JOURNAL OF BACTERIOLOGY, Oct. 1995, p. 5930–5936 Vol. 177, No. 20
0021-9193/95/$04.00+0

Transaldolase B of *Escherichia coli* K-12: Cloning of Its Gene, *talB*, and Characterization of the Enzyme from Recombinant Strains

GEORG A. SPRENGER,* ULRICH SCHÖRKEN, GERDA SPRENGER, AND HERMANN SAHM

Institut für Biotechnologie 1, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany

Received 7 June 1995/Accepted 7 August 1995

A previously recognized open reading frame (T. Yura, H. Mori, H. Nagai, T. Nagata, A. Ishihama, N. Fujita, K. Isono, K. Mizobuchi, and A. Nakata, Nucleic Acids Res. 20:3305–3308) from the 0.2-min region of the *Escherichia coli* K-12 chromosome is shown to encode a functional transaldolase activity. After cloning of the gene onto high-copy-number vectors, transaldolase B (D-sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate dihydroxyacetone transferase; EC 2.2.1.2) was overexpressed up to 12.7 U mg of protein^{-1} compared with less than 0.1 U mg of protein^{-1} in wild-type homogenates. The enzyme was purified from recombinant *E. coli* K-12 cells by successive ammonium sulfate precipitations (45 to 80% and subsequently 55 to 70%) and two anion-exchange chromatography steps (Q-Sepharose FF, Fractogel EMD-DEAE tentacle column; yield, 130 mg of protein from 12 g of cell wet weight) and afforded an apparently homogeneous protein band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a subunit size of 35,000 ± 1,000 Da. As the enzyme had a molecular mass of 70,000 Da by gel filtration, transaldolase B is likely to form a homodimer. N-terminal amino acid sequencing of the protein verified its identity with the product of the cloned gene *talB*. The specific activity of the purified enzyme determined at 30°C with the substrates fructose-6-phosphate (donor of C_3 compound) and erythrose-4-phosphate (acceptor) at an optimal pH (50 mM glycylglycine [pH 8.5]) was 60 U mg^{-1}. K_m values for the substrates fructose-6-phosphate and erythrose-4-phosphate were determined at 1,200 and 90 μM, respectively. Kinetic constants for the other two physiological reactants, D,L-glyceraldehyde 3-phosphate (K_m, 38 μM; relative activity [V_{rel}], 8%) and sedoheptulose-7-phosphate (K_m, 285 μM; V_{rel}, 5%) were also determined. Fructose acted as a C_3 donor at a high apparent K_m (≥2 M) and with a V_{rel} of 12%. The enzyme was inhibited by Tris-HCl, phosphate, or sugars with the L configuration at C_2 (L-glyceraldehyde, D-arabinose-5-phosphate).

Transaldolase (TAL; D-sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate dihydroxyacetonetransferase [EC 2.2.1.2]) is an enzyme of the nonoxidative pentose phosphate cycle (34) which was originally isolated from brewer's yeast cells (9, 10). The enzyme catalyzes the reversible transfer of a dihydroxyacetone moiety derived from fructose-6-phosphate (Fru-6-P) to erythrose-4-phosphate (Ery-4-P), forming sedoheptulose-7-phosphate (Sed-7-P) and releasing glyceraldehyde-3-phosphate (Ga-3-P). The enzyme requires no known cofactors and performs a base-catalyzed aldol cleavage reaction in which a Schiff base intermediate is formed, similar to class I aldolases. The reaction involves an active-site ε-amino group of lysine, as shown for the enzyme from *Candida utilis* (for a review, see reference 31). In bacteria, the enzyme is recruited for the catabolism of pentose sugars (e.g., D-xylose, D-ribose, and L-arabinose) and in the provision of Ery-4-P, a precursor of the shikimic acid and pyridoxine pathways, leading eventually to the aromatic amino acids and vitamins and to pyridoxine (7, 37). *Escherichia coli* mutants which lack TAL have not yet been reported, nor has a purification of the enzyme been reported for this organism. TAL-deficient mutants of *Saccharomyces cerevisiae* accumulated Sed-7-P but showed no auxotrophic traits (27). During the systematic sequencing of the *E. coli* K-12 genome region from 0 to 2.4 min (35), two open reading frames (ORFs) at 0.2 min (the first one starting at bp 7827) which showed significant amino acid identities (54.4 and 51.9% respectively) to the *TAL1*-derived sequence from *S. cerevisiae* were identified (27). Later, as a result of corrections in the sequence, a single ORF of 317 amino acids was established with overall similarity to the yeast protein (DDBJ accession number 10483). This led us to the assumption that this ORF might encode *E. coli* transaldolase, whose gene had not been previously identified. In a preliminary study, TAL A had been identified, after enrichment from crude extracts of *E. coli* K-12, by its reaction with TAL antibodies raised against the *S. cerevisiae* enzyme (29). Forty N-terminal amino acid residues (TAL A [29]) showing significant homology to the yeast enzyme were determined. TAL A, however, differed in several areas from the derived N-terminal sequence of the ORF at 0.2 min (hereafter termed *talB* [29]). A recent report on the cloning of the minor transketolase activity (*tktB*) of *E. coli* revealed the C-terminal end of an ORF just 5′ of *tktB* (mapping at 51.5 min on the linkage map [11]) which also showed amino acid identities with the yeast protein sequence. It was not clear, however, which of these two presumed *tal* genes encoded a functional TAL or whether they both contributed as isoenzymes to the overall TAL activity, as in the case of transketolase (11, 28). While the present study was being finished, another group showed that the ORF at bp 7827 of the *E. coli* map encoded a functional TAL both in *E. coli* and in *Zymomonas mobilis* (36), the latter being a bacterium which is thought to lack a genuine TAL activity (6).

We cloned the presumed *talB* gene from the 0.2-min region of the *E. coli* K-12 chromosome by a PCR approach and present evidence here that it encodes a TAL activity.

* Corresponding author. Mailing address: Institut für Biotechnologie 1, Forschungszentrum Jülich GmbH, P.O. Box 1913, D-52425 Jülich, Germany. Phone: 0049-2461-616205. Fax: 0049-2461-612710. Electronic mail address: sprenger@ibt013.ibt.kfa-juelich.de.

