

Stereospecific Production of the Herbicide Phosphinothricin (Glufosinate) by Transamination: Cloning, Characterization, and Overexpression of the Gene Encoding a Phosphinothricin-Specific Transaminase from *Escherichia coli*

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We have cloned the gene encoding a 43-kilodalton transaminase from *Escherichia coli* K-12 with a specificity for L-phosphinothricin [L-homoalanine-4-yl-(methyl)phosphinic acid], the active ingredient of the herbicide Basta (Hoechst AG). The structural gene was isolated, together with its own promoter, and shown to be localized on a 1.6-kilobase *DraI*-*Bam*HI fragment. The gene is subject to catabolite repression by glucose; however, repression could be relieved completely when 4-aminobutyrate (GABA) served as the sole nitrogen source. The regulation pattern obtained and a comparison of the restriction map of the initially cloned 15-kilobase *SaI*I fragment with the physical map of the *E. coli* K-12 genome suggest that the cloned gene is identical with *gabT*, a locus on the *gab* gene cluster of *E. coli* K-12 which codes for the GABA:2-ketoglutarate transaminase (EC 2.6.1.19). A number of expression plasmids carrying the isolated transaminase gene were constructed. With these constructs, the transaminase expression in transformants of *E. coli* could be increased up to 80-fold compared with that in a wild-type control, and the transaminase constituted up to 20% of the total soluble protein of the bacteria. Thus, the protein crude extracts of the transformants could be used, after a simple heat precipitation step, for the biotechnological production of L-phosphinothricin in an enzyme reactor.

In the accompanying paper we describe the isolation and characterization of an L-phosphinothricin [L-homoalanine-4-yl-(methyl)phosphinic acid]-specific transaminase from *Escherichia coli* K-12 (24). By substrate specificity studies, the enzyme was characterized as a 4-aminobutyrate:2-ketoglutarate transaminase (GABA:2-ketoglutarate transaminase) (EC 2.6.1.19). The transaminase was immobilized to the epoxy-activated carrier VA-Biosynth (Riedel de Haen) and used in a column reactor for the continuous production of L-phosphinothricin, the active ingredient of the herbicide Basta (Hoechst AG). The amounts of transaminase required for large-scale production of L-phosphinothricin by the immobilized-enzyme technique cannot be obtained from fermentation of wild-type *E. coli*, since the enzyme constitutes only roughly 1% of the total soluble proteins of the bacterium. To achieve an efficient and economic production of the phosphinothricin-specific transaminase, we therefore decided to engineer a transaminase-overproducing strain by using recombinant-DNA techniques. For this purpose, the corresponding gene had to be cloned and overexpressed in *E. coli*. Since the phosphinothricin transaminase has no specificity for protein amino acids other than glutamate (24), one could assume that an overexpression of the cloned gene would not interfere with the amino acid metabolism of the bacterial host strain. A stable replication of aminotransferase genes on expression plasmids and an overexpression up to 100-fold in *E. coli* has already been reported for the genes encoding aspartate transaminase (*aspC*) and the aromatic transaminase (*tyrB*) (9; S. B. Primrose and R. M. Edwards, European patent application 84100521.8, 1984), the imidazolylacetol phosphate:glutamate transaminase (*hisC*) (10), the branched-chain amino acid transaminase

(*ilvE*) (15), the alanine:valine transaminase (*avtA*) (26), and the 3-phosphoserine transaminase (*serC*) (8).

In this paper we describe the cloning, characterization, and overexpression of the gene coding for the phosphinothricin-specific transaminase from *E. coli* K-12.

MATERIALS AND METHODS

Bacterial strains, media, and enzymes. For the transformation and expression experiments, we used *E. coli* DH1 (18) and W3110i⁹ (4). The composition of the media used for bacterial cultivation is described by Maniatis et al. (19). Plasmid-carrying transformants were grown in Luria-Bertani (LB) medium with 50 mM Tris hydrochloride (pH 7.5) and 50 µg of the appropriate antibiotic per ml as the standard medium. The transaminase-overproducing strain was fermented in M9-mineral medium supplemented with 2% Casamino Acids (acid-hydrolyzed casein; Difco Laboratories, Detroit, Mich.). Restriction enzymes, T4 DNA ligase, T4 DNA polymerase, exonuclease III, and polynucleotide kinase were obtained from New England BioLabs, Inc., Beverly, Mass. Alkaline phosphatase, nuclease S1, and the large fragment of DNA polymerase I (Klenow enzyme) were obtained from Boehringer GmbH, Mannheim, Federal Republic of Germany.

