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Xylitol and D-Arabitol Toxicities Due to Derepressed Fructose, Galactitol, and Sorbitol Phosphotransferases of *Escherichia coli*

ALBEY M. REINER

Microbiology Department, University of Massachusetts, Amherst, Massachusetts 01003

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D-Arabitol was observed to be toxic to many laboratory strains of *Escherichia coli* K-12, and xylitol was found to be toxic to an existing *E. coli* C mutant strain. Fructose-specific components of the phosphoenolpyruvate:sugar phosphotransferase system are required for xylitol toxicity. Selection for xylitol resistance results in Fru$^-$ strains blocked in fructose phosphotransferase. Introduction of the *ptsF* or *ptsI* mutation into a xylitol-sensitive strain eliminates sensitivity. [^{14}C]fructose uptake experiments imply that the mutation to xylitol sensitivity, which is co-transducible with *ara* and *leu*, results in derepression of normally inducible fructose phosphotransferase. Wild-type strains also become xylitol sensitive if induced by (and then removed from) fructose. Xylitol toxicity is prevented by fructose in both wild-type and mutant strains. Circumstances causing xylitol, a new food additive, to become toxic to an otherwise insensitive wild-type organism have not been reported previously. The D-arabitol-sensitive laboratory strains are galactitol (dulcitol) utilizers, although most other strains are not. Selection for D-arabitol resistance results in Gat$^-$ strains blocked in a constitutive galactitol-specific component of the phosphotransferase system. A mutation causing D-arabitol sensitivity occurred many years ago in AB284, the parent of AB311, AB312, AB313, and many other strains. D-Arabitol sensitivity also occurs in sorbitol-constitutive strains and is shown, like the previous two instances of pentitol toxicities, to result from a constitutive phosphotransferase, which is blocked in mutants selected for resistance.

Investigations of pentitol catabolism in *Klebsiella* and *Escherichia coli* have provided models for studying acquisition of new catabolic pathways, intraspecies genetic polymorphism, and gene duplications (18, 20, 23, 24). One of the pentitols, xylitol, is being seriously considered as a sucrose substitute in foods, because it allegedly does not promote dental caries (26). During our studies concerning gene duplication, we observed that certain existing laboratory *E. coli* mutant strains are inhibited by xylitol, and that certain others are inhibited by D-arabitol. Wild-type *E. coli* strains are not inhibited by either. Neither toxicity effect has been reported previously.

Most previously characterized instances of carbohydrate toxicity in bacteria are due to lethal syntheses of phosphorylated intermediates. Generally, as for L-arabinose (7), fructose (8), galactose (28), galactitol (14), and mannitol (27), the cause is a block subsequent to a phosphorylation reaction in the normal catabolic pathway for that carbohydrate. For xylitol and D-arabitol toxicities, however, this is not the case, since toxicities may occur in *E. coli* K-12 strains that lack pentitol catabolic pathways (23).

Evidence presented here implicates certain components of the phosphotransferase system in toxic syntheses from xylitol and D-arabitol. The transport-phosphorylation of sugars via the phosphoenolpyruvate:sugar phosphotransferase system of bacteria involves the transfer of phosphoryl groups, from an intermediate common to several different sugar transports (P ~ HPr) to a particular sugar, via a pair of sugar-specific proteins called "phosphotransferases" (4, 13, 22, 25). The lethal components involved, fructose phosphotransferase for xylitol toxicity and galactitol and sorbitol phosphotransferases for D-arabitol toxicity, are constitutive in the pentitol-sensitive mutant strains and are lost by mutation in strains selected for resistance.

MATERIALS AND METHODS

Growth and properties of bacteria. Casein hydrolysate (CH) medium consisted of 5 g of vitamin-

free CH (Difco) per liter. Plates contained 15 g of agar (Difco) per liter. Sugars and sugar alcohols were sterilized by filtration and were incorporated into CH plates in either of two ways: by adding before pouring, or by spreading (0.1 or 0.2 ml of a 1 M solution) onto an already poured (ca. 30-ml) CH plate. Only in the former instances are concentrations specified (e.g., CH + 3 mM xylitol). Minimal medium contained (per liter): NH_4Cl, 1 g; $MgSO_4$, 250 mg; K_2HPO_4, 150 mg; NaCl, 1 g; and 1 M tris(hydroxymethyl)aminomethane (pH 7.3), 70 ml. Carbon and energy sources were present at 8 mM, except for fructose, which was present at 3 mM to prevent functioning of alternative fructose pathways (13). Growth in liquid was monitored by a Klett-Summerson photocolorimeter with a red filter. Properties and sources of bacterial strains are shown in Table 1.

