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# Genetic Evidence for the Physiological Significance of the D-Tagatose 6-Phosphate Pathway of Lactose and D-Galactose Degradation in *Staphylococcus aureus*[1]

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Mutants of *Staphylococcus aureus* were isolated which were unable to utilize D-galactose or lactose, but which were able to utilize all other carbohydrates tested. Growth of the mutants on a peptone-containing medium was inhibited by D-galactose. Of those mutants selected for further study, one (*tagI2*) was missing D-galactose 6-phosphate isomerase, one (*tagK3*) was missing D-tagatose 6-phosphate kinase, and one (*tagA4*) was missing D-tagatose 1,6-diphosphate aldolase. Each of these mutants accumulated the substrate of the missing enzyme intracellularly. Spontaneous revertants of each of the mutants simultaneously regained their ability to utilize D-galactose and lactose, lost their sensitivity to D-galactose, regained the missing enzymatic activities, and no longer accumulated intermediates of the D-tagatose 6-phosphate pathway. These data support our previous contention that the physiologically significant route for the metabolism of D-galactose and the D-galactosyl moiety of lactose in *S. aureus* is the D-tagatose 6-phosphate pathway. Furthermore, a mutant constitutive for all three enzymes of this pathway was isolated, indicating that the products of the *tagI*, *tagK*, and *tagA* genes are under common genetic control. This conclusion was supported by the demonstration that D-galactose 6-phosphate isomerase, D-tagatose 6-phosphate kinase, and D-tagatose 1,6-diphosphate aldolase are coordinately induced in the parental strain.

In *Staphylococcus aureus*, D-galactose and lactose are phosphorylated with phosphoenolpyruvate during transport into the cell to yield D-galactose 6-phosphate (15) and lactose phosphate (5–7), respectively. Lactose phosphate is hydrolyzed by a phospho-β-galactosidase to yield D-glucose and D-galactose 6-phosphate (5–7). We have shown (1) that D-galactose 6-phosphate derived either from lactose or D-galactose is metabolized further in this organism through a pathway involving D-tagatose derivatives: D-galactose 6-phosphate → D-tagatose 6-phosphate → D-tagatose 1,6-diphosphate → dihydroxyacetone phosphate + D-glyceraldehyde 3-phosphate. This pathway has been termed the D-tagatose 6-phosphate pathway of D-galactose metabolism (1, 2), in contradistinction to the Leloir pathway in which D-galactose is metabolized through D-galactose 1-phosphate to D-glucose 1-phosphate by means of nucleotide derivatives (11).

Our previous studies have indicated that certain species of *Streptococcus* possess enzymes of both the D-tagatose 6-phosphate pathway and the Leloir pathway (2), whereas *Escherichia coli* possesses enzymes only of the Leloir pathway and *S. aureus* possesses enzymes only of the D-tagatose 6-phosphate pathway (1, 2). Although other investigators have suggested that D-galactose is metabolized through 6-phosphogalactonate in *S. aureus* (15), we have been unable to detect this or any other route for D-galactose metabolism other than the D-tagatose 6-phosphate pathway (1). This communication reports the isolation and analysis of mutants of *S. aureus* deficient in enzymes of the D-tagatose 6-phosphate pathway (i.e., D-galactose 6-phosphate isomerase, D-tagatose 6-phosphate kinase, and D-tagatose 1,6-diphosphate aldolase; see Fig. 1). The data support our previous conclusion (1) that the D-tagatose 6-phosphate pathway functions instead of, rather than in addition to, other possible pathways of lactose and D-galactose metabolism in *S. aureus*, and further indicate that the genes coding for the enzymes of the D-tagatose 6-phos-

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phate pathway are under common genetic control.

## MATERIALS AND METHODS

**Organisms.** The wild-type organism was *S. aureus* NCTC 8511. The genealogy and description of the mutants (isolated as described below) are shown in Table 1.

**Media.** Induction of the enzymes of D-galactose metabolism was achieved by incubation of the cells in a peptone broth (10) containing 1% D-galactose (induction broth). Routine liquid cultivation was done in the same medium except that no D-galactose or other sugar was added and the peptone concentration was increased to 2% (routine broth). Carbohydrate utilization was determined in bromocresol purple broth (3). D-Galactose-negative mutants were isolated on the agar medium described by Morse and Alire (12) or modifications of it as specified below. Spontaneous revertants were isolated on the modified eosine-methylene blue agar described by McClatchy and Rosenblum (10). During the isolation of mutants, cells were washed with a mineral medium (14).