Cosmid cloning, gene isolation, and plasmid constructions. Genomic DNA from the *E. coli* K-12 derivative DH1 was isolated by the procedure of Meade et al. (20), partially digested with restriction endonuclease *Sau*3AI, and size fractionated on 1% agarose gels. The DNA fragments in a size range of 25 to 35 kilobases (kb) were ligated to the *Bam*HI-digested and dephosphorylated cosmid vector pTBE, packaged in vitro, and transduced into the host strain *E. coli* DH1. The methods used were essentially those described by Meyerowitz et al. (22).

For screening of the genomic library, two oligonucleotide

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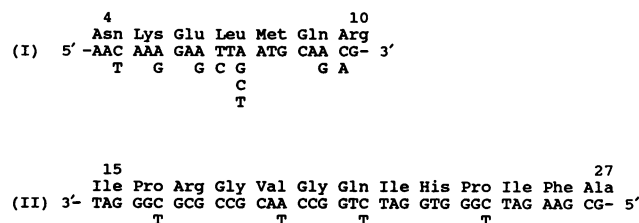


FIG. 1. Sequences of the two oligonucleotide mixtures used for screening of the cosmid library from *E. coli* DH1. The oligonucleotides correspond to amino acid sequences of the N-terminal region of the phosphinothricin transaminase protein given in the accompanying paper (24). I, Amino acids 4 to 10 (coding strand); II, amino acids 15 to 27 (noncoding strand).

mixtures (Fig. 1) corresponding to different parts of the N-terminal amino acid sequence of the transaminase (24) were synthesized in the Hoechst pharmaceutical department by automated phosphoramidite chemistry (2). Cosmid clones were isolated in pools of 12, bound to nitrocellulose filters (BA 85; Schleicher & Schuell, Dassel, Federal Republic of Germany), and hybridized independently to each of the two oligonucleotide mixtures after end labeling with [γ - 32 P]ATP. Cosmid pools hybridizing to both probes were rescreened individually by using the same technique.

The clones identified by this procedure were further characterized by restriction enzyme mapping and deletion analysis. Different restriction fragments from the original cosmid clones were subcloned, rehybridized to the oligonucleotide mixtures, and tested for their ability to confer elevated phosphinothricin transaminase activity. Deletions of subcloned DNA-fragments were constructed by digestion with exonuclease III and nuclease S1 by the method of Henikoff (12). The resulting series of subfragments were tested for the presence of the transaminase gene as described above. The vectors used for subcloning and the constructed recombinant plasmids carrying the transaminase gene are listed in Table 1. The hybridization and subcloning experiments were performed by standard procedures described by Maniatis et al. (19).

Assay of phosphinothricin transaminase activity. The phosphinothricin transaminase activity in cell extracts was determined by method 2 described in the accompanying paper (24). When the enzyme was assayed in intact cells, the following modifications were made. After cultivation at 37°C for the indicated time, the bacteria were washed twice in 10 mM sodium phosphate (pH = 7.5)–10 mM NaCl and then incubated in 100 μ l of reaction mix consisting of 0.1 M Tris

hydrochloride (pH = 8.0), 150 mM 2-oxo-4-[(hydroxy)(methyl)phosphinoyl]butyric acid (PPO), and 600 mM L-glutamate. Specific enzyme activities were expressed in nanokatal per milligram (wet weight) of cells (1 nkat = 1 nmol turnover per s).

