

Lactose Metabolism by *Staphylococcus aureus*: Characterization of *lacABCD*, the Structural Genes of the Tagatose 6-Phosphate Pathway

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The nucleotide and deduced amino acid sequences of the *lacA* and *lacB* genes of the *Staphylococcus aureus* lactose operon (*lacABCDFEG*) are presented. The primary translation products are polypeptides of 142 ($M_r = 15,425$) and 171 ($M_r = 18,953$) amino acids, respectively. The *lacABCD* loci were shown to encode enzymes of the tagatose 6-phosphate pathway through both *in vitro* studies and complementation analysis in *Escherichia coli*. A serum aldolase assay, modified to allow detection of the tagatose 6-phosphate pathway enzymes utilizing galactose 6-phosphate or fructose phosphate analogs as substrate, is described. Expression of both *lacA* and *lacB* was required for galactose 6-phosphate isomerase activity. LacC (34 kDa) demonstrated tagatose 6-phosphate kinase activity and was found to share significant homology with LacC from *Lactococcus lactis* and with both the minor 6-phosphofruktokinase (PfkB) and 1-phosphofruktokinase (FruK) from *E. coli*. Detection of tagatose 1,6-bisphosphate aldolase activity was dependent on expression of the 36-kDa protein specified by *lacD*. The LacD protein is highly homologous with LacD of *L. lactis*. Thus, the *lacABCD* genes comprise the tagatose 6-phosphate pathway and are cotranscribed with genes *lacFEG*, which specify proteins for transport and cleavage of lactose in *S. aureus*.

Growth of *Staphylococcus aureus* on lactose leads to induction of the lactose phosphoenolpyruvate phosphotransferase system (PEP-PTS) (26). The combined action of two sugar-specific components, enzyme II^{Lac} and enzyme III^{Lac}, results in the vectorial transport and phosphorylation of the lactose molecule in the 6 position of the galactose moiety. Subsequent action by the intracellular enzyme, phospho-β-galactosidase, results in the formation of glucose and galactose 6-phosphate.

In *Escherichia coli* and other organisms which do not transport lactose by a PEP-PTS, the most common pathway for the metabolism of lactose involves hydrolysis of the disaccharide by β-galactosidase to glucose and galactose, and further metabolism of galactose via the galactose 1-phosphate (Leloir) pathway (17): D-galactose → D-galactose 1-phosphate → D-glucose 1-phosphate → D-glucose 6-phosphate. In contrast, assimilation of lactose or galactose by the PEP-PTS of wild-type *S. aureus* cells ultimately results in the intracellular accumulation of galactose 6-phosphate. This phosphorylated carbohydrate, which is the actual intracellular inducer of the lactose (*lac*) genes (19), is metabolized to triose phosphates of the glycolytic pathway via tagatose phosphate intermediates (2) (D-galactose 6-phosphate → D-tagatose 6-phosphate → D-tagatose 1,6-bisphosphate → D-glyceraldehyde 3-phosphate plus dihydroxyacetone phosphate) and involves the enzymes galactose 6-phosphate isomerase (6), tagatose 6-phosphate kinase (4), and tagatose 1,6-bisphosphate aldolase (5), respectively. The isolation of

mutants deficient in these enzymes supports the conclusion that the D-tagatose 6-phosphate pathway is the sole route of lactose and D-galactose metabolism in *S. aureus* (3).

Our laboratory has reported the cloning and nucleotide sequences of the structural genes of the heptacistronic staphylococcal *lac* operon (8–10, 24; this report) as well as that of a closely linked gene, *lacR*, which encodes the repressor of the *lac* operon (22, 23). The organization of the operon is shown in Fig. 1. The terminal three genes, *lacFEG*, encode the sugar-specific transport components, enzyme III^{Lac} and enzyme II^{Lac} (8), and the hydrolytic enzyme, phospho-β-galactosidase (10), respectively. In this study, we demonstrate that the remaining determinants of the staphylococcal *lac* operon, *lacABCD*, specify enzymes of the D-tagatose 6-phosphate pathway in this organism.

MATERIALS AND METHODS

Bacterial strains, media, and reagents. *E. coli* JM83r [F^- *ara* Δ(*lac-proAB*) *rpsL*, *strA* *thi*⁻ φ80*dlacZ*Δ*M15* *hsdR4*, *zjy-202::Tn10*] (laboratory strain) and LE392 (20) were used for routine maintenance of plasmids in this study. *E. coli* JM101 (18) was used as the host for bacteriophages M13mp18 and M13mp19 (18). *E. coli* H8 [*fda-2*(Ts) *relA2* *fadL701* *fhuA22* *garB10* *ompF627* *pit-10* *spoT1*] (7), DF1010 [Δ(*pfkB*)201 Δ(*rha-pfkA*)200 *recA56* *relA1* *tonA22* *spoT1*, φT₂] (11), and LE392 [F^- *supE44* *supF58* *hsdR514*(r_K⁻ m_K⁺) *lacY1* *galK2* *galT22* *metB1* *trpR55* λ⁻] (20) were used as hosts for the determination of staphylococcal tagatose 1,6-bisphosphate aldolase, tagatose 6-phosphate kinase, and galactose 6-phosphate isomerase activities, respectively. The growth media used were L broth, tryptic soy broth, and minimal salts solution P (13) plus 0.2% (wt/vol) (NH₄)₂SO₄, 0.4% (vol/vol) glycerol, glucose, or lactose and, when required, 5 μg of thiamine per ml. Antibiotics used were ampicillin (100 μg/ml) and chloramphenicol (10 μg/ml).