**Other materials.** D-Tagatose 6-phosphate was prepared as described previously (1). Glass beads (88- to 125-$\mu$m diameter) were from LaPine Scientific Co., Chicago, Ill.; they were treated with 3 N HCl, washed free of acid, and dried prior to use. Crystalline (grade III) glucose 6-phosphate isomerase (EC 5.3.1.9), crystalline (type III) rabbit muscle fructose 6-phosphate kinase (EC 2.7.1.11), crystalline rabbit muscle fructose 1,6-diphosphate aldolase (EC 4.1.2.13), crystalline (type III) triose phosphate isomerase (EC 5.3.1.1)-$\alpha$-glycerol phosphate dehydrogenase (EC 1.1.1.8) mixture, D-cycloserine, penicillin G, streptomycin sulfate, D-fructose 6-phosphate, and D-fructose 1,6-diphosphate were from Sigma Chemical Co., St. Louis, Mo. Crystalline A-grade lactate dehydrogenase (EC 1.1.1.27), crystalline A-grade pyruvate kinase (EC 2.7.1.40), and A-grade glucose 6-phosphate dehydrogenase (EC 1.1.1.49) were from Calbiochem, Los Angeles, Calif. Ethyl methane sulfonate was from Eastman Organic Chemicals, Rochester, N.Y. Other chemicals were the highest purity available from standard commercial sources.

**Cell growth and enzyme induction procedures.** The incubation temperature was 37 C. The cultures were aerated by agitation on a shaker.

For most studies involving the determination of enzyme levels, cells were grown overnight in 7 ml of routine broth, harvested by centrifugation, suspended in 7 ml of induction broth, and incubated for 3 h. For coordinate induction studies, 1-liter volumes were used.

For the preparation of D-tagatose 6-phosphate pathway enzymes as reagents for metabolite assays, the same growth and induction procedures described above (1-liter volumes) were used.

Carbohydrate utilization tests were done in culture tubes containing bromocresol purple broth. A color change from purple to yellow indicated carbohydrate utilization with concomitant acid production. No color change within 16 h after inoculation (3) was recorded as negative. The lack of carbohydrate utilization in these cases was verified by the anthrone method (13).

**Preparation of cell extracts.** Cells were harvested by centrifugation, washed with 0.85% (wt/vol) NaCl, and suspended in 20 mM potassium phosphate buffer (pH 7.5) containing 0.2% (vol/vol) 2-mercaptoethanol and glass beads (five times the packed cell volume). The cells were disrupted by exposing the suspensions to sonic vibration (10,000 Hz) for 20 min at 0 to 4 C in

TABLE 1. *Genealogy and phenotype of the S. aureus strains*

| Strain | Parent | Genotype | Phenotype[a] |
|---|---|---|---|
| NCTC 8511 | | Wild type | Str-s32; Gal$^+$Lac$^+$; Gal6PI$^+$ Tag6PK$^+$ Tag1,6P$_2$A$^+$ |
| SR7 | NCTC 8511 | *str-7* | Str-r32; Gal$^+$Lac$^+$; Gal6PI$^+$ Tag6PK$^+$ Tag1,6P$_2$A$^+$ |
| L2 | SR7 | *str-7 tag-1* | Str-r32; Gal$^+$Lac$^+$; constitutive for Gal6PI, Tag6PK, and Tag1,6P$_2$A |
| GD29 | SR7 | *str-7 tagI2* | Str-r32; Gal$^-$Lac$^-$; Gal6PI$^-$ Tag6PK$^+$ Tag1,6P$_2$A$^+$ |
| GD29R | GD29 | *str-7* | Str-r32; Gal$^+$Lac$^+$; Gal6PI$^+$ Tag6PK$^+$ Tag1,6P$_2$A$^+$ |
| GB27 | SR7 | *str-7 tagK3* | Str-r32; Gal$^-$Lac$^-$; Gal6PI$^+$ Tag6PK$^-$ Tag1,6P$_2$A$^+$ |
| GB27R | GB27 | *str-7* | Str-r32; Gal$^+$Lac$^+$; Gal6PI$^+$ Tag6PK$^+$ Tag1,6P$_2$A$^+$ |
| GE26 | SR7 | *str-7 tagA4* | Str-r32; Gal$^-$Lac$^-$; Gal6PI$^+$ Tag6PK$^+$ Tag1,6P$_2$A$^-$ |
| GE26R | GE26 | *str-7* | Str-r32; Gal$^+$Lac$^+$; Gal6PI$^+$ Tag6PK$^+$ Tag1,6P$_2$A$^+$ |

