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Identification and Characterization of the *tktB* Gene Encoding a Second Transketolase in *Escherichia coli* K-12

AKIHIRO IIDA,[1]* SADAO TESHIBA,[1] AND KIYOSHI MIZOBUCHI[2]†

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Machida, Tokyo 194,[1] and Department of Biochemistry and Biophysics, The University of Tokyo, Hongo, Tokyo 113,[2] Japan

Received 23 April 1993/Accepted 15 June 1993

We isolated a transposon Tn*10* insertion mutant of *Escherichia coli* K-12 which could not grow on MacConkey plates containing D-ribose. Characterization of the mutant revealed that the level of the transketolase activity was reduced to one-third of that of the wild type. The mutation was mapped at 63.5 min on the *E. coli* genetic map, in which the transketolase gene (*tkt*) had been mapped. A multicopy suppressor gene which complemented the *tkt* mutation was cloned on a 7.8-kb *Pst*I fragment. The cloned gene was located at 53 min on the chromosome. Subcloning and sequencing of a 2.7-kb fragment containing the suppressor gene identified an open reading frame encoding a polypeptide of 667 amino acids with a calculated molecular weight of 72,973. Overexpression of the protein and determination of its N-terminal amino acid sequence defined unambiguously the translational start site of the gene. The deduced amino acid sequence showed similarity to sequences of transketolases from *Saccharomyces cerevisiae* and *Rhodobacter sphaeroides*. In addition, the level of the transketolase activity increased in strains carrying the gene in multicopy. Therefore, the gene encoding this transketolase was designated *tktB* and the gene formerly called *tkt* was renamed *tktA*. Analysis of the phenotypes of the strains containing *tktA*, *tktB*, or *tktA tktB* mutations indicated that *tktA* and *tktB* were responsible for major and minor activities, respectively, of transketolase in *E. coli*.

Transketolase (EC 2.2.1.1) catalyzes transfer of the glycol aldehyde moiety from a ketose or its phosphate to an aldose or its phosphate. The enzyme is found in animals, plants, and bacteria and is involved in the pentose phosphate pathway responsible for production of NADPH and several sugar phosphate intermediates such as ribose 5-phosphate, erythrose 4-phosphate, and sedoheptulose 7-phosphate. Ribose 5-phosphate is utilized for the biosynthesis of purine and pyrimidine nucleotides and histidine, erythrose 4-phosphate is used for the biosynthesis of aromatic amino acids, and sedoheptulose 7-phosphate is used for the biosynthesis of cell wall components in gram-negative bacteria. The reaction is also important in CO_2 fixation in photosynthetic organisms.

In *Escherichia coli* K-12, Josephson and Fraenkel (18, 19) first isolated mutants defective in transketolase activity. These mutants were unable to use L-arabinose or D-xylose as a sole carbon source and required shikimic acid or aromatic amino acids for growth on a minimal medium. The requirement for aromatic amino acids was shown to be slightly leaky, and the existence of low residual transketolase activity was suggested by the authors. The mutations were mapped around 62 min on the *E. coli* chromosome. A similar mutant was also isolated from *Salmonella typhimurium*, and the role of transketolase in supplying sedoheptulose 7-phosphate was examined (7).

In this report, we describe isolation of a transposon Tn*10* insertion mutant of *E. coli* defective in transketolase as a ribose-sensitive mutant on MacConkey plates containing D-ribose. By isolating the clone which complemented the mutation, we succeeded in identifying a second transketolase gene. We present the nucleotide sequence, characterization of the mutant phenotype, and expression of the second transketolase gene, which we designated *tktB*.

* Corresponding author.
† Present address: Department of Applied Physics and Chemistry, University of Electro-Communications, Chofu-shi, Tokyo 182, Japan.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages. Bacterial strains and plasmids used in this work are listed in Table 1. The bacterial strains were all derivatives of *E. coli* K-12. Bacteriophages used were λtc (N^+ *c*I857 *b*221 *Oam*21 *c*III::Tn*10*) (10) for Tn*10* insertion mutagenesis and P1*kc* for transduction.

Media. *E. coli* cells were grown in LB medium (29) or M9 minimal medium (29) with 0.2% D-ribose. Solid media for plates contained 1.5% agar. MacConkey plates contained 4% MacConkey agar base and 1% sugar. Isopropylthiogalactopyranoside (IPTG) or amino acids were supplemented at a final concentration of 1 mM when required. Ampicillin (75 μg/ml), tetracycline (15 μg/ml), or kanamycin (40 μg/ml) was added to the media when necessary.

Genetic procedures and DNA manipulation. Standard genetic procedures such as bacterial conjugation, preparation of phage lysates, and P1 transduction were performed as described by Miller (29). Transposon insertion mutagenesis of Tn*10* was performed as described previously (20). DNA manipulation (e.g., preparation, digestion, and ligation of plasmid DNA) and agarose gel electrophoresis were performed by standard procedures (27).

Determination of the site of Tn*10* insertion by Southern blot analysis. Determination of the site of Tn*10* insertion in the *E. coli* chromosome was carried out by the method of Yonetani (44). Chromosomal DNA from strain AI80 was prepared as described previously (15). After digestion of the chromosomal DNA with appropriate restriction enzymes, the DNA fragments formed were separated by agarose gel electrophoresis and transferred onto nitrocellulose filters (BA85; Schleicher & Schuell) as described previously (36). A 389-bp *Hpa*I-*Hin*dIII fragment of pTN203, which corresponded to

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Reference or source
E. coli strains		
W3110	F⁻ λ⁻ IN(*rrnD-rrnE*)1	1
EJ500	W3110 *cfs*	14
CSH57b	F⁻ *ara leu lacY purE gal trp his argG tsx rpsL xyl mtl ilvA metA* or *metB thi*	29
CSH64	Hfr *thi*	29
JC7623	F⁻ *recB recC sbcB15 thi-1 thr-1 leu-6 lacY1 mtl-1 xyl-5 ara-14 galK2 his-4 proA2 argE3 rpsL31 tsx-33 sup-37*	23
H677	F⁻ *his tyrA purC*	NIG[a]
PL1068	W3110 *guaA*	25
AI80	EJ500 *tktA*::Tn*10*	This study
AI1116	JC7623 *tktB*::*kan*	This study
AI1118	AI80 *tktB*::*kan*	This study
AI1122	EJ500 *tktB*::*kan*	This study
AI1156	AI80 *rbsK101*::Tn*5*	This study
Plasmids		
pUC19	Cloning vector	43
pUC4K	Cloning cartridge, Kan^r	41
pTN203	389-bp *Hpa*I-*Hin*dIII fragment of Tn*10* inserted between the *Sma*I and *Hin*dIII sites of pTZ19R (28)	44
pAI198	7.8-kb *Pst*I fragment carrying *tktB* inserted at the *Pst*I site of pUC19	This study
pAI210	3.8-kb *Pvu*II-*Pst*I fragment of pAI198 inserted between the *Hin*cII and *Pst*I sites of pUC19	This study
pAI218	1.3-kb *Hin*cII fragment of pUC4K inserted at the blunt-ended *Spl*I site of pAI210	This study

[a] NIG, National Institute of Genetics (Mishima, Japan).