(Some of the present results were previously published as an abstract [28a].)

MATERIALS AND METHODS

Materials. Sugar phosphates were purchased from Sigma, Deisenhofen, Germany. Aldehydes and erythrose were supplied by Fluka. Auxiliary enzymes (triosephosphate isomerase/glycerol-3-phosphate dehydrogenase, phosphoglucose isomerase, and glucose-6-phosphate dehydrogenase), restriction endonucleases, *Taq* DNA polymerase, and T4 DNA ligase were obtained from Boehringer, Mannheim, Germany. Sodium dodecyl sulfate (SDS) was purchased from Serva, acrylamide/bisacrylamide was purchased from Bio-Rad, chromatographic standards (Combithek) were purchased from Boehringer, and Q Sepharose FF was purchased from Pharmacia, Freiburg, Germany. Fractogel EMD-DEAE-650 ("tentacle columns") was obtained from Merck, Darnstadt, Germany; glycylglycine, NADH, and NADP(H) were purchased from Biomol, Hamburg, Germany. Antibiotics, other fine chemicals, and the enzymes transketolase and TAL (both from yeast cells) were supplied by Sigma. The gene-mapping membrane containing the ordered λ-DNA bank of *E. coli* was purchased from Tahara Shuzo Co., Kyoto, Japan, and bacterial media were from Difco. Other reagents were commercial products of the highest-purity grade available.

Bacterial strains, growth conditions, and recombinant DNA techniques. *E. coli* K-12 strains were grown under aeration at 37°C in Luria-Bertani (LB) medium (26) containing 100 mg of ampicillin liter^{-1} (LB-Amp). Techniques involving manipulation of DNA, such as amplification, restriction endonuclease analysis, cloning, and transformation, were performed by standard techniques (8, 21, 27). On the basis of the published sequence (35), two oligonucleotide primers, 5′ TGG CGA ATT CCC GTC TTG TCG GCG GT 3′ (5′ of presumable *talB* sequence) and 5′ TTC GAA TTC TGA TAC ACT GCG AAG GGA GT 3′ (3′ of *talB*), including *Eco*RI restriction sites were synthesized and used together with chromosomal DNA from wild-type strain CA8000 (5) to amplify the respective portion of the chromosome. By using *Taq* DNA polymerase and a standard PCR protocol (20), a 1,133-bp fragment (see Fig. 1) was amplified. The PCR fragment was cleaved with *Eco*RI and subcloned on the high-copy-number vector pUC19 (32). Strain DH5 (8) was used as a recipient in transformations. Clones were analyzed for the correct insert by restriction analysis and by DNA sequencing by the nonradioactive fluorescent technique in conjunction with the automated A.L.F. sequencer and the protocol of the supplier (Pharmacia).

Purification of TAL from recombinant strains. TAL from recombinant strain DH5/pGSJ451 was purified by the following procedure, with all operations carried out at 4°C in 50 mM glycylglycine buffer (pH 8.5) containing 1 mM dithiothreitol. A single colony of strain DH5/pGSJ451 was inoculated into 50 ml of LB + Amp and incubated overnight at 37°C. This culture served as a starter for the main culture, which was grown in five Erlenmeyer flasks (2-liter volume each, filled with 400 ml of medium) with shaking at 37°C but without isopropyl-β-D-thiogalactopyranoside (IPTG) addition, because we learned that cells soon stopped growing when TAL activity was further induced. Cells were collected by centrifugation (12 g [wet weight]). After being washed with glycylglycine buffer, the pellets were broken by ultrasonic treatment (Branson Sonifier, Danbury, Conn.) consisting of eight 30-s bursts at 40 W with cooling in an ethanol-ice bath. After centrifugation at 20,000 × *g*, the supernatant was used as a cell extract. The protein solution was diluted to 100 ml (10 mg of protein ml^{-1}), and crystalline ammonium sulfate was added slowly with constant stirring until a salt concentration of 277 g liter^{-1} (45%) was reached. The mixture was stirred for an additional 1 h and centrifuged at 20,000 × *g* for 45 min. The precipitate was discarded, and ammonium sulfate was added to give a final concentration of 561 g liter^{-1} (80% saturation). After stirring and centrifugation, the precipitate was dissolved in 100 ml of buffer (8 mg of protein ml^{-1}) and subjected to a second round of ammonium sulfate precipitation. This time, steps were performed from 0 to 351 and 351 to 472 g liter^{-1} (about 0 to 55% and 55 to 70% salt saturation, respectively). The precipitate of the 55 to 70% step, containing about 75% of the enzyme activity, was dissolved in 100 ml of glycylglycine buffer and desalted with an ultrafiltration cell. The protein solution was applied to a Q-Sepharose FF anion-exchange column (XK 26/20; 26 by 200 mm; Pharmacia). At a flow rate of 0.5 ml min^{-1}, TAL was eluted in a linear NaCl gradient (100 to 150 mM NaCl). At this point, the enzyme was already nearly homogeneous as judged by SDS-polyacrylamide gel electrophoresis (PAGE) (see Fig. 2). Fractions with enzyme activity were pooled, dialyzed against buffer, and passed over a column (XK 26/20; 26 by 200 mm) packed with Fractogel EMD-DEAE-650 tentacle material (anion-exchange). The protein was eluted in a linear NaCl gradient in buffer at a flow rate of 0.5 ml min^{-1}.

SDS-PAGE. SDS-PAGE was carried out in the presence of 1% SDS on 12% vertical polyacrylamide gels with the buffer system of Laemmli (17). Gels were run at room temperature in an HSI model SE 400 apparatus with an LKB 2297 Macrodrive 5 power supply at a constant voltage of 100 V. Protein bands were visualized by staining with Coomassie brilliant blue G-250. By using different reference marker proteins, the subunit mass of the *E. coli* TAL B was calculated from a plot of the log of the molecular mass versus the relative mobility on SDS-polyacrylamide gels.