[a] Abbreviations: Str-s32, sensitive to 32 $\mu$g of streptomycin sulfate per ml; Str-r32, resistant to 32 $\mu$g of streptomycin sulfate per ml; Gal$^+$, metabolizes D-galactose; Gal$^-$, does not metabolize D-galactose; Lac$^+$, metabolizes lactose; Lac$^-$, does not metabolize lactose; Gal6PI, D-galactose 6-phosphate isomerase; Tag6PK, D-tagatose 6-phosphate kinase; Tag1,6P$_2$A, tagatose 1,6-diphosphate aldolase.

a Raytheon sonic oscillator. The supernatant fluid obtained after centrifugation of the broken-cell suspension at 48,200 × *g* for 10 min was the cell extract.

**Enzyme and protein assays.** Assays for D-galactose 6-phosphate and D-glucose 6-phosphate isomerases, D-tagatose 6-phosphate and D-fructose 6-phosphate kinases, and D-tagatose 1,6-diphosphate and D-fructose 1,6-diphosphate aldolases were those described previously (1). All enzyme units are defined in terms of micromoles of product formed per minute. Protein was estimated by the method of Lowry et al. (9), with bovine serum albumin as the standard.

**Isolation of a streptomycin-resistant mutant (strain SR7).** A streptomycin-resistant mutant was isolated for the purpose of having a genetic marker in the parental strain used for the isolation of D-galactose-negative and constitutive mutants. An overnight culture of *S. aureus* NCTC 8511 in 7 ml of routine broth was harvested by centrifugation. The cells were washed twice with mineral medium, suspended in routine broth containing 0.14 M ethyl methane sulfonate (8), and incubated at 37 C for 2 h. The cells were then collected by centrifugation, washed three times with mineral medium, and suspended in 7 ml of routine broth. The cells were allowed to grow for 10 generations by serial transfer and then were plated on agar (12) containing 32 μg of streptomycin sulfate per ml. The streptomycin-resistant isolate selected for further use was designated SR7. This isolate, like the parent, utilized D-glucose, D-fructose, D-galactose, D-mannitol, and lactose, as determined by plating on an indicator medium (12).

**Isolation of a mutant, L2, constituve for the enzymes of the D-tagatose 6-phosphate pathway.** An overnight culture of strain SR7 in 7 ml of routine broth was mutagenized and grown for 10 generations as described above. The cells were plated on an indicator agar (12) containing 1% lactose. Mutant L2 appeared as an entirely red colony (the parent and strain NCTC 8511 form colonies with a red center and white periphery).

**Isolation of mutants GB27, GD29, and GE26 deficient in enzymes of the D-tagatose 6-phosphate pathway.** A culture of strain SR7 was mutagenized and grown for 10 generations as described above for strain NCTC 8511. The culture was then suspended in induction broth to a density of $10^8$ cells per ml. After one cell doubling, 2,000 U of penicillin G (4) per ml and D-cycloserine (16) to a concentration of 0.01 M were added. Incubation on a shaker at 37 C was continued for 10 to 15 h (i.e., until the optical density at 600 nm began to decrease), at which time the survival was about 1%. The culture was plated on agar (12) containing 0.01% D-glucose plus 1% D-galactose but no neutral red. The smallest colonies were selected and tested on neutral red agar (12) for their ability to utilize various carbohydrates. Mutants GB27, GD29, and GE26 were selected as representatives of those that formed red growth on D-glucose, D-fructose, and D-mannitol, but formed pale growth on D-galactose and lactose.

**Isolation of spontaneous revertants GD29R, GB27R, and GE26R.** Overnight cultures of mutants GD29, GB27, and GE26 on routine broth were plated heavily on modified eosin-methylene blue agar, which is inhibitory to non-lactose-utilizing strains (10). Revertants, which appeared as distinct colonies against the lawn of inhibited growth, were isolated. Strains GD29R, GB27R, and GE26R were isolated as revertants of strains GD29, GB27, and GE26, respectively.