TABLE 2. Activities of transketolase in cell extracts from strains carrying *tkt* mutations or *tkt* clones

Bacterial strain (plasmid)[a]	Relevant genotype	Transketolase sp act (U)
EJ500	*tktA*⁺ *tktB*⁺	0.10
AI80	*tktA*::Tn*10*	0.03
AI1122	*tktB*::*kan*	0.09
AI1118	*tktA*::Tn*10 tktB*::*kan*	0.03
AI80 (pAI198)	*tktA*::Tn*10*⁺/*tktB* clone	0.14
AI80 (pAI210)[b]	*tktA*::Tn*10*⁺/*tktB* clone	1.4

[a] Strains were grown for 16 h at 37°C in LB medium.
[b] IPTG (1 mM) was added as the inducer.

nucleotides 1884 to 2272 on the physical map of Tn*10* (21), was used as the probe. Labeling of probes, hybridization, and detection of hybridized DNA fragments were performed by using the DIG DNA Labeling and Detection kit (Boehringer Mannheim) as specified by the supplier. The size of hybridized DNA was estimated by using *Hin*dIII-digested λ DNA (40) as a standard, and a physical map of the chromosomal region starting from the right end of Tn*10* was constructed on the basis of the physical map of Tn*10* (21). The physical map was compared with that of the whole *E. coli* chromosome (22). The location which showed a similar pattern of restriction sites was surveyed by eye, and the site of insertion of Tn*10* was determined.

DNA sequencing. A series of deletions of pAI198 and pAI210 was constructed by using exonuclease III as described by Henikoff (12). Nucleotide sequences were determined for both strands by the dideoxy-chain termination method (31), using the Taq Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems, Inc.) with a universal primer or synthetic oligonucleotide primers. Samples were analyzed by an automated DNA sequencer (model 373A; Applied Biosystems, Inc.).

Protein analysis. Total proteins of *E. coli* cells were analyzed on a 9% polyacrylamide gel with 0.1% sodium dodecyl sulfate (SDS) (24). After polyacrylamide gel electrophoresis (PAGE), the gel was stained with Coomassie brilliant blue R-250. Molecular weight standards used were phosphorylase *b* (97,400), bovine serum albumin (66,200), ovalbumin (45,000), and carbonic anhydrase (31,000). Analysis of amino acid sequences containing the N-terminal amino acid of the *tktB* gene product was performed by the automated Edman degradation method (6, 13), using an Applied Biosystems 470A protein sequencer.

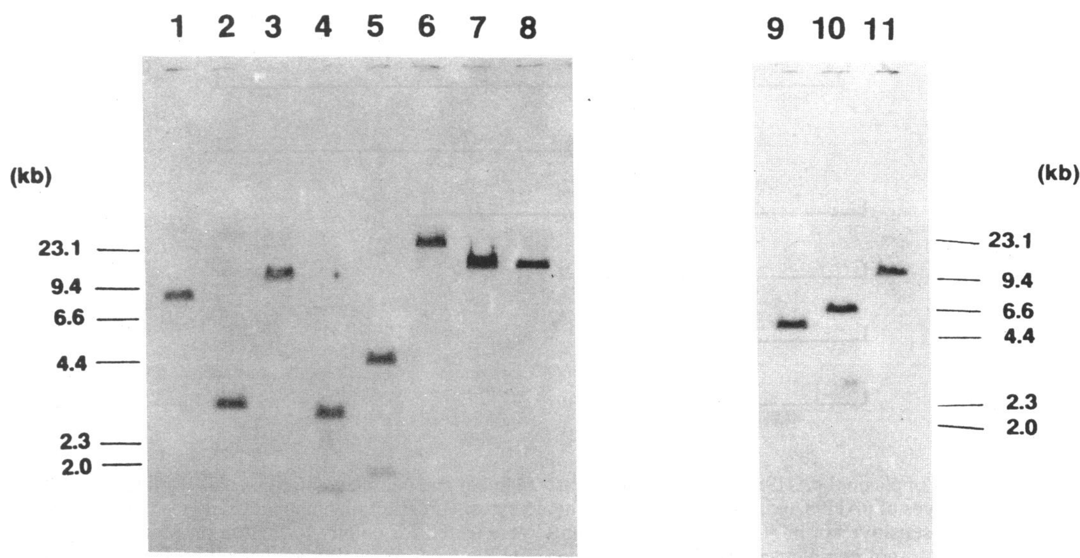
Disruption of the chromosomal *tktB* gene. Disruption of the chromosomal *tktB* gene was performed as follows. Plasmid pAI210 carrying the whole *tktB* gene was digested with *Spl*I, the two sites of which were located in the *tktB* gene, and blunt ended with T4 DNA polymerase. Then a 1.3-kb *Hin*cII fragment of pUC4K which contained the kanamycin resistance determinant of Tn*903* was ligated to the blunt-ended *Spl*I site of pAI210 to make pAI218. Integration of a 4.4-kb linearized *Eco*RI-*Sph*I fragment of pAI218 into the chromosome of *E. coli* JC7623 was performed as described by Winans et al. (42).