Other materials were obtained from the following sources:

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TABLE 1. Recombinant plasmids used for determination of *S. aureus* tagatose 6-phosphate pathway activities^a

Plasmid	Vector	Insert (kb)	Comment
pYA501	pBR322	<i>Bam</i> HI (5.1)	<i>S. mutans</i> tagatose 6-phosphate gene cluster
pER920	pUC13	<i>Eco</i> RI [Δ <i>Sph</i> I] (1.7)	LacC ⁻ , CAT
pER973	pUC13	<i>Eco</i> RI (1.5)	LacC
pER988	pUC19 Δ <i>Eco</i> RI	<i>Sph</i> I- <i>Ase</i> I (1.1)	LacD
pER990	pUC19 Δ <i>Eco</i> RI	<i>Sph</i> I- <i>Ase</i> I (1.9)	LacD ⁻ , CAT
pER2070	pUC19	<i>Sal</i> I- <i>Pvu</i> II (1.5)	LacA LacB
pER2072	pUC19	<i>Hinc</i> II- <i>Pvu</i> II (1.4)	LacA ⁻ LacB
pER2075	pUC19	<i>Sal</i> I- <i>Eco</i> RI (1.4)	LacA LacB ⁻

^a See Materials and Methods for details on plasmid constructions. CAT, chloramphenicol resistance gene cassette.

antibiotics, lysozyme, human serum aldolase kit (procedure no. 752), triose phosphate isomerase (EC 5.3.1.1), rabbit muscle fructose 1,6-bisphosphate aldolase (EC 4.1.2.13), and rabbit muscle fructose 6-phosphate kinase (EC 2.7.1.11), Sigma Chemical Co., (St. Louis, Mo.); restriction endonucleases, mung bean exonuclease, Klenow fragment of DNA polymerase I, and T4 DNA ligase, Promega Biotec and Pharmacia, Inc.; and [α -³²P]dATP, Dupont, NEN Research Products.

Recombinant DNA methods. Standard methods for recombinant DNA manipulations were generally as described by Maniatis et al. (16).

DNA sequencing. Plasmid pMK4 (29) and the replicative forms of coliphages M13mp18 and M13mp19 (18) were used as vectors for the subcloning of staphylococcal DNA restriction fragments encompassing the promoter-proximal region of the *lac* operon. Both strands from this region were sequenced by the dideoxy-chain termination method (25).

Plasmid constructions. Plasmids generated for the enzymatic analysis of LacABCD polypeptide functions in *E. coli* are described in Fig. 2 and 6 and Table 1. The chloramphenicol acetyltransferase (CAT) gene cassette was obtained from a plasmid, pER919a, which had been constructed previously by insertion of an 840-bp *Ssp*I-*Dpn*I fragment containing the CAT determinant of pC194 (15) into the *Hinc*II restriction site of pUC7 (16). Thus, this gene cassette is flanked by both *Dpn*I and *Eco*RI restriction sites, thereby facilitating direct insertion into the *lacC* or *lacD* determinants as described below.

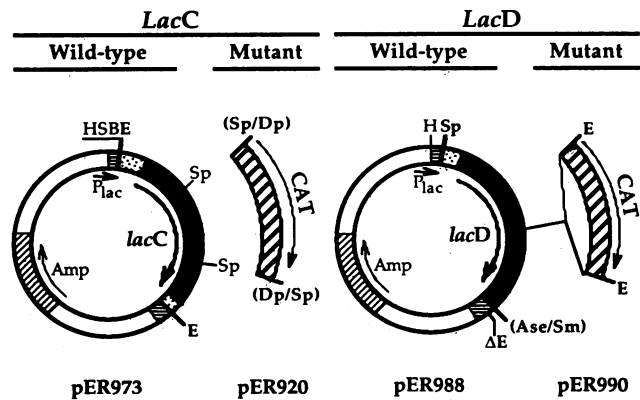


FIG. 2. Plasmids derived for elucidation of *lacC*- and *lacD*-encoded polypeptide functions. Plasmids pER973 and pER920 are pUC13 derivatives which contain a 1.5-kb *Eco*RI fragment expressing *lacC* under control of the *lacUV5* (P_{lac}) promoter. The 611-bp *Sph*I fragment is replaced by a CAT gene cassette in pER920. Similarly, pER988 contains a 1.1 kb *Sph*I-*Ase*I fragment encoding the *lacD* ORF as directed by P_{lac} in pUC19 Δ E. Truncated LacD (corresponding to amino acid 178) is expressed from pER990. For details, see Materials and Methods. Abbreviations: Amp, ampicillin resistance; CAT, chloramphenicol resistance gene cassette; Sp, *Sph*I; Dp, *Dpn*I; Ase, *Ase*I; Sm, *Sma*I; H, *Hind*III; S, *Sal*I; B, *Bam*HI; E, *Eco*RI; Δ E, disrupted *Eco*RI restriction site of pUC19.

