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 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+) and one resistant (Val−) 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+ 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 × 10⁶ 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 α-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
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-naphthol 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 Bgl+, Cya-.

### Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI261</td>
<td>thi-1, leu-8, ilvG605, ilvH612, ilvI614, ilvB619, ara; HfrH</td>
<td>This laboratory (De Felice et al., manuscript in preparation)</td>
</tr>
<tr>
<td>MI262b</td>
<td>thi-1, gba, ilvI614, ara; HfrH</td>
<td>Cross of MI262b and AB3590 (J. Guardiola, M. De Felice and M. Iaccarino, manuscript in preparation)</td>
</tr>
<tr>
<td>MI262H</td>
<td>thi-1, gba, ilvDAC115, ilvI614, ara; HfrH</td>
<td>J. Beckwith (7)</td>
</tr>
</tbody>
</table>

* Symbols for genetic markers are those used by Taylor and Trotter (17).

**When the ilvG gene is mutated the ilvG acetolactate synthase is found resistant to valine inhibition (M. De Felice 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.

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

### Table 2. Acetolactate synthetase activity in Ilv- strains as compared to an Ilv+ parental strain (MI261)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Acetolactate synthetase sp act*</th>
<th>Colour formation in the culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI261</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>&lt;0.3</td>
<td>+</td>
</tr>
<tr>
<td>No. 5</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>No. 8</td>
<td>26</td>
<td>+</td>
</tr>
<tr>
<td>No. 10</td>
<td>&lt;0.4</td>
<td>-</td>
</tr>
<tr>
<td>No. 12</td>
<td>&lt;0.1</td>
<td>-</td>
</tr>
</tbody>
</table>

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

The Ilv- strain M1262 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+, whereas the other two were Val+. (However, in the presence of the ilvH612 allele, the ilvG product was also Val+.) 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+ 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+ mutants is that in which a Val+ acetolactate synthase becomes Val+ (11, 14, and De Felice et al., manuscript in preparation). A third class of Val+ 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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LITERATURE CITED