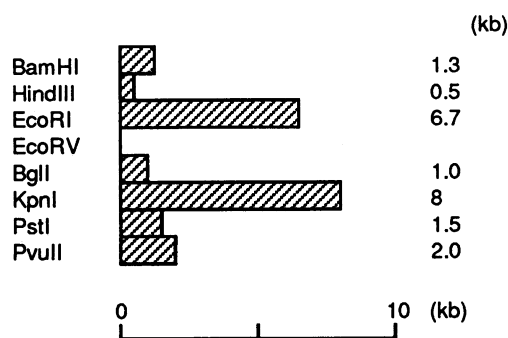
Transketolase assay. Cells grown to the stationary phase at 37°C in LB medium with appropriate antibiotics or IPTG were harvested by brief centrifugation, washed once with saline buffer, and suspended in 50 mM Tris-HCl (pH 7.5), adjusting the concentration of 100 mg (wet weight) of cells per ml. The suspension was cooled in an ice-salt bath and then sonicated for five 10-s intervals with a 15-s pause between each sonication. The sonicated samples were centrifuged at 12,000 × *g* for 30 min to remove unbroken cells. The supernatant fraction was used as crude cell extracts. Transketolase activity was assayed as described previously (34). The reaction mixture (1 ml) contained 50 mM Tris-HCl (pH 7.5), 0.24 mM $MgCl_2$, 10 μM thiamine pyrophosphate (TPP), 0.25 mM NADH, 3 U of glycerol 3-phosphate dehydrogenase, 10 U of triosephosphate isomerase (Boehringer Mannheim), 0.5 mM D-ribose 5-phosphate (Sigma Chemical Co.), and 0.5 mM D-xylulose 5-phosphate (Sigma). The reaction proceeded at 37°C, and the decrease in A_{340} was monitored with a Shimadzu UV2100 spectrophotometer. One unit of enzyme was defined as the amount of enzyme which oxidized 1 μmol of NADH per min. Specific activity was expressed as units per milligram of protein. Protein concentration was determined by the method of Bradford (2), using bovine gamma globulin as a standard.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under accession number D12473.

RESULTS

Isolation of a ribose-sensitive mutant. After mutagenesis of *E. coli* EJ500 with transposon Tn*10*, we isolated a mutant, designated as AI80, which could grow on LB plates but not on MacConkey plates containing 1% D-ribose. When the





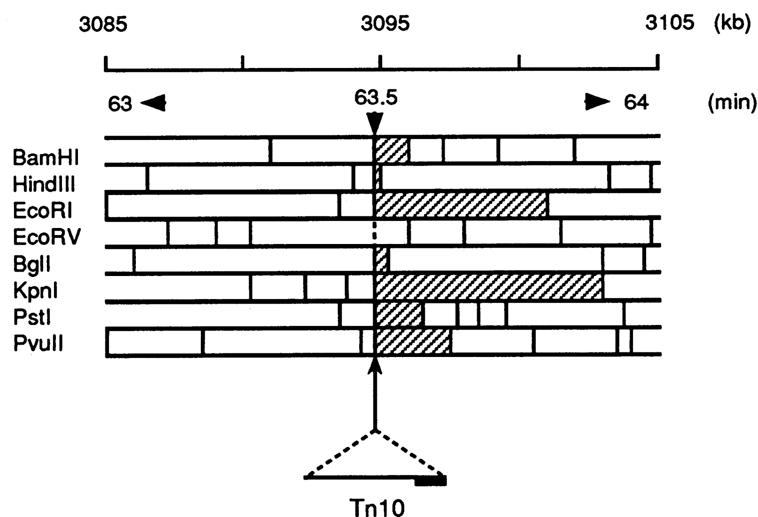


FIG. 1. Mapping of the Tn*10* insertion site in AI80 on the *E. coli* chromosome. (A) Southern hybridization analysis of AI80. Chromosomal DNA from *E. coli* AI80 was digested with various restriction enzymes, separated by agarose gel electrophoresis, transferred onto nitrocellulose filters, and hybridized with a digoxigenin-dUTP-labeled 389-bp *Hpa*I-*Hin*dIII fragment from pTN203 as a probe. Sizes of the restriction digests of λ DNA with *Hin*dIII are indicated at the right and left. Restriction enzymes used: lane 1, *Bam*HI; lane 2, *Hin*dIII; lane 3, *Eco*RI; lane 4, *Eco*RV; lane 5, *Bgl*I; lane 6, *Kpn*I; lane 7, *Pst*I; lane 8, *Pvu*II; lane 9, *Eco*RI-*Pst*I; lane 10, *Eco*RI-*Pvu*II; lane 11, *Eco*RI-*Kpn*I. (B) Sizes of chromosomal DNA fragments hybridized with the probe from pTN203. Sizes of fragments within Tn*10* were deduced from those obtained in the assay shown in panel A. Hatched boxes represent DNA fragments from the right end of Tn*10* to the restriction sites on the *E. coli* chromosome. The size of *Eco*RV was not determined because there is an *Eco*RV site in the IS*10*R region of Tn*10* (21). (C) Location of the Tn*10* insertion in the *E. coli* chromosome. The physical map shown in panel B was compared with that of the whole *E. coli* chromosome. As a result, we found that the physical map in panel B was identical to that around 63.5 min. Hatched boxes represent the pattern of the physical map around 63.5 min on the *E. coli* chromosome. The solid area within Tn*10* represent the location of a probe from pTN203 used for Southern blot analysis. The *Eco*RI restriction site at kb 3101.5 was not shown on the map of Kohara et al. (22), but it was reported to exist by Satishchandran et al. (32). The size of Tn*10* is not to scale.