(i) ***lacC* plasmids.** A 1.5-kb *Eco*RI fragment obtained from pBO4 (Fig. 1 [22]), which encodes the LacC polypeptide, was placed downstream of the relatively strong *lacUV5* (P_{lac}) promoter of pUC13, generating pER973 (Fig. 2). Sequence analysis confirmed that the orientation of this insert was such that expression of *lacC* is directed by the vector-borne *lacUV5* promoter. Plasmid pER973 was treated with *Sph*I, resulting in deletion of an internal 611-bp *Sph*I fragment specifying amino acids 79 to 282 of the 310-amino-acid *lacC* open reading frame (ORF). The resulting unique *Sph*I site was made blunt ended by treatment with mung bean exonuclease, and the intact CAT cassette was inserted at this site in the same orientation as the *lacC* ORF, thus generating pER920.

(ii) ***lacD* plasmids.** To generate plasmids expressing wild-type or allelic LacD polypeptides, a 1.1-kb *Sph*I-*Ase*I fragment encoding the entire 326-amino-acid residue *lacD* ORF was isolated from pER789 (Fig. 1). The *Ase*I site was blunt

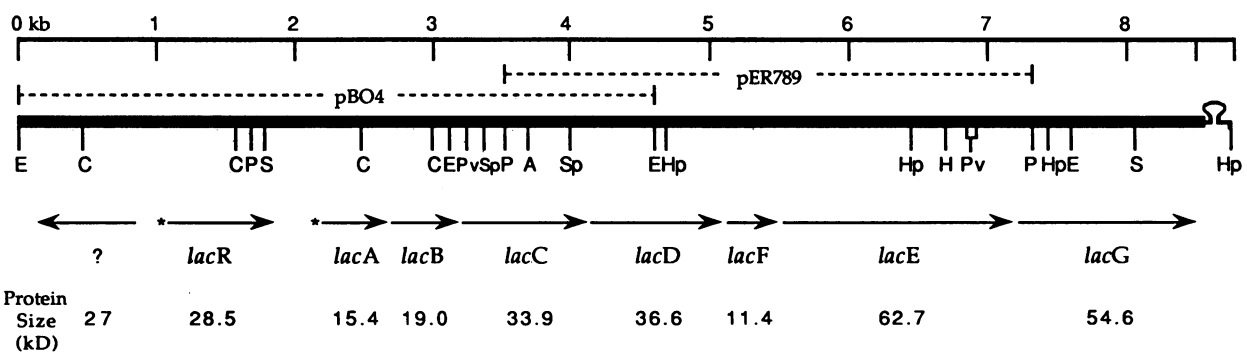


FIG. 1. Lactose operon of *S. aureus*. A heavy line represents chromosomal DNA followed by a potential transcription terminator sequence. Dashed lines denote the extent of inserts cloned into relevant plasmids for use in this study. The deduced molecular mass of each gene product is given. Putative promoter regions (*) are indicated. Abbreviations for restriction enzyme sites: A, *Ava*I; C, *Cla*I; E, *Eco*RI; H, *Hind*III; Hp, *Hpa*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; Sp, *Sph*I.

ended with Klenow enzyme plus appropriate nucleotide triphosphates, and the fragment was cloned into pUC19 which had been previously treated in the same manner to destroy the multiple-cloning linker's *EcoRI* site. The resulting plasmid, pER988, directs expression of *lacD* from the vector-borne *lacUV5* promoter and contains a unique *EcoRI* site (corresponding to amino acid residue 178) which was exploited in the insertional inactivation of *lacD* with a CAT cassette flanked by *EcoRI* restriction sites as shown for pER990 (Fig. 2). The orientations of the *lacD* and CAT ORFs were verified by sequence analysis.

(iii) *lacA* and *lacB* plasmids. Examination of the nucleotide sequence near the 5' end of the staphylococcal *lac* operon (23) revealed several *HincII* restriction sites clustered near the amino terminus of *lacA* and an *EcoRI* site near the carboxy terminus of *lacB* (Fig. 3). These restriction sites were exploited in the generation of mutant *lacA* or *lacB* alleles as shown in Fig. 6. A 1.5-kb *SalI*-*PvuII* fragment from pBO4 (Fig. 1) was gel purified and inserted into *SalI*-*SmaI*-digested pUC19 such that expression of wild-type LacA and LacB polypeptides is directed by the *lacUV5* promoter, thus generating pER2070. Digestion of pER2070 with *HincII* followed by religation under conditions favoring intermolecular ligation resulted in deletion of a 487-bp *HincII* fragment encoding the amino-terminal 39 amino acid residues of LacA, giving pER2072. Similar religation of pER2070 following digestion with *EcoRI* resulted in a plasmid, pER2075, which is interrupted at the *EcoRI* site present in the *lacB* ORF. The truncated LacB polypeptide lacks 11 carboxy-terminal amino acids of the mature protein. The generation of such deletion mutants from the parental plasmid allowed a direct assessment of the interdependence of the LacA and LacB proteins.