Chemicals. Sugars and sugar alcohols were obtained from Sigma. [^{14}C]fructose (100 mCi/mmol) was obtained from Amersham/Searle.

Genetic techniques. Conjugations and transductions were performed by standard methods, as described previously (23). No mutagenesis was used. To construct a *ptsF* derivative of strain 1278S and an *fpk* derivative of strain 1278S, strain 1278S was conjugated with Hfr strains KL16-21-23 and AT2243-11^c-25 bearing *ptsF* and *fpk*, respectively. Selection was for Sm^r Met$^+$ His$^+$ recombinants, with glycerol as carbon and energy source. A Fru$^-$ recombinant was designed 1278S *ptsF* in the one case and 1278S *fpk* in the other case.

RESULTS

Xylitol toxicity: Xylitol is toxic for *E. coli* C strain 1278. *E. coli* C strains and *Klebsiella* strains are naturally Rtl$^+$ (able to use ribitol as sole source of carbon and energy), and, in addition, become Xtl$^+$ if constitutive for ribitol dehydrogenase (4, 17, 23). A single *E. coli* C laboratory strain, however, was observed to be ribitol dehydrogenase constitutive, yet unable to utilize xylitol for growth. This strain, 1278 (3), also differed from the other *E. coli* C and K-12 strains in our laboratory in being unable to form colonies on CH plates supplemented with $\geq$2 mM xylitol. In liquid CH medium, xylitol inhibition is detectable at $\geq$0.1 mM. Other *E. coli* C and K-12 strains tested are unaffected at least to 30 mM xylitol. Our further work with 1278 was done using its streptomycin-resistant derivative 1278S (23).

Xylitol toxicity does not involve pentitol catabolic pathways. The genes for catabolism of pentitols (including xylitol) in *E. coli* C strains are tightly clustered, between *his* and *metG* (23). *E. coli* K-12 strains lack these genes (23). *E. coli* C strains may be "deleted" for the pentitol catabolic genes by replacing the *his-metG* portion of their chromosome by that of a K-12 strain. This is particularly easy in the

TABLE 1. *E. coli strains used*[a]

Strain	Type	Properties[b]	Source
1278	C	F$^-$, *metG his rha ara* Xtl^s	T. Blumenthal (3)
1278S	C	F$^-$, *metG his rha ara* Xtl^s Sm^r	Spontaneous Sm^r mutant (23) of 1278
PK191	K-12	Hfr (P066), *pro*	B. Bachmann
C600	K-12	F$^-$, *thr leu thi lac* Sm^r	Own collection
C600 Xtl^s	K-12-C	F$^-$, *thr, thi lac* Xtl^s Sm^r	P1 transduction from 1278S into C600
1278S *ptsF*	C-K-12	F$^-$, *rha ara* Xtl^s *ptsF* Sm^r	See Materials and Methods
1278S *fpk*	C-K-12	F$^-$, *rha ara* Xtl^s *fpk* Sm^r	See Materials and Methods
ctr-7	K-12	Hfr (P045), *ptsI relA bgl*	B. Bachmann
1278S *fpkA*$^+$ Xtl^s	C-K-12	F$^-$, *rha fpk* Xtl^s Sm^r	By transduction (see text)
1278S *fpkA*$^+$ XT	C-K-12	F$^-$, *rha fpk* Sm^r	By transduction (see text)
KL16-21-23	K-12	Hfr (P045), *thi ptsF ptsM*	B. Bachmann
AT2243-11^c-25	K-12	Hfr (P02A), *metB uhp pyrE fpk*	H. Kornberg (9)
AB313	K-12	Hfr (P013), *thr leu thi lac* Sm^r	B. Bachmann
AB284	K-12	F$^-$, *thr leu thi lac* Sm^r	B. Bachmann
W208	K-12	F$^-$, *thr leu thi lac*	B. Bachmann
Y10	K-12	F$^-$, *thr leu thi*	B. Bachmann
L141	K-12	F$^-$, *lac gal xyl mtlA his arg met* Sm^r Gat$^+$ D-Atl^s	J. Lengeler (14)
L141R	K-12	As L141, but Gat$^-$ D-Atl^r	Spontaneous D-Atl^r mutant of L141
L141R Mtl$^+$	K-12	As L141R, but Srl^c D-Atl^s	Spontaneous Mtl$^+$ mutant of L141R
L193	K-12	F$^-$, *lac gal xyl mtlA his arg met* Sm^r *gatD* D-Atl^s	J. Lengeler (15)

[a] See Fig. 1 legend and reference 2 for abbreviations.