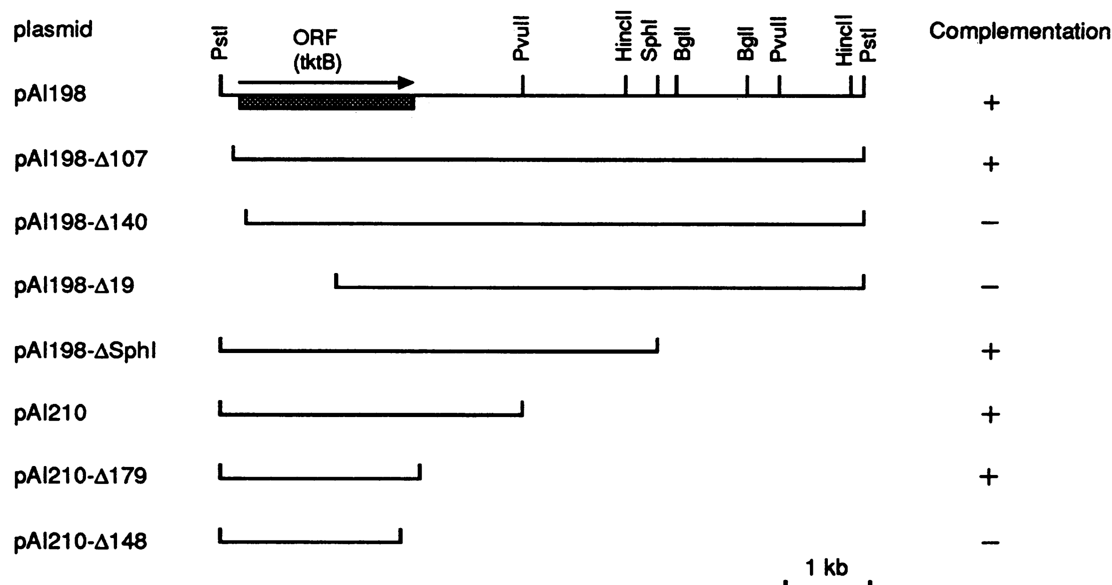


FIG. 2. Restriction map of plasmid pAI198 carrying a 7.8-kb *Pst*I fragment and complementation analysis of the *tktA*::Tn*10* mutant by *tktB* plasmids. Deletion derivatives of pAI198 and pAI210 were constructed by using restriction enzymes or exonuclease III, and each plasmid was introduced into the ribose-sensitive strain AI80. The transformants were tested for colony formation on MacConkey plates containing 1% ribose. +, complemented; −, not complemented. The arrow represents the location of *tktB* as defined by DNA sequence analysis.

linkage between the site of Tn*10* insertion and the ribose sensitivity in AI80 was examined by P1-mediated transduction, all of the tetracycline-resistant transductants showed the ribose-sensitive phenotype, indicating that the mutation resulted from the Tn*10* insertion. To examine whether ribose was a specific inhibitor to the mutant, we constructed a strain which carried both the Tn*10* insertion and *rbsK*::Tn*5* (16), the latter of which was defective in ribokinase activity but normal in the ribose transport system. This double mutant was not able to ferment ribose but showed a normal growth on MacConkey plates even in the presence of ribose. This observation indicated that ribose itself was not the cause of the ribose sensitivity of the mutant. We then examined the growth of the mutant on MacConkey plates containing several sugars. The mutant grew normally on the plate containing lactose or maltose but failed to grow on L-arabinose or D-xylose. These properties of the mutant were similar to those of the mutants reported by Josephson and Fraenkel (18), who showed that the mutations were defective in transketolase. Accordingly, we assayed the activity of transketolase in our mutant and found that the level of the enzyme activity was reduced to one-third of that of the wild type (Table 2).

Mapping of the mutation. To localize the mutation on the *E. coli* linkage map, two approaches were attempted: Hfr mapping using tetracycline resistance as the positive marker and Southern blot analysis using the fragment of Tn*10* as a probe. In the case of Hfr mapping, CSH64 (Hfr type KL14), which carried the Tn*10* insertion mutation, was used as the donor and strain CSH57b was used as the recipient. The frequency of the emergence of tetracycline-resistant transconjugants was lower than that of His$^+$, indicating that the mutation was located between 45 and 65 min on the linkage map (data not shown). For Southern blot analysis, the 389-bp *Hin*dIII-*Hpa*I fragment of pTN203, a subclone of Tn*10*, was used as the probe. Figure 1 shows the results of the Southern blot hybridization experiments. Comparison of the patterns of recognition sites for several restriction enzymes on the chromosome near the right end of Tn*10* with the physical map of the whole *E. coli* chromosome (22) identified the location of the mutation at 63.5 min on the linkage map of *E. coli*, or kb 3095 in the map of Kohara et al. (22) (Fig. 1C). No other location of the chromosome showed a similar pattern. While this study was in progress, Sprenger (37) mapped the gene for transketolase at the same location as that to which we mapped it.

Isolation of a suppressor gene. Mutant AI80 did not grow on M9 minimal plates containing 0.2% ribose. To isolate a DNA fragment(s) which supported growth of the mutant on minimal plates, chromosomal DNA of W3110 was digested with restriction enzyme *Bam*HI, *Pst*I, *Hin*dIII, or *Eco*RI, ligated with pUC19, and introduced into AI80 cells to select transformants which were able to grow on minimal medium in the presence of ribose and ampicillin. As a result, we isolated such a transformant. When the plasmid from the transformant was analyzed, a 7.8-kb *Pst*I fragment was found to be cloned. We designated the plasmid pAI198. Construction of a physical map of pAI198 showed that the location of the cloned fragment was different from the site of the Tn*10* insertion in AI80 (Fig. 2). To precisely localize the gene on the cloned fragment, several deletion derivatives of pAI198 were constructed by using restriction enzymes or exonuclease III, and complementation of the phenotype was examined. As shown in Fig. 2, pAI210-Δ179 complemented AI80 but pAI210-Δ148 did not, an indication that the gene was located in the 2.7-kb fragment of pAI210-Δ179.

Sequencing of the cloned gene. We determined a 2,668-bp nucleotide sequence from one end of the 7.8-kb *Pst*I fragment of pAI198 (Fig. 3). An open reading frame encoding 667 amino acid residues with a calculated molecular weight of 72,973 was found. A putative Shine-Dalgarno sequence, GGAG (35), was located upstream of the initiation codon, and one complete and three incomplete copies of REP sequences, which were reported to exist several hundred

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CTGCAGGAAAAAGTTTCGCCAGTGGTACGTAAATTAATCCCACCTTCTCAGACGTTCCCACGCCCAGCTCCCATGAGCGAAGCGGAGTTCCGTTGGGAGCACAATCAGGATGCGATGGCG  120

GTAGAAAAACTGTCTGAAGGCATTCGTCTGTTCGCCGTTGATCAACGCAAACTGGAAGATCTTCTTGCCGCCAAACTATAAACCAGCCACGGAGTGTTATATGTCCCGAAAAGACCTTGC  240
                                                                                                   M  S  R  K  D  L  A