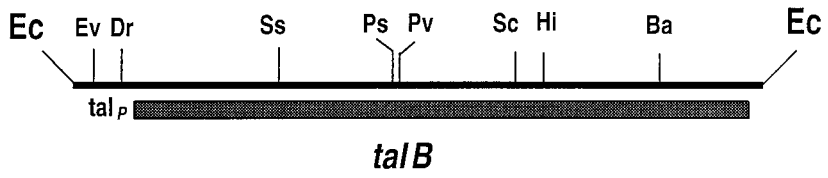
TAL assays. To determine the affinities of TAL toward its various substrates, two different assays for TAL activity were used. In assay I, the appearance of Ga-3-P from Fru-6-P and an acceptor substrate was monitored by the decrease in A_{340} of NADH, using triosephosphate isomerase and glycerol-3-phosphate dehydrogenase as auxiliary enzymes, essentially as described previously (31) with the modification that the buffer was at pH 8.5 and incubation was carried out at 30°C. In assay II, the formation of Fru-6-P from Ga-3-P and a donor was monitored by measuring the increase in NADPH concentration in the presence of phosphoglucose isomerase and glucose-6-phosphate dehydrogenase. A Shimadzu 160A UV spectrophotometer with a thermostated cuvette holder was used to detect the changes in UV absorption in both assays. Protein contents were determined by a dye-binding method (4).

RESULTS

Cloning of the *talB* gene. The *talB* gene was cloned as outlined in Materials and Methods. Both orientations of an *Eco*RI 1,133-bp DNA fragment (Fig. 1) were obtained and resulted in augmented TAL activities in crude extracts. The DNA sequence was determined and found to be identical to the one deposited in GenBank (one ORF; DDBJ no. 10483). Both types of inserts (pGSJ451 and pGSJ452) led to increased TAL activities in the crude extracts of recombinant *E. coli* DH5 (12 versus 4 U mg^{-1}), which could be further augmented by the addition, in the exponential phase, of the inducer IPTG to recombinant cells of DH5/pGSJ451 only (*talB* in the same orientation as *lac* promoter). We take this as evidence for the presence of an active promoter 5′ upstream of *talB* on the cloned *Eco*RI fragment. A typical ribosome-binding site was detected 10 bp upstream of the start codon (Fig. 1). The cloned *Eco*RI fragment was digoxigenin labeled and hybridized against an ordered λ-gene bank containing membrane-bound DNA of the whole *E. coli* chromosome (Tahara Shuzo Co.) (16). The first clone (λ101 or 9E4 of the Kohara bank) gave a strong hybridization signal, confirming the correct origin of the PCR-cloned fragment (data not shown).

Expression and purification of the plasmid-encoded TAL. Wild-type cells of *E. coli* K-12 displayed TAL activities in crude extracts ranging from 0.05 to 0.1 U mg of protein^{-1}. These activities were found after growth on LB or on minimal media with different carbon sources (glycerol, xylose, or glucose [data not shown]). The values are lower than those published previously (14), but accurate measurement in crude extracts is hampered by the coexistence of other enzymes reacting with either Fru-6-P or Ga-3-P. In crude extracts from DH5/pGSJ451, an extra protein band at 35,000 ± 1,000 Da appeared on SDS-PAGE (Fig. 2). We estimated that this prominent band constitutes up to 20% of the total soluble protein content (Fig. 2), thus being a suitable source for a rapid and high-yield enzyme purification. The purification started from strain DH5/pGSJ451, and the procedure is described in Materials and Methods. A total of about 130 mg of pure enzyme was obtained with an overall yield of 50%, corresponding to a purification factor of 4.7 (Table 1). Neither native PAGE nor SDS-PAGE displayed any protein contaminants in the DEAE tentacle fractions. No activity of other enzymes from the pentose phosphate pathway was detected in these fractions (data not shown).

N-terminal amino acid sequence. The purified enzyme was subjected to SDS-PAGE and blotted onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore) in a semidry blot apparatus. Staining was done with amido black. The first 10 amino acid residues were determined by an automated Edman degradation on an Applied Biosystems 470A gas liquid sequencer and analyzed by reversed-phase high-performance liquid chromatography. The sequence was determined as H_2N-Thr-Asp-Lys-Leu-Thr-Ser-Leu-Xaa-Gln-Tyr-. The N-terminal amino acid sequence was in agreement with the sequence deduced from the ORF at 0.2 min (35), except at position 8, where no unequivocal amino acid residue could be attributed




```
EcoRI
GAATTCCCGTCTTGTCGGCGGTTGCGCTGACGTTGCGTCGTGATATCATCAGGGCAGACCGGTTACATCC     70
                RBS
CCCTAACAAGCTGTTTAAAGAGAAAATACTATCATGACGGACAAATTGACCTCCCTTCGTCAGTACACCAC    140
                                  M  T  D  K  L  T  S  L  R  Q  Y  T  T

CGTAGTGGCCGACACTGGGGACATCGCGGCAATGAAGCTGTATCAACCGCAGGATGCCACAACCAACCCT    210
  V  V  A  D  T  G  D  I  A  A  M  K  L  Y  Q  P  Q  D  A  T  T  N  P

TCTCTCATTCTTAACGCAGCGCAGATTCCGGAATACCGTAAGTTGATTGATGATGCTGTCGCCTGGGCGA    280
  S  L  I  L  N  A  A  Q  I  P  E  Y  R  K  L  I  D  D  A  V  A  W  A

AACAGCAGAGCAACGATCGCGCGCAGCAGATCGTGGACGCGACCGACAAACTGGCAGTAAATATTGGTCT    350
K  Q  Q  S  N  D  R  A  Q  Q  I  V  D  A  T  D  K  L  A  V  N  I  G  L

GGAAATCCTGAAACTGGTTCCGGGCCGTATCTCAACTGAAGTTGATGCGCGTCTTTCCTATGACACCGAA    420
  E  I  L  K  L  V  P  G  R  I  S  T  E  V  D  A  R  L  S  Y  D  T  E

GCGTCAATTGCGAAAGCAAAACGCCTGATCAAACTCTACAACGATGCTGGTATTAGCAACGATCGTATTC    490
  A  S  I  A  K  A  K  R  L  I  K  L  Y  N  D  A  G  I  S  N  D  R  I

TGATCAAACTGGCTTCTACCTGGCAGGGTATCCGTGCTGCAGAACAGCTGGAAAAAGAAGGCATCAACTG    560
L  I  K  L  A  S  T  W  Q  G  I  R  A  A  E  Q  L  E  K  E  G  I  N  C

TAACCTGACCCTGCTGTTCTCCTTCGCTCAGGCTCGTGCTTGTGCGGAAGCGGGCGTGTTCCTGATCTCG    630
  N  L  T  L  L  F  S  F  A  Q  A  R  A  C  A  E  A  G  V  F  L  I  S

CCGTTTGTTGGCCGTATTCTTGACTGGTACAAAGCGAATACCGATAAGAAAGAGTACGCTCCGGCAGAAG    700
  P  F  V  G  R  I  L  D  W  Y  K  A  N  T  D  K  K  E  Y  A  P  A  E

ATCCGGGCGTGGTTTCTGTATCTGAAATCTACCAGTACTACAAAGAGCACGGTTATGAAACCGTGGTTAT    770
D  P  G  V  V  S  V  S  E  I  Y  Q  Y  Y  K  E  H  G  Y  E  T  V  V  M

GGGCGCAAGCTTCCGTAACATCGGCGAAATTCTGGAACTGGCAGGCTGCGACCGTCTGACCATCGCACCG    840
  G  A  S  F  R  N  I  G  E  I  L  E  L  A  G  C  D  R  L  T  I  A  P

GCACTGCTGAAAGAGCTGGCGGAGAGCGAAGGGGCTATCGAACGTAAACTGTCTTACACCGGCGAAGTGA    910
  A  L  L  K  E  L  A  E  S  E  G  A  I  E  R  K  L  S  Y  T  G  E  V

AAGCGCGTCCGGCGCGTATCACTGAGTCCGAGTTCCTGTGGCAGCACAACCAGGATCCAATGGCAGTAGA    980
K  A  R  P  A  R  I  T  E  S  E  F  L  W  Q  H  N  Q  D  P  M  A  V  D

TAAACTGGCGGAAGGTATCCGTAAGTTTGCTATTGACCAGGAAAAACTGGAAAAAATGATCGGCGATCTG   1050
  K  L  A  E  G  I  R  K  F  A  I  D  Q  E  K  L  E  K  M  I  G  D  L
                ..............  ..........
CTGTAATCATTCTTAGCGTGACCGGGAAGTCGGTCACGCTACCTCTTCTGAAGCCTGTCTGTCACTCCCT   1120
  L  *
            EcoRI
TCGCAGTGTATCAGAATTC                                                       1139
```