[b] *ptsF* confers xylitol tolerance to an Xtl^s strain. See Tables 2 and 3 for phenotypes of strains C600, 1278S, and L141 and their derivatives.

case of strain 1278S, which is *his metG*. His$^+$ Met$^+$ recombinants from a cross between *E. coli* K-12 Hfr strain PK191 and *E. coli* C strain 1278S were selected, the great majority of which were pentitol$^-$. Nevertheless, all recombinants from this cross remained sensitive to xylitol (Xtl^s). (See Table 1 for strain descriptions and Fig. 1 for a genetic map of *E. coli*.)

Xtl^s is highly co-transducible with *leu* and *ara*. It was advantageous to characterize the xylitol toxicity trait in certain *E. coli* K-12 strains as well as in strain 1278. Since strain 1278 is F$^-$, transfer could be easily accomplished only if the approximate location of the locus causing Xtl^s was known. Strain 1278 had been constructed 10 years earlier (3) by three sequential rounds of nitrosoguanidine mutagenesis. We reasoned that the Xtl^s mutation might have been induced by one of the nitrosoguanidine "hits" that caused a defect at a known genetic locus, in which case Xtl^s would map close to that defect (11).

The reported mutations in strain 1278 were *metG*, *his*, and *rha* (3). We noticed also an Ara$^-$ defect in this strain, which proved to be the significant one. Transductions showed that the Xtl^s and Ara$^-$ mutations were 75% linked by transduction (36 of 48 Ara$^+$ 1278S transductants became Xtl tolerant). Since *ara* maps near *leu*, we attempted to transfer the Xtl^s trait from 1278S to *E. coli* K-12 strain C600 (*thr leu*) by transduction with selection for Leu$^+$. Of 35 such transductants, 28 (80%) became Xtl^s. One strain was designated C600 Xtl^s and was used further below.

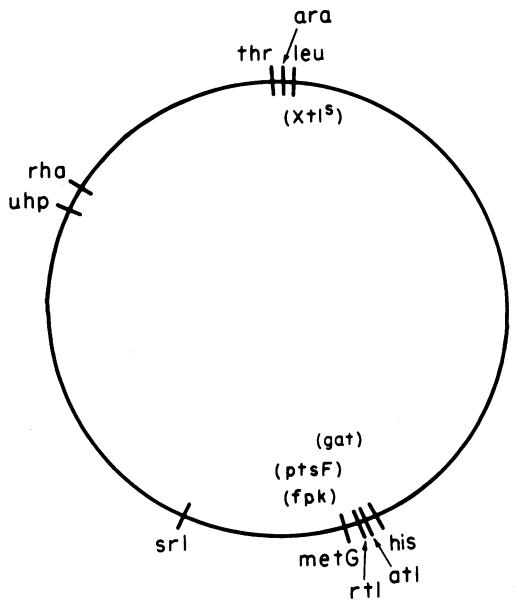

FIG. 1. *Genetic map of E. coli (2). Symbols ara, atl, rha, rtl, and srl are loci for catabolic pathways for* L-*arabinose,* D-*arabitol,* L-*rhamnose, ribitol, and sorbitol, respectively. uhp is uptake of hexose phosphate. Loci in parentheses (Xtl^s for xylitol sensitivity, gat for galactitol utilization, and also now ptsF for fructose phosphotransferase and fpk for fructose-1-phosphate kinase) are uncertain relative to neighboring loci (A. M. Reiner, unpublished data; B. Bachmann, personal communication).*

Xylitol-tolerant mutants are fructose$^-$. Xylitol-tolerant mutants are obtained readily by streaking an Xtl^s strain on CH plates supplemented with $\geq$1 mM xylitol. One hundred mutants so obtained from at least 10 independent clones of strain 1278S were tested for their abilities to grow on various carbohydrates. The great majority (95%) were Fru$^-$ and retained their abilities to grow on all other carbohydrates tested. The remaining mutants either retained their abilities to grow on all carbohydrates tested (possibly revertants of the original Xtl^s mutation) or lost their abilities to grow on many substrates, fructose included (suggestive of *ptsI* mutations generally affecting sugar phosphotransport [25]). Similar results were also obtained starting with strain C600 Xtl^s. These Frt$^-$ derivatives of strain 1278S, relieved of the Xtl^s trait, are now Xtl$^-$, as expected (see above), or ribitol dehydrogenase-constitutive strains.