CAATGCGATTCGCGCACTCAGTATGGATGCGGTACAAAAAGCCAACTCTGGTCATCCCGGCGCGCCGATGGGCATGGCTGATATTGCCGAAGTGCTGTGGAACGATTTTCTTAAACATAA  360
 N  A  I  R  A  L  S  M  D  A  V  Q  K  A  N  S  G  H  P  G  A  P  M  G  M  A  D  I  A  E  V  L  W  N  D  F  L  K  H  N

CCCTACCGACCCAACCTGGTATGATCGCGACCGCTTTATTCTTTCCAACGGTCACGCGTCGATGCTGCTCTACAGTTTGCTACATCTGACCGGTTACGACCTGCCGCTGGAAGAACTGAA  480
 P  T  D  P  T  W  Y  D  R  D  R  F  I  L  S  N  G  H  A  S  M  L  L  Y  S  L  L  H  L  T  G  Y  D  L  P  L  E  E  L  K

GAACTTCCGTCAGTTGCATTCGAAAACCCCAGGCCACCCGGAGATTGGCTATACGCCAGGCGTTGAAACCACCACCGGCCCGCTTGGACAAGGTTTGGCGAACGCCGTCGGGCTGGCGAT  600
 N  F  R  Q  L  H  S  K  T  P  G  H  P  E  I  G  Y  T  P  G  V  E  T  T  T  G  P  L  G  Q  G  L  A  N  A  V  G  L  A  I

AGCAGAGCGTACACTGGCGGCGCAGTTTAACCAGCCAGACCATGAGATCGTCGATCACTTCACCTATGTGTTTATGGGCGACGGCTGCCTGATGGAAGGTATTTCCCACGAAGTCTGTTC  720
 A  E  R  T  L  A  A  Q  F  N  Q  P  D  H  E  I  V  D  H  F  T  Y  V  F  M  G  D  G  C  L  M  E  G  I  S  H  E  V  C  S

GCTGGCAGGCACGCTGGGACTGGGCAAGCTGATTGGTTTTTACGATCACAACGGTATTTCCATCGACGGTGAAACAGAAGGCTGGTTTACCGACGATACGGCAAAACGTTTTGAAGCCTA  840
 L  A  G  T  L  G  L  G  K  L  I  G  F  Y  D  H  N  G  I  S  I  D  G  E  T  E  G  W  F  T  D  D  T  A  K  R  F  E  A  Y

TCACTGGCATGTGATCCATGAAATCGACGGTCACGATCCGCAGGCGGTGAAGGAAGCGATCCTTGAAGCGCAAAGCGTGAAAGATAAGCCGTCGCTGATTATCTGCCGTACGGTGATTGG  960
 H  W  H  V  I  H  E  I  D  G  H  D  P  Q  A  V  K  E  A  I  L  E  A  Q  S  V  K  D  K  P  S  L  I  I  C  R  T  V  I  G

CTTTGGTTCGCCGAATAAAGCAGGTAAGGAAGAGGCGCACGGCGCACCACTGGGGGAAGAAGAAGTGGCGCTGGCACGGCAAAAACTGGGCTGGCACCATCCGCCATTTGAGATCCCTAA 1080
 F  G  S  P  N  K  A  G  K  E  E  A  H  G  A  P  L  G  E  E  E  V  A  L  A  R  Q  K  L  G  W  H  H  P  P  F  E  I  P  K

AGAGATTTATCACGCCTGGGATGCCCGTGAAAAAGGCGAAAAAGCGCAGCAGAGCTGGAATGAGAAGTTTGCCGCCTATAAAAAGGCTCATCCGCAACTGGCAGAAGAGTTTACCCGACG 1200
 E  I  Y  H  A  W  D  A  R  E  K  G  E  K  A  Q  Q  S  W  N  E  K  F  A  A  Y  K  K  A  H  P  Q  L  A  E  E  F  T  R  R

GATGAGCGGTGGTTTACCGAAGGACTGGGAGAAAACGACTCAGAAATATATCAATGAGTTACAGGCAAATCCGGCGAAAATCGCTACCCGTAAGGCTTCGCAAAATACGCTTAACGCTTA 1320
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CGGGCCGATGCTGCCTGAGTTGCTCGGCGGTTCGGCGGATCTGGCTCCCAGCAACCTGACCATCTGGAAAGGTTCTGTTTCGCTGAAGGAAGATCCAGCGGGCAACTACATTCACTACGG 1440
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GGTGCGTGAATTTGGCATGACCGCTATCGCCAACGGCATCGCGCACCACGGCGGCTTTGTGCCGTATACCGCGACGTTCCTGATGTTTGTTGAATACGCCCGTAACGCCGCGCGGATGGC 1560
 V  R  E  F  G  M  T  A  I  A  N  G  I  A  H  H  G  G  F  V  P  Y  T  A  T  F  L  M  F  V  E  Y  A  R  N  A  A  R  M  A

GGCACTGATGAAAGCGCGGCAGATTATGGTTTATACCCACGACTCAATTGGCCTGGGCGAAGATGGTCCGACGCACCAGGCTGTTGAGCAACTGGCCAGCCTGCGCTTAACGCCAAATTT 1680
 A  L  M  K  A  R  Q  I  M  V  Y  T  H  D  S  I  G  L  G  E  D  G  P  T  H  Q  A  V  E  Q  L  A  S  L  R  L  T  P  N  F

CAGCACCTGGCGACCGTGCGATCAGGTGGAAGCGGCGGTGGGCTGGAAGCTGGCGGTTGAGCGCCACAACGGACCGACGGCACTGATCCTCTCAAGGCAGAATCTGGCCCAGGTGGAACG 1800
 S  T  W  R  P  C  D  Q  V  E  A  A  V  G  W  K  L  A  V  E  R  H  N  G  P  T  A  L  I  L  S  R  Q  N  L  A  Q  V  E  R

TACGCCGGATCAGGTTAAAGAGATTGCTCGTGGTGGCTATGTGCTGAAAGACAGCGGCGGTAAGCCAGATATTATTCTGATTGCCACCGGTTCAGAGATGGAAATTACCCTGCAAGCGGC 1920
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 V  A  V  E  A  G  I  A  D  Y  W  Y  K  Y  V  G  L  K  G  A  I  V  G  M  T  G  Y  G  E  S  A  P  A  D  K  L  F  P  F  F