**Determination of intracellular levels of D-galactose 6-phosphate, D-tagatose 6-phosphate, and D-tagatose 1,6-diphosphate.** Overnight routine broth cultures (7 ml) were inoculated into 300 ml of induction broth minus the D-galactose in Fernbach flasks. After 5 h on a rotary shaker at 37 C, D-galactose was added at a concentration of 1%. After incubation for an additional 5 h, the cells were harvested, washed with 0.85% NaCl, weighed, and then extracted with about 10 ml of 80% (vol/vol) ethanol per g of packed cells. The extracted cells were removed by centrifugation, the pellet was washed twice with water, and the three supernatant fractions were pooled. Most of the ethanol was removed with a rotary evaporator, and the remaining solution was freeze-dried. The residue was dissolved in 0.5 ml of water and clarified by centrifugation.

The resulting supernatant solution was assayed enzymatically for D-galactose 6-phosphate, D-tagatose 6-phosphate, and D-tagatose 1,6-diphosphate with a Gilford absorbance-recording spectrophotometer at 340 nm. The reaction volumes were 0.15 ml in microcuvettes with a 1.0-cm light path. The basic reaction mixture contained: 10 μmol of glycylglycine buffer (pH 7.5); 1.0 μmol of $MgCl_2$; 0.05 μmol of reduced nicotinamide adenine dinucleotide; nonlimiting amounts of fructose 1,6-diphosphate aldolase, triose phosphate isomerase, and α-glycerol phosphate dehydrogenase; and a limiting amount of the sample to be assayed. In addition, reaction A contained D-tagatose 1,6-diphosphate aldolase; reaction B contained 0.5 μmol of adenosine 5′-triphosphate and nonlimiting amounts of glucose 6-phosphate isomerase, fructose 6-phosphate kinase, and D-tagatose 1,6-diphosphate aldolase; and reaction C was like reaction B except that it also contained D-galactose 6-phosphate isomerase. During a preincubation period, all additional components except D-galactose 6-phosphate isomerase and D-tagatose 1,6-diphosphate aldolase were added to the basic reaction mixture. This preincubation resulted in the removal of any fructose 1,6-diphosphate present in the sample for reaction A and of any glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-diphosphate present in the samples for reactions B and C. D-Galactose 6-phosphate isomerase and D-tagatose 1,6-diphosphate aldolase were then added as indicated for reactions A, B, and C. Rabbit muscle fructose 6-phosphate kinase will phosphorylate D-tagatose 6-phosphate (18), but rabbit muscle fructose 1,6-diphosphate aldolase did not cleave D-tagatose 1,6-diphosphate under our conditions. Taking into account the twofold amplification obtained with these end-point assays, a change of 1.0 absorbance unit was equivalent to 12.2 nmol of hexose phosphate. The intermediates determined in each case were as follows: reaction A, D-tagatose 1,6-diphosphate; reaction B, D-taga-

tose 6-phosphate plus D-tagatose 1,6-diphosphate; reaction C, D-galactose 6-phosphate plus D-tagatose 6-phosphate plus D-tagatose 1,6-diphosphate. By appropriate subtraction, the level of each of the three intermediates was determined.

**Preparation of D-galactose 6- phosphate isomerase and D-tagatose 1,6-diphosphate aldolase.** These enzymes, when used as reagents for the determination of metabolites as described above, were prepared by chromatographing cell extracts on columns of Sephadex G-25 to remove small molecules. For reactions A and B, aldolase was prepared from strain GD29 (D-galactose 6-phosphate isomerase negative). For reaction C, the isomerase and aldolase were prepared from strain NCTC 8511 (wild type).