Xylitol-tolerant mutants are defective in fructose phosphotransferase. The two metabolic steps unique to fructose utilization in *E. coli*, a phosphotransferase reaction followed by a kinase reaction, are shown in Fig. 2 (9). When a strain also is able to transport fructose-1-P, the position of a mutation in fructose metabolism can be determined by testing the Fru$^-$ strain for growth on fructose-1-P, since the latter compound requires the second fructose-specific step, though not the first, for its catabolism (Fig. 2; 9). For this purpose, strains 1278S and C600 Xtl^s were spread on fructose-1-P plates to select fructose-1-P$^+$ mutants (hexose phosphate transport constitutives; 10) of each. These strains were then spread on CH + xylitol plates to select xylitol-tolerant mutants. All such mutants examined were impaired for growth on fructose, but not for growth on fructose-1-P, indicating that their blocks in fructose utilization occur at the phosphotransferase step. (Phenotypes of these and other C600 and 1278 derivatives are summarized in Table 2.)

The link between xylitol toxicity and fructose phosphotransferase was confirmed by using previously characterized fructose phosphotransferase (*ptsF*) and fructose-1-P kinase (*fpk*) mutations (9), each of which was introduced into strain 1278S by conjugation (see Materials

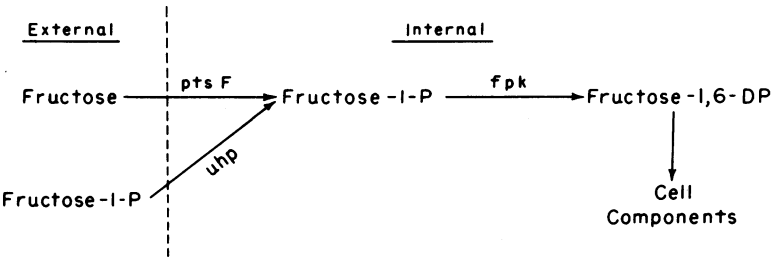

FIG. 2. *Fructose catabolism in E. coli. ptsF and fpk are genes specifying fructose phosphotransferase enzyme II and fructose-1-phosphate kinase, respectively. uhp specifies a transport system for hexose phosphates, which transports fructose-1-P when constitutive. For other routes of fructose catabolism not relevant to this paper, see reference 12.*

TABLE 2. *Relevant phenotypes of strains C600 and 1278S and their derivatives*

Strain	Growth				Sensitivity (xylitol)
	Fructose	Fructose-1-P	1-Arabinose	Xylitol	
C600	+	−	+	−	R
C600 Xtl^s	+	−	−	−	S
C600 Xtl^s F-1-P$^+$	+	+	−	−	S
C600 Xtl^s F-1-P$^+$ Xtl^r	−	+	−	−	R
1278S	+	−	−	−	S
1278S Xtl^r	−	−	−	+	R
1278S F-1-P$^+$	+	+	−	−	S
1278S F-1-P$^+$ Xtl^r	−	+	−	+	R
1278S *ptsF*	−	−	−	−	R
1278S *fpk*	−	−	−	−	S
1278S *fpkA*$^+$ XT	−	−	+	−	S
127S *fpkA*$^+$ XT	−	−	+	−	R

and Methods). The resulting 1278S *ptsF* strains became xylitol tolerant. The resulting 1278S *fpk* strains remained Xtl^s (Table 2).

To further confirm the role of the phosphotransferase system in xylitol toxicity, Hfr strain *ctr-7* of M. L. Morse, harboring a well-characterized *ptsI* mutation, was conjugated with strain 1278S, selecting for Met$^+$ His$^+$ Sm^r recombinants. Approximately half of the recombinants received the *ptsI* mutation from the donor. All those became xylitol tolerant, whereas all those that remained Pts$^+$ remained xylitol sensitive.

Xtl^s strains are derepressed for [^{14}C]fructose uptake. Both xylitol-tolerant wild-type strains and Xtl^s mutant strains have functional fructose phosphotransferase. To determine how these strains differ, we compared their uptake of [^{14}C]fructose. Uptake of [^{14}C]fructose at low fructose concentrations measures fructose phosphotransferase activity, which is approximately 10-fold inducible in wild-type *E. coli* (9).