Fermentation of *E. coli* and partial purification of the transaminase. The transaminase production strain, W3110i^a (pGT4), was inoculated in fermentation medium (M9-mineral medium with 0.5% glucose, supplemented with 2% Casamino Acids and 0.4% GABA) and cultivated for 24 h at 35°C in a 50-liter fermentor (P 80; Giavanola) with constant stirring (400 rpm), aeration (2 m³/h), and automated pH regulation at pH = 7.0. The bacterial growth rate and transaminase expression were monitored by analyzing culture samples for optical density, glucose content, and specific transaminase activity. At the end of the fermentation period, the bacteria were inactivated for 20 min with 0.5% *o*-cresol and concentrated about 10-fold by using a cell separator (SA1-02-575; Westfalia). After the suspension was adjusted with 20 mM sodium phosphate (pH = 7.0), 0.01 M pyridoxal phosphate, 5 mM 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride, the cells were disrupted in a microfluidizer (model M-110 TIV; Micro Fluids, Newton, Mass.) at 8×10^7 Pa. The crude extracts were subjected to heat precipitation at 70°C for 10 min, and cell debris and denatured proteins were removed by centrifugation for 20 min at $6,000 \times g$. After determination of the specific transaminase activity, the supernatant was used for enzyme immobilization (24).

Denaturing polyacrylamide gel electrophoresis. Protein samples were analyzed on sodium dodecyl sulfate–12.5% polyacrylamide gels by the method of Laemmli (16).

RESULTS

Isolation of the transaminase gene. For construction of a genomic library, partially *Sau*3A-digested DNA from the *E. coli* K-12 derivative DH1 was inserted into the *Bam*HI site of the cosmid vector pTBE, packaged in vitro, and transfected into the same host strain. A total of 2,000 cosmid clones (representing more than 10 genome equivalents) were hybridized to two transaminase-specific oligonucleotide mixtures (see Materials and Methods). Four clones hybridized to both oligonucleotide mixtures and exhibited a three- to fivefold-increased phosphinothricin transaminase activity compared with untransformed *E. coli*.

Restriction mapping of all four clones revealed an identical 15-kb *Sal*I fragment that hybridized to the oligonucleotides and conferred increased transaminase activity when re-cloned in the *Sal*I-digested vector pTBE (cosmid pGT1). DNA of the 15-kb *Sal*I fragment was digested with different restriction enzymes and subcloned into the vector pMLC12. Only *E. coli* DH1 transformants harboring a 3.8-kb *Bam*HI-*Sal*I-fragment (Fig. 2) showed about threefold-elevated transaminase activities.

The position of the transaminase gene on this fragment was further analyzed by constructing a series of deletions up to a length of 1.5 kb either from its *Bam*HI or from its *Sal*I terminus. Transaminase expression was not affected by deletions from the *Sal*I end of the fragment. On the other hand, the transaminase activity dropped to background levels in all subclones missing the *Bam*HI end, suggesting that the gene was located in the region adjacent to the *Bam*HI site of the fragment.

Further subcloning of restriction fragments from this region in the *Bam*HI-*Sma*I-digested vector pMLC12 resulted

TABLE 1. Plasmids used and constructed in this work

Plasmid	Description	Source or reference
pTBE	Cosmid vector, Amp ^r	11
pBR322	Plasmid vector, Amp ^r Tet ^r	3
pUC12	Plasmid vector, Amp ^r , <i>lac</i> promoter	25
pMLC12, pMLC13	Plasmid vector, Cm ^r , <i>lac</i> promoter, pUC12 polylinker in opposite orientations	17
pGT1	15-kb <i>Sal</i> I fragment in pTBE	This work
pGT2	1.6-kb <i>Dra</i> I- <i>Bam</i> HI fragment in pMLC12	This work
pGT3	3.8-kb <i>Bam</i> HI- <i>Sal</i> I fragment in pMLC13	This work
pGT4	3.8-kb <i>Bam</i> HI- <i>Sal</i> I fragment in pUC13	This work
pGT5	3.8-kb <i>Bam</i> HI- <i>Sal</i> I fragment in pBR322	This work

