Taylor and Trotter (17).

[b] When the *ilvH* gene is mutated the *ilvI* acetolactate synthase is found resistant to valine inhibition (M. DeFelice et al., manuscript in preparation). Its occurrence in strain MI261 and its derivative does not appear to affect the interrelationships of the *ilvG, B,* and *I* reported in this paper.

[c] Wild-type *E. coli* K-12 (*bgl*$^+$) does not ferment β-glucosides (phenotype Bgl$^-$). *bgl*$^-$ mutants ferment β-glucosides and show a Bgl$^+$ phenotype.

per mg of protein) of acetolactate synthase activity. This activity disappeared in the control tubes where the substrate was missing; it was resistant to inhibition by 1.5 mM valine (final concentration).

Strain MI262 (*leu, met*$^+$, *ilv*) was crossed with P1 phage grown on strain MI148a (*leu*$^+$, *metE, ilv*$^+$), and Ilv$^+$ transductants were selected. Among these transductants, two classes are interesting: Leu$^+$Met$^+$ (23%; 11/48) and Leu$^-$Met$^-$ (4%; 2/48). The presence of these two classes showed that strain MI262 became Ilv$^+$ upon introduction of either a gene(s) co-transduced with *metE* or a gene(s) co-transduced with *leu*. We believe that the former are *ilvB*$^+$ or (with lower frequency) *ilvG*$^+$ *ilv0603* transductants and the latter are *ilvI*$^+$ transductants. Strain MI262, therefore, contained a mutation contributing to an Ilv$^-$ phenotype and lying far from the other known Ilv$^-$ mutations previously described at 75 min on the *E. coli* map (17). Another cross was made by treating strain MI262b (*ilvG605, ilvI614, ilvB619, ara*) with P1 phage grown on strain Ca85 (*ilv*$^+$, *ara*$^+$) with selection of Ara$^+$ transductants. Sixty-nine percent of them (66/96) were Ilv$^+$ (this is the co-transduction frequency of *ara* with *ilvI*). The cultures of 20 of these Ilv$^+$ transductants (randomly chosen), but not of the Ilv$^-$ strain MI262b, developed a red color when incubated with α-naphtol as described above, thus suggesting the presence of an acetolactate synthase activity.

The Ilv$^-$ phenotype due to mutation(s) located at 75 min on the chromosome (15) was analyzed as shown in Fig. 1. A preliminary experiment showed that, upon introduction of an *ilvG*$^+$ allele, strain MI262 became Ilv$^+$. Therefore, strain MI262g (Ilv$^-$ because of mutations in the *ilvG, ilvB,* and *ilvI* genes) was treated with P1 grown on strain MI262h (Ilv$^-$ because of the *ilvDAC115* mutation), and Ilv$^+$ transductants were selected. None of them can be *ilvI*$^+$ transductants because both strains are *ilvI*$^-$. The evidence showing the *ilvG* gene location will be described elsewhere (Iaccarino et al., manuscript in preparation). The *ilvB* gene location has been reported (15). Of the Ilv$^+$ transductants, 62.5% (62/96) were Bgl$^-$, Cya$^-$ (genotype *bgl*$^+$, *cya*) and 27.5% (34/96) were

TABLE 2. *Acetolactate synthetase activity in Ilv$^-$ strains as compared to an Ilv$^+$ parental strain (MI261)*

| Strain | Acetolactate synthetase sp act[a] | Colour formation in the culture |
|---|---|---|
| MI261 | 26 | + |
| No. 1 | <0.3 | − |
| No. 5 | 25 | + |
| No. 8 | 26 | + |
| No. 10 | <0.4 | − |
| No. 12 | <0.1 | − |

[a] Enzymatic activity was assayed according to Stormer and Umbarger (16) and is expressed as nmol of product formed per min per mg of protein.

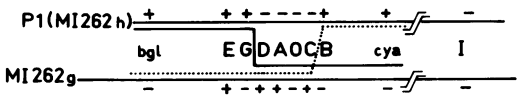


FIG. 1 *Cross between strain MI262g (recipient) and P1 grown on strain MI262h (donor). Ilv$^+$ transductants were selected. Details on the Bgl and Cya phenotype are found in Taylor and Trotter (17) and in Table 1.*

Bgl$^+$,Cya$^+$. The Bgl$^-$,Cya$^-$, Ilv$^+$ transductants were Val$^r$, thus confirming that they are ilvG$^+$ since the acetolactate synthase expressed in an *ilv0603 ilvG*$^+$ strain was Val$^r$ (see above). The Bgl$^+$,Cya$^+$ Ilv$^+$ transductants were Val$^s$. They show that there was a gene located between *ilvDAC115* and *cya* that permitted the expression of a Val$^s$ acetolactate synthase. This location coincides with that reported for *ilvB* (15).

The Ilv$^-$ strain MI262 can be made Ilv$^+$ by introduction of either the *ilvG* gene (in the presence of *ilv0603*), the *ilvB* gene, or the *ilvI* gene. This result suggests the presence in *E. coli* K-12 of three acetolactate synthase isoenzymes, each of which is capable of catalyzing the biosynthesis of isoleucine and valine intermediates. This situation seems analogous to the 3-deoxy-D-arabinoheptulosonic acid-7-phosphate synthetase isoenzymes (19) and to the aspartokinase isoenzymes (2). The *ilvG* acetolactate synthase was Val$^r$, whereas the other two were Val$^s$. (However, in the presence of the *ilvH612* allele, the *ilvI* product was also Val$^r$.) It is clear that the growth of *E. coli* K-12 was sensitive to valine inhibition, because the *ilvG* acetolactate synthase was not expressed in this strain unless there was an *ilvO* mutation such as *ilv0603* (see above). The *ilvG* product was expressed in strains *E. coli* W, *E. coli* B, and *S. typhimurium* (1, 9, 10), and this explains the Val$^r$ phenotype of these strains. Mutants of *E. coli* K-12 altered in the *ilvO* gene have been described (13, 14, and Iaccarino et al. manuscript in preparation) which are resistant to very high concentrations of valine. We believe that their resistance, as is that of the strain carrying the *ilv0603* mutation, is due to the expression of the *ilvG* gene. A second class of Val$^r$ mutants is that in which a Val$^s$ acetolactate synthase becomes Val$^r$ (11, 14, and De Felice et al., manuscript in preparation). A third class of Val$^r$ mutants consists of transport mutants (3, 5, 6, 7).

Experiments are in progress to determine if *ilvG* and *ilvB* are the structural genes for different acetolactate synthase isoenzymes and to study their regulation.

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