Regulation of Transaminase C Synthesis in Escherichia coli: Conditional Leucine Auxotrophy

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The regulation of synthesis of the valine-alanine-α-aminobutyrate transaminase (transaminase C) was studied in Escherichia coli mutants lacking the branched-chain amino acid transaminase (transaminase B). An investigation was made of two strains, CU2 and CU2002, each carrying the same transaminase B lesion but exhibiting different growth responses on a medium supplemented with branched-chain amino acids. Both had the absolute isoleucine requirement characteristic of ilvE auxotrophs, but growth of strain CU2 was stimulated by valine, whereas that of strain CU2002 was markedly inhibited by valine. Strain CU2002 behaved like a conditional leucine auxotroph in that the inhibition by valine was reversed by leucine. Results of enzymatic studies showed that synthesis of transaminase C was repressed by valine in strain CU2002 but not in strain CU2. Inhibition by valine in strain CU2002 appears to be the combined effect of repression on transaminase C synthesis and valine-dependent inhibition of α-acetohydroxy acid synthase activity, causing α-ketoisovalerate (and hence leucine) limitation. The ilvE markers of strains CU2 and CU2002 were each transferred by transduction to a wild-type genetic background. All ilvE recombinants from both crosses resembled strain CU2002 and were inhibited by valine in the presence of isoleucine. Thus, strain CU2 carries an additional lesion that allows it to grow on a medium containing isoleucine plus valine. It is concluded that conditional leucine auxotrophy is characteristic of mutants carrying an ilvE lesion alone.

The terminal reaction in each of the three interrelated biosynthetic pathways that lead to isoleucine, valine, and leucine involves transamination between an amino donor and the respective branched-chain α-keto acid (Fig. 1 and 2). In an early study of transamination by Rudman and Meister (11), three transaminase activities were separated from crude extracts: (i) transaminase B, which catalyzed the amination of each of the three keto acid precursors of the branched-chain amino acids; (ii) transaminase A, which catalyzed the terminal reactions of aspartate, leucine, tyrosine, and phenylalanine bioynthesis; and (iii) a third fraction that will be referred to here as transaminase C, which catalyzed only the terminal step in valine biosynthesis. In the first and second reactions, glutamic acid was the amino donor, whereas alanine and α-aminobutyric acid were the amino donors in the reaction catalyzed by transaminase C (1). That transaminase B is specifically involved in the synthesis of the branched-chain amino acids was revealed by

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the fact that the activity was missing in extracts of Escherichia coli mutants that were subsequently identified as ilvE mutants (1, 9, 11, 15). The activity is, thus, due presumably to a single enzyme. Transaminase A activities, on the other hand, are probably due to at least three proteins: one activity transferring amino groups from glutamate, tyrosine, phenylalanine, and leucine, which is under the control of the tyrR gene and is, therefore, repressed by tyrosine (12, 16); another activity which specifically transfers amino groups from phenylalanine and glutamate (2, 12); and a third, which is an aspartate-glutamate transaminase (2). Mutants lacking the three activities of transaminase A or that of transaminase C have not been described in E. coli or Salmonella typhimurium.

Because of this multiplicity of enzymes, mutants lacking transaminase B (ilvE mutants) have a phenotype distinct from other ilv auxotrophs. The earliest reports (11, 15) have listed these growth characteristics as follows. (i) They had an absolute requirement for isoleucine. (ii) They had a partial requirement for valine (because transaminase C activity is inadequate
Fig. 1. Biosynthetic pathways for isoleucine, valine, and leucine. The enzymes catalyzing the indicated steps are abbreviated as follows: TD, threonine deaminase; AHS I, endproduct-inhibited acetohydroxy acid synthase; AHS II, endproduct-noninhibited acetohydroxy acid synthase; IR, acetohydroxy acid isomeroreductase; DH, dihydroxy acid dehydrase; TRA, transaminase A; TRB, transaminase B; TRC, transaminase C; IPMS, α-isopropylmalate synthetase; ISO, isopropylmalate isomerase; IPMD, β-isopropylmalate dehydrogenase. The ilu and leu genes corresponding to these enzymes are indicated below the scheme. Genes iluP, iluQ, and leuO are repression recognition sites; iluQ is the induction recognition site; and iluY is a region specifying a positive control element needed for induction of isomeroreductase activity. It is probably part of the iluA gene. The gene order in each cluster is the reverse (i.e., left to right is counterclockwise) of the way it is usually represented on the E. coli chromosome map. Adapted from Taylor (14) and Pledger and Umbarger (8).