To assure that measurements reflect differences in uptake rather than in subsequent metabolism of fructose, we compared "isogenic" strains each blocked in *fpk*, the metabolic step immediately subsequent to fructose transport. These isogenic strains were constructed by transducing strain 1278S *fpk* to Ara$^+$ from a wild-type donor and choosing one xylitol-tolerant transductant (1278S *fpkA*$^+$ XT) and one Xtl^s transductant (1278S *fpkA*$^+$ Xtl^s). Comparison of fructose uptake by these strains (Fig. 3) shows that uninduced uptake of fructose is far greater in the xylitol-sensitive strain than in the xylitol-tolerant strain. These results were confirmed by similar data obtained by comparing strains C600 and C600 Xtl^s for fructose uptake (not shown).

Preinduction by fructose makes wild-type strains phenotypically Xtl^s. Wild-type strains grown on CH liquid or plates are not inhibited by xylitol. If a wild-type culture is grown on CH + fructose, however, and then transferred to CH + xylitol, growth ceases immediately. These results (Fig. 4) support the view that fructose phosphotransferase mediates xylitol toxicity, whether derepressed genotypically or phenotypically.

Fructose protects against xylitol toxicity. The presence of low levels of fructose prevents phenotypic or genotypic xylitol toxicity. In the phenotypic experiment just reported (Fig. 4), xylitol inhibition is not observed if xylitol is directly added to the CH + fructose culture, without first removing the cells from fructose-containing medium by centrifugation. To demonstrate this effect on genotypic Xtl^s strains, 1278S and C600 Xtl^s were streaked on CH + 3 mM xylitol plates supplemented with varying concentrations (to 10 mM) of each of the following carbohydrates individually: fructose, glucose, galactose, D-xylose, ribose, maltose, sorbitol, D-arabinose. Fructose at $\geq$0.3 mM permitted growth of the two Xtl^s strains in the presence of xylitol. Of the others, only plates supplemented with ribose or galactose at much higher concentrations (>6 mM) permitted growth.

Sorbitol-dependent xylitol resistance. Since sorbitol does not prevent xylitol toxicity, only Xtl^r mutants grow on CH + xylitol + sorbitol plates. However, we observed more such mutants on these plates than on unsupplemented CH + xylitol plates. Further investigation revealed that many of the Xtl^r mutants from CH + xylitol + sorbitol plates depend on sorbitol for Xtl resistance; i.e., they grow on CH + xylitol + sorbitol plates but not on CH + xylitol plates. Mutants whose fructose phosphotransferase is altered to enable sorbitol to be a competitive inhibitor of xylitol would have this property. These mutants were not investigated further.

Xtl^s strains also are sorbose sensitive. Sorbose does not support growth of *E. coli* and is not toxic to wild-type *E. coli* or to strain C600. Strain C600 Xtl^s, however, is inhibited on CH plates supplemented with sorbose. Sorbose-tolerant mutants of C600 Xtl^s, which can be selected on CH + ≥10 mM sorbose, are Xtl^r. Xtl^r mutants of C600 Xtl^s are sorbose^r. The structural similarity of sorbose, xylitol, sorbitol, and fructose is shown in Fig. 5.

D-Arabitol toxicity: D-arabitol is toxic for a family of *E. coli* K-12 mutant strains. D-Arabitol is not toxic to wild-type *E. coli* C or K-12. We observed, however, that strain AB313, from

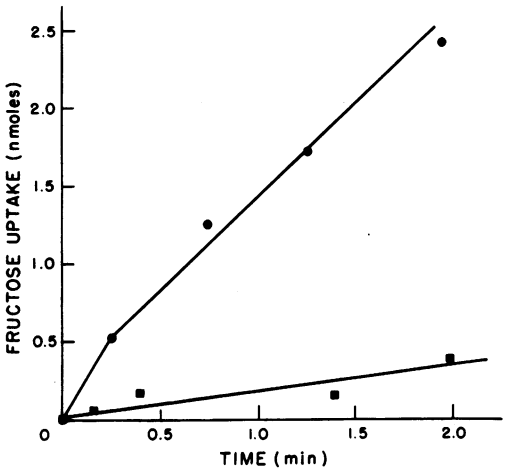

FIG. 3. *Uptake of [^{14}C]fructose by uninduced "isogenic" cultures. At t = 0, 5 × 10^6 cpm of [^{14}C]fructose (100 mCi/mmol) mixed with unlabeled fructose was added to 4-ml log-phase cultures (1.5 × 10^8 cells/ml) growing in CH medium in the absence of fructose at 37°C (final fructose concentration, 0.2 mM). At various times, 0.7-ml samples were removed and filtered by suction on 0.45 μm membrane filters (Millipore Corp.). Filters were rapidly exposed to two washes of 4 ml each of chilled CH medium, dried, and placed in scintillation vials for radioactivity determinations. Symbols: (●) strain 1278S fpkA$^+$ Xtl^s; (■) strain 1278S fpkA$^+$ XT.*