FIG. 1. Restriction map of the *Eco*RI fragment containing *talB* and its DNA sequence (including both *Eco*RI sites [35; also see the text]). Ec, *Eco*RI; Ev, *Eco*RV; Dr, *Dra*I; Ss, *Ssp*I; Ps, *Pst*I; Pv, *Pvu*II; Sc, *Sca*I; Hi, *Hin*dIII; Ba, *Bam*HI; tal_P, putative promoter region; RBS (double-underlined sequence), putative ribosome-binding site; ⋯, putative rho-independent terminator ($\Delta G^{\circ\prime} = -20$ kcal/mol [−83 kJ/mol]) ∗, stop codon. The deduced amino acid sequence (boldface letters) is given below the DNA sequence.

(Xaa). The formyl-methionine was cleaved off, thereby reducing the deduced protein subunit mass to 35,092.

Native and subunit molecular masses of TAL B. Examination of the comparative SDS-PAGE mobility of *E. coli* recombinant TAL with those of a number of known reference proteins indicated a subunit mass for the purified protein of 35,000 ± 1,000 Da. This was in good agreement with the mass calculated from the deduced protein sequence (without the initial f-Met) of 35,092 Da (see above). The molecular mass of native TAL B was determined by gel filtration on a Superdex 200 FPLC column (Pharmacia) calibrated with reference proteins with known molecular masses ranging from 12 to 400 kDa. Active TAL was eluted in 50 mM glycylglycine buffer (pH 8.5) containing 1 mM dithiothreitol. The enzyme eluted at a volume of 74.4 ml of buffer. In a logarithmic plot of elution volume versus molecular mass, an average mass of 70,000 ± 5,000 Da was calculated. This makes a homodimeric structure very likely.

Properties of TAL B. The influence of different buffer substances, pH, and temperature on the activity of the enzyme were analyzed by enzyme assay I (see Materials and Methods). Auxiliary enzymes were first checked for activity under the

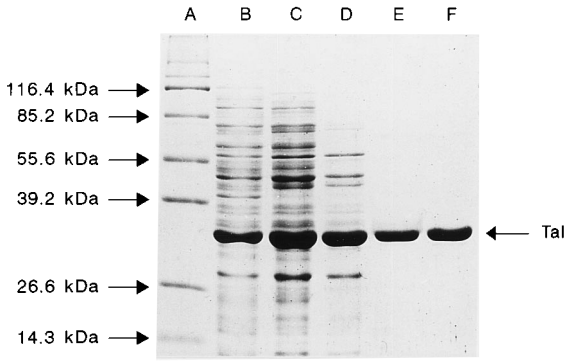


FIG. 2. SDS-PAGE analysis of the *E. coli* TAL B purification. The gel was run as described in Materials and Methods with the reference marker proteins in lane A (1, β-galactosidase, 116,400 Da; 2, phosphofructokinase, 85,200 Da; 3, glutamate dehydrogenase, 55,600 Da; 4, aldolase, 39,200 Da; 5, triosephosphate isomerase, 26,600 Da; 6, lysozyme, 14,300 Da). In lanes B to F, samples of the different purification steps (50 μg of protein each) were applied. TAL appears in all lanes at a molecular mass of 35,000 Da. Lane B contains crude extract after ultrasonication and centrifugation; lanes C and D contain samples of the ammonium sulfate precipitations as in Table 1; lane E contains extract after chromatography on Q-Sepharose FF; lane F contains extract after chromatography on DEAE tentacle column.

different reaction conditions and were then added to the reaction mixture in a great excess. As buffer substances, we tested glycylglycine, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and imidazole. TAL activity in the organic buffers was almost independent of the buffer concentration in the reaction mixture. TAL B was active in a rather broad pH range, with an optimum between pH 8.5 and 9.5 (Fig. 3). A significant loss of activity was found, however, in the presence of 50 mM Tris (at various pH values [Fig. 3]). At pH 9.0, 81% of relative activity (compared with that in glycylglycine buffer) was lost during 10 min of incubation (data not shown). This points to a reaction of Tris with residues of the catalytic center of TAL B. The enzyme was inhibited in a competitive manner by phosphate buffer. The mean K_i value at pH 8.5 determined from five different phosphate concentrations (between 4 and 100 mM) was 19 ± 3 mM. The phosphate ions may interact with the amino acids, which are necessary for fixing the phosphate groups of the substrates from the pentose phosphate pathway.