Complementation analyses. Assay of the ability of the staphylococcal LacC or LacD proteins expressed under control of the *lacUV5* promoter to complement *E. coli* phosphofructokinase (*pfk*) or fructose diphosphate aldolase (*fda*) mutants was as follows. Overnight tryptic soy broth starter cultures supplemented with 5 μ g of thiamine per ml (*E. coli* H8) were grown at either 30°C (*E. coli* H8) or 37°C (*E. coli* DF1010) with appropriate antibiotic selection. Nephelometer flasks containing 25 ml of a minimal medium consisting of basal salts solution P (1.34% Na_2HPO_4 , 1.36% KH_2PO_4 , 0.0011% CaCl_2 , 0.025% MgSO_4) plus 0.2% $(\text{NH}_4)_2\text{SO}_4$, 0.4% glycerol, glucose, or lactose and, when required, 5 μ g of thiamine per ml were then inoculated ($A_{600} = 0.05$), and growth was monitored spectrophotometrically (600 nm). Growth of *E. coli* DF1010 harboring pER973 or pER920 was monitored at 37°C for 24 h. *E. coli* H8 containing various test plasmids was incubated at 30°C for 4 h prior to shift-up to the nonpermissive temperature (40°C) at time zero. Growth was then monitored for up to an additional 24 h. Ampicillin and, for strains harboring pER920 or pER990, chloramphenicol were added for maintenance of plasmids during analysis. Following completion of growth assays, the presence of the appropriate plasmid was verified by restriction analysis.

In vitro enzyme assays. *E. coli* H8, DF1010, and LE392 harboring various test plasmids were grown in tryptic soy broth with antibiotic selection at either 30°C (*E. coli* H8) or 37°C. Cultures (35 ml) were grown from an initial optical density of $A_{600} = 0.05$ to $A_{600} = 0.9$, at which time a 25-ml aliquot was harvested (3,000 \times g, 10 min), washed with an equal volume of cold (4°C) 40 mM Tris-HCl, pH 8.0, and reharvested as described above. The cells were then resuspended in 5 ml of 0.3 M Tris-1.5 mM fluoride buffer, pH 7.0

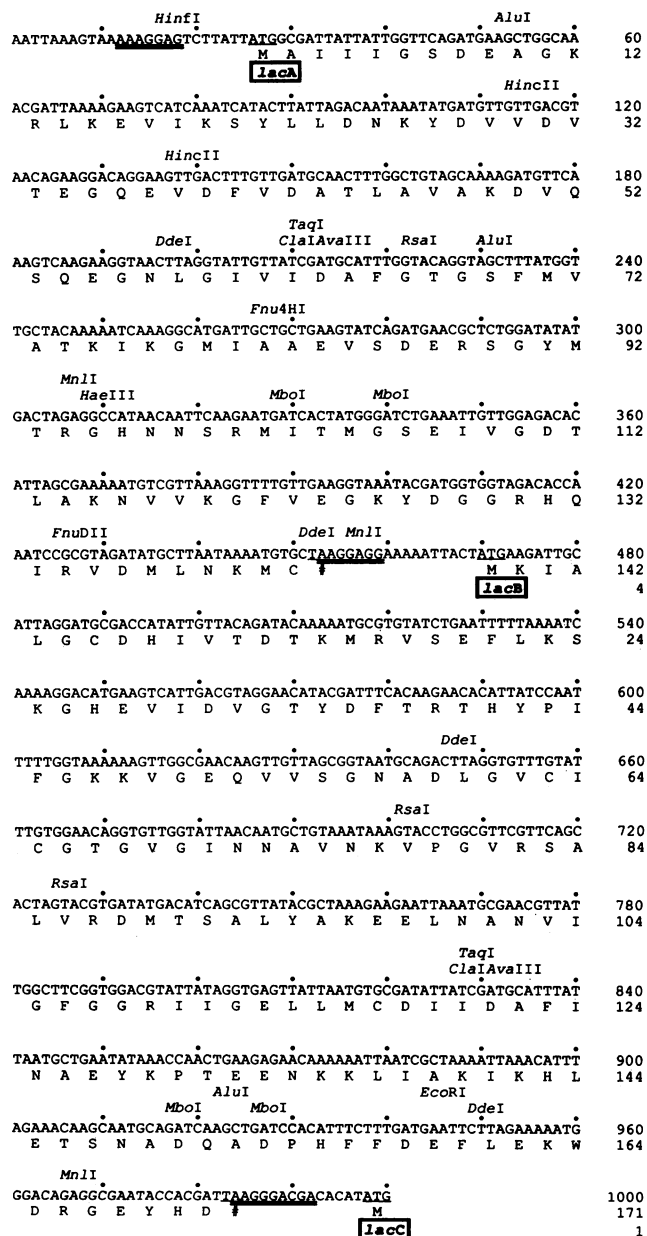


FIG. 3. Nucleotide sequence of the *lacAB* region of *S. aureus*. The nucleotide sequence of the nonsense strand and the corresponding amino acid sequences of *lacAB* are presented. Translation initiation and termination codons are underlined. Putative Shine-Dalgarno sequences are indicated by double underlines.