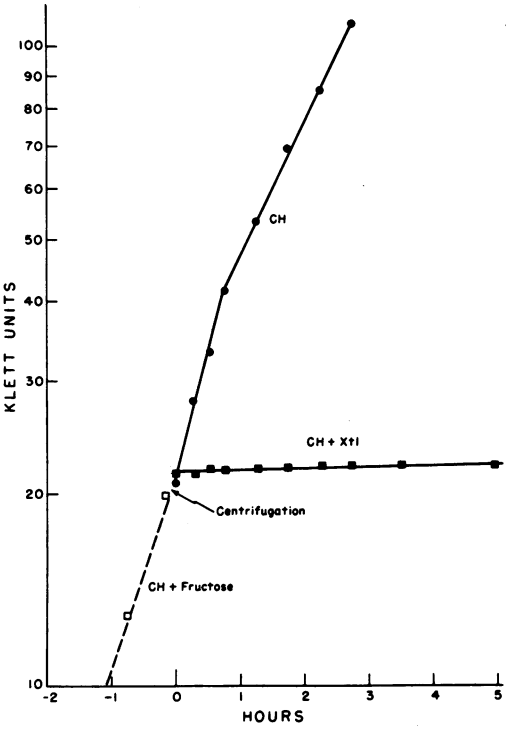

FIG. 4. *Inhibition of strain C600 after preinduction. Strain C600 was grown in CH + 5 mM fructose (□), washed by centrifugation, and suspended either into CH (●) or in CH + 10 mM xylitol (■).*

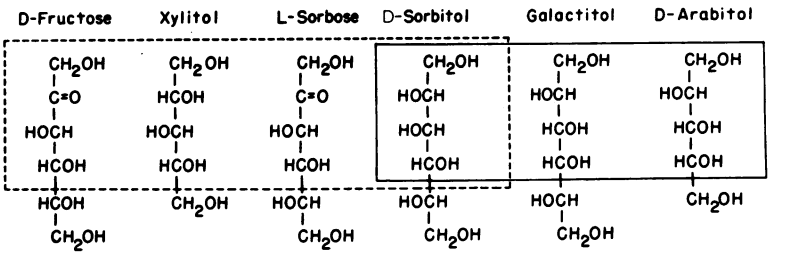

FIG. 5. *Structural similarities of sugars and sugar alcohols pertinent to pentitol toxicities.*

whose parent strain AB284 many common *E. coli* K-12 laboratory strains are derived (1), cannot form colonies on CH plates supplemented with D-arabitol. Strain AB284 is D-arabitol sensitive also, but its direct ancestor strains, Y10 and W208 (1), are D-arabitol insensitive.

D-Arabitol sensitivity and galactitol utilization. Comparison of AB313 and its D-arabitol-tolerant relative C600 for growth on many carbohydrates at 37°C revealed a single difference: AB313 segregated mutants capable of utilizing galactitol (dulcitol) for growth, but C600 did not. Further tests showed that at 30°C AB313 was Gat$^+$ and that C600 (as most *E. coli* strains) was Gat$^-$. Galactitol utilization, recognized as rare among *E. coli* K-12 strains, has been characterized in a single lineage, strain L141 and its derivatives (14), which also are Gat$^-$ at 37°C. We obtained strain L141 and found it also to be D-Atl^s. Furthermore, of the direct ancestor strains of AB313 (see above), AB284 (D-Atl^s) is Gat$^+$ but strains Y10 and W208 (both D-arabitol insensitive) are Gat$^-$.

The relationship between Gat utilization and D-arabitol sensitivity was confirmed by P1 transduction: all 24 recipients of strain 1278S transduced (from L141) to Gat$^+$ became D-Atl^s. The *gat*-D-Atl^s genes of strains AB313 and L141 may be, in fact, of single origin: L141's parent strain JC411 received *his* by conjugation with AB312, a sister strain of AB313 (1). In the transduction just reported, we found *gat* to be 75% (18/24) co-transducible with *metG*, between *metG* and *his*.