TGGCTTTACCGCCGAGAATATTGTGGCAAAAGCGCATAAGGTGCTGGGAGTGAAAGGTGCCTGATGGTGATTGCCGGATGCTGATTGCCGGATGCGACGCTGACGCGTCTTATCCGGCCT 2280
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ACAGCGTCTTATCCGGCCTACATGTCCCGCCATTTTGTTTAACGGGTGATCCACAACGTGGGCCAGGCGTCTGGCCCATGCCAGTTATCGCAGGTGGGTTCTGCGGCGTAACGCACCAGG 2400

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ACAGCTTAATCATCATCAGCGCTTTATT                                                                                             2668
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FIG. 3. Nucleotide and deduced amino acid sequences of the *tktB* gene. The DNA sequence of the sense strand is shown. One end of the *Pst*I site of pAI198 was considered coordinate +1. The potential ribosome binding site (the Shine-Dalgarno sequence) is marked by a double underline. The termination codon is marked by an asterisk. The REP sequence (39) is boxed, and incomplete REP sequences are underlined.

copies on the *E. coli* genome (39), were located downstream of the UGA termination codon. Comparison of the deduced amino acid sequence of the open reading frame with known protein sequences registered in the DDBJ, GenBank, EMBL, and NBRF data bases by using IDEAS (9) SEQFT, and SEQFP programs revealed high similarity to the sequences of the transketolases from *Rhodobacter sphaeroides* (3) and *Saccharomyces cerevisiae* (8), dihydroxyacetone synthase (DHAS) from *Hansenula polymorpha* (17), and the *recP* product from *Streptococcus pneumoniae* (30). Furthermore, the presence of the gene in multicopy increased the activity of transketolase (see below). From these data, we though that the cloned gene also encoded a transketolase which was different from the enzyme encoded by the *tkt* gene that is located at 63.5 min. Accordingly, in this report we designate the former *tktB* and the latter *tktA*.

Expression of *tktB*. Whole cell lysates of strains containing the *tktB* clone were analyzed by SDS-PAGE. As shown in Fig. 4, the strain carrying pAI198 or pAI210 expressed a protein of 72 kDa, a value comparable with the calculated molecular weight of the *tktB* gene product. The protein of the strain containing pAI210 was induced to more than 30% of

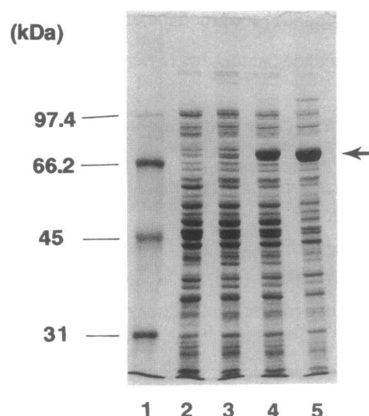

FIG. 4. Expression of the *tktB* gene. Strain AI80 carrying *tktB* plasmids was grown at 37°C in LB medium (lanes 2 to 4) or LB medium containing 1 mM IPTG (lane 5). Proteins were separated on an SDS–9% polyacrylamide gel and stained with Coomassie blue. Lanes: 1, molecular mass standards; 2, AI80; 3, AI80(pAI198); 4, AI80(pAI210); 5, AI80(pAI210).

total cellular proteins by adding IPTG, and inclusion bodies were observed in the cells under an optical microscope. This observation indicated that the gene was partly expressed under the control of the *lac* promoter in the vector. When the N-terminal sequence of the 72-kDa protein was analyzed, the order of the first seven amino acid residues was determined to be NH_2-Ser-Arg-Lys-Asp-Leu-Ala-Asn-COOH. Therefore, the AUG codon at nucleotide 221 was indeed the initiation codon of the *tktB* gene, although the first methionine residue was processed (Fig. 3). In addition, the transketolase activity of the strain containing pAI198 or pAI210 increased 1.4- or 14-fold, respectively, relative to that of EJ500 ($tktA^+$ $tktB^+$) (Table 2).

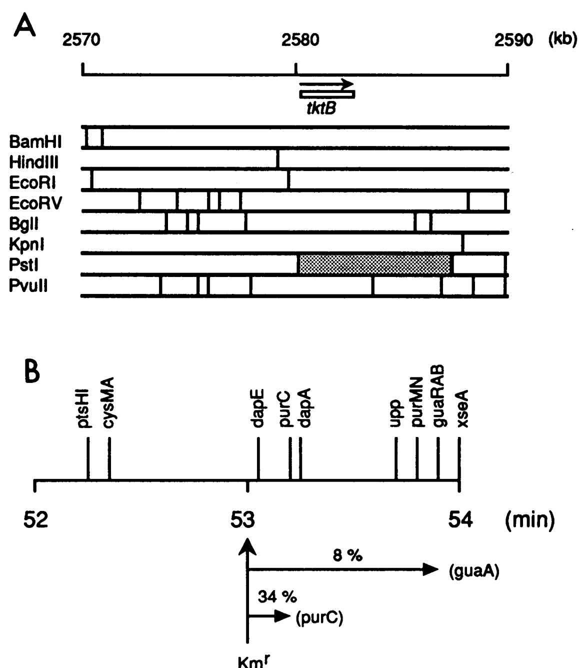

FIG. 5. Location of the *tktB* gene on the physical and genetic maps of *E. coli*. (A) Location of the cloned 7.8-kb *Pst*I fragment of pAI198 on the *E. coli* physical map. The cloned region is indicated by the dotted area. The arrow represents the location of *tktB* as defined by DNA sequence analysis. (B) Linkage of the *tktB* gene with the *purC* and *guaA* loci. P1 phage lysates prepared from AI1116 (*tktB*::*kan*) were transduced into *E. coli* ME5325 or PL1068. Selection was initially for kanamycin resistance followed by scoring for hypoxanthine (in the case of ME5325) or guanine (in case of PL1068) independence on minimal agar plates.

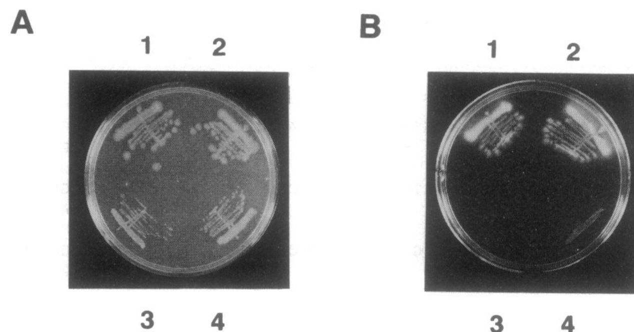

FIG. 6. Growth of strains carrying the *tkt* mutation. Strains were plated on LB plates (A) or MacConkey plates containing 1% ribose (B) and incubated at 37°C for 20 h. Strains: 1, EJ500; 2, AI1122 (*tktB*); 3, AI1118 (*tktA tktB*); 4, AI80 (*tktA*).