Subsequent studies, however, have indicated that an ilvE mutation leads to a triple auxotrophic requirement (isoleucine plus valine plus leucine) (4, 9). In these studies, the classifications were performed shortly after isolation, so that it seemed unlikely that the additional requirement for leucine could be due to additional, undetected mutations in each case. Rather, the possibility that the originally isolated ilvE mutant carried one or more additional mutations should be considered.

In the course of cloning a stock culture of the organism studied by Adelberg and Umbarger, cells with two distinct phenotypes could be isolated. Each carried the ilvE lesion, but differences were apparent in their growth requirements. One isolate, for which the strain designation CU2 was retained, had properties identical with those reported by Adelberg and Umbarger (1). The other strain, designated CU2002, grew with either isoleucine alone or with all three branched-chain amino acids, but was inhibited by valine in the presence of isoleucine. Upon further investigation, it was found that the phenotypic differences between the strains were due to differences in their levels of transaminase C, and that the conditional
leucine auxotrophy observed with strain CU2002 is characteristic of an authentic ilvE mutant.

**MATERIALS AND METHODS**

**Organisms.** The organisms used in this study were all derivatives of *E. coli* K-12. They were: strain CU2 (formerly 11A16), which exhibited an absolute requirement for isoleucine but required both valine and isoleucine to approach the wild-type growth rate; strain CU2002, a variant found in the CU2 culture, which grew as well as strain CU2 on isoleucine but which required leucine if valine was present; and strain CU2118, a Rb- derivative of strain CU2002. All strains bore the primary ilvE12 lesion.

**Media and culture methods.** The minimal medium employed was that of Davis and Mingioli (3), modified by the omission of citrate and by the use of 0.5% glucose or ribose as indicated. Supplements of the amino acids, where indicated, were 0.04 mM isoleucine and leucine and 1.2 mM valine. Nutrient broth was obtained from Difco. Solid medium was prepared with 1.5% agar (Difco). Liquid medium cultures (10 ml) were grown in 300-ml Belco Nephelo flasks. Growth was followed turbidimetrically with a Klett-Summerson colorimeter with a blue (no. 42) filter (Klett unit = 4.3 × 10^6 cells per ml). Inocula were cells grown overnight in nutrient broth and washed with sterile saline before use.

**Enzymatic assays.** The preparation of cell extracts was as described previously (13). Protein concentration was measured by the biuret method (5).