The enzyme displayed a narrow temperature optimum in the range of 15 to 40°C; with an increase in temperature of 10°C the activity was doubled. At temperatures above 50°C, activity was lost rapidly, and at 55°C, the enzyme was totally inactivated. The isoelectric point of the enzyme was determined by isoelectric focusing on precast Phast gels (pH range 3 to 9; broad pI-marker kit [Pharmacia]) and was found to be 4.6. This compares well with the calculated pI of 4.96 deduced from the protein sequence.

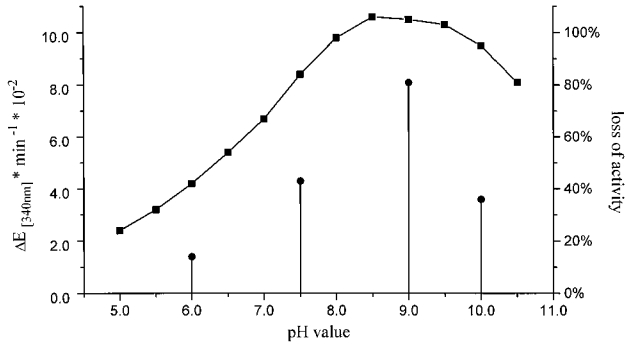


FIG. 3. pH optimum of TAL B and inhibitory effect of Tris. The TAL B activity in optimal organic buffers at different pH values (■) is given. The loss of activity per 10 min at different pH in Tris (50 mM; ●) is given below, showing a correlation of percent inhibition and activity at different pH values.

Kinetic studies on TAL B. TALs of both eukaryotic and prokaryotic origins are known to accept only a narrow range of acceptor and donor substrates other than their physiological substrates (34). The kinetic constants K_m and V_{max} were determined for a spectrum of sugars and sugar phosphates as possible substrates for TAL B from *E. coli* (Table 2). To compare TAL activity with different donor and acceptor compounds, the V_{max} values of the standard reaction pair Fru-6-P

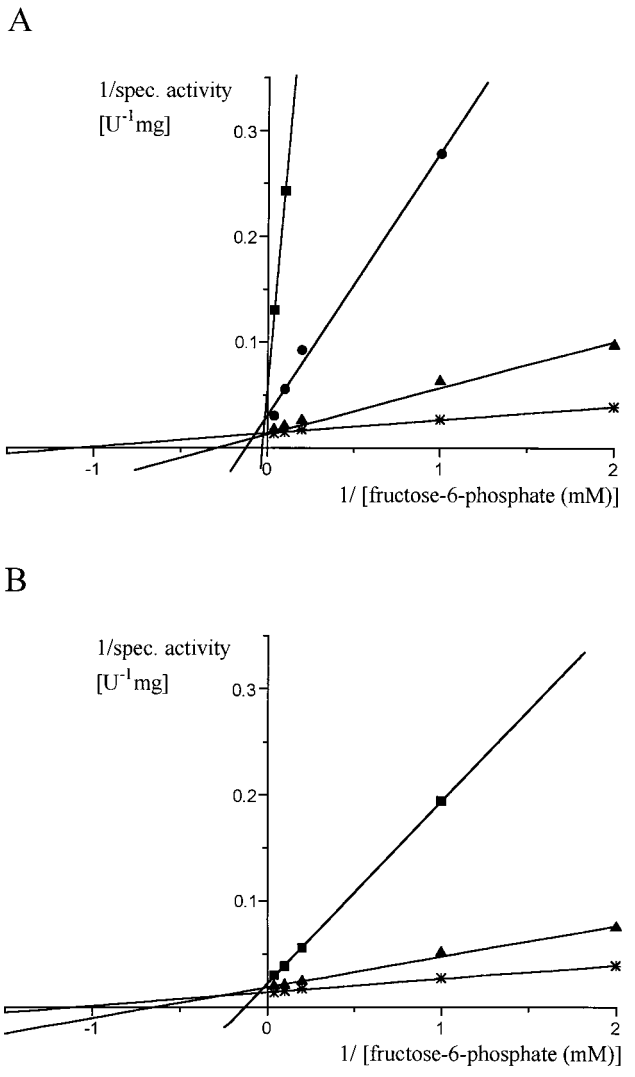


FIG. 4. Inhibitory effect of Ara-5-P and L-glyceraldehyde on TAL activity. (A) Inhibitory effect of Ara-5-P at various inhibitor concentrations, shown as a double-reciprocal Lineweaver-Burk plot. *, without inhibitor; ▲, 100 μM Ara-5-P; ●, 1 mM Ara-5-P; ■, 10 mM Ara-5-P (final concentrations). (B) Inhibition by L-glyceraldehyde at final concentrations of 10 mM (▲) and 50 mM (■), compared with the reaction without inhibitor (*). Ery-4-P and Fru-6-P were used as substrates. The concentration of Fru-6-P was varied and Ery-4-P was added at 1 mM (10-fold higher than the K_m). At high concentrations of Fru-6-P, the competitive inhibition of Ara-5-P on Ery-4-P results in intercepts on the *y* axis that do not coincide.

TABLE 1. Purification scheme for *E. coli* TAL B

Sample	Total activity (U)	Total protein (mg)	Spec act (U/mg)	Purification factor	Yield (%)
Crude extract	12,700	1,000	12.7	1.0	100
1.$(NH_4)_2SO_4$-prec. 40 to 80%[a]	9,500	800	13.3	1.05	75
2.$(NH_4)_2SO_4$-prec. 55 to 75%[a]	8,900	370	24.0	1.9	70
Q-Sepharose FF fractions	7,000	170	46.9	3.7	55
EMD-DEAE fractions	6,400	130	60.0	4.7	50

[a] prec., precipitate. Percentages indicate saturation (see the text).

and Ery-4-P (average, 60 U mg^{-1}) were determined each time as a control and were set at 100%. The pair Ery-4-P and Fru-6-P led to the highest reaction rates by far. The other two physiological substrates (Ga-3-P and Sed-7-P) gave less than 10% of relative activity, although the respective K_m values were below 1 mM. Relative rates (V_{rel}) of other compounds are given in Table 2.