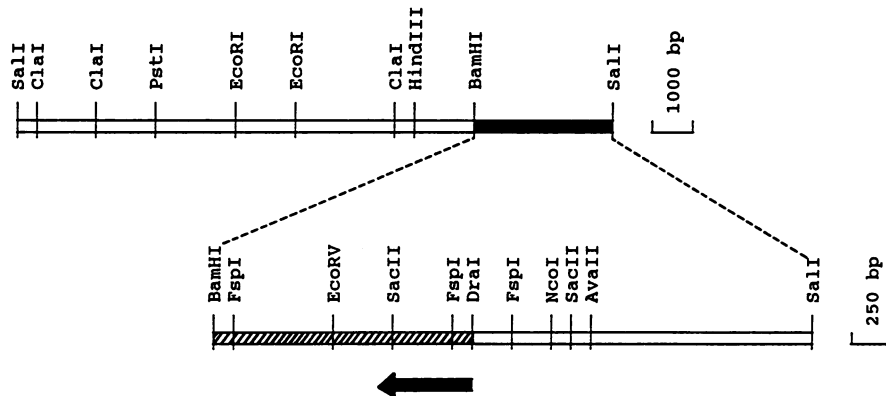


FIG. 2. Restriction maps of the 15-kb *SalI* and the 3.8-kb *BamHI-SalI* fragments containing the phosphinothricin transaminase gene. The smallest subfragment carrying the complete gene and the direction of transcription are indicated. bp, Base pairs.

first in a 2.3-kb *BamHI-AvaII* and subsequently in a 1.6-kb *BamHI-DraI* fragment (plasmid pGT2) conferring about 30-fold overexpression of the transaminase. The restriction maps of the 15-kb *SalI* and the 3.8-kb *BamHI-SalI* fragments are shown in Fig. 2.

When the 1.6-kb *BamHI-DraI* fragment was cloned in the reverse orientation with respect to the *lac* promoter of the vector (i.e., the orientation of the 3.8-kb fragment in pMLC12) into pMLC13 (plasmid pGT2r), the transaminase activity of the transformants was reduced to the level observed with the 3.8-kb fragment. Recombinant clones carrying an internal 1.45-kb *FspI* fragment (Fig. 2) inserted into *SmaI*-digested pMLC12 did not express any elevated transaminase level in either orientation of the subcloned fragment.

These results indicate that the complete gene, including the endogenous promoter, is located on the 1.6-kb *BamHI-DraI* fragment and that the gene is transcribed in the direction from the *DraI* site to the *BamHI* site (Fig. 2).

Expression of the transaminase gene in different plasmid vectors. To optimize the expression of the transaminase gene, the 3.8-kb *BamHI-SalI* fragment cloned into pMLC12 was inserted into pMLC13, pUC13, and pBR322, each digested with *BamHI* and *SalI*, resulting in the plasmids pGT3, pGT4, and pGT5, respectively. The constructs were introduced into *E. coli* DH1 by transformation, and the transaminase activity of the bacteria was determined (Table 2). The highest enzyme activity (50- to 80-fold elevated compared with the control) was obtained with both the pUC13 and pBR322 constructs.

TABLE 2. Overproduction of the phosphinothricin transaminase in *E. coli* DH1

Plasmid	Relative activity ^a
pTBE.....	100
pMLC13.....	60
pUC12.....	70
pBR322.....	120
pGT1.....	300
pGT3.....	2,990
pGT4.....	5,600
pGT5.....	5,700

^a Transaminase activities of transformants carrying either the cloned gene or the plasmid vector as a control. Bacteria were grown in standard medium with 50 μ g of the appropriate antibiotic per ml, and transaminase activities were measured after 24 h in intact cells.

Regulation of the transaminase expression. The pUC13 construct was transformed into *E. coli* W3110i^q, a strain that strongly represses transcription from the *lac* promoter in the vector by overproduction of the *lacI* gene product. The phosphinothricin transaminase activity in these transformants was similar to that obtained with the same plasmid in *E. coli* DH1, indicating that the transcription of the transaminase gene is driven mainly from the endogenous promoter.

In the accompanying paper (24) we provided evidence that the investigated transaminase is most probably identical with the GABA:2-ketoglutarate transaminase (EC 2.6.1.19). The corresponding gene, *gabT*, is part of the *E. coli gab* gene cluster (21) and is known to be regulated by a special type of catabolite repression (6). The gene activity is repressed in glucose-containing media, but can be restored specifically when GABA is added as the sole nitrogen source. Therefore, the phosphinothricin transaminase activity in W3110i^q carrying the plasmid pGT4 and in untransformed W3110i^q was determined after growth in M9-mineral medium containing 0.5% glucose as the carbon source and either 0.1% NH₄Cl or 0.4% GABA as the sole nitrogen source (Table 3). The data demonstrate that the activity of the cloned transaminase gene was strongly repressed by glucose to a level only slightly above the value measured with untransformed *E. coli*. When ammonia was substituted for GABA in the medium, however, complete relief from catabolite repression could be observed, and the gene expression was in the same range as obtained in glucose-free standard medium. This regulation pattern strongly supports the assumption that the isolated aminotransferase gene is coding for the GABA:2-ketoglutarate transaminase mentioned above.