Transaminase C activity was assayed at 37°C using a procedure based on the differential extraction of pyruvate produced by transamination between alanine and α-ketoisovalerate. The 1-ml reaction mixture contained 100 μmol of potassium phosphate buffer (pH 8.0), 10 μmol of α-ketoisovalerate (pH previously adjusted to 8.0), 10 μmol of L-alanine, 1 μmol of pyridoxal-5'-phosphate, and cell extract. After 30 min the reaction was terminated by the addition of 0.1 ml of 20% metaphosphoric acid. The precipitated protein was then removed by centrifugation, and the supernatant liquid was diluted in test tubes (18 by 150 mm) with an appropriate volume of water, depending on enzyme activity. The pyruvic acid formed during the transamination reaction and the remaining α-ketoisovalerate were converted to their dinitrophenylhydrazones by mixing 1 ml of the diluted solution with 1 ml of a 2,4-dinitrophenylhydrazine reagent. This reagent was prepared by trituration 600 mg of 2,4-dinitrophenylhydrazine with 34 ml of concentrated hydrochloric acid and diluting to 200 ml with distilled water. The reagent was stored in a light-proof bottle in the cold and was freshly prepared every 2 weeks. After addition of the reagent, the tubes were allowed to sit at 37°C for 10 min. Ethyl acetate (5 ml) was then added, and the content of each tube was mixed thoroughly by using a Vortex mixer (Scientific Products) until all precipitate had dissolved. A 4.5-ml volume of the ethyl acetate layer was then removed carefully, transferred to another tube, and shaken with 2 ml of 10% (wt/vol) Na₂CO₃, again by vigorous Vortex mixing. A 1-ml volume of the Na₂CO₃ layer was withdrawn to another tube, 2 ml of 1 N NaOH was added, and the optical densities at 445 nm (OD₄₄₅) were read against the blank. To correct for the slight amount of α-ketoisovalerate dinitrophenylhydrazone extracted into the Na₂CO₃ layer, a reaction mixture from which alanine was omitted was used as a control, and the OD yielded after the same analytical procedure was subtracted from the test values. Specific activities are expressed as the (increase in) OD₄₄₅ per hour per milligram of soluble protein.

**Genetic techniques.** Transduction was performed by the techniques of Lennox (6).

**Sampling experiments to measure transaminase C levels.** The inoculum for experiments in which derepression of transaminase C synthesis was demonstrated was an overnight culture, grown in nutrient broth, that had been harvested sterilely and suspended in saline. One liter of supplemented minimal medium in a baffled, 2-liter flask was inoculated with sufficient cells to give an OD₆₆₀ of approximately 0.200 (measured precisely) and was shaken throughout the experiment in a New Brunswick Gyraatory water bath shaker (model G76) at 37°C. A 100-ml volume of cell suspension was removed aseptically after inoculation and filtered immediately through a 4.7-cm membrane filter (0.45 μm pore size; Millipore Corp.). The membrane was transferred to 20 ml of ice-cold 25 mM potassium phosphate buffer (pH 7.0) in a centrifuge tube. After the solution was mixed to remove adhering cells, the membrane was discarded. The cells were centrifuged for 20 min at 27,000 × g, and all supernatant liquid was carefully removed. The packed cells were resuspended in exactly 1 ml of the pH 7.0 buffer, and the cell suspension was stored at −20°C until required for assay. Samples of cells were subsequently taken from the culture at various time intervals. The volumes removed at these times were adjusted so that a mass of cells equal to that of the zero time sample was taken. This adjustment in volume was based on the OD₆₆₀ of the culture; it had been demonstrated previously that there was direct proportionality between OD₆₆₀ of 1.0 and cell mass over the range of turbidities of samples taken (OD₆₆₀ = 1.8 × 10⁶ cells). Cell mass was kept constant because preliminary experiments had shown that permeability to enzyme substrates after the tolucene treatment of cells (see below) varied according to cell density.

The 1-ml volumes of frozen cells were thawed and treated at 37°C with 0.05 ml of toluene for 20 min. The tubes were occasionally shaken during this incubation. Duplicate samples (0.1 to 0.4 ml) of each toluene-treated suspension were transferred to test tubes and preincubated at 37°C for 5 min. The enzyme reaction was started by adding to each tube: 0.2 M potassium phosphate buffer (pH 8.0), 0.02 M α-ketoisovaleric acid (neutralized to pH 8.0), 0.2 M L-alanine, and 0.002 M pyridoxal-5'-phosphate. The reaction mixture was freshly prepared prior to each assay. A blank, prepared by adding 0.6 ml of the assay mixture to 0.4 ml of distilled water, was also incubated with the test samples. After 30 min of incubation, the reaction was terminated by the addition of 1 ml of 2,4-dinitrophenylhydrazine reagent. After a
further 10 min, 5 ml of ethyl acetate was added and each tube was Vortex-mixed thoroughly. Because of the protein content of the samples, an emulsion often formed at this stage, and centrifugation was required to separate the two phases. A 4.5-ml volume of the ethyl acetate layer was removed and treated as described above for color development; the OD_{465} were read against the blank. Enzyme activity was proportional to the volume of cell suspension, over the range of concentrations used, and the reaction rate was constant during the 30-min incubation period. One unit of transaminase C activity was defined as that amount of enzyme giving an OD_{465} of 0.100/h in the colorimetric assay. Since each sample taken contained a constant mass of cells and the toluene treatment was identical for each sample, changes in the calculated enzyme units were proportional to changes in cellular levels of transaminase C with time.