A comparison of the properties of *E. coli* TAL B with the enzymes from *S. cerevisiae* and the slime mold *Dictyostelium discoideum* and with *E. coli* transketolase A is presented in Table 2. K_m values for the physiological substrates are in the same order of magnitude for all three TALs.

TAL B was inhibited competitively by sugars with L-configuration at C_β, as shown for D-arabinose-5-phosphate (Ara-5-P) and L-glyceraldehyde (Fig. 4). Inhibitor studies were performed with the substrate pair Fru-6-P and Ery-4-P, with the Fru-6-P concentrations being varied. Inhibition of TAL B by Ara-5-P was more than 100-fold stronger (mean K_i, about 47 μM) than that exerted by L-glyceraldehyde (mean K_i about 5.6 mM [Fig. 4]). Ara-5-P at high concentrations (1 or 10 mM) also inhibited reaction with Ery-4-P, which was added in excess (1 mM or about 10-fold over K_m), in a competitive manner. Because of this inhibition of Ery-4-P, the V_{max} of TAL could not be reached even at saturating Fru-6-P concentrations. This double inhibition is reflected by the intercepts with the *y* axis ($1/V_{max}$) of a Lineweaver-Burk diagram (Fig. 4A) that do not coincide at different inhibitor concentrations, as would be expected if only one substrate is outcompeted.

Comparisons of the TAL B sequence with TALs of other origins. As the ORF on the cloned *Eco*RI fragment encoded an active TAL, namely, TAL B, a comparison of its derived amino acid sequence with other known TAL sequences was performed and is given in Fig. 5. TAL B shows 53% identical amino acid residues to the *S. cerevisiae* sequence (27), 55% identical to *Kluyveromyces lactis* (12), and a remarkable 55% identical to a human cDNA clone (2). A database search (TBLASTN program of EMBL HUSAR, Heidelberg, Germany) gave significant scores with these three sequences and additional partial homology with a cDNA clone from the plant *Arabidopsis thaliana* (21) displaying the typical peptide PGRx STEVDRAL (Fig. 5). Whether this *Arabidopsis* clone indeed encodes TAL remains to be shown (22).

A secondary-structure determination was performed by a system of neural networks, where a multiple sequence alignment is given as input for the prediction (24, 25). The sequences from *S. cerevisiae*, *K. lactis*, and the human clone showed an almost complete structural homology to the *E. coli* sequence. Only a less conserved region (amino acids 266 to 285) in the *E. coli* sequence differed in the secondary-structure prediction (Fig. 5). The sequences show over 60% helical areas, which are all highly conserved. All gaps in the sequence alignment are located within predicted loop regions, which should allow higher variability without inducing large structural differences. As TAL has a similar action to class I aldolases, the sequence of the *Staphylococcus carnosus* fructose bisphosphate aldolase (33) was also compared. As can be seen from Fig. 5, conserved amino acid residues occur in both aldolase types but the reactive lysine residue (19) of TAL is not conserved in the class I aldolase (see also reference 36).

TABLE 2. Kinetic constants for TAL B in comparison with yeast TAL and with *E. coli* transketolase A[a]

Substrate	TAL B		Transketolase A		Yeast[b] K_m (mM)	*D. discoideum* K_m (mM)
	K_m (mM)	V_{rel} (%)	K_m (mM)	V_{rel} (%)		
C_3 acceptor compounds						
D,L-Glyceraldehyde-3-phosphate	0.038	8	2.1	200	0.22	0.072
D-Erythrose-4-phosphate	0.090	100[c]	0.09	≥200	0.018	0.10
D-Ribose-5-phosphate[d]	31	0.8	1.4	100[e]	NA[e]	NA
D-Glyceraldehyde	28	7	10[f]	50[f]	NA	NA
C_3 donor compounds						
D-Xylulose-5-phosphate	ND[g]	ND	0.16	≥100[e]	NA	NA
D-Fructose-6-phosphate	1.2	100[c]	1.1	60	0.32	1.6
D-Sedoheptulose-7-phosphate	0.285	5	4.0	5	0.18	0.46
D-Fructose	>2000	12	ND	ND	NA	NA

[a] The kinetic constants for TAL were determined by enzyme assay I or II (see Materials and Methods). Lineweaver-Burk plots were used to determine the K_m values, and the cosubstrate was present in a great excess. Because of differences in reaction using the respective enzyme assays, the V_{max} of the reaction pair Fru-6-P and Ery-4-P is set as 100%. Data for transketolase A are taken from reference 30.

[b] Data for yeast (*S. cerevisiae*) are from compilations in reference 31.

[c] Ery-4-P, 80 U mg^{-1}; Fru-6-P, 60 U mg^{-1}.

[d] The product expected would be D-*glycero*-D-*altro*-octulose 8-phosphate (23).

[e] Rib-5-P, 55 U mg^{-1}; Xul-5-P, 110 U mg^{-1}. NA, not available.

[f] The D,L-racemate was used.

[g] ND, no donor compound.

FIG. 5. Alignment of partial and complete TAL sequences from different organisms. The sequence of TAL B (*Eco*B [35], DDBJ 10483 [this study]), partial sequences from TAL A (*Eco*A [29]), and C-terminal-derived sequence of *E. coli* (*ORF1*) preceding gene *tktB* [11] are shown. Sequences of TAL from *S. cerevisiae* (*Sce* [27]), *K. lactis* (Klac [12]), and humans (*Hum* [2]) and the sequence of an as yet unidentified cDNA clone from *A. thaliana* (*Ath* [22, 23]) are given. For comparison with bacterial class I aldolases, the sequence of fructose-1,6-bisphosphate aldolase (*ScFda* of *Staphylococcus carnosus* [33]) is aligned but not included in the determination of conserved residues. Boldface letters indicate highly conserved residues in all sequences, and an asterisk below the alignment marks unaltered residues in all transaldolases. +, end of known sequence; #, catalytic lysine residue involved in Schiff-base reaction of *S. cerevisiae* TAL (19), and @@ denote the His-Cys residues of the *S. cerevisiae* sequence discussed in the text. The amino acid residues are numbered according to the TAL B sequence. Above the amino acid numbering, a secondary-structure prediction (24, 25) for the TAL B sequence is given (H, α-helix; S, β-sheet; −, loop regions). All boldface letters denote conserved secondary-structure regions when compared with structure predictions for the *S. cerevisiae*, *K. lactis*, and human sequences.