## RESULTS

**Enzyme activities in cell extracts.** The specific activities of the enzymes of the D-tagatose 6-phosphate pathway (Fig. 1) and of the corresponding enzymes of the Embden-Meyerhof pathway are shown in Table 2. The specific activities of all six enzymes in strain SR7 (the streptomycin-resistant mutant from which all the other mutants were derived) were comparable to those in strain NCTC 8511 (wild type). Mutant GD29 (*tagI2*) was missing only D-galactose 6-phosphate isomerase, mutant GB27 (*tagK3*) was missing only D-tagatose 6-phosphate kinase, and mutant GE26 (*tagA4*) was missing only D-tagatose 1,6-diphosphate aldolase. Spontaneous revertants (strains GD29R, GB27R, and GE26R, respectively) regained the enzyme activities that were deficient in their parents. All of the D-galactose-negative mutants (GD29, GB27, and GE26) retained the corresponding enzymes of the Embden-Meyerhof pathway.

**Carbohydrate utilization tests.** Each of the mutants missing one of the enzymes of the D-tagatose 6-phosphate pathway (strains GD29, GB27, and GE26) failed to utilize D-galactose or lactose but did utilize the other six carbohydrates tested (Table 3). The wild type (NCTC 8511), the streptomycin-resistant mutant (SR7), and the revertants (GD29R, GB27R, and GE26R) utilized all eight carbohydrates (Table 3).

**Intracellular accumulation of metabolites.** The D-galactose-negative mutants (GD29, GB27, and GE26) were isolated on the basis of

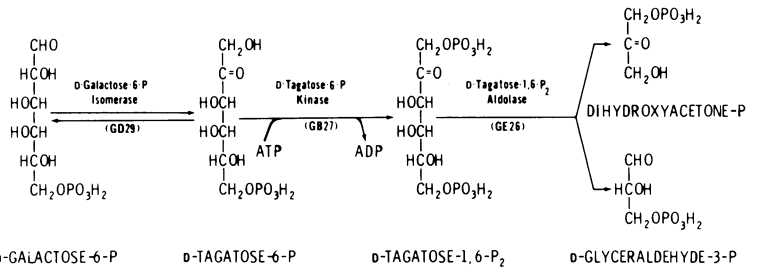


FIG. 1. *D-Tagatose 6-phosphate pathway as elucidated in reference 1. Designations in parentheses indicate mutants blocked in that reaction.*

TABLE 2. *Enzymes of the D-tagatose 6-phosphate (6-P) pathway and corresponding enzymes of the Embden-Meyerhof pathway in various strains of S. aureus*[a]

| Strain | Sp act (μmol of product formed per min per mg of protein) | | | | | |
|---|---|---|---|---|---|---|
| | D-Tagatose-6-P pathway enzymes | | | Embden-Meyerhof pathway enzymes | | |
| | D-Galactose-6-P isomerase | D-Tagatose-6-P kinase | D-Tagatose-1,6-$P_2$ aldolase | D-Glucose-6-P isomerase | D-Fructose-6-P kinase | D-Fructose-1,6-$P_2$ aldolase |
| NCTC 8511 | 1.20 | 0.034 | 0.064 | 0.454 | 0.025 | 0.046 |
| SR7 | 1.26 | 0.035 | 0.060 | 0.420 | 0.028 | 0.045 |
| GD29 | 0.000 | 0.032 | 0.063 | 0.194 | 0.021 | 0.049 |
| GD29R | 1.48 | 0.025 | 0.058 | 0.204 | 0.033 | 0.053 |
| GB27 | 0.93 | 0.000 | 0.052 | 0.397 | 0.011 | 0.037 |
| GB27R | 1.67 | 0.049 | 0.070 | 0.457 | 0.013 | 0.043 |
| GE26 | 0.30 | 0.014 | 0.000 | 0.444 | 0.032 | 0.030 |
| GE26R | 1.13 | 0.027 | 0.050 | 0.406 | 0.022 | 0.048 |
| L2 | 2.07 | 0.163 | 0.212 | 0.437 | 0.026 | 0.036 |

[a] All strains were induced with D-galactose except strain L2, which was grown in routine broth and not induced.

their sensitivity to D-galactose (see above). After 1 day on agar containing 0.01% D-glucose and 1% D-galactose, these strains produced colonies with diameters of approximately 0.3 mm, whereas D-galactose-positive strains had colony diameters of approximately 1.5 mm. Subsequent experiments revealed that the D-galactose-negative mutants also produced normal-sized colonies when D-galactose was replaced with 1% D-glucose, D-fructose, D-mannose, D-mannitol, maltose, or sucrose. Revertants produced normal-sized colonies on all eight of the carbohydrates tested.