TABLE 3. Effect of GABA on catabolite repression of the phosphinothricin transaminase by glucose

Medium ^a	Strain	Relative activity ^b
M9 + 0.5% glucose + 0.1% NH ₄ Cl	W3110i ^q	41
M9 + 0.5% glucose + 0.4% GABA	W3110i ^q	100
M9 + 0.5% glucose + 0.1% NH ₄ Cl	W3110i ^q (pGT4)	352
M9 + 0.5% glucose + 0.4% GABA	W3110i ^q (pGT4)	4,021

^a Cells were grown without antibiotic in unsupplemented M9-mineral medium.

^b Enzyme activities of *E. coli* W3110i^q and of the transformant W3110i^q (pGT4) carrying the cloned transaminase gene. Transaminase activities were measured after 24 h in intact cells.

TABLE 4. Effect of various carbon and nitrogen sources on the expression of the cloned phosphinothricin transaminase gene in *E. coli* W3110i^a(pGT4)

Carbon and nitrogen sources ^a	Relative activity ^b
1% sodium citrate, 0.1% NH ₄ Cl.....	100
1% sodium succinate, 0.1% NH ₄ Cl.....	67
1% maltose, 0.1% NH ₄ Cl.....	3
1% glycerol, 0.1% NH ₄ Cl.....	14
0.5% glucose, 0.1% NH ₄ Cl.....	6
0.5% glucose, 0.4% GABA.....	63

^a Cells were grown in M9-mineral medium supplemented with 2% Casamino Acids in the presence of 50 µg of ampicillin per ml.

^b Transaminase activities were measured after 21 h in intact cells.

Since the *E. coli lac* promoter cannot be relieved from catabolite repression by glucose in this specific manner, the results further confirm that the cloned gene is expressed under the control of its natural promoter. The identity of the L-phosphinothricin transaminase gene with *gabT* was confirmed by comparing the restriction map of the 15-kb *Sall* fragment obtained with *Bam*HI, *Hind*III, *Eco*RI, *Kpn*I, and *Pst*I with the physical map of the *E. coli* K-12 genome constructed by Kohara et al. (14). The restriction pattern of the *Sall* fragment matches the *E. coli* map at positions 2800 to 2815. This region is homologous to 57.6 min on the *E. coli* K-12 linkage map, the locus of the *gab* gene cluster (1).

Effects of medium composition on the transaminase expression. To produce large amounts of the phosphinothricin transaminase protein in the *E. coli* W3110i^a(pGT4) transformant by fermentation, the bacterial growth medium had to be optimized for high cell density as well as high specific enzyme activity. In a series of experiments (results not shown), the optimal growth medium for W3110i^a was found to be M9-mineral medium supplemented with 2% Casamino Acids. In this medium, however, the transaminase activity was low (ca. 0.03 nkat/mg of cells) owing to catabolite repression by glucose. Therefore, the effect of a variety of carbon sources on the expression of the cloned gene was tested (Table 4). Catabolite repression was also observed with maltose and glycerol, whereas much higher enzyme activities were obtained with succinate or citrate as the carbon source (up to 0.42 nkat/mg of cells). With the succinate and citrate, however, the bacterial growth rate was reduced about 50% compared with that in the medium containing 0.5% glucose as the carbon source. When NH₄Cl was substituted for GABA in this medium, the phosphinothricin transaminase activity increased about 10-fold, indicating that even in the presence of other organic nitrogen sources, the addition of GABA could partially reverse a catabolite repression of the transaminase gene.