(Sigma). Cell extracts were obtained by sonication with a Branson Sonifier 200 equipped with a 0.5-in. (ca 1.27-cm) tapped horn (80% duty cycle, continuous pulse, 90% output; VWR Scientific) for 10 s and then incubated on ice for 1 min to cool the suspension. Another 10-s treatment at the same settings resulted in a cleared suspension of lysed cells as determined by microscopic observation. The cell extract used for enzyme assays was the supernatant fluid resulting from a 10-min centrifugation of the broken cell suspension at 3,000 \times g.

(i) **Aldolase assay.** Generally, 0.8 to 1.2 ml of *E. coli* H8 cell extract was distributed into duplicate reaction tubes, and the

volume was adjusted to 1.8 ml with 0.3 M Tris–1.5 mM fluoride buffer, pH 7.0 (Sigma). After equilibration for 5 min at the assay temperature (40°C), nonlimiting amounts of the coupling enzyme triose phosphate isomerase (10 U) and fructose 1,6-bisphosphate (10 mM final concentration; Sigma) was added to one tube only. Both control and reaction samples were incubated in a 40°C stationary water bath for exactly 30 min to allow the enzymatic conversion of substrate to dihydroxyacetone phosphate (DHAP). Following this timed reaction, an equal volume (2 ml) of 10% trichloroacetic acid was added to quench the reaction, followed by addition of the components mentioned above to the control sample before separation of trichloroacetic acid-precipitable material by centrifugation at $3,000 \times g$ for 10 min.

A 2-ml aliquot of the resulting clear supernatant was then assayed for DHAP production by addition of 1 ml of 1.2 N NaOH and incubation for 20 min at room temperature to allow conversion of DHAP to hydroxypyruvic aldehyde. Subsequent incubation at 37°C for 30 min after addition of 1 ml of aldolase color reagent (0.1% 2,4-dinitrophenylhydrazine in 1.35 N HCl; Sigma) allowed formation of the corresponding hydrazone. Formation of an intense purple color following addition of 4 ml of 1.2 N NaOH was indicative of aldolase activity; cell extracts which did not demonstrate aldolase activity, or control samples which were not incubated in the presence of substrate before addition of trichloroacetic acid, remained light yellow (due to addition of aldolase color reagent) or became lavender to brown, indicating limited hydrazone formation. Enzyme activity was calculated by using a calibration curve relating the A_{560} versus aldolase activity (Sigma units per milliliter), using the control sample as a reference. One Sigma unit was defined as the amount of enzyme required for conversion of 1 nmol of substrate to DHAP per min under the conditions of these assays.

(ii) **6-Phosphokinase assay.** Conditions for kinase determinations were as described for aldolase assays, with the following modifications. Growth of *E. coli* DF1010 and enzymatic conversion of fructose 6-phosphate (10 mM final concentration; Sigma) to fructose 1,6-bisphosphate were done at 37°C. In addition to nonlimiting amounts of triose phosphate isomerase, the kinase assay included rabbit muscle fructose 1,6-bisphosphate aldolase (1.3 U), 7 mM $MgCl_2$, and 2 mM ATP in a 2-ml total reaction volume.

(iii) **Isomerase assay.** Galactose 6-phosphate isomerase assays were performed as described for kinase determinations with use of *E. coli* LE392 except that galactose 6-phosphate (10 mM final concentration; Sigma) was used as a substrate. Additionally, this assay was supplemented with rabbit muscle fructose 6-phosphate kinase (10 U) to facilitate complete conversion of tagatose 6-phosphate (via tagatose 1,6-bisphosphate) to DHAP in cell extracts exhibiting galactose 6-phosphate isomerase activity.

Nucleotide sequence accession number. The nucleotide sequence data shown in Fig. 3 have been assigned GenBank/EMBL accession number M64724.

RESULTS AND DISCUSSION

Nucleotide sequences of *lacA* and *lacB*. The sequences reported here represent the completion of the sequence of the *S. aureus lac* operon. The nucleotide and deduced amino acid sequences of *lacC* and *lacD* (24), *lacF* and *lacE* (8), and *lacG* (10) have been reported elsewhere. The complete nucleotide and deduced amino acid sequences of *lacA* and

lacB are presented in Fig. 3. The *lacA* ORF begins at position 26 and ends at position 452, while that of *lacB* extends from positions 470 to 983. The DNA sequence indicates that the *lacA* and *lacB* genes would encode proteins of 142 ($M_r = 15,425$) and 171 ($M_r = 18,953$) amino acids, respectively. This is in good agreement with the apparent molecular weights observed from sodium dodecyl sulfate-polyacrylamide gel analysis of extracts from maxicells harboring plasmids containing the 1.1-kb *Sall-EcoRI* fragment of pBO4 (22).