Presumably, the inhibition of growth by D-galactose was due to the intracellular accumulation of intermediates of the D-tagatose 6-phosphate pathway. The experimental determination of the intermediates is presented in Table 4. Strain GD29 (*tagI2*) accumulated D-galactose 6-phosphate, strain GB27 (*tagK3*) accumulated D-galactose 6-phosphate and D-tagatose 6-phosphate, and strain GE26 (*tagA4*) accumulated primarily D-tagatose 1,6-diphosphate. The levels of intermediates in the revertants (strains GD29R, GB27R, and GE26R) were similar to those in the wild type (NCTC 8511) and the parental strain (SR7).

**Common genetic control of the enzymes of the D-tagatose 6-phosphate pathway.** A mutant (strain L2) was isolated which simultaneously gained the ability to form the isomerase, kinase, and aldolase of the D-tagatose 6-phosphate pathway in the absence of an added inducer (Table 2). As would be expected, this constitutive mutant had the wild-type carbohydrate utilization pattern (Table 3) and did not accumulate intermediates of the D-tagatose 6-phosphate pathway (Table 4).

Coordinate induction of the isomerase, kinase, and aldolase in strain NCTC 8511 is shown in Fig. 2. There was a proportional induction of the isomerase with respect to the kinase (Fig. 2A), of the isomerase with respect to the aldolase (Fig. 2B), and of the kinase with respect to the aldolase (Fig. 2C).

## DISCUSSION

Previously we demonstrated that D-galactose 6-phosphate is metabolized in *S. aureus* through the pathway shown in Fig. 1 (1). The isomerase, kinase, and aldolase that catalyze the reactions of this pathway were shown to be induced by D-galactose or lactose, both of which are known to be metabolized to D-galactose 6-phosphate. Since we were unable to demonstrate any alternative metabolic route, we proposed that this D-tagatose 6-phosphate pathway is the physiologically significant route for the metabolism of D-galactose and the D-galactosyl moiety of lactose in *S. aureus*. The data in the present paper offer confirmatory evidence for this proposal.

Since mutants of *S. aureus* deficient in either D-galactose 6-phosphate isomerase, D-tagatose

TABLE 4. *Intracellular accumulation of intermediates of the D-tagatose 6-phosphate (6-P) pathway*

| Strain | Accumulated metabolite (μmol/g [wet wt] of cells) | | |
|---|---|---|---|
| | D-Galactose-6-P | D-Tagatose-6-P | D-Tagatose-1,6-$P_2$ |
| NCTC 8511 | 0.13 | 0.03 | 0.10 |
| SR7 | 0.19 | 0.04 | 0.08 |
| GD29 | 3.35 | 0.00 | 0.00 |
| GD29R | 0.15 | 0.04 | 0.40 |
| GB27 | 1.92 | 0.75 | 0.00 |
| GB27R | 0.10 | 0.02 | 0.10 |
| GE26 | 0.54 | 0.13 | 6.36 |
| GE26R | 0.20 | 0.07 | 0.06 |
| L2 | 0.07 | 0.02 | 0.03 |

TABLE 3. *Utilization of carbohydrates by various strains of S. aureus as determined in bromocresol purple broth*[a]

| Strain | Utilization of carbohydrates: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | D-Galactose | Lactose | D-Glucose | D-Fructose | D-Mannose | D-Mannitol | Maltose | Sucrose |
| NCTC 8511 | + | + | + | + | + | + | + | + |
| SR7 | + | + | + | + | + | + | + | + |
| GD29 | − | − | + | + | + | + | + | + |
| GD29R | + | + | + | + | + | + | + | + |
| GB27 | − | − | + | + | + | + | + | + |
| GB27R | + | + | + | + | + | + | + | + |
| GE26 | − | − | + | + | + | + | + | + |
| GE26R | + | + | + | + | + | + | + | + |
| L2 | + | + | + | + | + | + | + | + |

[a] +, Acid production; −, no acid production. Readings were made 16 h after inoculation.