The dependence of bacterial growth and specific transaminase activity on the glucose content of the medium was further investigated by determining the cell densities and the specific enzyme activities of cultures from W3110i^a(pGT4) after growth in an M9-Casamino Acids-GABA medium with different initial concentrations of glucose (Fig. 3). The cell density and the transaminase expression were at a high level up to 0.5% glucose. In the media of these cultures, no remaining glucose was detectable at the time of cell harvest (21 h). At initial glucose concentrations above 0.5%, cell growth was reduced and the specific transaminase activity dropped drastically. Accordingly, these cultures still contained between 0.5 and 1% glucose after 21 h, a level sufficient for maintaining the repression of the transaminase gene.

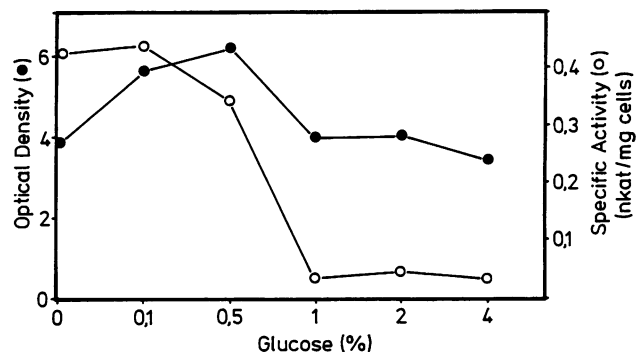


FIG. 3. Effect of glucose concentration on cell growth and specific phosphinothricin transaminase activity of *E. coli* W3110i^a(pGT4). Cells were cultivated in M9-mineral medium containing 2% Casamino Acids, 0.4% GABA, and 50 µg of ampicillin per ml, with the initial glucose concentrations as indicated. Optical densities (●) and specific transaminase activities (○) were determined after 21 h.

Production of the transaminase by fermentation. From the data presented above, we concluded that an ammonia-free M9-medium containing 0.5% glucose and supplemented with 2% Casamino Acids and 0.4% GABA should be used for fermentation of the transaminase-producing strain W3110i^a(pGT4). The bacteria were grown in a 50-liter fermentor at 35°C and harvested after 24 h at a specific transaminase activity of 0.35 nkat/mg of cells and an optical density of 22, equivalent to a cell mass of 2 kg (wet weight). Upon concentration and disruption of the cells, the transaminase was partially purified from the crude extracts by heat precipitation (70°C). After this procedure, the supernatant contained 16 g of protein in a total volume of 2 liters and with a specific phosphinothricin transaminase activity of 7480 nkat/mg of protein. Supernatants from untransformed *E. coli* W3110i^a prepared by the same procedure, however, exhibited a transaminase activity of only 200 nkat/mg of protein. Thus, the overproduction of the enzyme caused by plasmid pGT4 was ca. 40-fold. As demonstrated by sodium dodecyl sulfate-gel analysis, the transaminase was clearly the dominating protein in the supernatant fraction after heat precipitation (Fig. 4). Owing to its grade of enrichment, this material could be used directly for enzyme immobilization to 130 g of VA Biosynth and preparation of a column reactor (ca. 0.5 liter) as described in the accompanying article (24).

DISCUSSION

We have isolated the gene encoding the phosphinothricin: 2-ketoglutarate transaminase from *E. coli* that is described in the accompanying article (24). The complete structural gene is located on a 1.6-kb *Dra*I-*Bam*HI fragment, more than sufficient to encode a 43-kilodalton protein. Subcloning of the 1.6-kb fragment in both orientations with respect to the *lac* promoter in the expression vectors pMLC12 and pMLC13 resulted in large differences in transaminase expression. However, even in the wrong orientation, the transaminase activity was still threefold above background levels, indicating that the fragment contained the structural gene together with its own promoter, and expression did not rely only on readthrough from the *lac* promoter. That the expression of the gene was driven mainly from its endogenous promoter was demonstrated by transforming the recombinant plasmid pGT4 into the strain W3110i^a, in which

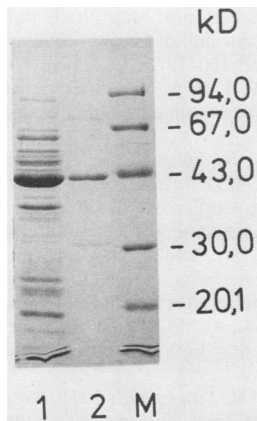


FIG. 4. Sodium dodecyl sulfate-gel analysis of protein from the transaminase-overproducing strain *E. coli* W3110i^q(pGT4). Lanes: 1, protein sample from W3110i^q(pGT4) supernatant after heat precipitation (25 μ g of protein); 2, purified transaminase (5 μ g of protein); M, molecular mass markers in kilodaltons (kD).

transcription from the *lac* promoter is strongly repressed but can be induced with isopropyl- β -D-thiogalactopyranoside (IPTG). In these transformants, the transaminase was expressed constitutively at high levels.