DISCUSSION

In this paper, we present evidence that a previously sequenced DNA fragment from the 0.2-min region of the *E. coli* chromosome (35) encodes a functional TAL. To our knowledge, *talB* constitutes the first complete gene encoding a TAL from a bacterial source. The cloned gene, in both orientations of a PCR-derived *Eco*RI fragment, led to a significant increase in the TAL activity of recombinant *E. coli* strains. This points to the existence of a promoter region between the first *Eco*RI site and the start codon on the cloned fragment. From cell extracts of a recombinant strain, TAL B activity was purified to apparent homogeneity with a yield of about 130 mg (50% of the initial total activity). The purification was accelerated by the availability of the cloned *talB* gene from this organism on a high-copy-number vector, leading to elevated activities even in the absence of IPTG. Two rounds of ammonium sulfate precipitation and two anion-exchange chromatography steps led to an apparently homogeneous protein. Evidence for the purity was provided (i) by visual inspection of Coomassie blue-stained SDS-PAGE gels and (ii) by the unambiguous determination of the N-terminal amino acid residues. The preparation was suitable for crystallization (13), underlining its purity. The enzyme most probably forms a dimer of identical subunits with molecular mass 35,000 ± 1,000 Da.

This is the first report on the detailed kinetics of a purified TAL from *E. coli* and verifies the existence of at least two genes encoding this enzyme. The gene for TAL A from *E. coli* (29) has not been found yet. It may be identical, however, to an ORF upstream of the *tktB* gene (11). It will be necessary to knock out both genes to study their relative contributions to the pentose phosphate pathway of *E. coli*. The substrate specificity of *E. coli* TAL B appeared to be as narrow as is also known for enzymes of other origin such as yeasts or the slime mold *D. discoideum* (1, 37). With regard to its physiological substrates, TAL competes with transketolase for the common intermediates Ga-3-P, Sed-7-P, Fru-6-P, and Ery-4-P. The affinities of both TAL B and transketolase A for Fru-6-P and Ery-4-P are about equal (Table 2). However, Ga-3-P and Sed-7-P (the products of transketolase A action on ribose-5-phosphate [Rib-5-P] and xylulose-5-phosphate) are better substrates for TAL than for transketolase (38 μM versus 2.1 mM and 285 μM versus 4.1 mM). This higher affinity may play a role in the regulation of carbon flux through the pentose phosphate pathway as the reactive advantage of transaldolase could favor the formation of Ery-4-P, an essential metabolite for the biosynthesis of aromatic amino acids, aromatic vitamins, and pyridoxine (37).

Inhibition by D-arabinose-5-phosphate (Ara-5-P) occurs with a relatively low K_i of about 50 μM. Ara-5-P is derived from ribulose-5-phosphate by isomerization and is a precursor for KDO-8-P in gram-negative bacteria (18). Its intracellular accumulation and inhibition exerted on TAL could constitute

a regulatory junction between cell growth and central metabolism. To assess whether Ara-5-P plays a significant regulatory role in vivo, the free intracellular Ara-5-P concentrations must be known, however.

TAL B from *E. coli* accepts unphosphorylated substrates, e.g., fructose, albeit with lower affinity and activity. Besides the four intermediates of the pentose phosphate cycle, an early report on a *Torula* TAL had shown fructose (no K_m value provided) and D-glyceraldehyde (K_m 7 mM) as substrates for the enzyme (3). TAL B could be used in C—C bonding and in enantiospecific enzymatic syntheses of novel sugars, as has previously been demonstrated for transketolase or aldolase from yeast cells (15). With the present report on a *talB* recombinant *E. coli* strain which overproduces the enzyme 100-fold and from which the enzyme could be purified with rather simple chromatography steps, an inexpensive source is now available.

The reactive lysine residue of the *S. cerevisiae* enzyme has recently been determined by mutagenesis (19); this lysine residue is conserved in all sequences shown in Fig. 5. An earlier report stated that a His-Cys dipeptide from *Candida* TAL was involved in the catalytic activity (31). Site-directed mutagenesis of the two residues on the *S. cerevisiae* gene led to drastically reduced enzyme activities but also to a nearly total loss of TAL protein; this was taken as evidence that the Cys residue is required for enzyme stability but not necessarily for catalytic activity (19). In this context, it is noteworthy that in the *E. coli* TAL B sequence the respective His residue is altered to an Asn residue and, moreover, there are no His residues conserved between the different sequences. Knowledge of the structure of TAL would certainly help us to understand whether a His residue is actually involved in the catalytic mechanism. The high phylogenetic conservation and the similarity in secondary structure between the proteins obtained from organisms ranging from a bacterium to humans suggest a high pressure to retain this structure. Structure analysis of the crystallized TAL B is under way (13).

ACKNOWLEDGMENTS

The skillful technical assistance of Doris Dohmen-Olma is gratefully acknowledged. We thank D. Kinzelt for help with the N-terminal amino acid sequencer, J.-J. Bongs for high-performance liquid, chromatography analyses, and I. Schaaff-Gerstenschläger for the gift of antibody material raised against yeast TAL. We thank G. Schneider, Uppsala, Sweden, for critically reading the manuscript and J. Carter-Sigglow for proofreading its English.

This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft through SFB380/B21.