Galactitol phosphotransferase causes D-arabitol toxicity. The first two steps in galactitol utilization are transport-phosphorylation by a constitutive phosphotransferase, followed by oxidation of galactitol-1-phosphate by a dehydrogenase (14, 15). We obtained a mutant of L141 blocked in the dehydrogenase step, strain L193 (15), and found that this mutation did not relieve D-arabitol sensitivity. Strain L193 is Gat^s as well, because the dehydrogenase block results in galactitol-1-phosphate accumulation. Mutation to galactitol resistance occurs by loss of the galactitol phosphotransferase (14, 15). We isolated eight independent L193 Gat^r mutants (on CH + galactitol plates) and found all to have become also D-Atl^r. (For phenotypes of these and other L141 and L193 derivatives, see Table 3.) Similarly, we isolated eight independent L193 D-Atl^r mutants and found all to have become Gat^r. We conclude that Gat phosphotransferase is the cause of D-arabitol sensitivity in the AB284 strain lines of *E. coli* K-12.

Preinduction of strain C600 by sorbitol leads to phenotypic partial D-arabitol sensitivity. Strain C600 is Gat$^-$ and is normally unaffected by D-arabitol. When shifted from CH + 5 mM sorbitol to CH + 10 mM D-arabitol, however, under identical conditions used to demonstrate its fructose-induced phenotypic xylitol sensitivity (Fig. 4), it becomes partially inhibited by D-arabitol (growth rate is reduced by half). Preinductions by galactitol, fructose, or mannitol do not lead to D-arabitol sensitivity, nor does preinduction by sorbitol lead to xylitol sensitivity.

Derepressed sorbitol phosphotransferase causes D-arabitol sensitivity. From the preinduction results, we suspected that derepressed sorbitol catabolic enzymes catalyze a toxic synthesis from D-arabitol. Strain L141 is useful for investigating this, because its defect in mannitol phosphotransferase (*mtlA*) enables selection of sorbitol-constitutive mutants. (Mannitol is a noninducing substrate of sorbitol phosphotransferase, and most L141 Mtl$^+$ mutants are sorbitol constitutives [14].)

We first eliminated the galactitol route of D-arabitol sensitivity by selecting D-Atl^r mutants of L141. One such strain, L141R (Gat$^-$ Mtl$^-$ Srl$^+$, see Table 3) was plated for Mtl$^+$ mutants. These strains (Gat$^-$ Mtl$^+$ Srl$^+$) indeed had become D-Atl^s. One was designated L141R Mtl$^+$.

Sorbitol-constitutive *mtlA* strains use sorbi-

TABLE 3. *Relevent phenotypes of strain L141 and its derivatives*

Strain	Growth				Sensitivity	
	Galactitol[a]	Mannitol	Sorbitol	D-Arabitol	D-Arabitol	Galactitol[a]
L141	+	−	+	−	S	R
L193	−	−	+	−	S	S
L193 Gat^r	−	−	+	−	R	R
L193 D-Atl^r	−	−	+	−	R	R
L141R	−	−	+	−	R	R
L141R Mtl$^+$	−	+	+[b]	−	S	R
L141R Mtl$^+$ D-Atl^r	−	−	−	−	R	R

[a] At 30°C. Gat$^+$ strains are Gat$^-$ and Gat^s and 37°C, because a late step in the pathway, keto-bisphosphate aldolase, is temperature sensitive (15).

[b] Constitutive.

tol phosphotransferase to transport both mannitol and sorbitol, after which separate metabolic routes are used for the two substrates (14). Only if the phosphotransferase were required for the lethal synthesis would D-Atl^r mutants lose the abilities to utilize both mannitol and sorbitol. Eight D-Atl^r mutants of L141R Mtl$^+$ were selected on CH + D-arabitol plates. All were Mtl$^-$ Sbl$^-$ (Table 3), while retaining abilities to utilize fructose and other substrates tested.

By testing strains L141 and L141R Mtl$^+$ on CH + 8 mM D-arabitol plates supplemented with 1 or 10 mM galactitol, we confirmed that separate routes of D-arabitol toxicity operate in the two strains. Galactitol at either concentration reverses D-arabitol toxicity in L141 but does not (at either concentration) reverse D-arabitol toxicity in L141R Mtl$^+$, as might be expected if lethal synthesis in L141 but not in L141R Mtl$^+$ is caused by galactitol phosphotransferase.