The fact that the expression of the transaminase gene was under the control of the endogenous promoter enabled us to study its regulation. The activity of the gene was repressed by glucose but could be released from catabolite repression when ammonium salts were substituted for GABA as the sole nitrogen source. This regulation pattern is consistent with the pattern described for the genes of the GABA utilization pathway of *E. coli* K-12 (5). This pathway includes four genes: *gabT*, encoding a GABA transaminase (EC 2.6.1.19); *gabD*, encoding a succinic semialdehyde dehydrogenase (EC 1.2.1.16); *gabP*, encoding a GABA permease; and a control gene, *gabC*, that coordinately regulates their expression. The relative positions of these genes have been mapped by Metzger et al. (21); they all form a cluster on the *E. coli* chromosome at 57.6 min linked to the *srl* and *recA* loci. It is unclear whether the structural genes of the *gab* cluster constitute an operon (7).

Our regulation experiments, as well as the comparison of the restriction map of the initially cloned 15-kb *SalI* fragment with the physical map of the *E. coli* K-12 genome, provide evidence that the cloned phosphinothricin transaminase gene described above corresponds to the *gabT* locus, encoding the initial enzyme of the GABA degradation pathway of *E. coli* K-12. In retrospect, it is clear that we also could have isolated the phosphinothricin transaminase gene by complementation of a *gabT* mutant (21).

From the work of Dover and Halpern (6) and Kahane et al. (13), it is known that the genes of the *gab* cluster are subject to dual physiological control: (i) by catabolite repression and (ii) by nitrogen availability. Glutamine synthetase (EC 6.3.1.2) was shown to act as a positive regulator in the *E. coli* GABA control system (27). A regulation by nitrogen availability has also been reported for histidase (EC 4.3.1.3) and proline oxidase (EC 1.4.3.2) of *Klebsiella aerogenes*, which catalyze the breakdown of histidine and proline, respectively (23). The aminotransferase gene characterized in this work is the first example for a cloned bacterial transaminase gene underlying this type of regulation.

To optimize transaminase overproduction in the strain W3110i^q(pGT4), we investigated the influence of various

carbon or nitrogen sources in the basic fermentation medium (see Materials and Methods) on transaminase expression. The use of citrate and succinate as carbon sources did not interfere with the expression of the transaminase. The generation time of the bacteria, however, was prolonged compared with that of bacteria cultivated in glucose-containing medium. This may be because glucose is more easily metabolized in the cells than citrate and succinate are. The catabolic repression of the transaminase gene in the presence of glucose could be partially overcome by the addition of GABA to the fermentation medium. A complete relief from catabolite repression was achieved when cells were grown in limiting glucose concentrations and harvested at least 3 h after the glucose was readily metabolized.

The derepressing effect of GABA on transaminase activity even in a glucose-containing medium with a nonlimiting nitrogen supply may be explained by diauxic utilization of the nitrogen sources. The cells probably metabolize GABA after the more easily accessible nitrogen compounds have been degraded. The fermentation medium that was finally chosen for the transaminase-producing strain W3110i^q(pGT4) contained 0.5% glucose and 0.4% GABA. The cells were harvested at an optical density of 22 after a fermentation time of 24 h, 3 h after the glucose in the medium had been completely utilized. In these cells, the production of the phosphinothricin transaminase was approximately 40-fold higher than in wild-type controls. A further improvement in transaminase production seems to be possible by expressing the gene under the control of an inducible promoter such as *lac* UV5 or λ p_R, which are not repressed by glucose. In these systems, higher cell densities are probably achievable since the bacteria could be cultured under non-limiting glucose concentrations and cell proliferation is not hindered by the constitutive expression of high levels of transaminase.

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