The *lacA* ORF is preceded by a potential ribosome-binding site sequence (28) (AAAGGAG; positions 12 to 18). The *lacB* ORF is preceded by a potential ribosome-binding site sequence (AAGGAG; positions 453 to 459) which overlaps by two nucleotides the ochre termination codon of *lacA*. A similarly arranged termination codon/ribosome-binding site sequence is apparent at the junction of the *lacB* and *lacC* ORFs (positions 1983 to 1992). These translational control elements have also been shown to overlap in the intercistronic regions of the *S. aureus lacC* and *lacD* (24) and *lacF* and *lacE* (8) genes, suggesting that translational coupling may play a role in expression of the proteins of the relatively large *lac* mRNA to help ensure that stoichiometric amounts of these coupled products are synthesized.

Assignment of *lacABCD* enzymatic activities. (i) Experimental rationale. The inavailability of tagatose phosphate derivatives for use as substrates prevented direct detection of the staphylococcal tagatose 6-phosphate enzymes based on their specificity for natural substrate. However, tagatose 1,6-bisphosphate aldolase and tagatose 6-phosphate kinase demonstrate enzymatic activity on the corresponding fructose phosphate analogs (fructose 1,6-bisphosphate and fructose 6-phosphate) (4, 5), and thus we utilized these sugar phosphates in our *in vitro* assay system. The presence of interfering Embden-Meyerhoff-Parnas (EMP) pathway fructose 1,6-bisphosphate aldolase and phosphofructokinase activities necessitated the use of appropriate *E. coli fda* or *pfk* mutants as hosts for determination of the staphylococcal tagatose 1,6-bisphosphate aldolase and tagatose 6-phosphate kinase enzymes. The functions of the LacABCD proteins were determined by using a human serum aldolase assay modified to allow the detection of enzymatic activity from *E. coli* cell extracts.

(ii) **Tagatose 1,6-bisphosphate aldolase is encoded by *lacD*.** The pUC19 derivative pER988 contains a 1.1-kb *SphI-AseI* fragment which expresses the *lacD* ORF under direction of the vector-borne *lacUV5* promoter (Fig. 2). When this plasmid was maintained in *E. coli* H8, it successfully complemented the thermolabile fructose 1,6-bisphosphate aldolase, thereby permitting growth at 40°C on the EMP pathway-dependent carbohydrate, glucose (Fig. 4B). Similar cultures maintained at the permissive temperature (30°C) grew somewhat faster, suggesting that the weaker cross-specificity of tagatose 1,6-bisphosphate aldolase for fructose 1,6-bisphosphate was growth limiting. Control strains harboring the vector only (pUC19ΔE) or pER990, which contains an insertionally inactivated *lacD* ORF, ceased growth within 1 h following the temperature shift to 40°C.

As a further step to test the ability to complement the *E. coli* H8 *fda-2*(Ts) allele *in vivo*, we constructed a strain harboring pYA501 for use in the growth studies described above. pYA501 is a pBR322 derivative containing a 5.1-kb *BamHI* fragment from the chromosome of *Streptococcus mutans*. This plasmid encodes galactose 6-phosphate isomerase, tagatose 6-phosphate kinase, and tagatose 1,6-bisphosphate aldolase (27). Surprisingly, cells harboring pYA501

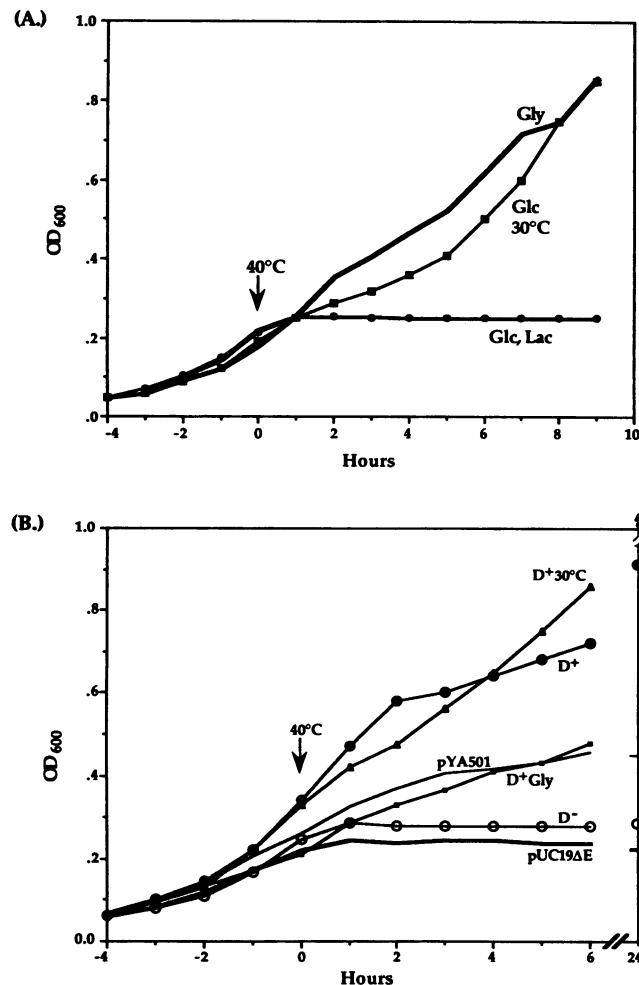


FIG. 4. In vivo complementation of *E. coli fda-2(Ts)* by tagatose 1,6-bisphosphate aldolase. Shown is representative growth of *E. coli* H8 at 30°C followed by a shift to the nonpermissive temperature (40°C) at time zero. The control culture, maintained at the permissive temperature, is indicated (30°C). (A) Sensitivity of strain H8 to the EMP pathway-dependent carbohydrates glucose and lactose. (B) Growth media contained glucose unless otherwise indicated. Abbreviations: D⁺, wild-type LacD protein expressed from pER988; D⁻, truncated LacD protein expressed from pER990; Gly, glycerol; Glc, glucose; Lac, lactose; OD₆₀₀, optical density at 600 nm.