### RESULTS

Growth experiments. Both strain CU2 and strain CU2002 grew equally well, with generation times of 68 and 60 min, respectively, in glucose minimal medium supplemented with all three of the branched-chain amino acids (Fig. 3), but failed to grow in unsupplemented media. With isoleucine alone, both grew after a pronounced lag of about 6 h, but the final limiting growth rates were different, with generation times of 120 min for strain CU2 and 186 min for strain CU2002. The inclusion of leucine with isoleucine markedly inhibited the growth of both strains and resulted in mean generation times of 270 min for strain CU2 and 384 min for strain CU2002. Provision of valine immediately reversed this inhibition, and growth commenced at the rate characteristic of the full complement of branched-chain amino acids (not shown). The most marked difference between the strains was observed when valine was present with isoleucine: strain CU2 grew almost as well as it did when all three branched-chain amino acids were present, whereas strain CU2002 was markedly inhibited and essentially unable to grow, with a mean generation time of more than 12 h. This effect of valine was immediately reversed when leucine was added to the culture. Since the inhibitory effect of valine upon growth with isoleucine in strain CU2002, and of leucine upon growth of both strains, is reversed by leucine and valine, respectively, the strains can be considered "conditionally auxotrophic" for these amino acids.

The above results were confirmed by growing the two strains on solid media. Under these conditions, colony appearance on isoleucine medium alone was delayed for more than 48 h. After prolonged incubation, several variants eventually grew on isoleucine-valine agar plates streaked with strain CU2002. Strain CU2002 was therefore routinely repurified before every experiment by single-colony isolation.

Growth inhibition of E. coli K-12 by leucine after "down-shift" transfer from nutrient broth to minimal medium has previously been reported by Rogerson and Freundlich (10), and the inhibition has been correlated with repression of synthesis of acetohydroxy acid synthase. Downshift was eliminated as being responsible for the leucine inhibition of strains CU2 or CU2002 by a demonstration of the inhibition using cells grown in a medium containing either isoleucine or isoleucine and leucine. The same effects were observed with both strains CU2 and CU2002, namely, a progressively developing inhibition by leucine when cells grown overnight in an isoleucine medium were transferred to an isoleucine-leucine medium (Fig. 4A), and relief of inhibition when the transfer was made from an isoleucine-leucine medium to an isoleucine medium (Fig. 4B). One observation in this experiment cannot be explained: when transfer was made to fresh medium of the same composition, growth was more rapid in the second culture than in the original.

**Effect of α-ketoisovalerate on strain CU2002.** It was thought that the growth inhibition of strain CU2002 in isoleucine-valine medium might be due to the combined effect of low transaminase C activity and the known feedback inhibition of acetohydroxy acid synthase.
by valine (15; Fig. 1). The net effect would be the curtailment of synthesis of \( \alpha \)-ketoisovalerate, and hence leucine, from both pyruvate and valine; the result would be the observed leucine deprivation. To test this hypothesis, \( \alpha \)-ketoisovalerate was added to a growth-inhibited culture of strain CU2002 in isoleucine and valine medium (Fig. 5). Rapid growth commenced immediately, indicating that conversion of valine to \( \alpha \)-ketoisovalerate had been growth limiting. The result also showed that the conditional leucine auxotrophy was not due to an effect on the enzymes unique to leucine biosynthesis.