DISCUSSION

Sugar-specific proteins (enzymes II) of the phosphotransferase system in *E. coli*, herein called "phosphotransferases," are known to lack strict specificities (6, 12–14, 16, 22). Sorbitol phosphotransferase, for example, acts on fructose (12), mannitol (16), and galactitol (14) as well as on sorbitol. These alternative substrates, however, normally are directly phosphorylated, and no novel toxic intermediates result.

Xylitol and D-arabitol, in contrast, normally are either oxidized to pentuloses before phosphorylation (*E. coli* C strains) or not catabolized (*E. coli* K-12 and B strains) (23). An expected result of phosphotransferases acting on xylitol and D-arabitol, therefore, is synthesis of phosphorylated intermediates with no route of further catabolism. We have presented evidence to show that derepressed fructose phosphotransferase results in toxic synthesis from xylitol, and that either derepressed galactitol phosphotransferase or derepressed sorbitol phosphotransferase results in toxic synthesis from D-arabitol. The structural relationships of these compounds are shown in Fig. 5. Instances of phosphotransferase action on 5-carbon compounds have not been reported previously.

The mutation that causes sensitivity to xylitol causes derepression of the normally inducible (10) fructose phosphotransferase. This mutation is co-transducible with both *ara* and *leu* and apparently represents a previously undescribed locus. Whether it codes for a regulatory protein for fructose catabolism or an enzyme that affects the intracellular concentration of the (unknown) inducer of fructose phosphotransferase is not known. Since a selection procedure exists for introducing *ara* mutations (19), the Xtl^s mutation readily can be introduced into any strain by transduction. This in turn enables the *ptsF* mutation to be introduced, by selection for xylitol resistance. Since wild-type strains are strongly inhibited by xylitol after their preinduction by fructose (Fig. 4), it also may be possible to select directly for *ptsF* mutations.

Many laboratory strains of *E. coli* K-12, descended or derived from AB284 or its derivatives (1), are Gat$^+$ and derepressed for a galactitol phosphotransferase, which is responsible for toxic synthesis from D-arabitol. The function of this phosphotransferase in Gat$^-$ (D-Atl^s) strains is not known (15). Although preinduction of xylitol-insensitive strains by fructose confers phenotypic xylitol sensitivity, D-arabitol-insensitive strains remain insensitive after preinductions by galactitol. We have found this Gat$^+$ marker, present in many common Hfr strains (including derivatives of AB311, AB312, AB313, and KL16), to be useful in conjugation selections (at 30°C), since many *E. coli* F$^-$ strains are Gat$^-$.

In addition to this route of D-arabitol toxicity, we have shown here that D-arabitol toxicity occurs in strains derepressed for sorbitol phosphotransferase. Furthermore, we have found two additional routes of toxic synthesis from D-arabitol in *E. coli*, resulting from constitutive kinases (G. A. Scangos and A. M. Reiner, manuscript in preparation). Because phosphorylated D-arabitol is apparently an especially toxic compound, and because pentitol-sensitive strains are fully inhibited by xylitol or D-arabitol even when they carry functional catabolic pathways for these substrates, we suspect that the intracellular concentrations required for toxicity may be quite low. We did not attempt to identify novel phosphorylated products in poisoned cells.

Not surprisingly, the levels of xylitol and D-arabitol required for toxicity are somewhat higher in strains with functional catabolic pathways than in strains lacking the pathways (data not shown). It is possible, therefore, that certain marginal catabolic pathways may be maintained for detoxification purposes as well as for energy-yielding purposes. The pentitol catabolic pathways in *E. coli*, present only in 5 to 10% of natural strains (23), may be examples of such marginal pathways.

We have focused here on those mutations that confer resistance by blocking the toxic

pathway, because these mutations identify the cause of the toxic synthesis. We believe that lower-level resistance is conferred also by mutations that cause increased levels of the "correct" catabolic pathway. These latter mutations are of interest to us, because they may provide a selection for gene duplications.

Acquisition of new catabolic capabilities by microorganisms most often occurs by derepression of existing catabolic pathways (18, 20, 21). Because of the general toxicity of phosphorylated sugars (7, 8, 14, 27, 28), derepressed phosphorylating enzymes pose risks. Derepressed phosphotransferases may pose the greatest risks, because they act on substrates before their cell entry. We expect that lethal syntheses impose a significant selective pressure for reestablishment of transcriptional control in the evolution of novel sugar catabolic pathways.

Xylitol recently has become a chewing-gum additive and is currently being tested as a sucrose substitute in foods, because it allegedly does not promote dental caries (26). Circumstances that cause it to become toxic to an otherwise insensitive wild-type organism have not been reported previously.

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