exhibited only limited complementation, since they were capable of undergoing only one mass doubling at 40°C before growth cessation (Fig. 4B). This low level of complementation is likely a manifestation of relative tagatose 1,6-bisphosphate aldolase expression from read-through of vector-borne pBR322 promoters, in contrast to the strong *lacUV5* promoter of pER988. Alternatively, the streptococcal tagatose 1,6-bisphosphate aldolase may only be partially active at the elevated temperature of this assay.

Crude cell extracts prepared from *E. coli* H8 harboring either pER988, pER990, or pYA501 demonstrated tagatose 1,6-bisphosphate aldolase activity (Table 2). The relative levels of aldolase activity detected from these lysates in vitro is in striking agreement with the ability of these plasmids to complement the *fda-2(Ts)* allele in vivo. As predicted from in vivo complementation analysis, lysates from cells harboring

TABLE 2. Specific activities of enzymes in *E. coli* cell extracts

Plasmid	Sp act (nmol of substrate hydrolyzed/min/mg [dry wt] of cells) ^a		
	Tagatose 1,6-bisphosphate aldolase	Tagatose 6-phosphate kinase	Galactose 6-phosphate isomerase
pUC19Δ <i>EcoRI</i>	0.3	— ^b	—
pYA501	0.9	5.8	6.4
pER988	96.1	—	—
pER990	0.1	—	—
pUC13	—	0.2	—
pER973	—	9.9	—
pER920	—	0.1	—
pUC19	—	—	0.2
pER2070	—	—	9.7
pER2072	—	—	3.5
pER2075	—	—	1.7

^a Determined under the assay conditions described in Materials and Methods.

^b —, not performed.

pER988 exhibited high tagatose 1,6-bisphosphate aldolase activity (specific activity, >80 Sigma units), while that detected from cells containing pER990 was negligible. Consistent with the previous in vivo complementation studies, we were able to detect only limited tagatose 1,6-bisphosphate aldolase activity (specific activity, 0.8 to 1 Sigma units) encoded by pYA501 in vitro. *S. aureus* LacD is 73% identical in amino acid sequence to the plasmid-borne LacD of *Lactococcus lactis* subsp. *lactis* MG1820 (30).

(iii) **Tagatose 6-phosphate kinase is encoded by *lacC* and shares homology with other phosphokinases.** Crude cell extracts prepared from *E. coli* DF1010 harboring pER973 or pER920 were analyzed for 6-phosphokinase activity in vitro, using fructose 6-phosphate as a substrate. As shown in Table 2, significant enzymatic activity (specific activity, 9.5 to 10 Sigma units) was encoded by the *lacC* ORF present in pER973, while truncated protein expressed from pER920 was completely inactive. In addition, the control plasmid, pYA501, exhibited 6-phosphokinase activity, although it was only approximately one-half that demonstrated by pER973. Again, this observation is in agreement with our earlier results in which we detected low tagatose 1,6-bisphosphate aldolase activity encoded by pYA501 in comparison with that encoded by the staphylococcal *lacD* gene of pER988.

Tagatose 6-phosphate kinase expressed from plasmid pER973 was capable of partially complementing *E. coli* DF1010. This strain lacks both the major (*ΔpfkA*) and minor (*ΔpfkB*) 6-phosphofruktokinase components of the wild-type *E. coli* glycolytic pathway (11). Growth was severely depressed when DF1010 harboring pER920 was propagated in a basal salts solution containing 0.4% glucose as the sole carbon source (Fig. 5). However, an isogenic strain expressing wild-type LacC (from pER973) exhibited a generation time approximately one-fourth that of DF1010 harboring the vector, pUC13, or the LacC⁻ plasmid (pER920) alone. Thus, the tagatose 6-phosphate kinase activity expressed from plasmid-borne *lacC* provides sufficient enzymatic activity on the fructose 6-phosphate produced during glycolysis in *E. coli* to allow at least the partial reconstitution of an intact EMP pathway, thereby supporting growth, albeit slow, of strain DF1010 on glucose.