**Transaminase C levels.** The indication that the transamination of valine is rate limiting in strain CU2002 grown with isoleucine and valine prompted an examination of the levels of transaminase C activity in the two strains (Table 1). Specific activities of the enzyme were measured in both strains grown in minimal medium with various supplements of branched-chain amino acids and in nutrient broth. The most important measurement, that of the enzyme level in strain CU2002 grown in an isoleucine-valine medium, could not of course be measured directly because of the very poor growth under these conditions. The demonstration of the effect of this combination of amino acids had to be done in a sampling experiment (see below; Table 2). With the enzyme level of cells grown with isoleucine alone as a reference, strain CU2 or CU2002 grown in nutrient broth or with an excess of all branched-chain amino acids had approximately 3- and 10-fold-repressed levels of transaminase C, respectively. When the isoleucine medium was supplemented with either leucine or valine separately, differences between the strains became apparent. Whereas leucine caused a twofold repression in strain CU2002, essentially no repression was evident in strain CU2. Valine similarly caused no repression in strain CU2, but as shown later (Table 2) did cause repression in strain CU2002.

Specific activities of the other enzymes of isoleucine and valine biosynthesis were mea-

![Fig. 4](image)

**Fig. 4.** (A) Leucine inhibition of strain CU2 after transfer of cells from isoleucine to isoleucine plus leucine medium. (B) Relief of leucine inhibition of CU2 after transfer of cells from isoleucine and leucine to isoleucine medium.

![Fig. 5](image)

**Fig. 5.** Effect of \( \alpha \)-ketoisovalerate (1 mM) on the growth of mutant CU2002 in isoleucine-valine medium. Supplement was added to one (O) of two duplicate flasks at time indicated by arrow.

**Table 1.** Levels of transaminase C in strains CU2 and CU2002 grown in minimal medium supplemented with branched-chain amino acids

<table>
<thead>
<tr>
<th>Supplement in growth medium</th>
<th>( \text{Sp act of transaminase C} )</th>
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<tbody>
<tr>
<td></td>
<td>CU2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>6</td>
</tr>
<tr>
<td>Isoleucine-leucine</td>
<td>5.8</td>
</tr>
<tr>
<td>Isoleucine-valine</td>
<td>5.9</td>
</tr>
<tr>
<td>Isoleucine-valine-leucine</td>
<td>2.4</td>
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<tr>
<td>Nutrient broth</td>
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</table>

* See Table 2.
Table 2. Transaminase C synthesis after transfer of strains CU2 and CU2002 from nutrient broth to isoleucine-valine medium*

<table>
<thead>
<tr>
<th>Cell generations</th>
<th>Transaminase C activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CU2</td>
</tr>
<tr>
<td>0*</td>
<td>0.301</td>
</tr>
<tr>
<td>1</td>
<td>0.546</td>
</tr>
<tr>
<td>1.2</td>
<td>0.232</td>
</tr>
<tr>
<td>2</td>
<td>0.951</td>
</tr>
</tbody>
</table>

* Cell generations were reached at the following times after transfer: 1, CU2 at 6 h and CU2002 at 7 h; 1.2, CU2002 at 12 h; 2, CU2 at 8 h.

OD₆₆₀ at transfer: CU2, 0.283; CU2002, 0.255.

Assured at the same time as transaminase C, and no differences between strains CU2 and CU2002 were found. In addition, the acetohydroxy acid synthase of both strains was inhibited markedly by valine. The amount of inhibition was the same as that previously reported for wild-type E. coli K-12 (7).

Transaminase C synthesis. Samples were removed from cultures of strains CU2 and CU2002 during adaptation to growth in isoleucine or isoleucine-leucine medium after transfer from nutrient broth. Cells were treated with toluene, and transaminase C was assayed as described above.