Location of *tktB*. To map the *tktB* gene, we compared the physical map of the chromosomal region of pAI198 with that of the whole *E. coli* chromosome (22) and found a similar map constructed by use of restriction enzymes *Bam*HI, *Hin*dIII, *Eco*RI, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I, and *Pvu*II at 53 min on the linkage map (Fig. 5). To further investigate its location on the chromosome, the *tktB* gene was disrupted by inserting the Km^r gene of Tn*903* as described in Materials and Methods, and the linkage of kanamycin resistance to the *purC* or *guaA* marker, which had been mapped at 53 min, was examined by P1-mediated transduction. As shown in Fig. 5, the kanamycin resistance marker was 8% linked to *guaA* and 34% linked to *purC*, confirmation of the location of *tktB* at 53 min on the chromosome.

Phenotypes of the *tktA* and *tktB* mutations. To examine the phenotypes of the *tktA* and *tktB* mutations, we constructed isogenic strains of EJ500 which carried either *tktA* or *tktB* or both by P1-mediated transduction. Growth on MacConkey plates containing ribose was normal in EJ500 (wild type) and AI1122 (*tktB*), while growth inhibition was observed in AI80 (*tktA*) and AI1118 (*tktA tktB*) (Fig. 6). Ribose sensitivity on the plate was leaky in AI80 but tight in AI1118. Strain AI1118 failed to grow on MacConkey plates containing other sugars such as L-arabinose, D-xylose, lactose, or maltose. Growth on LB plates was normal for EJ500 and AI1122, but slow development of colonies was observed for AI80 and AI1118 (Fig. 6). The level of transketolase activity in the strain carrying the *tktB* mutation was almost the same as that of the wild type, but reduced activity was observed in the *tktA tktB* and *tktA* strains (Table 2). These results indicated that although the activity of transketolase encoded by $tktB^+$ was weak compared with that of $tktA^+$, $tktB^+$ was functional in the cells.

Analysis of the DNA sequence upstream of *tktB*. We analyzed the sequences over 220 bp upstream of the initiation codon of *tktB* and found an open reading frame which continued from further upstream region of a cloned *Pst*I fragment of pAI198. A homology search indicated that the deduced amino acid sequence of this open reading frame showed a high similarity to the C-terminal region of transaldolase (TAL1) of *Saccharomyces cerevisiae* (33) (data not