That *lacC* specifies tagatose 6-phosphate kinase is also strongly supported by the high level of homology between its

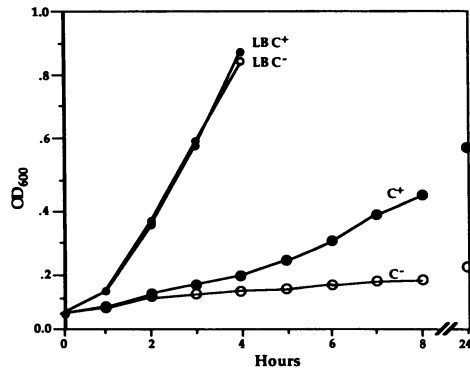


FIG. 5. Tagatose 6-phosphate kinase encoded by *lacC*. In an in vivo complementation analysis of *E. coli* DF1010 by pER973, cell growth in basal salts solution P plus 0.4% glucose or in Luria broth (LB) was monitored. Abbreviations: C⁺, wild-type LacC protein expressed from pER973; C⁻, truncated LacC protein expressed from pER920.

deduced amino acid sequence and that of the tagatose 6-phosphate kinase of *L. lactis* (61% identity [30]) as well as with other phosphokinases from *E. coli*, *Salmonella typhimurium*, and *Rhodobacter capsulatus*. The deduced LacC protein shares extensive (25% direct) homology throughout the 312-amino-acid 1-phosphofructokinase encoded by *fruK* from *E. coli* (21) and therefore also to the identical FruK of *S. typhimurium* (14). The 316-amino-acid 1-phosphofructokinase encoded by *fruK* from *R. capsulatus* (31) shares 28% identity with LacC. Ironically, LacC also shares 24% amino acid identity with the minor isoform of 6-phosphofructokinase, PfkB, from *E. coli* (12). This 308-amino-acid protein, which is only two residues smaller than LacC, has also been shown to catalyze the ATP-dependent phosphorylation of tagatose 6-phosphate (1), although its physiological role in *E. coli* remains unclear. It is therefore not completely unexpected that LacC expressed from pER973 is capable of partially complementing an *E. coli* strain (DF1010) devoid of functional PfkA and PfkB during growth on glucose.

(iv) *lacA* and *lacB* encode the multimeric galactose 6-phosphate isomerase. The plasmids shown in Fig. 6 were generated so as to allow direct determination of the interdependence of the staphylococcal *lacA* and *lacB* genes to encode galactose 6-phosphate isomerase. Cell extracts were prepared from *E. coli* LE392 (*galK2 galT22*) harboring various test plasmids and assayed for galactose 6-phosphate isomerase (Table 2). We could detect significant galactose 6-phosphate isomerase activity encoded only from the control plasmid (pYA501) or pER2070. Enzyme activity was decreased three- to fivefold in extracts from cells harboring plasmids pER2072 (LacA⁻B⁺) or pER2075 (LacA⁺B⁻), suggesting that both polypeptides are required for full galactose 6-phosphate isomerase activity. Partially purified staphylococcal galactose 6-phosphate isomerase is reported to have a native molecular mass of 100 kDa (6). Therefore, we conclude that galactose 6-phosphate isomerase is a heteromultimeric protein consisting of the 15- and 19-kDa components encoded by *lacA* and *lacB*, respectively. The finding that deletion of relatively few (11 of 171) amino acids from the carboxyl terminus of LacB (pER2075; Fig. 6) results in a marked (sixfold) decrease in enzymatic activity, further suggests this region may play a critical role in substrate binding or subunit interaction. These proteins are highly homologous to the LacA and LacB proteins of *L. lactis* (30),

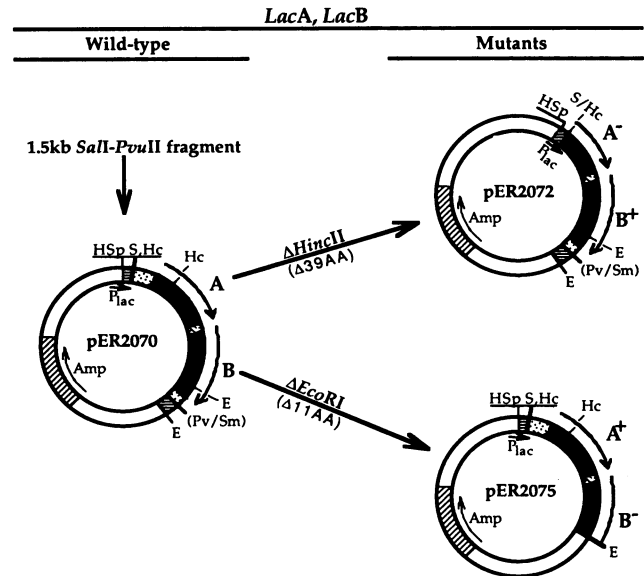


FIG. 6. Construction of *lacA* and *lacB* alleles. pUC19 derivatives containing appropriate alleles used in the determination of enzymatic activity of the LacAB polypeptides are illustrated (see Materials and Methods for details). Only relevant restriction sites are shown. Abbreviations are as for Fig. 2; Hc, *HincII*; Pv, *PvuII*.

with sequence identities of 70.4 and 85.4%, respectively. Thus, the *lacABCD* genes comprise the tagatose 6-phosphate pathway and are cotranscribed with genes *lacFEG*, which specify proteins for transport (enzyme III^{Lac} and enzyme II^{Lac}) and cleavage (phospho-β-galactosidase) of lactose in *S. aureus*.

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