Before the onset of logarithmic growth of strain CU2 in isoleucine medium, the transaminase level increased three- to fourfold (Fig. 6), and an almost identical pattern of derepression was found with strain CU2002. When leucine was included in the growth medium, there was a marked difference between the patterns of derepression in the two strains. Whereas the level of transaminase C in strain CU2 increased in the same manner as with isoleucine alone (fourfold increase in 10 h), essentially no synthesis occurred in strain CU2002 (50% increase in 18 h). A demonstration of the difference in rate of synthesis between the two strains can be found in the differential plot of Fig. 7.

Differences between the effects of valine on transaminase C synthesis in strains CU2 and CU2002 were demonstrated by a similar sampling experiment (Table 2). Isoleucine-valine medium was inoculated with nutrient-grown cells of either strain, and samples were removed for enzyme assay before and after one doubling of cell mass in each culture. During the 6 h required for this doubling to occur in the strain CU2 culture, the level of transaminase C increased 80%, whereas a 12% decrease was observed during the 7 h required for doubling of strain CU2002. The transaminase C level of strain CU2 continued to rise and, after the second doubling, was more than three times the level at inoculation. After 14 h of growth and 1.2 cell generations, transaminase C activity of strain CU2002 was still less than at inoculation. These data, in conjunction with the demonstration that in the presence of isoleucine alone derepression of transaminase C occurs readily, suggest that valine causes repression of the enzyme in strain CU2002 but not in strain CU2. They also support the hypothesis that valine inhibition of strain CU2002 in the presence of isoleucine could be due to low transaminase C activity.

Transductional analysis. At the outset of this analysis, it was presumed that strain CU2 was an authentic ilvE mutant and that strain CU2002 was a double mutant carrying the lesion in ilvE and an additional regulatory mutation affecting synthesis of transaminase C. In fact, it was later demonstrated that the reverse was true and that strain CU2002 was wild type with respect to regulation of transaminase C synthesis. For this reason the initial attempts to remove, by transduction, the conditional Leu⁻ marker of strain CU2002 using phage grown on wild-type strain K-12 were unsuccessful; strains K-12 and CU2002 were identical with respect to regulation of transaminase C synthesis. Moreover, in the course of selecting Leu⁺ recombinants from a cross with

![Fig. 6. Derepression of transaminase C synthesis in mutant CU2 during adaptation of nutrient-grown cells to growth in isoleucine medium.](http://jb.asm.org/...)
wild-type strain K-12 as donor and strain CU2002 as recipient on isoleucine-valine selective agar, introduction of an intact ilvE gene, and hence restoration of transaminase B activity, would also have given the Leu+ phenotype. For these two reasons, strain CU2 was used as donor and strain CU2002 was used as recipient in experiments to determine whether linkage existed between the locus responsible for the conditional Leu+ phenotype of strain CU2002 and the ilv region. Table 3 shows the results of such a cross. The donor phage had been freed from carry-over of wild-type phage by three successive growth cycles with strain CU2; and the recipient was strain CU2118, a derivative of strain CU2002 carrying a rbs marker that had previously been shown to be adjacent to ilvE.

Any measurable linkage between the leu marker and rbs would indicate the proximity of the former to the ilv region. In fact, no such linkage was found when either Leu+ or Rbs+ recombinants were selected.

The ilvE markers of both strains CU2 and CU2002 were transferred to a wild-type genetic background using rbs+ as the selected marker (Table 4). The recombinants were selected on ribose-isoleucine-valine-leucine agar plates to avoid selection against the CU2002 phenotype. rbs+ ilvE+ recombinants were identified by replica-plating onto ribose minimal agar and were further characterized as being of the CU2 or CU2002 phenotype by ability to grow on ribose-isoleucine-valine plates. Of more than 100 ilvE+ recombinants from each of the two crosses, all were like strain CU2002 (Table 4). Confirmation of this fact was obtained by picking several such recombinants and testing their growth characteristics in liquid culture (Fig. 8). In all respects (growth rates, inhibition by valine, and reversal of that inhibition by either leucine or α-ketoisovalerate), they could not be distinguished from strain CU2002.