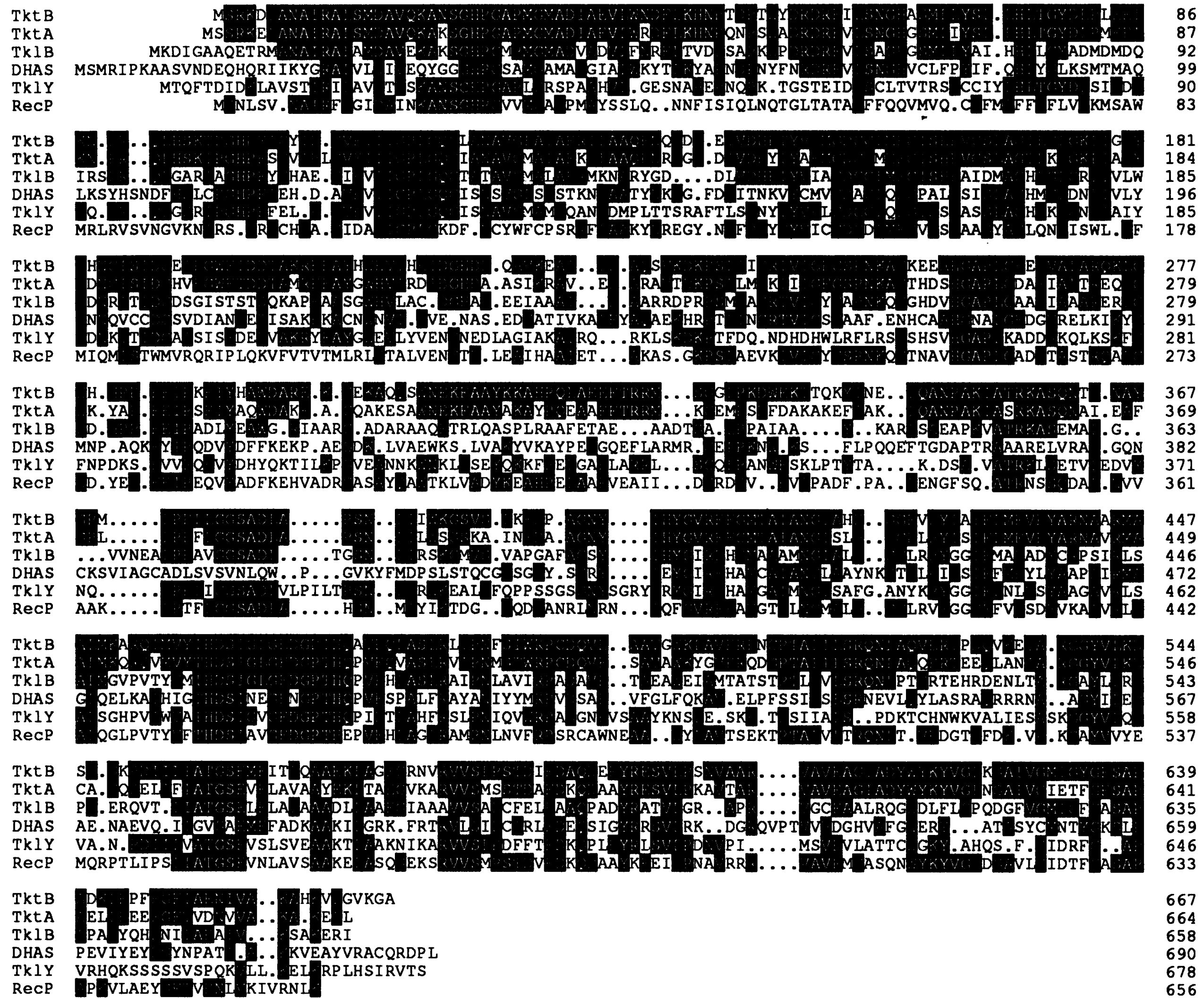

FIG. 7. Comparison of the deduced amino acid sequence of TktB with sequence of five proteins from various organisms. Abbreviations: TktB, transketolase II from *E. coli* (this study); TktA, transketolase I from *E. coli* (38); TklB, transketolase from *R. sphaeroides* (3); TklY, transketolase from *Saccharomyces cerevisiae* (8); DHAS, dihydroxyacetone synthase from *H. polymorpha* (17); RecP, *recP* product from *S. pneumoniae* (30). Numbers to the right of the sequences indicate amino acid positions relative to the start of each protein sequence. Amino acid residues which are identical with *E. coli* TktB at any particular sequence position are indicated by black boxes with white letters.

shown). In work to be described elsewhere, we concluded that the open reading frame, designated *talA*, was the structural gene for transaldolase in *E. coli*. The amino acid sequence of the *talA* gene product was 64% identical to that of the putative *tal* gene located at 0 min on the linkage map (45). Since there is only a 20-bp interval between the *talA* and *tktB* genes, and since both genes encode the enzymes of the nonoxidative pentose phosphate pathway, these two genes presumably form an operon.

DISCUSSION

A Tn*10* insertion mutant which lacked transketolase activity was isolated as a ribose-sensitive mutant on MacConkey plates containing ribose in *E. coli*. Ribose itself was not shown to be a substrate for the sensitivity. The putative sensitivity is due to the fact that accumulation of pentose phosphates or phosphoribosylpyrophosphate is inhibitory to the growth of cells or the fact that the limited supply of sedoheptulose 7-phosphate causes cells to be sensitive to bile acids included in MacConkey plates as reported for the transketolase mutant of *Salmonella typhimurium* (7) or both. The mutation was mapped at 63.5 min on the *E. coli* chromosome, where the transketolase mutation (*tkt*) had been mapped (37). Two groups reported the cloning of a 5- or 6-kb *Bam*HI fragment which contained the gene for transketolase (4, 5, 37). The strains carrying these clones overproduced a 72-kDa protein with a concomitant increase in transketolase activity. The physical maps of these clones were similar to that around 63.5 min on the chromosome. Recently the DNA sequence data from one of the clones have been deposited in the EMBL nucleotide sequence data base (38). These data suggest that the gene is the structural gene for a major transketolase.

The clone that complemented the Tn*10* insertion mutation was isolated and identified as a second gene for transketolase. We designated the gene as *tktB* (for transketolase II)

and the gene at 63.5 min as *tktA* (for transketolase I). The facts that the phenotypic suppression occurred only when the gene was in multicopy and that the *tktA* mutation alone caused a ribose-sensitive phenotype suggest to us that the transketolase activity of *tktB* was low in a single copy. This conclusion was supported by measuring the enzyme activity and characterizing the phenotype of the strain defective in the *tktB* gene. The reason why we were not able to clone the *tktA* gene was unclear. However, when we examined λ clones from the Kohara library which contained the *E. coli* chromosomal fragment around 63.5 min, clones 6C5 (miniset 472) and 1H10 (miniset 473) complemented the *tktA*::Tn*10* mutation of AI80, whereas the adjacent clones 1A2 (miniset 471) and 23G4S (miniset 474) failed to complement the mutation (data not shown). These results indicate that the chromosomal fragments around 63.5 min were able to complement the mutation of AI80 as well.

The deduced amino acid sequence of the *tktB* product (TktB) showed high similarity to transketolases from *E. coli* (TktA) (38), *R. sphaeroides* (TklB) (3), and *Saccharomyces cerevisiae* (TklY) (8), DHAS from *H. polymorpha* (17), and the *recP* product from *S. pneumoniae* (Fig. 7). We do not know whether the *recP* product from *S. pneumoniae* has transketolase activity, but aligned identities were found throughout the sequences. The TktB protein had amino acid identity to TktA, TklB, TklY, DHAS, and RecP at 497 (74%), 330 (49%), 241 (35%), 208 (31%), and 276 (41%) positions, respectively. A putative TPP-binding motif, Gly-Asp-Gly-(27 amino acids)-Asn-Asn, was postulated to exist in TPP-binding enzymes (11). Furthermore, the three-dimensional structure of transketolase from *Saccharomyces cerevisiae* was also determined at 2.5-Å (0.25-nm), resolution and the actual TPP-binding region was confirmed (26). The consensus motif was observed in the amino acid sequence of TktB at positions 153 to 155 (Gly-Asp-Gly) and 184 (Asn).

We constructed isogenic strains defective in *tktA* and/or *tktB*. In the strain carrying the *tktA* and *tktB* mutations, the growth of cells in LB medium was delayed at some extent compared with the wild type but was not lethal. This observation might be explained as suggesting that transketolase is dispensable to the cells or that the residual transketolase activity is sufficient for cell growth. To examine these possibilities, determination of the Tn*10* insertion site in the *tktA* gene is required, and the phenotype should be examined in the *tktA* and *tktB* null mutations. In this context, it is interesting that revertants which grew normally on MacConkey plates containing ribose were isolated spontaneously from the strain containing the *tktA*::Tn*10* mutation. The revertants were able to ferment ribose and still showed tetracycline resistance (data not shown). These revertants might be pseudorevertants of *tktA*::Tn*10* or derived by an increased activity of *tktB*. In addition, we found that when the *tktA*::Tn*10* mutation was introduced into several other *E. coli* strains by P1-mediated transduction, the degree of sensitivity to ribose was different from one strain to another. For example, strain KL96 (1) carrying the *tktA*::Tn*10* mutation did not show any ribose sensitivity (data not shown). This observation also might be explained by different activities of the second transketolase among *E. coli* strains.

Cloning and characterization of the genes for transketolase, *tktA* and *tktB*, enable not only study of the regulatory mechanism of the expression of these genes but also determination of the functions of these enzymes in regulation of interconversion of the glycolytic and pentose phosphate pathways under various conditions. Such experiments are in progress.

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