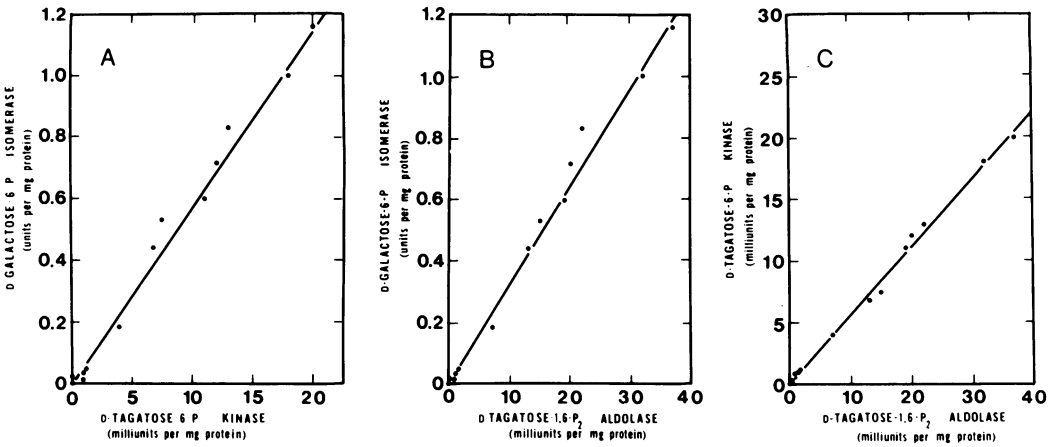


FIG. 2. *Coordinate induction of the enzymes of the D-tagatose 6-phosphate pathway in S. aureus NCTC 8511. Cells harvested from 1 liter of routine broth (containing no added carbohydrate) were suspended in 1 liter of induction broth (containing 1% D-galactose). Samples were removed immediately after resuspension and every 10 min for 2 h, extracts were prepared, and enzyme specific activities were determined.*

6-phosphate kinase, or D-tagatose 1,6-diphosphate aldolase were specifically unable to metabolize D-galactose or lactose, each of these enzymes must be essential in the metabolism of these two sugars. The demonstrated intracellular accumulation of the substrates of the missing enzymes indicated that the enzymes were indeed nonfunctional in vivo as well as being undetectable in cell extracts. The isolation of spontaneous revertants that simultaneously regained the deficient enzymes and the ability to metabolize D-galactose and lactose, and no longer accumulated intermediates of the D-tagatose 6-phosphate pathway, confirmed that the D-galactose-negative isolates were the results of single point mutations rather than deletions or multiple point mutations.

The Gal$^-$ Lac$^-$ mutants that were missing the D-tagatose 6-phosphate pathway enzymes nevertheless retained the corresponding enzymes of the Embden-Meyerhof pathway (i.e., D-glucose 6-phosphate isomerase, D-fructose 6-phosphate kinase, and D-fructose 1,6-diphosphate aldolase) and metabolized all carbohydrates tested except D-galactose and lactose. This is consistent with our earlier enzymological data (1) which indicated that the enzymes of the two pathways are distinct and separable, and not merely manifestations of nonspecificity of the glycolytic enzymes. This is of interest because it has been reported that mammalian D-fructose 6-phosphate kinase can phosphorylate D-tagatose 6-phosphate (18) and that mammalian D-fructose 1,6-diphosphate aldolase can cleave D-tagatose 1,6-diphosphate (18).

The isolation of a mutant (strain L2) that is constitutive for the enzymes of the D-tagatose 6-phosphate pathway indicated that the enzymes are under common genetic control. This was confirmed by demonstrating the coordinate induction of D-galactose 6-phosphate isomerase, D-tagatose 6-phosphate kinase, and D-tagatose 1,6-diphosphate aldolase in the wild-type strain. It is tempting to speculate that the genes coding for the synthesis of the D-tagatose 6-phosphate pathway enzymes reside in a single operon, but this needs to be verified by genetic mapping.

The genetic symbol *gal* has been used previously for enzymes of the Leloir pathway of D-galactose metabolism (17). To distinguish between the genes of the Leloir pathway and of the D-tagatose 6-phosphate pathway, and bearing in mind that some organisms possess enzymes for both of these pathways (2), we propose that *tag* be used as the genetic symbol for the D-tagatose 6-phosphate pathway of D-galactose metabolism. Furthermore, we propose the gene symbols *tagI* for D-galactose 6-phosphate isomerase, *tagK* for D-tagatose 6-phosphate kinase, and *tagA* for D-tagatose 1,6-diphosphate aldolase.

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