**DISCUSSION**

The findings reported above indicate that it is strain CU2002 that has the wild-type regulation of transaminase C synthesis and exhibits the phenotype of an ilvE auxotroph. Strain CU2 appears to have acquired an additional mutation (ilv-2025) that removes the repressive effect of valine on transaminase C synthesis, thus allowing growth in isoleucine and valine medium. The locus of the ilv-2025 allele is unknown.

The leucine auxotrophy of strain CU2002, which becomes apparent when isoleucine and valine are simultaneously present in the growth medium, can readily be explained by the regulatory effects of the latter compound upon the activity and synthesis of key enzymes in the isoleucine and valine biosynthetic pathway. The effect of (i) repression of transaminase C synthesis (Table 2) and (ii) feedback inhibition of acetohydroxy acid synthase would be to curtail synthesis of α-ketoisovalerate and, hence, leucine (Fig. 1). On the other hand,

![Fig. 7. Differential plot comparing rates of synthesis of transaminase C by strains CU2 and CU2002 during adaptation to growth in isoleucine-leucine medium. Washed cells grown in nutrient broth were used as inoculum. Symbols: ●, CU2; ○, CU2002.](image-url)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Selected phenotype</th>
<th>No. of recombinants</th>
<th>Unselected phenotype</th>
<th>No. of recombinants</th>
<th>Linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CU2</td>
<td>CU2118</td>
<td>Rbs+</td>
<td>120</td>
<td>Leu+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CU2</td>
<td>CU2118</td>
<td>Leu+</td>
<td>125</td>
<td>Rbs+</td>
<td>0</td>
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</tr>
</tbody>
</table>
strain CU2, which has lost the repressive effect of valine upon transaminase C synthesis, grows perfectly well in the presence of isoleucine and valine.

When leucine is present with isoleucine it, too, causes repression of transaminase C in strain CU2002. This repression would restrict the synthesis of valine from α-ketoisovalerate and generate the observed auxotrophy for this amino acid. However, the effect of leucine is probably not solely one of repression since, although it does not cause repression of transaminase C in strain CU2 (Table 1), it does cause growth inhibition (again reversible by valine) in this organism too. The most likely cause of this additional effect would be the leucine-dependent inhibition of transaminase C activity. This idea was tested, but no inhibition could be demonstrated; it may have been that the correct experimental conditions were not used, however.

The pattern of regulation of transaminase C synthesis occurring in strains CU2 and CU2002 deserves mention. Whereas leucine and valine repress independently in strain CU2002, in strain CU2 there is an apparent multivalent repression, with both leucine and valine being required for repression. The very low levels of transaminase C in cells grown in nutrient broth may indicate that compounds other than the branched-chain amino acids also repress its synthesis.

No linkage was detected between the gene responsible for the conditional Leu+ phenotype (i.e., the presumptive regulatory gene for transaminase C synthesis) and a rbs marker known to be linked to the ilv cluster of genes. Thus, the marker lies elsewhere on the chromosome. Since it is not linked to rbs (nor presumably to ilv), it would not have been co-transduced with ilv in the genetic crosses between strains CU2 and CU2002 and the strain in which control of transaminase C was like that in the wild-type strain K-12 (Table 4). The CU2002 mode of regulation of transaminase C shown to function in the recombinants must, therefore, be of the wild-type mode, and the CU2002 phenotype must be characteristic of ilv auxotrophs.

One reason that the conditional leucine auxotrophy of ilv mutants has not been reported previously is probably the readiness with which they acquire the second mutation, allowing growth on an isoleucine and valine medium. If one or more steps involving growth in this medium were included during the isolation, any ilv isolate would probably acquire such a mutation.

It should be pointed out that the wild-type phenotype exhibited by strain CU2002 resulted from reversion of the secondary lesion (ilv-2025) that had at some time arisen in strain CU2. Thus, it is quite possible that the locus that presumably controls the formation of transaminase C is different in strain CU2002 from that in the wild type at one or more base pairs and therefore should only be considered wild-type-like.
ACKNOWLEDGMENTS

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LITERATURE CITED