REFERENCES

1. **Albe, K. R.** 1991. Partial purification and kinetic characterization of transaldolase from *Dictyostelium discoideum*. Exp. Mycol. **15:**255–262.
2. **Banki, K., D. Halladay, and A. Perl.** 1994. Cloning and expression of the human gene for transaldolase. J. Biol. Chem. **269:**2847–2851.
3. **Bonsignore, A., S. Pontremoli, E. Grazi, and M. Mangiarotti.** 1959. The formation and cleavage of fructose catalyzed by transaldolase. Biochem. Biophys. Res. Commun. **1:**79–82.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. **72:**248–254.
5. **Brickman, E., L. Soll, and J. Beckwith.** 1973. Genetic characterization of mutations which affect catabolite-sensitive operons in *Escherichia coli*, including deletions of the gene for adenylate cyclase. J. Bacteriol. **116:**582–587.
6. **Feldmann, S. D., H. Sahm, and G. A. Sprenger.** 1992. Pentose metabolism in *Zymomonas mobilis* wild-type and recombinant strains. Appl. Microbiol. Biotechnol. **38:**354–361.
7. **Fraenkel, D. G.** 1987. Glycolysis, pentose phosphate pathway, and Entner-Doudoroff pathway, p. 142–150. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. ASM Press, Washington, D.C.
8. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
9. **Horecker, B. L., and P. Z. Smyrniotis.** 1953. Transaldolase: the formation of fructose-6-phosphate from sedoheptulose-7-phosphate. J. Am. Chem. Soc. **75:**2021–2022.
10. **Horecker, B. L., and P. Z. Smyrniotis.** 1955. Purification and properties of yeast transaldolase. J. Biol. Chem. **212:**811–825.
11. **Iida, A., S. Teshiba, and K. Mizobuchi.** 1993. Identification and characterization of the *tktB* gene encoding a second transketolase in *Escherichia coli* K-12. J. Bacteriol. **175:**5375–5383.
12. **Jacoby, J., C. P. Hollenberg, and J. J. Heinisch.** 1993. Transaldolase mutants in the yeast *Kluyveromyces lactis* provide evidence that glucose can be metabolized through the pentose phosphate pathway. Mol. Microbiol. **10:**867–876.
13. **Jia, J., Y. Lindqvist, G. Schneider, U. Schörken, H. Sahm, and G. A. Sprenger.** Crystallization and preliminary X-ray crystallography analysis of recombinant transaldolase B from *Escherichia coli*. Acta Cryst. Sec. D, in press.
14. **Josephson, B. L., and D. G. Fraenkel.** 1969. Transketolase mutants of *Escherichia coli*. J. Bacteriol. **100:**1289–1295.
15. **Kobori, Y., D. C. Myles, and G. M. Whitesides.** 1992. Substrate specificity and carbohydrate synthesis using transketolase. J. Org. Chem. **57:**5899–5907.
16. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
18. **Lim, R., and S. S. Cohen.** 1966. D-Phosphoarabinoisomerase and D-ribulokinase in *Escherichia coli*. J. Biol. Chem. **241:**4304–4315.
19. **Miosga, T., I. Schaaff-Gerstenschläger, E. Franken, and F. K. Zimmermann.** 1993. Lysine^{144} is essential for the catalytic activity of *Saccharomyces cerevisiae* transaldolase. Yeast **9:**1241–1249.
20. **Mullis, K. B., and F. A. Faloona.** 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. **155:**335–350.
21. **Newman, T.** 1994. Accession number EMBL AT545.
22. **Newman, T., F. J. de Bruijn, P. Green, K. Keegstra, H. Kende, L. McIntosh, J. Ohlrogge, N. Raikhel, S. Somerville, M. Thomashow, E. Retzel, and C. Somerville.** 1994. Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous *Arabidopsis* cDNA clones. Plant Physiol. **106:**1241–1255.
23. **Racker, E., and E. A. R. Schröder.** 1957. Formation and utilization of octulose-8-phosphate by transaldolase and transketolase. Arch. Biochem. Biophys. **66:**241–243.
24. **Rost, B., and C. Sander.** 1993. Prediction of protein structure at better than 70% accuracy. J. Mol. Biol. **232:**584–599.
25. **Rost, B., and C. Sander.** 1994. Combining evolutionary information and neural networks to predict protein secondary structure. Proteins **19:**55–72.
26. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Schaaff, I., S. Hohmann, and F. K. Zimmermann.** 1990. Molecular analysis of the structural gene for yeast transaldolase. Eur. J. Biochem. **188:**597–603.
28. **Sprenger, G. A.** 1993. Nucleotide sequence of the *Escherichia coli* K-12 transketolase (*tkt*) gene. Biochim. Biophys. Acta **1216:**307–310.

28a. **Sprenger, G. A.** 1994. Transketolase and transaldolase enzymes of *Escherichia coli* K-12: properties of the major enzymes and of defect mutants. Abstracts of the VAAM Meeting. Bio Engineering **10:**105.

29. **Sprenger, G. A.** Accession number P80218, and unpublished results.
30. **Sprenger, G. A., U. Schörken, G. Sprenger, and H. Sahm.** 1995. Transketolase A of *Escherichia coli* K-12: purification and properties of the enzyme from recombinant strains. Eur. J. Biochem. **230:**525–532.
31. **Tsolas, O., and B. L. Horecker.** 1972. Transaldolase, p. 259–280. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 7. Academic Press, Inc., New York.
32. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
33. **Witke, C., and F. Götz.** 1993. Cloning, sequencing, and characterization of the gene encoding the class I fructose-1,6-bisphosphate aldolase of *Staphylococcus carnosus*. J. Bacteriol. **175:**7495–7499.
34. **Wood, T.** 1985. The pentose phosphate pathway. Academic Press, Inc., Orlando, Fla.
35. **Yura, T., H. Mori, H. Nagai, T. Nagata, A. Ishihama, N. Fujita, K. Isono, K. Mizobuchi, and A. Nakata.** 1992. Systematic sequencing of the *Escherichia coli* genome: analysis of the 0–2.4 min region. Nucleic Acids Res. **20:**3305–3308.
36. **Zhang, M., C. Eddy, K. Deanda, M. Finkelstein, and S. Picataggio.** 1995. Metabolic engineering of a pentose metabolism pathway in ethanologenic *Zymomonas mobilis*. Science **267:**240–243.
37. **Zhao, G., and M. E. Winkler.** 1994. An *Escherichia coli* K-12 *tktA tktB* mutant deficient in transketolase activity requires pyridoxine (vitamin B_6) as well as the aromatic amino acids and vitamins for growth. J. Bacteriol. **176:**6134–6138.